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Ion Reporter™ Software 5.22

USER GUIDE

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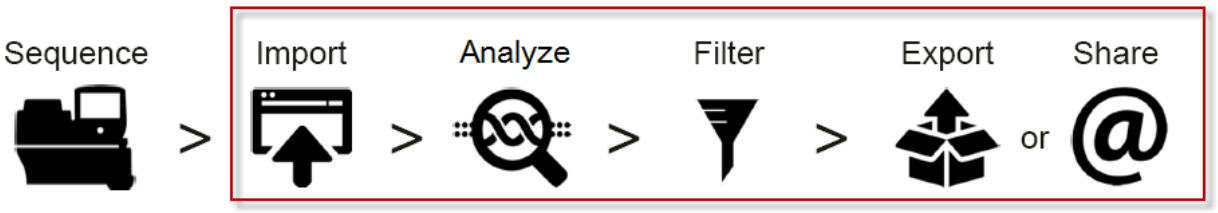
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About Ion Reporter™ Software



Ion Reporter Software functions in NGS workflow

Ion Reporter™ Software is a suite of bioinformatics tools that streamline and simplify the data analysis, annotation, and reporting of Ion Torrent™ semiconductor sequencing data.

With Ion Reporter™ Software, you have a variety of choices on how to import data into the software environment.

Ion Reporter™ Software performs analysis on BAM files that are output from Torrent Suite™ Software. VCF output files, that result from using the variantCaller plugin, can also be transferred and used for Ion Reporter™ Software analyses, provided that an annotation-only analysis workflow is used to process the files in Ion Reporter™ Software. You can also upload a BAM or VCF file manually. You can download the IonReporterUploader command-line utility if you want to import data from a command-line operating system.

Note: Ion Reporter™ Software is available under separate license and is not included with Torrent Suite™ Software.

After the data is imported, or transferred from a sequencing instrument, you can easily customize the software environment. For example, you can view a table of analysis results that contains only the data that is relevant to your research, and drag and drop columns to change the order in which the columns of data are arranged. If you download the analysis results, the columns are presented in the same order

in a TSV file. You can create preferences for these views, and toggle back and forth between different views, or use a view depending on which data you are currently viewing.

Sample data that is in Ion Reporter™ Software can be customized.

You can customize how Ion Reporter™ Software analyzes data through the analysis workflows. You can start with a predefined analysis workflow that includes optimized settings, and then change one or more settings to refine the results. You can further fine tune how the data is analyzed with parameters, filters, and copy number baselines.

You can select from extensive lists of search filters in Ion Reporter™ Software to narrow searches and refine search results to quickly find the sample, analysis workflow, or analysis results of interest. After you search, your selections remain active for your next search, or you can choose to reset the filters.

Visualizations of analysis results can be launched in Ion Reporter™ Software with Ion Reporter™ Genomic Viewer (IRGV), or with Integrative Genomics Viewer (IGV), depending on a preference that you set. You can also determine how many rows of analysis results are shown in tables within either of these applications.

You can also create customized reports that show details about the data, and annotations and details about the sequencing run or runs that were used to obtain data. Report options allow you to choose which sections you want to include, drag-and-drop to reorder those sections, preview a PDF report, then save and print the report.

Ion AmpliSeq™ and Ion AmpliSeq™ HD panel types

AmpliSeq.com offers the following design and ordering options for Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels.

- Ion AmpliSeq™ On Demand panels of optimized amplicons for germline analysis. These panels are configurable to a specific human disease area, and ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ Custom DNA panels for germline or somatic analysis of any genome, and Made-to-Order Ion AmpliSeq™ Custom RNA panels for analysis of human gene expression and gene fusion events. These panels are ordered in large reaction packs.
- Made-to-Order Ion AmpliSeq™ HD Custom ultra high-sensitivity DNA and RNA panels for liquid biopsy or somatic analysis of human DNA variants and RNA fusion events, including UMTs (Unique Molecular Tags) and dual barcodes.
- Oncomine™ tumor specific panels, optimized for use with FFPE-derived DNA and RNA. Cancer-type specific core panels can be customized by adding or removing gene targets from inventory. Each panel consists of 2 DNA primer pools and an optional RNA pool for fusion event detection.
- Ion AmpliSeq™ Kitted Panels, predesigned DNA and RNA panels for different applications. These panels are ordered in small reaction packs.

hg19 and GRCh38 human genome references

In Ion Reporter™ Software you can use human genome references hg19 or GRch38 for either predefined or user-defined analysis workflows. You can use the Ion GRch38 human reference when you create analysis workflows for panels that are designed based on this reference. The GRch38 reference genome in Ion Reporter™ Software is based on the latest GRC human reference assembly and is the first major update since 2009. Highlights include changes to chromosome coordinates, fixed errors in the former sequence, addition of mitochondria, and multiple loci for some highly variable genes.

Ion AmpliSeq™ Designer currently offers the GRch38 human reference and related target reference (BED) file for designs based on the GRch38 reference.

Note: If you edit earlier versions of analysis workflows that use the hg19 reference genome, you cannot change the reference genome to Ion GRch38.

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

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Purchasing and ecommerce

You can use your Thermo Fisher account credentials to manage your Ion Reporter™ Software and related third-party applications.

Note: You do not have to change your lifetechnologies.com user name and password to access Ion Reporter™ Software. All your legacy account options remain intact.

Create a Thermo Fisher account

Each Ion Reporter™ Software organization needs at least one user with a full Thermo Fisher account.

1. Go to **thermofisher.com**.
2. Click **Sign In** ▸ **Register**.
3. Complete the requested information, then click **Create account**.

You will receive a confirmation email after you create the account.

Ion Reporter™ Software on Connect

Ion Reporter™ Software on Connect, the cloud-based platform, is available at **https://ionreporter.thermofisher.com**.

Connect is the Thermo Fisher Scientific free, cloud-based solution. Use Connect to access scientific analysis applications, and peer and document collaboration tools. Additionally, when your lab's instruments are connected, you have access to the full breadth of features that can help boost productivity and efficiency in your lab. Connect is backed by our secure, cloud-based data storage (formerly Thermo Fisher Cloud) and includes 1 TB of storage at no charge.

Access to Ion Reporter™ Software on Connect is controlled by your **thermofisher.com** user name and password. Samples, data, and results in the software are controlled through "organizations". Members of an organization share data and results in Ion Reporter™ Software on Connect in the same way that users of a particular Ion Reporter™ Server organization share data and results.

To use Ion Reporter™ Software on Connect, you must first create a Thermo Fisher account at **thermofisher.com** with a user name and password, and then either create a new organization on Ion Reporter™ Software on Connect or be invited to join an existing organization. Each Ion Reporter™ Software user can belong to only one organization at a time. You can leave an organization at any time to join or create a different organization, but you lose access to the data and results of the organization that you left.

Ion Reporter™ Software storage

To use Ion Reporter™ Software on Connect, subscriptions are not required. Ion Reporter™ Software customers are given 1 TB of storage free on Connect. To obtain larger amounts of space, see the Connect Help for subscription information.

Create an organization on Ion Reporter™ Software on Connect

The first user on Ion Reporter™ Software on Connect can create an organization. An organization is more specific than an institute name. It defines a group of users who work together and share samples, data, analyses, and results.

When you create an organization, you automatically are the first administrator-level user of that organization. You can then begin entering or importing data into the software and invite other Ion Reporter™ Software on Connect users to your organization to share data and results. To join an existing organization, see “Join an existing Ion Reporter™ Software organization on Connect” on page 22.

You can belong to only one organization at a time. If you are already a member of an Ion Reporter™ Software organization on Connect, you will not be able to create a new organization until you are removed as a user from your current organization.

1. If you have not already done so, create a new account on **thermofisher.com**. Your user name and password are also used to sign in to Ion Reporter™ Software on Connect. Your user name is your email address.
2. Go to **https://ionreporter.thermofisher.com/**. Or, sign into the Connect, then under **My Apps**, click **Ion Reporter**.
3. On the software sign-in screen, click **Sign In**. If you are not already signed in to **thermofisher.com**, you are prompted to enter your user name and password.
4. When you sign in to Ion Reporter™ Software for the first time, you are prompted to create a new organization. Click **Yes**, then enter an organization name.

   Enter a name that describes your organization to other users whom you may invite to share data and results.

   The **User Manager** screen for Ion Reporter™ Software on Connect opens, and you are listed as an administrator-level user.
5. To return to the software, click the Connect **(Home)**, then under **My Apps** click **Ion Reporter**.
6. Accept the license agreement to begin using the software.

Invite a user to an organization on Connect

An administrator of Ion Reporter™ Software on Connect can invite other users on Connect to join their software organization.

1. In Ion Reporter™ Software on Connect, click the **Admin** tab.
2. In the **Users** screen, follow the link to **User management**.
3. In the **User management** screen, click **Action** ▸ **Invite User**.
4. In the **Invite User** screen, enter the email address of the user who you want to invite, then select one or more roles to assign to the user.
   - **Import**
   - **Analyze**
   - **Report**
   - **Admin**
5. Click **Invite**.

   The user receives an email invitation to join the organization. For more information, see “Join an existing Ion Reporter™ Software organization on Connect” on page 22.

Sign in to Ion Reporter™ Software on Connect

You need to have an account on **thermofisher.com** to sign in to Ion Reporter™ Software on Connect. The user name and password for the website are also used to sign in to the software.

You need to also be a member of an organization to use Ion Reporter™ Software on Connect. For more information, see "Create an organization on Ion Reporter™ Software on Connect" on page 20 or "Join an existing Ion Reporter™ Software organization on Connect" on page 22.

1. Go to **https://ionreporter.thermofisher.com**, then click **Sign In**.
2. Enter your user name and password, then click **Sign In**.
3. In the screen that opens, enter the two-factor authentication code from email that contains the code.
   a. *(Optional)* Select **Remember for 72 hours** to use the same computer for 72 hours to sign in with the same user ID without re-entering the authentication code. This setting applies only if you subsequently sign in using the same browser and computer that is used the first time you sign in. If another computer is used, or another user signs in, an authentication code must be entered.
   b. Click **Verify**.
      A message that the account has been verified is shown on the screen.
4. If the **Connect Terms of Use** opens, review the terms, then click **Accept** to approve the terms, then proceed in Ion Reporter™ Software.

Ion Reporter™ Software on Connect opens to the **Dashboard**. For more information, see "Ion Reporter™ Software dashboard" on page 26.

Join an existing Ion Reporter™ Software organization on Connect

You can join an existing Ion Reporter™ Software organization on Connect by invitation from a software administrator in that organization. For more information, see "Invite a user to an organization on Connect" on page 21.

When you join an organization, you can share data and results with other users in that organization. To create a new organization, see "Create an organization on Ion Reporter™ Software on Connect" on page 20.

You can belong to only one organization at a time. If you are already a member of an Ion Reporter™ Software organization on Connect, you cannot accept an invitation from another organization until you are removed as a user from your current organization.

1. If you have not already done so, create a new account on **thermofisher.com**. Your user name and password are also used to sign in to Ion Reporter™ Software on Connect. Your user name is your email address.
2. When an Ion Reporter™ Software administrator for the organization adds you as a new user, an email with an invitation to join the organization is sent to your email address.
3. Click the link in the email Invitation.

4. If you do not already have an account on **thermofisher.com** with the email address that the invitation was sent to, you are prompted to create one.

   The email address of your account must match the address that the invitation was sent to.

5. In the screen that opens, enter the two-factor authentication code from email that contains the code.

   a. *(Optional)* Select **Remember for 72 hours** to use the same computer for 72 hours to sign in with the same user ID without re-entering the authentication code. This setting applies only if you subsequently sign in using the same browser and computer that is used the first time you sign in. If another computer is used, or another user signs in, an authentication code must be entered.

   b. Click **Verify**.

      A message that the account has been verified is shown on the screen.

6. In the **Confirm Organization creation** dialog box, accept the invitation for the organization. Select the organization name to accept and click **Ok** to accept the invitation.

   - Click **No** to sign in with the verified account and join an existing organization.
   - Click **Yes** to create a new organization.

7. Accept the **Terms of Use** to start using Ion Reporter™ Software.

Ion Reporter™ Server

Always access Ion Reporter™ Server through HTTPS (*not* HTTP).

The URL depends on your server configuration, and is named `https://ion-reporter-server-ip-address`, where `ion-reporter-server-ip-address` is the IP address or hostname of your Ion Reporter™ Server.

Create an organization on Ion Reporter™ Server

The first user on Ion Reporter™ Server can create an organization. An organization is more specific than an institute name. It defines a group of users who work together and share samples, data, analyses, and results.

When you create an organization, you automatically are the first administrator-level user of that organization. You can then begin to enter or import data into the software and invite other Ion Reporter™ Server users to the organization to share data and results.

You can belong to only one organization at a time. If you are already a member of an organization on Ion Reporter™ Server, you will not be able to create a new organization until you are removed as a user from your current organization.

Note: You can create an organization on an Ion Reporter™ Server when you request a new user account on the server. You are not required to have a user account to make this request.

1. On the software sign-in screen click, click **Register new account**.
2. In the **Request Account** dialog box, enter the following information: organization name, organization address, your first and last name, email, phone number.

   If the Ion Reporter™ Server user is connected to the Internet, you must select the checkbox in the dialog box.
3. Click **Submit**.

   A new organization is created and you become the organization administrator. You will receive an email with a link to set the password.
4. Click the link in the email and set the password.
5. After the password is set, you can sign in using the new password. The first time you sign in, you must accept the **Terms and Conditions**.

You can now begin using the new Ion Reporter™ Server organization.

Sign in to Ion Reporter™ Software on Ion Reporter™ Server

Before you can sign in to Ion Reporter™ Software on Ion Reporter™ Server, you must have a user account on the server. An administrator-level user on the server can create an account for you. To sign in to the software on the server:

1. In a browser on the same network as the Ion Reporter™ Server, enter the server URL: `https://ion-reporter-server-ip-address`, where *ion-reporter-server-ip-address* is the IP address or host name of the server.
2. Enter your user name and password, then click **Sign In**.

User roles and permissions

In Ion Reporter™ Software, access is granted to specific functions based on roles that are assigned to users. Typically, an Ion Reporter™ Software organization has different team members performing different functions. Therefore, each user role within Ion Reporter™ Software has access to different areas of the software.

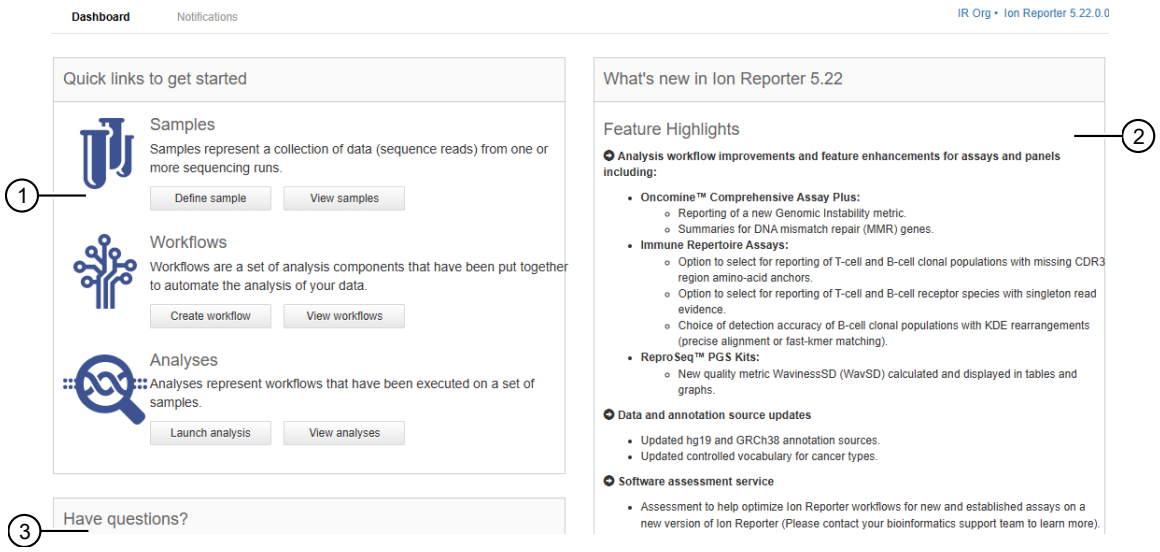
User role	Access
Import	The **Import** user is the person in the Ion Reporter™ Software organization who can import and define samples. Specific access includes the following tasks. • Upload data. • View samples. • Define samples. • View the analyses list (but not review results). • Launch analyses. • Access the **Samples** and **Analyses** tabs.
Analyze	The **Analyze** user is the person in the Ion Reporter™ Software organization who can create analysis workflows and launch analyses. Specific access includes the following tasks. • View samples. • Define samples. • Create or modify analysis workflows. • View analysis workflows. • Launch an analysis. • View analyses list. • Review and filter analysis results. • Access the **Samples**, **Analyses**, and **Workflows** tabs.
Report	The **Report** user is the person in the Ion Reporter™ Software organization who can generate reports. Specific access includes the following tasks. • View the analyses list (and view and classify results). • Generate reports and sign off on final and visualization reports using an electronic signature. • Select and classify variants. • Access the **Analyses** tab.

(continued)

User role	Access
Admin	The **Admin** user is the person in the Ion Reporter™ Software organization who can manage user access. Specific access includes the following tasks. • View samples. • View analysis workflows. • View the analyses list (but not view results). • Access the **Samples**, **Analyses**, **Workflows**, and **Admin** tabs. • Create and modify users. • Download and install plugins. • Update software on Ion Reporter™ Server. For more information about software upgrades, contact your Field Service Engineer (FSE).

Ion Reporter™ Software dashboard

You can open the dashboard in Ion Reporter™ Software, then navigate to various screens, view important information about the software, and access support resources. Click the **Home** tab, then click **Dashboard**.



① Quick links pane
② Announcements pane
③ Support pane

The **Quick links** pane includes:

- **Define sample**—Opens the **Define Samples** screen, where you can upload and define samples.
- **View samples**—Opens the **Samples** screen, where you can view, search, sort, or filter samples.
- **Create workflow**—Opens the **Create Workflow** wizard, where you can create a new analysis workflow.
- **View workflows**—Opens the **Workflows** screen, where you can view, search, sort, or filter analysis workflows.
- **Launch analysis**—Opens the **Launch Analysis** wizard, where you can select an analysis workflow, add samples and plugins, and launch an analysis.
- **View analyses**—Opens the **Analyses** screen, where you can view, search, sort, or filter analyses.

The **Announcements** pane includes a link to the latest Ion Reporter™ Software Release Notes and information on upcoming events.

The **Have questions?** pane includes:

- **Help section** link—Opens the software help menu.
- **Send feedback** link—Opens a help topic about how to send feedback regarding your experience with Ion Reporter™ Software.
- Send an email to **ionreporter@lifetech.com**—Opens Microsoft™ Outlook and creates a new email addressed to Ion Reporter™ Software support.
- Contact **Web Support**—Opens the **Services & Support** web page.

The **What's new in Ion Reporter** pane describes new features.

Note: Only the tabs and quick links corresponding to your user role are displayed. For more information, see “User roles and permissions” on page 25.

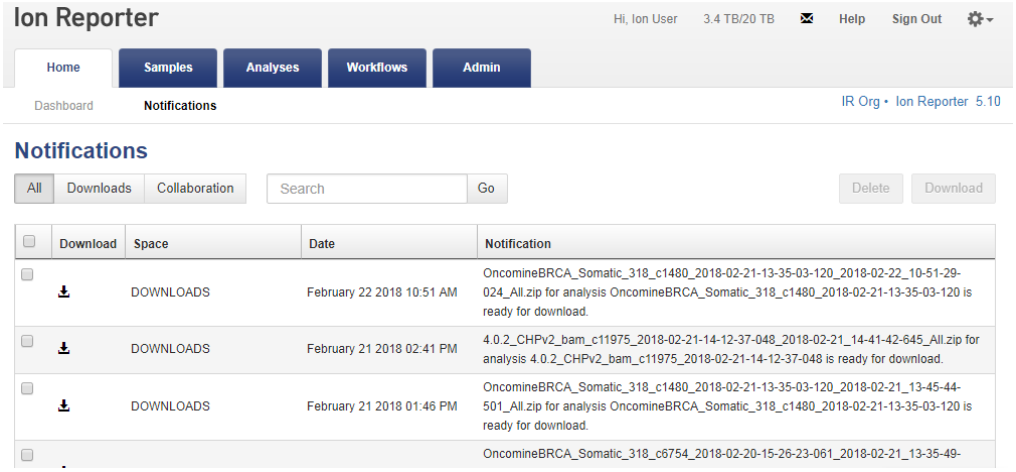
View notifications

The **Notifications** screen lists notifications for files that are generated by the Ion Reporter™ Software and for files that have been shared. Notifications are shown both for files that are shared by you with another user and for files that you share with another user.

From the **Notifications** screen, you can download completed analyses, reports, and files that have been shared with you by another user.

1. To view the **Notifications** screen, select one of the following actions.
   - In the **Home** tab, select **Notifications**.
   - From any screen, click ✉ **(Notifications)** in the menu bar.

   The **Notifications** screen opens.



The filename and status of a given file are shown in the **Notification** column.

2. *(Optional)* Click one of the following to change the list view.

Subtabs for Notifications window	Description
All	Show only notifications for all files that are available for download and files that have been shared.
Downloads	Show only notifications for all files generated within the software that are available for download.
Collaboration	Show only notifications for all files that have been shared.

3. *(Optional)* Enter a term in the search box to search for a file.

4. Select one of the following options.

Option	Description
Download a file	Do one of the following: • Click the **(Download)** link in the row of a notification. • Select the checkbox of a notification, then click **Download**.
Delete the notification	Select the checkbox for a notification, then click **Delete**. **Note:** Deleting a notification deletes only the notification from the notification list and does not delete the file. To delete a file, see the appropriate procedures for that file type.

File type	Topics
Analyses	Analysis files contain the results of sequencing runs. For more information, see Chapter 6, “Analysis results”.
Samples	Sample files contain a list of all samples from a sequencing run that are to be analyzed. For more information, see Chapter 3, “Import and manage samples”.
Reports	Reports are summaries of sequencing runs. For more information, see Chapter 9, “Create and view reports” for details.

Status alert emails

Ion Reporter™ Software sends an email to the user who is signed in and completes any of the following actions.

- Completes a sequencing run on an instrument that transfers run report files through the IonReporterUploader plugin. For more information, see *Torrent Suite™ Software Help*.

  Note: A single email is sent if multiple samples or barcodes are transferred from a single Torrent Suite™ Software run report. Additional emails are sent when analyses are deleted from the batch, or if some analyses remain in the Pending or Running state, then complete at a later time.

- Creates an Ion Reporter™ Software account
- Resets a password
- Deletes an account
- Shares an analysis
- Unshares an analysis
- Creates a new organization
- Generates a report generation request

Users with limited storage space will also receive a status email.

Dear Users,

Your Ion Reporter account is currently using all of the allocated 20 TB. You will not be able to import new data or run an analyses until you either purchase a larger storage subscription or remove data from your account.

Reference material and support resources are available at the following websites:
https://www.thermofisher.com/us/en/home/products-and-services/promotions/ion-community.html?icid=ThBan-IonBrand-IonCommunity-1216

https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html

If you have questions, contact your local bioinformatics team:
ngs-amsupport@thermofisher.com (Americas)
ngs-eusupport@thermofisher.com (EMEA)
ngs-gcsupport@thermofisher.com (Greater China)
ngs-sasiasupport@thermofisher.com (South Asia)
jptech@thermofisher.com (Japan)

APAC customers, please contact your local sales representative, Field Service Engineer or Field Bioinformatics Specialist, send an email to ionreporter@thermofisher.com.

Thank you,

Example of a storage warning message

Dear All,

Chintan Vora has completed the analysis for Demo VCF_c191_2017-06-03-14-06-544 and has requested a report be generated.

Reference material and support resources are available at the following websites:
https://www.thermofisher.com/us/en/home/products-and-services/promotions/ion-community.html?icid=ThBan-IonBrand-IonCommunity-1216

https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html

If you have questions, contact your local bioinformatics team:
ngs-amsupport@thermofisher.com (Americas)
ngs-eusupport@thermofisher.com (EMEA)
ngs-gcsupport@thermofisher.com (Greater China)
ngs-sasiasupport@thermofisher.com (South Asia)
jptech@thermofisher.com (Japan)

APAC customers, please contact your local sales representative, Field Service Engineer or Field Bioinformatics Specialist, or send an email to ionreporter@thermofisher.com.

Thank you,
Your Ion Reporter Software team

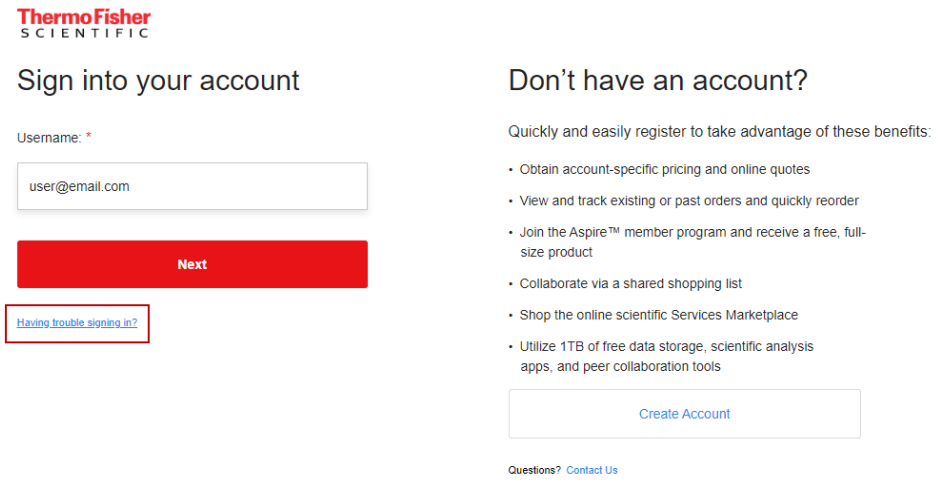
Example of a message that is generated when an analysis is complete and a request is sent to the person in the report role to generate a report.

Sign-in troubleshooting and support contacts for Ion Reporter™ Software

This section addresses questions that are related to how to troubleshoot sign-ins and get support for the Ion Reporter™ Software.

What can I do if I am locked out of my account?

- If you are a server user and have been locked out of your account (system locks you out after five attempts to login in with an incorrect password), you must contact your administrator to reset your password. For details on password reset, see “Set or reset the password on a user account on Ion Reporter™ Server” on page 512.
- If you use Ion Reporter™ Software on Connect, click **Sign In**, then click **Having trouble signing in?** on the sign in form to reset your password.



How do I contact support if I have additional questions?

Use one of the following options if you need help with Ion Reporter™ Software.

- Send an email to **ionreporter@lifetech.com**.
- For ordering and commerce issues, contact your local Technical Support representative at **https://www.thermofisher.com/us/en/home/technical-resources/order-support.html**.

What special characters are allowed in names?

- Most names in Ion Reporter™ Software, including names used for analyses, samples, user-defined analysis workflows, users, other text fields, and BAM files that are used to import samples into the software, allow these characters:
- – Alphanumeric characters: A-Z a-z 0-9
  – Underscore: _
  – Space: ' '
  – Dash: -
  – Period: .
  The period character (.) is not allowed at the beginning of the names the following elements: Project name, Workflow name, Sample name, any analysis Workflow Preset name, Analysis name, baseline name.

If unsupported characters are entered, the field appears in red with a bold outline.

Set default number of rows displayed in tables

You can set a default number of rows displayed in all tables in the Ion Reporter™ Software.

1. In the upper right corner of the screen, click ⚙ **(Settings)** ▸ **Account Preferences**
2. In the account Preference dialog box, select the number of rows to display in all tables from the **Default rows per page** dropdown menu, then click **Save**.
   The new settings are applied to all tables within the Ion Reporter™ Software.
3. *(Optional)* To override the default setting for a specific table, navigate to the table, then select the number of rows you want to display from the **items per page** dropdown menu located below the table.

IonReporterUploader command-line utility

The IonReporterUploader command-line utility is an command-line alternative to the IonReporterUploader plugin that is included with Torrent Suite™ Software. You can download the IonReporterUploader command-line utility at: **http://iru.ionreporter.thermofisher.com/**.

You can use the IonReporterUploader command-line utility to transfer analysis results files to Ion Reporter™ Software and to set up samples, based on the transferred files. The IonReporterUploader command-line utility uses the login ID to transfer run data from Torrent Suite™ Software to Ion Reporter™ Software. The utility supports the following types of transfers.

- Transfers of a single BAM or VCF file.
  The name of a BAM file that is uploaded to Ion Reporter™ Software cannot exceed 200 characters.
- Transfers of all results files for a Torrent Suite™ Software analysis.
- Transfers of results files that are in a single flat folder.
- Transfers of multiple files that are not restricted to a single folder.

You can later analyze the samples with Ion Reporter™ Software. The IonReporterUploader command-line utility can be run on the Torrent Server, or can be run on any standard (and current) computer that runs Linux™, macOS™, or Windows™ Operating System. You can enter IonReporterUploader command-line utility options through command line arguments, or through a properties file.

Note: IonReporterUploader command-line utility should not be used for uploading samples from references other than hg19 and GRCh38. Although E-coli and animal reference genomes can be used in Torrent Suite™ Software, they are not supported in Ion Reporter™ Software.

IonReporterUploader command-line utility supports the upload of combined run results that are output by using the **Combine Alignments** option in the Torrent Suite™ Software. Uploads of combined run results are not supported when the IonReporterUploader plugin is used in Torrent Suite™ Software.

Java™ 11 is required to run IonReporterUploader command-line utility without errors.

Note: Java™ is included with Ion Reporter™ Software. If you are running Java™ 9 or later, you need to also have Java™ 8 for the JNLP to act correctly to visualize variants from Ion Reporter™ Software with the Broad Institute Integrative Genomics Viewer (IGV).

For more information about IonReporterUploader command-line utility, see the *IonReporterUploader Command-Line Utility User Guide* (Pub. No. MAN0017648).

Download IonReporterUploader command-line utility

This procedure explains how to download and extract the IonReporterUploader command-line utility. The procedure can vary, based on the operating system of the target computer. In general, decompress the ZIP file on the target machine, then copy the folder `IonReporterUploader-cli` to a convenient location.

IMPORTANT!

- Download the utility onto the computer where it is to be run. At a minimum, download the utility to a computer with the same operating system as the computer where it is to be run.
- Use only the decompression utility available on the local computer. Do not decompress files on one operating system, then copy those files to a computer that uses a different operating system.

The IonReporterUploader command-line utility is an alternative to the IonReporterUploader plugin that is included with Torrent Suite™ Software.

1. To access the utility, go to **http://iru.ionreporter.thermofisher.com/**.
2. Click the file name `IonReporterUploader-cli.zip`, then download the file to the target computer.
3. Extract the downloaded `IonReporterUploader-cli.zip` file, then copy the `IonReporterUploader-cli` folder to a convenient location on the target computer.

Run IonReporterUploader command-line utility

The IonReporterUploader command-line utility `irucli` is ready to run after you extract it. Run the IonReporterUploader command-line utility from the `IonReporterUploader-cli` bin directory (with the `irucli.bat` or `irucli.sh` script).

Instructions to use the IonReporterUploader command-line utility on a standard Linux™ computer are downloaded with the utility.

For more information, see the *IonReporterUploader Command-Line Utility User Guide* (Pub. No MAN0017648.)

3 Import and manage samples

- Samples tab overview 35
- Sample attributes overview 36
- Sample import options 44
- Sample definition 49
- Find samples 58
- Customize the Samples table 60
- Transfer samples to users in other organizations 62
- Archive samples to an external storage device 64
- Restore archived samples from an external device to Ion Reporter™ Server 65
- Delete a sample 66

In Ion Reporter™ Software, the data and attributes that characterize the genomic data are called samples.

Samples originate as either data files in BAM format, or a single variants file in VCF format. Both types of files contain metadata, including attributes that characterize the samples. For example, a human DNA sample can include a gender attribute to indicate whether the sample originated from a male, a female, or whether the gender is unknown.

Samples can be automatically transferred from the sequencer through Torrent Suite™ Software to Ion Reporter™ Software using the IonReporterUploader plugin, or imported manually as individual BAM or VCF files. For more information see, “Sample import options” on page 44.

A set of analysis results is available in Ion Reporter™ Software when an analysis workflow is used to analyze a sample. You can view analysis results as tabular data, or visually in graphs, interactive charts, plots, and histograms. To view analysis results in a genomic context, you can use Ion Reporter™ Genomic Viewer (IRGV), a visualization tool that is included with Ion Reporter™ Software.

You can also download analysis results and visualizations. For more information, see “Download analysis files” on page 186 and Chapter 8, “Visualize analysis results with Ion Reporter™ Software”. Data and graphics from the visualizations can also be added to PDF reports that you generate in Ion Reporter™ Software. For more information, see “Visualization reports” on page 412.

Samples tab overview

You can access the list of available samples in the **Samples** table, which is located in the **Samples** tab, in the **Overview** screen.

① **Samples** tab

② The **Overview** screen in the **Samples** tab. Click the screen name to toggle between the different screens.

③ The **Presets** screen. For more information, see “Sample presets” on page 42.

④ The **Analyzed: All** filter is the default filter that is set on the **Samples** tab. For more information, see “Filter samples” on page 59.

⑤ **More Filters** allows you to add additional filters to the **Samples** table.

⑥ **Refresh** refreshes the list of samples.

⑦ Apply **Preferences** to customize how the **Samples** table is organized. For more information, see “Customize the Samples table” on page 60.

⑧ **Define Samples** allows you to assign a unique sample name to a collection of one or more BAM files and the corresponding sample attributes. You can also add or remove sample attributes and edit existing sample attribute values. For more information, see “Sample definition” on page 49.

⑨ You can enter keywords or text strings in the **Search** box to narrow the list of samples. For more information, see “Search for samples” on page 58.

⑩ Additional filters, added by using **More Filters**.

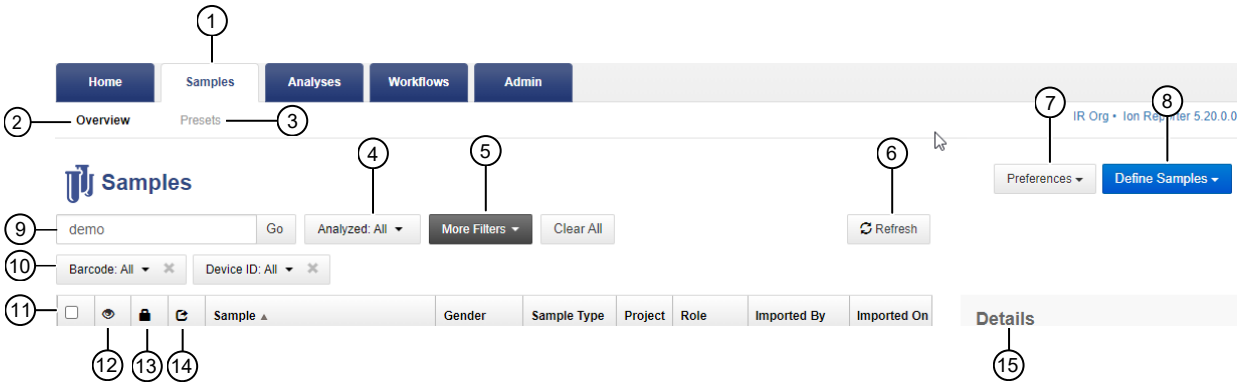
⑪ Table columns with information about the sample. For more information, see “Sample attributes” on page 37.

⑫ **(Analyzed sample)** Indicates that a sample has been analyzed.

⑬ **(Locked sample)** Indicates that a sample is locked and cannot be edited.

⑭ **(Transferred sample)** Indicates that the sample was transferred to a user in another organization

⑮ **Details** provides additional information about the selected sample.



Sample attributes overview

Samples contain attributes that are relevant to genetic research, such as information about gender, tumor, or a sample type such as RNA or DNA.

Sample attributes in Ion Reporter™ Software often originate in a Torrent Suite™ Software Planned Run. In Ion Reporter™ Software, you can add attributes and add values to those attributes that were not included in the Planned Run. You can also edit the values of existing attributes. For more information, see “Define samples with a CSV file” on page 53, “Edit a sample” on page 56 and “Edit a sample used in an analysis” on page 57.

Sample attributes are available as search filters in the **Samples** table. You can filter samples to find samples of interest, and then use the search results to launch analyses for a sample or a group of samples. For more information, see “Filter samples” on page 59 and “Launch an analysis” on page 165.

View sample attributes

When you select a single sample that is listed in the **Samples** table in Ion Reporter™ Software, you can view details for its attributes in the **Details** pane. Each column in the table represents a single sample attribute.

1. In the **Samples** tab, click **Overview**.
2. Select the row that contains the sample of interest.

Details that describe sample attributes, such as gender, role, or type, are listed in the **Details** pane.

Note: If you select more than one sample with checkboxes in the row, the **Details** pane shows only the sample details for the sample that you select last.

Sample attributes

User-defined and predefined sample attributes can be viewed in the **Samples** table in Ion Reporter™ Software. Each column in the **Samples** table represents a single sample attribute.

A set of default columns is always visible in the table. You can also add attributes to the table. To add more attribute columns to the table, or create a reusable table preference, see “Customize the Samples table” on page 60. After you add more attributes to the table, you can further customize the table with filters that you can use to find the attributes. For more information, see “Find samples” on page 58.

Several sample attributes can be edited in Ion Reporter™ Software. For more information, see “Edit a sample” on page 56, “Edit a sample used in an analysis” on page 57, and “Define samples with a CSV file” on page 53.

Each description indicates which attributes cannot be changed.

Attribute	Description
Default columns—These columns are selected by default when you open the Samples table if no table preferences are applied.	
☐	Selection checkbox—Use to select samples to include in analyses.
	Analyzed sample—Indicates that the sample has already been analyzed.
	Locked sample—Indicates that the sample attributes cannot be edited. After a sample is locked, the sample attributes and the files that make up the sample cannot be edited.
	Transferred sample—Indicates that the sample was transferred to a user in another organization.
Sample	The name of the sample.

(continued)

Attribute	Description
Gender	Information about the gender of the sample. • **Female** • **Male** • **Unknown** **IMPORTANT!** Several analysis workflows in Ion Reporter™ Software (for example, the Ion AmpliSeq™ IDP Trio analysis workflows) are limited when the gender is unknown. The analysis workflows can return unexpected results when the gender is incorrectly specified for a sample.
Sample Type	Information about the type of sample. • **DNA** • **DNA NTC** (No Template Control) • **Metagenomics** • **RNA** • **RNA NTC** (No Template Control) • **MSI On-Chip Control** • **TNA (Total Nucleic Acid)**
Project	A list of one or more project names that have been associated with a sample.
Role	The role in a pair or a group of samples, such as mother, father, proband, or unknown.
Imported By	The user name of the person who imported the sample.
Imported On	The date that the sample was imported.
Available columns—These attributes can be added to the Samples table.	
Bacterial Marker Type	Only one bacterial marker type can be associated with each barcode in a sample. The list of available bacterial marker types is divided into two categories: • **16S rRNA Gene** • **Target Species**
Barcode	The name of the specific barcode in the selected barcode kit. This attribute cannot be changed.
Biopsy Days	The time point post-fertilization at which the sample was taken from an embryo biopsy.
Cancer Type	The type of cancer of the sample.
Cell Num	The cell count of the biopsy material.
Chip ID	The number assigned to the chip. This attribute cannot be changed.

(continued)

Attribute	Description
Chip Type	The chip type, such as Ion 550™ Chip. This attribute cannot be changed.
Control Sequencing Kit	The control kit used when preparing the sample. This attribute cannot be changed.
Couple ID	An identifier for use with the Reproductive research application.
Device ID	The number of the sequencing instrument. This attribute cannot be changed.
Embryo ID	The number or name assigned to the embryo.
Instrument	The name of the sequencer. This attribute cannot be changed.
Instrument Type	The type of sequencer, such as Ion GeneStudio™ S5 Sequencer. This attribute cannot be changed.
IR Uploader Version	The software version number for the IonReporterUploader plugin. This attribute cannot be changed.
Library Kit Name	The name of the library kit used to prepare the sample. This attribute cannot be changed.
Mouse Strain	If a mouse sample was used, the strain of the mouse sample. • **129/Sv** • **A/J** • **BALB/c** • **C57BL/6** • **I/St** • **MLR/pr** • **NFS** • **NZB** • **Unknown** For use with the T-cell and B-cell receptor sequencing analyses. Samples that use the mouse strain attribute can be added to a user-defined analysis workflow. Available options are based on the most common mouse strains in the IRGT database.
Percentage Cellularity	The percent of tumor cells in the sample. **Note:** The **Percentage Cellularity** sample attribute is required for DNA samples. **IMPORTANT!** Do not change this setting for workflows that are used with the Oncomine™ TagSeq Breast DNA Regions, Oncomine™ TagSeq Lung DNA Regions, and Ion AmpliSeq™ HD assays. These assays are targeted to cell free DNA (cfDNA) and do not estimate tumor cellularity because calls are based on P values and cannot be quantified as a ploidy value.

(continued)

Attribute	Description
Plan Name	The name of the Planned Run used in Torrent Suite™ Software. This attribute cannot be changed.
Population	The ethnicity indicator for the sample. If **European** is selected, the haplotype of a T-Cell receptor is included in the analyses results for an Ion AmpliSeq™ Immune Repertoire Assay Plus—TCRβ assay. You can download a corresponding Haplotype Group Assignment plot. For more information about a TCRB-LR assay, see "Immune repertoire analysis results" on page 240 **Note:** The haplotype assignment compares a query sample against a reference database that is derived from European ethnicity samples. You must therefore select the **European** population value to assign a haplotype. The haplotype assignment will be blank if other population values are selected. The super populations from the 1000 Genomes Project are available as values. • **Unknown** • **African** • **Ad Mixed American** • **East Asian** • **European** • **South Asian** For information about super populations, see **www.1000genomes.org**.
Run Date	The date of the sequencing run. This attribute cannot be changed.
Run ID	The number of the sequencing run. This attribute cannot be changed.
Run Name	The name of the Planned Run used in Torrent Suite™ Software. This attribute cannot be changed.
Sample Collection Date	The date that the sample was collected from the biological subject. The **Sample Collection Date** must precede the **Sample Receipt Date**.
Sample Receipt Date	The date that the sample was received by the processing laboratory. The **Sample Receipt Date** must be later than or the same as the **Sample Collection Date**.

(continued)

Attribute	Description
Sample ID	A unique identification code (SampleID) for each barcode in a sample. This helps to track samples or possibly identify misassignment between samples and barcodes in a sequencing run. If you manage samples in an external system (for example, a LIMS), you can use the identifier from that system. This attribute cannot be changed.
Sample Preparation Kit	The name of the sample preparation kit used in Torrent Suite™ Software. This attribute cannot be changed.
Sequencing Kit Name	The name of the sequencing kit used in Torrent Suite™ Software. This attribute cannot be changed.
Sequencing Run Type	This value defines the type of sequencing run, for example, **Single End** or **Paired End**. In single-end reading, the sequencer reads a fragment from only one end to the other, generating the sequence of base pairs. In paired-end reading, it starts at one read, finishes this direction at the specified read length, and then starts another round of reading from the opposite end of the fragment. In the software, "FRAGMENT" appears for Single End and "PE" appears for Paired End. This attribute cannot be changed.
Templating Kit Name	The name of the templating kit used in Torrent Suite™ Software. This attribute cannot be changed.
Torrent Suite Hotspot File	The name of the hotspots file used in Torrent Suite™ Software. This attribute cannot be changed.
Torrent Suite Region File	The name of the target regions file used in Torrent Suite™ Software. This attribute cannot be changed.
Torrent Suite Results Name	The name of the Torrent Suite™ Software run report. This attribute cannot be changed.
Torrent Suite Version	The Torrent Suite™ Software version number. This attribute cannot be changed.
Attributes defined by the user.	
*	User-defined attributes are denoted with an asterisk (*) in the available columns list. Each user-defined attribute is listed in the **Samples** table and included in a CSV file of sample attributes.

Gender attribute

The gender attribute is a unique sample attribute that is used in various ways. The gender attribute is used by some analysis workflows in Ion Reporter™ Software, including the Ion AmpliSeq™ Exome trio and Ion AmpliSeq™ IDP trio analysis workflows

Several analysis workflows, such as the Ion AmpliSeq™ IDP trio analysis workflows, are limited when the sample gender is **Unknown**. These analysis workflows return unexpected results when gender is incorrectly specified. For example, in the Ion AmpliSeq™ IDP trio analysis workflow, when the gender of the proband is not known, variants cannot be assigned in the categories HasMaleMaternalX and HasUnknownX.

Gender can be supplied by the Planned Run in Torrent Suite™ Software. You can also add or change the gender attribute when you import a sample, or edit a sample in Ion Reporter™ Software. For example, if a sample that was transferred from Torrent Suite™ Software to Ion Reporter™ Software does not include the gender attribute or includes an attribute value that is not correct, you can edit the sample to add the gender attribute, or change its value.

To edit the gender attribute, first define new samples from the BAM or VCF data files. Then you can add the correct gender information to the new sample. For more information, see “Define samples with one or more BAM files” on page 50 and “Define a single VCF file as a sample” on page 52.

Note: **Called Gender** is another sample attribute that is calculated for some types of analyses and shown in the analysis results. The **Called Gender** sample attribute cannot be edited or defined manually. The software calculates **Called Gender** using an algorithm based on the analysis of sex chromosome data.

Sample presets

You can create user-defined sample attributes, which are known as sample presets, to describe samples that are used in research.

Ion Reporter™ Software supports the following types of attributes:

- Boolean—Creates a list of these two choices: True, False.
- Text—Accepted values: general text.
- Integer—Accepted values: integer numbers. Examples: 0, 9, –44.
- Float—Accepted values: decimal numbers Examples: 0.1, 9.0, –44.3.
- Controlled Vocabulary—Creates a list of these choices that you define with the preset terms.
- Date—Accepted values: provides a calendar tool to select a date.

Ion Reporter™ Software gives you the option to encrypt the internal storage for a sample attribute.

Create a user-defined sample attribute

User-defined sample attributes, which are a type of sample preset, allow you to add various kinds of pertinent metadata information to your samples.

Do not create a user-defined sample attribute that has the same name as a predefined sample attribute.

1. In the **Samples** tab, click **Presets**.
2. Click **Create Preset**, then select the data type of the preset that you want to create.
   - **Boolean**
   - **Text**
   - **Integer**
   - **Float**
   - **Controlled Vocabulary**
   - **Date**
3. Based on the data type of the preset, complete the preset dialog box:

Preset type	Steps
Boolean, Text, Integer, Float, or Date	a. Enter a **Name** and **Description** for the sample preset. b. *(Optional)* If you want the internal storage for the attribute to be encrypted, select **Yes** in the **Encrypted** list.
Controlled Vocabulary	a. Enter a **Name** and **Description** for the sample preset. b. *(Optional)* If you want the internal storage for the attribute to be encrypted, select **Yes** in the **Encrypted** list. c. In **Terms**, enter the first entry for the **Values** for the preset. Click **Add** to include an extra value in the sample preset. In the new field, enter the second entry. Repeat these steps for the desired number of preset values.

4. Click **Save**.
5. Ensure that the new preset is available in Ion Reporter™ Software.
   - In the **Samples** tab, click **Presets** to view the new sample attribute preset in the **Sample Presets** table. You can select a sample attribute that is listed in the **Sample Presets** table to view details that describe the sample attribute in the **Details** pane.
   - To add the user-defined attribute to a sample, edit the attributes for the sample when you define the sample. For more information, see “Define a sample manually” on page 50.

Sort the Sample Presets table

You can sort the **Sample Presets** table to organize the information by **Sample Preset Name**, **User**, or **Date of Creation**.

1. In the **Samples** tab, click **Presets**.
2. In the **Sample Presets** table, click the **Name**, **Created By**, or **Created On** column heading to sort the list.
3. Click the column heading again to reverse the order.

Sample import options

The following options are available to import sample data into Ion Reporter™ Software.

- Sample data are typically transferred from Torrent Suite™ Software when the sequencing run is complete. Sample data can be transferred automatically based on the IonReporterUploader plugin settings that are in the Torrent Suite™ Software Planned Run. For more information, see “Automatic transfers of sequencing run data” on page 45.
- Data from instrument sequencing runs can be transferred manually. You can also upload sample data directly from a BAM or VCF data file. Sample files in VCF format are typically uploaded to annotate the variants in the file, with an Annotate Variants analysis workflow. After you upload the sample files, you need to complete the steps to define the sample before it is analyzed in Ion Reporter™ Software. For more information, see “Upload a BAM file to create a sample or samples” on page 45 and “Upload a VCF file to create a sample” on page 47.
- You can also use the following options to import sample data directly into Ion Reporter™ Software.
  - A CSV file. For more information, see “Define samples with a CSV file” on page 53.
  - An Ion PGM™ Dx System. For more information, see “Import samples from Torrent Suite™ Dx Software” on page 49.
  - The IonReporterUploader command-line utility. For more information, see “IonReporterUploader command-line utility” on page 48.
  - An external data file. For more information or help with this option, contact your Field Bioinformatics Specialist (FBS).

Automatic transfers of sequencing run data

Samples are typically transferred to Ion Reporter™ Software based on the settings that are selected in Torrent Suite™ Software. These settings, which are in the **Ion Reporter** step of the Planned Run in Torrent Suite™ Software, determine how the sample data file is transferred.

- If the **Upload Only** option is selected, the IonReporterUploader plugin automatically transfers the sample file, or files, for the completed sequencing run from the IonReporterUploader plugin to Ion Reporter™ Software without running data through an analysis workflow. A BAM file is transferred for all sequencing runs. If the variantCaller plugin was set up in the Planned Run, a VCF file is also generated, and transferred. After the transfer is complete, you must add the sample file or files to a sample definition in Ion Reporter™ Software and manually launch an analysis before you can review analysis results. For more information, see “Sample definition” on page 49 and “Manually launch an analysis” on page 168.
- If instead, the **Select an Ion Reporter™ Software workflow for your sample type** option and an analysis workflow are selected in Torrent Suite™ Software, the transfer occurs based on which additional **Ion Reporter Upload** option is selected:
  - The **Automatically upload to Ion Reporter after run completion** option allows you to go directly to the analysis results in Ion Reporter™ Software. In this case, the sample data and analysis workflow settings are used to analyze the data.
  - The **Review results after run completion, then upload to Ion Reporter** option allows you to first review the sequencing run report in Torrent Suite™ Software, then manually upload the BAM file, the VCF file, or both files. With this option, you can evaluate the sequencing run before you transfer sample data and analyze the data in Ion Reporter™ Software. After you upload the BAM file, you must add one or more files to a sample definition in Ion Reporter™ Software, then launch an analysis to review analysis results. For more information, see “Sample definition” on page 49 “Launch an analysis” on page 165.

Upload a BAM file to create a sample or samples

You can manually upload one or more BAM files into Ion Reporter™ Software.

BAM files are available as output files from Torrent Suite™ Software. These BAM files contain sample reads that can be mapped to the reference genome, or unmapped. If the output file is mapped, the software makes both BAM and BAI files available as output files in Torrent Suite™ Software. If the file is unmapped, only the BAM file is available. If you upload BAM files that are unmapped into Ion Reporter™ Software, the software adds the mapping. Also, if files require additional reference mapping, those reference sequences are added. You can use these BAM output files to manually upload files to Ion Reporter™ Software.

Note: Ion Reporter™ Software will automatically re-map BAM files if there is a mismatch between tmap advanced parameters that are stored in the BAM file and the parameters that are set for the analysis workflow with which the BAM file will be used. If you want mapped BAM files that you upload to be

remapped, use the following guidelines to set the `Tmap Mapped Files Enable Re-map` parameter to ***True***.

- Set `Tmap Mapped Files Enable Re-map` to true for cases in which the incoming BAM file was mapped with an older version of TMAP but the analysis workflow requires a newer TMAP version (that includes later improvements).
- Set `Tmap Mapped Files Enable Re-map` to ***True*** for cases in which the TMAP parameter changes in the BAM do not trigger the software to do the automatic re-mapping.
- For more information, see “Customize tuning parameters” on page 94.

The name of a BAM file that is uploaded to Ion Reporter™ Software cannot exceed 200 characters.

1. In the **Samples** tab, click **Overview**.
2. Click **Define Samples** ▸ **Manual**.
3. In the **Samples** step, click **Upload BAM**.
4. In the **Upload BAM Sample File** dialog box, click **Select file** to navigate to the file that you want to upload, then click **Upload BAM**.
   The BAM file is added to the list of files in the **Samples** step.
5. Click **Close** to close the dialog box.
6. In the **Samples** step, select one or more BAM files, then click **Add to Sample**.
7. In **Sample Name**, enter a descriptive and unique name, then click **Add to Sample List**.
   Sample names can be any combination of alphanumeric characters plus spaces, periods (.), dashes (-), and underscores (_). There is a 255-character limit.
8. Click **Next**.
9. *(Optional)* In the **Attributes** step, set the value for a new or existing attribute.

Option	Description
Set the value of a pre-existing attribute.	a. Click the field in the column of the attribute heading. b. Enter a value or select a value from a list.
Add an attribute and set its value.	a. Click **Add Attribute**, then select an attribute from the list. b. Click the field in the column of the attribute heading. c. Enter a value or select a value from a list.
Add a new attribute.	For more information, see “Create a user-defined sample attribute” on page 43.

10. Click **Next**.
11. In the **Review** step, click **Save**.

The new sample or samples are added to the **Samples** table.

The sample or samples are now ready for analysis. For more information, see “Launch an analysis” on page 165.

Upload a VCF file to create a sample

You can upload a single VCF file and define it as a sample so that the variants that are listed in the file can be annotated with Ion Reporter™ Software. A sample can contain only one VCF file. It cannot include multiple VCF files.

Ensure that the VCF file meets the following criteria so the file is successfully imported into Ion Reporter™ Software.

- Annotate Variants analysis workflows accept only VCF files that conform to the 4.1 VCF standard.
- Annotate Variants analysis workflows also accept a compressed VCF file that use BCF, VCF, GZ, and BCF.GZ file formats. Ion Reporter™ Software decompresses the GZ file and uses the VCF or BCF file.
- Use ***CHROM*** as a column heading name to designate chromosome position in the VCF file.

Note: If you plan to use an uploaded VCF and the GRCh38 reference with the Annotate Variants analysis workflow in Ion Reporter™ Software, ensure that you include the following text in the header line of the VCF file: ***##reference=GRCh38***.

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1. In the **Samples** tab, click **Overview**.
2. Click **Define Samples** ▸ **Manual**.
3. In the **Define Samples** screen, click **Upload VCF**.
4. In the **Upload VCF Sample File** dialog box, click **Select file**, navigate to the file you want to upload, then click **Upload VCF**.
   The VCF file is added to the list of files in the **Samples** step.
5. Click **Close** to exit the **Upload VCF** dialog box.
6. In the **Samples** step, select the VCF file that you uploaded, then click **Add to Sample**.
7. In **Sample Name**, enter a descriptive and unique sample name, then click **Add to Sample List**.
   Sample names are limited to 255 characters, and can be any combination of alphanumeric characters plus spaces, periods (.), hyphens (-), and underscores (_).
   Your VCF file now appears in the **Files Included** list and the number of data files is shown in the field with dotted lines.
8. Click **Next**.

9. *(Optional)* In the **Attributes** step, set the value for a new or existing attribute:

Option	Description
Set the value of an existing attribute.	a. Click the field in the column of the attribute heading. b. Enter a value or select a value from a list.
Add an attribute and set its value.	a. Click **Add Attribute** and select an attribute from the list. b. Click the column of the attribute heading. c. Enter a value or select a value from a list.
Add a new attribute.	Use this option to add a new user-defined sample attribute. For more information, see “Create a user-defined sample attribute” on page 43.

10. Click **Next**.

11. In the **Review** step, click **Save**.

The new sample is added to the **Samples** table.

The sample or samples are now ready for analysis with the Annotate Variants analysis workflow. For more information, see “Launch an analysis” on page 165.

IonReporterUploader command-line utility

You can use the IonReporterUploader command-line utility to transfer or upload sequencing run results files to Ion Reporter™ Software, then define samples that are based on the transferred files. The IonReporterUploader command-line utility uses your Ion Reporter™ Software account credentials to transfer run data from Torrent Suite™ Software to the organization on the specified Ion Reporter™ Server. The utility, which is available at **http://iru.ionreporter.thermofisher.com/**, supports these types of file transfers:

- Transfers of a single BAM or VCF file.
  The name of a BAM file that is uploaded to Ion Reporter™ Software cannot exceed 200 characters.
- Transfers of all results files for a Torrent Suite™ Software analysis.
- Transfers of results files that are in a single flat folder.
- Transfers of multiple files that are not restricted to a single folder.
- Uploads of samples from hg19 and GRCh38 reference genomes only. Although the E-coli and animal reference genomes can be used in Torrent Suite™ Software, these reference genomes are not supported in Ion Reporter™ Software.
- To upload samples from other reference genomes, you can build a plugin that includes any reference genome. For more information, see Appendix C, “Programming guidelines for Ion Reporter™ Software plugins”.

You can later analyze the samples with Ion Reporter™ Software. The IonReporterUploader command-line utility can be run on your Torrent Server, or can be run on any standard (and current) computer that runs Linux™, macOS™, or Windows™ Operating System. You can enter IonReporterUploader command-line utility options through command-line arguments, or through a properties file.

The IonReporterUploader command-line utility supports the upload of combined run results that are output by using the **Combine Alignments** option in the Torrent Suite™ Software. Uploads of combined run results are not supported when the IonReporterUploader plugin is used in Torrent Suite™ Software.

For more information on the IonReporterUploader command-line utility, see the IonReporterUploader Command-Line Utility User Guide (Pub. No. MAN0017648).

Import samples from Torrent Suite™ Dx Software

This procedure applies to Ion Reporter™ Server only.

You can import samples that originate in the Ion PGM™ Dx System and are processed by Torrent Suite™ Dx Software to an Ion Reporter™ Server. Only samples that are signed off in Torrent Suite™ Dx Software can be transferred to an Ion Reporter™ Server. The samples are imported in a BAM file.

1. In the **Samples** tab, click **Overview**.
2. Click **Define Samples ▸ Import from PGM™ Dx TS™ Sw**.
3. In the **Servers** step of the workflow bar, click **Add Server**.
4. Enter the name, IP address, user name, and password for the Ion PGM™ Dx System account. Click **Save**, then click **Next**.
5. In the **Samples** step, select the samples that you want to import, then click **Next**.
6. Ensure that the information in the **Review** step of the workflow bar is correct, then click **Import**.

The samples appear in the **Samples** table in Ion Reporter™ Software.

The sample or samples are now ready for analysis. For more information, see “Launch an analysis” on page 165.

Sample definition

Before samples can be analyzed in Ion Reporter™ Software, samples must first be defined if any of the following is true:

- Data are from BAM files that are uploaded manually to Ion Reporter™ Software from Torrent Suite™ Software, using the **Upload Only** option.
- Data are imported manually through BAM or VCF files to Ion Reporter™ Software.
- Data are contained in a CSV file of samples that was uploaded to Ion Reporter™ Software.

The sample definition process assigns a unique name to a collection of one or more sample files and the corresponding sample attributes. After samples are defined, sample data can be included when you launch an analysis in Ion Reporter™ Software.

Note: If data are transferred automatically from Torrent Suite™ Software and an analysis workflow is launched in Ion Reporter™ Software, the samples are already defined and available in the **Samples** tab. In general the data that is transferred to Torrent Suite™ Software through the IonReporterUploader

plugin or IonReporterUploader command-line utility is automatically defined as a single sample for each BAM file, or is based up on the configuration of the plugin or the command-line utility.

Define a sample manually

You can define a new sample manually from imported data files or existing samples.

BAM files from multiple sequencing runs can be combined into a single sample for analysis. For example, you can combine BAM files if you are sequencing the same sample multiple times, and you want to combine those multiple samples into a single sample for greater read depth. Or, you can combine samples from different sequencing runs for sample comparisons. In Ion Reporter™ Software, you can also define a sample from a single VCF file.

Note: This procedure is not required if data are transferred automatically from Torrent Suite™ Software and an analysis workflow is launched in Ion Reporter™ Software. In this case, the samples are already available in the **Samples** tab. For more information, see "Sample import options" on page 44.

Before you manually define a sample, upload BAM or VCF data files into Ion Reporter™ Software, or use the demonstration data files available in Ion Reporter™ Software. For more information, see "Upload a BAM file to create a sample or samples" on page 45, "Define samples with a CSV file" on page 53, and "Demonstration samples" on page 167.

IMPORTANT! You cannot combine a VCF file and a BAM file in a sample. You also cannot combine multiple VCF files into a single sample.

Define samples with one or more BAM files

Sequence results from multiple libraries prepared from the same sample or multiple sequencing results from the same library can be combined for increased analytical power. You can also use a single BAM file to define the sample.

Note: Analyses might fail if the BAM files have different values for the SM (Sample Name) key in the RG (Read Group) (@RG) tag when you create single fusion sample from multiple BAM files. The multiple BAM files that are used to define a sample in Ion Reporter™ Software must each have the same Sample Name as is defined in the Read Group tag.

1. In the **Samples** tab, in the **Overview** screen, click **Define Samples** ▸ **Manual**.
   The name of your organization is shown on the left of the screen, and sample files for your organization are listed in the table.

2. *(Optional)* Click ⓘ **Upload BAM**, click **Select File**, then navigate to and select the desired BAM file, then click **Open**.

3. Click **VCF** above the table to filter for VCF files.
   Files that are transferred by the IonReporterUploader plugin in Torrent Suite™ Software appear under `data/IRU_Uploads`, in time-stamped folders. Demonstration data files appear under the **demodata** folder.

4. To view the files in either the upload directory or the `demodata` directory, expand the menu on the left that matches the organization name, then expand the data file directory that contains data files of interest.

5. Use these steps to select the data files that will be used as samples in the software.
   a. Select one or more BAM files from the list of available files, then click **Add to Sample**.
   b. Enter a **Sample Name**, then click **Add to Sample List**.
   c. *(Optional)* Repeat steps a and b to define more samples.

6. Click **Next**.

7. In the **Attributes** step, you can set sample attribute values or add a new attribute and set its value.

Option	Description
Set the value of a pre-existing attribute.	a. Click the field in the column of the attribute heading. b. Enter a value or select a value from a list.
Add an attribute and set its value.	a. Click **Add Attribute** and select an attribute from the list. b. Click the field in the column of the attribute heading. c. Enter a value or select a value from a list.

   You can create user-defined sample attributes that can be used for future analyses. For more information, see “Create a user-defined sample attribute” on page 43.

8. Review the samples listed under **Samples ready to import!**
   - If the sample name and the data files are correct, click **Save**.
   - To change the sample, click **Previous**.

9. If the sample information is correct, click **Next**.

The **Samples Overview** tab opens, with the new sample listed in the **Samples** table.

The sample or samples are now ready for analysis. For more information, see “Launch an analysis” on page 165.

Define a single VCF file as a sample

You can define a new sample manually from an imported data file that uses VCF format.

You can use only one VCF file for this procedure, and you cannot combine multiple VCF files into a single sample. To combine samples from multiple files into a single sample, see "Define samples with one or more BAM files" on page 50.

1. In the **Samples** tab, in the **Overview** screen, click **Define Samples** ▸ **Manual**.
   The name of your organization is shown on the left of the screen, and sample files for your organization are listed in the table.

2. *(Optional)* Click **Upload VCF**, click **Select File**, then browse to, then select the desired VCF file, then click **Open**.

3. Click **VCF** above the table to filter for VCF files.
   Files that are transferred by the IonReporterUploader plugin in Torrent Suite™ Software appear under `data/IRU_Uploads`, in time-stamped folders. Demonstration data files appear under the `demodata` folder.

4. To view the files in either the upload directory or the `demodata` directory, expand the menu on the left that matches the organization name, then expand the data file directory that contains data files of interest.

5. Select the checkbox for the data file that contains the sample that you want to use, then click **Add to Sample**.
   When a data file directory is selected, the contents of the file list changes to reflect the files from the directory that you select. The **Files included** area lists the files for the sample, and the count of data files is displayed.

6. At the top of the sample list, enter a descriptive name for the new sample. When the sample pane contains the correct data file and sample name for the sample, click **Add to Sample List**.
   Sample names can be any combination of alphanumeric characters plus spaces, periods (.), hyphens (-), and underscores (_). There is a 255-character limit.

7. Click **Next**.

8. In the **Attributes** step, you can set sample attribute values or add a new attribute and set its value.

Option	Description
Set the value of a pre-existing attribute.	a. Click the field in the column of the attribute heading. b. Enter a value or select a value from a list.
Add an attribute and set its value.	a. Click **Add Attribute** and select an attribute from the list. b. Click the field in the column of the attribute heading. c. Enter a value or select a value from a list.

You can create user-defined sample attributes that can be used for future analyses. For more information, see “Create a user-defined sample attribute” on page 43.

9. Review the samples listed under **Samples ready to import!**
   - If the sample name and the data files are correct, click **Save**.
   - To change the sample, click **Previous**.

The **Samples Overview** tab opens, with the new sample listed in the **Samples** table.

The sample or samples are now ready for analysis. For more information, see “Launch an analysis” on page 165.

Define samples with a CSV file

When the uploaded data files in BAM or VCF formats are available in Ion Reporter™ Software, you can then use a CSV file to define the data as samples in Ion Reporter™ Software. The CSV file defines sample names and attributes for an associated BAM or VCF file.

If there are editable sample attributes that you want to redefine, you can update the attributes in the CSV file, then upload the file into Ion Reporter™ Software as a new sample. You can then the new sample to launch a new analysis. For more information about which sample attributes can be edited, see “Sample attributes” on page 37.

You can also edit an existing CSV file to add attributes to existing samples. For example, you can add attributes to the samples that were not included in the Torrent Suite™ Software Planned Run, or add new attributes that are required to reuse the samples in a different analysis.

You can download an example sample definition CSV file from the **Define Samples** screen. Add the file location for the related sample BAM file and customize the example sample definitions CSV file to create a CSV file for upload. For more information, see “Create a sample CSV file to define samples” on page 54.

1. In the **Samples** tab, in the **Overview** screen, click **Define Samples ▸ Import from CSV**.
2. Click **Select File**, then browse to the CSV file.
3. Ensure that the displayed file name is correct, then click **Upload**.
   The software checks that the sample is valid.
4. In the **Samples** step, click **Next**.
5. In the sample list, ensure that the sample information is correct.

6. *(Optional)* In the **Attributes** step, set the value for a new or pre-existing attribute:

Option	Description
Set the value of a pre-existing attribute.	a. Click the field in the column of the attribute heading. b. Enter a value or select a value from a list.
Add an attribute and set its value.	a. Click **Add Attribute** and select an attribute from the list. b. Click the field in the column of the attribute heading. c. Enter a value or select a value from a list.
Add a new attribute.	For more information, see "Create a user-defined sample attribute" on page 43.

7. Click **Next**.

8. In the **Review** step, click **Save**.

The **Samples** tab opens, with the new sample listed in the Samples table.

The new samples are ready for analysis. For more information, see "Launch an analysis" on page 165.

Create a sample CSV file to define samples

You can define multiple samples in Ion Reporter™ Software, or update existing samples and edit sample attributes with a CSV file. If you do not yet have a sample file, you can create a new file from a template that is available in Ion Reporter™ Software.

1. In the **Samples** tab, click **Overview**.

2. Click **Define Samples** ▸ **Import from CSV**.

3. In the **Samples** step, click **download example**.
   The `SampleDefinition.csv` file is downloaded.

4. Open the `SampleDefinition.csv` file, then enter the sample information. Include the following information:
   - Relative path to the sample file
     You need to include a relative path to the sample BAM file from the root folder for the Ion Reporter™ Software organization of the user. For example, if the organization of the user is: ***/data/IR/data/OrganizationName/data/IRU_Uploads/example.bam***
     Include the following relative path:
     /data/IRU_Uploads/example.bam
   - Sample name
     Sample names can be any combination of alphanumeric characters plus spaces, periods (.), hyphens (-), and underscores (_). There is a 255-character limit.
   - Sample attributes, such as gender.

5. Save the file.

After you have entered all the relevant sample information into the CSV file, use the CSV file to define the samples. For more information, see “Define samples with a CSV file” on page 53.

Define samples as no-template controls

No-template control (NTC) samples can be defined in Torrent Suite™ Software or Ion Reporter™ Software. No-template controls are samples that intentionally leave out any nucleic acid, and are used to monitor contamination or primer-dimer formations that could produce false-positive results.

If samples are defined as NTCs in Torrent Suite™ Software, the sample attributes transfer to Ion Reporter™ Software automatically.

If you define NTCs with a sample type that is not correct, Oncomine™ DNA and Fusions analyses and Oncomine™ DNA analyses might not complete successfully in Ion Reporter™ Software due to insufficient reads.

1. In the **Samples** tab, in the **Overview** screen, click the sample name that you want to designate as an NTC.
2. Click **Define Samples** ▸ **Manual**
3. In the **Define Samples** screen, enter a sample name and select one or more samples to include in the sample, then click **Add to Sample**.
4. Click **Attributes** in the workflow bar. If the **Sample Type** attribute is not shown, click **Add Attribute** ▸ **Sample Type** to add the attribute to the table.
5. In the **Sample Type** column, select **DNA NTC** or **RNA NTC**.
6. If you define the sample as **DNA NTC**, you must also add the **Percentage Cellularity** attribute, then set the percentage cellularity to 100 percent.

   The **Percent Cellularity** attribute is not required for RNA samples.

   a. Click **Add Attribute** to add the **Percentage Cellularity** attribute.

   b. Enter ***100*** in the **Percentage Cellularity** column.
7. When the sample information is complete, click **Next**.
8. In the **Review** step, check the sample names and number of samples. If the sample information is correct, click **Save**.

The sample or samples are now ready for analysis. For more information, see “Launch an analysis” on page 165.

Edit a sample

When you edit an existing Ion Reporter™ Software sample, you can add or remove BAM data files, and add, remove, or change sample attributes.

You cannot edit the preinstalled demonstration samples that are provided with Ion Reporter™ Software. For more information, see “Demonstration samples” on page 167.

You cannot edit locked samples. For more information, see “Lock a sample” on page 57.

1. In the **Samples** tab, click **Overview**.
2. In the **Samples** table, select the sample that you want to edit, then click **Edit**.
3. Add the BAM data files from the table to the sample.
   a. Select the checkbox for the data file that contains the sample that you want to edit, then click **Add to Sample**.

      The **Add to Sample** tab on the right is active after you select at least one data file in the table.

      The **Files included** area lists the files for this sample, and the count of data files is displayed. A new sample box appears and **Next** is available.
4. Click **Next** to move to the **Attributes** step in the workflow bar, then edit the attributes that are associated with the sample.

Option	Description
Set the value of a preexisting attribute.	a. Click the field in the column of the attribute heading. b. Enter a value or select a value from a list.
Add an attribute and set its value.	a. Click **Add Attribute** and select an attribute from the list. b. Click the field in the column of the attribute heading. c. Enter a value or select a value from a list.

   You can create a sample preset that can be used for future analyses. For more information, see “Create a user-defined sample attribute” on page 43.
5. Click **Next**.
6. In the **Review** step, click **Save**.

The sample or samples are now ready for analysis. For more information, see “Launch an analysis” on page 165.

Edit a sample used in an analysis

A sample that has been previously used in an analysis can be edited. To edit a sample, you must first define a new sample from the BAM or VCF file that is associated with an analyzed sample.

1. Define a new sample from the BAM or VCF file that is associated with the analyzed sample. For more information, see “Define a sample manually” on page 50.
2. To change any attributes after the sample is defined, see “Edit a sample” on page 56.

The sample is now ready for analysis. For more information, see “Launch an analysis” on page 165.

Lock a sample

You can lock a sample to prevent any user from editing the sample in Ion Reporter™ Software.

IMPORTANT! After you lock a sample, you can no longer edit the sample or the attributes that are associated with the sample.

---

1. In the **Samples** tab, in the **Overview** screen, select the sample in the table, then click **Actions** ▸ **Lock**.
2. In the **Lock Sample** dialog box, click **Yes** to confirm that you want to lock the sample.

The locked sample is designated with a (Lock) in the **Samples** table.

Find samples

You can search, sort, and filter the list of samples in the **Samples** table in Ion Reporter™ Software.

Note: You can add attributes that are not shown in the Sample table, and later search for those attributes. For more information, see “Customize the Samples table” on page 60.

Search for samples

You can search the **Samples** table for all sample attributes that are shown in the **Samples** table at the time of the search. Both predefined and user-defined sample attributes are included in the search results.

IMPORTANT! Search terms must adhere to the following rules.

- An asterisk (*) is not allowed in the search field for use as a wildcard.
- Searches are case-insensitive, that is, both upper-case and lower-cases letters are found regardless the case of search term letters.
- Searches find every occurrence of a continuous string. For example, a search for ***demo*** in analysis names returns a list of all analyses with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and therefore are not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

1. In the **Samples** tab, click **Overview**.
2. In the **Search** box, enter a keyword or text string, then click **Go** to return a list of samples that match the keyword.

   If the search string is invalid, the search field is outlined in red. Correct the search term to proceed.

The search results are returned in the **Samples** table.

Sort the Samples table

You can sort the **Samples** table to make it easier to find the samples that you are looking for in Ion Reporter™ Software. You can also sort the table to customize the list of samples. For more information, see “Customize the Samples table” on page 60.

1. In the **Samples** tab, in the **Overview** screen, click a column heading to sort the table based on sample attributes.
2. Click the column again to reverse the order.
3. Click the column a third time to stop sorting for the column, then return the column to the order that was used before to the sort.

Filter samples

You can apply filters to the **Samples** table to narrow search results, or shorten the list of samples to make it easier to find the samples of interest. You can also add filters, and then find samples. When you use sample attributes as filters to search for samples, you can then easily launch analyses for a group of samples from the search results.

1. In the **Samples** tab, click **Overview**.
2. *(Optional)* You can add more columns to the table, then filter on those columns.
   a. Click **Preferences** ▸ **Select Columns** to add more columns.
   b. Add user-defined sample attributes or predefined sample attributes as filters:
      - Click **More Filters**, then select one or more filter categories from the list.
      - Click **More Filters**, type in a search string into the **Find Filters** field, then select one or more filter categories.
      - Click **More Filters**, then click **Select All** to select all filter categories.

   More filters are added to the **Samples** table. You can now use these filters to narrow your search results and make it easier to find samples of interest.
3. Apply the filters.
   - Click the filter to expand the list, then select one or more filters. For example: select **Chip Type** to filter for sequencing data from an instrument that uses a specific type of sequencing chip.
   - Click the **Analyzed: All** filter, then click **Yes** to view the samples that have been analyzed, or click **No** to view the samples that have not been analyzed.
   - Click the filter to expand the list, then enter a search string into **Find Filters**. Select one or more filters. For example: select **530** to filter for sequencing data from an instrument that uses a Ion 560™ Chip.
   - Click the filter to expand the list, then click **Select All** to select all filters in a specific filter category.

   The contents of the **Samples** table change each time that you select a filter or set of filters.

The filtered list of samples appears in the **Samples** table.

Remove filters

You can remove individual filters from the **Samples** table in Ion Reporter™ Software.

1. Remove filters from the **Samples** table.
   - Click ✕ **(Remove)** next to the filter list to remove the filter from the **Samples** table.
   - Click **More Filters**, then click the checkmark of the filter that you want to remove the filter from the **Samples** table.
   - Click **More Filters**, then click **Clear** to remove all the selected filter categories from the list.
2. Click **Clear All** to remove all filters, then view all samples in the **Samples** table.

Customize the Samples table

The **Samples** table is organized into columns of sample attributes that are initially organized in a default order. You can customize the table with table preferences to determine which columns appear and where the columns are placed. Use table preferences to include or exclude columns, then drag and drop each column to the desired position in the table. You can also use the column headings to resize the default width of each column.

Add attributes to the Samples table

You can add sample attributes to the **Samples** table that you view in Ion Reporter™ Software.

1. In the **Samples** tab, click **Overview**.
2. Click **Preferences** ▸ **Select Columns**, then select the sample attributes that you want to add to the **Samples** table, or deselect the sample attributes that you want to remove from the table.
   User-defined attributes are denoted with an asterisk (*) in the available columns list.

The selected sample attributes are added to the **Samples** table until you close the table. If you create a sample CSV file before you close the table, each user-defined attribute is included in the CSV file.

To save the table with the attributes that you added, see “Create a Samples table preference” on page 60.

Create a Samples table preference

You can create **Samples** table preferences to make it easier to view the sample attributes and information. Table preferences allow you to include or exclude sample attributes, and drag then drop each column to the desired position in the table. When you create a **Samples** table preference, you can apply it at any time to the **Samples** table. You can also use **Samples** table preferences to toggle between different views of the **Samples** table.

1. In the **Samples** tab, click **Overview**.
2. Click **Preferences** ▸ **Select Columns**.
3. In the **Select Columns** dialog box, then select the sample attributes that you want to add to the **Samples** table, or deselect the sample attributes that you want to remove from the table.
4. Click **Preferences** ▸ **Save Table Preference As**.
5. In the **Save Table Preference** dialog box, enter a name for the table preference, then click **Save**.

Your sample table preference is saved.

For information about how to use a table preference, see “Apply a preference to the Samples table” on page 61.

Rearrange columns in the Samples table

You can change the order of sample attribute columns in your **Samples** table in Ion Reporter™ Software. You can use the rearranged table temporarily, or to save the column order and use it later, you can create a table preference.

1. In the **Samples** tab, click **Overview**.
2. Click the column heading that you want to move, then drag and drop the heading to the position you prefer.
3. Repeat to move additional columns.
4. Click **Preferences** ▸ **Save Table Preference As**.
5. In **Save Table Preference**, enter a name for the table preference, then click **Save**.

The sample table preference is saved.

To use the table preference to return to the saved view of the **Samples** table, or toggle between different views of the table. see “Apply a preference to the Samples table” on page 61.

Apply a preference to the Samples table

You can apply saved table preferences to the **Samples** table in Ion Reporter™ Software.

1. In the **Samples** tab, click **Overview**.
2. Click **Preferences** ▸ **Table Preferences**.
   A menu of saved preferences appears.
3. Select the preference from the menu.

The table preference is applied to the **Samples** table. The last saved table preference remains until another preference is selected or default settings are restored.

Delete a Samples table preference

You can delete a table preference that is no longer needed in Ion Reporter™ Software.

1. In the **Samples** tab, click **Overview**.
2. Click **Preferences** ▸ **Table Preferences**.
   A menu of saved preferences appears.
3. Select the preference that you want to delete from the menu.
4. Click **Preferences** ▸ **Delete Table Preference**.
5. In the **Confirm Delete**, click **Yes** to delete the table preference.

The table preference is deleted and the **Samples** table returns to the default order of columns.

Restore table preference default settings

You can return the **Sample** table to default settings in Ion Reporter™ Software.

1. In the **Samples** tab, click **Overview**.
2. Click **Preferences** ▸ **Restore Defaults**.

The table is returned to the default column settings. For the list of default columns, see “Sample attributes” on page 37.

Transfer samples to users in other organizations

You can share samples with other users in different Ion Reporter™ Software organizations. To share a sample, you can transfer either a single sample or a batch of samples. Any user-defined attributes that are associated with the samples are also transferred with each sample. After a sample is transferred, you can delete the sample to free up storage space.

Transfer a sample to users in another organization

You can transfer samples to other users in different Ion Reporter™ Software organizations. All user-defined sample attributes are also transferred with the sample.

1. In the **Sample** tab, click **Overview**.
2. Click the row of the sample that you want to transfer, then click ⚙ **Actions** ▸ **Transfer**.
3. In the **Transfer Sample** dialog box, enter the address of one or more intended recipients, then click **Transfer**.
   A transfer initiation message appears.

The recipient receives a notification and can download the transferred sample from the **Notifications** screen.

To clear storage space, you can delete the sample after the transfer. For more information, see “Delete a sample” on page 66.

IMPORTANT! To avoid data loss, ensure that the user with whom you share the samples has accepted the transfer of the samples before you delete any samples.

Transfer multiple samples to a user in another organization

To share multiple samples with a user in another Ion Reporter™ Software organization, you can transfer a batch of samples to the user.

1. In the **Samples** tab, click **Overview**.
2. Select multiple samples, then click **Actions** ▸ **Transfer**.
3. In the **Batch Transfer** dialog box, enter the email address of the intended recipient, then click **Transfer**.

   A status appears stating your samples transfer has completed.

The recipient receives a notification and can download the transferred samples from the **Notifications** page. To free up storage space, you can delete the samples after transfer. For more information, see “Delete a sample” on page 66.

Accept a sample from a user in another organization

You can accept samples that are transferred from a user in another Ion Reporter™ Software organization.

The Ion Reporter™ Software account that you use for this procedure must include the Import, Analyze, or Admin roles.

1. In the **Home** tab, click **Notifications**.
2. Find the transferred sample, then click (Download).
3. Click the **Samples** tab, then find the transferred sample.
4. To accept the sample, in the **Details** pane, click **Actions** ▸ **Accept**.

The sample is available for use in the **Samples** table.

Archive samples to an external storage device

This information applies only to Ion Reporter™ Server.

You can archive samples from an Ion Reporter™ Server to an external storage device to increase disk space.

Note: Samples that are archived can later be restored to an Ion Reporter™ Server that uses of the same version, or an earlier version of Ion Reporter™ Software from which the samples were archived.

Available options for external storage devices include the following.

- Torrent Storage™ NAS (Network Attached Server) device (Network Attached Server)—For more information, see **https://www.thermofisher.com/order/catalog/product/A32198**
- DataSafe™ Solution—For more information, see **https://www.thermofisher.com/order/catalog/product/A32633**

IMPORTANT! Before you start this procedure, ensure that all users are signed off. Ion Reporter™ Server is not available to users during the archive process.

- You need to have an external storage device that is mounted to your Ion Reporter™ Server to archive samples to the device.
- If you have a new Ion Reporter™ Server or upgrade from a previous version, you need to specify a storage location for archiving samples and analyses in Ion Reporter™ Server.

  IMPORTANT! Change `archivalMountPath=/tmp/` to `archivalMountPath=/storage IP address/` in the `ionreportermanager/server/server.properties` file, then restart the Tomcat server. To check the data that you archived, go to the storage location of the archived data, which is set up in `/share/apps/IR/ionreportermanager/server/server.properties` file as `archivalMountPath`. If you have questions about the Tomcat server, contact your Field Support Engineer or Field Bioinformatics Support representative.

- You can only archive samples if no processes, such as an analysis is running, or new sample uploads in progress, are active on the server.

1. In the **Samples** tab, in the **Overview** screen, select the sample or samples to be archived.
2. Complete one of the following actions.

Option	Action
Archive multiple samples	In the **Selected Samples** section, click **Actions** ▸ **Archive**.
Archive a single sample	In the **Details** section, click **Actions** ▸ **Archive** .

3. Indicate whether the archives should be retained on the server or deleted in the **Confirm Batch Archive** dialog box.
   - To delete the samples after the archival process is complete, select the option **do you want to delete the data from path post archive?**.
   - To retain the samples after the archival process is complete, deselect the option **do you want to delete the data from path post archive?**.

4. Click **Yes** in the **Confirm Batch Archive** dialog box to begin the archive process for the selected samples.
   The **Archival Manager** screen shows the progress of the archive.

5. (Optional) To stop the archive process, click **Abort**.

   To prevent a loss of data, if you archive multiple analysis you can only abort the archive process if none of the archival processes are complete. For example, if there are 3 archives that are in progress, and 2 archives are completed, but the third item is in progress, the abort does not occur.

When the archival process is successful, there is a message in the **Confirm Batch Archive** dialog box to indicate that the process is complete. The samples are listed in the **Archives** screen. A successful archive has a **Status** of **Restored**. The table shows the name, type, size, status and any error message for the restoration.

Restore archived samples from an external device to Ion Reporter™ Server

This information applies only to Ion Reporter™ Server.

Note: Archived samples can be restored to an Ion Reporter™ Server that uses of the same version, or an earlier version of Ion Reporter™ Software from which the samples were archived.

- The external storage device that contains archived samples must be mounted to the Ion Reporter™ Server.
- If you have a new Ion Reporter™ Server or upgrade from a previous version, you need to specify a storage location for archiving samples and analyses in Ion Reporter™ Server.

  IMPORTANT! Change `archivalMountPath=/tmp/` to `archivalMountPath=/storage IP address/` in the `ionreportermanager/server/server.properties` file, then restart the Tomcat server.

  To check the data that you archived, go to the storage location of the archived data, which is set up in the `/share/apps/IR/ionreportermanager/server/server.properties` file as `archivalMountPath`.
  If you have questions about the Tomcat server, contact your Field Support Engineer or Field Bioinformatics Support representative.

- You can only archive samples if no processes are active on the server.
- After the archive process starts, users cannot use the Ion Reporter™ Server until the archive process is complete.

IMPORTANT! Before you start this procedure, ensure that all users are signed off. Ion Reporter™ Server is not available to users during the restoration process.

1. In the **Admin** tab, click **Archives**.
2. Select the path to the directory on the mounted device from which the samples will be restored.
   You can enter text for the sample name in the **Search** box to narrow the list, and select **Specimen** as the archive type in the dropdown list to view only archived samples.
3. Click **Restore** to return the sample back to the Ion Reporter™ Server from which the samples will be restored.
4. In the **Restore Manager** dialog box, do the following.
   a. Select the option to **delete the data from the archival path post restore** to delete the samples from the archive directory when the sample restoration is complete. Or, de-select the option to keep the samples on the external archive device.
   b. Click **Yes** to confirm that you want to restore the selected samples.

   If any processes are running on the server, you will receive a message that contains information about the number of processes that are in progress. Wait until the processes are complete before you proceed.
5. *(Optional)* To stop the restore process, click **Abort**.
   To prevent a loss of data, if you restore multiple samples you can only abort the restore process if none of the restoration processes are complete. For example, if 3 restorations are in progress and 2 restorations are complete but the third restoration remains in progress, the abort does not occur.

When the restoral process is successful, there is a message in the **Confirm Batch Archive** dialog box to indicate that the process is complete. The restored samples are listed in the **Samples** screen.

Delete a sample

You can delete one or more samples in Ion Reporter™ Software. For example, you can remove low-quality samples or remove samples that were transferred to other organizations or archived. Storage space can increase when you delete samples.

IMPORTANT! When you delete a sample, this action also deletes the data files that are associated with the sample. If the BAM or VCF file that contains the data are associated with more than one sample, you cannot delete the sample.

---

1. In the **Samples** tab, click **Overview**.
2. Select the sample (or samples), then click **Actions** ▸ **Delete**.
3. Review the **Confirm Delete** message, then click **Yes** to delete the sample.

The sample is deleted and is removed from the **Samples** table.

4 Manage analysis workflows

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About analysis workflows

Analysis workflows in Ion Reporter™ Software are sets of instructions that determine how analysis results are produced.

The predefined analysis workflows include settings for common genetic research analysis applications. You can use a predefined analysis workflow as is, or you can copy a predefined analysis workflow, customize its settings, then save and reuse it in the future. After a predefined analysis workflow is copied and saved, it is known as a *user-defined analysis workflow*. You can also copy and edit, or edit a user-defined analysis workflow.

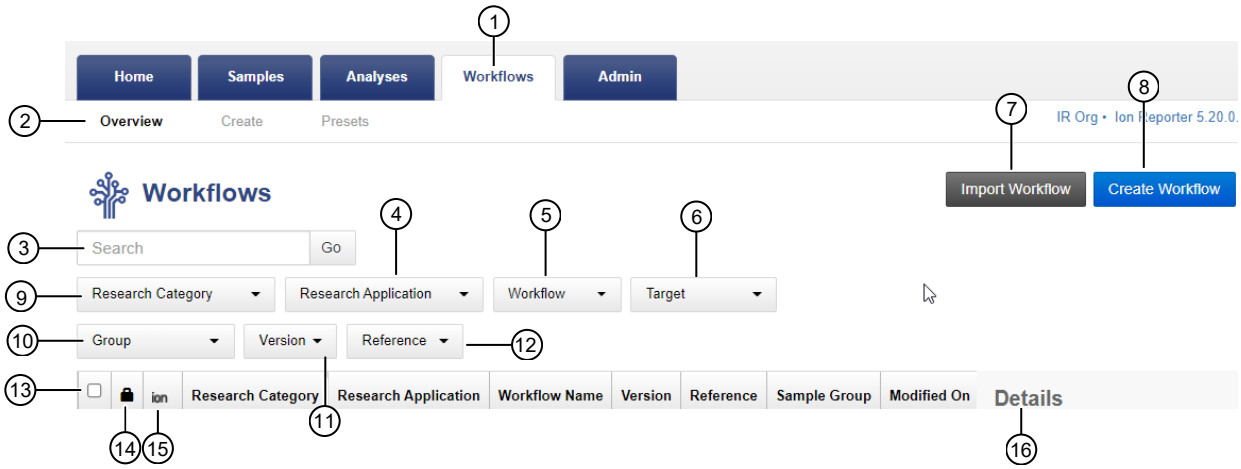
When an analysis workflow that contains samples is launched, Ion Reporter™ Software generates an analysis. Each analysis contains analysis results in various formats, including tables and visualizations in the software, and data files that can be downloaded.

In Torrent Suite™ Software, you can create a Planned Run that transfers data automatically to the appropriate Ion Reporter™ Server or to an Ion Reporter™ Software on Connect account, and use any of the available analysis workflows to create an analysis.

All analysis workflows that are available to the Ion Reporter™ Software organization are listed under the **Workflows** tab.

Workflow tab overview

You can access the list of available analysis workflows in the **Workflows** table, which is located in the **Workflows** tab, in the **Overview** screen.



① **Workflows** tab.

② The **Overview** screen in the **Workflows** tab. Click the screen name to toggle between the different screens.
The **Create** screen. For more information, see “Copy and edit an existing analysis workflow” on page 77.
The **Presets** screen. For more information, see “Workflow presets” on page 130.

③ The **Search** box enables you to enter keywords or text strings to narrow the list of analysis workflows. For more information, see “Search for analysis workflows” on page 69.

④ The **Research Application** filter enables you to search by application type.

⑤ The **Workflow** list enables you to filter by analysis workflows that are predefined, user-defined, or tagged for IonReporterUploader plugin.

⑥ The **Target** list enables you to filter by sequencing technology.

⑦ **Import Workflow** enables you to upload a file to create an analysis workflow.

⑧ **Create Workflow** enables you to create a new analysis workflow.

⑨ The **Research Category** filter allows you search by application category.

⑩ The **Group** list enables you to filter by sample grouping type.

⑪ The **Version** filter enables you to search by Ion Reporter™ Software version.

⑫ The **Reference** filter enables you to search by GRCh38 or hg19 references.

⑬ Table columns with information about the analysis workflows.

⑭ **(Locked)** Indicates that the analysis workflow is locked.

⑮ **Ion Factory Shipped** If present, indicates that the analysis workflow is predefined with parameters for use with specific products.

⑯ **Details** provides more information about the selected workflow.

Find analysis workflows

You can sort, search and filter information to narrow the **Workflows** list, then find a specific analysis workflow in Ion Reporter™ Software.

Search for analysis workflows

You can use keywords or text strings to search for data in the **Workflow Name** column of the **Workflows** table.

IMPORTANT! Search terms must adhere to the following rules.

- An asterisk (*) is not allowed in the search field for use as a wildcard.
- Searches are case-insensitive, that is, both upper-case and lower-cases letters are found regardless the case of search term letters.
- Searches find every occurrence of a continuous string. For example, a search for ***demo*** in analysis names returns a list of all analyses with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and therefore are not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

---

1. In the **Workflows** tab, click **Overview**.
2. In the **Search** box, enter a search term, then click **Go** to return a list of workflows that match the keyword.

   If the search string is invalid, the search field is outlined in red. Correct the search term to proceed.

The search results are returned in the **Workflows** table.

Sort the Workflows table

You can sort the **Workflows** table to make it easier to find the analysis workflows that you are looking for in Ion Reporter™ Software.

1. In the **Workflows** tab, in the **Overview** screen, click a column heading to sort the list of analysis workflows.
2. Click the column again to reverse the order.
3. Click the column a third time to return the column to the order that was used before the sort and stop sorting for the column.

Filter analysis workflows

You can apply filters to the **Workflows** table to narrow search results, or shorten the list of analysis workflows to make it easier to find the analysis workflows of interest.

1. In the **Workflows** tab, click **Overview**.
2. Apply the filters.
   - Click a filter to expand the list, then select one or more filters. For example: select **Research Application**, then select **Fusions**.
   - Click a filter to expand the list, then click **Show All** to select all filters in a specific filter category.

The contents of the **Workflows** table changes each time that you select a filter or set of filters.

3. Use the following guidelines to find Ion AmpliSeq™ HD analysis workflow templates or analysis workflows that have been copied:
   - To filter for Ion AmpliSeq™ HD analysis workflow templates, or copy and edited Ion AmpliSeq™ HD analysis workflows that include saved panel files, search for **Target ▸ AmpliSeq HD**.
   - To filter for tumor Ion AmpliSeq™ HD analysis workflow templates, use a combination of the filters, such as **Research Category ▸ Oncology – Solid Tumor**, and **Target ▸ AmpliSeq HD**.
4. Use the following guidelines to find predefined TagSeq analysis workflows or analysis workflows that have been copied.
   - To filter for all TagSeq analysis workflows, search for **Research Application ▸ Oncology – Liquid Biopsy**, or search for **Target ▸ TagSequencing**.
   - To filter for Liquid Biopsy and Tumor TagSeq analysis workflows, but *only* the Liquid Biopsy Ion AmpliSeq™ HD analysis workflows (copy and edited analysis workflows that include saved Ion AmpliSeq™ HD panel files), search for **Research Category ▸ Oncology – Liquid Biopsy**.

The filtered list of analysis workflows is returned in the **Workflows** table.

Remove filters

You can remove filters from a filter category from the **Workflows** table in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Overview**.
2. Click the filter category to expand the list, then select **Show All** at the top of the list.

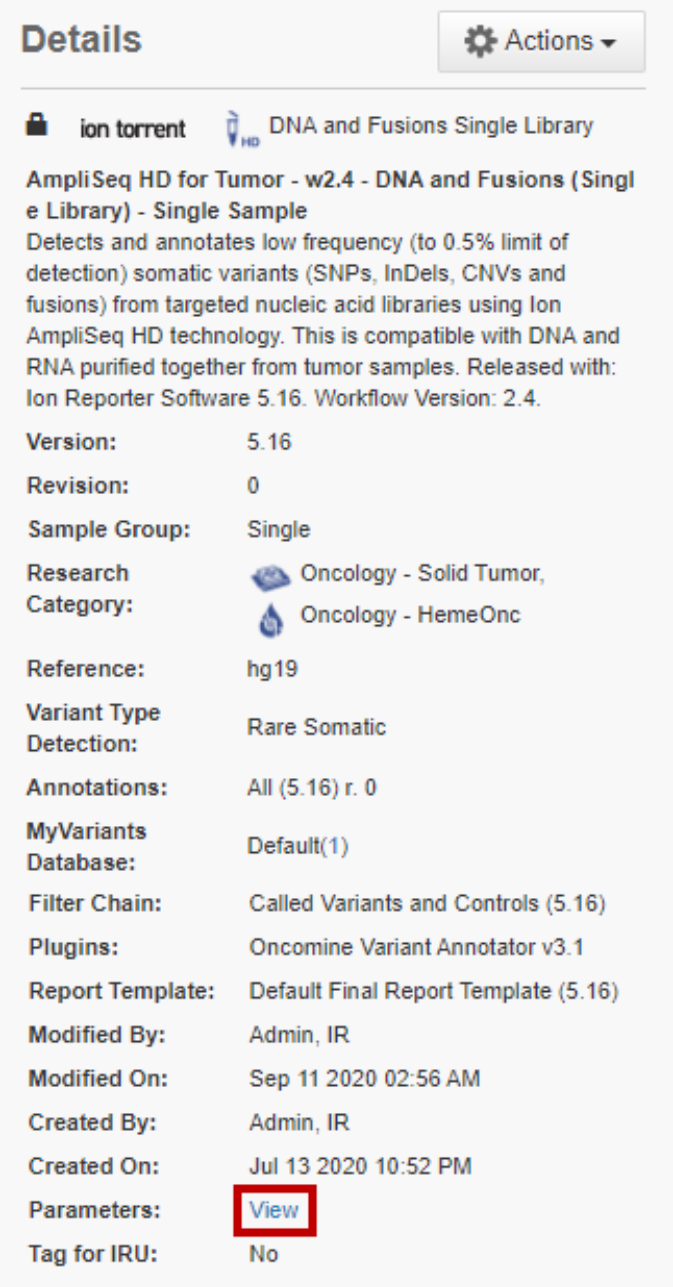
View analysis workflow details

When you select a single analysis workflow that is listed in the **Workflows** table in Ion Reporter™ Software, you can view information about its components in the **Details** section.

1. Click the **Workflows** tab.
2. On the **Overview** screen, in the **Workflows** table, select a row.

3. In the **Details** section, view the analysis workflow details.

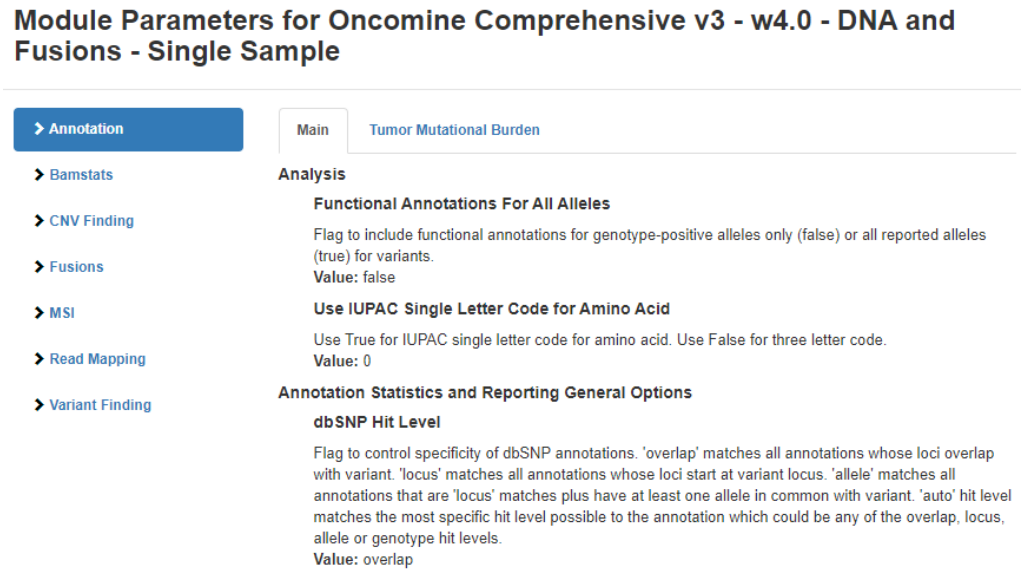
   The **Details** section is displayed.



The information that is available in the **Details** section depends on how you defined the analysis workflow. For more information, see “Analysis workflow details” on page 72.

Custom analysis workflows can be edited from the **Details** section. Predefined analysis workflows cannot be edited.

4. In the **Details** section, click the **View** link to open a dialog box that displays the workflow parameters.



Analysis workflow details

When you select a single analysis workflow that is listed in the **Workflows** table in Ion Reporter™ Software, you can view information about its components in the **Details** section. The information that is available depends on the settings in the analysis workflow.

Custom analysis workflows can be edited from the **Details** section. Predefined analysis workflows cannot be edited. For more information, see “Edit a user-defined analysis workflow” on page 93.

Analysis workflow detail	Description
Research Application	The research application in use in the analysis workflow.
Workflow name	The name and version of the analysis workflow.
Description	A short description of the analysis workflow.
Version	The version number of Ion Reporter™ Software.
Revision	The revision number of the analysis workflow.
Sample Group	The sample group in use in the analysis workflow.
Research Category	The research category selected for the analysis workflow.
Reference	The reference selected for the analysis workflow.
Target Regions	The file name of the target regions file used in the analysis workflow.
Variant Type Detection	The variant type detection selected for the analysis workflow.
Hotspot Regions	The file name of the hotspots file used in the analysis workflow.

(continued)

Analysis workflow detail	Description
Fusion Panel	The name and version of the fusion panel.
MSI Baseline File	The file name of the MSI baseline file used in the analysis workflow. This file contains baseline information that accounts for variations in sample preparation or run conditions to support robust MSI detection.
MSI Marker Regions	The file name of the MSI marker regions file used in the analysis workflow. The MSI marker regions file contains information about the genomic position of the MSI markers, and more information that is used by the MSI detection algorithm.
TMB Filter Chain	The TMB Filter Chain that is used in the analysis workflow.
TMB Calculation Version	The version of the tumor mutational burden algorithm that is available in the analysis workflow is listed. The algorithm version can provide information about how the data was analyzed and can be useful if earlier versions of the software were used, or if troubleshooting is required.
Sequence Variant Baseline	The file name of the Sequence Variant Baseline file used in the analysis workflow.
Annotations	The annotations that are used in the analysis workflow, based on the annotation set that is selected.
MyVariants Database	The MyVariants database in use in the analysis workflow.
Filter Chain	The filter chain that is in use for the analysis workflow.
Copy Number	The copy number baseline control in use in the analysis workflow. The copy number baseline identifies the copy number variants (CNVs) in samples.
Plugins	The name and version number of the plugin or plugins in use in the analysis workflow.
Report Template	The name of the report template used in the analysis workflow.
Modified By	The Ion Reporter™ Software user who modified the analysis workflow.
Modified On	The date and time that the analysis workflow was modified.
Created By	The Ion Reporter™ Software user who created the analysis workflow.
Created On	The date and time that the analysis workflow was created.
Parameters	Click the **View** link to open a window to see the parameters that are defined for the selected analysis workflow.
Tag for IRU	Indicates whether the analysis workflow is tagged for use with IonReporterUploader plugin.

Predefined analysis workflows

Analysis workflows are sets of analysis components that you can use to automate the analysis of data. Predefined analysis workflows are optimized with parameters for use with specific products.

These predefined analysis workflows are organized into the following research categories:

Research category	Description
Exome	For use with Ion AmpliSeq™ Exome and TargetSeq Exome panels.
16S rRNA Profiling	For use with 16S Metagenomics panels and Ion AmpliSeq™ Microbiome Health Research panels.
Oncology–Liquid Biopsy	For use with a variety of Ion AmpliSeq™ and Ion AmpliSeq™ HD, and Oncomine™ cancer panels and assays.
Reproductive	For use with Ion ReproSeq™ and other PGS whole genome assays, and the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel.
Immunology	For use with a variety of Ion AmpliSeq™ cancer panels and Oncomine™ immunology assays. For use with a variety of Ion AmpliSeq™ and Ion AmpliSeq™ HD, and Oncomine™ cancer panels and assays.
Oncology–ImmunoOncology	For use with a variety of Ion AmpliSeq™ and Oncomine™ cancer panels and assays.
Annotate Variants	For use with VCF samples as the input to annotate variants.
Oncology–HemeOnc	For use with a variety of Ion AmpliSeq™ and Ion AmpliSeq™ HD, and Oncomine™ cancer panels and assays.
Oncology–Solid Tumor	For use with a variety of Ion AmpliSeq™ and Ion AmpliSeq™ HD, and Oncomine™ cancer panels and assays.
Carrier Screening	For use with the Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel.
Inherited Disease	For use with a variety of Ion AmpliSeq™ and Oncomine™ cancer assays.
Plugin	Inserts plugin code into a workflow and allows a plugin to be included as the last step or the only step in when an analysis workflow is launched.

Tumor-normal pair research

The predefined analysis workflows for tumor-normal research generate analysis results that identify reads of both a tumor sample and reads of the related normal sample. These predefined analysis workflows are optimized to find somatic variants, which appear in the tumor sample and *do not appear in the normal sample*. These predefined analysis workflows also perform a statistical evaluation of the likelihood that the tumor allele is *not present in the normal sample* and calculates a P value that represents the statistical confidence of that call.

At each position within a variant in the tumor research sample, the evidence for that allele in the normal sample is examined as part of the analysis. If the tumor allele is detected in the reads of the normal research sample in levels that are higher than the error rate, it is not considered to be a tumor-specific (somatic) variant. Therefore the variant is rejected (not called).

Tumor variants in which the same position in the normal sample has no coverage, or has low coverage, are designated as nonconfident. Nonconfident variants are not assigned a p-value, and are flagged with NC-LC (nonconfident because of low-coverage) in the adjacent field in the output VCF file.

Some nonconfident variants receive the NC-LF (nonconfident because of low-frequency) flag instead of a p-value. This occurs with variants for which both of these conditions are true:

- The allele frequencies for the variant are <10% in both the tumor and normal sample.
- The variant has a nonzero allele frequency in the normal sample.

A Non-Confident variant call means that the variant might appear *not only* in the tumor sample, but also in the normal sample. This could indicate that either a germline variant or a systematic error is present in both samples.

In the VCF file, ***./.*** means a no-call in the normal sample and ***0/0*** means a homozygous reference call.

The tumor-normal pair predefined analysis workflows are run on a pair of research samples from the same individual. Ideally both research samples are sequenced on the same chip.

Note: When you create a user-defined analysis workflow for an Ion AmpliSeq™ Exome tumor-normal pair analysis, it is recommended that you do one of the following to help ensure that the correct parameters that are applied:

1. Make a copy of the predefined analysis workflow or a user-defined analysis workflow for use with Ion AmpliSeq™ Exome tumor-normal pairs and edit any desired parameters. For more information, see “Copy and edit an existing analysis workflow” on page 77.
2. Use the predefined BED file to create a new user-defined analysis workflow. For more information, see “Create a new analysis workflow” on page 79.

It is recommended that you *not* import the Ion AmpliSeq™ Exome panel BED file through either the import function in Ion Reporter™ Software, or with a manual import.

Flow Space Alternate Allele Calculation (FAO) calculation

Flow Space Alternate Allele Calculation (FAO) is calculated with the following formula.

Key for formula below

AF: Allele frequency

AO: Alternate allele depth at position

RO: Reference allele depth at position

DP: Total depth at position

FAO: Flow space alternate allele depth at position

FRO: Flow space reference allele depth at position

FDP: Flow total depth at position

1. FAO is usually equal to AO; however, due to complex alleles and/or downsampling*, FAO may differ from AO.
2. AF = FAO / (FAO + FRO) and not FAO / FDP. This is because FDP may include reads that do not fit the flow space profile of any hypothesis; in such cases, FDP ≥ FAO + FRO and this is not used in allele frequency calculation.

Exception: When flow correction is not performed and there are no F tags in the VCF file, then DP = AO + RO and AF = AO / DP.

*FAO along with all the F tags are subject to downsampling but AO/DP/RO/SAF/SAR/SRF/SRR are not. So when total coverage is higher than the downsampling cutoff, FAO tends to be smaller than AO.

CNV detection in aneuploidy analysis workflows

CNV detection is used in all predefined low-pass whole genome detection analysis workflows, including Ion ReproSeq™ analysis workflows for single-sample and 2-sample Ion AmpliSeq™ panels.

These predefined analysis workflows support chromosome and subchromosome-level aneuploidy detection down to submegabase resolution.

These low-pass whole genome detection aneuploidy detection analysis workflows that are intended for PreImplantation Genetic Testing (PGT) based on low-pass whole genome preparation include a CNV detection module, and correct read coverage for GC bias. Corrected coverage is compared to a baseline coverage from control samples of regions with known expected normal ploidy. That is, 2 on autosomes and X in females, and 1 on sex chromosomes in males.

The following information applies to the predefined analysis workflows for CNV aneuploidy detection and user-defined analysis workflows that you create from the predefined analysis workflows:

- The input data are only a test research sample. A control research sample is not necessary, because a precomputed Informatics Baseline Control is used as a copy number reference.
- The input sample is from a whole genome amplified library.
- The volume of the sample can be small.
- The average coverage can be small, in the order of 0.01x.
- These analysis workflows identify regions of the genome that are duplicated or deleted. The variant length detectable is typically from ~10 Mb up to a whole chromosome.
- These analysis workflows overcome the variations in coverage that are typical with amplified data.
- With these analysis workflows, the coverage is typically too low to call SNPs or INDELs.

User-defined analysis workflows

There are three ways to create user-defined analysis workflows in Ion Reporter™ Software for use with ready-to-use Ion AmpliSeq™ and Ion AmpliSeq™ HD panels.

- Copy an existing analysis workflow, then edit its settings to create an analysis workflow. For more information, see “Copy and edit an existing analysis workflow” on page 77. This procedure is typically used to create or edit the parameters of the analysis workflow for an expansion panel, based on the analysis workflow for the pre-existing panel. This procedure is also used to edit the settings for a ready-to-use panel.
- Create a new analysis workflow that contains unique settings. For more information, see “Create a new analysis workflow” on page 79. This procedure is typically used to create an analysis workflow for use with a custom Ion AmpliSeq™ panel.
- For Ion AmpliSeq™ HD panel files that are imported from **AmpliSeq.com**, copy the appropriate Ion AmpliSeq™ HD analysis workflow template, then use the template to make further changes to the user-defined analysis workflow, then save the analysis workflow before it is used for analysis. For more information, see “Copy and edit an analysis workflow template” on page 80. This procedure is required to create an analysis workflow for use with a custom Ion AmpliSeq™ HD panels.

Copy and edit an existing analysis workflow

You can copy and edit an existing analysis workflow to save time on analysis in Ion Reporter™ Software. This is the recommended way to create a user-defined analysis workflow.

Use a predefined analysis workflow or a user-defined analysis workflow to start with an optimized set of parameters.

When you create a user-defined analysis workflow, you can change details such as:

- variant annotation filters
- final report settings
- analysis plugins
- parameter settings

You can copy predefined analysis workflows and user-defined analysis workflows from the current software and from previous versions of the software. When you copy analysis workflows from an earlier version of the software, you must use target regions files, hotspots files, and fusion panel files from the same version of the software. You can view the analysis workflow version in the **Details** pane. For more information, see “View analysis workflow details” on page 70.

This procedure is typically used to create or edit the parameters of the analysis workflow for an expansion panel that is used with user-defined Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels. Edits are made to an analysis workflow for the pre-existing research panel. This procedure is also used to edit the settings for a ready-to-use Ion AmpliSeq™ and Ion AmpliSeq™ HD research panel.

1. In the **Workflows** tab, click **Overview**.
2. In the **Workflows** table, click the row for the analysis workflow that you want to copy, then click **(Actions)** ▸ **Copy**.
   The workflow bar opens to the **Research Application** step.

When you copy an analysis workflow, some settings and fields are defined by the analysis workflow and remain selected.

3. In the **Research Application** step, ensure the research application and sample group, then click **Next**.
4. In the **Reference** step, ensure that the required files are selected, then click **Next**.
5. In the **Annotation** step, ensure that one annotation set is selected, then ensure or select a MyVariants database associated with the analysis workflow, then click **Next**.
6. In the **Filters** step, ensure or select a filter chain, then click **Next**.
7. In the **Copy Number** step, ensure or select a copy number baseline, then click **Next**.
8. In the **Exon Tile Fusion** step, for analysis workflows for use with assays that include RNA Exon Tiling, ensure that the appropriate Exon Tile Fusion Baseline for the analysis workflow is selected, then click **Next**.
9. In the **Plugins** step, ensure or select plugins, then click **Next**.
10. In the **Final Report** step, ensure or select the final report template, then click **Next**.
11. In the **Parameters** step, ensure or edit parameters, then click **Next**.
12. In the **Confirm** step, name the analysis workflow, enter an optional description, then click **Confirm** and **Save Workflow**.

To view the copied analysis workflow, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name then find the custom analysis workflow listed in the **Workflows** table.

Create a new analysis workflow

You can create a user-defined analysis workflow in Ion Reporter™ Software that is not based on an existing analysis workflow that uses predefined settings.

This procedure is typically used when creating a workflow for a user-defined Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels.

Note: If you are creating a new analysis workflow, compare its parameters with the parameters of a predefined analysis workflow to optimize performance.

1. In the **Workflows** tab, click **Create Workflow**.
   The workflow bar opens.

2. In the **Research Application** step, select the research application and sample group, then click **Next**.
   If you are working with a custom Ion AmpliSeq™ research panel, you can use the following research applications, depending on the type of panel, the application and the genomic endpoints.
   - **AmpliSeq Germline DNA** (optionally with CNV)
   - **AmpliSeq Somatic DNA** (optionally with CNV)
   - **AmpliSeq Somatic** (Fusions)
   - **AmpliSeq Somatic** (DNA+Fusions)

   If you are working with a custom Ion AmpliSeq™ HD research panel, see instead “Copy and edit an analysis workflow template” on page 80.

3. In the **Reference** step, select the required files, then click **Next**.

4. In the **Annotation** step, select an annotation set and a MyVariants database, then click **Next**.

5. In the **Filters** step, select a filter chain, then click **Next**.

6. In the **Copy Number** step, select a copy number baseline, then click **Next**.

7. In the **Exon Tile Fusion** step, for analysis workflows that apply to assays that include RNA Exon Tiling, confirm that the appropriate **Exon Tile Fusion Baseline** for the analysis workflow is selected, then click **Next**.

   Note: Custom Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels do not typically include RNA Exon Tiling.

8. In the **Plugins** step, select plugins, then click **Next**.

9. In the **Final Report** step, select the final report template, then click **Next**.

10. In the **Parameters** step, select parameters, then click **Next**.

11. In the **Confirm** step, name the analysis workflow, enter an optional description, then click **Save Workflow**.

To check that the analysis workflow was created, click the **Workflows** tab, then click **Overview** and search for the analysis workflow name. You can now use the analysis workflow to launch an analysis. For more information, see “Launch an analysis” on page 165. You can also optionally download the analysis results and view them visually in the `cn_results.png`.

Copy and edit an analysis workflow template

To analyze Ion AmpliSeq™ HD sequencing data in Ion Reporter™ Software, you need to create a user-defined analysis workflow for use with Ion AmpliSeq™ HD panels. To create a user-defined analysis workflow, first copy a predefined Ion AmpliSeq™ HD analysis workflow template, then add a target regions file, and any available hotspots file or CNV baseline. For RNA samples, you need to also add fusion panel files to the user-defined analysis workflow. This procedure must be used when creating an analysis workflow for custom Ion AmpliSeq™ HD research panels.

After you create the user-defined analysis workflow, you can select the analysis workflow in Torrent Suite™ Software to transfer data automatically to the appropriate Ion Reporter™ Server and use the analysis workflow for the data analysis in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Overview**.

   For information about available Ion AmpliSeq™ HD analysis workflow templates, see instead “Analysis workflow templates for Ion AmpliSeq™ HD” on page 84.

2. In the **Workflows** table, select the Ion AmpliSeq™ HD analysis workflow template that you want to copy, then click **Actions** ▸ **Copy**.

   The **Edit** workflow bar opens to the **Research Application** step. Review the **Research Application** and **Sample Group**.

   When you copy analysis workflow templates, you cannot change the **Research Application** or **Sample Group**.

3. Click **Next**, then in the **Reference** step, select or upload the appropriate files.

Option	Description
Select a file that was previously uploaded to Ion Reporter™ Software.	Select the **Target Regions** and, if needed, **Hotspot Regions** files from the dropdown lists. **IMPORTANT!** The **Target Regions** and **Hotspot Regions** files that are used in an analysis workflow must each have a unique file name. To review the file names in the **Workflows** tab, go to **Workflow Presets** ▸ **Hotspot Region Files** and review the names of the hotspots files. If any of the names are identical to the **Target Regions** that is to be added to the analysis workflow, delete the entry in the list and upload a renamed version of the **Hotspot Regions** file.
Upload the entire package of the Ion AmpliSeq™ HD panel files directly from **AmpliSeq.com**. When you use this option, a target regions file and any available hotspot regions or fusion files specific for the panel are uploaded and available in the **Workflow Presets** screen.	Click **AmpliSeq Import**. a. In the **Import for AmpliSeq** dialog box, select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order, or Ion AmpliSeq™ On Demand panel files, or select the **Fixed Panel** tab for Community or Ready-to-Use panel files. b. Enter an account user name. c. In the Password field, enter the access code. For more information, see List item. on page 81. d. Click **List My AmpliSeq Panels**. e. From the list, select the fusion panel file that you want to import. f. Click **Import**.
Import a target regions file that you previously downloaded from **AmpliSeq.com** and saved to the local storage.	a. Under the **Target Regions** list, click **Upload**. b. Click **Select file**, browse to, then select the target regions BED file, then click **Open**. c. Select **Ion AmpliSeq™ HD**, then click **Upload**.
(Optional) Upload a hotspot regions file that was previously uploaded from **AmpliSeq.com** and saved to the local storage.	a. Under the **Hotspots Regions** list, click **Upload**. b. Click **Select file**, browse to, then select the hotspot regions BED file, then click **Open**. c. Select **Ion AmpliSeq™ HD**, then click **Upload**.
If you use a panel that detects fusions, upload a ZIP file that was previously downloaded from **AmpliSeq.com** and saved to the local storage.	a. Under the **Fusion Panel** list, click **Upload**. b. Click **Select file**, browse to, then select the fusions ZIP file, then click **Open**. c. Select **Ion AmpliSeq™ HD**, then click **Upload**.

4. Click **Next**.

5. In the **Annotation** step, ensure or select an annotation set and, if applicable, select a MyVariants database, then click **Next**.

6. In the **Filters** step, select a filter chain from the **Filter Chains** list to change the default filter chain for analyses that use this analysis workflow. If you do not change the filter chain, the default filter chain is saved for the new analysis workflow.

Option	Description
Called Variants and Controls	This filter chain reports all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, FLT3-ITD, and RNA Exon Tiles.
Called Hotspot Variants and Controls	This filter chain reports all hotspot variants that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, and RNA Exon Tiles.
Variant Matrix Summary	Select this filter chain for analysis results that contain the same set of variants that are included in the Variant Matrix Analysis visualization for Ion AmpliSeq™ HD analysis results. The **Variant Matrix Summary** filter chain filters in: • Positive hotspot calls • Positive gene-level CNV GAIN or LOSS calls • FLT3-ITD variants • Non-wild-type intragenic structural variations • For TagSeq Breast and TagSeq Pan-Cancer assays: non-hotspot variants in TP53 with QUAL >50 and Allele Frequency > 1.0 (Breast) or 0.5 (Pan Cancer) This filter chain allows analysis results to be exported. You can then import the results into another software application for further analysis. Filter variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants. If you use the Oncomine™ Myeloid MRD analysis workflow, a FLT3-ITD variant subtype named **Combined** can be reported in analysis results. The **Combined** subtype is a value for the sum of all reported FLT3-ITD variant allele frequencies.
Oncomine™ Variants	Select this filter chain to show only the variants that are annotated with the Oncomine™ Variant Annotator plugin. For more information, see Appendix D, “Oncomine™ Variant Annotator plugin criteria”.

7. Click **Next**.

8. In the **Copy Number** step, select a copy number baseline from the **Baseline** list, if applicable, then click **Next**.

   IMPORTANT! If you need help to add, create or copy a copy number baseline, contact your Field Bioinformatics Specialist (FBS).

9. In the **Plugins** step, select a plugin, then click **Next**.

   In the **Plugins** step, the Oncomine™ Variant Annotator plugin is selected by default. This plugin adds annotations for variants that are relevant to cancer with Oncomine™ Gene Class and Oncomine™ Variant Class information. This plugin is included by default with the Ion AmpliSeq™ HD analysis workflow templates. If you import the VCF file of analysis results into Oncomine™ Reporter, these annotations are included in a report that is generated from that software.

10. In the **Final Report** step, select or confirm the final report template that is selected in the list, then click **Next**.

11. In the **Parameters** step, make any required changes, then click **Next**.

    IMPORTANT! If you are using the Ion AmpliSeq™ HD test panel, consult your field support representative and other training materials before you change the parameters. If you designed a white glove panel, consult your white glove representative or field support representative to determine if parameter changes are required.

12. In the **Confirm** step, name the analysis workflow, enter an optional description, then click **Save Workflow**.

The newly created analysis workflow is added to the list of analysis workflows in the **Workflows** tab in the **Overview** screen.

Use of target regions BED files with a copied or edited analysis

When you copy an analysis workflow in Ion Reporter™ Software, in most cases, the parameters used for the Torrent Variant Caller (TVC) software component are the same as those contained in the analysis workflow which was copied. However, in a few cases, if you select a new target regions BED file that was imported from **AmpliSeq.com**, the TVC parameters settings are based on the values that are contained in the imported files. Panels that can include TVC parameters are Ready-to-Use Ion AmpliSeq™ panels, and custom panels and community panels imported from **AmpliSeq.com**.

In these cases, when you copy an analysis workflow from a customized workflow, and then change the target regions BED file, the parameter settings depend on how the target regions BED file is created.

The choices and information about the parameter settings are as follows.

Choice	Information about TVC parameter settings
Target regions BED file associated with a predefined analysis workflow	TVC parameters and the values associated with the BED file (if any) are used to update the TVC parameters of the edited workflow.
Target regions BED file from AmpliSeq.com	If a new target region bed file that was imported from AmpliSeq.com is selected, the TVC parameters can be set based on the values that are contained in the imported files.
All other target regions BED files	Parameters that are associated with the copied analysis workflow are used to update the parameters of the analysis workflow that is edited (with the selection of a target regions BED file).

To use a new target regions BED file, copy the workflow, then adjust the parameters, instead of editing a workflow. That is, use **Actions ▸ Copy** instead of **Actions ▸ Edit** to ensure that the correct TVC parameters are used.

Analysis workflow templates for Ion AmpliSeq™ HD

Ion Reporter™ Software supports predefined analysis workflow templates for use with Ion AmpliSeq™ HD panels. The Ion AmpliSeq™ HD predefined analysis workflow templates must be activated by an administrator before use. To activate the templates, contact Technical Support or the support representative. After activation, select the template appropriate for the application and use it to create a user-defined analysis workflow. For more information, see "Copy and edit an analysis workflow template" on page 80.

IMPORTANT! Ensure that the analysis workflow template name that you use matches the type of Ion AmpliSeq™ HD panel files that you include in the analysis. For example, if you use panel files for a liquid biopsy fusions single sample, copy the Ion AmpliSeq™ HD for Liquid Biopsy - w2.6 - Fusions - Single Sample analysis workflow template to create the user-defined analysis workflow.

Use the following guidelines to find Ion AmpliSeq™ HD analysis workflow templates or analysis workflows that have been copied in the **Workflows** tab.

- To filter for Ion AmpliSeq™ HD analysis workflow templates, or copy and edited Ion AmpliSeq™ HD analysis workflows that include saved panel files, search for **Target: AmpliSeq HD**.
- To filter for Tumor Ion AmpliSeq™ HD analysis workflow templates, use a combination of the **Workflow** filters **Research Category: Oncology – Solid Tumor**, and **Target: AmpliSeq HD**.

Note: Workflow versions 2.7 are released with Ion Reporter™ Software 5.22 for use with the Ion 560™ Chip.

Research Application	Analysis workflow template	Description
HD for Tumor (DNA)	Ion AmpliSeq™ HD for Tumor - w2.7 - DNA - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) variants (SNPs, INDELs and CNVs) from targeted DNA libraries using Ion AmpliSeq™ HD technology. Compatible with DNA that is purified from tumor samples.
HD for Tumor (Fusions)	Ion AmpliSeq™ HD for Tumor - w2.7 - Fusions - Single Sample	Detects and annotates gene fusions from targeted RNA libraries using Ion AmpliSeq™ HD technology. Compatible with RNA that is purified from tumor samples.
HD for Tumor (DNA+Fusions)	Ion AmpliSeq™ HD for Tumor - w2.7 - DNA and Fusions (Single Library) - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) variants (SNPs, INDELs, CNVs and fusions) from targeted nucleic acid libraries using Ion AmpliSeq™ HD technology. Compatible with DNA and RNA that is purified together from tumor samples.
HD for Tumor (DNA+Fusions)	Ion AmpliSeq™ HD for Tumor - w2.7 - DNA and Fusions (Separate Libraries) - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) somatic variants (SNPs, INDELs and CNVs) from targeted DNA libraries, and gene fusions from matching targeted RNA libraries using Ion AmpliSeq™ HD technology. Compatible with DNA and RNA that is purified separately from tumor samples.

(continued)

Research Application	Analysis workflow template	Description
HD for Liquid Biopsy (DNA)	Ion AmpliSeq™ HD for Liquid Biopsy - w2.7 - DNA - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) variants (SNPs, INDELs and CNVs) from targeted DNA libraries using Ion AmpliSeq™ HD technology. Compatible with DNA that is purified from cell-free liquid biopsy samples.
HD for Liquid Biopsy (Fusions)	Ion AmpliSeq™ HD for Liquid Biopsy - w2.7 - Fusions - Single Sample	Detects and annotates gene fusions from targeted RNA libraries using Ion AmpliSeq™ HD technology. Compatible with RNA that is purified from cell-free liquid biopsy samples.
HD for Liquid Biopsy (DNA+Fusions)	Ion AmpliSeq™ HD for Liquid Biopsy - w2.7 - DNA and Fusions (Single Library) - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) variants (SNPs, INDELs, CNVs and fusions) from targeted nucleic acid libraries using Ion AmpliSeq™ HD technology. Compatible with DNA and RNA that is purified together from cell-free liquid biopsy samples.
HD for Liquid Biopsy (DNA+Fusions)	Ion AmpliSeq™ HD for Liquid Biopsy - w2.7 - DNA and Fusions (Separate Libraries) - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) somatic variants (SNPs, INDELs and CNVs) from targeted DNA libraries, and gene fusions from matching targeted RNA libraries using Ion AmpliSeq™ HD technology. Compatible with DNA and RNA that is purified separately from cell-free liquid biopsy samples.

Create a user-defined analysis workflow for use with Metagenomics research application

The metagenomics analysis workflow provides access to 2 preinstalled reference databases for mapping: the curated MicroSEQ™ ID database and the curated GreenGenes database. You can customize the analysis workflow to make any of the following changes:

- Upload user-defined reference files, then map samples to any combination of user-defined and preinstalled reference databases for metagenomics research.

  If multiple reference databases are selected, data are first mapped against the first selected reference in the list. Next, reads that were not mapped against the first selected database are mapped against the next database that is selected in descending order, and so on, until the sample is mapped against the entire list of selected databases.
- Add primer sequences that were used to prepare the samples for metagenomics analysis workflows.

Note: You can create an analysis workflow that does not include any primer information, however, it is recommended that you always add primer information to a metagenomics analysis workflow. When the primer information is absent, no trimming is performed on your reads. A warning message appears during analysis review when primer information is missing.

1. In the **Workflows** tab, click **Overview**.

2. In the **Workflows** table, select the metagenomics analysis workflow that you want to copy, then click **Actions** ▸ **Copy**.
   The analysis workflow is copied and the Edit workflow bar opens to the **Research Application** step. Ensure that **Metagenomics** is selected in the **Research Application** section, then review the selection in **Sample Groups**, then click **Next**.

3. *(Optional)* To upload a user-defined reference database in FASTA format, in the **Reference** step, click **Upload**, then browse to, select, then upload the user-defined reference database file.

4. Select one or more reference databases in the **Available References** list, then click the arrow to add the reference to the **Selected References** list. Click **Next** when you have added all references that you want to use for the analysis.
   The order of the **Selected References** determines the order in which the sample is mapped against reference databases.

5. In the **Primers** step, select a primer option, then click **Next**.

Option	Description
Use No Primers	Select if no primers were used in library preparation.
Use Custom Primers	Select to provide your own primers. If primer sequences are provided, the sequences are trimmed from the reads before mapping occurs in the software. For more information about the file format of the primers, see "Custom primer sequences for Metagenomics analysis workflows" on page 87.
User Default Primers	Select to use proprietary primers that are included by default.

6. If you select **Use Custom Primers**, do one of the following to enter custom primer sequences:
   - Enter individual primer sequences directly into the **Paste FASTA Sequences** text box.
     For example: `>MyFavoriteV5_forward ACTCGGTCCARACTGAGACT >MyFavoriteV5_Rev TTACCGRGGCGTATGCGG>MyFavoriteV8_FwdCCARAACTCGGTCTGSGACT >MyFavoriteV8_rRGGCGTATGCSTACCGGG`
   - The names of forward primers must end in ***_f****. Reverse primer names must end in ***_r****. Primers in a pair must have identical names so that the software can match the primers during the analysis.
   - Upload a FASTA file that contains primers. For more information about the file format of the primers, see "Custom primer sequences for Metagenomics analysis workflows" on page 87.

   a. Click **Select File**.

   b. Browse to the folder containing the FASTA file, select it, and click the **Choose**, **Open**, or **Save** .

   c. Ensure that the correct file name appears in the **Upload FASTA File** field, then click **Upload**.
      The primer sequences are uploaded and you can optionally edit them in the **Paste FASTA File** field.

7. Click **Next**.

8. In the **Parameters** step, make any desired changes to the Metagenomics parameters, then click **Next**.

9. Enter a name for the analysis workflow, and an *(optional)* description, then click **Save Workflow**.

Custom primer sequences for Metagenomics analysis workflows

You can upload a set of primer sequences that were used to prepare your samples for metagenomics analysis workflows. Primer sequences can be uploaded from a FASTA file or entered individually into the software. If primer sequences are provided, the sequences are trimmed from the reads before mapping occurs in the software. The names of forward primers must end in ***_f**** and reverse primer names must end in ***_r****. Primers in a pair must otherwise have identical names so that the software can match the primers during the analysis.

IMPORTANT! The header for each custom reference sequence must include at least the following information, where `sequence` is the base-pair sequence:

```
>mg|Genus|Species|

sequence
```

The header can include the following additional information, if available:

```
>mg|Genus|Species|Subspecies/
Strain|Accession#|Kingdom|Phylum|Class|Order|Family|PubMed#|LibraryID#|

sequence
```

Enable tumor mutational burden calculation in existing analysis workflows

Tumor mutational burden (TMB) is a calculation of somatic mutations per megabase (Mb). You can enable the calculation in any DNA – Single Sample, or DNA and Fusions – Single Sample analysis workflow. When enabled, tumor mutational burden and other data values are included in analysis results from that analysis workflow. To enable the tumor mutational burden calculation, copy and edit any predefined analysis workflow, or edit an existing user-defined analysis workflow that is not locked.

An analysis workflow that is enabled for tumor mutational burden calculates mutations per megabase (Mb) and adds graphics and other information about the mutations to Ion Reporter™ Software analysis results and visualizations.

1. In the **Workflows** tab, click **Overview**.

2. In the list of analysis workflows, select the row for the DNA – Single Sample, or DNA and Fusions – Single Sample analysis workflow that you want to copy.
   The Details pane shows information about the selected workflow.

3. Click ⚙ **(Actions)** ▸ **Copy**.
   The **Edit** workflow bar opens to the **Research Application** step with the **Research Application** and **Sample Groups** preselected. When you copy an analysis workflow template, you cannot change these settings.

4. Click **Next** multiple times to proceed to the **Parameters** step. Alternatively, click each step in the workflow bar to go to the parameter step.

5. To enable the tumor mutational burden calculation, ensure that the **Tumor Mutational Burden Filter Chain** parameter is set.
   a. In the **Parameters** step, under annotation, select the **Tumor Mutational Burden** tab.
   b. Find the **Tumor Mutational Burden Filter Chain** parameter, then change the value to **TMB (Non-germline Mutations**).

   You can review the algorithm version that is used to calculate tumor mutational burden in the **Tumor Mutational Burden Calculation Version** parameter.

   For parameter descriptions see “Annotation parameters” on page 94.

6. Change other tumor mutational burden parameters, if needed.

   IMPORTANT! The parameter settings of an Oncomine™ Tumor Mutation Load are optimized. If you are using these parameters for another type of analysis workflow, change the parameters if needed. Contact your Field Bioinformatics Specialist (FSB) for help.

7. Click **Next**, then enter a **Workflow Name** and an optional **Description** for the analysis workflow.

8. Click **Save Workflow**.
   The user-defined analysis workflow is saved and is added to the **Workflows** table.

To verify that the analysis workflow was saved, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name. To analyze samples with the new tumor mutational burden calculation enabled analysis workflow, see “Manually launch an analysis” on page 168.

Reduce the impact of deamination in low-quality FFPEs

Samples of low quality that exhibit deamination can be analyzed if you increase the minimum allele frequency in an analysis workflow. For example, a minimum allele frequency of 10% for the tumor mutational burden (TMB) calculation can reduce the impact of deamination on the reported TMB value. It is important to consider the tumor content of a given sample when you increase the minimum allele frequency of the TMB calculation.

- Samples with low tumor content can have many true somatic mutations that are excluded from the tumor mutational burden calculation when a higher minimum allele frequency filter is included in the analysis workflow.
- The tumor mutational burden values cannot be reliable if you adjust the minimum allele frequency parameters for samples with a high estimated SNP proportion consistent with deamination, (primarily an FFPE number) or a high deamination score, (for example >100).
- The mean depth and uniformity of the sample as determined by the coverageAnalysis plugin can also indicate sample quality. For more information, see *Torrent Suite™ Software Help*.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Customize an Oncomine™ Tumor Mutation Load - w3.1 - DNA - Single Sample analysis workflow

The Oncomine™ Tumor Mutation Load - w3.1 - DNA - Single Sample analysis workflow calls variants at ≥5% allelic frequency at positions with sufficient read coverage (≥60) for tumor mutational burden calculation. Poor sample fixation can artificially increase the determined tumor mutational burden due to deamination. Deamination is reported as the **Deamination Score** under the **Sample QC** tab. Samples with low tumor content can have true biological somatic C:G>T:A mutations that are counted towards deamination.

You can modify the Oncomine™ Tumor Mutation Load - w3.1 - DNA - Single Sample analysis workflow to reduce the affect of deamination in low-quality FFPEs and achieve a higher minimum allele frequency for a tumor mutational burden calculation.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

1. In the **Workflows** tab, click **Overview**.
2. In the **Overview** screen, you can use the **Oncology-ImmunoOncology** Research Category filter and **DNA** Research Application filters to limit the list of analysis workflows.
3. In the list of analysis workflows, select the row for the DNA – Single Sample, or DNA and Fusions – Single Sample analysis workflow that you want to copy, then click **(Actions)** ▸ **Copy**.
   The **Edit** workflow bar opens to the **Research Application** step with the **Research Application** and **Sample Groups** preselected. When you copy analysis workflow templates, you cannot change these settings.
4. Click **Next** multiple times to proceed to the **Parameters** step. Alternatively, click each step in the workflow bar to go to the parameter step.
5. In the **Parameters** step, click the **Annotation** tab, then select the **Tumor Mutational Burden** tab, scroll to the **TMB Variant Minimum Allele Frequency** parameter and set the allele frequency value to a higher value.
   For example, set **From =** value to ***0.05*** for ≥5% allele frequency, and to ***0.1*** for ≥10% allele frequency.
   IMPORTANT! Do not change other parameters, which can negatively affect analysis results.
6. Click **Next**.
7. In the **Confirm** step, enter an analysis workflow name and optional description, then click **Save Workflow**.

The modified analysis workflow is now available for use in the **Workflows** tab, **Overview** screen.

Analysis workflows for aneuploidy research

To create a new analysis workflow for use with Ion ReproSeq™ aneuploidy research, you can copy and edit a predefined analysis workflow.

You can enable mosaicism detection, a software setting that allows non-integer ploidy calls and reports a CNV event as a decimal ploidy value instead of an integer value. For each tile that is shown in analysis results, the algorithm fits the data to all ploidy with a step of 0.05. You can further customize mosaicism detection with the Expected Normal Ploidy Buffer (ENPB) filter. For more information, see "Predefined filters" on page 455 and "Create an Expected Normal Ploidy Buffer filter chain" on page 437.

You can also improve the detection of small segmental CNV events in Ion ReproSeq™ analysis workflows for use with aneuploidy research. The `CNV Transition Penalty` parameter establishes the trade-off between false-positive and false-negative rates. The transition penalty is a probability that the copy-number state changes for any given random tile. For more information, see "Improve detection of small segmental CNV events" on page 91.

IMPORTANT! Do not change parameters from the default settings unless you understand how the change can affect the analysis.

Create a user-defined analysis workflow for mosaicism, No Gender, or different tile sizes

To create a new analysis workflow for use with Ion ReproSeq™ aneuploidy research, you can copy and edit a predefined analysis workflow.

Mosaicism detection, a software setting that allows non-integer ploidy calls and reports a CNV event as a decimal ploidy value instead of an integer value, is enabled by default in the Mosaic aneuploidy analysis workflows. For each tile that is shown in analysis results, the algorithm fits the data to all ploidy with a step of 0.05.

You can decrease tile size to further improve sensitivity to detect small segmental CNV events. This change must be accompanied by selection or creation of a CNV Baseline with a corresponding smaller tile size. CNV baselines that correspond to smaller tile sizes are included in Ion Reporter™ Software and are ready to copy and edit to create user-defined aneuploidy analysis workflows.

You can further customize mosaicism detection if you use a filter chain that includes the Expected Normal Ploidy Buffer (ENPB) filter.

For example, you can copy the Ion ReproSeq™ No Gender PGS w1.1 analysis workflow, which has mosaicism detection that is enabled by default, and improve the detection of small segmental CNV events if you adjust the CNV Transition Penalty parameter. For more information, see "Improve detection of small segmental CNV events" on page 91. You can also select a baseline to increase sensitivity through tile sizes.

IMPORTANT! Do not change parameters from the default settings unless you understand how the change can affect your analysis.

The **CNV Gender Caller Enable Flag** parameter value can be set to **True** in only the Ion ReproSeq™ No Gender PGS w1.1 analysis workflow. You cannot enable gender hiding in other Ion ReproSeq™ analysis workflows because the **Hide called gender** parameter is locked as **False**. That is, gender is called in analyses that use other Ion ReproSeq™ analysis workflows.

1. In the **Workflows** tab, click **Overview**.
2. In the list of analysis workflows, select **Aneuploidy** from the **Research Application** list to narrow the list of analysis workflows. Select the Ion ReproSeq™ No Gender PGS w1.1 analysis workflow, then click **(Actions)** ▸ **Copy**.
3. To change the default sensitivity of 2-Mbp tile size, browse to the **Baseline** step, then select a baseline.
   - **Ion ReproSeq Low-Coverage Whole-Genome Baseline 1 Mbp**,
   - **Ion ReproSeq Low-Coverage Whole-Genome Baseline .5 Mbp**,
   - or a user-defined baseline to increase sensitivity for tile sizes in analysis results.
4. Click **Next** .
5. In the **Confirm** step, enter a name and an optional description for the new analysis workflow, then click **Save Workflow**.
   The user-defined analysis workflow is added to the list of available analysis workflows. To see a visualization of analysis results for which a custom analysis workflow for use with aneuploidy research was used, see “Smoothing, no gender, and mosaicism results in IRGV” on page 325.

Improve detection of small segmental CNV events

You can improve the detection of small segmental CNV events if you adjust the `CNV Transition Penalty` parameter in Ion ReproSeq™ analysis workflows for use with aneuploidy research. The CNV Transition Penalty parameter establishes the trade-off between false-positive and false-negative rates. The transition penalty is a probability that the copy-number state changes for any given random tile. A smaller probability results in calling of only larger CNV segment sizes, or calling of only segments that include greater support for the changed state, that is, a greater difference in copy number value from the current ploidy state.

Low sensitivity results in fewer false positives, but more false negatives. High sensitivity results in fewer false negatives, but more false positives. High sensitivity is required to make segmental aneuploidy calls of ~20 Mbp. **Custom CNV Sensitivity** enables you to change the CNV Transition Penalty parameter to detect even smaller segmental CNV events more sensitively.

IMPORTANT! Do not change parameters from the default settings unless you understand how the change can affect your analysis.

You can change the CNV Transition Penalty parameter to any value that does not exceed the highest allowable for this parameter:

- –1.05 for nonmosaic analysis workflows
- –2.31 for mosaic analysis workflows

1. In the **Workflows** tab, click **Overview**.
2. In the list of analysis workflows, select **Aneuploidy** from the **Research Application** list, select the software version that you use, then sort the list by predefined analysis workflows.
3. Select the analysis workflow of interest, such as the Ion ReproSeq™ PGS analysis workflow, then in the **Workflows** tab, click **Overview**, then in the **Workflows** table, click the row for an analysis workflow that you want to copy, then click **(Actions)** ▸ **Copy**.
4. In the **Create Workflow** screen, advance to the **Parameters** step, then click the **Cnv Finding** link. In the **Analysis** section **Main** tab, change the **CNV Sensitivity** setting to **Custom**, then enter new values for the parameter.
5. Click **Advanced**, scroll to the **CNV Transition Penalty** parameter, then enter the desired value.

   The approximate recommended values are:

   - -2.33 for mosaic ploidy calling
   - -15 for low sensitivity
   - -5 for medium sensitivity
   - -2.0 for nonmosaic ploidy calling
   - -3.0 for high sensitivity in mosaic and nonmosaic analysis workflows
6. In the **Confirm** step, click **Save Workflow**.

   The following results show a sample analyzed twice with 2 user-defined analysis workflows—one with the CNV Transition Penalty parameter set to -3, and a second with the parameter set to -2.

   In this visualization, a 7.9-Mb deletion that is called on chromosome 6 is visible with the slightly higher transition penalty. 

Transfer a user-defined analysis workflow

Ion Reporter™ Software allows you export a user-defined analysis workflow, then import that workflow to another Ion Reporter™ Server or Ion Reporter™ Software on Connect account. Exporting and importing an analysis workflow that has the settings you require saves time, because you can reuse the user-defined analysis workflow, instead of creating a new analysis workflow for each server or account.

You need to use an Ion Reporter™ Software account with the Analyze role to export and import an analysis workflow. An analysis workflow can only be exported from, then imported to, a single Ion Reporter™ Software organization. Each ZIP file that is exported can include only one analysis workflow.

The target regions, hotspot regions, and CNV baseline files, are not included in the ZIP file that is exported. Presets for the analysis workflow, including presets for the annotation set, filter chain, copy number baseline, final report template, fusion panel, target regions file, and hotspot regions file are also not included in the ZIP file. The files must be uploaded separately, and the workflow presets must be present in the organization into which the analysis workflow is imported.

1. In the **Workflows** tab, click **Overview**.
2. In the **Workflows** screen, find the user-defined analysis workflow that you want to transfer, select the checkbox in the first column, then click **Actions** ▸ **Export**.

3. Browse to the directory where you want to download the ZIP file that contains the files used for the workflow, then click **Export**.

   A ZIP file is saved to the hard drive.

4. Sign into the Ion Reporter™ Server or Ion Reporter™ Software on Connect account that you want to use to import the workflow.

5. In the **Workflows** tab, click **Overview**, then click **Import Workflow**.

6. Click **Select File**, browse to find, then select the ZIP file. The file name of the workflow directory is `workflow name.zip`.

   The workflow directory contains 2 files: a file with metadata to set the workflow parameters, and a separate parameters file.

7. Click **Open**, then click **Import**.

8. Refresh the **Workflows** list, or search for the workflow by name, then verify that the workflow is in the list for the Ion Reporter™ Server or Ion Reporter™ Software on Connect account on which you imported the workflow.

You can start to use the analysis workflow immediately, if the target regions, hotspot regions, and CNV baseline files, and presets are available in the Ion Reporter™ Server or the Ion Reporter™ Software on Connect account.

Edit a user-defined analysis workflow

You can edit a user-defined analysis workflow to change the settings or parameters that are associated with the analysis workflow.

You cannot edit predefined analysis workflows or locked analysis workflows. After an analysis workflow is locked, that action cannot be undone.

1. In the **Workflows** tab, select the analysis workflow that you want to edit, then in the **Details** section click ⚙ **(Actions)** ▸ **Edit**.

2. In the **Workflows** table, click the row for the analysis workflow that you want to copy, then click ⚙ **(Actions)** ▸ **Edit**.

   When you edit an analysis workflows, some settings and fields are defined by the workflow and remain selected.

3. In the **Research Application** step, confirm or edit the research application and sample group, then click **Next**.

4. In the **Reference** step, you can confirm that the required files are selected, or change the files. You cannot change the **Reference** or **Variant Type Detection** when you edit an analysis workflow. then Click **Next**.

5. In the **Annotation** step, confirm that one annotation set is selected, confirm or change the MyVariants database that is associated with the analysis workflow, then click **Next**.

6. In the **Filters** step, confirm or select a different filter chain, then click **Next**.

7. In the **Copy Number** step, confirm or change the copy number baseline, then click **Next**.
8. In the **Exon Tile Fusion** step, for analysis workflows that apply to assays that include RNA Exon Tiling, confirm that the appropriate Exon Tile Fusion Baseline for the analysis workflow is selected, then click **Next**.
9. In the **Plugins** step, confirm or edit the list of selected plugins, then click **Next**.
10. In the **Final Report** step, confirm or change the final report template, then click **Next**.
11. In the **Parameters** step, confirm or edit parameters, then click **Next**.
12. In the **Confirm** step, name the analysis workflow, enter an optional description, then click **Confirm** and **Save Workflow**.

To verify that the edited analysis workflow was saved, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name to confirm that the user-defined analysis workflow is listed in the **Workflows** table.

Customize tuning parameters

You can modify tuning parameters in the **Parameters** workflow step during analysis workflow creation in Ion Reporter™ Software.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

All analysis workflow types allow some parameter user-defined settings. The parameters that you can customize vary by analysis workflow. Parameter categories include: annotation, bamstats, CNV finding, MSI, read mapping, and variant finding.

In the **Parameters** workflow step, select the parameter category on the left, then confirm the settings or change the settings if needed.

Annotation parameters

You can modify the following annotation parameters to optimize analysis results when you create or edit Ion Reporter™ Software analysis workflows.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Parameter	Description
Main tab	
Analysis options	
Functional Annotations For All Alleles	Flag to include functional annotations for genotype-positive alleles only (false) or all reported alleles (true) for variants. **Allowed values: True** or **False**

(continued)

Parameter	Description
Use IUPAC Single Letter Code for Amino Acid	Use True for IUPAC single letter code for amino acid. Use False for three letter code. **Allowed values: True** or **False**
Annotation Statistics and Reporting General Options	
dbSNP Hit Level	Flag to control specificity of dbSNP annotations. 'overlap' matches all annotations whose loci overlap with variant. 'locus' matches all annotations whose loci start at variant locus. 'allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'auto' hit level matches the most specific hit level possible to the annotation which can be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto**
ClinVar Hit Level	Flag to control specificity of ClinVar annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Auto' hit level matches the most specific hit level possible to the annotation which can be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto**
COSMIC Hit Level	Flag to control specificity of COSMIC annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Auto' hit level matches the most specific hit level possible to the annotation which can be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto**

(continued)

Parameter	Description
VariantDB Hit Level	Flag to control specificity of VARIANTDB annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Genotype' matches all annotations that are 'allele' matches where the genotypes also match. 'Auto' hit level matches the most specific hit level possible to the annotation which can be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto**
ExAC Hit Level	Flag to control specificity of ExAC annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Auto' hit level matches the most specific hit level possible to the annotation which can be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto**
Gene Extension Size	Gene extension is the number of bases upstream and downstream of the start and end positions in a transcript that should include the regulatory and control regions. **Allowed values:** 0 to unlimited
Splice Site Size	The 5' splice site of an exon is the small intronic region immediately upstream, which depends on the strand. Its size in bases is given by splice site size. **Allowed values:** 0 to unlimited **Suggested trial value:** 2

(continued)

Parameter	Description
Tumor Mutational Burden tab	
Tumor Mutational Burden Filter Chain	Filters out the potential germline variants and retains the somatic variants for tumor mutational burden calculation after the variant calling and variant annotation is complete for the analysis. To enable tumor mutational burden calculations on DNA samples, you must also edit, or copy and edit, either: any DNA–Single Sample, or DNA and Fusions–Single Sample analysis workflow. For more information, see “Enable tumor mutational burden calculation in existing analysis workflows” on page 87. To enable the tumor mutational burden calculation for any other analysis workflow, you must select an available filter chain options. **Allowed values:** • TMB (Non-germline Mutations). If you use an analysis workflow for a Tumor Mutation Load Assay, TMB (Non-germline Mutations) is selected by default. • Mutation Load (Somatic Mutations) • More user-defined filter chains (if created on the server) **IMPORTANT!** Do not enable the filter chain parameter for other workflow types other than a DNA–Single Sample, or DNA and Fusions–Single Sample analysis workflow.
Tumor Mutational Burden Calculation Version	The version of the tumor mutational burden algorithm that is available in the software is listed. The algorithm version can provide information about how the data was analyzed and can be useful if earlier versions of the software were used, or for troubleshooting.
Minimum Base Coverage	The minimum depth of base coverage required for a variant to be counted for TMB calculation. **Allowed values:** 0 to unlimited
TMB Variant Minimum Allele Frequency	The minimum alternate allele frequency for a variant to be included for TMB calculation. **Allowed values:** 0 to 1 You can use this parameter to reduce the affect of deamination in low-quality FFPEs and obtain a higher minimum allele frequency for a tumor mutational burden calculation. For more information, see “Reduce the impact of deamination in low-quality FFPEs” on page 88.
TMB Variant Region Type	The type of region to include for TMB calculation. **Allowed values:** • Exonic regions only • Total target regions (exonic + intronic regions)

(continued)

Parameter	Description
TMB Variant Type	The variant types to be included for TMB calculation. **Allowed values:** • SNV • INDEL • MNV
TMB Variant Effect Type	The variant effect types to be included for TMB calculation. **Allowed values:** • missense • frameshiftDeletion • frameshiftInsertion • nonframeshiftDeletion • nonframeshiftInsertion • nnsense • stoploss • synonymous • unknown
Deamination QC Threshold	The deamination value above which a sample is deemed failed for TMB reporting. **Allowed values:** 0 to unlimited
Calibration Minimum Cutoff	The lower limit of the range at which the germline calibration is applied. **Allowed values:** 0 to unlimited
Calibration Maximum Cutoff	The upper limit of the range at which the germline calibration is applied. **Allowed values:** 0 to unlimited
TMB Germline-Filter Calibration Factor: Slope	The user-supplied value for the slope of the linear curve to which the number of somatic variants is calibrated. **Allowed values:** 0 to unlimited
TMB Germline-Filter Calibration Factor: Intercept	The user-supplied value for the intercept of the linear curve to which the number of somatic variants is calibrated. **Allowed values:** –2,000 to 2,000
TMB-Low Threshold	The TMB (mut/mb) threshold below which a sample is defined as TMB-Low. **Allowed values:** 0 to unlimited
TMB-High Threshold	The TMB (mut/mb) threshold above which a sample is defined as TMB-High. **Note:** A TMB score that is above the TMB-Low Threshold and below the TMB-High Threshold is defined as Intermediate.

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Parameter	Description
TMB Standardization	Apply the standardization of the observed TMB value to fit a linear curve. **Allowed values: On** or **Off**
TMB Standardization Factor: Slope	The user-supplied value for the slope of the linear curve to which the observed final TMB value is adjusted. **Allowed values:** 0 to unlimited
TMB Standardization Factor: Intercept	The user-supplied value for the intercept of the linear curve to which the observed final TMB value is adjusted. **Allowed values:** -2,000 to 2,000

Bamstats parameters

You can adjust the following Bamstats parameters when you create or edit Ion Reporter™ Software analysis workflows to optimize the quality of the BAM results file that is generated during analysis.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Parameter name	Description
Main tab	
Maximum Coverage	The maximum coverage of locations in the reference. Locations with coverage more than the maximum coverage values are ignored during coverage calculations. **Allowed values:** 100 to 1,000,000
Maximum read length	The maximum read length. **Allowed values:** 700 to 20,000
Advanced tab	
Max mapping qv	The maximum mapping quality value. Any alignment with mapping quality value more than the specified value is ignored. **Allowed values:** 0 to 255
Max mismatches	The maximum number of mismatches allowed in the alignments. Any alignment with more than the specified number of mismatches are ignored when reports that are related to the number of mismatches are generated. **Allowed values:** 0 to 500
Max base qv	The maximum base quality values. Any base with a base quality value of more than the specified value are ignored when reports are generated. **Allowed values:** 0 to 100

(continued)

Parameter name	Description
Maximum Medium Coverage (QC)	The maximum medium coverage value. Any base with a coverage value of more than the specified value are ignored when reports are generated. The value of **Maximum Medium Coverage (QC)** should be greater than **Maximum Low Coverage (QC)**. **Allowed values:** 2 to unlimited
Minimum Target Overlap Forward	The fraction of an alignment that must be overlapped by a target to be classified as on target. **Allowed values:** 0.000001 to 1
Maximum Low Coverage (QC)	The maximum low coverage value. Any base with a coverage value of more than the specified value is ignored when reports are generated. **Value of Maximum Low Coverage (QC)** should be less than **Maximum Medium Coverage (QC)**. **Allowed values:** 1 to unlimited
Maximum Target Overlap Reverse	The fraction of an alignment that must be overlapped by a target to be classified as on target. **Allowed values:** 0.0001 to 1

CNV Finding parameters

You can adjust Copy Number Variant (CNV) finding parameters to optimize analysis results when you create or edit Ion Reporter™ Software analysis workflows.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Parameter Name	Description
Main tab	
Analysis (apply only to Anueploidy analysis workflows)	
CNV Sensitivity	Sensitivity. The value of editable parameter **Transition Penalty** is used by the algorithm only when **CUSTOM** option is selected, This option is available in **Advanced** tab for CNV parameters. **Allowed values:** Low, Medium, High, or Custom
CNV Somatic	**IMPORTANT!** The somatic gene-level CNV calling parameter is for beta use only, and requires BED files and a copy number informatics baseline containing gene and pooling information. This parameter is not for use for germline copy number calling such as in ReproSeq workflows or other analysis workflows, which are designed to detect low pass whole genome aneuploidy events. **Allowed values:** True or False

(continued)

Parameter Name	Description
Advanced tab	
Gender calling	
CNV Gender Caller Enable Flag	Flag to indicate whether Gender caller should be invoked. **Allowed values:** True or False This flag is valid only for analysis workflows that are used to detect Low Pass whole genome aneuploidy events, such as Ion ReproSeq™ analysis workflows and other low pass whole genome analysis workflows that are used for aneuploidy research.
CNV Gender Threshold	Specifies threshold ratio of chrY to Autosomes for taking male/female call. **Allowed values:** 0 to unlimited
CNV Gender Min Mapping QV	Specifies minimum mapping qv of reads to consider in gender calling. **Allowed values:** 0 to 255
CNV Gender Min Autosomes Count	Specifies minimum number of required filtered reads in autosomes. **Allowed values:** 0 to unlimited
CNV CHRM to Autosomes Ratio Min Mapping QV	Specifies minimum mapping qv of reads to consider in calculating chrM A Ratio. **Allowed values:** 0 to 255
Sample Filtering (applies only to VCIB CNV finding algorithm)	
Read Count	User to enter a threshold number (integer). **Allowed values:** 10,000 to unlimited
min-mapping-qv	Minimum mapping quality value required for a read to be counted. **Allowed values:** 0 to unlimited
Percent Non Zero Amplicons. Percent of reads aligning to an amplicon in the target regions file.	User to enter a threshold number (integer). **Allowed values:** 50 to 100
MAPD threshold Sample will fail if MAPD is above this threshold.	User to enter a threshold number (float). **Allowed values:** 0 to unlimited
Analysis (applies only to VCIB CNV finding algorithm)	
NPC	Number of Principal Components used for correction. **Allowed values:** 0 to 12

(continued)

Parameter Name	Description
CNV Shift Type	**Allowed values:** MEDGENE, MEDAMP, VALUE_BASED_ON_MEDAMP, MAXGENE, or FLATGENE. The following CNV shift methods are available. • **Median Amplicon** – The median copy number of autosomal amplicons is set to 2. • **Median Gene** – The median copy number of autosomal genes is set to 2. • **VALUE_BASED_ON_MEDAMP** – The subtraction value applied to the log2 ratio is based on the result of the CNV shift method used. • **MAXGENE** – The log2-normalized counts for each gene in the panel are adjusted by first setting the median of the gene with the highest counts to the expected normal value (log2N = 0), while maintaining the relative copy number of the two BRCA genes. This method assumes that at least one gene has a normal copy number internally, which can reduce false-positive calls caused by small copy-number differences between genes in germline samples. • **FLATGENE** – The log2-normalized counts for each gene are adjusted by independently setting the median of each gene to the expected normal value (log2N = 0). This method assumes that both genes have a normal internal copy number, enabling detection of germline exon deletion variants in somatic whole-gene deletion samples.
CNV Shift Value	The amount of subtraction in log2 ratio to the result of the CNV Shift method used. May be used to fix an error in the CNV Shift method. Shift Value is only used when the Shift Type is set to VALUE_BASED_ON_MEDAMP. **Allowed values:** -2 to 2
Tumor Cellularity Selection	Select a value to use tumor calculated tumor fraction or user-specified Percent Tumor Cellularity in sample. **Allowed values:** Manually input Tumor Cellularity input per user or Auto-calculated tumor cellularity. The minimum amount that cellularity can be adjusted is 40%, for both auto-calculated and manual input tumor cellularity.
Analysis (applies only to aneuploidy analysis workflows) [1]	
Enable Mosaicism Detection	Enable Mosaicism Detection **Allowed values:** True or False
Enable Smoothing	Enable Smoothing **Allowed values:** True or False
Set Tile Size for aneuploidy Workflow	Set the tile size for Ion ReproSeq™ analysis workflows, designed for use with aneuploidy research. The tile size used for creating the aneuploidy baseline must match the tile size selected here. **Allowed values:** 1 to 10,000,000

(continued)

Parameter Name	Description
Hide called gender	Hide gender called by CNV gender calling. **Allowed values:** True or False
Analysis	
Plot Y chromosome for Female or Unknown Gender?	Plot Y chromosome for Female or Unknown Gender. **Allowed values:** True or False
Analysis (applies only to Exon Deletions)	
Cutoff for non-integer CN calls	Specifies the cutoff for making CN #calls that are not precisely of integer values. **Allowed values:** 0 to 0.50
Max Calls	Specifies the number of non-contiguous exon variant calls above which the sample will fail QC. **Allowed values:** 0 to 47
Min Quality	Specifies the quality score below which a CNV variant is classified as a NOCALL. **Allowed values:** 0 to 100
Analysis (applies only to Liquid Biopsy and Ion AmpliSeq™ HD)	
Minimum Tag Family Size	Minimum number of reads with the same tag required to form a functional family. **Allowed values:** 0 to unlimited
Max Fold Difference for Loss	Maximum fold difference relative to reference for calling a loss. **Allowed values:** 0 to 1
Min Fold Difference for Gain	Minimum fold difference relative to reference for calling a gain. **Allowed values:** 1 to unlimited
P-value for maximum calls	P value for maximum calls. **Allowed values:** 0 to 1
Analysis (applies to all CNV finding algorithm types except VCIB CNV algorithm)	
CNV Transition Penalty	Transition Penalty dictates the likelihood that the algorithm will call a different ploidy state between two adjacent data points. Transition Penalty is logarithm (to the base 10) of Transitional Probability. Lower (more negative) values will make it less likely that the algorithm will call adjacent data points as ploidy states that are different from each other. The Transition Penalty parameter edited here will only take effect when using the CUSTOM CNV Sensitivity setting. When CNV MOSAICISM parameter is not enabled, the maximum value supported for Transition Penalty is -1.05. When CNV MOSAICISM parameter is enabled, the maximum value supported for Transition Penalty is -2.31. **Allowed values:** -1,000,000 to -1.05

(continued)

Parameter Name	Description
Analysis (applies only to VCIB CNV finding algorithm when custom panel is focal amplification)	
CNV Gain Threshold	Threshold value (≥0) for calling GAIN in autosomes **Allowed values:** 0 to unlimited
CNV Gain Threshold XY	Threshold value (≥0) for calling GAIN in X or Y for males. **Allowed values:** 0 to unlimited
Gain Confidence Level	Confidence level value (>0 and <1) to be used to compare to gain-threshold or gain_threshold_xy. **Allowed values:** 0 to 1
Analysis (applies only to Hidden Gender aneuploidy analysis workflow) [2] These settings apply only to aneuploidy workflows which provide only information whether the sex chromosomes look Normal or Abnormal, based on what is expected for the Expected Normal Ploidy of sex chromosomes on a normal XX female or a normal XY male.	
Male Normal Ploidy Lower Bound	Male Minimum Normal Ploidy for Hidden Gender aneuploidy analysis workflow. **Allowed values:** 0 to 100,000
Male Normal Ploidy Upper Bound	Male Maximum Normal Ploidy for Hidden Gender aneuploidy analysis workflow **Allowed values:** 0 to 100,000
Female Normal Ploidy Lower Bound	Female Minimum Normal Ploidy for Hidden Gender aneuploidy analysis workflow. **Allowed values:** 0 to 100,000
Female Normal Ploidy Upper Bound	Female Maximum Normal Ploidy for Hidden Gender aneuploidy analysis workflow **Allowed values:** 0 to 100,000

[1] Currently, these parameters apply to the Ion ReproSeq™ PGS w1.1, Ion ReproSeq™ No Gender PGS w1.1, and Ion ReproSeq™ Mosaic PGS w1.1. analysis workflows.

[2] Currently, these parameters apply only to the Ion ReproSeq™ No Gender PGS w1.1 analysis workflow.

Fusions parameters

You can adjust the following fusions parameters to optimize analysis results when you create or edit Ion Reporter™ Software analysis workflows.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Parameter	Description
Main tab	
Sensitivity	Sensitivity. **Allowed values:** Fixed values, only one of can be applied. • High–the algorithm requires 60% overlap between reads and reference sequence with at-least 50% exact matches in the overlap. • Medium– the algorithm requires 70% overlap between reads and reference sequence with at-least 66.66% exact matches in the overlap. • Low–the algorithm requires 80% overlap between reads and reference sequence with at-least 75% exact matches in the overlap.
Minimum Read Counts for Fusions	Threshold on the minimum number of valid reads aligned to specific fusion isoform sequence to call the isoform as present, if the normalized read count is also greater than the threshold. Example : If count of a target is >20, the target is called present. **Allowed values:** ≥0 Integers only
Minimum Normalized Read Counts for Fusions	Threshold on minimum normalized read counts threshold required to call a fusion isoform as present. **Allowed values:** ≥0 Float values
Minimum Total Valid mapped reads	Minimum number of total valid mapped reads required to qualify a sample as valid and to proceed with the analysis. **Allowed values:** ≥0 integers only
Make calls based on Imbalance Score	If this flag is set to true, Imbalance scores are used to make fusion presence, absence, or nocall calls. **Allowed values:** True or False (boolean)
Minimum number of Valid pools	For multipool RNA pools, specify the minimum number of pools in a sample that have to pass QC to qualify that sample as valid and proceed with the analysis. Example: If a panel has two pools, use value = 2 to specify that both pools needs to have sufficient number of reads to qualify that sample. Similarly use value = 1 to proceed with the analysis even if one pool fails. **Allowed values:** ≥1 integers only
Minimum Total Valid mapped reads Per Pool	Minimum number of total valid mapped reads in each pool (if there are multipool RNA panels) to qualify that primer pool as valid. **Allowed values:** ≥0 integers only

(continued)

Parameter	Description
Advanced tab	
Minimum Read Counts for Non-Targeted Fusions	Threshold on minimum number of valid reads aligned to a nontargeted fusion sequence to call the fusion as present. Example : If the count of a non-targeted isoform is >250, it is reported as present-nontargeted. **Allowed values:** ≥0 integers only
Minimum Read Counts for Controls	Threshold on minimum number of valid reads aligned to specific expression control sequence required to call it as present. Example : If the read count of an expression control is >15, it is called present. **Allowed values:** ≥0 integers only
Minimum Total Control reads	Minimum number of housekeeping control reads required to calculate Imbalance scores for 5p3p assays. **Allowed values:** ≥0 integers only
Maximum Imbalance for Negatives	If the Imbalance score of any driver gene is less than this value, the sample is called fusion negative for that gene. **Allowed values:** Text field. String value in specific format as shown in the default value. This verifies the input of a Regular expression.
Minimum Imbalance for Positives	If the imbalance score of any driver gene is greater than this value, the sample is called fusion positive for that gene. However, there is a grey zone between maximum and minimum values where scores are called nocall. If they are equal, there is no grey zone. **Allowed values:** Text field. String value in a specific format as shown in the default value. This verifies the input of a Regular expression.
Minimum Isoform Counts for Imbalance	If the sum of counts from all the isoforms of that driver gene is greater than this number, thresholds set by **Maximum Imbalance for Negatives with evidence from Isoforms** and **Minimum Imbalance for Positives with evidence from Isoforms** are used for the imbalance scores. **Allowed values:** Text field. String value in a specific format as shown in the default value. This verifies the input a Regular expression.
Maximum Imbalance for Negatives with evidence from Isoforms	If the imbalance score of any driver gene is less than this value, the sample is called fusion negative for that gene. **Allowed values:** Text field. String value in a specific format as shown in the default value. This verifies the input of a Regular expression.

(continued)

Parameter	Description
Minimum Imbalance for Positives with evidence from Isoforms	If the imbalance score of any driver gene is greater than this value, the sample is called fusion positive for that gene. However, there is a grey zone between maximum and minimum values where scores are called nocall. If they are equal, there is no grey zone. **Allowed values:** Text field. String value in a specific format as shown in the default value. This verifies the input of a Regular expression.
Estimate max crosstalk	Maximum percentage of spill-over reads that could be seen in any sample due to reasons like barcode crosstalk. **Allowed values:** ≥0 (% values) float values
Analysis configuration file	A tab-separated file specific to each panel that enables users to set individual target specific thresholds for the following properties, as applicable for that type: • Minimum read count • Minimum normalized read count • Minimum wild type ratio • Make calls • Do not report • Max read count negative **Allowed values:** Path to a tab-separated file
Keep Intermediate files	Turn this flag on to keep the intermediate files generated when using the fusions analysis. **Allowed values:** True or False
Report non-targeted fusions	If this flagged is turned off, any nontargeted fusions detected are not reported in the output VCF file. **Allowed values:** True or False
Minimum Read Counts for Gene Expression targets	Threshold on minimum number of valid reads aligned to specific gene expression target to call it as present. **Allowed values:** ≥0 integers only
Minimum mean read length for valid SampleQC	If the average read length computed from all the reads in the sample is less than the value specified, that sample is not qualified to be valid and results are not reported. This is an additional SampleQC metric. Other QC metrics are minimum total valid mapped reads and minimum number of valid pools. For example, a recommended value is 50 bp. **Allowed values:** Integers only
Use pool Specific normalization	For multipool RNA panels, use this flag to specify whether read counts are to be normalized to total reads in each pool separately or to total reads in the sample. This also applies to calculation of wild type ratio and normalized count in gene metrics for RNAExonVariant targets. **Allowed values:** True or False

(continued)

Parameter	Description
Minimum Molecular Family Consensus Size	Minimum number of reads with same tag required to form a functional family. Suggested value between 3 and 7. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** Integers only
Minimum Molecular Family Count	Minimum number of variant supporting functional families required to make a call. Impact: Increasing values make calls less sensitive but more specific. **Allowed values:** Integers only
Minimum Family Coverage per Strand	Minimum required coverage of reads on each strand in a bidirectional molecular tag family. **Allowed values:** Integers only
Minimum Number Of PC Amplicons Required To Pass QC	Minimum number of process (or expression) control amplicons containing equal or more families than **fusions.min.fam.count** required to pass quality control. **Allowed values:** Integers only
Minimum Number Of PC Amplicons Required To Fail QC	Maximum number of process (or expression) control amplicons containing equal or more families than **fusions.min.fam.count** required to fail quality control. **Allowed values:** Integers only
Minimum read counts for RNAExonVariants	Minimum number read counts for RNAExonVariant targets to be called as present. This value is used only in cases where present/absent calls are made for RNAExonVariant targets. **Allowed values:** Integers only
Minimum Molecular Family Count for RNAExonVariant	Minimum number of variant supporting functional families required to make a call for RNAExonVariant targets. This value is used only in the cases where present/absent calls are made for RNAExonVariant targets. **Allowed values:** Integers only
Minimum Imbalance Score for the RNAExon Tile Assays	Minimum imbalance score for calling 'imbalance positive' from RNA exon tiling assays in a driver gene. Positive calls also depend on the P value for imbalance. **Allowed values:** ≥0 float values **Note:** Gene-specific parameters are enabled in the properties.txt file.[1]
Minimum p-value for Imbalance Score for the RNAExon Tile Assays	Maximum statistical significance P value for calling 'imbalance positive' from RNA exon tiling in a driver gene. Positive calls also depend on imbalance scores. **Allowed values:** 0-1 float values **Note:** Gene-specific parameters are enabled in the properties.txt file that is included in downloaded analysis files.[1]

(continued)

Parameter	Description
Minimum average read counts for all the Exon Tiling assays in a Driver Gene	Mean coverage of a driver gene with RNA exon tiling assays. Measured per gene, as the total valid mapped reads counts from all exon-tiling assays divided by the number of exon-tiling assays **Allowed values:** ≥0 float values **Note:** Gene-specific parameters are enabled in the properties.txt file.[1]
Number of weak amplicons	The RNA Exon Tiling amplicons beyond the breakpoint are required to have sufficient expression. This parameter defines an upper boundary to allow a defined number of exon tiling amplicons that fail to reach a coverage threshold. (Coverage that is below than 20x coverage the maximal amplicon in the gene).[1]
Minimum number of exon tiling amplicons flanking predicted breakpoints	A parameter that restricts the position of the breakpoint by the minimum number of flanking exon tiling amplicons. Samples in which breakpoints are predicted at very close proximity to the 3' and 5' – without sufficient number of exon tiling amplicons on both sides – will result in No Calls.[1]
Minimum number of Wild type assays detected for RNAExonVariants	Minimum number of wild type assays detected for RNAExonVariants. This parameter is for use with Ion AmpliSeq HD and TagSeq analysis results and analysis workflows only.[1] **Allowed values:** ≥0 integers only
Use either family counts only or read counts only to make calls	Use this flag to make calls based on either family counts (molecular counts) or read counts. Turn this flag on to enable dynamic calls using either metric, with molecular counts analyzed first, followed by read counts. Turn this flag off to require both metrics to make calls. This parameter is for use with Fusion, RNAExonVariant, and ProcessControl fusion types. For more information, see "Data types for gene fusions analyses" on page 535. **Allowed values:** True or False

[1] For more information, see "Analysis configuration file for gene fusion analysis" on page 531.

MSI parameters

You can adjust the following MSI parameters to optimize your analysis results when you create or edit analysis workflows in Ion Reporter™ Software. MSI parameters are available only for some Oncomine™ analysis workflows.

Parameter name	Description
Main tab	
Enable MSI Detection	When set to **True**, this parameter enables detection of MSI markers for the workflow. These markers can identify a form of genomic instability in the replication of repetitive DNA. MSI often occurs in tumor cells. It leads to the appearance of multiple alleles at microsatellite loci, which can be easily identified. This parameter is set to **True** by default for Oncomine™ Comprehensive Assay Plus, DNA Ion Reporter™ Software analysis workflows, and is intended for use only in select Oncomine™ analysis workflows. If you do not want to include MSI results in an Oncomine™ analysis, you can set to the parameter to **False**. Do not change the default setting of this parameter for other analysis workflows unless you understand how the change can affect your analysis.
MSI Algorithm Version	The version of the algorithm that is used to generate MSI results. The algorithm version can provide information about how the data was analyzed and can be useful if earlier versions of the software were used, or in the case of troubleshooting. This parameter is listed for informational purposes only and cannot be changed.
MSI Control Type	Allows the selection of a control for use in MSI calculation. Select RMC, or an MSI On-Chip control sample, from the same chip that is uploaded at the same time as the sample that is analyzed. **MSI Control Type** is available only for analysis results from sequencing runs that use Oncomine™ Comprehensive Assay Plus analysis workflows on the Ion 560™ Chip. The RMC control captures sample and library preparation variations. The on-Chip Control, which is generally a designated normal sample on the chip, is used to help determine sequencing based variations. The MSI signal generated by the on-chip control is used to evaluate the MSI signal generated for all DNA samples that are sequenced on the same chip to generate an MSI score. With use of the MSI on-chip control samples, the addition of RMC controls is still required during the library preparation.
MSI Marker Regions	The MSI Marker regions file contains information about the genomic position of the MSI markers, the version of the MSI algorithm and additional information that is used by the MSI detection algorithm. It is blank by default until the release of analysis workflows that are designed for use with MSI detection. The MSI marker regions file is set as the default for some Oncomine™ analysis workflows. The MSI marker regions file contains information about the genomic position of the MSI markers, and additional information that is used by the MSI detection algorithm. Data in the file includes chromosome name, chromosome start and end positions, and the name of the MSI markers.
MSI Baseline File	Select an MSI baseline file for use in MSI analysis. This file contains baseline information that accounts for variations in sample preparation or run conditions to support robust MSI detection.

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Parameter name	Description
MSI Marker Threshold	The MSI Marker Score (Forward or Reverse) above which the marker will be considered in the Total Marker Score (Forward or Reverse)
Minimal MSI Marker Coverage	The minimum number of filter reads per direction that is required for an MSI Marker Score (Forward or Reverse) to be calculated
MSI-High Threshold	The MSI score above which a sample is considered MSI-High. This score is reported in analysis results. [1,2]
MSS Threshold	The MSI score below which a sample reports MSS in analysis results.[1,2]
Advanced tab	
Enable Tumor Cellularity Percentage	When enabled (value = 1), the MSI algorithm uses the tumor cellularity percentage in its calculation. We recommend that you do NOT change this parameter.

[1] If the MSI Score falls between the **MSI-High Threshold** and the **MSS Threshold**, a No Call is reported in the analysis results.

[2] The MSI-High and MSS thresholds are optimized based on the MSI baseline of the workflow. Do not change parameters from the default settings unless you understand how the change can affect your analysis.

Read Mapping parameters

You can adjust the following read mapping parameters to optimize your analysis results when you create or edit analysis workflows.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Variant Name	Description
Main tab	
Tmap Mapped Files Enable Re-map	Flag to indicate whether mapped BAM files should be remapped. **Allowed values:** True or False
Advanced tab	
Mismatch Penalty	Specifies the mismatch penalty. **Allowed values:** 1 to 100
Soft Clipping Type	Specifies the type of soft-clipping to perform. **Allowed values:** 0 to 3
Gap Open Penalty	Specifies the gap open penalty. **Allowed values:** 1 to 100
Match Score	Specifies the match score. **Allowed values:** 1 to 100

(continued)

Variant Name	Description
Gap Extension Penalty	Specifies the gap extension penalty. **Allowed values:** 1 to 100
Context	Realign with context-dependent gap scores. **Allowed values:** True or False
Maximum Amplicon Overrun Large INDEL Rescue	The maximum number of bases allowed for a read to overrun the end of the amplicon. **Allowed values:** 0 to unlimited
Flow Score Penalty	Specifies the flow score penalty. **Allowed values:** 1 to 100
Max adapter bases for soft clipping	Specifies to perform 3' soft-clipping (via -g) if at most this # of adapter bases were found. **Allowed values:** 0 to unlimited
Local Band Width	Specifies the Local band width. **Allowed values:** 1 to 100
Do Repeat Clip	Trim repetitive sequence at the ends of alignment. **Allowed values:** True or False
End Repair	Specifies to perform 3' end repair. **Allowed values:** 0 to unlimited
Use BED file	Use bed file to capture INDELs near amplicon edges. **Allowed values:** True or False
Output Filter	Specifies the output filter for the mapping. **Allowed values:** 0 to 3
Maximum INDEL Size to Rescue	The maximum INDEL size to rescue with one large INDEL algorithm. **Allowed values:** 0 to unlimited

Variant Finding parameters

You can adjust the following Variant Finding parameters to optimize your analysis results when you create or edit analysis workflows.

IMPORTANT! Use the default parameter settings unless you are an advanced user.

Parameter Name	Description
Main tab	
Analysis	
Data Quality Stringency	Filter: Phred-scaled minimum average evidence per read or no-call. Related VCF field: MLLD. **Allowed values:** 0 to unlimited
Downsample to Coverage	Reduce coverage in over-sampled locations to this value. **Allowed values:** 1 to unlimited
SNP Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited
SNP Min Variant Score	Filter: Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited
SNP Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals)
SNP Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited
SNP Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1.0 (decimals)
INDEL Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited
INDEL Min Variant Score	Filter: Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited (integers)
INDEL Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals)
INDEL Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers)

(continued)

Parameter Name	Description
INDEL Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1.0 (decimals)
Hotspot Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited (integers
Hotspot Min Variant Score	Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited (integers)
Hotspot Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals)
Hotspot Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers)
Hotspot Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1.0 (decimals)
PPA	Reports Possible Polyploidy Alleles (PPA) in the INFO FIELD of the VCF file. Related VCF field: PPA. Allowed values: 1 = report PPA, 0 = do not report. 1 is recommended for somatic-only and experimental research. If PPA is set to True and **Variant View** advanced parameter is set to Allele View, a PPA column is included in the **Analysis Results** screen. A value of Yes indicates variants that are PPA alleles. No indicates variants that are not PPA alleles. **PPA** must be set to **True** for the Possible Polyploidy Alleles filter to function. For more information, see “Locus View versus Allele View of variants” on page 197. **Allowed values:** True or False
Prediction Precision	Number of pseudo-data-points suggesting our predictions match the measurements without bias. **Allowed values:** 0 to unlimited (decimals)
Outlier Probability	Prior probability that a read comes from some other distribution. **Allowed values:** 0 to 1 (decimals)
Heavy Tailed	How heavy the t-distribution tails are to allow for unusual spread in the data. **Allowed values:** 0 to unlimited (decimals)
Filter Unusual Predictions	Filter: predictions are distorted to fit the data more than this distance (relative to the size of the variant). Related VCF fields: FWDB, REVB [RBI = sqrt(FWDB ^ 2 + REVB ^ 2)]. **Allowed values:** 0 to unlimited (decimals)

(continued)

Parameter Name	Description
Filter Insertion Predictions	Filter: observed clusters deviate from predictions more than this amount (relative to the size of the variant). Related VCF fields: VARB, REFB. **Allowed values:** 0 to unlimited (decimals)
Filter Deletion Predictions	Filter: observed clusters deviate from predictions more than this amount (relative to the size of the variant). Related VCF fields: VARB, REFB. **Allowed values:** 0 to unlimited (decimals)
HP Max Length	Filter: homopolymer length involved in an in/del. Related VCF field: HRUN. **Allowed values:** 1 to unlimited (integers)
Do SNP Realignment	Realign reads in the vicinity of SNP candidates. Impact: True = do not realign, False = realign. **Allowed values:** True or False
Suppress Recalibration	Recalibration values from pipeline used or not (experimental). No related fields, changes basecalling behavior. Allowed values: True = allow recalibration, False = don't allow recalibration. **Allowed values:** True or False
SSE Probability Threshold	Filter out variants in motifs with error rates above this. **Allowed values:** 0 to 1
MNP Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited (integers)
MNP Min Variant Score	Filter out MNPs with a QUAL score less than or equal to this Phred-scaled value. **Allowed values:** 0 to unlimited
MNP Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1
MNP Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers)
MNP Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1
MNP Strand Bias Pval	Filter out mnps with pval below this [1.0] given strand bias > mnp-strand-bias. **Allowed values:** 0 to 1
SNP Strand Bias Pval	Filter out snps with pval below this [1.0] given strand bias > snp-strand-bias. **Allowed values:** 0 to 1

(continued)

Parameter Name	Description
INDEL Strand Bias Pval	Filter out INDELs with pval below this [1.0] given strand bias > INDEL-strand-bias. **Allowed values:** 0 to 1
Hotspot Strand Bias Pval	Filter out hotspot variants with pval below this [1.0] given strand bias > hotspot-strand-bias. **Allowed values:** 0 to 1
Position Bias Reference Fraction	Skip position bias filter if (reference read count)/(reference + alt allele read count) less than or equal to this. **Allowed values:** 0 to 1
Position Bias	Filter out variants with position bias relative to soft clip ends in reads > position-bias. **Allowed values:** 0 to 1
Position Bias Pvalue	Filter out if position bias is above the Position Bias given pval less than Position Bias Pvalue. **Allowed values:** 0 to 1
Use position bias	Enable the position bias filter. **Allowed values:** True or False
INDEL As HPINDEL	Apply INDEL filters to non HP INDELs. **Allowed values:** True or False
Do MNP Realignment	Realign reads in the vicinity of candidate mnp variants. **Allowed values:** True or False
Realignment Threshold	Maximum allowed fraction of reads where realignment causes an alignment change. **Allowed values:** 0 to 1
FD Nonsnp Min Var Cov	Override min_var_coverage of the flow-disrupted variants that are not SNPs (0 to disable the override). Impact: Decreasing values make variant calls less specific but more sensitive. **Allowed values:** 0 to 10
Read Mismatch Limit	Do not use reads with number of mismatches (where 1 gap open counts 1) above this value. **Allowed values:** 0 to unlimited
Min Cov Fraction	Do not count reads with fraction of covering any amplicons below this threshold. **Allowed values:** 0 to 1
Use Input Allele Only	Only consider provided alleles in the hotspots file. 0 = generate de novo candidates, 1 = hotspots only. **Allowed values:** 0 to 1

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Parameter Name	Description
Liquid Biopsy and Ion AmpliSeq™ HD	
ampliseq-hd	Sets defaults for counting reads in liquid biopsy runs with ampliseq-hd using "tvc consensus". **Allowed values:** 0 to 1
Min Family Size	Minimum number of reads with same Unique Molecular Tag required to form a functional family. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 0 to unlimited
min-mapping-qv	Minimum mapping quality value required for a read to be counted. **Allowed values:** 0 to unlimited (integer)
Poisson	Use Poisson parameter estimation to estimate count of functional families. **Allowed values:** 0 to 1
SNP Min Var Coverage	Minimum number of variant supporting functional families required to make a SNP call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10
MNP Min Var Coverage	Minimum number of variant supporting functional families required to make a MNP call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10
INDEL Min Var Coverage	Minimum number of variant supporting functional families required to make a INDEL call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10
Hotspot Min Var Coverage	Minimum number of variant supporting functional families required to make a hotspot call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10
INDEL Func Size Offset	Require family of size >= (min_tag_fam_size + this value) to be functional for calling HP-INDEL. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 0 to 4
Tag Sim Max Cov	Check the similarity of UID of variant families if the variant molecular coverage is less than or equal to this value. Related VCF field: TGSM. **Allowed values:** 0 to unlimited
Minimum Family Coverage per Strand	Minimum required coverage of reads on each strand in a bi-directional molecular tag family. **Allowed values:** 0 to unlimited

(continued)

Parameter Name	Description
Minimum Callable Probability	Minimum callable probability for LOD calculation. **Allowed values:** 0 to 1
Suppress called allele LOD	Suppress the LOD reporting of a variant allele that is called. This metric is useful to investigate possible false negatives. Do not use this metric as a filtering criteria. **Allowed values:** True or False
Advanced tab	
Analysis	
Allow INDELs	Enable INDELs in FreeBayes hypothesis generator. Allowed values: True = generate INDEL hypotheses, False = don't generate. **Allowed values:** True or False
Allow SNPs	Enable SNPs in FreeBayes hypothesis generator. Allowed values: True = generate SNP hypotheses, False = don't generate. **Allowed values:** True or False
Allow MNPs	Enable MNPs in FreeBayes hypothesis generator. Allowed values: True = generate MNP hypotheses, False = don't generate. **Allowed values:** True or False
Allow Complex	Enable complex variants in FreeBayes hypothesis generator. Allowed values: True = generate MNP hypotheses, False = don't generate. **Allowed values:** True or False
Minimum mapping qv	Minimum mapping QV value required for reads to be allowed into the pileup (both FreeBayes and evaluator). **Allowed values:** 0 to unlimited (integer)
Read SNP Limit	Read mismatch limit on number of mismatches: filter potential mis-mapped reads. **Allowed values:** 0 to unlimited (integer)
Read Max Mismatch Fraction	Read maximum mismatch fraction of mismatches in length of read: filter potential mis-mapped reads. **Allowed values:** 0 to 1 (decimals)
Generate Min Alt Allele Freq	Generate variants with at least this frequency in the pileup. **Allowed values:** 0 to 1 (decimals)
Generate Min INDEL Alt Allele Freq	Generate INDEL variants with at least this frequency in the pileup. **Allowed values:** 0 to 1 (decimals)
Generate Min Coverage	Generate variants in locations with at least this depth of coverage. **Allowed values:** 0 to unlimited

(continued)

Parameter Name	Description
Kmer Len	Size of the smallest k-mer used in assembly. Impact: Increasing values make INDEL calls less sensitive but more specific. **Allowed values:** 5 to unlimited (integers)
Min Var Count	Minimum support for a variant to be evaluated. Impact: Increasing values make INDEL calls less sensitive but more specific. **Allowed values:** 1 to unlimited (integers)
Short Suffix Match	Minimum assembled sequence match on both sides of the variant. Impact: Increasing values make INDEL calls less sensitive but more specific. **Allowed values:** 2 to unlimited (integers)
Min INDEL Size	Minimum size INDEL reported by assembly. Impact: Increasing values make INDEL calls less sensitive but more specific. **Allowed values:** 1 to unlimited (integers)
Max HP Length	Variants containing HP larger than this are not reported. Impact: Increasing values make INDEL calls more sensitive but less specific. **Allowed values:** 1 to unlimited (integers)
Min Var Freq	Minimum frequency of the variant to be reported. Impact: Increasing values make INDEL calls less sensitive but more specific. **Allowed values:** 0 to 1 (decimals)
Relative Strand Bias	Variants with strand bias above this are not reported. Impact: Increasing values make INDEL calls more sensitive but less specific. **Allowed values:** 0 to 1 (decimals)
Output MNV	Enables reporting of complex variants. Impact: 1 = report complex variants, 0 = don't report. **Allowed values:** 0 to 1 (decimals)
Variant View	Represent variants with Locus view or Allele view. For more information, see “Locus View versus Allele View of variants” on page 197. **Allowed values:** Locus view or Allele view
Analysis SSE File	Analysis strand specific error file. Select a mask from the list or upload a mask from **Upload**.

Import user-defined variantCaller parameters

If you would like to use user-defined variantCaller plugin variant finding parameters in Ion Reporter™ Software, export the parameters JSON file from Torrent Suite™ Software. Then, import this file from the **Parameters** screen when creating an analysis workflow.

1. Export the variantCaller parameters from Torrent Suite™ Software and store the JSON file on your computer or network.
2. In the **Parameters** step, select **Variant Finding**.
3. Click **Select File**, then browse to the exported variantCaller parameters JSON file and click **Import**.
   The variantCaller parameters replace the default settings. Error messages appear if any of the imported parameters are out-of-range or if a JSON file with an incompatible format is imported.

Steps in analysis workflow creation

Ion Reporter™ Software provides a workflow bar that includes steps to guide you through analysis workflow creation.

The selections that you make as you go through the workflow bar are documented in the **Summary** pane.

Some analysis workflows have fewer setup steps than others. The following analysis workflow creation steps are available.

Analysis workflow step	Description
Research Application	Use this analysis workflow step to select a research application and a sample group to use in a custom analysis workflow. The research application and sample group settings affect the options that are available in subsequent analysis workflow creation steps. This step applies to all analysis workflow types. If you copy an existing analysis workflow, you cannot change its research application.
Reference	Use this analysis workflow step to select a reference genome type and to upload the appropriate reference files for the analysis to use to focus the analysis in the custom analysis workflow. The predefined analysis workflows that use a human reference are designed for use with the hg19 human reference genome. Custom Ion AmpliSeq™ DNA panels can be designed for use with the GRCh38 human reference genome. For more information about uploading panel files, see “Import panel files from AmpliSeq.com” on page 125

(continued)

Analysis workflow step	Description
Primers	Use this analysis workflow step to select the primers used in the library preparation in a metagenomics custom analysis workflow. For more information, see “Create a user-defined analysis workflow for use with Metagenomics research application” on page 85 and “Custom primer sequences for Metagenomics analysis workflows” on page 87.
Annotation	Use this analysis workflow step to select annotation sets that are specific for the custom analysis workflow. Annotation sets include various annotation sources that provide information about the biological meaning of variants and allow variant sorting and prioritization. Many annotation sources are available in this step that are derived from public and private annotation databases for hg19 and GRCh38. If the annotation set that you need is not available to select, you can create a new annotation set from the **Workflow Presets** screen. For more information, see “Create annotation set workflow presets” on page 133.
Filters	Use this analysis workflow step to establish a default filter chain to use for the custom analysis workflow. All future analyses that are run through this analysis workflow will use this filter chain. Filter chains are sets of filters that you apply to variants that are identified in an analysis. Filter chains allow you to remove or include variants in analysis results. For more information, see Chapter 10, “Filters and filter chains”.
Copy Number	Use this analysis workflow step to select a copy number baseline to apply to the custom analysis workflow. The copy number baseline is used by the algorithm to identify copy number variants (CNVs) in samples. This analysis workflow step applies only to DNA, aneuploidy, and some Oncomine™ analysis workflows. You need to create a copy number baseline to call CNVs in these analysis workflows. Analysis workflows that use copy number baseline controls can provide copy number detection as an alternative to paired sample analysis workflows. Copy number baseline choices are limited to copy number baselines that were created using the hotspots and target regions file that is selected in the **Reference** step of the workflow bar. For more information, see “Apply a copy number baseline workflow preset to an analysis workflow” on page 127

(continued)

Analysis workflow step	Description
Fusion	Use this analysis workflow step to detect fusions. If the panel supports expression imbalance fusion detection, then you need to use the fusion baseline. Select a baseline that provides a reference point against which fusion calls that are based on an expression imbalance are made with the RNA Exon Tiling assays that are included in a panel. If the panel contains at least one RNAExonTile target, you need to select an RNA baseline that corresponds to the panel content. **Note:** Custom Ion AmpliSeq™ panels do not typically support RNA exon tiling fusion detection. Therefore most custom Ion AmpliSeq™ panels do not require a fusion baseline.
Plugins	Use this analysis workflow step to apply available plugins to a custom analysis workflow. Plugins provide added functionality and content to an analysis workflow. Use of plugins is optional. This analysis workflow step applies only to DNA, DNA and fusions, fusions, annotate, and aneuploidy analysis workflows. The Oncomine™ Variant Annotator plugin is selected by default for some Oncomine™ analysis workflows and all Ion AmpliSeq™ HD analysis workflows. Analysis workflows for Oncomine™ assays and analysis workflow templates for Ion AmpliSeq™ HD panels in Ion Reporter™ Software include the Oncomine™ Variant Annotator plugin. The plugin integrates into analysis results data from more than 24,000 exomes across solid tumor and hematological cancer types, and annotates variants relevant to cancer with Oncomine™ Gene class and Oncomine™ Variant class information. For more information and a full list of annotation rules for each Oncomine™ assay, see Appendix D, “Oncomine™ Variant Annotator plugin criteria”, or contact your local support representative, Field Bioinformatics Specialist (FBS), or Clinical Account Consultant (CAC). Use the Oncomine™ Variant Annotator plugin with Oncomine™ panels only.
Final Report	Use this analysis workflow step to specify the final report template that reports the variants used in the analysis in the custom analysis workflow. If a final report template is not available, you can set up a new template. Final report templates are available to select in this step. If the final report template that you need is not available to select, you can create a new final report template from the **Workflow Presets** screen. For more information, see “Create a final report template workflow preset” on page 135.

(continued)

Analysis workflow step	Description
Parameters	Use this step to review or change runtime parameters to refine and optimize parameters for an analysis workflow. The default analysis workflows for use with custom Ion AmpliSeq™ panels provide optimized variant calling parameters. Many fixed and community panels imported from AmpliSeq.com also include variant calling parameters that are optimized for use with each specific panel. Parameter categories include: annotation, bamstats, CNV finding, MSI, read mapping, and variant finding. All analysis workflow types allow some parameter custom settings. The parameters that you can customize vary by analysis workflow. **IMPORTANT!** Use the default parameter settings unless you are an advanced user. For more information, see “Customize tuning parameters” on page 94
Confirm	This analysis workflow step is the final step that you need to complete to create custom analysis workflow. All analysis workflows include this step.

Research applications

Ion Reporter™ Software supports research applications to use when setting up analysis workflows. The research application selections work with sample group selections, and affect the options that are available in the following workflow steps.

The default options are recommended. Advanced users may change these settings if needed.

Research application	Description
Aneuploidy	Detect human chromosomal large structural abnormalities in low-pass whole-genome sequencing research samples.
Annotate Variants	Annotate the variants from a VCF file for research use.
DNA	Detect and annotate variants in human DNA research samples.
DNA and Fusions	Detect and annotate variants in human DNA and fusions research samples.
Immune Repertoire [1]	Detect and analyze T-cell receptor beta (TCRB) and B-cell receptor (BCR) rearrangements for research use.
Metagenomics [1]	Determine population diversity in polymicrobial research samples using detection of 16S gene variable regions.
Mutation Load [1]	Calculates mutation load and displays associated graphs and tables in human DNA samples. **Note:** This research application applies only to the Oncomine™ Tumor Mutation Load - w1.0 - DNA - Single Sample analysis workflow.

(continued)

Research application	Description
Oncology-Liquid Biopsy[1]	Detects and annotates low frequency variants including SNPs and INDELs (down to 0.1% limit of detection), fusions, and CNVs from targeted nucleic acid libraries (DNA or RNA).
Fusions	Detect and annotate gene fusions in human DNA research samples.

[1] This research application is for use only with a copy and edit of an analysis workflow. This research application is not an option when you create a custom analysis without predefined settings.

Sample groups

Ion Reporter™ Software supports sample groups to use when setting up analysis workflows. The sample group selections work with research application selections, and affect the options that are available in subsequent workflow steps.

The default options are recommended. Advanced users may change these settings if needed.

Sample group	Description	Research application
Paired	Analyze and compare two samples.	DNA
Single	Analyze a single sample.	Aneuploidy, Annotate Variants, DNA, Fusions, DNA and Fusions, Metagenomics, Oncology-Liquid Biopsy
Single Fusions	Analyze a single fusions sample.	Fusions
Single/Multi	Determine the microbial diversity of a 16S sample in one or more samples.	Metagenomics
Trio	Analyze a trio of a mother, father, and proband.	DNA
Tumor—Normal	Identify somatic mutations using advanced statistical approaches.	DNA

Add a filter chain to an analysis workflow

You can apply a filter chain during analysis workflow creation in Ion Reporter™ Software. Filter chains are sets of filters that you apply to variants identified in your analysis. Filters allow you to remove or include variants in your analysis results. For more information, see Chapter 10, “Filters and filter chains”.

1. In the **Filters** step, select a default filter chain from the list.
   After you select a filter chain, the filters that are contained in the filter chain are shown.
2. (*Optional*) Click the **Workflow Presets** link to configure a custom filter chain.
   For more information, see “Create a user-defined filter chain” on page 433.
3. Click **Next**.

The filter chain is established as the default filter chain for the analysis workflow. All future analyses that are run through this analysis workflow will use this filter chain.

Import panel files from AmpliSeq.com

You can import files for Ion AmpliSeq™ and Ion AmpliSeq™ HD Made-to-Order, On-Demand, or some Ready-to-Use, and Oncomine™ tumor specific research panels from **AmpliSeq.com** into Ion Reporter™ Software during analysis workflow creation. For more information, see “Ion AmpliSeq™ and Ion AmpliSeq™ HD panel types” on page 17.

Only ordered and quoted panel files are available for import into Ion Reporter™ Software.

You must have an access code to sign in to AmpliSeq.com when you import custom panel files. For more information, see “Generate an access code for AmpliSeq.com” on page 126.

1. In the **Research Application** step, select an appropriate research application and sample group for the panel, then click **Next**.

2. In the **Reference** step, import a target regions file.

Option	Description
Custom Panel	a. Under **Target Regions**, click **AmpliSeq Import**. b. In the **Import for AmpliSeq** dialog box, select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order panel files, or Ion AmpliSeq™ On Demand panel files. c. Enter an account user name. d. In the Password field, enter the access code. For more information, see “Generate an access code for AmpliSeq.com” on page 126. e. Click **List My AmpliSeq Panels**. f. Select the panel files that you want to import from the dropdown list, then click **Import**.
Fixed Panel	a. Under **Target Regions**, click **AmpliSeq Import**. b. In the **Import for AmpliSeq** dialog box, select the **Fixed Panel** tab for Community or Ready-to-Use panel files. c. From the list, select the panel file that you want to import. d. Click **Import**.

3. If the analysis includes fusions, you can import fusion panel files.

Option	Description
Custom Panel	a. Under **Fusion Panel**, click **AmpliSeq Import**. b. In the **Import for AmpliSeq** dialog box, select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order, or Ion AmpliSeq™ On Demand fusion panel files. c. Enter an account user name. d. In the Password field, enter the access code. For more information, see "Generate an access code for AmpliSeq.com" on page 126. e. Click **List My AmpliSeq Panels**. f. From the list, select the fusion panel file that you want to import, then click **Import**.
Fixed Panel	a. Click **AmpliSeq Import** under the Fusion Panel field. b. In the **Import for AmpliSeq** dialog box, select the **Fixed Panel** tab for Community or Ready-to-Use panel files. c. Enter an account user name. d. In the Password field, enter the access code. For more information, see "Generate an access code for AmpliSeq.com" on page 126. e. Click **List My AmpliSeq Panels**. f. From the list, select the fusion panel file that you want to import. g. Click **Import**.

4. Complete the remaining steps for analysis workflow creation.

   For more information, see "Create a new analysis workflow" on page 79.

The target regions files and fusion panel files are added to the list of files that can be selected in the **Reference** workflow step.

Generate an access code for AmpliSeq.com

Before you start this procedure, you need to have a valid **AmpliSeq.com** account.

Access codes have been added to **AmpliSeq.com** to improve security and compatibility with single sign-on features. The access codes increase security because they are unique to each **AmpliSeq.com** account.

You must generate an access code and sign in with the access code each time that you import files for Ion AmpliSeq™ and Ion AmpliSeq™ HD Made-to-Order, On-Demand, or some Ready-to-Use research panels from **AmpliSeq.com** into Ion Reporter™ Software. The imported files are used when you create an analysis workflow for use with the **AmpliSeq.com** panels.

You need to generate an access code for the **AmpliSeq.com** account just once, if you save the access code when it is generated. If you do not save the access code, you can regenerate a new one when you sign in again on **AmpliSeq.com**.

You need to set a new access code when the code expires.

1. Go to **AmpliSeq.com**, and sign in to the account.
2. Click **My Account** ▸ **Manage Access Code**.
3. Generate an access code.
   a. In the **Manage Access Code** dialog box, select an **Access Code Expiration** in the dropdown list.
   b. Click **Generate**.
   c. Click **Copy to Clipboard**.
      The access code is available for use on the clipboard.
   d. *(Optional)* Click **Save** to save the access code for use when you return to **AmpliSeq.com**.

You can now use the access code to import panel files from **AmpliSeq.com**. For more information, see “Import panel files from AmpliSeq.com” on page 125.

Apply a copy number baseline workflow preset to an analysis workflow

When you want to identify copy number variants (CNVs), you can select a copy number baseline to apply to the analysis workflow. These baselines are controls that you can apply to analysis workflows to determine copy number changes in the sample of interest, without the use of a matched control. You can apply a copy number baseline control only to DNA, aneuploidy, and some Oncomine™ analysis workflows.

IMPORTANT! When importing a custom copy number baseline, the target regions file used to generate the baseline must be present in the software; otherwise, the baseline is not shown in the list of available sequence variant baselines. When you add the baseline to an analysis workflow, the same target regions files that is used to create the baseline must also be used in the analysis workflow.

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1. In the **Workflows** tab, start to create or copy an analysis workflow.
   - Click **Overview**, then select an analysis workflow to copy, then click **Actions** ▸ **Copy**.
   - Click **Create**.
2. In the **Reference** workflow bar step, select the target regions file that was used to previously create the baseline.
   The target regions file used to generate the copy number baseline must be present in the software for the imported baseline to be shown in the list of available copy number baselines.
   Only baselines that were created with the same target regions file that is selected in the **Reference** step of the workflow bar are available.

3. In the **Copy Number** workflow bar step, select the baseline.

Option	Description
No Baseline–Don't call CNVs	If you do not want to identify CNVs, select this option.
Copy number baselines	Select an existing copy number baseline from the list.

   Make a choice to view details for the baseline in the **Summary** pane.

   Alternatively, you can click the **Workflow Presets** link, then configure a new copy number baseline preset. For more information, see “Create a Variability Correction Informatics Baseline (VCIB) workflow preset” on page 142.

4. Click **Next**.

5. Proceed through the remaining steps in the workflow bar.

   For more information, see “Steps in analysis workflow creation” on page 120.

6. In the **Confirm** step, click **Save Workflow**.

Apply a sequence variant baseline workflow preset to an analysis workflow

You can create a custom **Sequence Variant Baseline** workflow preset that you can use as a baseline control to detect somatic variants. These baselines are controls that you can apply to analysis workflows to detect somatic variants in the sample of interest. For more information, see “Sequence variant baselines” on page 148. You must add the custom baseline to an analysis workflow to use the baseline in an analysis.

1. In the **Workflows** tab, start to create or copy an analysis workflow.
   - Click **Overview**, then select an analysis workflow to copy, then click **Actions** ▸ **Copy**.
   - Click **Create Workflow**.

2. In the **Reference** workflow bar step, select the target regions file for the panel that is associated with the analysis workflow.

3. In the **Parameters** workflow bar step, click the link to open the **Variant Finding** parameters and click **Advanced**.

4. Scroll to select a sequence variant baseline that matches the panel associated with the analysis workflow from the **Analysis SVB File** dropdown list.

   After you make a selection, you can view details for the baseline in the **Summary** pane.

5. Click **Next**.

6. Proceed through the remaining steps in the workflow bar.

   For more information, see “Steps in analysis workflow creation” on page 120.

7. In the **Confirm** step, click **Save Workflow**.

Apply an exon tile fusion baseline workflow preset to an analysis workflow

An exon tile fusion baseline is created from fusion-negative samples to establish a control baseline. For analysis workflows that include RNA Exon Tiling, you can select an exon tile fusion baseline to apply to an analysis workflow.

1. In the **Workflows** tab, start to create or copy an analysis workflow.
   - Click **Overview**, then select an analysis workflow to copy, then click **Actions** ▸ **Copy**.
   - Click **Create**.
2. In the **Exon Tile Fusion Baseline** workflow bar step, select an option from the **Baseline** dropdown list.

Option	Description
No Exon Tile Fusion baseline selected	If you do not want to identify RNAExon Tiles, select this option.
Exon Tile Fusion baselines	Select an existing exon tile fusion baseline from the list.

   Alternatively, you can click the **Workflow Presets** link, then configure a new exon tile fusion baseline preset. For more information, see “Create an exon tile fusion baseline workflow preset” on page 151.
3. Click **Next**.
4. Proceed through the remaining steps in the workflow bar.
   For more information, see “Steps in analysis workflow creation” on page 120.
5. In the **Confirm** step, click **Save Workflow**.

Change the default filter chain for an analysis workflow

Many Ion Reporter™ Software analysis workflows include a default filter chain. You can change the default filter chain so that your selected settings are saved with the workflow and applied automatically when the workflow is launched.

Note: Only one default filter chain can be associated with an analysis workflow.

1. Click **Workflows** ▸ **Create Workflow**.
2. In the **Filters** step, select a different filter chain from the list.
3. Proceed through the remaining steps of the workflow bar, then click **Save**.

The newly saved filter chain is now the default filter chain for the analysis workflow. All future analyses that are run through the new version of the analysis workflow will use the new filter chain. Analyses that were previously launched with older versions of the analysis workflow are not affected when the default filter chain is changed.

Workflow presets

Workflow presets are the components that are used in creating an analysis workflow. You can create custom workflow presets, then later apply the presets to analysis workflows.

The following lists and describes the types of workflow presets that are available or can be created in Ion Reporter™ Software.

Copy Number Variant detection

Workflow preset type	Description
Annotation Sets	A set of annotation sources to use for variants in the **Annotation** step of creating an analysis workflow. For more information, see "Create annotation set workflow presets" on page 133.
Annotation Source	Annotation sources to use for variants in the **Annotation** step of creating an analysis workflow. For more information, see "Create annotation set workflow presets" on page 133.
Filter Chains	A set of filters to use for variants in the **Filter** step of creating an analysis workflow For more information, see "Create a user-defined filter chain" on page 433
Copy Number Baselines	A set of control samples that are used to create a baseline for detecting CNVs in single-sample workflows. The baselines are available in the **Copy Number** step when you create an analysis workflow. For more information, see "Copy Number Variant detection" on page 137. For more information, see "Copy Number Variant detection" on page 137.
Sequence Variant Baselines	Used in single sample analysis workflows for removing false positive variants that occur due to a sequencing error. For more information, see "Sequence variant baselines" on page 148.
Final Report Templates	Final report templates that are available to use in the **Final Report** step of creating an analysis workflow. For more information, see "Create a final report template workflow preset" on page 135.
Fusion Panels	The tab-delimited BED file that defines the coordinates of amplicons. Fusion panels are added in the **Reference** step when you create an analysis workflow that includes fusions.

Copy Number Variant detection *(continued)*

Workflow preset type	Description
Target Region Files	Target regions files restrict analysis to only regions specified in the file. These BED files are available to use in the **Reference** step of creating an analysis workflow. For more information, see "Upload a target regions BED file workflow preset" on page 152.
Hotspot Region Files	Hotspot files cause the hotspot positions to be listed in the analysis results, even if a variant is not called at those positions. These BED files are available to use in the **Reference** step of creating an analysis workflow. For more information, see "Upload a hotspots BED file workflow preset" on page 153.

Find workflow presets

You can search and filter information in the **Workflow** ▸ **Presets** screen in Ion Reporter™ Software to narrow the list of workflow presets, then find a specific workflow preset.

Sort the workflows presets list

You can sort the workflows presets list to make it easier to find the workflow presets that you are looking for in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Presets**.
2. Click a column heading to sort the list based on the column category.
3. Click the column heading a third time to return the column to the order that was used before the sort and stop sorting for the column.

Search for workflow presets

You can use keywords or text strings to search for data in the **Workflow Presets** table.

1. In the **Workflows** tab, click **Presets**.
2. In **Search**, enter a search term, then click **Go**.

   The search field is outlined in red if the search string is invalid. The following rules apply to all search fields:

   - An asterisk (*) is not allowed in the search field.
   - Searches are not case-sensitive.
   - Searches match your search string in any location in the target list. For example, a search on "demo" in analysis workflow names matches analysis workflows with "demo" anywhere in their name.

The search results are returned in the **Workflow Presets** table.

Filter workflow presets

You can apply filters to the **Workflow Presets** table to narrow search results, or shorten the list of workflow presets to make it easier to find the workflow presets of interest.

1. In the **Workflows** tab, click **Presets**.
2. Click the filter category to expand the list, then select a filter from the list.

Option	Description
Annotation Sets	Filter by: • **Annotation Sets** • **Annotation Source** • **Filter Chains** • **Copy Number Baselines** • **Sequence Variant Baselines** • **Final Report Templates** • **Fusion Panels** • **Target Region Files** • **Hotspot Region Files**
Version	Versions of Ion Reporter™ Software.
Reference	Filter by: • **GRCh38** • **hg19**

The filtered list of workflow presets is returned in the **Workflow Presets** table.

Remove filters

You can remove filters from the **Version** or **Reference** filter categories in the **Workflow Presets** table in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Presets**.
2. Click the **Version** or **Reference** filter category to expand the list, then select **Show All** at the top of the list.

   The first filter category does not have a **Show All** option. You must select a type of workflow preset.

Custom annotation sources

You can create custom annotation sets in Ion Reporter™ Software. A custom annotation set is a type of workflow preset that you can add to an analysis workflow so that you can launch analyses that use the custom annotation set.

Ion Reporter™ Software includes 2 gene models: RefGene and Ensembl. Only one gene model can be added to any custom annotation set.

Use the following guidelines to create custom annotation sets.

- To use an Ion Reporter™ Software canonical transcript set, you need to use the compatible gene model when you select an annotation source. For example, use the RefGene gene model with the Refseq canonical transcript set.
- To create a custom annotation set with Polyphen and SIFT scores, ensure that you use the corresponding RefSeq or Ensembl gene model. For more information, see “Effect of Ensembl and RefSeq sources on Polyphen and SIFT scores” on page 495.
- To create a custom annotation set that includes any of the following predefined annotation sources in the custom annotation set, a gene model must be also included in the annotation set.

  IMPORTANT! A custom annotation source that uses the following annotation sources without a gene model (RefGene and Ensembl) results in unexpected analysis results because the custom annotation source is unable to annotate variants.

  Variants are annotated with the genes in the gene model for the predefined annotation sources in the following order:
  - OMIM
  - DRUGBANK
  - DRA
  - GO
- A gene model must be also included in the annotation set that is used to create a custom annotation source, such as a gene set or custom transcript set, if the annotation set includes any of the following predefined annotation sources:
  - Custom geneset
  - Custom Transcripts

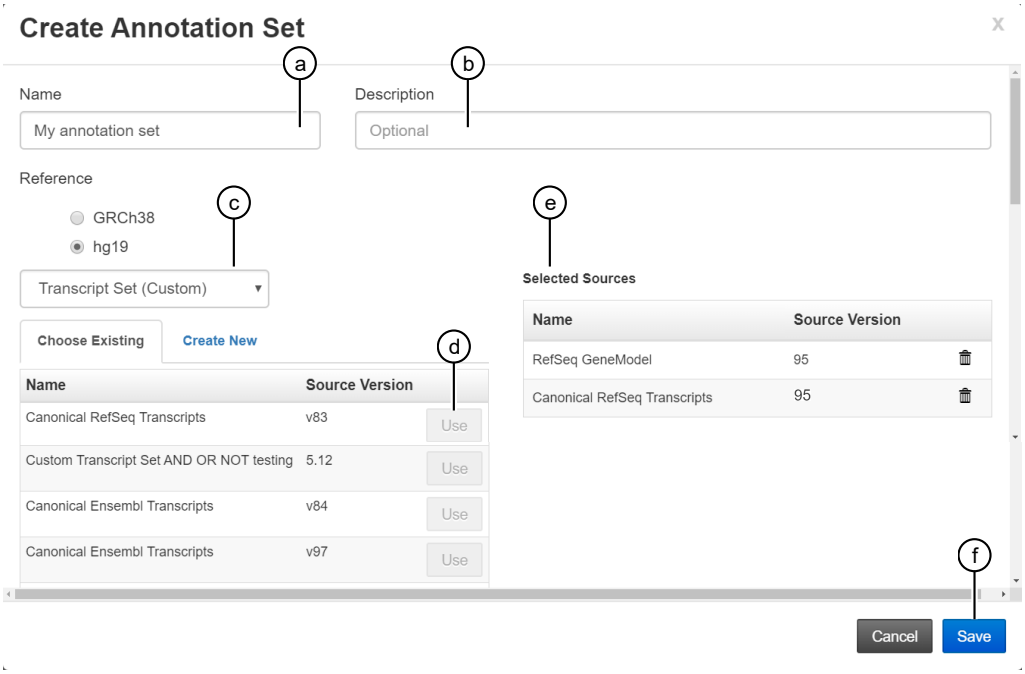
Create annotation set workflow presets

You can create custom annotation sets in Ion Reporter™ Software. A custom annotation set is a type of workflow preset that you can add to an analysis workflow so that you can launch analyses that use the custom annotation set.

Before you begin this procedure, review the guidelines in “Custom annotation sources” on page 133.

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Annotation Set**.

3. In the **Create Annotation Set** screen:



   a. Enter a descriptive name.

      IMPORTANT! You can use the words AND, NOT or, OR in an annotation set name in Ion Reporter™ Software 5.16 and later.

   b. *(Optional)* Enter a description of the annotation set.

   c. From the **Choose Type** list, select the annotation source or sources that you want to add to the preset.

   d. Click **Use** next to the annotation source that you want to use.

      The source is added to the **Selected Sources** list in your annotation set.

   e. Repeat this process for each annotation source that you want to add to your annotation set until your list of **Selected Sources** is complete.

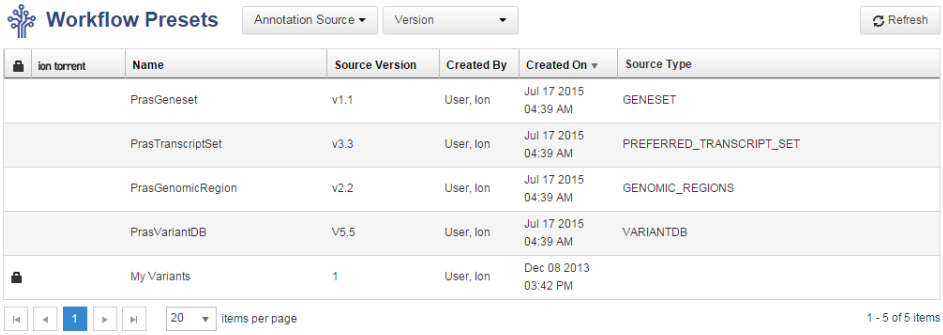
   f. Click **Save** to save the annotation set.
      The annotation set appears in the **Workflow Presets** table and is available in the **Annotation** step when you create an analysis workflow.

4. *(Optional)* When your preset works as you intend, the analysis workflow is ready for use in production. Select the preset in the **Workflow Presets** table, then click **Actions** ▸ **Lock** to lock the analysis workflow. You cannot undo a lock action.

View custom annotation sources

Annotation sets created by users are displayed in the **Annotation Source** list. You can mix customer annotation sources with Ion Reporter™ Software predefined annotation sources to create your own custom annotation set. For more information, see “Create annotation set workflow presets” on page 133.

1. In the **Workflows** tab, click **Presets**.
2. Click **Annotation Set ▸ Annotation Source**.
   A table listing the custom annotation sources appears. You can use these custom annotation sources to create custom annotation sets.



Create a final report template workflow preset

You can predefine a final report template as an Ion Reporter™ Software workflow preset. The final report template can then be added to an analysis workflow.

Note: The options that you can add to a final report template with a workflow preset are limited. You can also create a report template that has more customization options from your analysis results. For more information, see “Create a final report template from analysis results” on page 407.

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1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset ▸ Final Report Template**.
3. Complete the **Create Final Report Template** dialog box.
   a. *(Required)* Enter a name for the report template.
   b. *(Optional)* Enter a description.
   c. *(Optional)* Click **Add Image Section** to add a section to upload images to reports that are generated from the template. To add multiple image sections to the template, click **Add Image Section** again until you are satisfied with the number of image sections that are added. Scroll to the bottom of the **Create Final Report Template** to preview the number of **Image** sections added.
   d. *(Optional)* Enter an organization name.

e. *(Optional)* Enter the organization address.

f. *(Optional)* To add a logo to the report header, click **Upload** . In the **Upload Header Logo** dialog box, click **Select File** to find, and then select the logo file. Acceptable formats are: PNG, GIF, and JPG.

g. Complete the sections that are to be included in the report template, or click the **Exclude** to remove the section from the report template:

Section	Description
Background (Optional)	Enter background information describing the purpose of the report that will be generated by the analysis workflow.
Analysis Information (Optional)	This section provides summary information about the analysis that was run. Information includes the Ion Reporter™ Software version number, report generation date, name of person who launched the report, the analysis workflow used, the name of person who analyzed the data, a list of annotations, the date the information was imported, reference information, and the name and version of any copy number baseline that is used.
Samples Overview (Optional)	This section provides detailed information about each sample used in the analysis. Information includes the sample attributes that are included in the **Samples** table when the data is viewed in Ion Reporter™ Software.
QC Metrics (Optional)	This section provides coverage metrics for hotspots, amplicons, and genes. For a complete list of the metrics that are included, see “Quality control (QC) metrics” on page 423. You can also view and download these metrics in a separate report. For more information, see “View a Quality Control (QC) report” on page 418.
Reported Variants (Optional)	If included, select the columns of data to include in the report, then drag-and-drop the selected columns to configure the sort order on the report. This section generates a table of reported variants, and includes columns of variant details that you select.
Variant Details (Optional)	This section provides details about the occurrence of each reported variant in the samples. Information includes the name and description of variants, and which samples they came from. In addition, it lists annotations and notes for each variant.
Comments (Optional)	This section displays an open text field in the report where notes can be entered before publishing the report.
Sign–Off (Optional)	This section provides lines with the name and qualifications of people who are required to provide a handwritten signature for the report. Enter the name and title for a handwritten signature, then click **Add**. Continue to add the information for all more required signatures, until all designated signers are added.
Disclaimer (Optional)	This section provides a customizable legal disclaimer that is placed at the end of the report. If the organization requires a legal disclaimer on the report, enter the text of a legal disclaimer that you want to appear.

4. Click **Save** to create your report template preset.

5. Add your report template to an analysis workflow.
   For more information, see “Add a report template to an analysis workflow” on page 410.

6. Launch an analysis from your analysis workflow.
   For more information, see “Launch an analysis” on page 165.

Copy Number Variant detection

There are known sources of variability in analysis results, including pool imbalance (if an assay has more than one pool of amplicons), total number of reads and per-amplicon attributes of GC proportion. In practice, other variability exists that is not associated with known attributes, yet is systematic.

A copy number baseline, when added to an analysis workflow can correct the variability. Copy number baselines can be added only to assays that support CNV detection.

A copy number baseline is a set of control samples that are used to create a baseline for the detection of copy number variants (CNVs). CNV analysis is based on reads counts for each amplicon. Copy number estimates are made using a proprietary algorithm.

Analysis workflows that use baseline controls allow you to determine copy number changes in the sample of interest, without the use of a matched control.

In Ion Reporter™ Software, copy number baseline workflow presets are the components that you can add to analysis workflows as baseline controls. There are 6 types of copy number baselines in the software. The **Baseline Type** is based on the type of libraries that you use.

- **AmpliSeq**—Use to create copy number baselines for Ion AmpliSeq™ and Oncomine™ libraries (not Exome).
- **AmpliSeq HD**—Use to create copy number baselines for Ion AmpliSeq™ HD libraries.

  Note:
  Only **hg19** reference genome is supported for a **Baseline Type** of **AmpliSeq HD**.

- **AmpliSeq Exome**—Use to create copy number baselines for Ion AmpliSeq™ Exome libraries.
- **TagSeq**—Use to create copy number baselines for Ion TagSeq libraries.
- **TargetSeq-Exome**—Use to create copy number baselines for Ion TargetSeq™ Exome or other targeted libraries.

There are 2 algorithms that detect CNVs in the software for Ion AmpliSeq™ and Ion AmpliSeq™ HD custom panels. You can select a **CNV Detection Type** based on the types of variants that the panel detects. The **CNV Detection Type** that you select applies a CNV detection algorithm to the analysis workflow, based on the panel that you use.

- The **Somatic (CNV VCIB)** detection type is designed for use with panels used to detect somatic variation. The **Somatic (CNV VCIB)** detection type is recommended for use with somatic variant detection.

  Note: The **Somatic (CNV VCIB)** detection type can also be used in germline variant calling.

- The **Germline (CNV Informatics Baseline)** is designed for use with panels and samples used to detect germline variation.

Samples are added to the baseline, based on recommended guidelines. For more information, see “CNV Informatics Baseline workflow presets” on page 138 and “Variability Correction Informatics Baseline (VCIB) baseline workflow presets” on page 141.

Administrators and **Analyze** users can create new copy number baselines, create copies of existing copy number baselines, and augment existing copy number baselines. When a baseline is augmented, an existing copy number baseline is copied and saved with a new name. More samples can then be added to the new baseline.

Design guidelines for CNV detection in Ion AmpliSeq™ and Ion AmpliSeq™ HD custom panels

For any genomic region for which you want to detect CNVs, it is recommended that samples include at least 10 amplicons. That is, if the goal is to detect CNVs in a gene, at least 10 amplicons should be in or next to that gene, ideally 12 amplicons or more. If fewer than 10 amplicons are present, only CNVs with greater copy numbers may be detectable.

For robust detection of CNVs, most amplicons in a panel should be in normal diploid regions. If more than 30 percent of the amplicons are in a CNV region, it may be difficult to detect the CNV. In practice, if a panel contains at least 5 genes widely spread across the genome, the panel typically meets these criteria.

CNV Informatics Baseline workflow presets

If you use a single sample analysis workflow, you need to use a copy number baseline to detect CNVs. A copy number baseline can be associated with a panel file in the software. If a copy number baseline is not shipped with a panel, you must create a baseline and add it to an analysis workflow.

A **CNV Informatics Baseline** is used in Ion Reporter™ Software to detect CNVs in samples when using a custom panel. The **CNV Informatics Baseline** should include at least 20 samples that are not expected to have germline CNVs. Replicate samples can be used but at least 10 unique samples from diverse populations, and preferably more, are recommended if available.

You can either create a baseline and use that baseline as is, or you can augment an existing baseline with more samples then rerun the create baseline step in the **Presets** screen of the **Workflow** tab. When a baseline is augmented with samples, new samples are run, the size of each systematic effect that is encoded in the baseline is estimated, and a correction is applied to remove the effect. The added samples must be diverse to capture likely systematic variation.

To use a **CNV Informatics Baseline** in Ion Reporter™ Software, you can select the baseline preset when you create an analysis workflow. For more information, see “Apply a copy number baseline workflow preset to an analysis workflow” on page 127. You can then use the analysis workflow to call CNVs in the samples.

CNV Informatics Baseline calculations

Copy number estimates are made using a proprietary algorithm.

The algorithm for **CNV Informatics Baselines** normalize some known sources of variability include pool imbalance, median mean base coverage of all tiles, and per tile attributes of GC proportion.

In Ion AmpliSeq™ assays, germline copy number estimates are performed in small units called tiles. Tiles are short segments of sequences that are about 100bp long. Contigs that are formed by overlapping amplicons are divided into disjointed tiles of equivalent size closest to 100bp.

When Ion Reporter™ Software calculates the mean base coverage for each tile, the same normalization that was done by the **CNV Informatics Baseline** creation step is performed on the test sample. The software then compares the mean coverages to expected mean coverage for the tiles in the baseline samples. The software further derives for every tile, posterior likelihood of its being every possible ploidy value (0 to 10), given the coverage observation.

The algorithm uses a model for the whole chromosome to determine the ploidy value of every tile, instead of the most likely ploidy value for a CNV segment tile by tile. A transition probability between 2 adjacent tiles to model the likelihood that two adjacent tiles are having the same ploidy value is used. The likelihood of an assignment of ploidy values to a list of tiles is the multiplication of the likelihood of the assigned ploidy of every tile times all the transitional probabilities between adjacent tiles based on the assigned ploidy values. The software finds the most likely assignment, and that is reported as the CNV result.

For details about CNV call results shown in analysis results, see the user guide for the assay that you use.

Create a CNV Informatics Baseline workflow preset

You can create a **CNV Informatics Baseline** workflow preset in Ion Reporter™ Software to determine copy number changes in the sample of interest, without the use of a matched control. You can later add the **CNV Informatics Baseline** to an analysis workflow that you can use as a baseline control for analyses.

Use the following guidelines when you select samples to use in the baseline.

- Select a cohort of diverse sample types. This includes germline samples and cell-lines.
- Select samples with no known CNV aberrations. If this information is not available, a large cohort size can compensate any CNV aberrations.

If you import a custom copy number baseline, the target regions file that was used to create the copy number baseline must be available in the software. Then the imported copy baseline is shown in the list of available copy number baselines.

1. In the **Workflows** tab, click **Presets**, then click **Create Preset** ▸ **Copy Number Baseline**.
   The **Create Copy Number Baseline** screen opens.

2. In the **Baseline Type** step, make the following selections.
   a. Select **AmpliSeq**, **TargetSeq-Exome**, or **AmpliSeq-Exome** for the baseline type.

   b. Select a reference genome.
      GRCH38
      hg19

c. Select the **Target Regions** file that corresponds to the panel.

- If you have already uploaded a germline Target Regions `<panelID>_Designed.bed` file for the custom Ion AmpliSeq™ panel, select it from the dropdown list.
- If the target regions file is not available in the dropdown list, click (Upload), then select the `<panelID>_Designed.bed` file of the panel that you imported from Ion AmpliSeq™ Designer to upload the file from your computer.
- If the **Target Regions** is not imported from Ion AmpliSeq™ Designer Use the following option to import AmpliSeq libraries and panel files.

1. Click **AmpliSeq Import**.
2. In the **Import for AmpliSeq** dialog box, select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order, or Ion AmpliSeq™ On Demand panel files, or select the **Fixed Panel** tab for Community or Ready-to-Use panel files.
3. Enter an account user name.
4. In the Password field, enter the access code. For more information, see “Generate an access code for AmpliSeq.com” on page 126.
5. Click **List My AmpliSeq Panels**.
6. From the list, select the fusion panel file that you want to import.
7. Click **Import**.

3. Click **Next**.

4. In the **CNV Detection Type** workflow bar step, verify that **Germline (CNV Informatics Baseline)** is selected, to apply the CNV detection algorithm to the analysis workflow.

   Based on the selections in the previous step, the **Germline (CNV Informatics Baseline)** is automatically selected.

5. In the **Samples** step, select the required number of samples for the baseline. For accurate baselines, ensure that the samples selected for the baseline have been run with the same panel as the baseline.

   If you do not select the minimum number of samples that are required for the baseline, you cannot proceed.

   Note: Only samples designated in the software as male will be available to be selected. to be used to create the CNV Informatics Baseline.

   ---

6. Click **Next** to advance to the **Confirm** step.

7. In the **Confirm** step, enter a name for the baseline, or use the default name, and enter an optional description for the baseline.

8. Click **Create Baseline**.

   The baseline creation job is started and its status is shown in the **Presets** screen.

When the baseline creation is complete, it is locked and shown in the **Presets** screen with a **State** of **Successful** and is available for use in the software. The new baseline can be added to an analysis workflow. For more information, see “Create a new analysis workflow” on page 79“Copy and edit an

existing analysis workflow" on page 77, and "Apply a copy number baseline workflow preset to an analysis workflow" on page 127.

If the **State** of a copy number baseline is **Failed**, place the pointer over the row of the copy number baseline and click **View Log** to open the log in a separate browser tab, or click **Download Log** to download the log. This record can help you troubleshoot the failure.

Variability Correction Informatics Baseline (VCIB) baseline workflow presets

In Ion AmpliSeq™, Ion AmpliSeq™ HD, and Oncomine™ assays, somatic copy number estimates are made when Ion Reporter™ Software counts reads for each amplicon, and makes adjustments to account for specific types of variability. The software then compares the read counts to expected counts for the amplicons and then makes further adjustments in a set of normal samples. That is, for plasma or cell-line samples that do not have known CNVs, or in baseline samples with no known copy number variants.

Known sources of variability include pool imbalance (when the assay has more than one pool of amplicons), total number of reads and, for each amplicon, attributes of GC proportion, and length of the amplicon insert. In practice, we observe other variability that does not associate with known attributes yet is systematic. The method that is used by the software to identify copy number variations is based on many diverse samples, captures systematic effects, and encodes the samples into a file that contains the baseline information.

You can create a **Variability Correction Informatics Baseline (VCIB)** to detect copy number variation in samples. You can either create a baseline and use that baseline as is, or you can augment an existing baseline with samples. The VCIB baseline must include a minimum of 48 samples, and at least 6 of the samples must be designated as normal. All samples that are used to create the baseline must have been analyzed in Ion Reporter™ Software with the same custom panel.

Runs included in the baseline should cover the technical variability that is present in the research laboratory, such as the number of PCR cycles, multiple Ion Chef™ Instruments if present, laboratory technicians, variations of protocols, and so on. Typically a VCIB baseline is created for one chip type, for example, the Ion 550™ Chip.

For capturing sample to sample variability, it is recommended to select a cohort of diverse sample types. Including multiple samples of every sample type to which the CNV baseline are applied. This always includes clinical research samples such as FFPE. It can also include control samples, cell lines, and so on.

For FFPE samples, it is recommended to select samples with no known CNV aberrations in the region covered by the panel, if possible. If this information is not available, it is recommended that more than 48 distinct libraries be used with no more than 2 library replicates.

When a baseline is augmented with samples, new samples are run, the size of each systematic effect that is encoded in the baseline is estimated, and a correction is applied to remove the effect. The added samples must be diverse to capture likely systematic variation.

Note: If you use a single sample analysis workflow, you need to use a copy number baseline to detect CNVs. You need to create a custom baseline if a baseline is not shipped with a custom panel.

To use a VCIB baseline in Ion Reporter™ Software, you can select the baseline preset when you create an analysis workflow. For more information, see "Apply a copy number baseline workflow preset to an analysis workflow" on page 127.

Variability Correction Informatics Baseline (VCIB) calculations

Copy number estimates are made using a proprietary algorithm.

The Variability Correction Informatics Baseline (VCIB) algorithm uses a bioinformatics baseline, which is created using at least 48 diverse samples, to allow the assessment of corrected log2ratios of amplicons of identified CNV regions (usually genes) in input sample data. This algorithm is followed by a correction algorithm for the percent tumor cellularity recorded for the sample to give copy number and confidence interval data for the identified CNV regions. The algorithms used to calculate the corrected log2 ratios and the correction for tumor fraction are proprietary.

For details about CNV call results in the **Analysis Results** screen, see the user guide for the assay that you use.

Create a Variability Correction Informatics Baseline (VCIB) workflow preset

You can create a **Variability Correction Informatics Baseline (VCIB)** workflow preset in Ion Reporter™ Software to determine copy number changes in the sample of interest, without the use of a matched control. You can later add the custom copy number baseline to an analysis workflow that you can use as a baseline control for analyses.

You must use a minimum of 48 samples, and at least 6 of the samples must be normal, to create a **Variability Correction Informatics Baseline (VCIB)** workflow preset.

If you import a custom **Variability Correction Informatics Baseline (VCIB)**, the target regions file that was used to create the copy number baseline must be available in the software to ensure that the imported copy baseline appears in the list of available copy number baselines.

1. In the **Workflows** tab, click **Presets**, then click **Create Preset** ▸ **Copy Number Baseline**.

2. In the **Baseline Type** step, make the following selections.
   a. Select the **Baseline Type** that corresponds to the type of libraries that you use. For more information, see “Variability Correction Informatics Baseline (VCIB) baseline workflow presets” on page 141.

Baseline Type	Description
AmpliSeq HD	Create VCIB baselines for Ion AmpliSeq™ HD libraries.
AmpliSeq	Create VCIB baselines for Ion AmpliSeq™ and Oncomine™ libraries (not Exome).
AmpliSeq-Exome	Create VCIB baselines for Ion AmpliSeq™ Exome libraries.
TagSeq	Create VCIB baselines for Ion TagSeq libraries.
TargetSeq-Exome	Create VCIB baselines for Ion TargetSeq™ Exome or other targeted libraries.

   b. Select a reference genome.
   GRCH38
   hg19

c. Select the **Target Regions** file that corresponds to the panel.
   - If you have already uploaded a germline Target Regions `<panelID>_Designed.bed` file for the custom Ion AmpliSeq™ panel, select it from the dropdown list.
   - If the target regions file is not available in the dropdown list, click (Upload), then select the `<panelID>_Designed.bed` file of the panel that you imported from Ion AmpliSeq™ Designer to upload the file from your computer.

d. If the **Target Regions** is not imported from Ion AmpliSeq™ Designer Use the following options to import AmpliSeq libraries and panel files.
   1. Click **AmpliSeq Import**.
   2. In the **Import for AmpliSeq** dialog box, select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order, or Ion AmpliSeq™ On Demand panel files, or select the **Fixed Panel** tab for Community or Ready-to-Use panel files.
   3. Enter an account user name.
   4. In the Password field, enter the access code. For more information, see “Generate an access code for AmpliSeq.com” on page 126.
   5. Click **List My AmpliSeq Panels**.
   6. From the list, select the fusion panel file that you want to import.
   7. Click **Import**.

3. Click **Next**.
   The **CNV Detection Type** step of the **Create Copy Number Baseline** workflow bar opens. Ensure that the **Somatic (CNV VCIB)** algorithm is selected for use with VCIB workflow presets.

4. In the **CNV Detection Type** step, make the following selections to make a new baseline, or start from an existing baseline.
   - To create a new baseline, deselect the **Start with an existing CNV baseline** checkbox.
   - To use an existing baseline and augment the baseline with more samples, select **Start with an existing CNV baseline**. The target region file selected in the **Baseline Type** step is shown in the dropdown list. For more information, see “Augment an existing VCIB baseline workflow preset” on page 145.

5. Click **Configure parameters** to configure the parameters if needed, then click **Done**. For more information about these parameters, see the user guide for the panel that you use, or contact your Field Bioinformatics Specialist.

6. Click **Next**.

7. In the **Samples** step, select the 48 samples to be used in the CNV baseline creation.

8. Click **Next**.

9. In the **Confirm** step, enter a name, or accept the default name, and enter an optional description for the baseline.

10. Click **Create Baseline**.

When the baseline creation is complete, it is locked and shown in the **Presets** screen with a **State** of **Successful** and is available for use in the software. The new baseline can be added to an analysis workflow. For more information, see “Create a new analysis workflow” on page 79“Copy and edit an existing analysis workflow” on page 77, and “Apply a copy number baseline workflow preset to an analysis workflow” on page 127.

If the **State** of a copy number baseline is **Failed**, place the pointer over the row of the copy number baseline and click **View Log** to open the log in a separate browser tab, or click **Download Log** to download the log. The log file can help you troubleshoot the failure.

Use gender to call gains from expected copy number changes

You can use the following approach to call gains from expected copy number changes with analyses that are performed on Oncomine™, Ion AmpliSeq™, or Ion AmpliSeq™ HD cancer research panels that use analysis workflows that include the Variability Correction Informatics Baseline (VCIB).

A copy number baseline has a gender, either male or female. A sample also has a gender: male, female, or unknown (unknown is interpreted as female). Expected genomic copy number regions for these genders are defined in the ploidy files that are supplied by Ion Reporter™ Software within analysis workflows.

For example, create a copy number baseline with "male" normal human samples and chromosome X non-Pseudo Autosomal Regions (non-PAR) amplicons in the panel. The baseline copy number for the non-PAR chromosome X amplicons is expected to be 1. If a male sample is run using this baseline the relative copy number to the baseline should be 1:1 and is expected (normal) and should be reported accordingly. A female sample using this baseline should have a relative copy number of 2:1 as is expected (normal) and should be reported accordingly. Using a ploidy file, each amplicon is assigned to the baseline expected copy number found in the baseline ploidy file (male), in this example non-PAR chromosome X amplicons would have a copy number of 1. Running a sample with a female ploidy file, the non-PAR chromosome X amplicons has a copy number of 2, so the final copy number relative to the baseline is adjusted appropriately.

More precisely, if for amplicon *, expected baseline copy number = `B`, and expected sample copy number = `S`, after variability corrections, set `l2r` = log2 ratio of sample observation to the baseline.

`Final CN` = 2**`(lrr)` * `B`

For purposes of reporting at the CNV_ID level, S and B are used to determine how different Final CN is from expected for that sample. The actual algorithm is proprietary.

Note: Analyses results in Ion Reporter ™ Software reflect the following:

- FASTA files in Torrent Suite™ Software hard mask all Pseudo Autosomal Region (PAR) region values in chromosome Y for hg19 and GRch38 reference genomes. The hard mask removes the PAR for the Y chromosome to improve variant calling in Ion Reporter™ Software.
- The variantCaller plugin in Torrent Suite™ Software defaults to use only reads that include mapping quality value scores >0, that is, reads that map to only one location in the reference. When FASTA files originate in Torrent Suite™ Software, Ion Reporter™ Software removes the PAR from chrY to prevent the reads that map to the PAR in chrX from mapping to both the chrY and chrX locations. As a result, all reads in the chrX PAR that do not also map to yet another location will be included for making variant calls.

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1. Create a normal male and chrX PAR CNV baseline.

   For more information, see “Create a Variability Correction Informatics Baseline (VCIB) workflow preset” on page 142.

2. Add the CNV baseline to an Oncomine™, Ion AmpliSeq™, or Ion AmpliSeq™ HD analysis workflow.

   For more information, see “Apply a copy number baseline workflow preset to an analysis workflow” on page 127.

   Note: The name of the target regions BED file in the CNV baseline must match the target regions BED file that is used in the analysis workflow.

3. Launch the analysis.

   For more information, see “Launch an analysis” on page 165.

4. Visualize the analysis.

   For more information, see “IRGV & Generate Report tab” on page 351 or Chapter 8, “Visualize analysis results with Ion Reporter™ Software”.

Copy number gains are reported on the **Analysis Results** screen and the **Analysis Visualization** screen, **Variant Matrix** tab. For more information about the results, see “Reasons for NOCALL in a gene-level CNV” on page 227.

Augment an existing VCIB baseline workflow preset

You can augment an existing Variability Correction Informatics Baseline (VCIB) workflow preset with additional samples.

When baseline is augmented, new samples are run, the size of each systematic affect encoded in the baseline is estimated, and a correction is applied to remove the effect. These added samples need not be normal and should be diverse so as to capture likely systematic variation.

1. In the **Workflows** tab, click the **Presets** screen, then click **Create Preset** ▸ **Copy Number Baseline**.

2. Select a **Baseline Type**, then select your **Target Regions** file, then click **Next**.

3. In the **Algorithm Type** step, select the **Start with an existing CNV Baseline** checkbox, then select a baseline from the list.

   The list of baselines will be limited to baselines that were created with the Target Regions BED file that was selected in the **Baseline Type** step.

4. Click **Configure Parameters**.

5. In the **Configure Parameters** dialog box, click **Cnv Baseline Creation**, then **Advanced**. Set the **Minimum number of samples required to add to an existing baseline** parameter to reflect the number of samples that you add.

6. In the **Samples** step, select samples to use in the baseline.

   By default, the software prompts you to add another 48 samples. However, you can set the number to 1 or more.

To distinguish between *non-normal* and *normal* samples, you can use the **Normal** designation in the analysis workflow. This designation has no effect on the analysis. Only the original **Normal** samples in the first baseline creation are treated as Normal samples in the augmented baseline.

7. Click **Next**.

8. Enter a name for your baseline, or accept the default name, then enter an optional description, then click **Create Baseline** to save the updated workflow preset.

You can now add the augmented baseline to an analysis workflow. For more information, see "Create an analysis workflow that contains a copy number baseline" on page 147.

Create a copy number baseline for low-coverage whole-genome libraries

Users with the analyze role can create a copy number baseline for use with whole genome libraries with whole genome amplification and low coverage, such as Aneuploidy. This type of copy number baseline is for use with Ion ReproSeq™ PGS Kits.

IMPORTANT! Use the default parameter settings unless you are an advanced user. Contact a Field Bioinformatics Specialist (FBS) for help.

1. In the **Workflows** tab, click **Presets**, then click **Create Preset** ▸ **Copy Number Baseline**. The **Create Copy Number Baseline** screen opens.

2. Make the following selections in the **Create Copy Number Baseline** screen.
   a. Select **Low-coverage Whole-Genome**.
   b. Select a reference genome.
      - **GRCH38**
      - **hg19**

3. Click **Next**.

4. In the **CNV Detection Type** step, under **Germline (CNV Informatics Baseline)**, click Configure parameters.

5. In the **Configure Parameters** screen, accept the default settings or adjust the appropriate parameters, then click **Done**.

6. Click **Next**.

7. In the **Samples** step, select the required number of samples for the baseline. For accurate baselines, ensure that the samples selected for the baseline have been run with the same panel as the baseline.

   If you do not select the minimum number of samples that are required for the baseline, you cannot proceed.

   Note: Only samples designated in the software as male are available to be selected to be used to create the CNV Informatics Baseline.

8. Click **Next** to advance to the **Confirm** step.
9. In the **Confirm** step, enter a name for the baseline, or use the default name, and enter an optional description for the baseline.
10. Click **Create Baseline**.
    The baseline creation job is started and its status is shown in the **Presets** screen.

When the baseline creation is complete, it is locked and shown in the **Presets** screen with a **State** of **Successful** and is available for use in the software. The new baseline can only be added to analysis workflows that are used for whole genome libraries with whole genome amplification and low coverage.

If the **State** of a copy number baseline is **Failed**, place the pointer over the row of the copy number baseline and click **View Log** to open the log in a separate browser tab, or click **Download Log** to download the log file. This record can help you troubleshoot the failure.

Create an analysis workflow that contains a copy number baseline

IMPORTANT! The **Target Regions** and **Hotspot Regions** files that are used in an analysis workflow must each have a unique file name. To review the file names in the **Workflows** tab, go to **Workflow Presets** ▸ **Hotspot Region Files** and review the names of the hotspots files. If any of the names are identical to the **Target Regions** that is to be added to the analysis workflow, delete the entry in the list and upload a renamed version of the **Hotspot Regions** file.

Ion Reporter™ Software provides a workflow bar to guide you through the process to create or copy an analysis workflow that contains a **Germline (CNV Informatics Baseline)** or **Somatic (CNV VCIB)** workflow preset.

1. In the **Workflows** tab, click the **Overview** screen, then select an appropriate Oncomine™ analysis workflow.
2. Click **Actions** ▸ **Copy**, then click **Next** .
3. In the **Reference** step, select the applicable **Reference**, then select the **Target Regions**, **Hotspot Regions**, and **Fusions** BED files, if needed, then click **Next**.
4. In the **Annotation** step, select an **Annotation Set** from the dropdown list, then click **Next**.
5. In the **Filters** step, select a **Filter Chain** from the list, then click **Next**.
6. Select the baseline that you want to use from the **Baseline** dropdown list.
7. Click **Next**.
8. In the **Plugins** step, ensure that all **In-Analysis** plugins are deselected, then click **Next**.
9. In the **Final Reports** step, select a **Final Report Template** from the list, then click **Next**.

10. In the **Parameters** tab, review the default settings, then click **Next**.

    Note: Although **Read Mapping** parameters are exposed when you create an analysis workflow that includes a **VCIB** baseline, it is not necessary to change any settings.

11. In the **Confirm** tab, enter a **Workflow Name** and description, then click **Save Workflow**.

You can now use the analysis workflow to launch an analysis. For more information, see "Launch an analysis" on page 165. Alternatively, you can download the analysis results and view them visually in the `cn_results.png`. For more information, see "Visualize an analysis workflow that contains a CNV baseline" on page 332.

Sequence variant baselines

The filters that are used in Ion Reporter™ Software remove most sequencing errors. Although variant calling filters in the software suppress sequencing errors, it is possible that some errors can pass through the filters and be reported as false positives. However, in somatic panels, sequencing errors that survive filtering are reported as false positives that require investigation.

To decrease the number of potential false positives, you can create a sequence variant baseline for panels that detect somatic variants.

A sequence variant baseline is BED file that contains a list of alleles that are known sequencing errors. The baseline file records detailed properties for each sequencing error. The errors and properties are based on known germline normal samples. When added to a workflow, the baseline detects and filters out the errors in sequencing that can lead to false positive variant calls.

Ion Reporter™ Software includes predefined sequence variant baselines for use with Oncomine™ panels and other ready-to-use panels. You can also create a user-defined sequence variant baseline, which is especially useful for custom Ion AmpliSeq™ panels that do not include a predefined sequence variant baseline.

Create a Sequence Variant Baseline workflow preset

You can create a custom **Sequence Variant Baseline** workflow preset in Ion Reporter™ Software to help reduce noise. You can later add the custom baseline to an analysis workflow that you can use as a baseline control for analyses.

If you import the custom sequence variant baseline, the target regions file that was used to create the baseline must be available in the software to ensure that the imported baseline appears in the list of available sequence variant baselines. When you add the baseline to an analysis workflow, the same target regions files that is used to create the baseline must also be used in the analysis workflow.

IMPORTANT! A sequence variant baseline can be used with Ion AmpliSeq™ somatic samples only. Do not use a custom baseline for the detection of germline variants, as a germline variant can occasionally be misclassified as a sequencing error.

The sequencing run or runs that are used to create the sample data must use the same Ion Torrent chip type and same Ion AmpliSeq™ panel. For example, if an SVB will be applied to a sequencing run that used a Ion 540™ and custom Ion AmpliSeq™ panel, then the samples that were used to create the SVB must have been sequenced with the Ion 540™ and same custom Ion AmpliSeq™ panel.

1. In the **Workflows** tab, click **Presets**, then click **Create Preset** ▸ **Sequence Variant Baseline**.
2. In the **Baseline Type** step of the **Create Sequence Variant Baseline** workflow bar, select the baseline type that corresponds to the type of libraries that you use.

Baseline type	Description
AmpliSeq Somatic	Ion AmpliSeq™

3. Select a reference genome.
   - **GRCH38**
   - **hg19**
4. Select the **Target Regions** file that corresponds to the panel. If the target regions file is not available in the panel, you can upload the file.
5. Click **Next**.
6. In the **Parameters** step, do not change parameters from the default settings unless you understand how the change can affect your analysis. Click **Next**.
7. In the **Samples** step, do the following:
   a. Select the samples to be used in the baseline creation.

      You need to add at least 40 samples, up to a maximum of 100 samples, to create the custom sequence variant baseline. All samples must be normal (non-tumor). Samples can be of either gender. The software examines gender data for each sample.

      If you do not see the samples in the table, see “Sample definition” on page 49 for information about how to upload or define a sample.

   b. Select the **Chip Type** that was used for the sample data.

      All samples that you select for the sequence variant baseline must use the same chip type.
8. Click **Next**.
9. In the **Confirm** step, enter a name, or accept the default name, then enter an optional description for the baseline.
10. Click **Launch**.

The baseline creation job is started and the baseline with its status now appears on the screen. When the job completes, the baseline is added to the **Workflow Presets** screen and is available to add to an analysis workflow. For more information, see “Apply a sequence variant baseline workflow preset to an analysis workflow” on page 128.

Exon tile fusion baselines

When an exon tile fusion baseline is applied to an analysis workflow, Ion Reporter™ Software can model the amplicon expression variation within a gene. The exon tile fusion baseline is used to normalize a test sample, correct the coverage in exon-tile amplicons, and identify the presence or absence of expression imbalance in a gene.

An exon tile fusion baseline is a TXT file that contains:

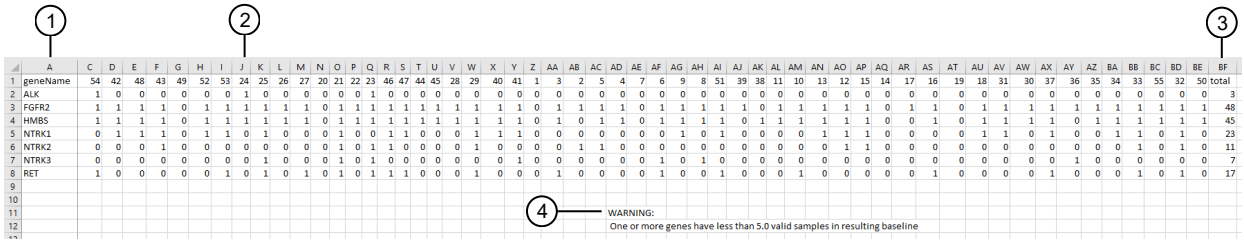
- the normalized expression values of exon-tile amplicons for genes with imbalance assays
- the median normalized expression for each amplicon

Ion Reporter™ Software includes predefined exon tile fusion baselines. You can also create an exon tile fusion baseline workflow preset in the software. You can later add the exon tile fusion baseline to an analysis workflow and use it as a baseline control for analyses.

An exon tile fusion baseline must include at least 48 fusion-negative RNA samples. Various parameters and thresholds used in the baseline computation determine the inclusion or exclusion of samples in the baseline for each of the genes, for example, ALK, RET, NTRK1, that are assayed in exon-tiling analysis workflows. The primary criteria for inclusion in the baseline is sufficient coverage across the exon-tile amplicons of a given gene. Samples with low coverage across the exon-tiling amplicons for one of the genes are excluded from the baseline.

Note: A sample can qualify for inclusion in a baseline of one gene but can be excluded from the baseline of another gene. For example, if a gene is not expressed in the tissue of the sample, the sample is excluded for that gene.

A summary table of the samples that are included and excluded in the exon tile fusion baseline for each gene is contained in a log file called createBaseline.ReadCounts.summary. For more information about file downloads, see “Variants file downloads” on page 208. Here is an example exon tile fusion baseline summary file.



	A	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF
1	geneName	54	42	48	43	49	52	53	24	25	26	27	20	21	22	23	46	47	44	45	28	29	40	41	1	3	2	5	4	7	6	9	8	51	39	38	11	10	13	12	15	14	17	16	19	18	31	30	37	36	35	34	33	55	32	50	total
2	ALK	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
3	FGFR2	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	48
4	HMBS	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1	0	1	1	1	1	1	45
5	NTRK1	0	1	1	1	0	1	1	0	1	0	0	0	1	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	0	1	1	0	1	0	23
6	NTRK2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	11
7	NTRK3	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	7
8	RET	1	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	17
9																																																											
10																																																										
11																																																										
12																																																										

1. Genes that are included in exon-tiling imbalance analysis workflows. Each row summarizes the imbalance baseline composition status of a gene.
2. Each sample is represented by a column. In this example, sample 24 is included in the baseline for ALK, FGFR2, and HMBS, but excluded for NTRK1, NTRK2, NTRK3, and RET.
3. The last column, with the header **total**, lists the total number of valid baseline samples per gene.
4. When a baseline results in fewer than 5 valid samples for one or more genes, a warning message is shown. In this example, the warning message applies to ALK.

Create an exon tile fusion baseline workflow preset

You must use at least 48 fusion-negative RNA samples to create an exon tile fusion baseline workflow preset.

1. In the **Workflows** tab, click **Presets**, then click **Create Preset** ▸ **Exon Tile Fusion Baseline**.
2. In the **Baseline Type** step of the **Create Exon Tile Fusion Baseline** workflow bar, select the **Fusion Panel** to use for the exon tile fusion baseline.

   Note:
   - The baseline type is set to **Exon Tile Fusion Baseline** and cannot be changed.
   - The reference genome is set to **hg19** and cannot be changed.
3. Click **Next**.
4. In the **Parameters** step, do not change parameters from the default settings unless you understand how the change can affect the analysis. Click **Next**.
5. In the **Samples** step, select the samples to be used in the baseline creation.

   You must select at least 48 or more fusion-negative RNA samples. If you do not see samples of interest in the table, see “Sample definition” on page 49 for information about how to upload or define a sample.
6. Click **Next**.
7. In the **Confirm** step, enter a name, or accept the default name, then enter an optional description for the baseline.
8. Click **Launch**.

The exon tile fusion baseline creation job is started and the baseline with its status now appears on the screen. When the job completes, the exon tile fusion baseline is added to the **Workflow Presets** screen and is available to add to an analysis workflow. For more information, see “Apply an exon tile fusion baseline workflow preset to an analysis workflow” on page 129.

Upload a target regions BED file workflow preset

You can upload a target regions BED file from a copied analysis workflow, then create a target regions BED file workflow preset for use in other analysis workflows.

IMPORTANT! Use only BED file names that do not contain spaces. If a file name includes spaces, the analysis fails.

1. In the **Workflows** tab, click **Create**.
2. In the **Research Application** column, click **DNA**. Click any type of sample group.
3. For **Reference**, select **GRCh38** or **hg19**.
4. In the **Target Regions** section, click **Upload**.
5. In the **Upload Target Regions File** dialog box:
   a. Select your library type or technology.
   b. Click **Select File** , then browse to your BED file.
   c. Ensure that the correct BED file name appears in the display field, then click **Upload**.
      Ion Reporter™ Software uploads and verifies your BED file. When verification is complete, the progress bar changes to green, and a message confirms that the files are ready for use.
   d. Click **Close**.

   The new BED file preset appears in the **Target Regions** section of the **Reference** step.
6. You can now upload additional BED files, continue to create an analysis workflow, or cancel the analysis workflow creation.
   In the **Workflows** tab, click **Presets**, then select **Annotation Sets** ▸ **Target Regions Files**. The new BED file is listed in the **Workflow Presets** table.
7. *(Optional)* When you have run a set of samples to validate that your preset works as you intended, select the preset in the **Workflow Presets** table, then click **Actions** ▸ **Lock** to lock the analysis workflow.
   You cannot undo a lock action.

Upload a hotspots BED file workflow preset

You can upload a hotspots BED file from a copied analysis workflow and then create a hotspots BED file workflow preset for use in other analysis workflows.

IMPORTANT! Use only BED file names that do not contain spaces. If a file name includes spaces, the analysis fails.

1. In the **Workflows** tab, click **Create**.
2. In the **Research Application** column, click **DNA**. Click any type of **Sample Group**.
3. In the **Reference** step, in the **Hotspot Regions** section, click **Upload**.
4. In the **Upload Hotspot File** dialog box:
   a. Click **Select File**, then browse to your BED file.
   b. Confirm that the correct BED file name appears, then click **Upload**.
      Ion Reporter™ Software uploads and verifies your BED file. When verification is complete, the progress bar changes to green and you see a message to indicate that the preset is available for use.
   c. Click **Close**.

   The new BED file preset appears in the **Hotspot Regions** section of the **References** step.
5. You can now upload additional BED files, continue to create an analysis workflow, or cancel the analysis workflow creation.
   In the **Workflows** tab, click **Presets**, then select **Annotation Sets ▸ Hotspot Regions Files**. The new BED file is listed in the **Workflow Presets** table.
6. *(Optional)* When you have run a set of samples to confirm that your new preset works as you intended, select the preset in the **Workflow Presets** table, then click **Actions ▸ Lock** to lock the analysis workflow.
   You cannot undo a lock action.

Analysis workflow revision autonumbering

Ion Reporter™ Software displays revision numbers of analysis workflows, and automatically increases the revision numbers when you copy or edit an analysis workflow.

To view analysis workflow version numbers, click **Workflows ▸ Overview**, then select the desired analysis workflow. The analysis workflow version number, for example w2.2, appears in two places.

- In the **Workflow Name** column at the end of the analysis workflow name.
- In the **Details** section at the end of the analysis workflow name.

The version number increases if you copy a predefined analysis workflow, or copy and edit a user-defined analysis workflow and use the name of the previous analysis workflow as the name for the new analysis workflow.

You can copy predefined analysis workflows and user-defined analysis workflows from the current version of the software or from previous versions of the software.

Lock an analysis workflow

You can lock an analysis workflow to ensure that the settings are not changed. After an analysis workflow is locked, it cannot be edited or unlocked. However, it can be copied.

1. Click the **Workflows** tab.
2. Select the analysis workflow that you want to lock, then click **Actions** ▸ **Lock**.
3. Click **Yes** to confirm that you want to lock the analysis workflow.
   Due to a database update latency, it can take a few minutes for the analysis workflow to lock.

When the workflow is locked, a **(Lock)** is shown beside the name of the workflow in the list of analysis workflows. The analysis workflow is now locked and cannot be edited.

5 Analyses

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About analyses

When one or more samples are successfully analyzed using an analysis workflow in Ion Reporter™ Software, the output is a set of analysis results. Analyses provide you links to tabular analysis results, and access to report generation and the visualization tools Ion Reporter™ Genomic Viewer (IRGV) and Integrative Genomics Viewer (IGV).

One or more samples from a sequencing run can be automatically analyzed in Ion Reporter™ Software. For this automatic analysis, set up the IonReporterUploader plugin, and select an Ion Reporter™ Software analysis workflow in Torrent Suite™ Software. For more information, see the Torrent Suite™ Software Help.

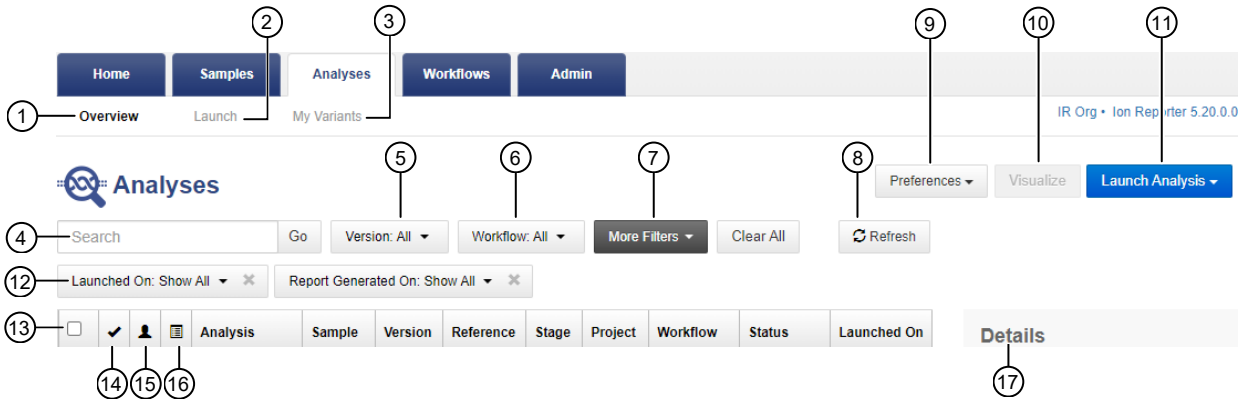
You can also generate, or *launch*, one or more analyses manually in Ion Reporter™ Software. Manual launches allow you to select an analysis workflow and one or more previously uploaded sample or samples for the analysis. For single-sample analysis workflows, you can select multiple samples for multiple single-sample analyses to be created with the selected analysis workflow. In this case, each analysis delivers an independent set of analysis results.

If you have numerous analyses to launch, you can launch analyses with different samples by batch with a CSV file. Batch launches of multiple analyses can use one or more analysis workflows.

Any time that an analysis is successfully completed, Ion Reporter™ Software sends you an email notification that provides a link to the analysis. You can use the link to review, edit, and interpret analysis results. Or you can access analyses and open analysis results in the **Analysis** tab of the software.

Analyses tab overview

You can access the list of available analyses in the **Analyses** table, located on the in the **Overview** screen in the **Analyses** tab.



1. The **Overview** screen in the **Analyses** tab. Click the screen name to toggle between screens.
2. The **Launch** screen. For more information, see "Launch an analysis" on page 165.
3. The **MyVariants** screen. For more information, see "MyVariants" on page 172.
4. You can enter keywords or text strings in the **Search** box. For more information, see "Search for analyses" on page 163.
5. The **Version All** filter allows you to search for specific versions of analyses.
6. The **Workflow All** filter allows you to search for specific analysis workflows.
7. **More Filters** allows you to add additional filters to the **Analyses** tab. For more information, see "Filter analyses" on page 164.
8. **Refresh** refreshes the list of samples.
9. Apply **Preferences** to customize how the **Analyses** table is organized. For more information, see "Customize the Analyses table" on page 157.
10. **Visualize** allows you to view the data in Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV). For more information, see Chapter 8, "Visualize analysis results with Ion Reporter™ Software".
11. **Launch Analyses** allows you to manually launch one or more analyses (batch). For more information, see "Launch an analysis" on page 165.
12. Additional filters, added by using **More Filters**.
13. Table columns with information about the analysis and data for analysis attributes. Several columns are included by default, and you can add more attributes with table preferences. For more information, see "Analyses table column options" on page 159.
14. ✓ **(Checked Out)** Indicates that edits to the analysis are in progress. Open the analysis in view-only mode to view analysis results and see the name of the user who is making edits to the analysis.
15. **(Analyze role)** Indicates that the analysis is shared by an Analyze role user.
16. **(Notes)** Notes about the analysis.

⑰ **Details** provides additional information about the selected analysis.

Customize the Analyses table

The **Analyses** table includes details that describe the samples, analysis workflows, and software versions that were used to create the analyses in Ion Reporter™ Software.

The information that is shown in the table depends on which analysis workflow and annotation set are applied when the analysis is launched. You can customize the table to reflect the information that is most relevant to your research. To customize the table, create a table preference, or drag and drop the columns to change the order in which they appear.

Add columns to the Analyses table

You can add columns to the **Analyses** table that you view in Ion Reporter™ Software. Columns in the **Analyses** table show values for information about the analysis. The information can be associated with samples that are used in the analysis, with the analysis workflow, or with the analysis itself. Sample attributes can be inherited from Torrent Suite™ Software and include information such as chip type, sequencing run details, and user-defined attributes.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences** ▸ **Select Columns**.
3. In the **Select Columns** dialog box, then select the sample attributes that you want to add to the **Samples** table, or deselect the sample attributes that you want to remove from the table.

The selected attributes are shown the **Analyses** table until you close the table. To save the table with the attributes that you added, create a preference. For more information, see “Create an Analyses table preference” on page 158.

Change the order and width of columns in the Analyses table

Default columns are included for all analyses. You can sort the **Analyses** table to more easily find analyses of interest, either by the date that the analysis was run, or by sample, workflow, or analysis attributes. You can also add other available columns to customize the table.

You can change the order and width of the columns in the **Analyses** table in Ion Reporter™ Software.

1. In the **Analyses** tab, select **Overview**.
2. Click any column heading that you want to move, then drag and drop each column to the position that you prefer. Change the width of each column, as desired.
   The column order and width remain as you have arranged them until you sign out of the software.
3. (Optional) To use the new column order in the future, create a table preference. Save the order of the columns as a table preference.
   a. Click **Preferences** ▸ **Save Table Preference As**.
   b. Enter a name for the table preference, then click **Save**.

The **Analyses** table preference is saved.

Create an Analyses table preference

You can create a table preference setting in Ion Reporter™ Software. The table preference enables you to view only the columns of data that are relevant to your research in an **Analyses** table.

The user-defined table preference setting is included as output when you download a variants file.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences** ▸ **Select Columns**, then select the sample attributes that you want to add to the **Samples** table, or deselect the sample attributes that you want to remove from the table.
   The selected columns are added on the right side of the table.
3. Save the custom table as a preference.

Option	Description
Preferences ▸ **Save Table Preference As**	This setting enables you to name the table preference. The preference setting is then selectable from **Preferences** ▸ **Table Preferences**.
Preferences ▸ **Save Table Preference**	This setting becomes the standard view for the user account. To restore default table columns, click **Preferences** ▸ **Restore Defaults**.

The table preference remains until you apply another table preference or click **Restore Defaults**.

Apply an Analyses table preference

You can apply table preferences to change the view of information in an **Analyses** table. Table preferences allow you to view information that is relevant to your research in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences** ▸ **Table Preferences**.
3. Select any available table preference that you want to apply to the table.
   The view in the **Analyses** table changes to show your selected preferences.
4. *(Optional)* Create a new **Analyses** table preference based on the table preference that you selected.
   a. Click **Preferences** ▸ **Select Columns**, select any available columns that you want to add, then click **Apply**.
   b. Click the column heading that you want to move, then drag and drop the column to the position that you prefer.
   c. Click **Preferences** ▸ **Save Table Preference As**.
   d. In the **Save Table Preference** dialog box, enter a name for the table preference, then click **Save**.

The new **Analyses** table preference is saved.

Restore default settings for the Analyses table

You can restore default table column headings in the **Analyses** table in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences** ▸ **Restore Defaults**.

The default table settings are restored.

Delete an Analyses table preference

You can delete a user-defined **Analyses** table preference setting in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences** ▸ **Table Preferences**, then select the user-defined table preference that you want to delete.
3. Click **Preferences** ▸ **Delete Table Preferences**.
4. In the **Confirm Delete** dialog box, click **Yes** to confirm the deletion.

The user-defined table preference is removed from the **Table Preferences** list.

Analyses table column options

When you open the **Analyses** table, a default set of columns is included. The table columns are based on various factors, including the samples that are used, the analysis name, the analysis workflow that is used, other analysis attributes, and the analysis workflow and annotation set that is applied when the analysis is launched. You can customize the **Analyses** table to more easily find analyses of interest, either by the date that the analysis was run, or by sample, analysis workflow, or analysis attributes.

The following columns are included in the **Analyses** table in Ion Reporter™ Software. Default columns are included for all analyses. You can add other available columns to customize the table.

Column	Description
Default columns—These columns are available by default when you open the Analyses table if no table preferences are applied.	
☐	Selection box—Use to select the analysis in the row that contains the selection box. Select one or more analyses to view analysis details, visualize analysis results, or perform other actions on the selected analysis or analyses.
✓ **(Checked Out)**	Checked out—Indicates that edits to the analysis are in progress. Open the analysis in view-only mode to view analysis results and see the name of the user who is making edits to the analysis.
👤	Analyze role—Indicates that the analysis is shared by a user who has the Analyze role.

(continued)

Column	Description
+ **(Add)**	Add notes—Use to add a new note to analysis. Analysis notes are time-stamped written notes to capture comments or observations for an analysis that has successfully completed. An analysis can contain multiple notes from different users.
(Note)	Add/Delete Notes—Use to add a note to analysis, or delete an analysis note.
Analysis	The name of the analysis.
Sample	The name of the sample.
Version	The Ion Reporter™ Software version number of the analysis workflow.
Reference	Indicates the human or other genomic or database reference used in the analysis.
Stage	The stage of the analysis.
Project	The name of the project. Can be inherited from Torrent Suite™ Software.
Workflow	The name of the analysis workflow used in the analysis.
Launched On	The date and time that the analysis was launched.
Status	The status of the analysis. • **Success** • **Aborted** • **Failed**
Available columns — These columns can be added to customize the Analyses table.	
Analyzed	Analysis is complete for the samples shown in the **Analyses** table. • **True** • **False**
Analyzed By	The user name of the person who analyzed the data.
Analyzed On	The date of the analysis.
Annotations	The variant annotations that are added.
Barcode	The name of the specific barcode in the selected barcode kit.
Biopsy Days	The time point post-fertilization at which the sample was taken from an embryo biopsy.
Cancer Type	The type of cancer in the sample.
Cell Num	The cell count of the biopsied material.
Chip ID	The identifying number assigned to the chip.

(continued)

Column	Description
Chip Type	The chip type that was used when preparing the sample, such as Ion 550™ Chip.
Control Sequencing Kit	The control kit that was used when preparing the sample.
Copy Number Baseline	The set of control samples that were used to create a baseline for detecting copy number variants (CNVs).
Couple ID	The identifier of the couple for use with the Reproductive research application.
Device ID	The identifying number of the sequencing instrument.
Embryo ID	The identifying number or name assigned to an embryo.
Filter Chain	The name of filter chain that was applied.
Fusion Panel	The name of fusion panel that was used.
Gender	Information about the gender of the sample.
Grouping Type	The grouping type. • **Single** • **Paired** • **Trio**
Hotspots Regions	The name of the hotspots file that was used in Torrent Suite™ Software.
Imported By	The name of the user who imported the sample.
Imported On	The date that the sample was imported.
Instrument	The name of the sequencer that was used.
Instrument Type	The type of the sequencer, such as Ion GeneStudio™ S5 Sequencer.
IR Uploader Version	The software version number of the IonReporterUploader plugin that was used in Torrent Suite™ Software.
Launched By	The name of the user who launched the analysis.
Library Kit Name	The name of the library kit that was used in the analysis.
Locked Workflow	Indicates whether the analysis workflow used in the analysis is locked. A locked analysis workflow cannot be edited or unlocked. However, it can be copied. • **True** • **False**
Mouse Strain	The strain of the mouse sample.
MyVariants Database	The name of the MyVariants database that was used.
Percentage Cellularity	The percentage of tumor cells in the sample.

(continued)

Column	Description
Plan Name	The name of the plan that was used in Torrent Suite™ Software.
Plugin	The name of the plugin or plugins used in the analysis.
Population	The ethnicity indicator for the sample.
Report Generated By	The name of the user who generated the report.
Report Generated On	The date that the report was generated.
Research Application	The research application that applies to the analysis, such as DNA, Aneuploidy, and so on.
Research Category	The research category that applies to the analysis, such as exome, reproductive, oncology, inherited disease, and so on.
Role	The role in a pair or a group of samples, such as mother, father, proband, or unknown.
Run Date	The date of the sequencing run.
Run ID	The identifying number of the sequencing run.
Run Name	The name of the run that was used in Torrent Suite™ Software.
Sample Collection Date	The date that the sample was collected.
Sample ID	*(Optional)* If you manage samples in an external system (for example, a LIMS), you can enter the identifier from that system in this column.
Sample Preparation Kit	The name of the sample preparation kit used in Torrent Suite™ Software.
Sample Receipt Date	The date that the sample was received.
Sample Transferred	An indication of whether the sample has been transferred to a user in another organization. • **Transferred**—The sample has been transferred to an organization. A sample can be transferred to more than one organization. Click **Transfer** to see when and to whom the sample was transferred. • **Available**—The sample is available to be transferred.
Sample Type	Information about the relationship between individual samples that are analyzed.
Sequencing Kit Name	The name of the sequencing kit that was used in Torrent Suite™ Software.
Sequencing Run Type	The type of the sequencing run that was used in Torrent Suite™ Software
Tagged for IRU	Indicates whether the analysis workflow used in the analysis is tagged for use with IonReporterUploader plugin. • **No** • **Yes**

(continued)

Column	Description
Target Regions	The name of the target regions file that was used in Torrent Suite™ Software
Templating Kit Name	The name of the templating kit that was sed in Torrent Suite™ Software.
Torrent Suite Hotspots File	The name of the hotspots file that was used in Torrent Suite™ Software.
Torrent Suite Region File	The name of the target regions file that was used in Torrent Suite™ Software.
Torrent Suite Results Name	The name of the Torrent Suite™ Software run report.
Torrent Suite Version	The version number of the Torrent Suite™ Software that was used for the analysis.
Variant Type Detection	The type of variant cells detected in the analysis. • **Germline** • **Rare Somatic** • **Somatic**
Workflow Revision	The number of the revision of the analysis workflow.

Find analyses

You can search for, sort, or filter, to find analyses in Ion Reporter™ Software.

Search for analyses

You can search the **Analyses** table for attributes that are shown in the **Analyses** table at the time of the search. To add more attributes, see “Add columns to the Analyses table” on page 157. You can also search for a word or phrase that is in an analysis note.

IMPORTANT! Search terms must adhere to the following rules.

- An asterisk (*) is not allowed in the search field for use as a wildcard.
- Searches are case-insensitive, that is, both upper-case and lower-cases letters are found regardless the case of search term letters.
- Searches find every occurrence of a continuous string. For example, a search for ***demo*** in analysis names returns a list of all analyses with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and therefore are not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

---

1. In the **Analyses** tab, click **Overview**.
2. In the **Search** box, enter a keyword or text string, then click **Go** to return a list of analyses that match the keyword.

   If the search string is invalid, the search box is outlined in red. Correct the search term to proceed.

The search results are returned in the **Analyses** table.

Sort the Analyses table

Many of the columns in the **Analyses** table can be sorted to make it easier to find the analysis that you are looking for in Ion Reporter™ Software.

1. In the **Analyses** tab, in the **Overview** screen, click a column heading to sort the table based on sample attributes.
2. Click the column heading again to reverse the order.

Filter analyses

You can use filters on the **Analyses** table to narrow search results, or shorten the list of analyses.

1. In the **Analyses** tab, click **Overview**.
2. *(Optional)* You can add more columns to the table, then filter on those columns.
   a. Click **Preferences** ▸ **Select Columns** to add more columns.
   b. Add user-defined sample attributes or predefined analyses attributes as filters:
      - Click **More Filters**, then select one or more applicable filter categories from the list.
      - Click **More Filters**, type in a search string into the **Find Filters** field, then select one or more applicable filter categories.
      - Click **More Filters**, then click **Select All** to select all filter categories.
3. Apply the filters:
   - Click the filter to expand the list, then select one or more specific filters. For example, select **Chip Type** to filter for sequencing data from an instrument that uses a specific type of sequencing chip.
   - Click the **Version** filter to view all analyses or select from the list to filter for specific versions.
   - Click the **Workflow** filter to view all analysis workflows or select from the list to filter for specific versions.
   - Click the filter to expand the list, then type search string in **Find Filters**. Select one or more specific filters. For example: select **530** to filter for sequencing data from an instrument that uses a Ion 560™ Chip.
   - Click the filter to expand the list, then click **Select All** to select all filters in a specific filter category.

   The contents of the **Analyses** table changes each time you select a filter or set of filters.

The filtered list of analyses is returned in the **Analyses** table.

Remove filters

You can remove filters from the **Analyses** table in Ion Reporter™ Software.

- Click ✕ **(Remove)** next to the filter list to remove the filter from the **Analyses** table.
- Click **More Filters**, then click the filter to remove the filter from the **Analyses** table.
- Click **More Filters**, then click **Clear** to remove all of the selected filter categories from the list.
- Click **Clear All** to remove all filters, then show all samples in the **Analyses** table.

Launch an analysis

An analysis is *launched* when samples are combined with an analysis workflow in Ion Reporter™ Software. Analysis results are generated when the analysis completes successfully.

You can select which samples to analyze and select the analysis workflow to apply, then launch the analysis to generate analysis results, or reanalyze a set of samples.

1. In the **Analyses** tab, click **Launch Analysis**, then select **Manual**.

   This step is the fastest way to get to the **Launch Analysis** screen. However, the screen can also be accessed from the **Analysis Results** screen and the **Workflows** tab.

2. In **Launch Analysis** screen, in the **Workflow** step, click the row of the analysis workflow to select it, then click **Next**.

3. *(Optional)* You can search, sort or filter the list of samples in the **Samples** step to find samples of interest. For more information, see “Search for samples” on page 58, “Filter samples” on page 59 and “Sort the Samples table” on page 58.

4. In the **Samples** step, select the samples.

Option	Description
Select single or multiple samples (for use with single-sample analysis workflows).	Click the row or rows of samples to select them. If you select multiple samples, a separate analysis is launched for each sample for single-sample analysis workflows.
Select tumor-normal samples (for use with tumor-normal analysis workflows).	a. Select a Normal and Tumor sample pair. b. Click **Add Samples**. c. Enter a **Relationship Name** for the sample pair. d. Click **Add to Analysis**.
Select a control sample for CNV analysis workflows. (for use with both tumor-normal and paired analysis workflows, which detect CNVs).	CNVs are reported based on their copy number relative to the control sample used. It is recommended to use a control sample confirmed to have no known CNVs in the regions covered by the Ion AmpliSeq™ panel. If most or all test samples report the presence of a CNV in the same region, one possible cause for the unexpected result is that the control sample does has a CNV in that region. a. Select a Sample and Control pair. b. Click **Add Samples**. c. Enter a **Relationship Name** for the sample pair. d. Click **Add to Analysis**.
Select trio samples (for use with trio analysis workflows).	a. Select the three samples for the trio (**Mother**, **Father**, **Child**). b. Click **Add Samples**. c. Enter a name for the sample trio. d. Click **Add to Analysis**.

5. Click **Next**.

6. *(Optional)* In the **Plugins** step, select a plugin, then click **Next**.

7. In the **Confirm & Launch** step, enter a name, and an optional description, then click **Launch Analysis**.

The analysis is launched. You receive an email notification when the analysis completes.

Demonstration samples

You can use the predefined analysis workflows with the demonstration samples that are included in Ion Reporter™ Software. You can use these samples to practice the steps to launch analyses and view analysis results.

Analysis workflow to use with the demonstration sample	Demonstration sample name	Detection type
Ion AmpliSeq™ CCP single sample	Demo Ion AmpliSeq™ CCP tumor	Somatic
Ion AmpliSeq™ CCP tumor-normal pair	Demo Ion AmpliSeq™ CCP normal and Demo Ion AmpliSeq™ CCP tumor	Somatic
Ion AmpliSeq™ CCP paired sample	Demo Ion AmpliSeq™ CCP CNV control and Demo Ion AmpliSeq™ CCP CNV case, or Demo AmpliSeq CCP normal and Demo Ion AmpliSeq™ CCP tumor	Somatic
Ion AmpliSeq™ Exome paired sample	Demo Ion AmpliSeq™ Exome CNV control and Demo Ion AmpliSeq™ Exome CNV case	Germline
Ion AmpliSeq™ Exome single sample (Germline)	Demo Ion AmpliSeq™ Exome CNV case	Germline
Ion AmpliSeq™ Exome single sample (Somatic)	Demo Ion AmpliSeq™ Exome CNV case	Somatic
Ion AmpliSeq™ IDP single sample	Demo Ion AmpliSeq™ IDP Daughter	Germline
Ion AmpliSeq™ IDP trio	Demo Ion AmpliSeq™ IDP Daughter, Demo Ion AmpliSeq™ IDP Father, and Demo Ion AmpliSeq™ IDP Mother	Germline
Ion AmpliSeq™ Colon Lung v2 with RNA Lung Fusion single sample	Demo Ion AmpliSeq™ RNA Lung Fusion	Somatic
Ion AmpliSeq™ RNA Lung Fusion single sample	Demo Ion AmpliSeq™ RNA Lung Fusion	Fusions and Expressions Control
Aneuploidy	Demo Aneuploidy	CNV only
Metagenomics 16S w1.1	Demo Metagenomics Mock Community	Bacterial Identification
Annotate variants single sample	Demo Ion AmpliSeq™ Exome VCF	Variant Annotation only

Manually launch an analysis

1. Launch the analysis.

From the…	Directions
Home tab	a. In the **Dashboard** screen, click **Launch analysis**. b. In the **Launch Analysis** screen, select a **Research Application**. c. In the **Research Category** list, select a row with an analysis workflow name. d. Click **Next**.
Analyses tab	a. Click **Launch Analysis**, then select **Manual** from the list. b. In the **Launch Analysis** screen, select a **Research Application**. c. Click in the analysis workflow row, then click **Next**.
Workflows tab	a. Select a **Research Application**. b. Click in the analysis workflow row, then in the **Details** pane select **Launch Analysis** from the **Actions** list.

2. Search by any unique identifier you used to label your samples during setup. Select one or more of the samples to include in the analysis, then click **Next**.

3. *(Optional)* Select plugins to run with your analysis.

   Note: The Oncomine™ Variant Annotator plugin is applied by default and cannot be edited or removed from the analysis workflow.

   a. Click ⚙ **(Actions)** adjacent to the selected plugin.

   b. Configure the plugin parameters as necessary, click **Submit**, then click **Close**.

4. Click **Next**.

5. Enter an **Analysis Name** and an optional **Description**, then click **Launch Analysis**.

The Ion Reporter™ Software performs the analysis and the results appear on the **Analyses** page.

Launch analyses by batch

You can launch multiple analyses by using a CSV file that you upload to Ion Reporter™ Software.

1. Prepare a CSV file to launch analyses by batch. For more information, see “Batch launch analyses CSV file guidelines” on page 169 .
   a. In the **Analyses** tab, click **Overview**, then click **Launch Analysis** ▸ **Batch**.

   b. Click **download example** to download the example CSV file.

   c. Open the example CSV file, then enter information for the analyses that you want to launch by batch in file.

d. Save the CSV file to a directory that you can access from Ion Reporter™ Software.

2. In the **Batch Launch Analyses** screen, click **Select file**, navigate to your CSV file, then click **Open**.

3. Ensure that the correct file name appears, then click **Upload**.
   The file is verified and the analyses are created. If the analyses are successfully created, the analyses are listed in the **Batch Launch Analyses** screen.

4. Click **Start Batch Analyses**.

The analyses that are launched by batch are added to the list in the **Analyses** screen.

Batch launch analyses CSV file guidelines

Use these guidelines to edit the example CSV file, or create your own CSV file, to launch analyses by batch.

IMPORTANT! The following column headings are required. Do not remove or edit this line in the CSV file. If you are creating a CSV file in plain text format, ensure that you include each of the commas as shown.

```
type, workflow name, version, IsIonDefault, samples, plugins
```

- Enter the attributes in the order that you want the columns to appear in the analysis. The order that the attributes are entered in the spreadsheet is the order that is used for columns in the analyses.
- For multiple samples, use a colon to add the role to each sample name. For example: `Demo AmpliSeq IDP Daughter:proband`

Ensure that you include the following contents in the batch analysis file.

Column name	Example	Required
Type Enter one of the following values. • `DNA_RNA_FUSION` • `multi` • `paired` • `paired_tumor_normal` • `single` • `SINGLE_RNA_FUSION` • `trio` To upload analyses with fusions, enter `DNA_RNA_FUSION` for DNA. If you use RNA-only samples, you enter ***SINGLE_RNA_FUSION***.	`single`	Yes
Workflow name	`AmpliSeq CHPv2 single sample`	Yes

(continued)

Column name	Example	Required
Version	`5.18`	Yes
IsIonDefault Enter `Yes`, if a predefined analysis workflow is provided, or `No` if a predefined analysis workflow is not included.	`Yes`	Yes
Samples	`Demo AmpliSeq CHPv2 tumor:`	Yes
Plugins	`RNASeqAnalysis_5.18.0.1-IR1;ampliSeqRNA_5.18.0.1-IR1`	No. If no plugins are used in the analysis, you can leave this field empty.

Add an analysis note

One or many analysis notes can be added to an analysis in Ion Reporter™ Software. An analysis note is any written comment or observation that you want to add to an analysis that has successfully completed. Anyone who is assigned Admin, Analyze, Import, or Report roles can add analysis notes.

You cannot edit analysis notes. However, you can add additional analysis notes to an analysis. You can also add a description to an analysis. For more information, see “Launch an analysis” on page 165.

Analyses notes can be shared within the same organization. Analysis notes are not shared when an analysis is shared with another Ion Reporter™ Software organization.

1. In the **Analyses** tab, select **Overview**.
2. In the row of an analysis of interest, click + **(Add)** to add a new note, or **(Note)** to add a note to the list of existing notes.
3. In the **Analysis Notes** dialog box, click the **Notes** tab and enter a note in the text field.
4. Click **Add**, then click **Done**.

The analysis note appears in the **Details** pane of a selected analysis on the **Analyses Overview** screen.

View an analysis note

You can view the analysis notes that are included in Ion Reporter™ Software. Analysis notes are time-stamped written notes to capture comments or observations for an analysis that has successfully completed. An analysis can contain multiple notes from different users.

You cannot edit analysis notes. However, you can add additional notes to an analysis. You can also add a description to an analysis. For more information, see “Launch an analysis” on page 165. Analyses notes can be shared within the same organization. Analysis notes are not shared when an analysis is shared with another Ion Reporter™ Software organization.

1. In the **Analyses** tab, select **Overview**.
2. To review analysis notes, on the **Analyses Overview** screen:
   - Click **(Note)** in the row for an analysis of interest.
   - Select the row of an analysis of interest, and then hover the mouse over the **Notes:** link in the **Details** pane of a selected analysis.

Search for an analysis note

You can search for the text in a specific analysis note or notes in Ion Reporter™ Software.

Analysis notes record any written comments or observations for an analysis that has successfully completed.

In the **Analyses** tab, click **Overview**.

Option	Description
To find one or more analysis notes that contain a specific word or phrase	In the **Search** field, enter a word or phrase that is used in the analysis note, then click **Go**.
To find all analysis notes	*(Optional)* In the **Search** field, enter ***Note***, then click **Go**.

Delete an analysis note

You can delete an analysis note in Ion Reporter™ Software.

Analysis notes record any written comments or observations for an analysis that has successfully completed.

1. In the **Analyses** tab, select **Overview**.
2. In the row of an analysis of interest, click **(Note)**.
3. In the **Analysis Notes** dialog box, select the note that you want to delete, then click **(Delete)**.
4. Click **Done**.

MyVariants

You can label variants as relevant to your research in Ion Reporter™ Software, and either track significant variants or ignore insignificant variants, such as known false positives. You can then share the information for these variants with other researchers through a TSV file or PDF report that you create in Ion Reporter™ Software. MyVariants complements variant annotations that are already applied by the various annotation sources in Ion Reporter™ Software.

When you apply the MyVariants flags **Important** or **Ignore** to the variants that are included in analysis results from predefined analysis workflows, the variants are automatically added to the default MyVariants database for the organization in Ion Reporter™ Software. This database is named **MyVariantsDb_hg19** and all users in the organization can apply it to their analysis results.

You can also apply notes and classifications to MyVariants. For more information, see “Classifications and Notes” on page 174.

Each analysis workflow can be associated with only one MyVariants database.

Custom MyVariants databases can be created for specific research projects or individual researchers. For example, you can create a separate MyVariants database for breast cancer research and another for lung cancer research. The same variants can have different flags and classifications in the different research areas.

MyVariants table

You can view MyVariants when you open the **Analyses** tab, then click **MyVariants**. The **MyVariants** table includes specific information such as genome location and mutation type. The following columns of information are available in the **MyVariants** table.

Information type	Description
(Variant Flag)	MyVariants flag options: • **Important**—To identify variants that are relevant to the research. • **Ignore**—To denote known false positives or variants that are not associated with the research area. • **None**—To remove a variant marked as **Important** or **Ignore** from the MyVariants database. This column can be edited.
(MyVariants Notes)	Information that researchers add to the MyVariants in note format. This column can be edited.
Locus	The location of the variant on a specific chromosome. This column can be sorted.
Classification	Classifications that can be set for MyVariants. For more information, see “Apply flags, notes, or classifications to variants of interest” on page 175. This column can be edited.

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Information type	Description
Gene(s)	The gene or genes where the variant is found. This column can be sorted.
Genotype	The genotype sequence (ATCG). This column can be sorted.
Ref	The reference sequence. This column can be sorted.
Type	The type of variant or call, such as SNV, CNV, Fusion, or REF. This column can be sorted.
Analysis Name	The name of analysis where the variant was identified.
Sample Name	The name of sample where the variant was identified.
Observed Allele	The observed allele variation. This column can be sorted.
Copy Number	The copy number variation. This column can be sorted.
Subtype	The copy number subtype. This column can be sorted.
Variant ID	The identifier of the variant. This column can be sorted.

MyVariants database

A MyVariants database is a repository of identified variants that is shared by an entire Ion Reporter™ Software organization, a specific research project, or an individual researcher. When you label the variants that are included in analysis results from predefined analysis workflows as **Important** or **Ignore**, the variants are automatically added to the default MyVariants database for the organization in Ion Reporter™ Software. This database is named **MyVariantsDb_hg19**. You can also create a different MyVariants database for a specific research project and then select this MyVariants database when you create an analysis workflow.

Other ways to use MyVariants databases include:

- Export MyVariants between different MyVariants databases by using a TSV file. For more information, see “Export MyVariants database” on page 180 and “Import MyVariants” on page 180 .
- Use a MyVariants database to create various filter chains to narrow analysis results. For more information, see “MyVariants filter” on page 469.
- Apply the latest MyVariants information to older analysis results. For more information, see “Get the latest updates from the MyVariants database” on page 178.
- Create a MyVariants report in PDF format. For more information, see “Create a PDF report of MyVariants” on page 181.

Classifications and Notes

Variants that use the **Important** or **Ignore** flags are often variants that can also be categorized with **Classifications** and include other information that can be captured in **Notes**. Ion Reporter™ Software includes an optional setting to add **Classifications** and **Notes** that were used in a previous occurrence of a variant automatically to subsequent occurrence of the same variant. Variants in a MyVariants database that include the setting, when reported in a new set of analysis results, automatically add the **Classifications** and **Notes**. The analysis workflow that is used for the analysis must be associated with a MyVariants database.

For example, in a MyVariants database, you can set a variant to always be flagged as **Important**, classified as **Benign**, and include an informational note.

MyVariants **Flags**, **Classifications**, and **Notes** are all stored in the MyVariants database. The preference to add variant **Classifications** and **Notes** automatically is disabled by default. For more information, see “Automatically add classifications and notes to variants” on page 175.

IMPORTANT! The latest **Classifications** and **Notes** are added to a variant and the MyVariants database when this setting is enabled. If you change the **Classifications** and **Notes** in the future, the variants for subsequent analyses reflect those changes.

Automatically add classifications and notes to variants

You can set a preference to add notes and classifications automatically to MyVariants in Ion Reporter™ Software. Use the setting to add **Classifications** and **Notes** that were used in a previous occurrence of a variant automatically to subsequent occurrence of the same variant. Variants in a MyVariants database that include the setting, when reported in a new set of analysis results, automatically add the **Classifications** and **Notes** to the variant. The analysis workflow that is used for the analysis must be associated with a MyVariants database.

1. In the **Analyses** tab, in the **MyVariants** screen, click **Preferences**.
2. In the **Preferences** dialog box, select one or both options to be added to variants.

Option	Description
Notes	Information for the variant that is entered by a user. The last note added to that same variant is added automatically to new instances of that same variant in a new analysis.
Classifications	A category that users can add to a variant. Classification options include: **Unclassified**, **Pathogenic**, **Likely Pathogenic**, **VUS**, **Uncertain Significance**, **Likely Benign**, **Benign**, **Unknown**, **Suspected Benign**, **Suspected Deleterious**, **Deleterious**, **Technical Artifact**, **Oncogenic**, and **Likely Oncogenic**. By default, classifications for each variant are blank until a value is selected.

Apply flags, notes, or classifications to variants of interest

You can contribute to the MyVariants databases for the organization by applying flags to variants in analysis results in Ion Reporter™ Software. It is recommended that you first apply classifications and notes before selecting the MyVariants flags.

1. Click the **Analyses** tab to open the list of analyses, then click an analysis of interest.
   The **Analysis Results** table opens.
2. (*Optional*) In the **(Note)** column, click **(Add)** , click the **Notes** tab, then enter a note for the variant.
3. When you are finished, click **Add Note**.
4. (*Optional*) In the **Classification** column, you can select a classification for each variant of interest:
   - **Unclassified**
   - **Pathogenic**
   - **Likely Pathogenic**
   - **VUS**
   - **Uncertain Significance**
   - **Likely Benign**
   - **Benign**
   - **Unknown**
   - **Suspected Benign**

- **Suspected Deleterious**
- **Deleterious**
- **Technical Artifact**
- **Oncogenic**
- **Likely Oncogenic**

5. In the **(Flag)** column, expand **(Flag list)**, then select the appropriate flag.

Option	Description
Important	The flag icon becomes orange in the row of the variant and the variant is added to the MyVariants database. **Important** is used to track variants that are relevant to the research.
Ignore	The flag icon becomes gray in the row of the variant and the variant is added to the MyVariants database. **Ignore** is often used to indicate known false positives or false negatives, or variants that are not associated with the research area.
None	All variants are marked **None** by default. Select **None** to remove an **Important** or Ignore flag and to remove a variant from the MyVariants database.

6. To check that the variants were added to the MyVariants database, select the **Analyses** tab, then click **MyVariants**. The latest additions appear at the top of the table.

Variants that are labeled as **Important** or **Ignore** are added to the default MyVariants database, **MyVariantsDb_hg19**, if the analyses were run through predefined analysis workflows. To add variants to a different MyVariants database, create or select a custom MyVariants database in the **Annotation** step when you create, copy, or edit an analysis workflow.

Search or filter the MyVariants table

You can search and filter the **MyVariants** table to help you find a variant of interest. For example, you can filter the table by locus, gene, sample name, and other variant characteristics.

In the **Analyses** tab, click **MyVariants**, then perform the following actions in the **MyVariants** table.

To...	Do this...
Search the list	In the **Search** box, enter a search term, then click **Go**. If the search string is invalid, the search box is outlined in red. The following rules apply to all searches: • An asterisk (*) is not allowed in the search field. • Searches are not case-sensitive. • Searches match the search string in any location in the target list. For example, a search on "demo" in analysis workflow names matches analysis workflows with "demo" anywhere in their name.
Filter the list by category	Click the filter category to expand the list, then select a specific filter.
Filter the list by date of analysis	• Click **(Calendar)** next to **From :** and **To :** above the **MyVariants** table, select the date range from the calendar menu, or enter the dates in `mm/dd/yyyy` format, then click **Go**. • To remove the date range filter, delete the **From :** and **To :** entries.

Sort MyVariants

You can sort the MyVariants list to make it easier to find the MyVariants that you are looking for in Ion Reporter™ Software.

1. In the **Analyses** tab, click **MyVariants**.
2. Click a column heading to sort the list based on the column category type (numerical, alphabetical, date).
3. Click the column heading again to reverse the order.

Edit a MyVariants database

You can edit a MyVariants database in Ion Reporter™ Software.

IMPORTANT! Edits to MyVariants can affect the MyVariants settings that are made by other members of the organization if you are sharing a MyVariants database.

You can change the **Important** or **Ignore** flags that are associated with variants in a MyVariants database.

If you delete an analysis that includes a variant that is labeled as **Important** or **Ignore**, the MyVariants database is unaffected and the variant flags, notes, and classifications remain.

You can also add notes to variants in a MyVariants database.

1. In the **Analyses** tab, click **MyVariants**.
2. In the **MyVariants** table, select a specific MyVariants database from the list.
3. Review the flags, notes and classifications for each variant, then update each option, if needed.
   a. In the **(Flag)** column, expand **(Flag list)**, then select the appropriate flag.

Option	Description
Important	The flag icon becomes orange in the row of the variant and the variant is added to the MyVariants database. **Important** is used to track variants that are relevant to the research.
Ignore	The flag icon becomes gray in the row of the variant and the variant is added to the MyVariants database. **Ignore** is often used to indicate known false positives or false negatives, or variants that are not associated with the research area.
None	All variants are marked **None** by default. Select **None** to remove an **Important** or **Ignore** flag and to remove a variant from the MyVariants database.

   b. In the **(Note)** column, click **(Add)** , click the **Notes** tab, then enter a note for the variant. When done, click **Add Note**

c. In the **Classification** column, you can select from the following classifications for each variant of interest.
   - **Unclassified**
   - **Unknown**
   - **Benign**
   - **Suspected Benign**
   - **Likely Benign**
   - **Deleterious**
   - **Suspected Deleterious**
   - **Pathogenic**
   - **Likely Pathogenic**
   - **VUS** (Variant of Unknown Significance)
   - **Uncertain Significance**
   - **Technical Artifact**

Get the latest updates from the MyVariants database

You can get the latest additions, deletions, or changes to the MyVariants database for an organization by synchronizing the analysis results to the MyVariants database in Ion Reporter™ Software. For example, you can open older analysis results and apply the updates for any MyVariants that were added, deleted, or changed, to the older analysis results.

IMPORTANT! MyVariants flags are updated only when an analysis is successfully completed or when **MyVariants** is clicked. In previous versions of the software, the MyVariants flags are updated automatically when analysis results are opened.

---

1. In the **Analyses** tab, click **Overview**.
2. Click the link of a new or existing analysis to open the **Analysis Results**.
3. Click **MyVariants** to download flags from the MyVariants database into the analysis results.
4. *(Optional)* Manually edit **Important** or **Ignore** flags within the analysis results.

View the variants in a MyVariants database

You can view a list of variants that are contained in a specific MyVariants database in Ion Reporter™ Software. When you view variants, you can also add notes, synch the MyVariants database, generate a report, and select variants and perform batch actions.

1. In the **Analyses** tab, click **MyVariants**.
2. In the database list, select the MyVariants database of interest.
3. View the list.

View a MyVariants database associated with an analysis workflow

You can determine which MyVariants database is selected for an analysis workflow in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Overview**.
2. Click the row for the analysis workflow of interest.
3. In the **Details** pane, look for **MyVariants Database**.

Note: If MyVariants Database is not in the **Details** pane, then no MyVariants database was selected during analysis workflow creation. To change the MyVariants database, select a different MyVariants database in the **Annotation** step of analysis workflow creation or revision. For more information, see “Associate a MyVariants database with an analysis workflow” on page 180.

Create a MyVariants database

You can create a MyVariants database when you create an analysis workflow. A MyVariants database can be used research projects for various reasons. For example, separate databases can be useful for projects with interpretations of genomic variants that appear to conflict. If a breast cancer study includes specific gene mutations that are suspected to cause tumors, and a lung cancer study includes the same mutations that are not suspected to cause tumors, two MyVariants databases can be used.

1. In the **Workflows** tab, click **Create**.
2. In the **Research Application** step, select the research application and sample group, then click **Next**.
3. In the **Reference** step, select the required files, then click **Next**.
4. In the **Annotation** step, under **MyVariants Database**, click **Create here**.
   a. In the **Create MyVariant Database** dialog box, enter a database name, then click **Save**. The newly created database is now available for selection in the Annotation step.
   b. Select the new MyVariants database, then click **Next**.
5. In the **Filters** step, select a filter chain from the dropdown list, then click **Next**. .
6. In the **Copy Number** step, select a copy number baseline from the dropdown list, then click **Next**.
7. In the **Plugins** step, select plugins, then click **Next**.
8. In the **Final Report** step, select the final report template in the list, then click **Next**.
9. In the **Parameters** step, select parameters, then click **Next**.
10. In the **Confirm** step, name the analysis workflow, enter an optional description, then click **Save Workflow**.

To check that the analysis workflow was created, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name.

Associate a MyVariants database with an analysis workflow

You can apply MyVariants that are included in a MyVariants database to the analysis results in Ion Reporter™ Software if you select a MyVariants database when you create an analysis workflow. MyVariants allows you to track variants that are important to your research and to ignore other variants that are not relevant to your research.

Each analysis workflow can be associated with only one MyVariants database in the **Annotation** workflow step. To check that the MyVariants database was associated with an analysis workflow, see "View a MyVariants database associated with an analysis workflow" on page 179.

Export MyVariants database

You can export a MyVariants database to share it with another user, project, or organization in Ion Reporter™ Software.

1. In the **Analyses** tab, click **MyVariants**.
2. Click **MyVariants**, then select **Show All**, **Important**, or **Ignore**.
3. Click **Export TSV**.
   A compressed file appears.
4. When the download is complete, a compressed directory that is named `myvariants_results.zip` is downloaded in the browser. Save the directory to a hard drive.
5. Extract the MyVariants directory.
   The TSV file contains the information for each variant, such as Locus, Gene, Type, Flag Type, and Analysis Name. You can also view additional details for each variant such as the list of analyses in which the variant was called.

   Note: Sorting on the **Gene(s)** column factors for each gene in a multigene result. Therefore, the order of the genes in one row is by chromosomal order, not alphabetical order.

Import MyVariants

You can import MyVariants from a VCF or a TSV file that is exported from another MyVariants database, or from a TSV file that you create. For more information, see "Export MyVariants database" on page 180.

1. In the **Analyses** tab, click **MyVariants**, then click **Import MyVariants**.
2. In the **Upload MyVariants File** screen, click **Select file** , then browse to the VCF or TSV file.
3. Click **Submit**.

When the import is complete, a message indicates that the file has been uploaded and lists the number of records that were uploaded, and the number of records that are valid and invalid.

Create a PDF report of MyVariants

You can create a PDF report of MyVariants in Ion Reporter™ Software.

1. In the **Analysis** tab, click **Overview**.
2. Click an analysis link to open the **Analysis Results** screen.
3. Select the MyVariants that you want to include in the report.
   MyVariants are denoted by flags in the **Analysis Results** screen. You can also use filter chains to filter the analysis results for only flagged variants. For more information, see “MyVariants filter” on page 469 .
4. Click **Generate Report**. If a report template is associated with the analysis workflow, use the **Select Final Report Template** dialog box to:
   - Select a template, then click **OK.**
   - Click **Create Report Template** to create a new template. For more information, see “Create a final report template from analysis results” on page 407.
5. Enter required information into the sections. For more information, see “Available report sections” on page 421.
6. (*Optional*) Scroll to the **Reported Variants** section, then click **Select Columns**. Rearrange the columns, if needed, then click **Next**.
   A preview of the PDF report opens. Click **Previous** to go back and change the report if needed.
7. When you are satisfied with the report sections, click **Lock and Publish**, then review the PDF. If edits are needed click **Cancel**, then make edits.
8. Click **Publish** to create the final report.
9. Click **Download** to download the published report.

Delete an analysis

IMPORTANT! Ensure that you want to delete the analysis or analyses before you start this procedure. When an analysis is deleted, the action cannot be undone.

If you have Analyze role privileges, you can delete one or more analyses from Ion Reporter™ Software. You can use this procedure to free up storage space or to remove obsolete or erroneous analyses from being used again.

1. In the **Analyses** tab, select the checkbox to select the analysis (or analyses) in the table that you want to delete, then in the **Details** pane click **Actions** ▸ **Delete**.
2. Click **Yes** in the dialog box to confirm that you want to complete the deletion.

The **Analyses** table refreshes and no longer includes the deleted analysis. To delete an archived analysis, you must first restore it and then delete it. For more information, see “Restore archived analyses and samples” on page 517.

Copy analyses to storage on Connect

You can copy VCF files from successful Ion Reporter™ Software analyses to storage on Connect to store and share analyses with other users. You can also upload analyses to use with other software applications that are available on Connect. For more information, see “Increase storage space on Connect” on page 182.

You need to have a Connect account to use this procedure.

1. In the **Analyses** tab, click **Overview**.
2. Select the row that contains the successful analysis that you want to copy to storage on Connect.
3. In the **Details** pane, click **Actions** ▸ **Copy to DataConnect**.
4. In the **Export to Data Manager** dialog box, click **Confirm**.
   A message at the top of the **Analyses** screen indicates that the files have been copied to storage on Connect.
5. To view the files, sign in to Connect, then click **View my files** ▸ **Personal Files**.

Increase storage space on Connect

You can buy extra storage space as a user of Ion Reporter™ Software on Connect. The administrator of the Ion Reporter™ Software organization can assign the storage subscription to the organization. When a subscription is assigned to an organization, all members of the organization have access to the storage space. For more information, see Connect Help.

Monitor analyses

There are two ways to monitor analyses in Ion Reporter™ Software.

- In the **Analyses** tab, click **Overview**, then review the **Status** column.
  The analysis is reported as **Pending**, **% complete**, **Successful** , or **Failed**.
- You can also check for email notifications. Ion Reporter™ Software sends an email notification when the analysis is complete, and indicates whether the analysis was successful or failed.

Reanalyze aborted analysis results

You can reanalyze results from any failed or aborted analysis that uses an analysis workflow that is supported in the version of Ion Reporter™ Software that you use. To view details about an analysis workflow, see “View analysis workflow details” on page 70.

1. In the **Analyses** tab, select an analysis of interest that has a status of **Aborted**.
2. Click **Actions** ▸ **Reanalyze**.

Share data from an analysis

Ion Reporter™ Software provides a simple mechanism to share analysis data with anyone. They do not have to be users of Ion Reporter™ Software.

By default, all data in your organization is visible only to users within your organization. You can share data with users from other organizations, even though they do not have access to all of your data. Users whose permissions include the Analysis role can also share analysis reports.

When data from an analysis is shared:

- The people with whom you share data or analysis reports receive an email notification with links to download the variant information (filtered and unfiltered) for DNA datasets and species quantification data for metagenomic datasets. Registered users of Ion Reporter™ Software can view the shared analysis in their organization.
- You are notified about the status of the share in your Ion Reporter™ Software **Home** page. If you share data with registered Ion Reporter™ Software users, they also receive notifications.
- You are sent an email notification to confirm that the share is complete. If you shared the data with multiple users at the same time, you receive just one email for the entire share.
- When the share is complete, this icon → appears beside the report that contains shared data on the **Analyses Overview** page for you and the recipients of the shared data. When a share is in progress, you see this icon: ⟳.
- The status in the messages that are shown at the top of the **Analyses** page are as follows:

Status	Description
Sharing/Unsharing	A share is in progress.
Shared	The share is successful.
Failed	The share failed. The notification includes the reason for the failed action. The **Analyses Overview** page does not show the latest status. It shows failed status if any shares for that analysis has failed.

Share analyses data

You can share analysis data from Ion Reporter™ Software with anyone, whether or not they are users of the software.

Variants cannot be flagged when an analysis report has been shared by a user in another organization. The flag column on the **Analysis** tab and the option to flag multiple variants are not available for shared analysis reports.

When a share is in progress, the analysis and the samples associated with it cannot be edited, archived, or deleted.

1. In the **Analyses** tab, select the analysis that contains the data that you want to share, then in the analysis **Details** click **Actions** ▸ **Share** .
2. In the **Share Analysis** dialog box, enter the email address of the person with whom you want to share data, then click **Share**.
   A message about the status of the share opens briefly. When a share is in progress, you see this icon: . You can continue with other tasks in Ion Reporter™ Software while the share is in progress. When the sharing is complete, you and the recipients of the shared data see this icon beside the report that contains shared data: .
3. Click the **Home** tab, then click **Notifications** to view notifications about the share.

Unshare analyses data

When you unshare analyses data, the links to the data contained in the email notifications are inactivated. After you unshare the data, subsequent emails notifications with information about the unshare action are sent to both you and your destination users. For Ion Reporter™ Software users, the copied analysis is removed from their organization.

IMPORTANT! Unsharing analyses data cannot be undone.

1. Click the **Analyses** tab.
2. Select the analysis that you want to unshare, then in the **Details** pane click **Actions** ▸ **Share**.
   This icon appears next to analyses that are shared in the **Analyses** list: .
3. To unshare the dataset, click the trash can icon next to the user or users.
4. In the **Unshare** column, click **Confirm** to complete the unshare.

Send an analysis to the Report role

You can send an analysis to someone in the organization who has the Report role in Ion Reporter™ Software. A person with the Report role can then generate a report.

1. In the **Analyses** tab, select **Overview**.
2. Click an analysis link to open the **Analysis Results** screen.

3. Click **Actions** ▸ **Send to Report Role**.

   Note: The **Send to Report Role** button activates the same function.

4. In the **Send to Report Role** window, click **Send**.

The analysis results are sent to the person in the organization who has the Report role. The person with the Report role can find the results on the **Analyses Overview** screen. The results are at the **Pending Report** stage.

Download BAM files

You must have an Ion Reporter™ Software account on an Ion Reporter™ Server to complete this procedure. You cannot download BAM files from Ion Reporter™ Software on Connect.

IMPORTANT! If you have data from BAM files that you intend to store long term, you can archive the data. For more information, see "Archive analyses to an external storage device" on page 515.

You can download a BAM file that is generated by Ion Reporter™ Software to review the file, or open the file and inspect the data with a visualization tool outside of the software. The BAM index file (BAI) is included in the download.

The software gives you the option to download the BAM files, if one or more BAM files are available for an analysis. The following BAM files are available in Ion Reporter™ Software for completed analyses:

- If only RNA samples are analyzed, a mapped BAM is available in for download.
- For DNA samples, two BAM files can be available for download: a mapped BAM and a processed BAM.

A mapped BAM is a file that maps the BAM file that is uploaded to Ion Reporter™ Software to the reference genome and based on selected alignment parameters. The processed BAM file, that is available only for DNA samples, is the BAM file that contains the reads processed by Variant Caller module in Ion Reporter™ Software, where read trimming and read filtering can be applied.

Note: The processed BAM file is much smaller than the mapped BAM file. For DNA samples, download the processed BAM to a computer for review to save time and computer hard drive disk space.

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Select one of the following options:
   - Click **Actions** ▸ **Download Processed BAM** to download the BAM file that was processed by the Variant Caller in Ion Reporter™ Software.
   - Select **Actions** ▸ **Download Mapped BAM** download the BAM file that is mapped to the reference genome, based on selected alignment parameters.
3. To download the BAM file from Ion Reporter™ Software:
   a. Click the **Home** tab, then click **Notifications**.

b. Find the download file in the list. You can click **Downloads** to narrow the list or see the timestamp.

c. Click **(Download)**.

A file that used the naming convention `analysisName_bamFileType_bam.zip` is downloaded. The file contains the BAM files and the associated BAI file. The mapped BAM file is named ***merged.bam*** and the processed BAM is named ***merged.bam.ptrim.bam***. An unmapped BAM file is named the same as the file name that was used to define the samples used in the analysis launch.

Download analysis files

You can download files for analyses in Ion Reporter™ Software that do not include variants. Examples are Metagenomics analysis results, Microbiome analysis results and immune repertoire analyses.

1. In the **Analyses** tab, click **Overview**.
2. Select an analysis to download, then in the **Details** section, click **Actions** ▸ **Download Results**.
   A notice may appear that states that the request is in progress and that downloaded files are available on the **Notifications** screen.
3. To download the file from Ion Reporter™ Software:
   a. Click the **Home** tab, then click **Notifications**.
   b. Locate the download notification in the list. You can click **Downloads** to narrow the list, or refer to the timestamp.
   c. Select the analysis notification that you want to download, then click **(Download)**.
4. Extract the contents of the ZIP file to access the folders and files.
   A ZIP file named `analysis name_All.zip` is downloaded. If the Ion Reporter™ Software analysis includes notes, the notes are included in the VCF file contained in this directory.
   Depending on the analysis you selected, the ZIP file may contain the following:

Folder	Files
`Plugin outputs`	Contains files related to the plugins used, including: CSV, FASTA, JSON, PNG, and HTML files.
`Results`	Contains files related to the analysis. The folder may include TXT, FASTA, PDF, CSV, or PNG files.
`QC`	Contains files related to Quality Control metrics that are run for the analysis. • <bam_file_basename>.ionstats_alignment.json • analysis.bfmask.stats • explog_final.txt • raw_peak_signal • InitLog.txt

(continued)

Folder	Files
QC	The basecaller_results folder contains the following JSON files: • BaseCaller.json • datasets_basecaller.json • ionstats_tf.json • TFStats.json
Workflow_ Settings	• Analysis_Settings—Folder that contains a text file of the settings used for the analysis workflow. • Module_Configuration_Files—Folder that contains tertiary analysis files, including: INI and PLN (plan) files.
RNACountsActor-00	driver_WT_assays_Level.txt A tab-delimited text file with 4-columns: • column1: Gene name (only driver genes, not partners) • column2: The type of assay used to compute the RNA levels (This could be either RNAExonTile or RNAExonVar) • column3: A number which is the sum of read-counts from the WT amplicons of the gene (for example, all of the RNAExonTile) divided by the number of WT amplicons for the gene • column4: A number which is the proportion of the ratio in column3 from the Total Valid RNA Mapped reads

Download detailed analysis logs

You can download logs that contain details for each analysis in Ion Reporter™ Software. You can share the detailed analysis results, analyze them in another software application, or provide the results to Technical Support for troubleshooting.

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Click **Actions** ▸ **Download Logs**.
   A status message prompts you to find the compressed directory, available for download from the **Notifications** screen.
3. Click the link for the notification in the status message, or click **Home** ▸ **Notifications** to view messages.
4. Find the analyses on the **Notifications** screen, then click **(Download)**.
   The analyses results directory is downloaded to the browser or a file folder, depending on the browser settings.

5. Extract the contents of the directory to access the folders and files.

Folder or file name	Description
`Analysis`	This folder contains details about the algorithmic pipeline that was used in the analysis workflow and details about the analysis. The following files and folders are included: • analysis.log—Contains output from the logs console about the analysis workflow modules executed for the analysis. • analysis.status—Contains detailed information about the execution of an analysis. The details include status, percent completion, time taken for each analysis workflow module to complete, version of the analysis workflow module. • AnalysisData.json—Contains information about the analysis workflow configuration that is required for the analysis. • analysisSamples.json—Contains information about sample or specimen details that are required for the analysis. • log folder—Contains logs for analysis workflow module-level information. • .ini files folder—Contains parameter information for each execution of an analysis workflow module that is required for the analysis. • summary.log—Start and end time for each time an analysis workflow module is executed for the analysis. • analysis stderr.log—When an analysis failure has occurred, this log contains critical analysis errors that occurred for the analysis. • analysis primary-failure.log—When an analysis failure has occurred, this log contains specific errors that occurred for each execution of the analysis workflow modules for the analysis.
`Summary`	This file contains details about the analysis process and the start and finish time for each analysis workflow module.

6 Analysis results

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In Ion Reporter™ Software, the variants identified after an analysis workflow has been launched and completed are summarized in **Analysis Results**. You can view analysis results as tabular data in multiple formats, or visually in graphs, interactive charts, plots, and histograms. Detailed QC metrics, audit logs, and several types of reports generated from the analysis results are available.

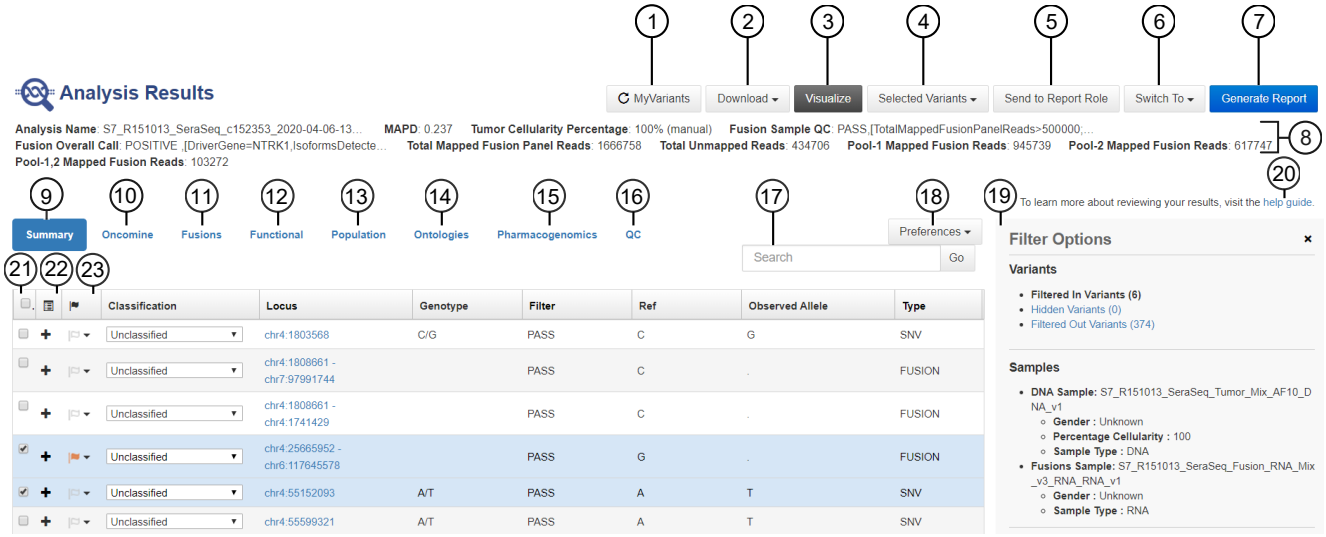
Customization of the view and layout of the tables and the application of filters and column rearrangements allows you to view a table of analysis results that contains only the data that is of interest to you. You can sort, view, and flag relevant variants, track significant variants, and ignore insignificant variants, such as known false positives. Variants from all analyses that are designated as **Important** are listed in the **My Variants** database.

Classifications, such as **Benign** or **Likely Pathogenic**, can be added to variants that are in analysis results so that you can organize, group, and track variants. You can customize variant classifications and store those classifications.

Visualization tools in the software include time series graph creation, comparisons between multiple analyses displayed in tables and graphs, such as heat maps, the Ion Reporter™ Genomic Viewer (IRGV), and the Integrative Genomics Viewer (IGV).

Analysis Results table overview

The **Analysis Results** table lists the annotated variant results from an analysis. The views that are available for an analysis result depend on the workflow used in the analysis. This example shows the **Analysis Results** view for a sample analyzed with an Oncomine™ cancer panel and assay. For more information, see "Display different views of analysis results" on page 193.



① **MyVariants** updates the customized MyVariants database.

② **Download** allows you to download variant files. Files that you can download:

- Compressed file that contains data files including a VCF file with all variants.
- Compressed file that contains data files including a VCF file with filtered-in variants.
- TSV file of the current results shown.
- Compressed file that contains data files including a VCF file with only selected variants.

For more information, see "Variants file downloads" on page 208.

③ **Visualize** allows you to view the data in Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV). For more information, see Chapter 8, "Visualize analysis results with Ion Reporter™ Software".

④ Options in the **Selected Variants** dropdown menu:

- Add or remove variants from the **MyVariants** database.
- Order a TaqMan™ assay for SNP genotyping the variant directly from **thermofisher.com**. Only assays specific for the selected locus are displayed.
- Classify variants. For more information, see "Classify variants in analysis results" on page 194.
- Order capillary electrophoresis primers specific for the selected variant or variants directly from **thermofisher.com**. Only primers specific for the selected locus are displayed.
- Hide the selected variants. For more information, see "Hide or Unhide variants in Analysis Results list" on page 194.

The **Selected Variants** dropdown menu is only enabled when at least one variant is selected.

⑤ **Send to Report Role** allows you to send an analysis to someone in the organization who has the **Report** role in Ion Reporter™ Software. A person with the **Report** role can then generate a report. For more information, see "Send an analysis to the Report role" on page 184.

⑥ **Switch to** options:

- **Audit log** opens the audit log. For more information, see "Audit logs" on page 427.
- **QC Report** opens the quality control report. For more information, see "Quality Control (QC) reports" on page 417.
- **Coverage Report** opens the **Amplicon Coverage Report**. For more information, see "Amplicon coverage reports" on page 415.

⑦ **Generate Report** allows you to create a final report. For more information, see "Final reports" on page 400.

⑧ Analysis details, including the analysis name, are listed. You can hover over the listed parameters and values for definitions and to show values if they are cropped due to space limits. The details that are shown depend on the workflow used.

⑨ The **Summary** provides a summary of the analysis results.

⑩ **Oncomine™** provides Oncomine™ variant class annotations.

⑪ **Fusions** provides a fusions view of the analysis results. For more information, see "Display different views of analysis results" on page 193.

⑫ **Functional** provides a functional view of the analysis results. For more information, see "Display different views of analysis results" on page 193.

⑬ **Population** provides a population view of the analysis results. For more information, see "Display different views of analysis results" on page 193.

⑭ **Ontologies** provides an ontologies view of the analysis results. For more information, see "Display different views of analysis results" on page 193.

⑮ **Pharmacogenomics** provides a pharmacogenomics view of the analysis results. For more information, see "Display different views of analysis results" on page 193.

⑯ **QC** provides a quality control view of the analysis results. For more information, see "Display different views of analysis results" on page 193.

⑰ The **Search** box allows you to enter keywords or text strings to limit the analyses listed.

⑱ Table preferences. For more information, see "Apply an Analysis Results table preference" on page 206.

⑲ **Filter Options**:

- Change to view to show only **Filtered In Variants**, **Hidden Variants**, or **Filtered out Variants**. For more information, see "Apply a filter chain to analysis results" on page 195.
- Show variants for a single chromosome. For more information, see "Show variants for a single chromosome" on page 195.
- Apply a filter chain and save the filter chain so that when you reopen the analysis again, the newly saved filter chain is applied. For more information, see "Apply a filter chain to analysis results" on page 195.

⑳ The **help guide** link opens the software help menu.

㉑ **(Checkbox)**—Used to select variants.

㉒ **(Variant Details)**—Used to view a variant detail card.

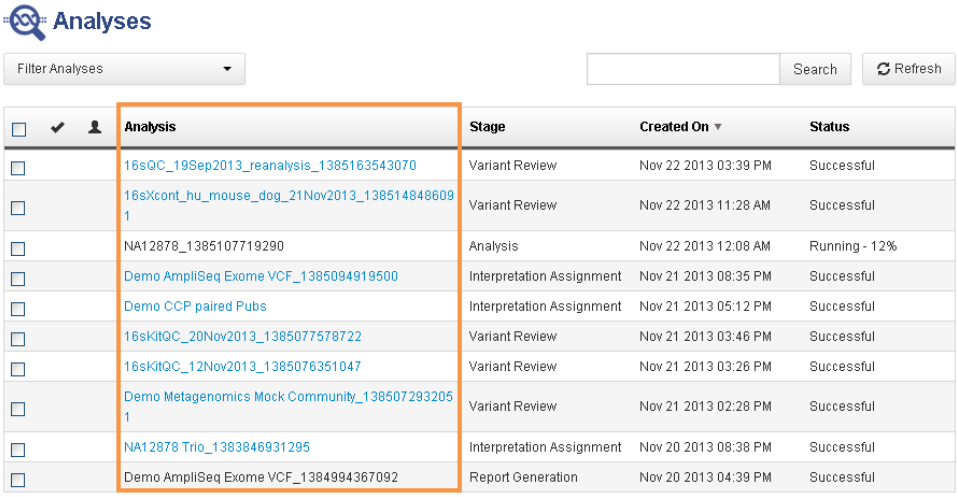
㉓ **(Flag)**—Used to flag and select variants for the MyVariants database. For more information, see "Apply flags, notes, or classifications to variants of interest" on page 175.

Open and review analysis results

To open analysis results:

1. In the **Analysis** tab, click **Overview**.
2. In the **Analyses** table, click the name of the analysis for which you want to view analysis results.

   Analyses that are available to view have an active link. You cannot view analyses that are still running.



   The **Analysis Results** table opens to show a list of variants and detailed information about the variants. You can customize the view of the analysis results. For more information, see “Customize the Analysis Results table” on page 204 “Edit Analysis Results” on page 207.

   The **Allele Coverage** column reports counts for all reported alleles, not only genotype alleles. In contrast, the **Allele Read Count** filter sets the minimum count of the genotype alleles.

3. Click an option above the list of variants to show a different view of variants, annotations and other analysis results data, such as the following for results from analysis workflows that use a **Research Application** of DNA.

   - Summary
   - Functional
   - Population
   - Ontologies
   - Pharmacogenomics
   - Somatic
   - QC

   For more information about the data contained in the **Analysis Results** table, see Chapter 7, “Detailed analysis metrics”.

Display different views of analysis results

You can see several different views of analysis results in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click an analysis name to open the **Analysis Results** table.
3. Select from the following views.

Option	Description
Summary	Provides a summary of the analysis results. Includes columns for Classification, Genotype, Locus, Filter, Reference, Observed Allele, Type, No Call Reason, Genes, Location, Length, Info, Variant ID, Variant Name, and Allele Frequency % information.
LOD	Provides analysis results for analyses that use Ion AmpliSeq™ HD or TagSeq analysis workflows.
Oncomine	Provides analysis results for analyses that use the Oncomine™ or Ion AmpliSeq™ HD analysis workflows.
Functional	Provides a functional view of the analysis results. Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, Strand, Exon, Transcript, Coding, Amino Acid Change, Variant Effect, and PhyloP information.
Population	Provides a population view of the analysis results. Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, dbSNP, DGV, MAF, EMAF, AMAF, GMAF, and UCSC Common SNPs. If the analysis includes the ExAC annotation source, the following columns are included: ExAC LAF, ExAC EAAF, ExAC OAF, ExAC EFAF, ExAC SAAF, ExAC ENFAF, ExAC AAF, and ExAC GAF information. If the analysis uses a custom gnomAD annotation source, the following columns are included: gnomAD AAF, gnomAD AJAF, gnomAD EAAF, gnomAD EFAF, gnomAD FEM, gnomAD FNFAF, gnomAD GAF, gnomAD LAF, gnomAD MAF, gnomAD OAF, gnomAD SAAF information.
Ontologies	Provides an ontologies view of the analysis results. Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, COSMIC, OMIM, Gene Ontology, and DRA information.
Pharmacogenomics	Provides a pharmacogenomics view of the analysis results. Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, DrugBank, and ClinVar information.
Somatic	Provides a somatic view of the analysis results. Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, Length, Amino Acid Change, Allele Coverage, Allele Ratio, Allele Frequency %, and Information.
QC	Provides a QC view of the analysis results,

Option	Description
	Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, p-value, Phred QUAL Score, Raw Coverage, Coverage, Allele Coverage, Allele Ratio, Ref+/Ref-/Var+/Var-, Homopolymer Length, and Subset of information.

Hide or Unhide variants in Analysis Results list

You can hide or unhide variants that are listed in analysis results in Ion Reporter™ Software. It is useful to hide variants if you do not want to include them in visualizations of the analysis results, in downloads of the results, or in reports that you generate from the results.

1. Click the **Analyses** tab to open the list of analyses, then click an analysis of interest.
   The **Analysis Results** table opens.

2. To hide variants, select the variants that you want to hide.

3. Click **Selected Variants** ▸ **Hide**.
   The selected variants are removed from the **Analysis Results** screen and are listed in the count of **Hidden Variants** in the **Filter Options** pane.

4. To unhide variants, click **Hidden Variants** in the **Filter Options** pane, then click **Selected Variants** ▸ **Unhide**.

Hidden variants are not visible on the **Analysis Results**, and are not included in the results files when you download variants, or in visualized results.

Classify variants in analysis results

You can add a classification to one or more of the variants that are in analysis results in Ion Reporter™ Software. Classifications can be added to individual variants, or to a list of selected variants. When a variant is classified, the classification for the variant can be viewed in analysis results that include the variant. Variant classifications are also added to final reports that are published from the analysis.

1. Click the **Analyses** tab to open the list of analyses, then click an analysis of interest.
   The **Analysis Results** table opens.

2. Classify variants individually, or apply the same classification to multiple variants.

   - To classify variants individually, in the Classification column, use the dropdown menu to select one of the following classifications for each variant of interest: Unclassified, Unknown, Benign, Suspected Benign, Likely Benign, Deleterious, Suspected Deleterious, Pathogenic, Likely Pathogenic, VUS, Uncertain Significance, Technical Artifact.
   - To classify multiple variants with the same classification, select the variants to which you want to add the classification, then click **Selected variants** ▸ **Classify variants**. Then, select a classification from the dropdown list and click **Confirm**.

Apply a filter chain to analysis results

You can apply a filter chain to the analysis results to further refine the view to only variants of interest in Ion Reporter™ Software. Filter chains are preinstalled or user-defined sets of one or more filters that remove variants from analysis results. You can apply only one filter chain at a time. For more information, see Chapter 10, “Filters and filter chains”.

When you apply a filter chain to analysis results, the list of variants is filtered in real-time. You need to save the filter chain to have the filter chain immediately change the heat maps, other visualizations, and exported results of your variants of interest. If you do not save the filter chain on the **Analysis Results**, then the heat maps and other multiple analysis visualizations show the entire results.

1. In the **Analyses** tab, click **Overview**.
2. Select the link to the analysis of interest.
3. In the **Analysis Results** screen, in the **Filter Options** pane, navigate to the **Filter Chains** list, then select a filter chain to apply it.
   The filtered variants results change.
4. In the **Filter Options** pane, review the **Variants** counts.
   - **Filtered In Variants**—The number of variants that are included in the final report, if you publish the report at this point.
   - **Hidden Variants**—The number of variants that are hidden.
   - **Filtered Out Variants**—The number of variants that are removed from the final report, if you publish the report at this point, because of filter chain settings.
5. *(Optional)* To save these changes to the analysis results, click **Save Filter Chain**.
   Saving the filter chain ensures that when reopening the same analysis, the newly saved filter chain is applied to the analysis results.

Show variants for a single chromosome

You can refine analysis results to show only those variants that are called on a single chromosome in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. In the **Analyses** screen, select the row of your analysis of interest.
3. In the **Analysis Results** screen, in the **Filter Options** pane, navigate to the **Chromosome** list, then select the chromosome of interest.

The number of **Filtered In Variants** reflects only the variants on the selected chromosome. Because variants called on other chromosomes are considered to be "not displayed" instead of "filtered out", their variant totals are not added to the **Filtered Out Variants** for the analysis results.

View notes attached to a variant

Variants with notes attached display in the **Variant Details** column.

1. In the **Analysis** tab, click **Overview**.
2. Click an analysis name to open the **Analysis Results** screen.
3. In the **Analysis Results** screen, click in the **Variant Details** column for the variant of interest.
4. View the notes in the **Variant Details** screen.

Add flag labels to variants of interest

You can add labels (flags) to variants of interest to add them to the default MyVariants database.

Variants that have **Important** or **Ignore** flags are automatically added to the default MyVariants database, **MyVariantsDb_hg19**, if the analyses were run through predefined analysis workflows. To add variants to a different MyVariants database, create or select a custom MyVariants database in the **Annotation** step when you create, copy, or edit an analysis workflow.

1. Click the **Analyses** tab to open the list of analyses, then click an analysis of interest.
   The **Analysis Results** table opens.
2. In the **(Flag)** column, expand **(Flag list)**, then select the appropriate flag.

Option	Description
Important	The flag icon becomes orange in the row of the variant and the variant is added to the MyVariants database. **Important** is used to track variants that are relevant to the research.
Ignore	The flag icon becomes gray in the row of the variant and the variant is added to the MyVariants database. **Ignore** is often used to denote known false positives or false negatives, or variants that are not associated with the research area.
None	All variants are marked **None** by default. Select **None** to remove an **Important** or Ignore flag and to remove a variant from the MyVariants database.

Add a note to a variant

1. In the **Analysis** tab, click **Overview**.
2. Click an analysis name to open the **Analysis Results** screen.
3. In the **Analysis Results** screen, click + or in the **Variant Details** column (second from the leftmost column).

Note:

- The + icon indicates that the variant does not have any notes that are associated with that variant.
- The icon indicates that the variant already has a note that is associated with that variant.

4. In the **Variant Details** screen, click **Notes**.
   A appears.

5. In a new **Variant Details** screen, in **Note text**, enter the note.

6. Click **Add Note**.
   - You are returned to the **Analysis Results** screen.
   - If the variant previously did not have a note that is attached, the icon changes from + to .

Locus View versus Allele View of variants

When you set workflow parameters, you can set the view that is used for variants in analysis results with the **Variant View** parameter. You can view variants per position with **Locus View**, or variants per allele with **Allele View**. For more information about how to set this view, see "Customize tuning parameters" on page 94 and "Variant Finding parameters" on page 113. The VCF files for both the **Locus View** and **Allele View** include one line for each variant row that is displayed in the Ion Reporter™ Software **Analysis Results** table.

The FDP (Flow Evaluator read depth at the locus) and DP (Total read depth at the locus) are subfields inside the INFO field of the VCF file. The values of these two fields remain the same for both the **Locus View** VCF file and the **Allele View** VCF file. These two fields, FDP and DP, are based on the TOTAL SUM of ALL the FAO (Flow Evaluator Alternate allele observations) and AO (Alternate allele observations) fields, which are based on the following formulas:

FDP=SUM(FAO)+FRO or DP=SUM(AO)+RO

Note: In Ion Reporter™ Software, the **Allele View** VCF file includes values of the FDP and DP fields that remain the same, although each allele is displayed as a single line of record in the **Allele View** VCF file.

Locus View

Locus View is the default variant view that appears in analysis results in Ion Reporter™ Software, which is a locus-centric view. In **Locus View**, there can be multiple alternate alleles for a given locus in each line or row.

Allele View

Allele View is an allele-centric view that displays each allele as a single row in the Ion Reporter™ Software and in a separate line in the VCF file output.

Allele subset information

In Ion Reporter™ Software, a data column labeled **Subset** can be used to determine whether alleles are present at a variant locus in either analysis results that are viewed as either **Allele View** or **Locus View**.

In **Locus View**, the following applies:

The subset column indicates that an allele, which is part of a larger variant that has been called, is present (the superset). The format is subset index (superset index). The larger variant can be found by using the value of the superset index counting into the alleles listed in the **Observed Allele** column. The subset ID can be a hotspot ID or a number. If it is a number and not a hotspot ID then it is also an index count of the alleles listed in the **Observed Allele** column. The subset allele always will match one of

alleles without a genotype. A special case is that two superset indices might be present, in which case both indices are displayed and are separated by a forward slash (/).

In **Allele View**, the following applies:

The subset column indicates that an allele, which is part of a larger variant that has been called, is present (the superset). The format is "SupersetID" which will be a name for the superset variant. A special case is that two superset indices might be present, in which case both indices are displayed and are separated by a forward slash (/).

Evaluate analysis results

You can evaluate analysis results in Ion Reporter™ Software by comparing multiple analyses. For example, you might compare new analysis results to known accurate analysis results.

Note: Comparison and visualization are not supported for Metagenomics analyses, or for analyses with a status of **Failed** or **In-Progress**. Tumor-normal analyses, which has only one set of variants for the tumor sample, and does not report variants for the normal sample, also cannot be visualized.

Visualization of a single trio or paired analysis is supported, because trio and paired analyses each contain more than one set of variants.

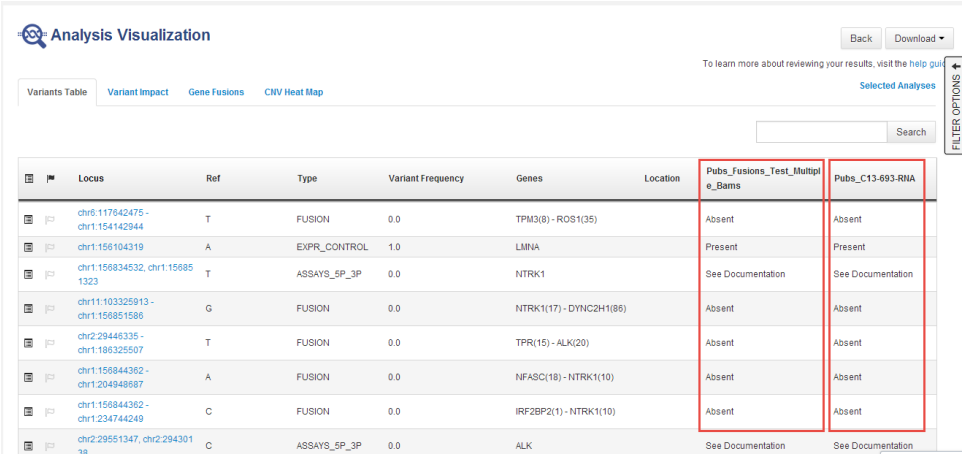
1. In the **Analyses** tab, click **Overview**.
2. Select multiple analyses, then click **Actions** ▸ **Visualize**.

You can review the results in various ways. For more information, see “Compare results of single or multiple analyses” on page 198.

Compare results of single or multiple analyses

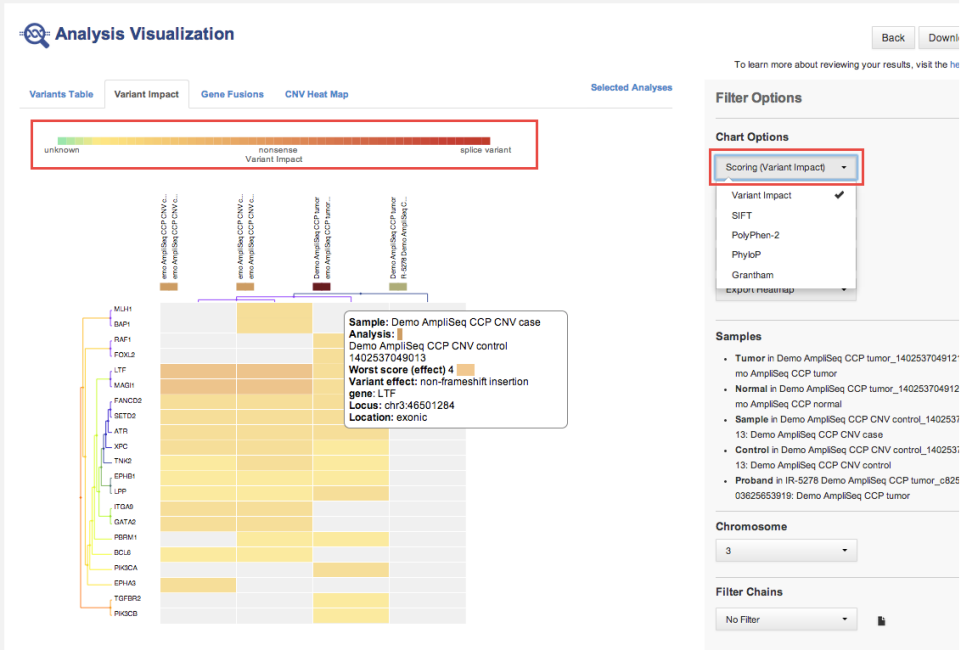
You can review results for single analyses, or compare the results of multiple analyses in the **Analysis Visualization** screen. Visualization for multiple analyses supports the following views, depending on analysis workflow type.

- In the **Variants Table** tab, the table view shows side-by-side columns to compare variant calls in different analyses:

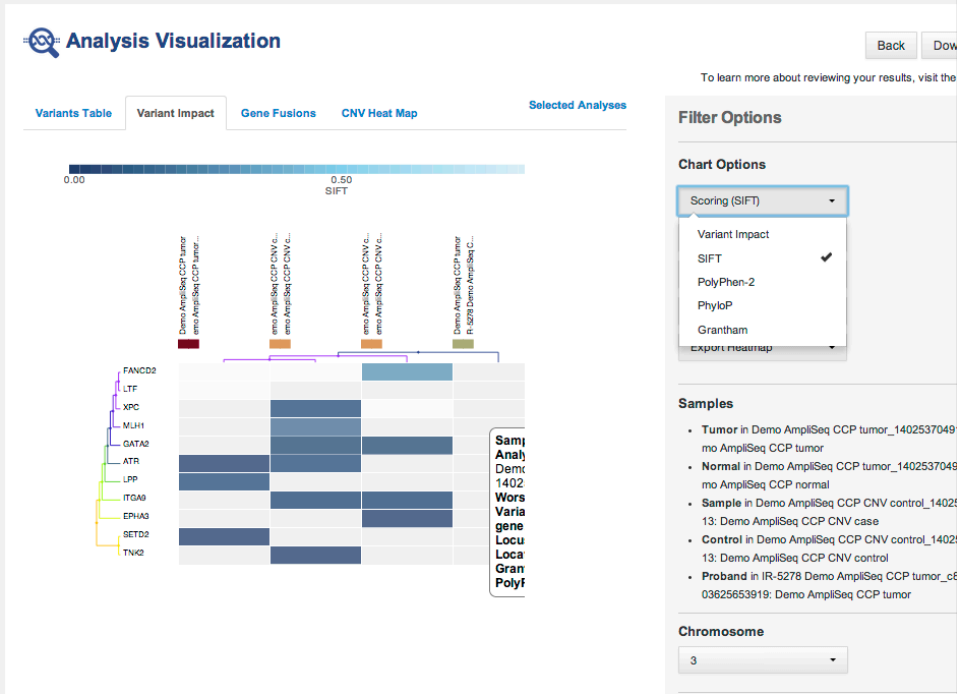


- In the **Variant Impact** tab, the variant impact heat map that displays is based on the predicted variant impact:

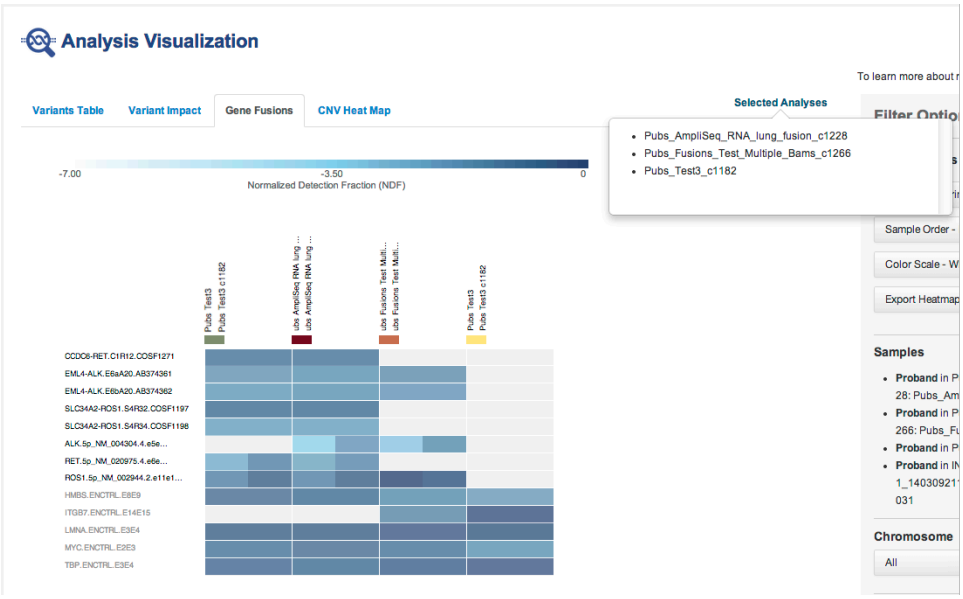
  The following is a variant impact heat map that is based on the predicted variant impact.



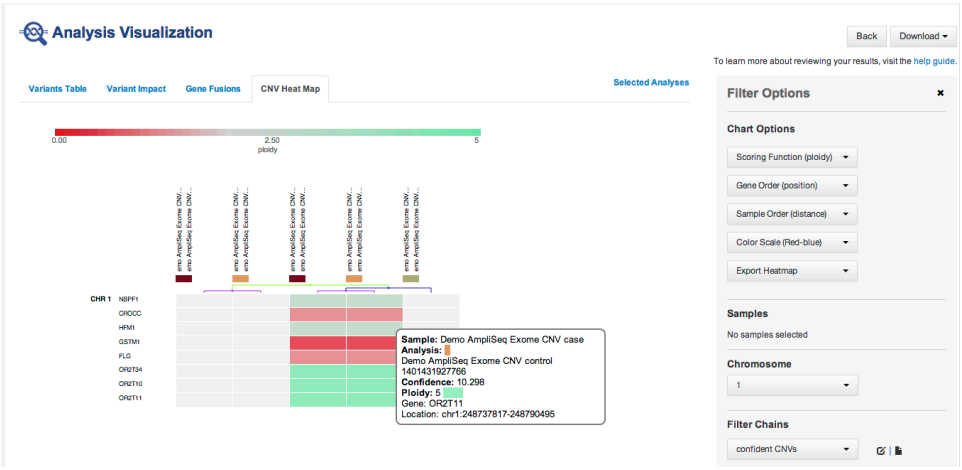
  The following is a variant impact heat map that is based on other scoring.



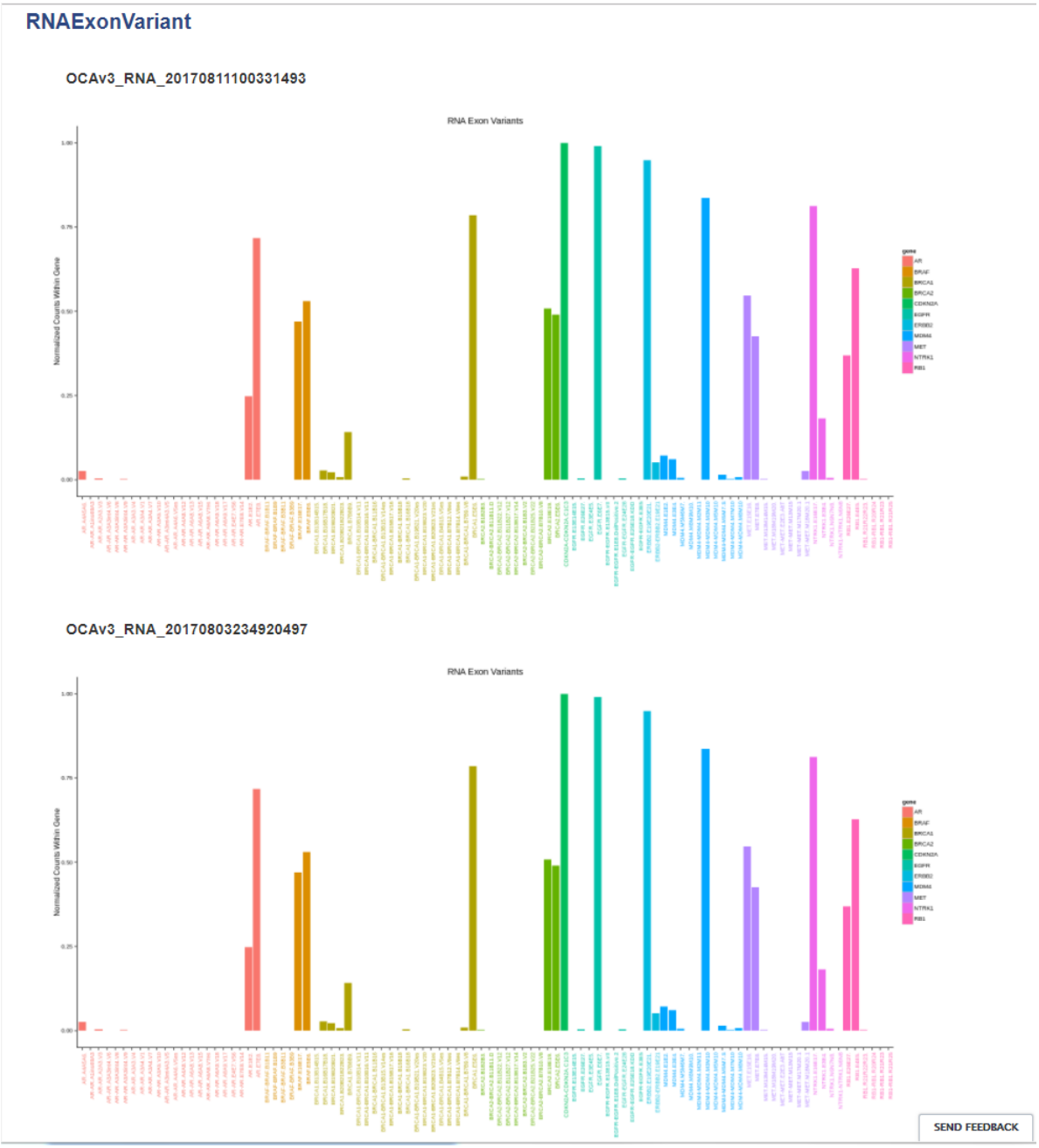
  The following is a gene fusions heat map.



The following is a CNV heat map.

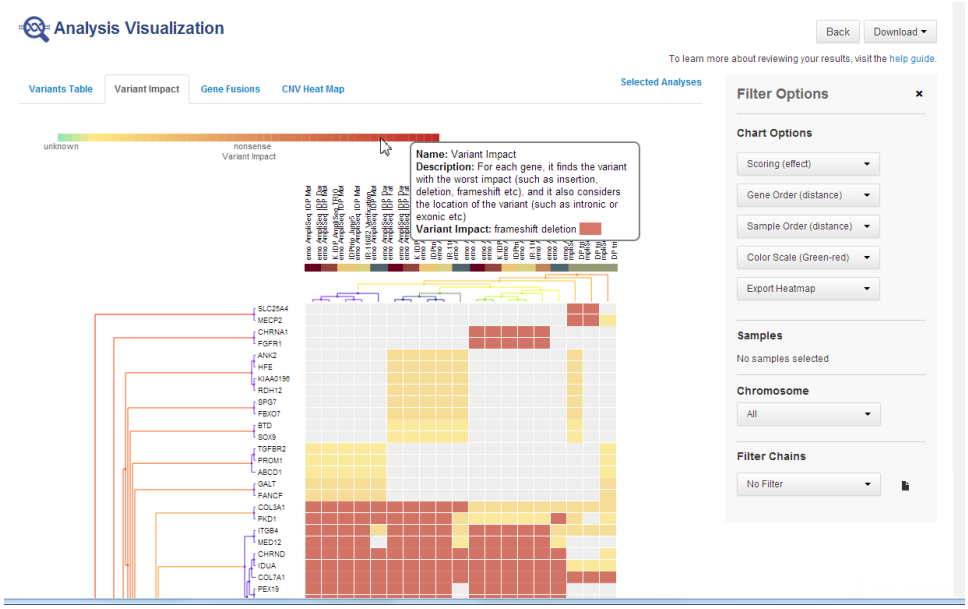


- RNA Exon Variants:

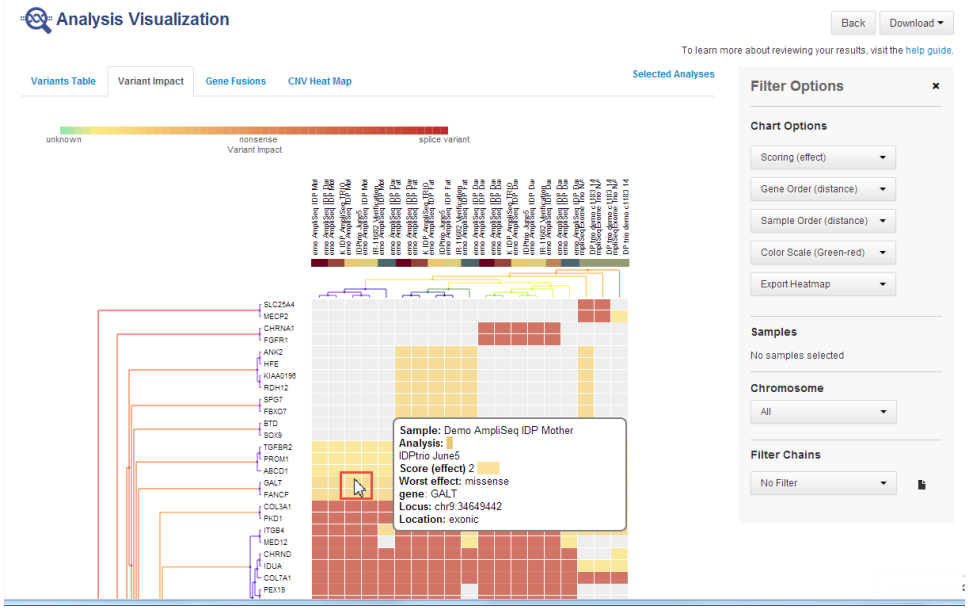


Note:

- In most cases, similar analyses are compared. However, Ion Reporter™ Software does not restrict comparisons to only similar analyses.
- You can compare one trio or one paired analysis without selecting multiple analyses. Each trio or paired analysis contains more than one set of variants.
- Place the pointer over the legend of a heat map:



Hover over the legend of a cell to see more information:



View original source of annotation information

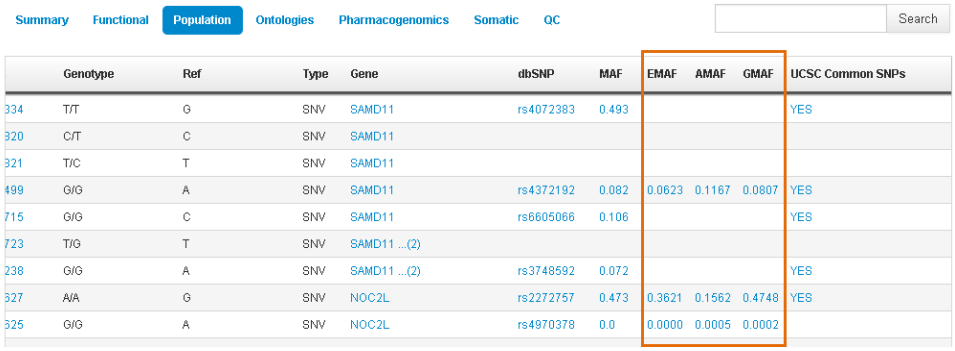
You can view the original source of annotation information in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click the name of the analysis to open the **Analysis Results** table.
3. In the **Genes** column, click the link.
   The link opens to the HUGO Gene Nomenclature Committee (HGNC) website.

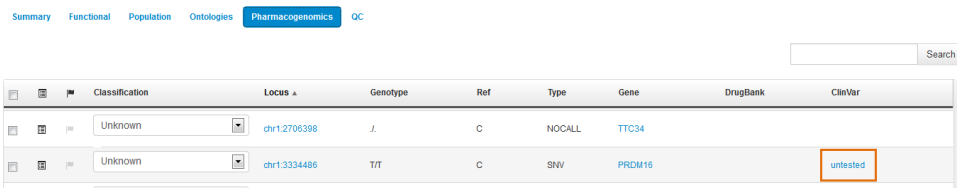
Links to external databases

In the following cases, the links to external databases that are included in the **Analysis Results** screen can lead to database pages that are not specific, contain an error, or are blank.

- The 5000 Exomes database link in Ion Reporter™ Software uses gene information. Because the 5000 Exomes site does not provide a unique identifier for each record, if the website does not have specific information about the gene of a variant, the link in Ion Reporter™ Software leads to a blank page. In this case, try to search for the rsID of the variant on the 5000 Exomes site. (Not all 5000 Exomes records contain rsIDs.) This image shows example 5000 Exome links in the Population tab of the variant table:



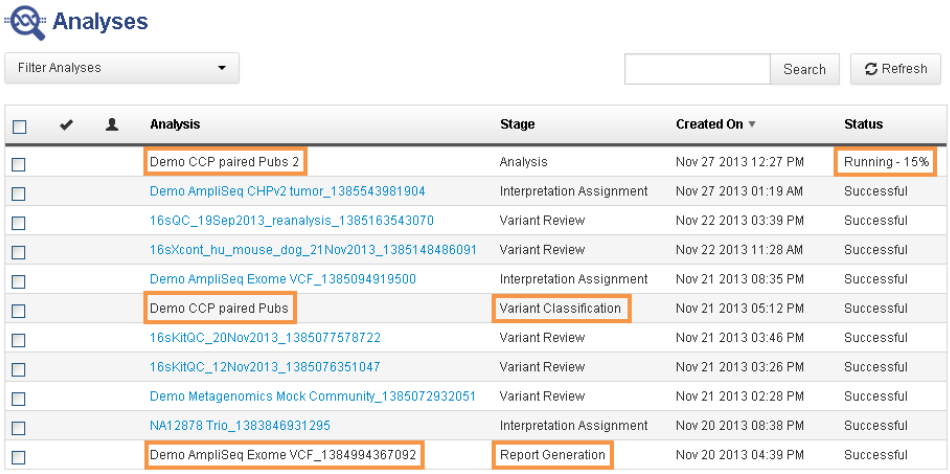
- **When a ClinVar annotation does not have a CLNACC ID associated with it, the annotation string is "untested" and the link leads to a generic ClinVar screen instead of to the specific variant page.**



- Some COSMIC records' link-outs may no longer be displayed on the COSMIC website. In such cases, the link-out leads to a generic COSMIC page that displays "no entry found".

Inactive links in the Analyses screen

If an analysis name in the **Analyses** screen is in plain text, it does not provide a link to open the analysis.



The link to an analysis name can be inactive for the following reasons:

- The analysis is still running. The analysis can be edited when the processing is complete.
- The next stage is **Variant Classification**, and you do not have the required Report role.
- The final report is published for the analysis, and cannot be edited. In this case, the stage is **Report Generation**.

Customize the Analysis Results table

The **Analysis Results** table includes details that describe the results of the analysis, samples, analysis workflows, and software versions that were used to create the analyses in Ion Reporter™ Software.

When you open an analysis, the information that is shown in the **Analysis Results** table depends on which workflow and annotation set is applied when the analysis is launched. You can customize the table to reflect the information that is most relevant to your research. To do so, create a table preference, or drag and drop the columns to change the order in which they appear.

Analysis Results table column options

When you open the **Analysis Results** table in Ion Reporter™ Software, a default set of columns is included. The table columns are based on various factors, including the samples that are used, analysis and workflow name, and other analysis attributes, and the workflow and annotation set that is applied when the analysis is launched.

Add attribute columns to the Analysis Results table

You can add columns to the **Analysis Results** table that you view in Ion Reporter™ Software.

Note: In the **Summary** tab, you can add any of the available columns from any subtab in the **Analysis results** table. You can move or deselect existing columns in the other tabs, such as the Oncomine tab, Functional tab, Population tab, Ontologies tab, Pharmacogenetics tab, and QC tab, but cannot add more columns to these tabs.

1. In the **Analyses** tab, click **Overview**.
2. Click the link for an analysis of interest to open the **Analysis Results** table.
3. Click **Preferences** ▸ **Select Columns**, then select the columns that you want to add to the **Analysis Results** table, or deselect the columns that you want to remove from the table.

The selected columns are added to the **Analysis Results** table until you close the table. To save the table with the attributes that you added, see “Create an Analysis Results table preference” on page 205.

Change the order and width of columns in the Analysis Results table

You can change the order and width of columns in the **Analysis Results** table in Ion Reporter™ Software.

1. In the **Analyses** tab, select **Overview**.
2. Click the link for an analysis of interest to open the **Analysis Results** table.
3. Click any column heading that you want to move, then drag and drop each column heading to the position that you prefer. Change the width of each column, as desired.

   The column order and width remains until you close the screen, or can optionally be saved as a table preference.
4. (Optional) To save the order and width of the columns as a table preference:
   a. Click **Preferences** ▸ **Save Table Preference As**.
   b. Enter a name for the table preference, then click **Save**.

   The **Analysis Results** table preference is saved.

Create an Analysis Results table preference

You can create a table preference for the **Analysis Results** table in Ion Reporter™ Software. The table preference enables you to view only the columns of data that are relevant to your research.

The user-defined table preference setting is included as output when you download a variants file.

1. In the **Analyses** tab, click **Overview**.
2. Click the link for an analysis of interest to open the **Analysis Results** table.

3. Click **Preferences** ▸ **Select Columns**, then select the analysis attributes that you want to add to the **Analysis Results** table, or deselect the analysis attributes that you want to remove from the table.
   The selected columns are added on the right side of the table.

4. To save the table as a preference:

Option	Description
Preferences ▸ **Save Table Preference As**.	This setting enables you to name the table preference. The preference setting is then selectable from **Preferences** ▸ **Table Preferences**.
Preferences ▸ **Save Table Preference**	This setting becomes the standard view for the user account. To restore default table columns, click **Preferences** ▸ **Restore Defaults**.

The table preference remains until you change it by clicking **Restore Defaults** or by applying another table preference.

Apply an Analysis Results table preference

You can apply an **Analysis Results** table preference to change the way that you view the information in an **Analysis Results** table. Table preferences allow you to view information that is relevant to your research in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.

2. Click the link for an analysis of interest to open the **Analysis Results** table.

3. Click **Preferences** ▸ **Table Preferences**.

4. Select any available **Table Preference** that you want to apply to the table.
   The view in the **Analysis Results** table changes that are based on the selected table preference.

5. *(Optional)* Create a new **Analysis Results** table preference based on the table preference that you selected.
   a. Click **Preferences** ▸ **Select Columns**, then select any **Available Columns** that you want to add, then click **Apply**.
   b. Click the column header that you want to move, then drag, then drop it to the position you prefer.
   c. Click **Preferences** ▸ **Save Table Preference As**.
   d. Enter a name for the table preference in the **Save Table Preference** dialog box, then click **Save**.

   The new **Analysis Results** table preference is saved.

Delete an Analysis Results table preference

You can delete a user-defined table preference setting for the **Analysis Results** table in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click the link for an analysis of interest to open the **Analysis Results** table.
3. Click **Preferences** ▸ **Table Preferences**, then select the user-defined table preference that you want to delete.
4. Click **Preferences** ▸ **Delete Table Preferences**.
5. In the **Confirm Delete** dialog box, click **Yes** to confirm the deletion.

The user-defined table preference is removed from the **Table Preferences** list.

Restore default settings for the Analysis Results table

You can restore default column headings in the **Analysis Results** table Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click the link for an analysis of interest to open the **Analysis Results** table.
3. Click **Preferences** ▸ **Restore Defaults**.

The default table is restored.

Edit Analysis Results

You can edit analysis results to capture, save, and share the most relevant interpretations of analysis results. You can then generate reports, download files, and share analyses that include the edited results. Available options to edit analysis results depend on the user role. See “User roles and permissions” on page 25 for more information. You cannot edit an analysis when the **Status** is **running** or **Report Generated**.

1. In the **Analyses** tab, click **Overview**.
2. There are 2 ways to edit analysis results in Ion Reporter™ Software:
   - In the **Analyses** table, click the name of the analysis to edit.
   - Select the checkbox next to the analysis of interest, then click **Edit**.

   The **Analysis Results** table opens in edit mode.

3. Edit the analysis results.

Note: You can customize the view of the analysis results to show only the variant information that is most relevant to you. For more information, see the following sections:

- "Customize the Analysis Results table" on page 204
- "Display different views of analysis results" on page 193
- "Apply an Analysis Results table preference" on page 206

Option	More information
Refine and save the list of variants that is displayed. Apply then save the applied filter chain to ensure that the newly saved filter chain persists when you reopen the analysis results.	"Apply a filter chain to analysis results" on page 195
Interpret variant results, then categorize the variants with user-defined- or system-installed classifications.	"Classify variants in analysis results" on page 194
Flag important variants, then track them in a database in the software or ignore insignificant variants, such as known false positives.	"MyVariants" on page 172
Add a written comment or observation to a variant.	"Apply flags, notes, or classifications to variants of interest" on page 175

4. When you are satisfied that you have displayed, flagged, and classified the variants of interest to you, lock and publish the analysis results to create the final report. For more information, see "Create a final report" on page 404.

Note: You need to have the **Report** role to generate final reports. Only one final report for each analysis can be published for later use. After a report has been locked and published, the **Analysis Results** table for the analysis displays in a view-only mode. You cannot change flags for **MyVariants**, classifications, or notes that are associated with the analysis results in the view-only mode.

Variants file downloads

You can download a ZIP file that contains files from a successful analysis and includes variants results in a VCF file. You can use the files to analyze Ion Reporter™ Software data in a software application that requires VCF files. You can view the extracted files individually, or upload a VCF file to the software application.

Each downloaded ZIP file also includes files with other analysis results information for variants that are not contained in the VCF file.

You cannot download a variants file for analyses that do not include a VCF file in the results package. For example, analysis results that are launched from the immune repertoire or 16 S Metagenomics analysis workflows do not include variants. If a plugin is run, it must generate a VCF file for this procedure to apply.

The contents of each folder that is available for download in Ion Reporter™ Software are as follows.

- **Filtered variants**—A VCF file and other files, all which contains variants, or information for the variants, which were *filtered IN* for the analysis. Variants that are in the VCF file are results that are *filtered IN* by either the filter chain that is used by default in the analysis workflow, or the filter chain that is saved to the analysis workflow. See Chapter 10, "Filters and filter chains" for more information.
- **All variants**—A VCF that contains *all* variants that are included in the analysis, and other files that contain information for the variants. Variants in this file include those that are called, uncalled and controls.
- **Selected variants**—A VCF that contains *selected* variants from the analysis, and other files that contain information for the variants. Each variant to be included in the selected variants VCF file must be selected in the **Analysis Results** before the files are downloaded. Variants in this file include those that are called, uncalled and controls.

Downloads of results files can be automated with the web services API that is included with Ion Reporter™ Software. For more information, see Appendix B, "Ion Reporter™ Software web services API".

Download filtered variants

You can download a VCF file and other files, each of which contains variants, or information for the variants, which were *filtered IN* for an analysis. You can also download a batch of filtered variant files from multiple analyses simultaneously.

1. In the **Analyses** tab, click **Overview**, then select the analysis or analyses that contains the filtered variants.

Task	Steps
Download a set of files with data for variants that are filtered from a single analysis.	• Select the row for the analysis of interest, then click **Actions** ▸ **Download Filtered Variants**. • Click the hyperlink for the name of the **Analysis** to open the **Analysis Results** screen, then click **Download** ▸ **Filtered Variants**. **Note:** The set of downloaded filtered variants files are identical for both of these options.
Download a set of files with data for variants that are filtered from multiple analyses.	Select rows for multiple analyses of interest, then click **Actions** ▸ **Export Filtered** next to **Selected Analyses**.

   For a single analysis, a compressed folder that is named `analysis name_Filtered.zip` is downloaded. For multiple analyses, a ZIP file that is named `batch_analysis_results_date_system-generated-number_Filtered.zip` is downloaded.

2. To download the ZIP file from Ion Reporter™ Software:
   a. Open the **Home** tab, then click **Notifications**.
   b. Find the download file in the list. You can click **Downloads** to narrow the list, or see the timestamp.

c. Click ⬇ **(Download)**.

3. Extract the contents of the folder to access the folders and files.

Folder	Description of contents
`CNV_VCIB`	• `cn_results.png`— A PNG file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. • `amplicon_data.txt`— A file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor.
`CnvActor`	`Gene_deletions.xls`, a file that contains information about whole gene deletions and amplicon coverages that are found in BRCA analyses. Values in the third column of the file indicate the following information for the BRCA1 and BRCA2 genes in the sample. • `S`—Stable. • `SQCFAIL`—Calculated overall variation between amplicon coverage is too high. • `LGD=<score>`—Large Gene Deletion with Phred scale score = <score>. • `WGD=<score>`—Whole Gene Deletion with Phred scale score = <score> (max score = 100). The Phred scale score is must >=40, for the WGD (subtype = GeneCNV) to be reported in the VCF file. Large gene deletions are not reported in the VCF file.
`FusionImbalancePlots`	• `<sample name>_covPlot.png`—Fusion imbalance coverage plots for the sample. • `<sample name>_ImbalanceCalls.png`—Plot that shows whether fusion imbalance coverage calls are positive or negative. • `<sample name>_SampleQC.png`—Plot that shows the per amplicon QC mean read counts for fusion imbalance calls.
`RNACountsActor-00`	• driver_WT_assays_Level.txt—Information for the fusion wild type assays. Lists the ExpressionControl, RNAExonTile and RNAExonVariant details for the genes. • <barcode>_rawlib_RNAExonTile_TotalCounts.txt—Lists the read counts for the RNA Exon tiles.
N/A	`fusions_RNAExonVariants_normCounts.png`—A coverage plot for the normalized RNA Exon Variants read counts. The data is available in text format in the file <barcode>_rawlib_RNAExonTile_TotalCounts.txt, which is available in the `RNACountsActor-00` folder.
`HRR_Results`	`hrr_summary.tsv`—A TSV file that contains a summary of the HRR results.
`MRR_Results`	`mmr_summary.json`—A JSON file that contains a summary of the MRR results.
`signature_prediction`	The signature_prediction folder contains: • A PDF report of the mutational signature prediction results • Image files that graphically represent the results (Only available if a mutational signature match is found.) • A TXT file of the trinucleotide distribution of variants

(continued)

Folder	Description of contents
QC	• report.pdf • Torrent Suite™ Software Quality Control (QC) files. For example, bam_file_basename>.ionstats_alignment.json, analysis.bfmask.stats, explog_final.txt, raw_peak_signal, InitLog.txt, basecaller_results/BaseCaller.json, basecaller_results/ datasets_basecaller.json, basecaller_results/ionstats_tf.json, basecaller_results/TFStats.json • mapd.txt—output of the copy number variation. • diffCoverage.seg—output of CNV module. • RNAQCAndCalls.txt—contains information about the QC for fusion data. • TotalMappedFusionPanelReads.txt—provides number of total mapped fusion panel reads. • qc_cnv_display.txt—CNV sample QC for BRCA analyses. • <analysis name>_QC.pdf—Report of QC metrics. The same QC metrics are available in the Final Report of an analysis.
	StatsActor • – amplicons_low_no_coverage_statistics.txt—A tab-delimited report file that contains detailed coverage metrics for genomic regions (amplicons, genes, or hotspots) that are analyzed during quality control assessment for sequencing runs. – analysis_low_no_coverage_statistics.txt—A file that contains coverage statistics for genomic regions below acceptable coverage thresholds. Each row in the file represents a genomic region and tracks the distribution of bases across different coverage levels. – genes_low_no_coverage_statistics.txt—A file that identifies problematic genomic regions where sequencing coverage is insufficient for reliable variant calling and analysis.
	• AnnotatorActor— – variome-stats.csv—The variome-stats.csv file is a Quality Control (QC) report that provides comprehensive variant-level statistics and metrics for a genomic sample.

(continued)

Folder	Description of contents
`Variants`	Contains VCF and TSV files for the variants, and fusion data if the sequencing run includes a DNA and Fusions or Fusions analysis workflow. You can open these files in Microsoft™ Excel™. **Note:** If the Ion Reporter™ Software analysis includes notes, the notes are included in the files in a row named `#analysisnotes`. • `<sample name>_Filtered_<timestamp>.oncomine.tsv`—VCF file that contains information in tab-separated format. • `<sample name>_Filtered_<timestamp>.vcf`—VCF file that contains all, filtered, or selected variants based on user action. • `<sample name>-full.tsv`—Tab-separated file for all the variants with annotation. • `SmallVariants.filtered.vcf`—An intermediate output VCF file from VarinatCallerActor of the Ion Reporter Software. The VCF file is primarily for use in troubleshooting. It contains variants that are blocked by a Sequence Variant Baseline (SVB) or filtered out by a specific filter reason. • `SmallVariants.vcf`—An intermediate output VCF file from VarinatCallerActor software component. The file contains the variants to be reported to the user. The file is further processed and annotated by Ion Reporter™ Software. • `Variant.filtered.genome.vcf`—An output file from VariantCallerActor software component. The VCF file uses the **Genomic Variant Call Format (GVCF)** format and contains the coverage information of the targeted regions. Variants in the file are reported in the Locus View VCF format in Ion Reporter™ Software.
`Workflow_Settings`	• `Analysis_Settings`—Contains a text file that describes settings used for the analysis. Open the file with a text editor. • `Module_Configuration_Files`—Contains configuration files for workflows, including `VariantCallerActor.json`, a JSON file that contains variant finding parameters used to customize variant calling in Ion Reporter™ Software and the settings used in the analysis. The file shows changes made to the parameters in the software. The file is present only for analyses that include variants.
`MSI`	Files with data about microsatellite instability (MSI) markers. These markers can identify a form of genomic instability in the replication of repetitive DNA. MSI often occurs in tumor cells. It leads to the appearance of multiple alleles at microsatellite loci, which can be easily identified. A folder for each sample contains the following files. • `MSIQC.json`—File contains "flags" and warning messages associated with MSI status that are used by customer support representatives. • `Classify.json` —Provides a summary of the MSI status and coverage.

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Folder	Description of contents
`RESULTS`	If the `Tumor Mutational Burden` parameter is enabled, or an analysis workflow for the Tumor Mutation Load Assay is used, a Results folder is generated that contains: • `filter_variants.tsv` and `somatic_variants.tsv`—TSV files that include post-filter and somatic variants. • `<tmb report>.pdf`—Report that contains tumor mutational load results. • `statistic.txt`—contains tumor mutational burden statistics. • PNG files that include images of: – allele frequency distribution of germline and somatic variants – allele frequency distribution of only somatic variants – bar plot of signature type and context of somatic mutations – pie chart of substitution type of somatic mutations – pie chart of signature pattern of somatic mutations • `base_change_file.txt` and `somatic_mutation_substitution_context_summary.txt`— four files that include the data used to generate the substitution type and context of somatic mutations and substitution type and signature pattern of somatic mutations plots • `deamination_metric.txt` — contains the TMB score and other TMB metrics
`Plugin outputs`	Contains ZIP file of the plugin output folder.
`Immune repertoire output`	The CSV, PNG and PDF files that show the data for Immune repertoire analyses. • `<analysis name><timestamp>.csv` • `<analysis name><timestamp>.png` • `<analysis name><timestamp>.pdf`
`Metagenomics Output`	Files from a single metagenomics sample, or results from multiple samples that the software compares for Beta diversity, and files that contains reads for the analysis in FASTA format, and other TXT files for the analysis. To download all results files for metagenomics analyses, use instead the **Download results files for all samples** link, shown in the **Visualization/Downloads** section of the metagenomics analysis results. For more information, see “View interactive taxonomy charts for metagenomics analyses” on page 284.

You can view the extracted files individually, or upload a VCF file to a software application that requires VCF files.

Download all variants

You can download a VCF file that contains all variants that are included in an analysis, and other files that include information for the variants. You can also download a batch of variant files from multiple analyses simultaneously.

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Use one option to download a ZIP file of files that contains data for all variants in the analysis:

Task	Steps
Download a set of files that include data for all variants from a single analysis.	• Select the row for the analysis of interest, then click **Actions** ▸ **Download All Variants**. • Click the hyperlink for the name of the **Analysis** to open the **Analysis Results** screen, then click **Download** ▸ **All Variants**. • Click the hyperlink for the name of the **Analysis** to open the **Analysis Results** screen, then click **Visualize**, In the **Analysis Visualization** click **Download** ▸ **All Variants**. **Note:** The set of downloaded variants files are identical for each of these options.
Download a set of files that include data for all variants from multiple analyses.	Select rows for multiple analyses of interest, then click **Actions** ▸ **Export** next to **Selected Analyses**.

   A compressed folder that is named `analysis name_All.zip` is downloaded.

3. To download the ZIP file from Ion Reporter™ Software:
   a. Open the **Home** tab, then click **Notifications**.
   b. Find the download file in the list. You can click **Downloads** to narrow the list, or see the timestamp.
   c. Click **(Download)**.
4. Extract the contents of the folder to access the folders and files.

Folder	Description of contents
`CNV_VCIB`	• `cn_results.png`— A PNG file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. • `amplicon_data.txt`— A file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor.

(continued)

Folder	Description of contents
CnvActor	Gene_deletions.xls, a file that contains information about whole gene deletions and amplicon coverage that are found in BRCA analyses. Values in the third column of the file indicate the following information for the BRCA1 and BRCA2 genes in the sample. • S—Stable. • SQCFAIL—Calculated overall variation between amplicon coverage is too high. • LGD=<score>—Large Gene Deletion with Phred scale score = <score>. • WGD=<score>—Whole Gene Deletion with Phred scale score = <score> (max score = 100). The Phred scale score is must >=40, for the WGD (subtype = GeneCNV) to be reported in the VCF file. Large gene deletions are not reported in the VCF file.
FusionImbalancePlots	• <sample name>_covPlot.png—Fusion imbalance coverage plots for the sample. • <sample name>_ImbalanceCalls.png—Plot that shows whether fusion imbalance coverage calls are positive or negative. • <sample name>_SampleQC.png—Plot that shows the per amplicon QC mean read counts for fusion imbalance calls.
RNACountsActor-00	• driver_WT_assays_Level.txt—Information for the fusion wild type assays. Lists the ExpressionControl, RNAExonTile and RNAExonVariant details for the genes. • <barcode>_rawlib_RNAExonTile_TotalCounts.txt—Lists the read counts for the RNA Exon tiles.
N/A	fusions_RNAExonVariants_normCounts.png—A coverage plot for the normalized RNA Exon Variants read counts. The data is available in text format in the file <barcode>_rawlib_RNAExonTile_TotalCounts.txt, which is available in the RNACountsActor-00 folder.
HRR_Results	hrr_summary.tsv—A TSV file that contains a summary of the HRR results.
MRR_Results	mmr_summary.json—A JSON file that contains a summary of the MRR results.
signature_prediction	The signature_prediction folder contains: • A PDF report of the mutational signature prediction results • Image files that graphically represent the results (Only available if a mutational signature match is found.) • A TXT file of the trinucleotide distribution of variants

(continued)

Folder	Description of contents
QC	• report.pdf • Torrent Suite™ Software Quality Control (QC) files. For example, bam_file_basename>.ionstats_alignment.json, analysis.bfmask.stats, explog_final.txt, raw_peak_signal, InitLog.txt, basecaller_results/BaseCaller.json, basecaller_results/ datasets_basecaller.json, basecaller_results/ionstats_tf.json, basecaller_results/TFStats.json • mapd.txt—output of the copy number variation. • diffCoverage.seg—output of CNV module. • RNAQCAndCalls.txt—contains information about the QC for fusion data. • TotalMappedFusionPanelReads.txt—provides number of total mapped fusion panel reads. • qc_cnv_display.txt—CNV sample QC for BRCA analyses. • <analysis name>_QC.pdf—Report of QC metrics. The same QC metrics are available in the Final Report of an analysis.
	StatsActor • – amplicons_low_no_coverage_statistics.txt—A tab-delimited report file that contains detailed coverage metrics for genomic regions (amplicons, genes, or hotspots) that are analyzed during quality control assessment for sequencing runs. – analysis_low_no_coverage_statistics.txt—A file that contains coverage statistics for genomic regions below acceptable coverage thresholds. Each row in the file represents a genomic region and tracks the distribution of bases across different coverage levels. – genes_low_no_coverage_statistics.txt—A file that identifies problematic genomic regions where sequencing coverage is insufficient for reliable variant calling and analysis.
	• AnnotatorActor— – variome-stats.csv—The variome-stats.csv file is a Quality Control (QC) report that provides comprehensive variant-level statistics and metrics for a genomic sample.

(continued)

Folder	Description of contents
`Variants`	Contains VCF and TSV files for the variants, and fusion data if the sequencing run includes a DNA and Fusions or Fusions analysis workflow. You can open these files in Microsoft™ Excel™. **Note:** If the Ion Reporter™ Software analysis includes notes, the notes are included in the files in a row named `#analysisnotes`. • `<sample name>_Non-Filtered_<timestamp>.oncomine.tsv`—VCF file that contains information in tab-separated format. • `<sample name>_Non-Filtered_<timestamp>.vcf`—VCF file that contains all, filtered, or selected variants based on user action. • `<sample name>-full.tsv`—Tab-separated file for all the variants with annotation. • `SmallVariants.filtered.vcf`—An intermediate output VCF file from VarinatCallerActor of the Ion Reporter Software. The VCF file is primarily for use in troubleshooting. It contains variants that are blocked by a Sequence Variant Baseline (SVB) or filtered out by a specific filter reason. • `SmallVariants.vcf`—An intermediate output VCF file from VarinatCallerActor software component. The file contains the variants to be reported to the user. The file is further processed and annotated by Ion Reporter™ Software. • `Variant.genome.vcf`—An output file from VariantCallerActor software component. The VCF file uses the **Genomic Variant Call Format (GVCF)** format and contains the coverage information of the targeted regions. Variants in the file are reported in the Locus View VCF format in Ion Reporter™ Software.
`Workflow_Settings`	• `Analysis_Settings`—Contains a text file that describes settings used for the analysis. Open the file with a text editor. • `Module_Configuration_Files`—Contains configuration files for workflows, including `VariantCallerActor.json`, a JSON file that contains variant finding parameters used to customize variant calling in Ion Reporter™ Software and the settings used in the analysis. The file shows changes made to the parameters in the software. The file is present only for analyses that include variants.
`MSI`	Files with data about microsatellite instability (MSI) markers. These markers can identify a form of genomic instability in the replication of repetitive DNA. MSI often occurs in tumor cells. It leads to the appearance of multiple alleles at microsatellite loci, which can be easily identified. A folder for each sample contains the following files. • `MSIQC.json`—File contains "flags" and warning messages associated with MSI status that are used by customer support representatives. • `Classify.json` —Provides a summary of the MSI status and coverage.

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Folder	Description of contents
`RESULTS`	If the `Tumor Mutational Burden` parameter is enabled, or an analysis workflow for the Tumor Mutation Load Assay is used, a Results folder is generated that contains: • `filter_variants.tsv` and `somatic_variants.tsv`—TSV files that include post-filter and somatic variants. • `<tmb report>.pdf`—Report that contains tumor mutational load results. • `statistic.txt`—contains tumor mutational burden statistics. • PNG files that include images of: – allele frequency distribution of germline and somatic variants – allele frequency distribution of only somatic variants – bar plot of signature type and context of somatic mutations – pie chart of substitution type of somatic mutations – pie chart of signature pattern of somatic mutations • `base_change_file.txt` and `somatic_mutation_substitution_context_summary.txt`— four files that include the data used to generate the substitution type and context of somatic mutations and substitution type and signature pattern of somatic mutations plots • `deamination_metric.txt` — contains the TMB score and other TMB metrics
`Plugin outputs`	Contains ZIP file of the plugin output folder.
`Immune repertoire output`	The CSV, PNG and PDF files that show the data for Immune repertoire analyses. • `<analysis name><timestamp>.csv` • `<analysis name><timestamp>.png` • `<analysis name><timestamp>.pdf`
`Metagenomics Output`	Files from a single metagenomics sample, or results from multiple samples that the software compares for Beta diversity, and files that contains reads for the analysis in FASTA format, and other TXT files for the analysis. To download all results files for metagenomics analyses, use instead the **Download results files for all samples** link, shown in the **Visualization/Downloads** section of the metagenomics analysis results. For more information, see “View interactive taxonomy charts for metagenomics analyses” on page 284.

You can view the extracted files individually, or upload a VCF file to a software application that requires VCF files.

Download selected variants

You can download a VCF file that contains variants that you select, and other files that include information for the selected variants. Use this option to download files that focus on the variants that are relevant to research.

1. In the **Analyses** tab, click **Overview**, then select the analysis that contains the selected variants.
2. Use an option to download a ZIP file that contains files with data for variants that are selected.
   - a. Click the hyperlink for the name of the **Analysis** to open the **Analysis Results** screen.
     b. Select each checkbox for one or more variants of interest to download.
     c. Click **Download** ▸ **Selected Variants**.
   - a. Click **Visualize**.
     b. Click **Download** ▸ **Selected Variants**.

   A ZIP file that is named `analysis name_SelectedVariants.zip` is downloaded.
3. To download the ZIP file from Ion Reporter™ Software:
   a. Open the **Home** tab, then click **Notifications**.
   b. Find the download file in the list. You can click **Downloads** to narrow the list, or see the timestamp.
   c. Click ⬇ **(Download)**.
4. Extract the contents of the ZIP file to access the folders and files.

Folder	Description of contents
`CNV_VCIB`	• `cn_results.png`— A PNG file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. • `amplicon_data.txt`— A file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor.
`CnvActor`	`Gene_deletions.xls`, a file that contains information about whole gene deletions and amplicon coverage that are found in BRCA analyses. Values in the third column of the file indicate the following information for the BRCA1 and BRCA2 genes in the sample. • `S`—Stable. • `SQCFAIL`—Calculated overall variation between amplicon coverage is too high. • `LGD=<score>`—Large Gene Deletion with Phred scale score = <score>. • `WGD=<score>`—Whole Gene Deletion with Phred scale score = <score> (max score = 100). The Phred scale score is must >=40, for the WGD (subtype = GeneCNV) to be reported in the VCF file. Large gene deletions are not reported in the VCF file.

(continued)

Folder	Description of contents
`FusionImbalancePlots`	• `<sample name>_covPlot.png`—Fusion imbalance coverage plots for the sample. • `<sample name>_ImbalanceCalls.png`—Plot that shows whether fusion imbalance coverage calls are positive or negative. • `<sample name>_SampleQC.png`—Plot that shows the per amplicon QC mean read counts for fusion imbalance calls.
`RNACountsActor-00`	• driver_WT_assays_Level.txt—Information for the fusion wild type assays. Lists the ExpressionControl, RNAExonTile and RNAExonVariant details for the genes. • <barcode>_rawlib_RNAExonTile_TotalCounts.txt—Lists the read counts for the RNA Exon tiles.
N/A	`fusions_RNAExonVariants_normCounts.png`—A coverage plot for the normalized RNA Exon Variants read counts. The data is available in text format in the file <barcode>_rawlib_RNAExonTile_TotalCounts.txt, which is available in the `RNACountsActor-00` folder.
`HRR_Results`	`hrr_summary.tsv`—A TSV file that contains a summary of the HRR results.
`MRR_Results`	`mmr_summary.json`—A JSON file that contains a summary of the MRR results.
`signature_prediction`	The signature_prediction folder contains: • A PDF report of the mutational signature prediction results • Image files that graphically represent the results (Only available if a mutational signature match is found.) • A TXT file of the trinucleotide distribution of variants
`QC`	• `report.pdf` • Torrent Suite™ Software Quality Control (QC) files. For example, `bam_file_basename>.ionstats_alignment.json`, `analysis.bfmask.stats`, `explog_final.txt`, `raw_peak_signal`, `InitLog.txt`, `basecaller_results/BaseCaller.json`, `basecaller_results/ datasets_basecaller.json`, `basecaller_results/ionstats_tf.json`, `basecaller_results/TFStats.json` • `mapd.txt`—output of the copy number variation. • `diffCoverage.seg`—output of CNV module. • `RNAQCAndCalls.txt`—contains information about the QC for fusion data. • `TotalMappedFusionPanelReads.txt`—provides number of total mapped fusion panel reads. • `qc_cnv_display.txt`—CNV sample QC for BRCA analyses. • `<analysis name>_QC.pdf`—Report of QC metrics. The same QC metrics are available in the Final Report of an analysis.

(continued)

Folder	Description of contents
QC	StatsActor • – amplicons_low_no_coverage_statistics.txt—A tab-delimited report file that contains detailed coverage metrics for genomic regions (amplicons, genes, or hotspots) that are analyzed during quality control assessment for sequencing runs. – analysis_low_no_coverage_statistics.txt—A file that contains coverage statistics for genomic regions below acceptable coverage thresholds. Each row in the file represents a genomic region and tracks the distribution of bases across different coverage levels. – genes_low_no_coverage_statistics.txt—A file that identifies problematic genomic regions where sequencing coverage is insufficient for reliable variant calling and analysis.
	• AnnotatorActor – variome-stats.csv—The variome-stats.csv file is a Quality Control (QC) report that provides comprehensive variant-level statistics and metrics for a genomic sample.
Variants	Contains VCF and TSV files for the variants, and fusion data if the sequencing run includes a DNA and Fusions or Fusions analysis workflow. You can open these files in Microsoft™ Excel™. **Note:** If the Ion Reporter™ Software analysis includes notes, the notes are included in the files in a row named #analysisnotes. • <sample name>_Selected_<timestamp>.oncomine.tsv—VCF file that contains information in tab-separated format. • <sample name>_Selected_<timestamp>.vcf—VCF file that contains all, filtered, or selected variants based on user action. • <sample name>-full.tsv—Tab-separated file for all the variants with annotation. • SmallVariants.selected.vcf—An intermediate output VCF file from VarinatCallerActor of the Ion Reporter Software. The VCF file is primarily for use in troubleshooting. It contains variants that are blocked by a Sequence Variant Baseline (SVB) or filtered out by a specific filter reason. • SmallVariants.vcf—An intermediate output VCF file from VarinatCallerActor software component. The file contains the variants to be reported to the user. The file is further processed and annotated by Ion Reporter™ Software. • Variant.genome.vcf—An output file from VariantCallerActor software component. The VCF file uses the **Genomic Variant Call Format (GVCF)** format and contains the coverage information of the targeted regions. Variants in the file are reported in the Locus View VCF format in Ion Reporter™ Software.

(continued)

Folder	Description of contents
`Workflow_Settings`	• `Analysis_Settings`—Contains a text file that describes settings used for the analysis. Open the file with a text editor. • `Module_Configuration_Files`—Contains configuration files for workflows, including `VariantCallerActor.json`, a JSON file that contains variant finding parameters used to customize variant calling in Ion Reporter™ Software and the settings used in the analysis. The file shows changes made to the parameters in the software. The file is present only for analyses that include variants.
`MSI`	Files with data about microsatellite instability (MSI) markers. These markers can identify a form of genomic instability in the replication of repetitive DNA. MSI often occurs in tumor cells. It leads to the appearance of multiple alleles at microsatellite loci, which can be easily identified. A folder for each sample contains the following files. • `MSIQC.json`—File contains "flags" and warning messages associated with MSI status that are used by customer support representatives. • `Classify.json` —Provides a summary of the MSI status and coverage.
`RESULTS`	If the `Tumor Mutational Burden` parameter is enabled, or an analysis workflow for the Tumor Mutation Load Assay is used, a Results folder is generated that contains: • `filter_variants.tsv` and `somatic_variants.tsv`—TSV files that include post-filter and somatic variants. • `<tmb report>.pdf`—Report that contains tumor mutational load results. • `statistic.txt`—contains tumor mutational burden statistics. • PNG files that include images of: – allele frequency distribution of germline and somatic variants – allele frequency distribution of only somatic variants – bar plot of signature type and context of somatic mutations – pie chart of substitution type of somatic mutations – pie chart of signature pattern of somatic mutations • `base_change_file.txt` and `somatic_mutation_substitution_context_summary.txt`— four files that include the data used to generate the substitution type and context of somatic mutations and substitution type and signature pattern of somatic mutations plots • `deamination_metric.txt` — contains the TMB score and other TMB metrics
`Plugin outputs`	Contains ZIP file of the plugin output folder.

(continued)

Folder	Description of contents
`Immune repertoire output`	The CSV, PNG and PDF files that show the data for Immune repertoire analyses. • `<analysis name><timestamp>.csv` • `<analysis name><timestamp>.png` • `<analysis name><timestamp>.pdf`
`Metagenomics Output`	Files from a single metagenomics sample, or results from multiple samples that the software compares for Beta diversity, and files that contains reads for the analysis in FASTA format, and other TXT files for the analysis. To download all results files for metagenomics analyses, use instead the **Download results files for all samples** link, shown in the **Visualization/Downloads** section of the metagenomics analysis results. For more information, see "View interactive taxonomy charts for metagenomics analyses" on page 284.

You can view the extracted files individually, or upload a VCF file to a software application that requires VCF files.

Searches on the Analysis Results screen

Advanced searches, such as those that use OR and AND, can be performed on the **Analysis Results** screen with a controlled vocabulary query language. The searches are available in both a single-analysis variant review table and a multi-sample visualization table. However, the searches have been tested only on the single-analysis variant review table.

Note: Most searches on the **Analysis Results** screen can be done effectively with filters, instead of with the search field. For most searches, use a filter instead.

The following filter terms are supported:

Notation	Meaning
key:value1,value2	key=value1 OR key=value2
key:[min,max]	min <=key <=max
key:(min,max)	min < key < max
key:[min,]	min <= key
key:[,max]	key <= max
key:(min,)	min < key
key:(,max)	key < max
key:value*	key contains value
key:*	key exists (key has any value)
-key:value	key != value

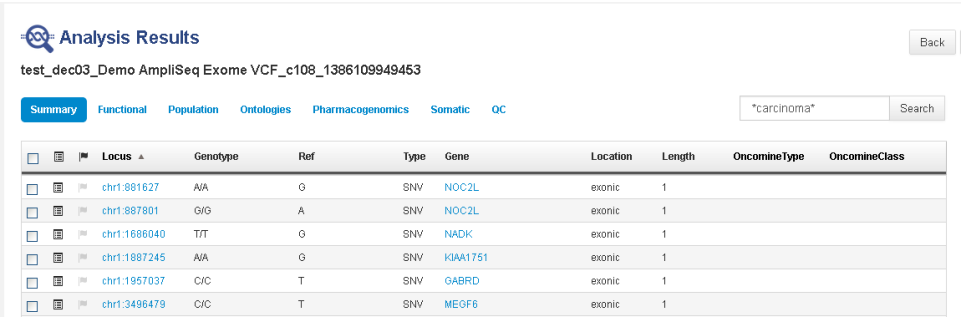
The following keys are supported:

Key	Example
locus	chr2:123456
function	missense
location	exonic
cosmic, omim, pfam, drugbank, go	glioma
dbsnp	rs12345
gene, transcript	TP53, NM_01010.1
maf	[0.0,0.05]
coverage	[1000']
sift, polyphen, grantham	['0.05]
type	INDEL
comment	something*
vkb "vkb" stands for MyVariants.	*

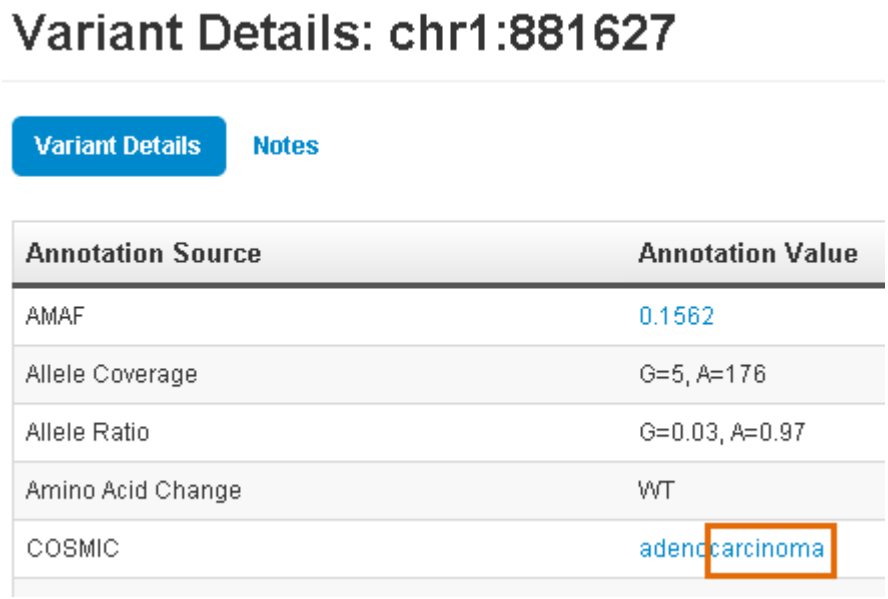
Search field behavior is different on the variant table in the **Analysis Results** screen, than for searches on other screens.

Asterisks are useful to search for matches in any annotation source. (By contrast, a filter search matches only one annotation source.) **Asterisks (*) for *some* variant table searches on the Analysis Results screen is used in the following ways:**

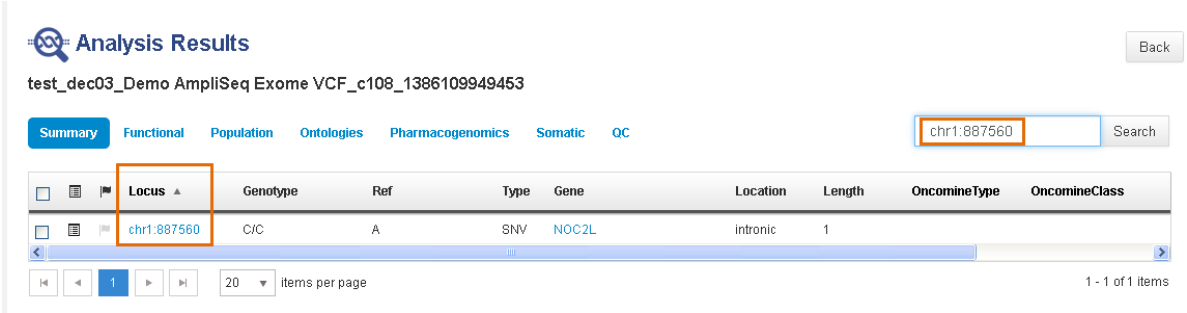
- An asterisk (*) in the search field is allowed only on the **Analysis Results** screen.
- An asterisk (*) is required for some searches, but is not allowed for other searches. The differences are due to how the different types of information are stored.
- The asterisk is a search wildcard. Without the asterisk, searches match only the exact string entered. With asterisks both before and after the search string, matches at the beginning, middle, and end are all found.
- This example shows a search for *carcinoma*.



This search lists all variants that have an OMIM, COSMIC, ClinVar, DrugBank, and so on, annotation that contains "carcinoma" anywhere in the annotation. Click **(Details)** for one **(Flag)** of the matched variants to open its variant detail card. Scroll to find the entries that include "carcinoma".

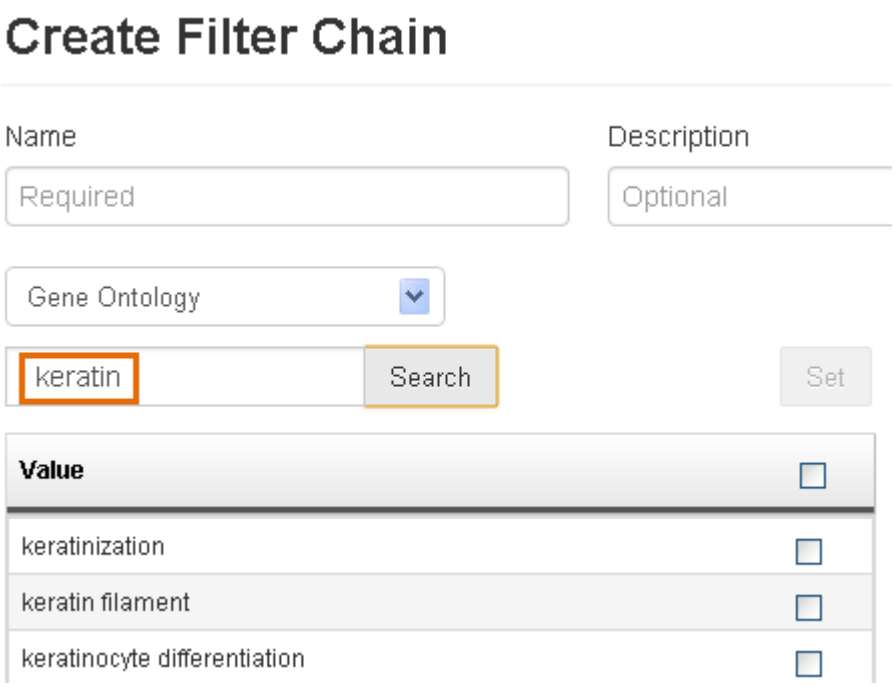


- To search for a locus, enter the complete locus entry, with chromosome number and full position number.



- Do not use an asterisk (*) for a locus search. Searches on a chromosome number by itself or with a partial position number also are not supported.
- For other information, use a filter chain.

- Asterisks cannot be used in search terms during creation of a filter chain.



Example searches:

- ```
  (gene:TP53 OR (function:missense,nonsense AND (maf:[0.0,0.05} OR
  -dbsnp:*))) OR type:CNV
  ```

  This retrieves all variants that fall in TP53, all CNVs, plus variants that have a functional impact of missense or nonsense AND either have a minor allele frequency <0.05 or are novel (not found in dbSNP).

- ```
  cosmic:carcinoma* AND ((type:SNV AND sift:[0.0,0.10]) AND coverage:
  [300,] OR locus:chrX
  ```

  This retrieves all SNV variants annotated with COSMIC histology terms containing "carcinoma" with a deleterious SIFT score (<0.10) and high coverage(>300), plus any variant that falls on chromosome X.

CNV subtypes in the Analysis Results screen

Some CNV subtypes in the **Analysis Results** screen are specific for Oncomine™ research assays. For more information, see the user guide for the Oncomine™ assay.

Reasons for NOCALL in a gene-level CNV

CNV_IDs are used in the target regions BED file to identify one or more amplicons that represent a single genomic region; most often CNV_IDs are gene symbols.

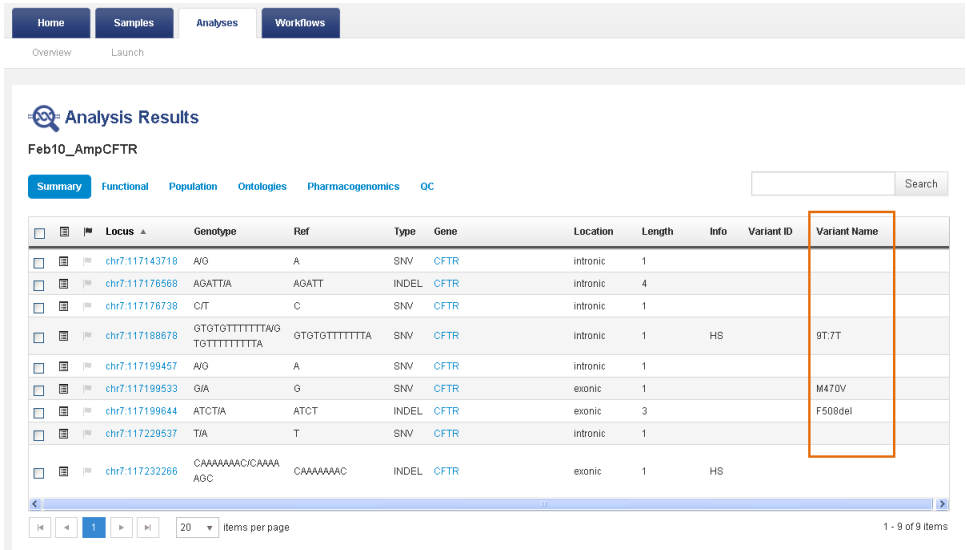
The CNV algorithm looks for heterogeneity and trends at the CNV_ID level, which may potentially, be indicative of data artifacts, and annotates the calls for CNV_IDs displaying these effects as SUSPECT as it is unclear whether the data for this CNV_ID indicates a true biological focal amplification.

- The annotation SEVERE_GRADIENT indicates an increasing or decreasing trend in calculated copy number above a specific threshold (range of values > 4*MAPD or > 0.5) across adjacent amplicons. This is calculated as a moving average and is compared to the local MAPD of the CNV_ID.
- The annotation DIFFERENT_MEAN_SIGNAL relies on a calculation, which looks at differences in groups of amplicons co-located within a CNV_ID. The amplicons are divided into two groups and if the mean difference in the log read ratio is greater than 0.5 this flag is raised.
- The annotation LOCAL_AVERAGE_SIGNAL_VARIATION is made if any pairwise mean in log2 read ratio of adjacent amplicons within a CNV_ID has a value of less than -4 and another such pairwise mean has a value greater than -2. A CNV_ID with partial total loss usually is flagged by this heuristic.

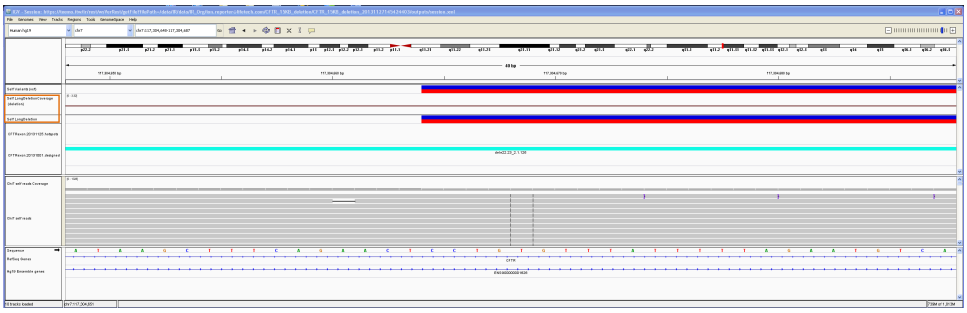
CFTR analysis results

Review the following examples for information about how to review data in the analyses results from CFTR workflows:

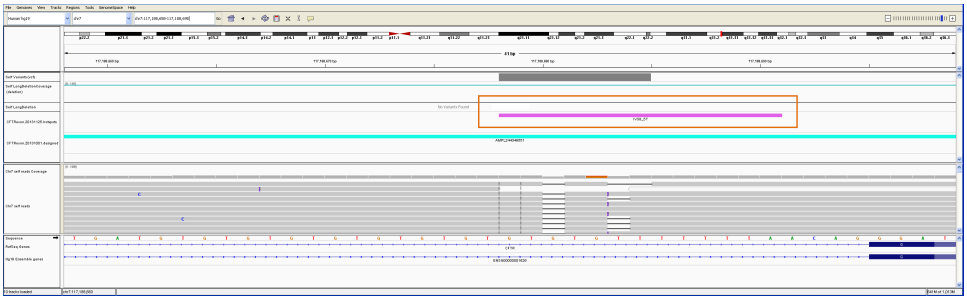
- The **Variant Name** column reports matches with the Named Variants annotation source.



- The following IGV visualization for CFTR analysis results include tracks for long deletions:



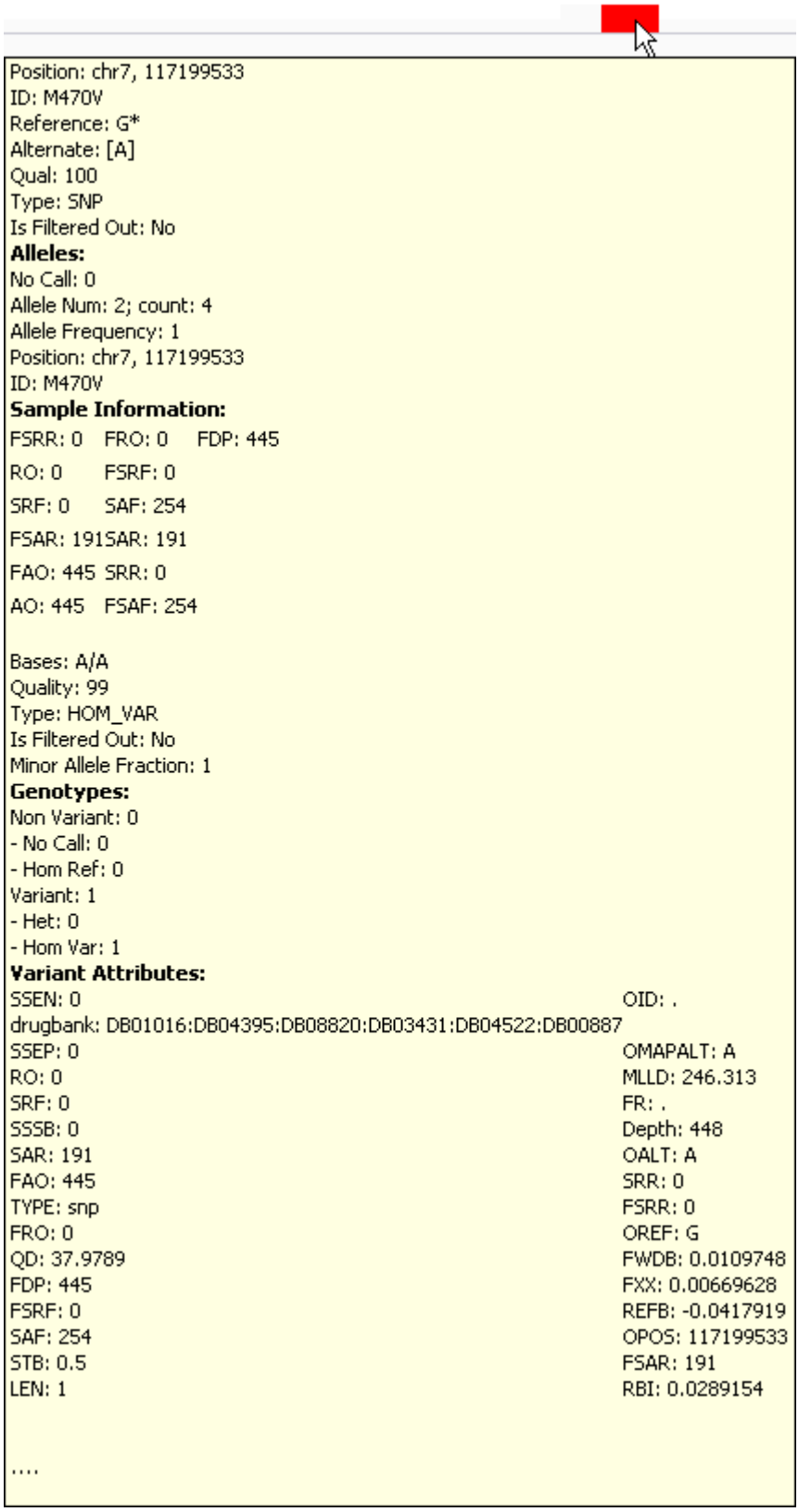
- The following IGV visualization for CFTR analysis results include a named variant:



- The following IGV visualization for CFTR analysis results include a hotspot position:



- Hover the mouse over a position in the IGV visualization to view details such as the following:



Position: chr7, 117199533
ID: M470V
Reference: G*
Alternate: [A]
Qual: 100
Type: SNP
Is Filtered Out: No
Alleles:
No Call: 0
Allele Num: 2; count: 4
Allele Frequency: 1
Position: chr7, 117199533
ID: M470V
Sample Information:
FSRR: 0 FRO: 0 FDP: 445
RO: 0 FSRF: 0
SRF: 0 SAF: 254
FSAR: 191SAR: 191
FAO: 445 SRR: 0
AO: 445 FSAF: 254

Bases: A/A
Quality: 99
Type: HOM_VAR
Is Filtered Out: No
Minor Allele Fraction: 1
Genotypes:
Non Variant: 0
- No Call: 0
- Hom Ref: 0
Variant: 1
- Het: 0
- Hom Var: 1
Variant Attributes:
SSEN: 0 OID: .
drugbank: DB01016:DB04395:DB08820:DB03431:DB04522:DB00887
SSEP: 0 OMAPALT: A
RO: 0 MLLD: 246.313
SRF: 0 FR: .
SSSB: 0 Depth: 448
SAR: 191 OALT: A
FAO: 445 SRR: 0
TYPE: snp FSRR: 0
FRO: 0 OREF: G
QD: 37.9789 FWDB: 0.0109748
FDP: 445 FXX: 0.00669628
FSRF: 0 REFB: -0.0417919
SAF: 254 OPOS: 117199533
STB: 0.5 FSAR: 191
LEN: 1 RBI: 0.0289154

....

The factory-provided CFTR workflow includes long deletion detection. You can copy/edit the CFTR workflow and preserve long deletion detection. If you create your own CFTR workflow (even with the

CFTR regions file), your workflow includes the standard Ion AmpliSeq™ workflow template and does not include the long deletion detection module.

Allele calls for hotspot positions

For hotspot positions, sometimes an allele with zero coverage is reported in the **Allele Coverage** and **Allele Ratio** columns of the **Analysis Results** table.

Allele Coverage	Allele Ratio
G=0, A=1991	G=0.0, A=1.0
T=349, C=1650	T=0.17, C=0.83
T=658, A=1341, C=0	T=0.33, A=0.67, C=0.0
G=471, C=1510, T=0	G=0.24, C=0.76, T=0.0

This is by design and occurs only with hotspot positions. For hotspot positions, the **Analysis Results** table reports all alleles that have any evidence in either basespace or in flowspace. Alleles with zero coverage appear in the **Analysis Results** table for a hotspot position if the second alternate allele has nonzero reads reported in basespace (even though zero reads are reported in flowspace).

RefGene GeneModel and Ensembl GeneModel transcript versions

You can view the details about the annotations that Ion Reporter™ Software supports for a specific gene model transcript in the list of **Variant Details**, including the versions for the RefGene GeneModel and Ensembl GeneModel transcripts.

1. In the **Analysis** tab, click **Overview**.
2. Click an analysis name to open the **Analysis Results** screen.

3. In the **Analysis Results** screen, click **(Variant Details)** to open the **Variant Details** screen. The transcript version is listed in the **Annotation Value** column.

Variant Details: chr20:54961541

Variant Details | Notes

Annotation Source	Annotation Value
Coding	c.91T>A
Codon	ATT
Coverage	290
DrugBank	1-[5-(2-(1-methyl-1H-pyrazol-3-ylaminodipyrimidin-7-ylaminoethyl)-1,3-thiazol-2-yl)-3,3-(trilluoromethyl)phenylurea …(17)
EMAF	0.2169
ExAC AAF	1.414E-1 (pos.54961541)
ExAC EAAF	6.784E-1 (pos.54961541)
ExAC EFAF	2.705E-1 (pos.54961541)
ExAC ENAF	2.172E-1 (pos.54961541)
ExAC GAF	2.735E-1 (pos.54961541)
ExAC LAF	3.687E-1 (pos.54961541)
ExAC OAF	2.941E-1 (pos.54961541)
ExAC SAAF	3.112E-1 (pos.54961541)
Exon	3
Filter	PASS
GMAF	0.1882
Gene Ontology	ATP binding …(61)
Genes	AURKA
Genotype	A/T
Grantham	21.0
Homopolymer Length	3
Info	HS
Length	1
Linear-scaled quality score	0.00022
Location	AURKA:exonic:NM_003600.4
Locus	chr20:54961541
MAF	0.31
Observed Allele	T
Oncomine Gene Class	Gain-of-Function

4. Alternatively, open the TSV variants file in the `Variants/sample_name` subdirectory, to see the transcript versions.



```
#chr  pos   type  ref   length      genotype    pvalue      coverage    allele_coverage   maf   iscn  confidence  precision   gene  transcript  location
      function    codon exon  protein     coding      sift  polyphen    grantham    5000Exomes  Oncomine    clinvar     cosmic      dbsnp drugbank    go    omim
      pfam  phylopMyVariants
1     324100      SNV   T     1     A/A                                                   LOC100132287|LOC100132062|LOC100133331    NR_028322.1|NR_028325.1|NR_028327.1
      intronic_nc|intronic_nc|intronic_nc ||    ||    ||    ||    ||    ||    ||    ||
1     985955      SNV   G     1     G/C                                                   AGRN  NM_198576.3 exonic      missense    CGG   29    p.Gly1709Arg
      c.5125G>C   0.0   1.0   125.0             pathogenic        rs199476396       clustering of voltage-gated sodium channels:axon guidance:extracellular
space:neurotransmitter receptor metabolic process:structural constituent of cytoskeleton:cell surface:laminin binding:extracellular region:G-protein coupled
acetylcholine receptor signaling pathway:synapse organization:signal transduction:receptor clustering:extracellular matrix:protein binding:plasma membrane
organization:positive regulation of neuron apoptosis:acetylcholine receptor regulator activity:synapse assembly:positive regulation of transcription from RNA
polymerase II promoter:regulation of synaptic growth at neuromuscular junction:synapse:basal lamina   Agrin EGF-like domain:SEA domain:Laminin G domain:Kazal-type
serine protease inhibitor domain:Laminin EGF-like (Domains III and V):Agrin NtA domain    2.04
1     2488068     CNV         118059986                                 1p36.32p12(2488068-120548054)x2     0     143.56      LOC100133445|TTC34|FLJ42875|MIR4251
|MIR551A|TP73-AS1|LOC388588|LOC100133612|LOC728716|LOC284661|MIR4417|MIR4689|MIR4252|PLEKHG5|ENO1-AS1|MIR34A|C1orf200|MTOR-AS1|CLCN6|NPPA-AS1|MIR4632
|SNORA59B|SNORA59A|PRAMEF3|PRAMEF15|PRAMEF9|LRRC38|C1orf126|FLJ37453|CLCNKB|CROCCP3|MIR3675|NBPF1|CROCCP2|MST1P2|ESPNP|MST1P9|MIR4695|AKR7L|LOC100506730|LOC339505
|LOC100506801|LINC00339|MIR4684|MIR4253|LOC729059|MIR3115|C1orf213|MDS2|LOC100506963|MIR378F|PNRC2|LOC284632|RCAN3AS|LOC646471|MIR3917|TRIM63|MIR1976|NUDC|SLC9A1
|LOC644961|SCARNA1|SNHG3|SNHG12|SNORD99|SNORA61|SNORA44|SNORA16A|RNU11|LOC100129196|MIR4420|SNORD103A|SNORD103B|SNORD85|PRO0611|LOC149086|LOC284551|MIR4254
|MTMR9LP|LOC100128071|MIR3605|LOC402779|LOC653160|MIR4255|LOC728431|MIR5581|SNIP1|CDCA8|MIR3659|LOC339442|GJA9-MYCBP|PPIEL|SNORA55|ZMPSTE24|LOC100130557
|MIR30E|MIR30C1|CITED4|LOC100507178|LOC100129924|FLJ32224|LOC100132774|SLC6A9|MIR5584|SNORD55|SNORD46|SNORD38A|SNORD38B|EIF2B3|LOC400752|CCDC163P|RPS15AP10
|LOC729041|LOC100507423|LOC100130197|CYP4Z2P|PDZK1IP1-AS1|MGC12982|LOC388630|SKINTL|MIR761|TXNDC12|LOC100507564|SLC25A3P1|MIR4781|HEATR8-TTC4|LOC100507634|MIR4422
|LOC100131060|HSD52|MIR4711|LINC00466|DLEU2L|MIR4794|MIR3671|MIR101-1|DNAJC6|MIR3117|GNG12-AS1|MIR1262|PIN1P1|ZRANB2-AS1|MIR186|ZRANB2-AS2|NEGR1-IT1
|SNORD45C|SNORD45A|SNORD45B|MGC27382|MIR548AP|UOX|C1orf180|MIR4423|LOC646626|CLCA3P|LOC339524|LOC100505768|GBP1P1|FLJ27354|GEMIN8P4|HSP90B3P|SNORD21|SNORA66
|LOC100131564|BCAR3|LOC100129046|MIR760|SLC44A3|LOC729970|FLJ31662|DPYD-AS1|MIR137HG|MIR2682|MIR137|LOC729987|LOC100129620|MIR548D1|MIR548AA1|MIR553|LOC100128787
|EXTL2|RNU6-6|DNAJA1P5|ACTG1P4|LOC100129138|VAV3-AS1|SLC25A24|NBPF6|AKNAD1|SRG7|SCARNA2|MIR197|AMPD2|LOC440600|CYMP|RP11-165H20.1|CHIA|PGCP1|LOC100129269
|LOC100506343|MIR4256|AKR7A2P1|LOC643441|LOC100287722|ATP1A1OS|MIR320B1|MIR942|HSD3BP4|LOC644242

1     2615740     SNV,MNV     GGTCG 4     GAACC/GGTTG                                           TTC34 NM_001242672.1    intronic
                                          rs71490543:rs80062728:rs76497889:rs76926500          binding             Tetratricopeptide repeat      0.5,-1.76,-
2.75,0.5,-1.85
1     2706398     NOCALL      C           ./.                                             TTC34 NM_001242672.1    upstream
                                          rs113477241       binding                 0.73
1     3334486     SNV   C     1     T/T                                                   PRDM16  NM_022114.3 exonic      missense    CTC   11    p.Pro929Leu
      c.2786C>T   0.0   0.002 98.0  AMAF=0.001:EMAF=0.0:GMAF=3.0E-4                 untested    malignant_melanoma      rs145632008       tongue development:zinc ion
binding:somatic stem cell maintenance:positive regulation of transcription, DNA-dependent:transcriptional repressor complex:metal ion binding:transcription
coactivator activity:palate development:negative regulation of transcription from RNA polymerase II promoter:negative regulation of granulocyte
differentiation:regulation of cellular respiration:protein binding:neurogenesis:sequence-specific DNA binding:white fat cell differentiation:brown fat cell
differentiation:SMAD binding:negative regulation of transcription, DNA-dependent:intracellular:negative regulation of transforming growth factor beta receptor
signaling pathway:nucleus     PR domain-containing protein 16     Zinc finger 2.29
1     7527892     SNV   C     1     G/A                                                   CAMTA1      NM_015215.2 exonic      missense|nonsense TGG|TGA     6
      p.Cys147Trp|p.Cys147*   c.441C>G|c.441C>A 0.0|  0.968|      215.0|                                                 calmodulin binding:regulation of transcription,
DNA-dependent:cytoplasm:nucleus     Calmodulin-binding transcription activator 1    IPT/TIG domain:CG-1 domain    2.25
1     7804961     INDEL T     2     TAA/TAA                                                     CAMTA1      NM_015215.2 exonic      nonsense    TAA   17    p.Phe1417*
      c.4249_4250insAA                                                  calmodulin binding:regulation of transcription, DNA-dependent:cytoplasm:nucleus
      Calmodulin-binding transcription activator 1    IPT/TIG domain:CG-1 domain    2.06
1     7826549     INDEL T     1     T/TC                                                  CAMTA1      NM_015215.2 exonic      stoploss    TCG   23    p.*1674Ser  c.5020
_5021insC                                       calmodulin binding:regulation of transcription, DNA-dependent:cytoplasm:nucleus     Calmodulin-
```

5. Use these version numbers with the transcript IDs in the preferred transcript file.

Phred QUAL Score

Phred quality score column is added to the **Analysis Results** table. For more information, see “Q score” on page 639.

Review analysis results for Ion AmpliSeq™ HD panels

1. In the **Analyses** tab, click **Overview**.
   The **Analyses** table lists all available analyses results.

2. Click the column headings to sort the results. Alternatively, use the available filters or the **Search** function to limit the list of analyses.

3. In the **Analysis** column, click the link to open an analysis of interest.
   The **Analysis Results** screen opens to the results for the selected analysis.

4. *(Optional)* In the **Filter Options** section, select a filter chain from the **Filter Chains** list to view analyses results as described.

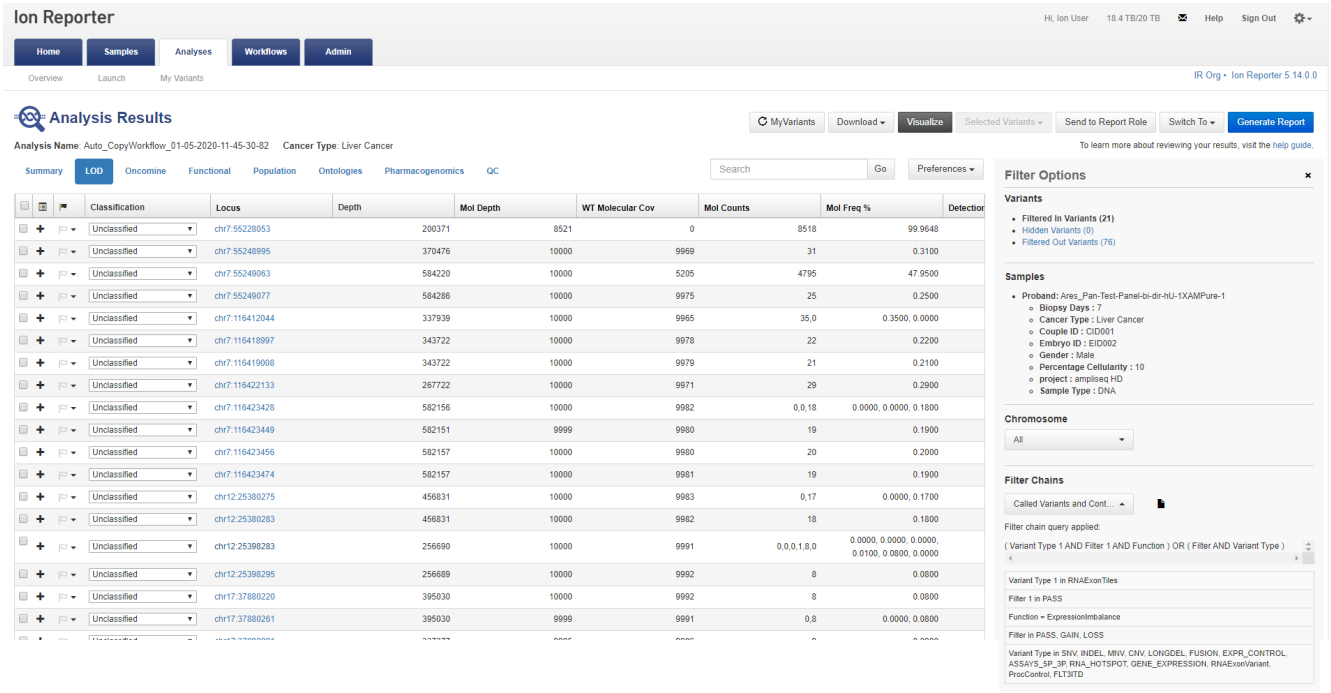
   If you download filtered variants, the downloaded files reflect the filter chain that is applied and *saved* to the analysis. Ensure that you click **Save Filter Chain** to save an applied filter chain to the analysis before you download filtered variants.

Option	Description
Called Variants and Controls	This filter chain reports all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, FLT3-ITD, and RNA Exon Tiles.
Called Hotpot Variants and Controls	This filter chain reports all hotspot variants that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, and RNA Exon Tiles.
Variant Matrix Summary	Select this filter chain for analysis results that contain the same set of variants that are included in the Variant Matrix Analysis visualization for Ion AmpliSeq™ HD analysis results. The **Variant Matrix Summary** filter chain filters in: • Positive hotspot calls • Positive gene-level CNV GAIN or LOSS calls • FLT3-ITD variants • Non-wild-type intragenic structural variations • For TagSeq Breast and TagSeq Pan-Cancer assays: non-hotspot variants in TP53 with QUAL >50 and Allele Frequency > 1.0 (Breast) or 0.5 (Pan Cancer) This filter chain allows analysis results to be exported. You can then import the results into another software application for further analysis. Filter variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants. If you use the Oncomine™ Myeloid MRD analysis workflow, a FLT3-ITD variant subtype named **Combined** can be reported in analysis results. The **Combined** subtype is a value for the sum of all reported FLT3-ITD variant allele frequencies.
Oncomine Variants	Apply the **Oncomine Variants** filter chain to show only the variants that are annotated with the Oncomine™ Variant Annotator plugin. For more information, see Appendix D, “Oncomine™ Variant Annotator plugin criteria”.

The analysis results update immediately to reflect the filtered results.

5. *(Optional)* Create final reports from analysis results and publish a final report or save the report as a final report template. Or, create reports from visualizations in Ion Reporter™ Software, and enable electronic signatures for reports. For more information, see “Create a final report” on page 404, “Create a visualization report” on page 413 and “Generate a final report and approve with an electronic signature” on page 412.

Example of Analysis Results screen, LOD view, and Filter Options pane



Use filter chains to change analysis results

You can use filter chains in Ion Reporter™ Software to change the view of analysis results.

This example shows how to review Oncomine™ analysis results for a sequencing run that uses a TagSeq analysis workflow, then use filter chains to change the results.

1. In the **Analyses** tab, click the link in the **Analysis** column to open an analysis of interest.
   The analysis results appear with the Oncomine™ Variants filter chain applied.

2. *(Optional)* In the **Filter Options** pane, select a different filter chain from the list.

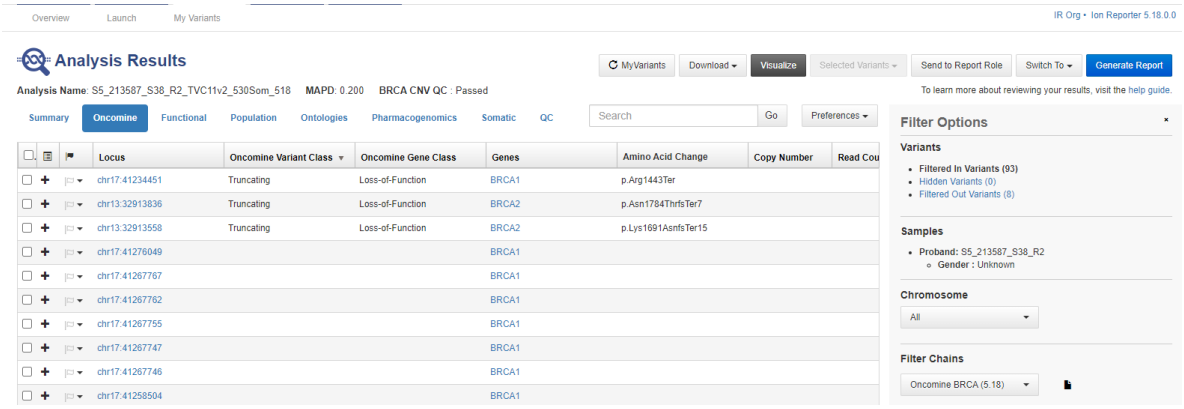
Option	Description
Called Variants and Controls	This filter chain reports all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, FLT3-ITD, and RNA Exon Tiles.
Called Hotspot Variants and Controls	This filter chain reports all hotspot variants that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, and RNA Exon Tiles.
Variant Matrix Summary	Select this filter chain for analysis results that include the same set of variants that are included in the Variant Matrix Analysis visualization for Ion AmpliSeq™ HD analysis results. The **Variant Matrix Summary** filter chain filters in: • Positive hotspot calls • Positive gene-level CNV GAIN or LOSS calls • FLT3-ITD variants • Non-wild-type intragenic structural variations • For TagSeq Breast and TagSeq Pan-Cancer assays: non-hotspot variants in TP53 with QUAL >50 and Allele Frequency > 1.0 (Breast) or 0.5 (Pan Cancer) This filter chain allows analysis results to be exported. You can then import the results into another software application for further analysis. Filter variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants. If you use the Oncomine™ Myeloid MRD analysis workflow, a FLT3-ITD variant subtype named **Combined** can be reported in analysis results. The **Combined** subtype is a value for the sum of all reported FLT3-ITD variant allele frequencies.

3. Review the results.

View Oncomine™ BRCA analysis results

Some Oncomine™ assays used with Ion Reporter™ Software include BRCA1 and BRCA2 amplicons that enable detection and visualization of whole exon and multiple exon deletion in BRCA1 and BRCA2 genes in somatic and germline samples with high sensitivity.

1. In the **Analyses** tab, click **Overview**.
2. Search, sort, or filter the **Analyses** table to find the sample result of interest.
3. In the **Analysis** column, click the hyperlink for the analysis of interest to open **Analysis Results** screen for the BRCA analysis.



4. The following data for the BRCA results is available in the **Analysis Results** screen.
   - A summary of called variants and their genotypic and functional properties. Variants that are listed include SNVs, INDELs, long exon deletions, duplications, and whole gene deletions and duplications.

- Metrics and information at the top of the screen.

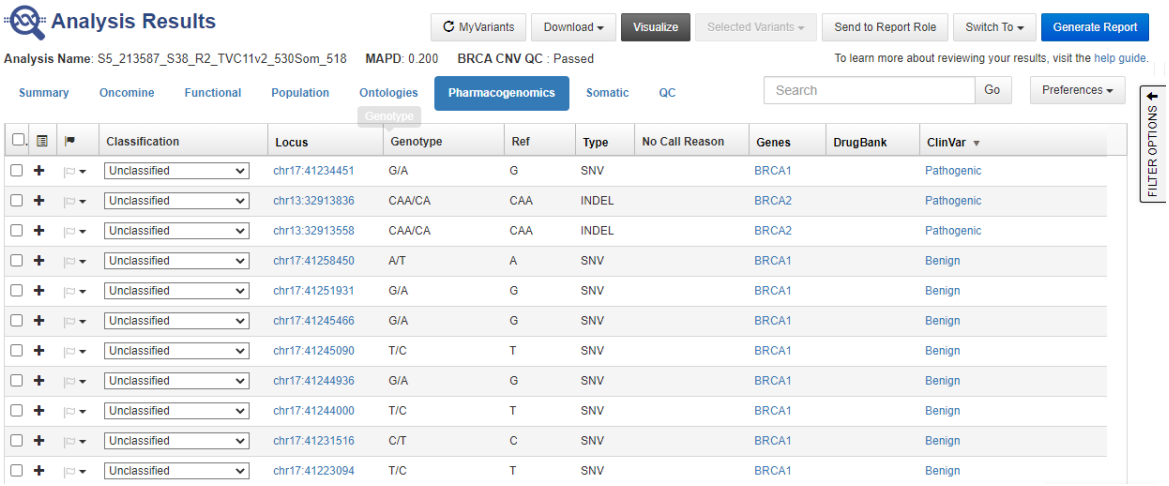
Item	Description
Analysis Name	The name of the analysis.
Cancer Type	The type of cancer, as defined by the sample attribute in Torrent Suite™ Software, if sample data are transferred by Torrent Suite™ Software.
MAPD	The MAPD (Median of the Absolute values of all Pairwise Differences) metric is an estimate of coverage variability between adjacent amplicons. The default threshold is 0.5. As a result, sample results with a MAPD above this value should be viewed with lower confidence.
BRCA CNV QC	A quality control score that is based on the MAPD threshold, and quality scores for the percent of non-zero amplicons, total number of reads, and the number of calls. **BRCA CNV QC** is PASSED or FAILED. If the **BRCA CNV QC** fails, a reason is provided.
Sample ID	**Note:** If the sample data is transferred from Torrent Suite™ Software and the **SampleID** plugin was run, a Sample ID is displayed at the top of the **Analysis Results** screen, and on the **IRGV & Generate Report** tab in the **Sample Information** section.

- Click **Summary** to view a summary of the called variants. Select a classification from the dropdown list to assign a classification to a variant. The following variant types are available for a BRCA analysis.

Type	CNV Subtype	Description
CNV	BigDel	Deletion of at least one exon
	BigDup	Duplication of at least one exon
	GeneCNV	Whole BRCA1/BRCA2 gene deletion or duplication
	NOCALL	Read count differs from baseline by non-integer amount; evidence for a BigDel or BigDup call is weak
	REF	Read count matches reference baseline
LongDel	—	A specific 40 bp deletion in BRCA1 (c.1176_1214del)
SNV	—	Single nucleotide substitution
MNP	—	Multiple nucleotide polymorphism at adjacent nucleotide positions
INDEL	—	Single or multiple nucleotide insertion or deletion

- Click **Functional** to view other functional annotations and use the annotations to classify, sort, and filter variants.

- Click **Pharmacogenomics** to view the **ClinVar** column. Click the link in the **ClinVar** column for a selected variant to open an NCBI ClinVar website where information about the ClinVar variant annotation is available.



5. Click **Visualize** to view a **BRCA Report** that shows read counts of BRCA1 and BRCA2 exons that are normalized to the Oncomine BRCA DNA Baseline. For more information, see "Visualize the BRCA report" on page 327.

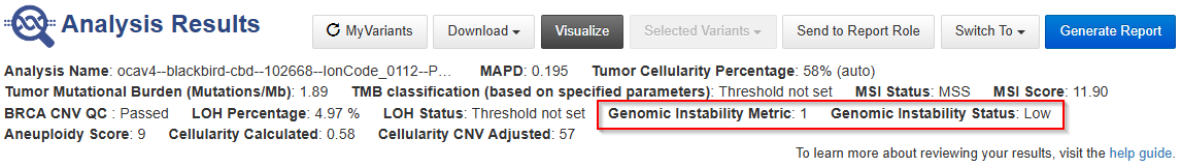
View Genomic Instability Metric (GIM) in analysis results

The Genomic Instability Metric (GIM) is a proprietary measurement that quantifies genomic scarring associated with Homologous Recombination Deficiency (HRD). Starting with Ion Reporter™ Software 5.20, Oncomine™ Comprehensive Assay Plus analyses report GIM.

GIM summarizes unbalanced copy number changes in autosomes that are determined using genomic segmentation, and the software generates a value between 0–100. Higher GIM values correlate with the observation of more genomic instability in the sample.

In addition to GIM, the Genomic Instability Status is also reported. Genomic Instability Status compares GIM to a threshold. Genomic Instability Status is reported as high if GIM is equal to or greater than the threshold or low otherwise. By default, the threshold is set at 16, which is based on ovarian cancer. If a threshold is not set, *Threshold not set* is reported. If GIM cannot be calculated, a value of *no call* is set.

1. In the **Analyses** tab, click **Overview**.
2. Search, sort, or filter the **Analyses** table to find the sample result of interest.
3. In the **Analysis** column, click the hyperlink for the analysis of interest to open **Analysis Results** screen.
   GIM and Genomic Instability Status are listed below the **Analysis Results** screen header (red box).



Immune repertoire analysis results

Analyses that are performed in Ion Reporter™ Software with Oncomine™ immune repertoire analysis workflows report the frequency and sequence information for clonotypes, and provide other data results, such as clone frequency and Jaccard Similarity indices. There are interactive graphs and plots that you can use to adjust views of the data and access details about clones in the software. You can also download the graphs and plots that you have adjusted, or other static graphics that are available in PNG or PDF file formats. You can download static plots in PDF format that can be added to slides for presentations.

The immune repertoire analysis results also include sample and quality control results for each sample that is included in the analysis.

View immune repertoire analysis results

If you use an immune repertoire analysis workflow, you can view the analysis results in Ion Reporter™ Software.

To perform a multi-sample analysis, see “Compare the immune repertoire between samples” on page 261.

Note: You can search analyses from the Oncomine™ TCR Beta-LR Assay and the Oncomine™ BCR IGH-LR Assay by analysis name, sample name, and project. You cannot search these analyses by barcode.

---

1. In the **Analyses** tab, click **Overview**.
2. In the **Workflow** list, select an immune repertoire analysis workflow to limit the list of results to immune repertoire analyses.

   You can further refine the list of analyses with other filters, or click column headings to sort the list.
3. Click the analyses name hyperlink.
4. In the **Immune Repertoire Results** screen, click the hyperlinked **Sample** name to open the **Immune Repertoire Results** for that sample.
5. Select the **Sample Results**, **Sample QC**, or **Plugin Results** tab, then select the graphical representation of the data from the **Views** list.

Sample results for immune repertoire

Immune repertoire results are represented graphically in various plots and graphs on the **Sample Results** tab in the immune repertoire analysis results.

Select a results from the **Views** dropdown list.

Spectratyping plots

The immune repertoire within a sample is represented in each spectratyping plot by the range of CDR3 lengths and their pattern of distribution. Reads for identified clones are arranged along the X-axis according to the variable gene identity and the Y-axis according to the CDR3 nucleotide length. The variable gene order reflects the gene position within the IGH locus. Dots are separated vertically along the Y-axis by 3 nucleotides (one codon), the higher up the Y-axis the longer the CDR3 region. Circle size indicates the frequency of a particular variable gene-CDR3 nucleotide length combination within the dataset. Circle color represents a fourth metric specific to each graph (for example, Shannon Diversity, evenness, clone frequency). Key repertoire metrics are displayed along the lower margin of the plot.

You can visualize immune repertoire results in Ion Reporter™ Software with spectratyping plots for:

Spectratyping plot	More information
V-gene Mutation	"Example V-gene Mutation plots" on page 242
Evenness	"V-gene usage and evenness plot examples" on page 246
Shannon Diversity	"V-gene usage and Shannon Diversity plot example" on page 247
Largest Clone Frequency	"V-gene usage and largest clone frequency plot example" on page 248
Number of Clones	"V-gene usage and number of clones plot example" on page 249

In Ion Reporter™ Software, the spectratyping plots are interactive, allowing you to adjust the data and access clone details. For more information, see "Adjust data and access clone details in spectratyping plots" on page 242.

Note: T-cell Leukemia (Jurkat) Total RNA is derived from a cell line consisting of a single T-cell clonotype. Running the Oncomine™ TCR Beta-LR Assay on Jurkat Total RNA should detect a single clonotype (a single spot on spectratyping plot).
Sequencing of a B cell line such as Ramos will reveal a single dominating clonal lineage, indicated by a single spot on the spectratyping plot.

Adjust data and access clone details in spectratyping plots

You can adjust data and access clone details in the interactive spectratyping plots in Ion Reporter™ Software. You can also download a static image of the plot shown in the screen. When you adjust the plot view and download an image of the plot, the adjusted plot is downloaded.

1. Adjust the view of the data in the plot.

Option	Description
Limit the region (v-genes) that appear in the plot	Drag the ends of the horizontal bar below the X-axis.
Limit the clones that appear	Drag the ends of the color range up or down.

2. Access or close the clone details.

Option	Description
View the details of an individual clone	Place the pointer over a dot of interest.
Restore the default plot view	Click ↻.

3. Click ⬇ to download a static image of the plot.

Example V-gene Mutation plots

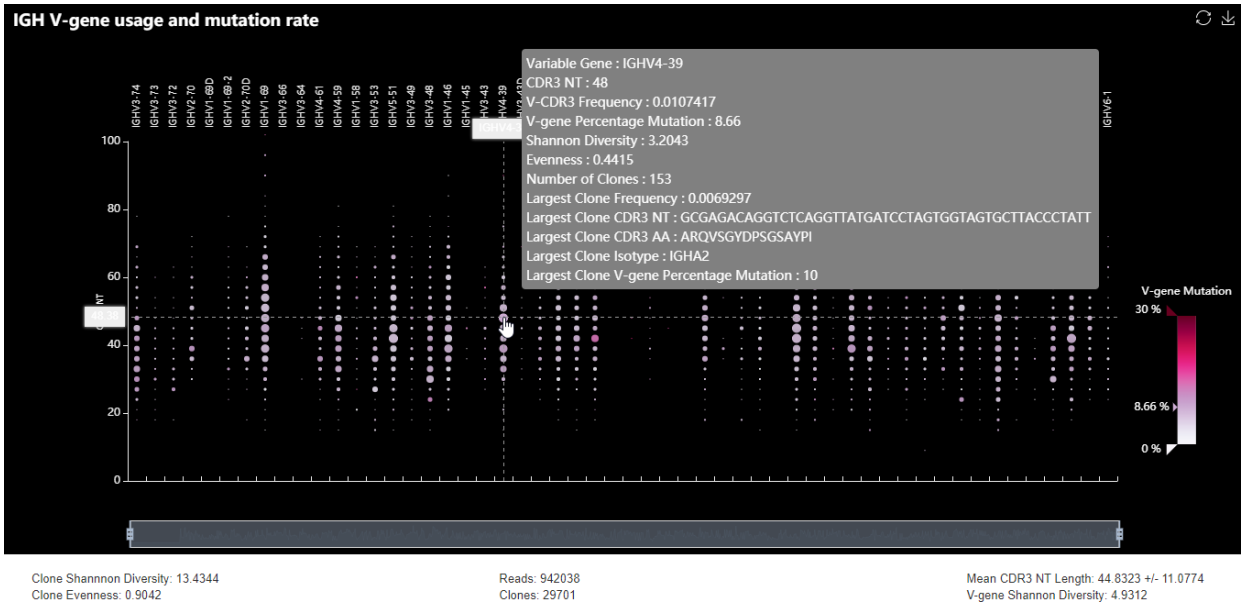
The following examples are **V-gene Mutation** plots for analyses from three different immune repertoire analysis workflows.

- Oncomine™ BCR IGH-LR Assay
- Oncomine™ BCR IGKL-SR Assay
- Oncomine™ TCR Gamma-SR Assay

Each analysis workflow generates spectratyping plots when you visualize analysis results it in Ion Reporter™ Software.

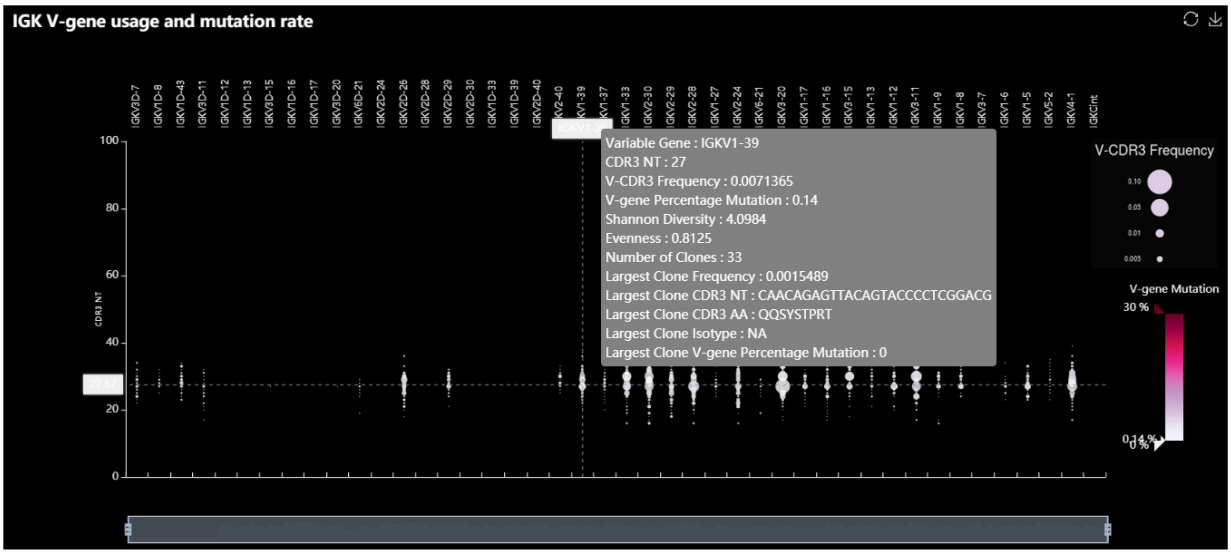
Select **V-gene Mutation** for a view of a spectratyping plot that highlights the frequency of mutated bases over the variable gene of identified clones.

IGH V-gene usage and mutation rate



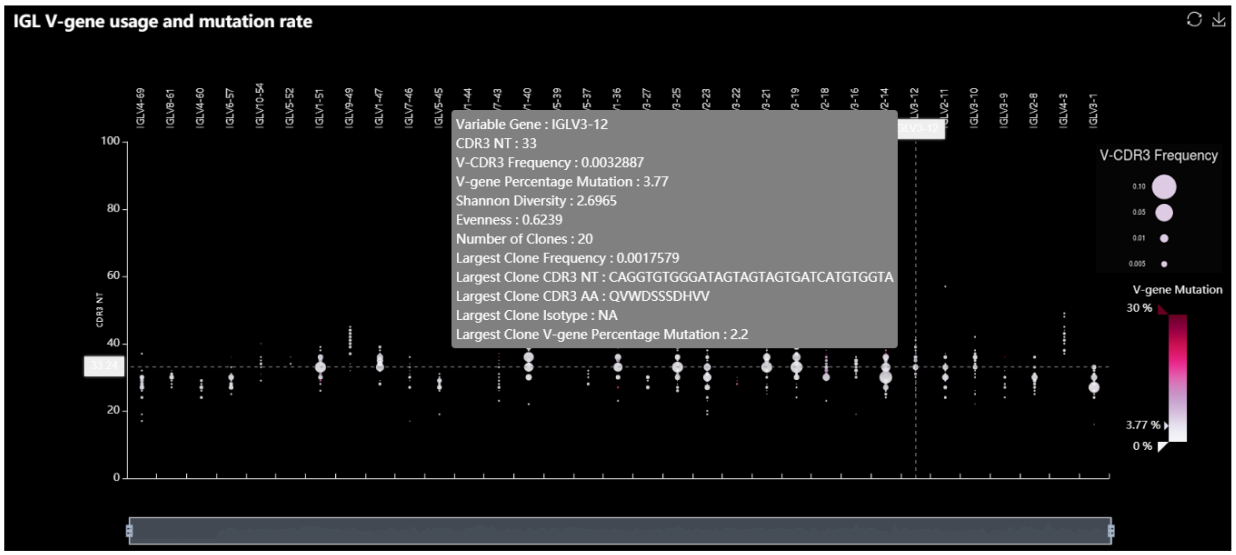
This is an example of an immunoglobulin heavy chain (IGH) spectratyping plot from an Oncomine™ BCR IGH-LR Assay analysis. Circle color indicates the average frequency of mutated bases for clones having a particular variable gene-CDR3 nucleotide length combination. B cells that have undergone isotype switching tend to have a higher frequency of somatic hypermutation than B cells expressing IgM or IgD isotypes, which tend to represent naive B cells. Systematic differences with respect to reference may indicate the presence of polymorphism within the variable gene that is not captured by the IMGT database. You can further partition the clones by isotype in Ion Reporter™ Software, with the buttons that are above the interactive spectratyping plot.

IGK V-gene usage and mutation rate



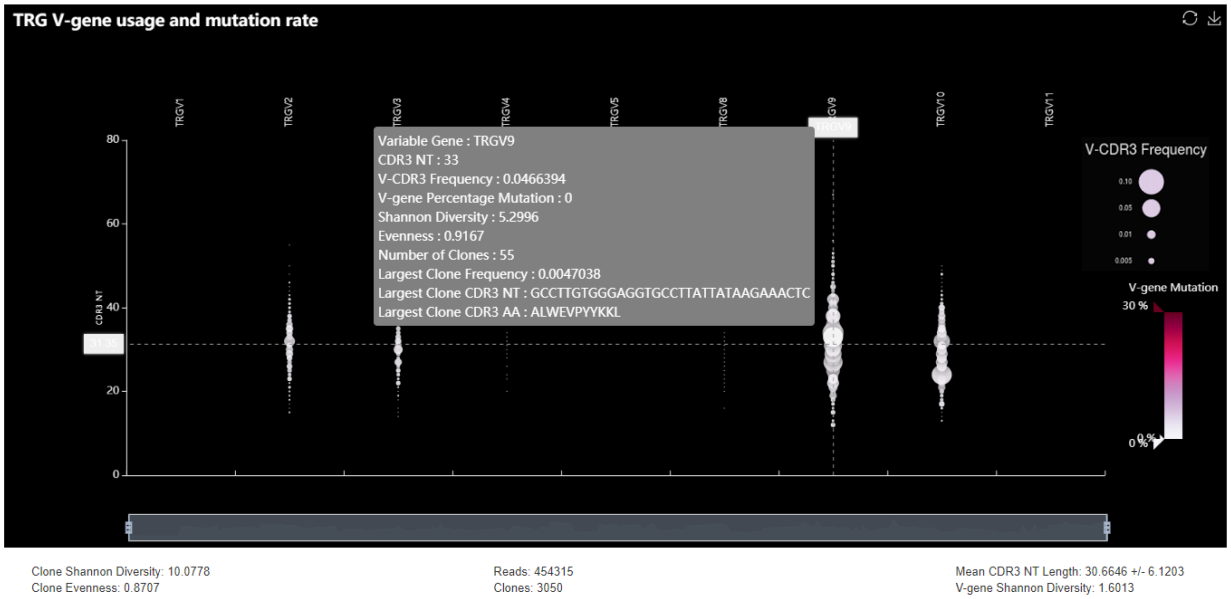
This is an example of an immunoglobulin light chain kappa (IGK) spectratyping plot from an Oncomine™ BCR IGKL-SR Assay analysis. The plot highlights the frequency of mutated bases over the variable gene of identified clones. Flanking region mutations that occur in the intron between the joining genes and the constant gene are shown in the immunoglobulin kappa constant intron (**IGKCint**) column. The circle color indicates the average frequency of mutated bases for clones that have a particular variable gene-CDR3 nucleotide length combination.

IGL V-gene usage and mutation rate



This is an example of an immunoglobulin light chain lambda (IGL) spectratyping plot from an Oncomine™ BCR IGKL-SR Assay analysis. The plot highlights the frequency of mutated bases over the variable gene of identified clones. The circle color indicates the average frequency of mutated bases for clones that have a particular variable gene-CDR3 nucleotide length combination.

TRG V-gene usage and mutation rate

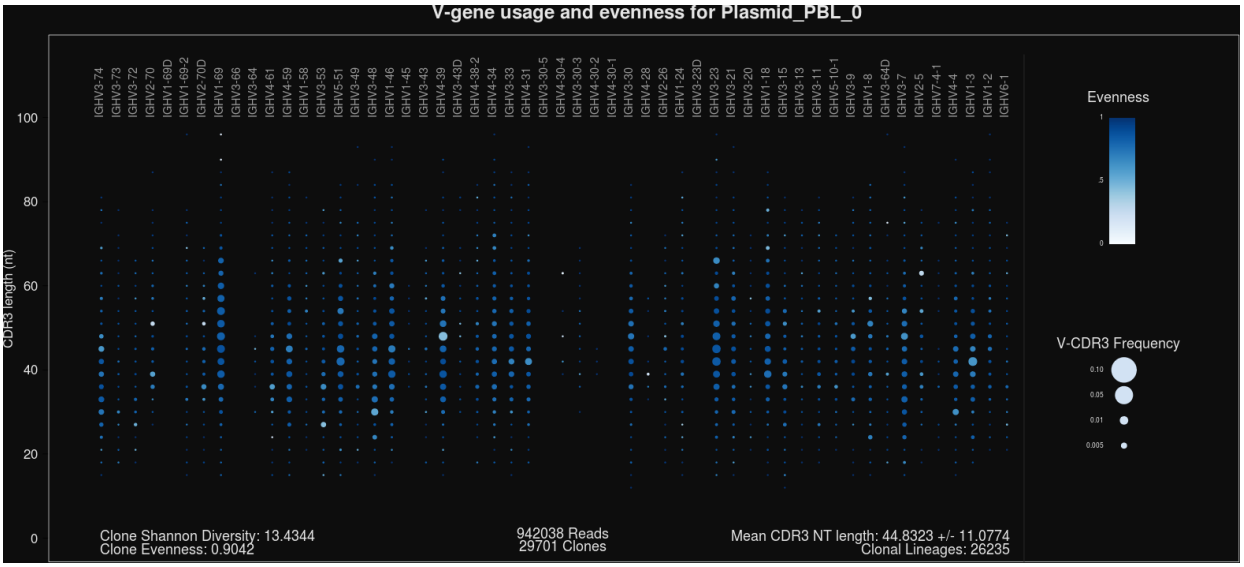


This is an example of a T-cell receptor gamma (TRG) spectratyping plot from an Oncomine™ TCR Gamma-SR Assay analysis. The plot highlights the frequency of mutated bases over the variable gene of identified clones. The circle color indicates the average frequency of mutated bases for clones that have a particular variable gene-CDR3 nucleotide length combination.

V-gene usage and evenness plot examples

IGH V-gene usage and evenness

Select **Evenness** for a view of a spectratyping plot that highlights evenness of identified clone sizes (Normalized Shannon Entropy).



This is an example of an immunoglobulin heavy chain (IGH) V-gene usage and evenness spectratyping plot from an Oncomine™ BCR IGH-LR Assay analysis. Circle color indicates the evenness of clone sizes for clones having a particular variable gene-CDR3 nucleotide length combination. Values range from 0 to 1, with 1 indicating most even clone sizes. In this representation, portions of the repertoire containing highly expanded clones appear white. You can further partition the clones by isotype in Ion Reporter™ Software, with the buttons that are above the interactive spectratyping plot.

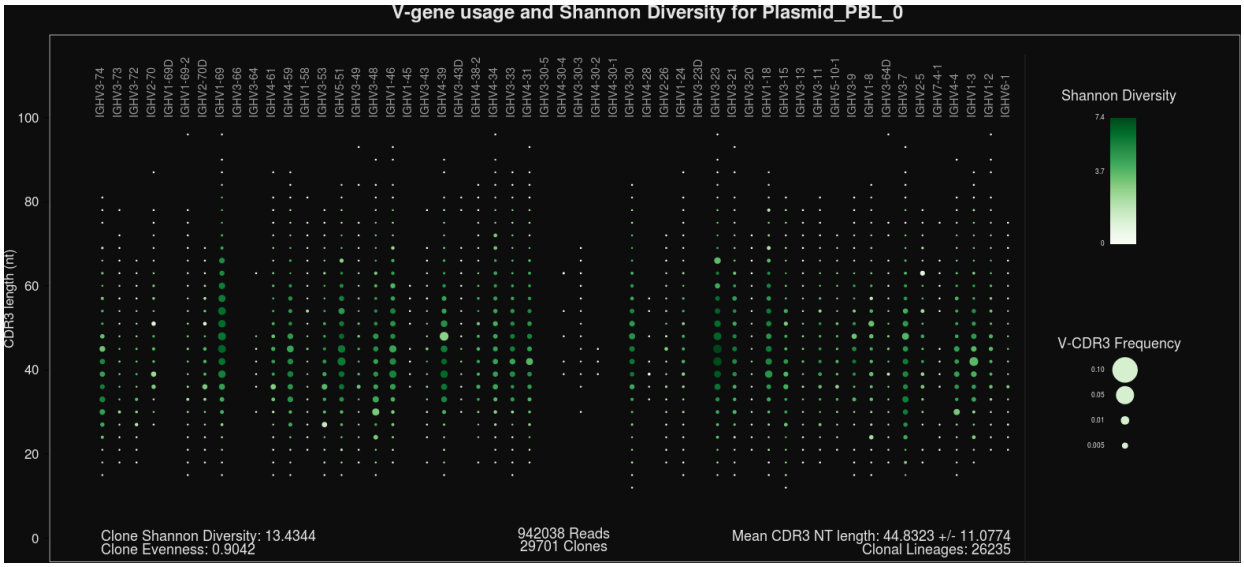


This is an example of an IGH V-gene usage and evenness spectratyping plot from an Oncomine™ IGHV Leader-J Assay analysis.

V-gene usage and Shannon Diversity plot example

IGH V-gene usage and Shannon Diversity

Select **Shannon Diversity** for a view of a spectratyping plot that highlights the Shannon Diversity (entropy) of identified clones.

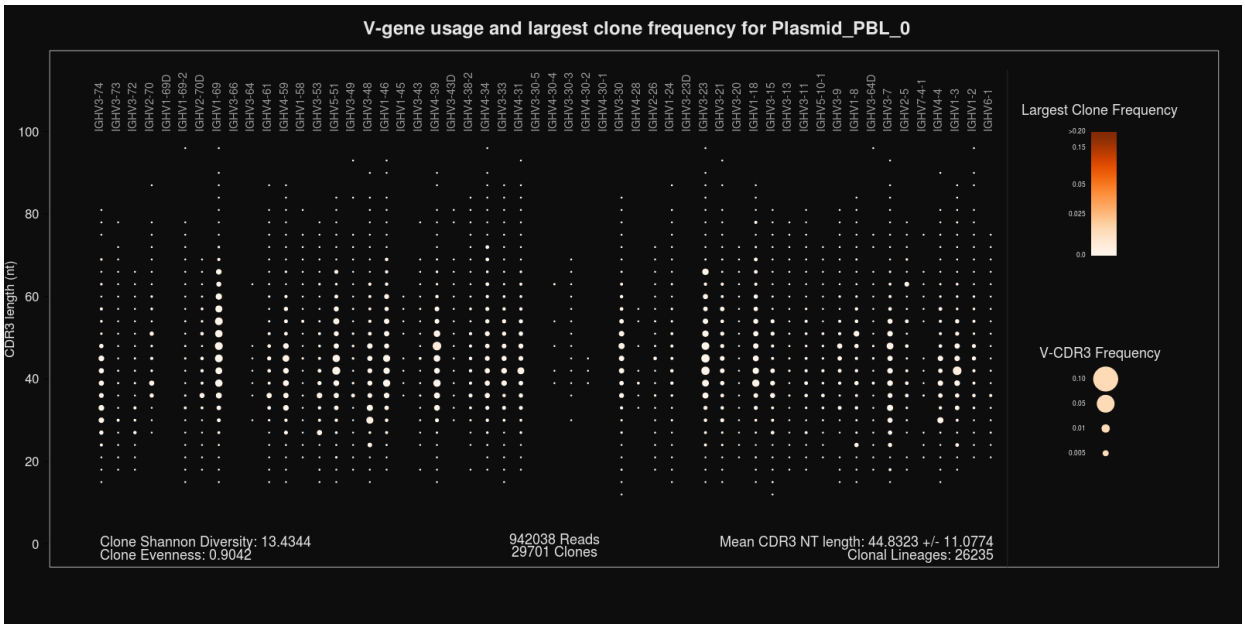


This is an example of an IGH spectratyping plot from an Oncomine™ BCR IGH-LR Assay analysis. Circle color indicates the Shannon Diversity of clones having a particular variable gene-CDR3 nucleotide length combination. Portions of the repertoire containing highly expanded clones typically have a corresponding low Shannon Diversity value. You can further partition the clones by isotype in Ion Reporter™ Software with the buttons that are above the interactive spectratyping plot.

V-gene usage and largest clone frequency plot example

IGH V-gene usage and largest clone frequency

Select **Largest Clone Frequency** for a view of a spectratyping plot that highlights the frequency of the largest clone for each variable gene-CDR3 nucleotide length combination.

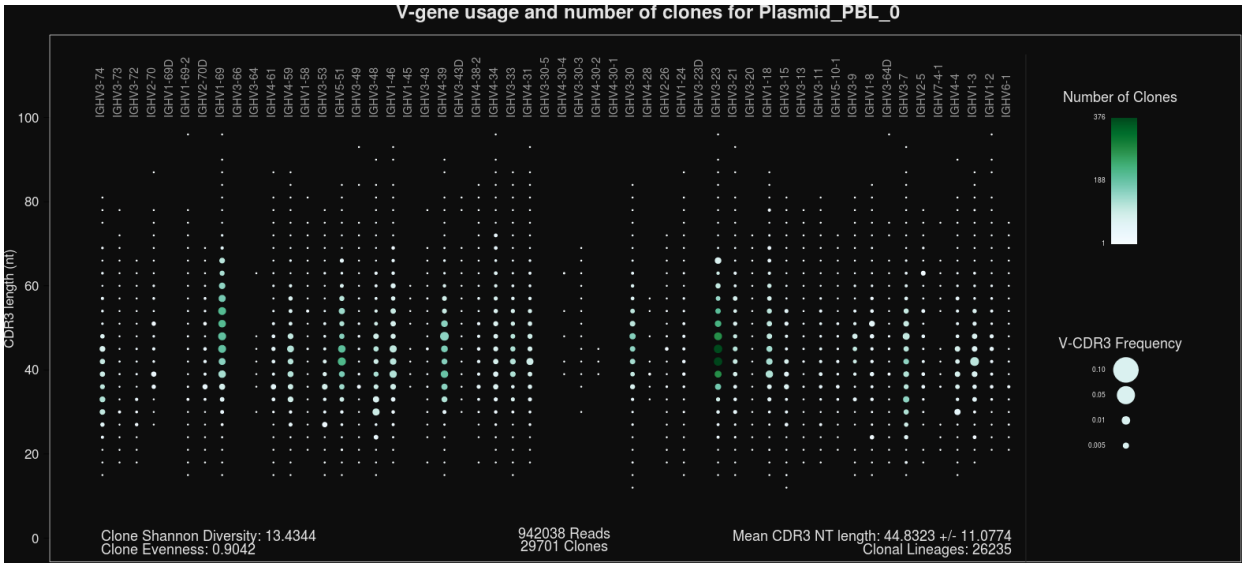


This is an example of an IGH V-gene usage and largest clone frequency spectratyping plot from an Oncomine™ BCR IGH-LR Assay analysis. Circle color indicates the frequency of the largest clone having a particular variable gene-CDR3 nucleotide length combination. Dark color indicates the presence of expanded clones. This is a polyclonal repertoire that lacks highly expanded clones. You can further partition the clones by isotype in Ion Reporter™ Software, with the buttons that are above the interactive spectratyping plot.

V-gene usage and number of clones plot example

IGH V-gene usage and number of clones

Select **Number of Clones** for a view of a spectratyping plot that highlights the number of clones that are identified for each variable gene-CDR3 nucleotide length combination.



This is an example of an IGH V-gene usage and number of clones spectratyping plot from an Oncomine™ BCR IGH-LR Assay analysis. Circle color indicates the number of clones having a particular variable gene-CDR3 nucleotide length combination. Specific variable genes may more frequently participate in VDJ recombination, leading to an enrichment in distinct clones for those variable genes. You can further partition the clones by isotype in Ion Reporter™ Software, with the buttons that are above the interactive spectratyping plot.

Spectratyping overview plot

Spectratyping overview plots highlight the variable gene mutation, evenness, diversity, number of clones, and largest clone frequency for each variable gene-CDR3 nucleotide length combination, with further subdivision of the data by isotype. For the Oncomine™ BCR IGH-LR Assay analysis workflow, ordering of the isotypes reflects their position within the IGH locus.

Overview plots are generated for all immune repertoire assays used with the analysis workflow, and are available only in the compressed folder of analysis results. For more information, see “Download spectratyping overview plots” on page 250.

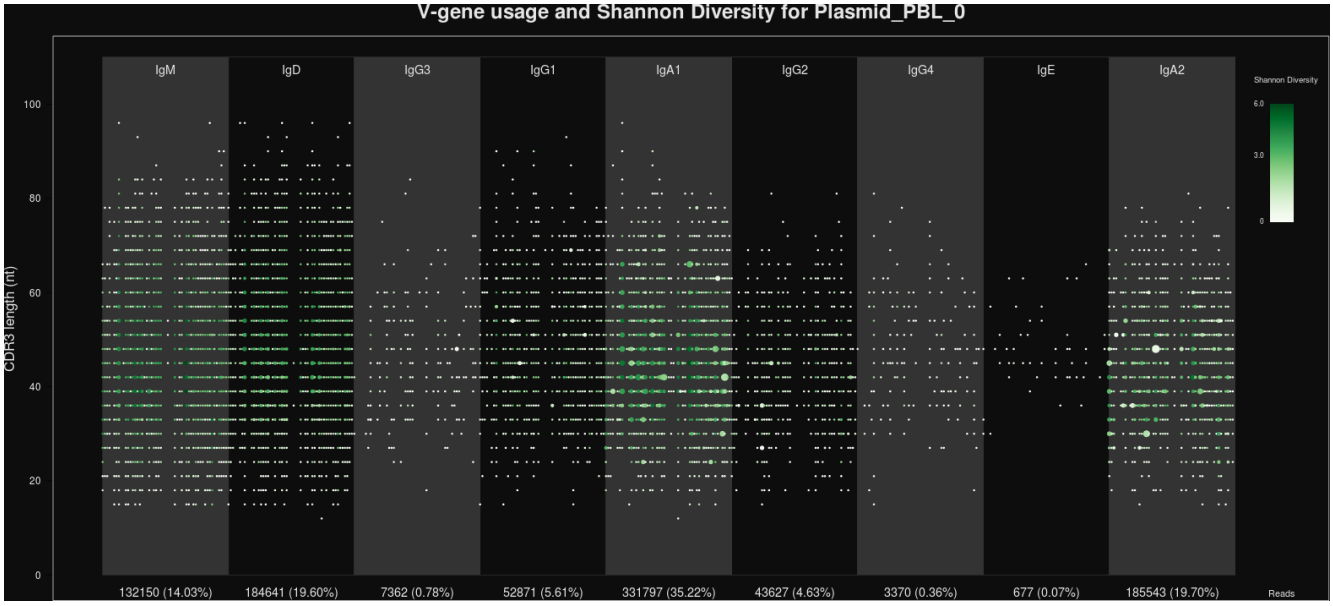


Figure 1 IGH V-gene usage and Shannon Diversity spectratyping overview plot

Download spectratyping overview plots

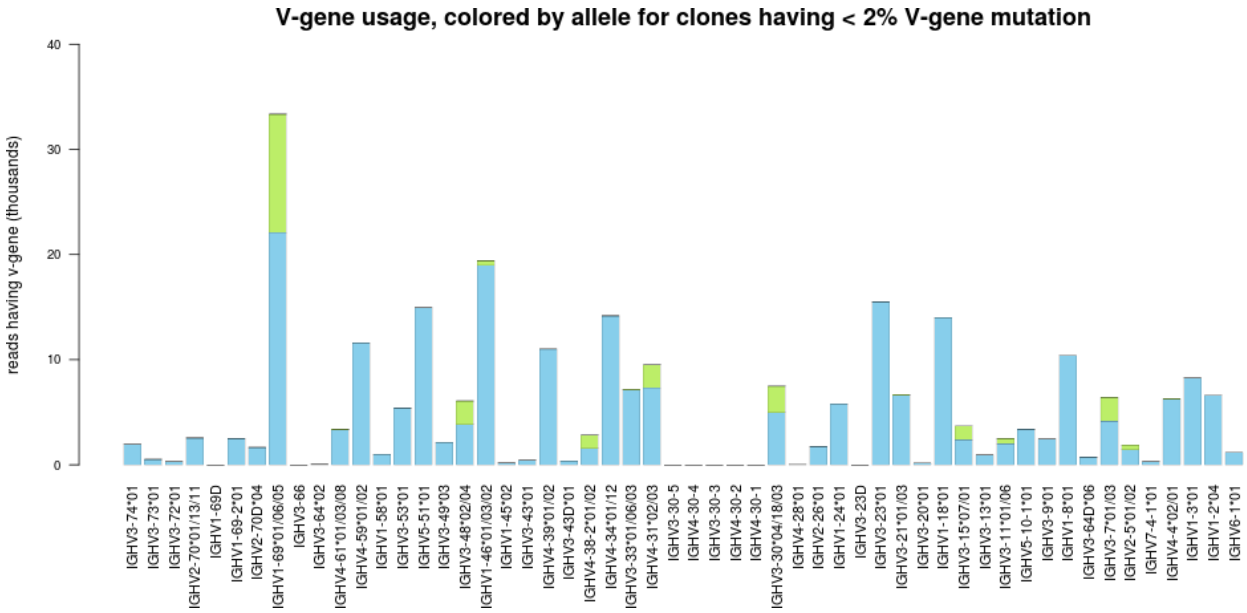
Spectratyping overview plots are generated for all immune repertoire assays, and are available only in the compressed folder of analysis results.

1. In the **Analyses** tab, click **Overview**.
2. Click the analysis name hyperlink.
3. In the **Immune Repertoire Results** screen, in the **Actions** column for the analysis of interest, click **Download**.
   A ZIP file that contains PNG and PDF files of the plots and other results files is downloaded.

V-gene usage

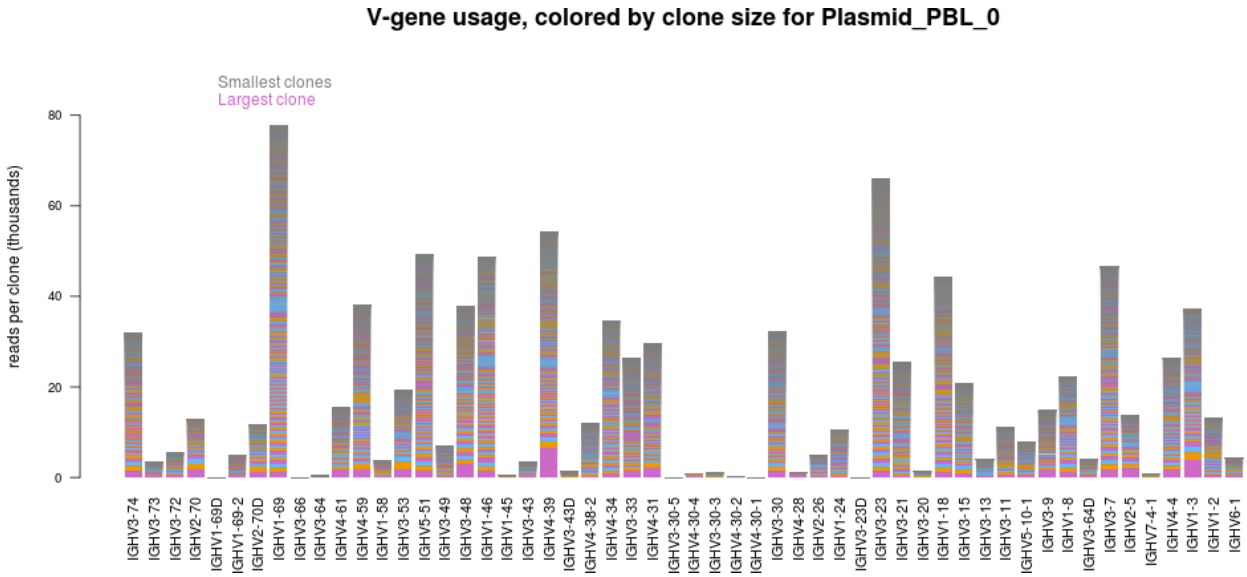
Immune repertoire analysis results report the frequency of variable genes and the variable gene allele. Select **V-gene usage** from the **Views** list to see graphs that represent the results. Stacked barplots indicate the representation of variable genes among identified clones.

V-gene usage for alleles



Color segments within each bar indicate the frequency of particular variable gene alleles, arranged by frequency from rarest (top) to most common (bottom), for clones having <2% variable gene mutation. This cutoff is used to avoid noise in allele identification caused by somatic hypermutation.

V-gene usage for clone sizes

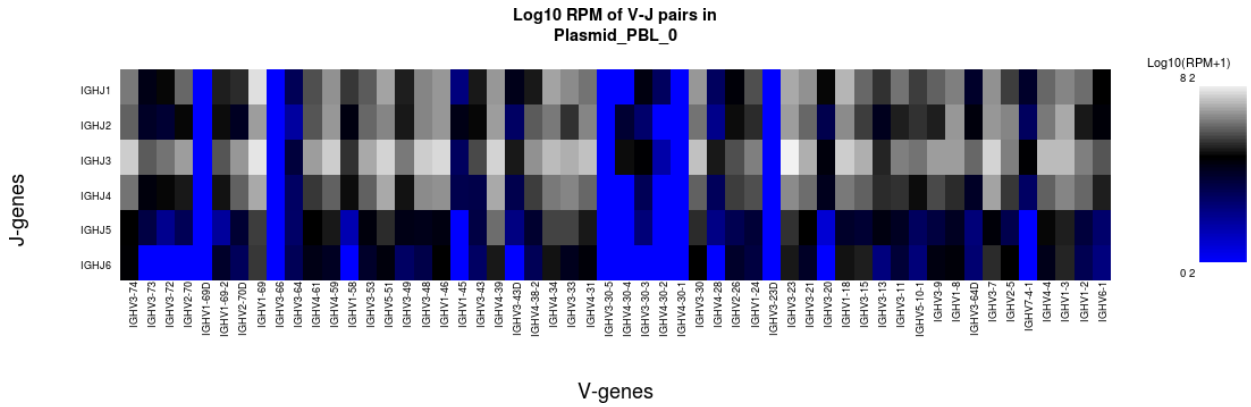


Color segments in each bar indicate the contribution of individual clones, arranged from smallest clones (top) to largest clones (bottom).

VJ-gene usage heatmap

Immune repertoire analysis results report Variable gene-Joining gene (VJ-gene) combinations for identified clones. Select **VJ- gene usage heatmap** from the **Views** list to see heatmaps that represent the results.

VJ-gene usage heatmap example

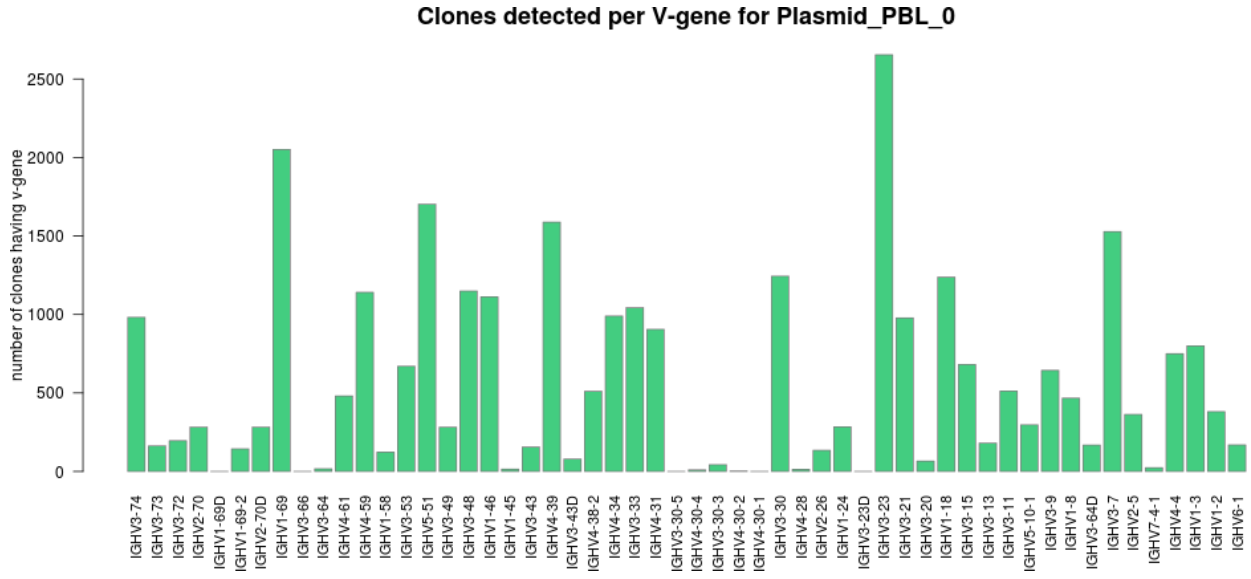


Heatmap illustrating the frequency of particular Variable gene-Joining gene combinations for identified clones. Frequencies are log transformed with pseudocount added. Over-represented Variable-Joining gene combinations (white) can indicate the presence of a highly expanded clone.

Clones detected per variable gene

Immune Repertoire analysis results are represented graphically in the **Sample Results** tab of the results screen for the selected analysis.

Select **Clones detected per variable gene** from the **Views** list.

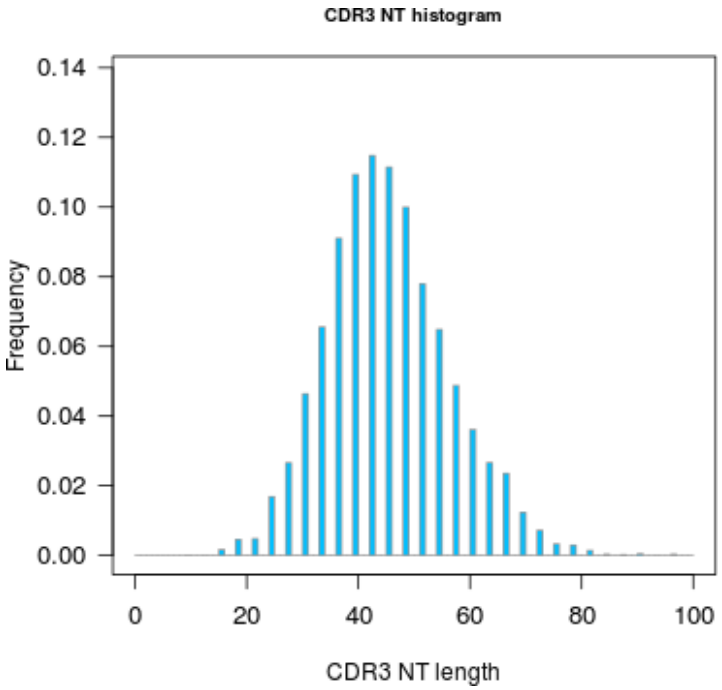


The bar plot indicates the number of identified clones having a particular variable gene. Ordering of variable genes reflects position within the IGH locus.

CDR3 histogram

Immune repertoire analysis results report the CDR3 lengths of clones that are identified in a sample. Select **CDR3 histogram** from the **Views** list to see a histogram that represents the data.

Relative frequency (Y-axis) of identified clones with a given CDR3 nucleotide length (X-axis)



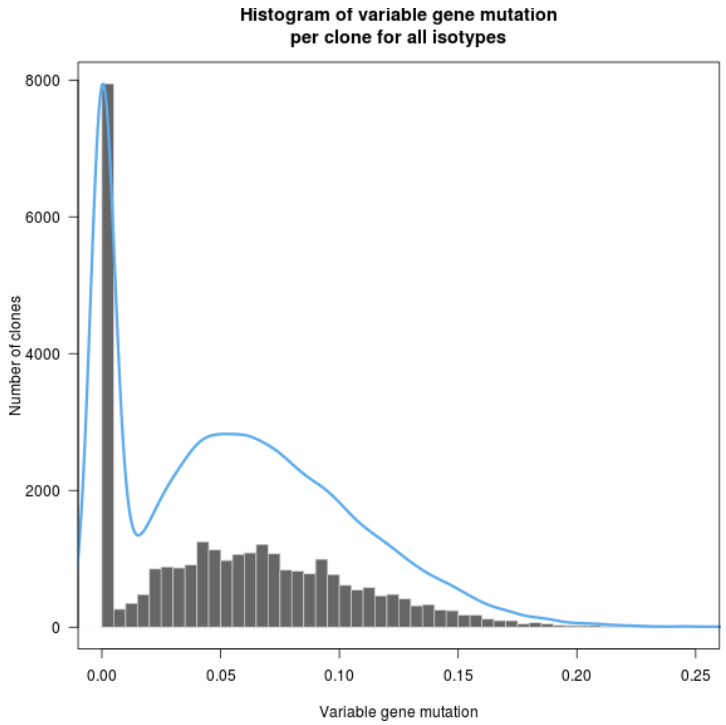
The histogram indicates the distribution of CDR3 lengths for clones that are identified in the sample.

Somatic hypermutation profile

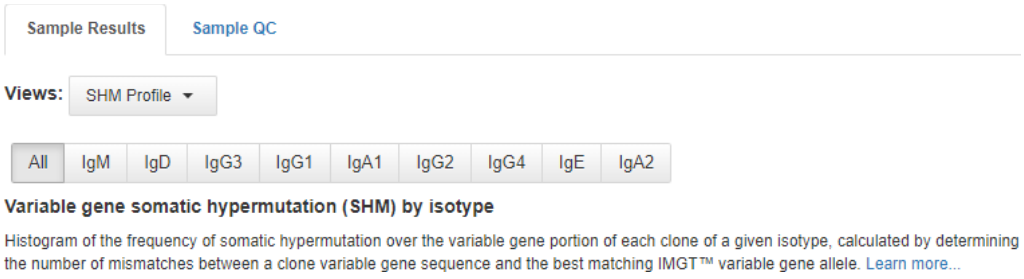
Select **SHM profile** from the **Views** list to see a histogram that represents the data. Immune repertoire analysis results report the frequency of somatic hypermutation (SHM) over the variable gene portion of each clone of a given isotype. This view is available only for the Oncomine™ BCR IGH-LR Assay and Oncomine™ BCR IGH-SR Assay.

Somatic hypermutation (SHM) is determined through a calculation of the number of mismatches between a clone variable gene sequence and the closest matching IMGT® variable gene allele.

Variable gene somatic hypermutation (SHM) by isotype



Click the isotype above the **SHM profile** plot to further partition the data by isotype.

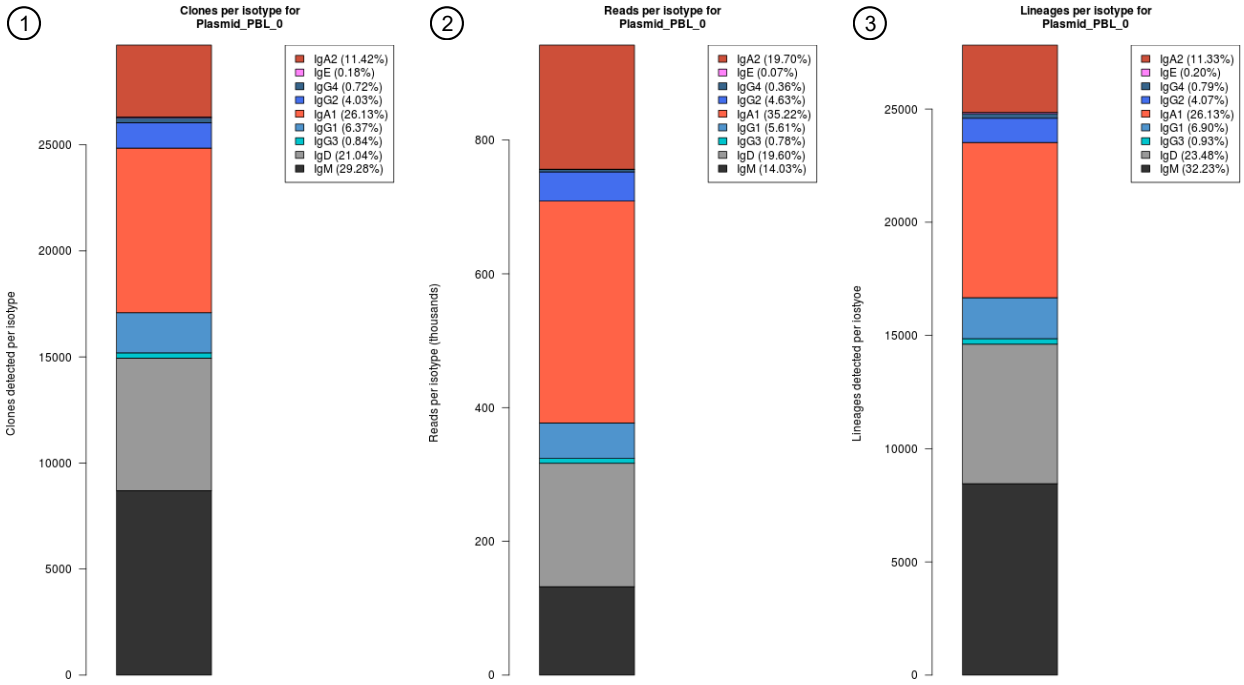


Isotype usage

Immune repertoire analysis results report isotype representation within a sample, which is calculated through either the number of reads per isotype, the number of clones per isotype, or the number of lineages per isotype. This view is available only for the Oncomine™ BCR IGH-LR Assay. Select **Isotype usage** from the **Views** list to see the graphs that represent the data.

Ordering of the isotypes reflects their position within the IGH locus. B cells of plasmablast or plasma cell type express the BCR at a higher level than memory or naïve B cells. Therefore these cell types have an outsized contribution to the reads per isotype plot, but less so for calculations made at the clone or lineage level.

Isotype usage example



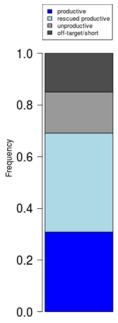
① Reads per isotype
② Clones per isotype
③ Lineages per isotype

QC Metrics for immune repertoire results

In the **Sample QC** tab, select a QC metric from the **Views** list to see a graphical representation of QC metrics.

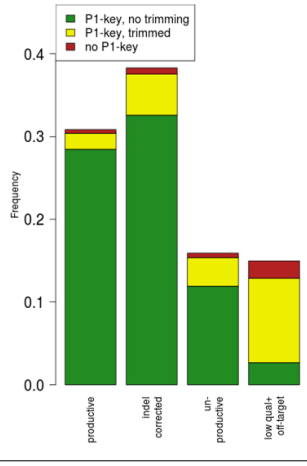
Read classification

After the first stage of data processing, raw sequencing reads are classified and proportionally represented in a stacked bar plot. Actual read counts for each classification are listed below the figure in the results report.

Read classification	Description	Example
Off-target/low-quality (dark gray)	Reads that are of low quality or represent the product of an off-target amplification.	
Unproductive (gray)	Reads that have uncorrectable sequencing or PCR errors that lead the rearrangement to have out-of-frame variable and joining genes or a premature stop codon.	
Rescued productive (light blue)	Reads that have an in-frame variable and joining gene, and no stop codons after INDEL error correction.	
Productive (blue)	Reads that have an in-frame variable and joining gene, and no stop codons.	

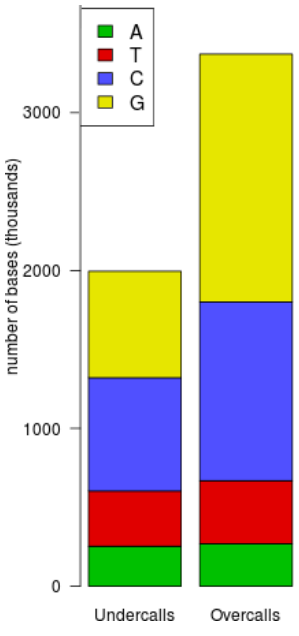
Proportion of full length, quality trimmed and reads lacking P1-key, by read classification

Stacked bar plot indicating the frequency of quality trimming for reads classified as productive, rescued productive, unproductive, and off-target/low-quality. Full length reads categorized as low quality/off-target likely represent off-target amplifications.

Color	Description	Example
Red	Quality trimmed reads lacking an identifiable P1-key.	
Yellow	Quality trimmed reads having an identified P1-key.	
Green	Full length reads having an identified P1-key, and no quality trimming.	

Base composition of overcalled and undercalled homopolymers

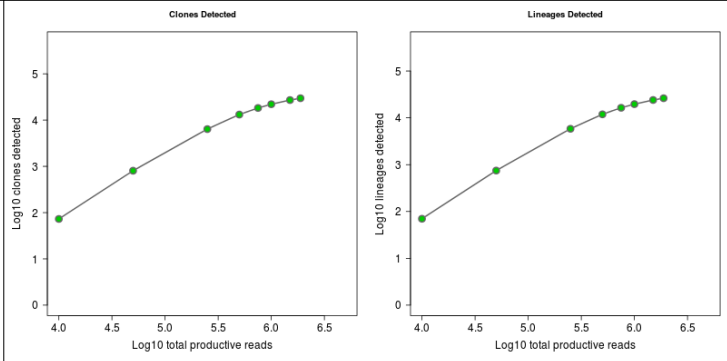
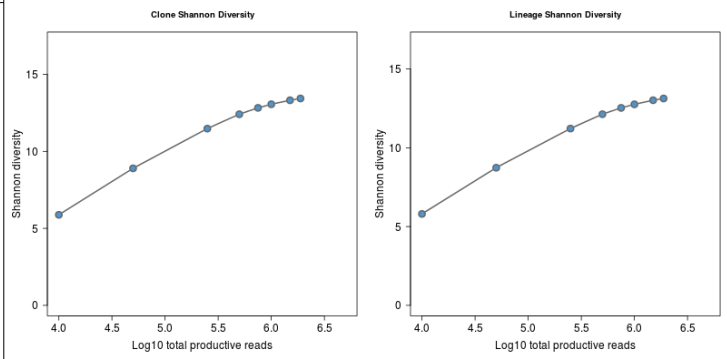
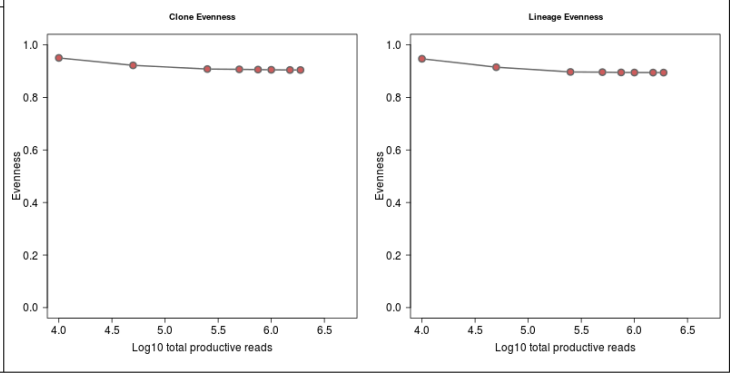
Stacked bar plot indicating the nucleotide composition of overcalled bases (base insertion sequencing errors) and undercalled bases (base deletion sequencing errors). Highly skewed nucleotide composition can indicate lower quality sequencing or low library diversity.

Color	Description	Example
Yellow	Guanidine (G)	
Blue	Cytosine (C)	
Red	Thymidine (T)	
Green	Adenosine (A)	

Downsampling analysis

Downsampling is achieved by repeating clone identification and measurement of repertoire features using 10 K, 50 K, 250 K, 500 K, 750 K, 1 M, 1.5 M, 2 M, and 5 M randomly selected productive and rescued productive reads, contingent on sequencing depth. The graphs show the effect of sequencing depth on select repertoire features: number of clones detected, lineages detected, clone & lineage evenness, and the clone & lineage Shannon diversity. Values for these repertoire metrics that are displayed in this plot are provided in the metrics file.

Clone summary and lineage summary files that are derived from downsampled data are provided in the 'downsampling' subdirectory of the zipped results download file. If insufficient reads are available for a particular downsampling depth, the corresponding fields are assigned a 'NA' value in the metrics file.

Color	Description	Example
Green	Clones & Lineages detected	
Blue	Clone & Lineage Shannon diversity	
Orange	Clone & Lineage Evenness	

QC metrics

The QC metrics include the read classification counts and strand QC metrics.

Category	Description
Read classification	
Total productive reads	Productive + rescued productive reads.
Productive reads	Reads having an in-frame variable and joining gene, and no stop codons.
Rescued productive reads	Reads having an in-frame variable and joining gene, and no stop codons after INDEL error correction.
Unproductive reads	Reads that have uncorrectable sequencing or PCR errors that lead the rearrangement to have out-of-frame variable and joining genes or a premature stop codon.
Off-target/ low-quality	Reads which are of low quality or represent the product of an off-target amplification.
Strand QC metrics	
Plus strand (v-side) read counts	Number of sequence read counts from the plus (+) strand.
Minus strand (c-side) read counts	Number of sequence read counts from the minus (–) strand.
Plus strand CDR3 avg PHRED	Average PHRED score for plus (+) strand reads.
Minus strand CDR3 avg PHRED	Average PHRED score for minus (–) strand reads.

Clonal lineage identification

Immune repertoire analysis results report B cell clonal linages. This view is available for both the Oncomine™ BCR IGH-LR Assay and Oncomine™ BCR IGH-SR Assay. The Oncomine™ BCR IGH-LR Assay provides additional information on the isotype representation among lineage members.

A B cell clonal lineage represents a set of B cells that are related by descent, arising from the same VDJ rearrangement event. B cells in a clonal lineage can differ at the sequence level owing to somatic hypermutation or isotype switching. Members of a clonal lineage are more likely to have a shared antigen specificity than members of different clonal lineages. Analysis of patterns of somatic hypermutation within clonal lineages can be used to infer patterns of isotype switching and identify IGH residues important for antigen recognition. For these reasons, the clonal lineage is a fundamental unit of B cell repertoire analysis. Ion Reporter™ Software automatically groups B cell clones into clonal lineages such that lineage members:

- Have the same variable and joining genes, excluding allele information.
- Have CDR3 regions of the same nucleotide sequence length.
- Have ≥85% CDR3 region sequence homology with one another.

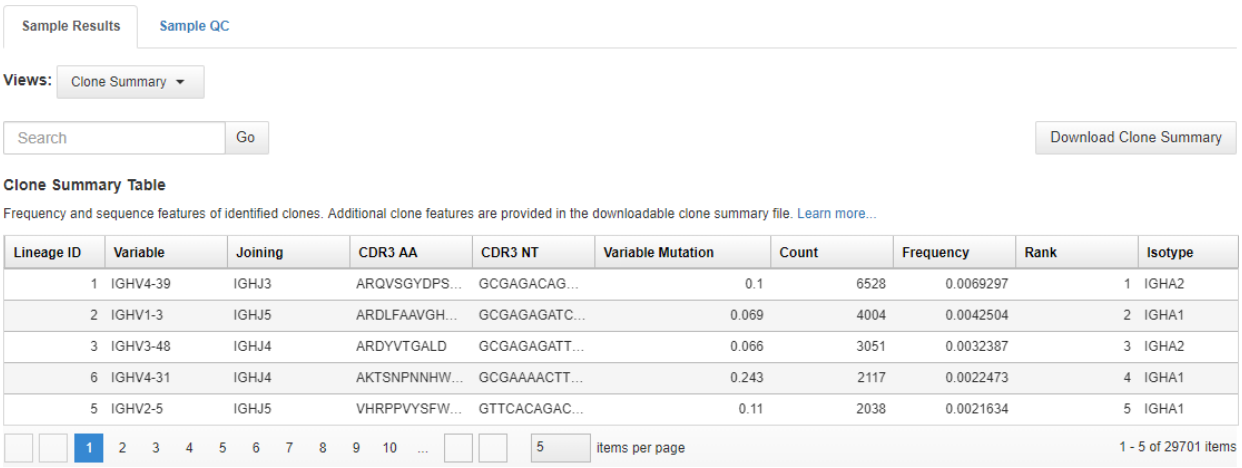
Each clone is assigned a Lineage ID. The ordering of the Lineage ID reflects the abundance ranking of the clonal lineage, which is calculated by determining the sum of the frequencies of all members of the clonal lineage. Lineage 1 corresponds to the most abundant lineage, followed by Lineage 2, until the least abundant lineage is reached.

The Lineage ID is displayed in the leftmost column of the clone summary and lineage summary views.

Clone Summary table

The **Clone Summary** table lists the identified clones in rank order from the most frequently occurring to least frequent. Each row represents an individual clone. Select **Clone Summary** from the **Views** list to see the table.

For each clone, the identified variable and joining region are listed as well as the amino acid (**CDR3 AA**) and nucleotide (**CDR3 NT**) sequences of the CDR3 region. The variable gene mutation, the isotype (Oncomine™ BCR IGH-LR Assay only), and the clonal lineage assignment (Lineage ID) are also listed.



Click the column heading cells to sort the table. Frequency = # of reads for the identified clone (**Count**) / total reported reads (sum of **Count** column).

For multi-sample analyses the **Clone Summary** table lists the frequency of each clone that is identified in any of the samples. The table is sorted in descending order that is based on the frequency of the clones in the leftmost column for each sample in the analysis. Although you can compare across multiple repertoire analysis workflows (that is, BCR IGH-SR versus BCR IGH-LR), in some instances BCR IGH-SR analysis results can include multiple variable gene assignments.



① Sample 1 ② Sample 2

The following table lists and describes the information that is available in the **Clone Summary** table.

Column name	Description
Lineage ID [1]	Lineage ID represents the rank order of the clonal lineage abundance. Calculated as the sum of the frequencies of all members of the clonal lineage. Lineage 1 corresponds to the most abundant lineage, followed by Lineage 2, until the least abundant lineage is reached.
Variable	The IMGT® variable gene determined by the software to have the highest sequence similarity to the rearranged sequence.
Joining	The closest matching IMGT joining gene of the rearrangement.
CDR3 AA	The CDR3 amino acid sequence of the rearrangement, denoted using the IMGT definition of the CDR3 region.
CDR3 NT	The CDR3 nucleotide sequence of the rearrangement, denoted using the IMGT definition of the CDR3 region.
Variable Mutation	The fraction of bases within the variable (V) gene that differ from the closest matching IMGT reference V gene, as determined by the highest alignment score assigned by the software.
Count	The total number of reads mapping to the rearrangement after quality filtering.
Frequency	The frequency of the rearrangement as a proportion of total reads passing quality filtering.
Rank	The frequency rank of the rearrangement.
Isotype	The isotype identified for the clone[2].

[1] This data is available only for the Oncomine™ BCR IGH-LR Assay and Oncomine™ BCR IGH-SR Assay.

[2] Isotype identification with the Oncomine™ BCR IGH-LR Assay only.

Note: Additional details are available by downloading the **Clone Summary** table.

Download clone summary and lineage summary files

- To download the **Clone Summary** metrics file.
  a. In the **Immune Repertoire Results** screen, **Sample Results** tab, select **Clone Summary** from the **Views** list.
  b. Click **Download Clone Summary**.
  The clone summary CSV file downloads automatically.
  c. Open the `.clone_summary` CSV file to view the additional information that is included in the spreadsheet.
- To download the **Lineage Summary** metrics file.
  a. In the **Immune Repertoire Results** screen, **Sample Results** tab, select **Lineage Summary** from the **Views** list.
  b. Click **Download Lineage Summary**.
  The lineage summary CSV file downloads automatically.

c. Open the `.lineage_summary` CSV file to view the additional information that is included in the spreadsheet.

Compare the immune repertoire between samples

Ion Reporter™ Software can perform multisample (or cross-sample) analysis to compare the immune repertoire between samples.

1. Under the **Analyses** tab, in the **Overview** screen, select the appropriate analysis workflow from the **Workflow** filter.
2. Select the adjacent checkboxes to select 2 or more results files from the available list of analyses, then click **Visualize** to generate a clone summary table.
3. Select samples for a multisample analysis to generate comparison plots.

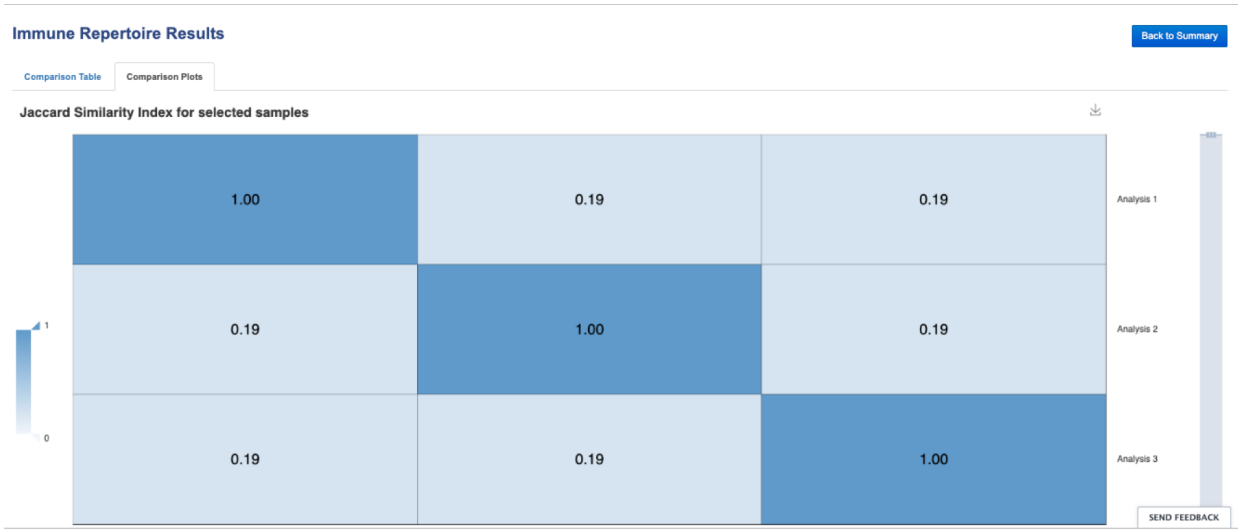
Option	Description
Select 2 samples, then click **Compare Samples**.	An interactive scatter plot for the selected samples is shown in the **Comparison Plots** tab.
Select 2 or more samples, then click **Compare Samples**.	A Jaccard Similarity Index for the selected samples is shown in the **Comparison Plots** tab.

- The visualized multisample analysis results are reported side-by-side in a clone summary table in rank order from most frequent to least frequent. For more information, see “Clone Summary table” on page 259.

  You can also download the multisample clone summary table or the multisample metrics file. For more information, see “Download clone summary and lineage summary files” on page 260.
- The interactive scatter plot indicates the frequency of clones across 2 samples. For more information, see “Example correlation and proportion of shared clones” on page 263.
- The Jaccard similarity index is determined for each pairwise comparison and is displayed in heat map form. For more information, see “Example Jaccard Similarity Index for selected samples” on page 262.

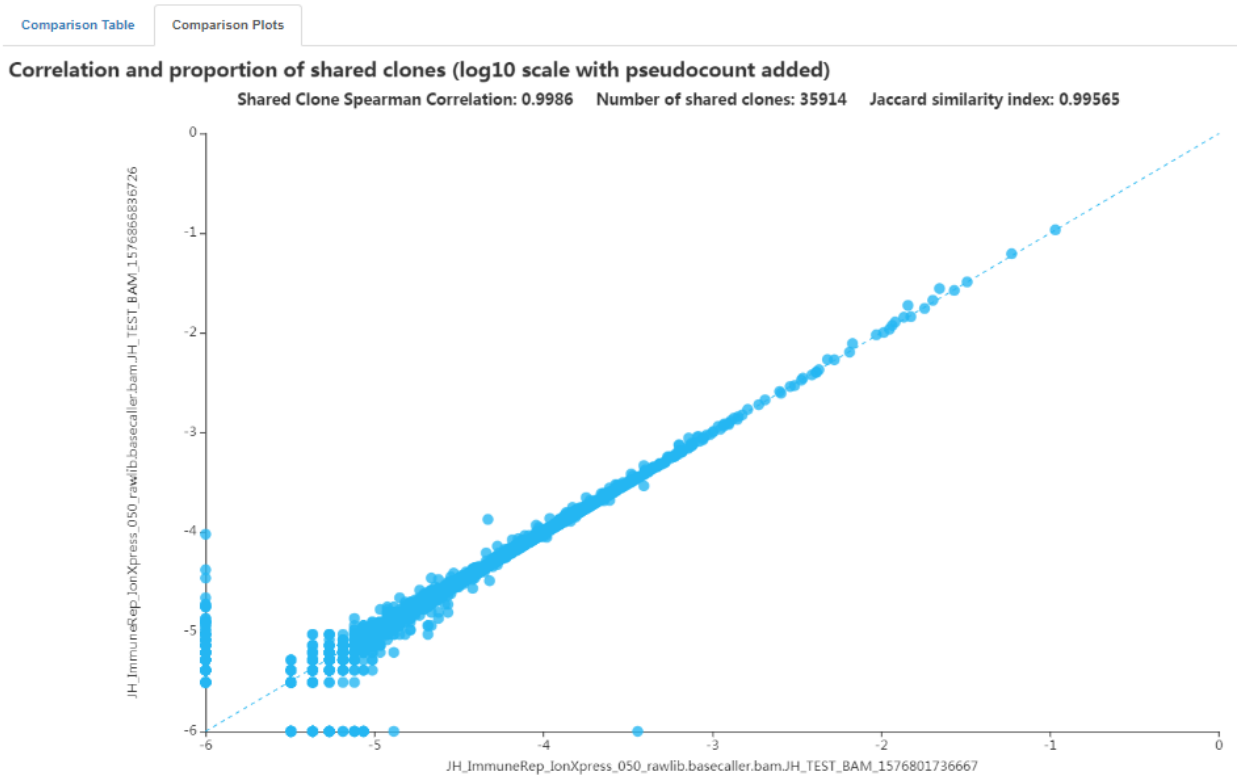
Example Jaccard Similarity Index for selected samples

This is an example of a **Comparison Plots** tab showing the Jaccard Similarity Index for selected samples. The Jaccard similarity index is determined for each pairwise comparison and displayed in heatmap form. The Jaccard Similarity Index ranges from 0 to 1 and is calculated as the total number of shared clones divided by the total number of distinct clones across two samples.



Example correlation and proportion of shared clones

This is an example of a **Comparison Plots** tab scatterplot. The illustration indicates the frequency of clones across two samples. Frequency values are log10 transformed with a pseudocount frequency of 1E-6 added to each value. Place the pointer over a point to see the CDR3NT and AA sequence of a clone, the variable mutation, and the frequency in either sample.



Microbiome health research analysis results

Analyses that are performed in Ion Reporter™ Software with the AmpliSeq Microbiome Health – w1.1 – Single Sample analysis workflow show analysis results from the detection of 73 bacterial species, and identification of bacterial species taxonomy for the microbial species that are present.

Microbiome health research analysis results are designed to help you study the potential impacts of the microbiome on human health, such as research on disease and chronic conditions, and the maintenance of a healthy immune system.

View microbiome health research analysis results

If you use the AmpliSeq Microbiome Health analysis workflow, you can view the analysis results in Ion Reporter™ Software.

To visualize multiple analyses, see “Visualize microbiome health research analysis results” on page 316.

1. In the **Analyses** tab, click **Overview**.
2. Click **Workflow** filter, then select an AmpliSeq Microbiome Health analysis workflow to narrow the list to microbiome health research analysis results.

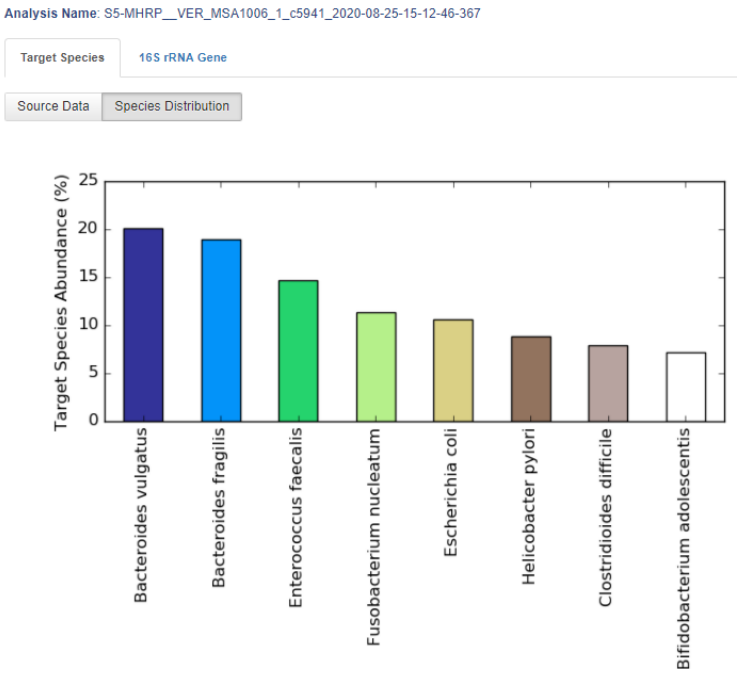
   You can further refine the list of analyses with other filters, or click column headings to sort the list.
3. Click the analyses name link.

   The **Analysis Results** screen opens.

   The following parameters are displayed.
   - Pool QC—Pool QC is determined to be either PASS or FAIL. If the total number of valid mapped reads is greater than the threshold applied and the mean read length of all the reads from the BAM file is greater than the threshold, pool QC is determined to be PASS, otherwise FAIL. If pool QC is determined to be FAIL, it means that library pool failed and the results are not valid.
   - Total Reads—The total number of filtered and trimmed reads independent of length reported in the output BAM file.
   - Total Valid Mapped Reads—Total number of reads mapped to the reference sequences with good mapping quality (reads with alignment score greater than the min local alignment score threshold).
   - Mean read length—The average length of all reads from the BAM file.
4. Select the **Target Species** tab, then click **Source Data** or **Species Distribution**.
   - The Ion AmpliSeq™ Microbiome Health Research – Target Species Pool detects 73 bacterial species that are associated with human disease. Click **Download Results** to download a file with a list of the 73 species.

Note: Some of the primer pairs designed to detect the 73 targeted species can sometimes amplify strains of other non-targeted species. As a result, an additional 16 species can be reported as well. When such species are detected the species names are reported with (*), indicating that it is one of the non-targeted species.

- The **Species Distribution** view displays a bar chart of the abundance of the species that are detected.

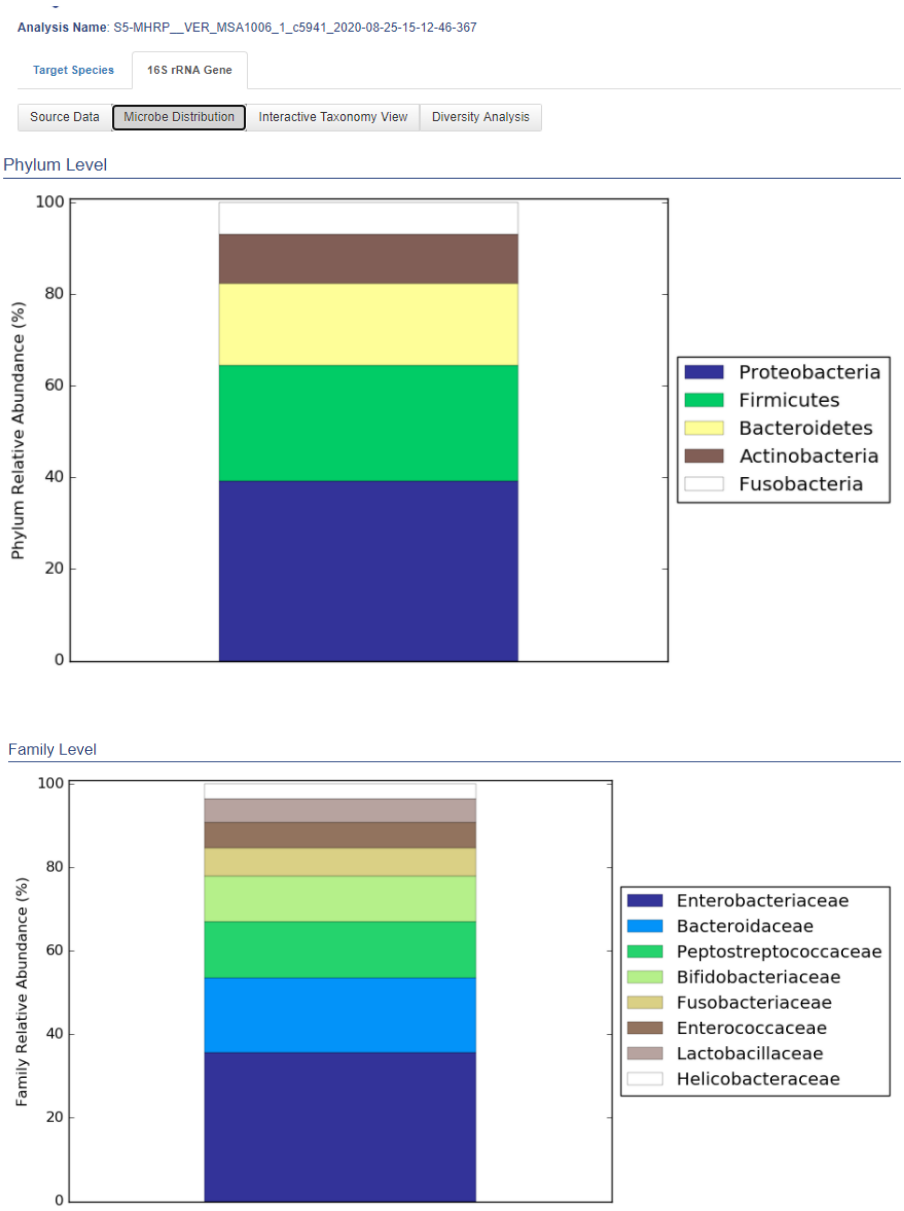


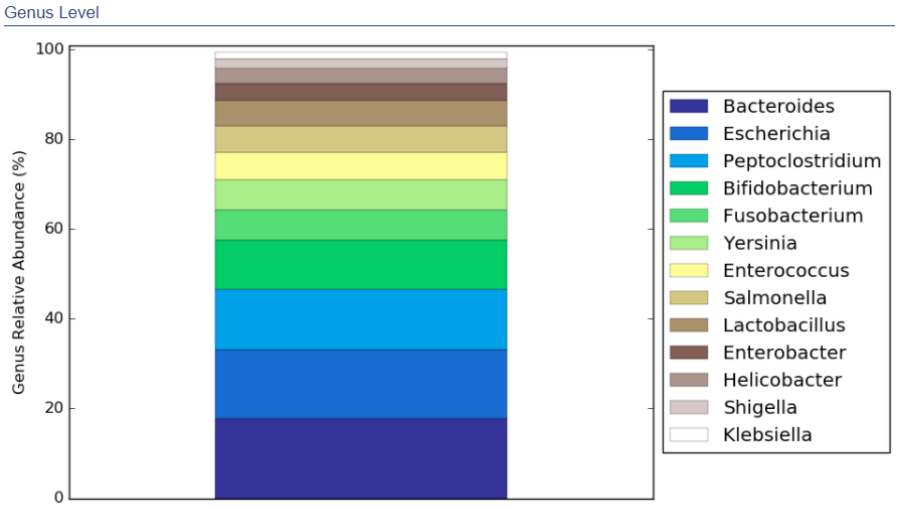
- The **Source Data** view displays the parameters in the following table for each detected species.

Parameter	Description
Species name	Bacterial species.
Detected Target species abundance	Percentage abundance of the species in the sample.
Read Count	Raw read count.
Normalized read count	Read counts are normalized by the number of amplicons per sequence and the sampling depth per sample. Normalized read counts are comparable across different microbes and samples.

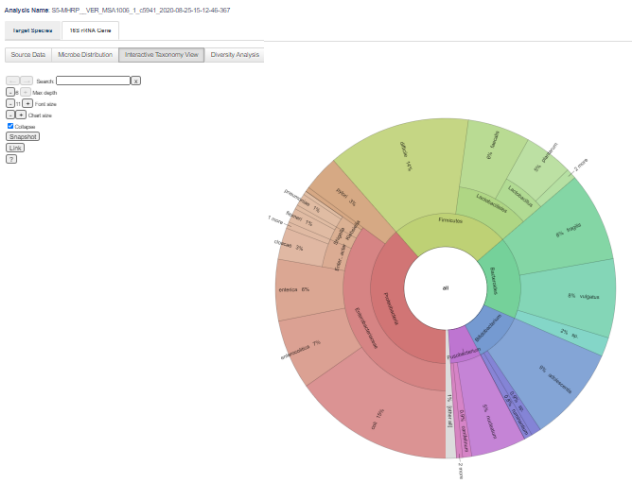
5. Click **16s rRNA Gene tab**, then select an option to view taxonomy data.
   - To view a summary of the taxonomy data, click **Source Data**.

- To view bar charts of the abundance of detected species at the phylum, family and genus levels, click **Microbe Distribution**.





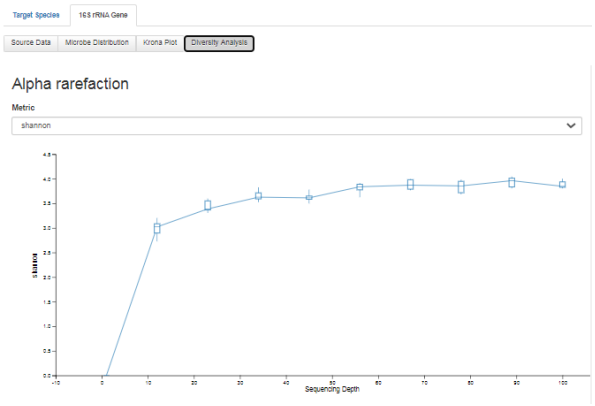
- To view a interactive taxonomy view of the detected species, click **Interactive Taxonomy View**.



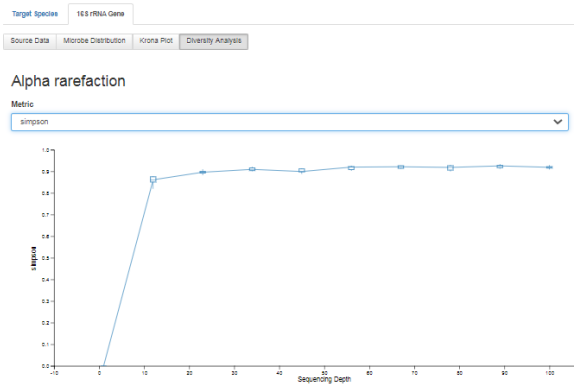
You can adjust the taxonomy view as follows.

- Enter a species to highlight that species in the **Search** field.
- Click **Max depth** to adjust the number of taxonomy classes that are displayed.
- Click **Font size** to change the font size of the characters.
- Click **Chart size** to change the size of the plot.
- Click **Collapse** to toggle the number of displayed taxonomy classes between 4 and 6.
- Click **Snapshot** to display the plot in a new window to enable saving the plot as a graphic.
- Click **Link** to display a link that can be copied for sharing or bookmarking
- Click **?** to open the help system for generating the interactive taxonomy view

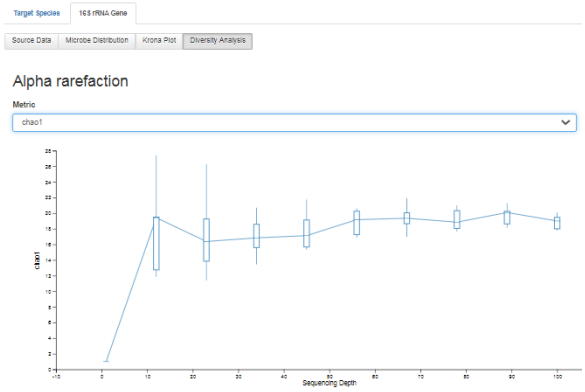
- To view an Alpha rarefaction plot, click **Diversity Analysis** then select an option from the **Alpha Rarefaction Metric** dropdown menu.
  - **shannon**



  - **simpson**



  - **chao1**



6. To download data files, click **Download Results**.
   A ZIP file containing the following files is downloaded to your local drive.

File	File extension	Description
Target Species Panel	XLSX	A list of the 73 bacterial species detected by Ion AmpliSeq™ Microbiome Health Research – Target Species Pool. Not all of the species are detected in a particular sample.
Interactive Taxonomy View	HTML	A taxonomy visualization for the sample that is interactive.
Folders for alpha diversity tests	various	Data for Alpha diversity tests.

MSI analysis results

You can use the analysis workflows for Oncomine™ Comprehensive Assay Plus to determine whether microsatellite instability (MSI) is present. You can apply parameters that detect MSI markers in these analysis workflows, to identify a form of genomic instability in the replication of repetitive DNA.

The Oncomine Comprehensive Plus - 550 - w3.2 – DNA (and DNA and Fusions) analysis workflow uses the RMC control and a system-installed baseline for analyses; the Oncomine Comprehensive Plus - 560 - w3.2 - DNA (and DNA and Fusions) workflow uses the MSI On-Chip control sample with RMC control.

The RMC control captures sample and library preparation variations. The on-Chip Control, which is generally a designated normal sample on the chip, is used to help determine sequencing based variations. The MSI signal generated by the on-chip control is used to evaluate the MSI signal generated for all DNA samples that are sequenced on the same chip to generate an MSI score. With use of the MSI on-chip control samples, the addition of RMC controls is still required during the library preparation.

To view MSI analysis results, see “Visualize MSI analysis results” on page 391.

View tumor mutational burden analysis results

You can view the results and calculated tumor mutational burden (TMB mutations/Mb) when you open analysis results that used a workflow with tumor mutational burden enabled.

Note: Microsoft™ Excel™, or other spreadsheet tool, is required for viewing VCF, CSV, and TSV files.

1. Click the **Analyses** tab.
2. In the **Overview** screen, use the **Workflow** filter to limit the list of analyses to limit the list of analyses that use an Oncomine™ Tumor Mutation Load workflow.
3. Click a row in the **Analyses** table to view the **Details** of the analysis.
   In the **Details** pane you can view **Workflow Details**, and access the **Actions** list.

4. Click the link for the name of the **Analysis** to open the **Analysis Results** screen.

   The **Analysis Results** screen opens to the **Oncomine** tab that displays Oncomine™ annotated variants that are relevant to cancer with Oncomine™ Gene Class and Oncomine™ Variant Class information.

   Information for tumor mutational burden that is displayed at the top of the **Analysis Results** screen is as follows.

Item	Description
Tumor Mutational Burden (Mutations/Mb)result	The value of the result for the tumor mutational burden calculation. For more information, see "Example: Tumor Mutational Burden calculation" on page 273.
TMB classification	A score of **High**, **Intermediate**, or **Low**. Thresholds to determine the **TMB classification** are set with the **TMB-Low Threshold** and **TMB-High Threshold** parameters. For more information, see "Annotation parameters" on page 94. A classification status of **Undefined** indicates that either the TMB classification thresholds settings are not set in the analysis workflow or were set incorrectly. **Note:** Threshold values are not provided in the predefined analysis workflow. The analysis workflow always reports **Undefined** as the TMB classification status unless a user sets the thresholds in the parameter settings.

5. In the **Analysis Results** table, sort or filter the data using the Oncomine™-specific annotations.
   a. The default **Filter Chain** is **Oncomine Variants**, which runs the Oncomine™ Variant Annotator plugin, a plugin that integrates data from more than 24,000 exomes across solid tumor and hematological cancer types and annotates variants relevant to cancer research with Oncomine™ Gene Class and Oncomine™ Variant Class information. For a variant to be filtered in on the results, the variant that is called must meet all the criteria for the filter chain. For more information, see "Predefined filter chains" on page 449.
      - Select **No Filter** to view all the variant calls attempted by the variant caller.
      - If you save a filter chain other than **Oncomine Variants (5.12)**, changes can occur to the variant calls that are saved in the VCF file and can affect results for downstream processes.

   b. In the **Oncomine** tab, click the column headings to sort the list of variants by **Oncomine Variant Class** or **Oncomine Gene Class**.

6. To download a results file.
   a. Click **Download** , then select **All Variants**, **Filtered Variants**, **Selected Variants** or **Current Results TSV**.

      For more information on the downloads, see "Download filtered variants" on page 209.

   b. In the **Home** tab, click **Notifications**, then click ⬇ next to the file name. Alternatively, select one or more rows, then click **Download**.

The software generates a ZIP file with 4 folders: RESULTS, QC, Variants, and Workflow_Settings.

Folder	Contents of folder
RESULTS	**Note:** The Variant Details TSV file can be downloaded separately. For more information see "Visualize tumor mutational burden analysis results" on page 333.. If the `Tumor Mutational Burden` parameter is enabled, or an analysis workflow for the Tumor Mutation Load Assay is used, a Results folder is generated that contains: • `filter_variants.tsv` and `somatic_variants.tsv`—TSV files that include post-filter and somatic variants. • `<tmb report>.pdf`—PDF report that contains tumor mutational load results. • `statistic.txt`—contains tumor mutational load statistics. • PNG files that contain images of: – allele frequency distribution of germline and somatic variants – allele frequency distribution of only somatic variants – bar plot of signature type and context of somatic mutations – pie chart of substitution type of somatic mutations – pie chart of signature pattern of somatic mutations
QC	Contains a PDF of the QC report, and a folder containing coverage statistics files.
Variants	• Contains intermediate and Oncomine™ annotated VCF files. • TSV files that include Oncomine™-filtered and all somatic variants.
Workflow_Settings	Contains folders with: • A text file that describes settings used for the analysis. Open the file with a text editor. • Configuration files used by the Ion Reporter™ Software in the workflow settings.

View the tumor mutational burden statistics

The tumor mutational burden value is calculated automatically when you launch select Oncomine™ analysis workflows, such as analysis workflows for Oncomine™ Comprehensive Assay Plus, DNA or Oncomine™ Tumor Mutation Load Assay. You can download the tumor mutational burden statistics to view more details of the analysis, such as the TMB algorithm version that is used, and information about how the tumor mutational burden is calculated.

Note: Refer to the assay user guide to determine whether the tumor mutational burden value can be calculated for a specific assay.

1. Download, then extract a results file to your hard drive.

   For more information, see “Download analysis files” on page 186.

2. In the **RESULTS** folder, open the `statistic.txt` file in a compatible text editor. The tumor mutational burden and the data that are used to calculate its value are listed.

   In this example, the tumor mutational burden value is calculated from the values for `Total Exonic Bases with Sufficient Depth of Base Coverage`, and `Total Somatic Filtered Variants Count (numerator for TMB calculation)`. TMB Standardization is off by default, that is, `0.0TMB Standardization='Not Applied'`. Observed TMB score was set to ***25***, which is below the calibration threshold, so that the germline calibration was also not applied. That is `Germline Calibration='Not Applied'`.

```
IR Sample Name='JH_TMB_colon1_4_N.bam.JH_TEST_BAM'IR
Analysis Name='JH_TMB_colon1_4_N.bam.JH_TEST_BAM_1564544352260'Released with Ion
Reporter 'ir512'.
TMB Algorithm Version='V4.0'Minimum Read Depth of Base Coverage required=60.0
Maximum Deamination Score allowed=100.0
Minimum Variant Allele Frequency required=0.05
Deamination=16 (QC: PASS; observed (16) < threshold (100.0))
Total Bases (sequenced bases in the panel / in the design bed file) with
Sufficient Depth of Base Coverage=1652887
Average Coverage=1300.0
Total Exonic Bases with Sufficient Depth of Base Coverage=1198355
Denominator used for TMB Calculation (as specified in the workflow
parameters)=1198355
Total Somatic Filtered Variants Count (numerator for TMB calculation)=19
('missense=18,frameshiftDeletion=0, frameshiftInsertion=0,
nonframeshiftDeletion=0, nonframeshiftInsertion=0, nonsense=1')
Germline Calibration='Not Applied'
Germline Calibration Slope=1.4637
Germline Calibration Intercept=0.0
TMB Standardization='Not Applied'
TMB Standardization Slope=1.0
TMB Standardization Intercept=0.0
Mutation Load (Mutations/Mb)=15.86
TMB classification (based on specified parameters)='Intermediate
'Variant count (Germline + somatic)=1098
Variant count: 19COSMIC Annotated Somatic Variants=2
Variant count=19
```

Example: Tumor Mutational Burden calculation

Tumor Mutational Burden calculation

Ion Reporter™ Software uses TMB Algorithm v4.0:

$$\text{precalibration TMB} = \frac{\text{SM} \times 10^6}{\text{Total Exonic Bases with Sufficient Coverage}}$$

where

- TMB is Tumor Mutational Burden
- SM is Somatic Mutations

You can control how somatic mutations are calculated. You can also select between Exonic bases or all of the genomic bases that are covered by the panel. Similarly, the 'Sufficient Coverage' threshold can be modified. By default, the TMB calculation are done using Total Exonic Bases with ≥60bp coverage.

The following settings apply:

- applyTMBStandardization: Customizable parameter (default value: off)
- standardization intercept: TMB Standardization Factor: Intercept (default value: 0)
- standardization slope: TMB Standardization Factor: Slope (default value: 1)

It is apparent from the values that even if the standardization is enabled with the current default values, the TMB score value are not be adjusted until a value is provided for the intercept and the slope.

Note: Standardization is independent of the germline calibration.

View Oncomine™ Myeloid Research Assay analysis results

The dedicated Myeloid_FLT3_LongITD algorithm in Ion Reporter™ Software Oncomine™ Myeloid Research Assay analysis workflows identify large internal tandem duplications (ITD) in exons 14 and 15 of the FLT3 gene.

The analysis workflow is for use with Oncomine™ Myeloid Research Assay DNA sequencing results.

In-frame insertions in exons 14 and 15 are detected by both the FLT3 Long ITD algorithm, and the variant calling algorithms in Ion Reporter™ Software. Therefore FLT3-ITD variants can be called by one or both types of variant calling. We recommend obtaining the results from the Myeloid_FLT3_LongITD algorithm for ITDs of 8 bases or larger. For smaller variants review the Ion Reporter™ Software variant calling results. The Ion Reporter™ Software variant calling module can sometimes call ITDs larger than 8 base pairs, but its sensitivity to variants in that size range is lower than the Myeloid_FLT3_LongITD algorithm.

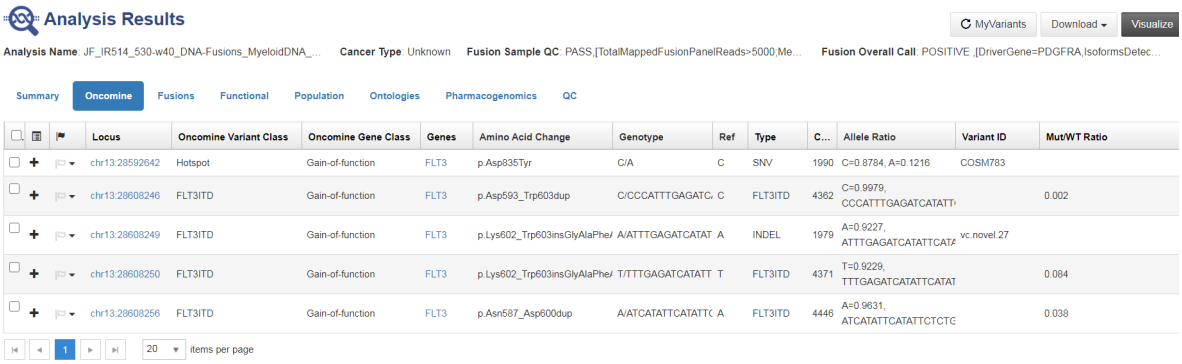
See the following table for guidelines in obtaining FLT3 ITD calls from the Oncomine™ Myeloid Research Assay analysis workflows.

FLT3- ITD variant Length	Called by Myeloid_FLT3_LongITD algorithm	Called by variant calling module in Ion Reporter™ Software	Annotated by Oncomine™ Reporter
≥8 bp	Yes	Yes (with lower sensitivity than the FLT3 algorithm)	Yes
<8 bp	No	Yes	Yes

Algorithm description: For standard INDEL detection, long inserts at the ends of reads can cause a partial alignment, resulting in soft-clipping of the alignment. Since this can soft-clip the FLT3-ITD sequence, and eliminate downstream anchoring sequence, the standard INDEL parameters might not detect all FLT3-ITD calls. The Myeloid_FLT3_LongITD algorithm analyzes 3' regions of trimmed reads for presence of anchor sequences, and determines the likely position and size of the duplication by looking for copies of sequence in the mapped and trimmed regions.

To view the analysis results:

1. In the Ion Reporter™ Software **Home** tab, click **View analyses** or click the **Analyses** tab. Search, filter, or scroll to find the analysis of interest in the list of **Analyses**, then click the analysis link. The **Analysis Results** table opens to the list of Oncomine variants.

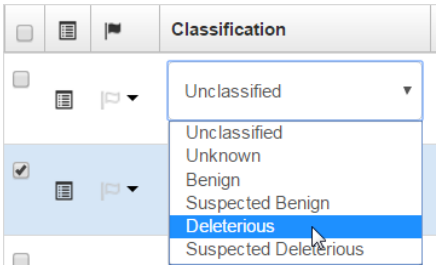


- Variants that are called by the Myeloid_FLT3_LongITD algorithm appear in the default **Oncomine** view, and are identified as **FLT3ITD Oncomine Variant Class** and **Type** in the **Analysis Results** table and as **SVTYPE** in the output VCF file.
- Both the variant calling module in Ion Reporter™ Software and the Myeloid_FLT3_LongITD algorithm can detect the same variant, which appear adjacent in the Ion Reporter™ Software **Analysis Results** as **SNV** or **Indel** and **FLT3ITD** variant types, respectively.
- **Mut/WT** is the ratio of mutant FLT3-ITD variants to the number of the wild type FLT3 read count.

  Note: Values in the **Mut/WT Ratio** column appear only for variants that have FLT3ITD as the **Type**.

  A FLT3-ITD variant subtype named **Combined** can be reported in Oncomine™ Myeloid MRD analysis results. The **Combined** subtype is a value for the sum of all reported FLT3-ITD variant allele frequencies.

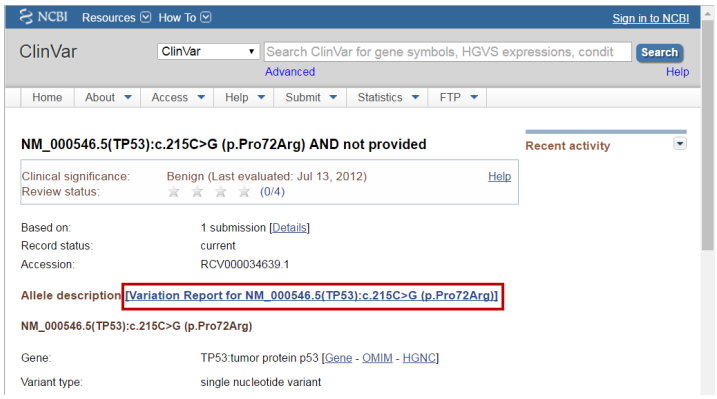
2. *(Optional)* In the **Classification** column, classify variants by selecting a classification from the dropdown list.



3. Click **Pharmacogenomics**.

4. In the **ClinVar** column, click the ClinVar link to open the NCBI ClinVar variant website where information about the ClinVar variant annotation is maintained.

5. In the variant-specific ClinVar website, click the **Variation Report** link to view more information about the variant.



View metagenomics analysis results

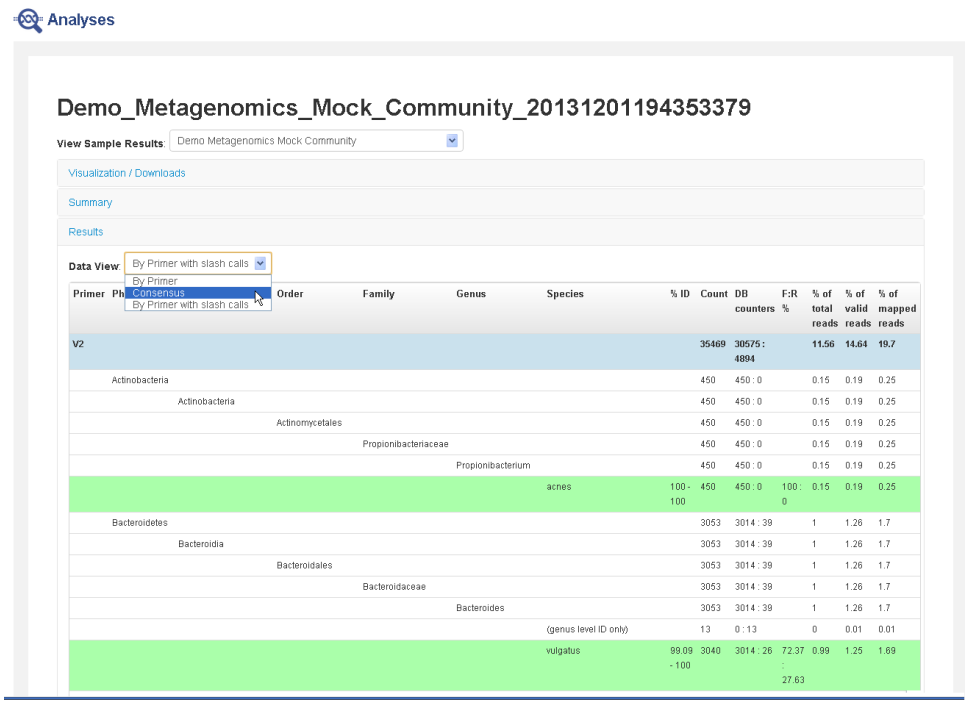
Sequences from different 16S databases may use different taxonomic hierarchies. For example, Greengenes may have *de novo* classified sequences labeled as Archaea>Crenarchaeota> Thaumarchaeota, where *Thaumarchaeota* is a class of *Crenarchaeota*. Records from other databases may treat both *Thaumarchaeota* and *Crenarchaeota* as phyla. Therefore, you may see differences in taxonomies when you compare 16S metagenomics analysis results from the two databases used in Ion Reporter™ Software to other, external databases.

1. In the **Analyses** tab, click **Overview**.
2. In the **Analyses** table, click the name of your metagenomics analysis to view the analysis results.
3. Review the **Visualization / Downloads**, **Summary**, or **Results** sections.

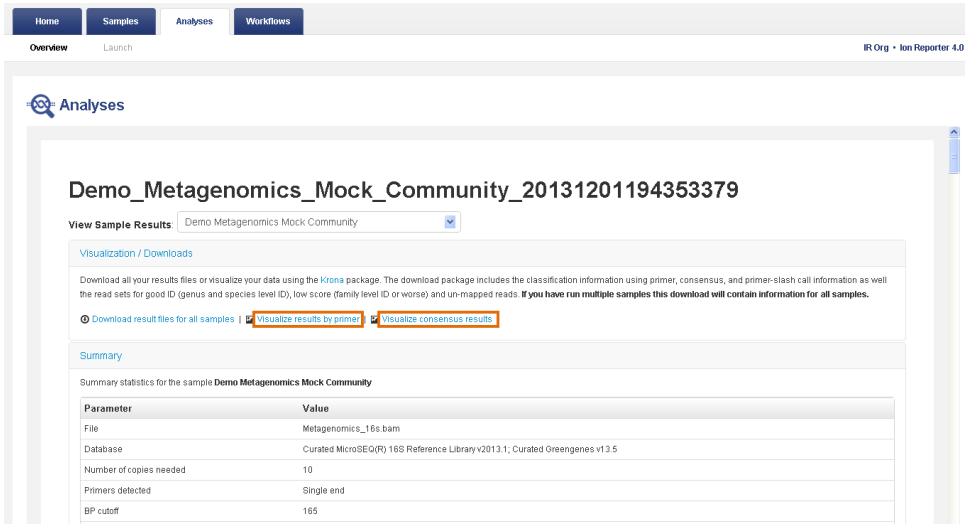
Note: In the **Analyses Results** table, Total Mapped Reads in a sample differs from the sum of the bottom species-level counts. The reason is that the Total Mapped Reads are filtered by genus cutoff, species cutoff, and minimum alignment coverage. The topmost number in the "count" column differs from the sum of the taxonomy levels below it, because the reads that did not satisfy the filters are also included in the total mapped reads.

- **Visualization/Downloads** section—Contains links to view interactive taxonomy charts and download results files. For more information, see “View interactive taxonomy charts for metagenomics analyses” on page 284 and “Reference output files for Metagenomics” on page 279.
- **Summary** section—Contains analysis and parameter information and read metrics by primer.
- **Results** section—Contains either consensus data (combined from all primers) or data broken out by primer. Reads that can be identified down to the species level are marked in green.

4. In the **Results** section, click **Data View** to display either consensus data (combined from all primers) or data broken out by primer, with or without slash calls.

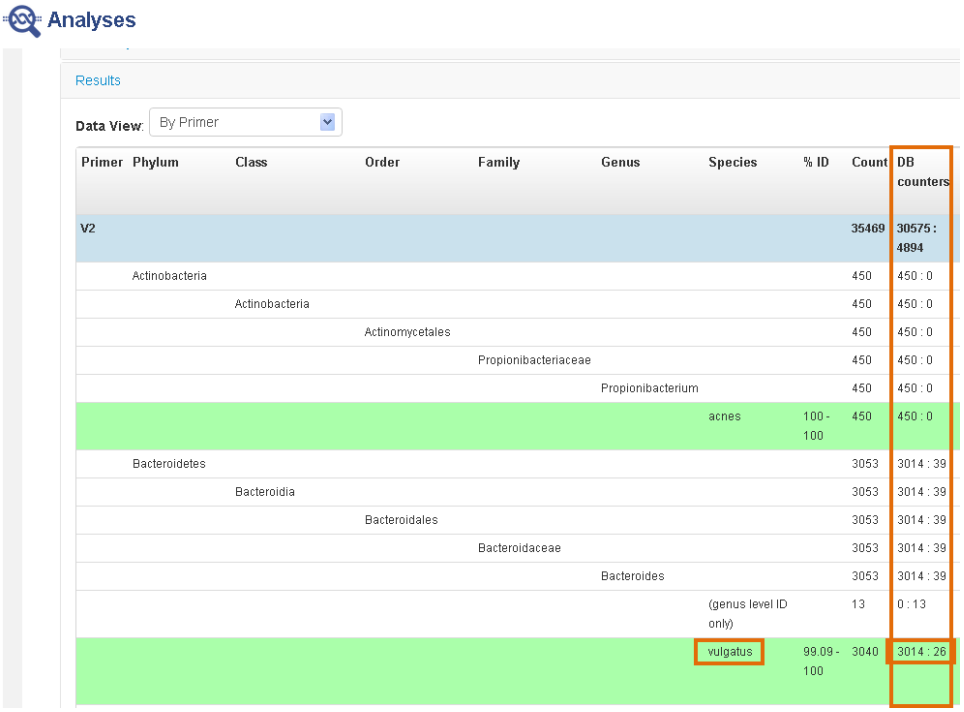


5. In the **Visualization/Downloads** section, click one of the visualization links to view your results with interactive taxonomy views.



Reads included in Metagenomics analysis results

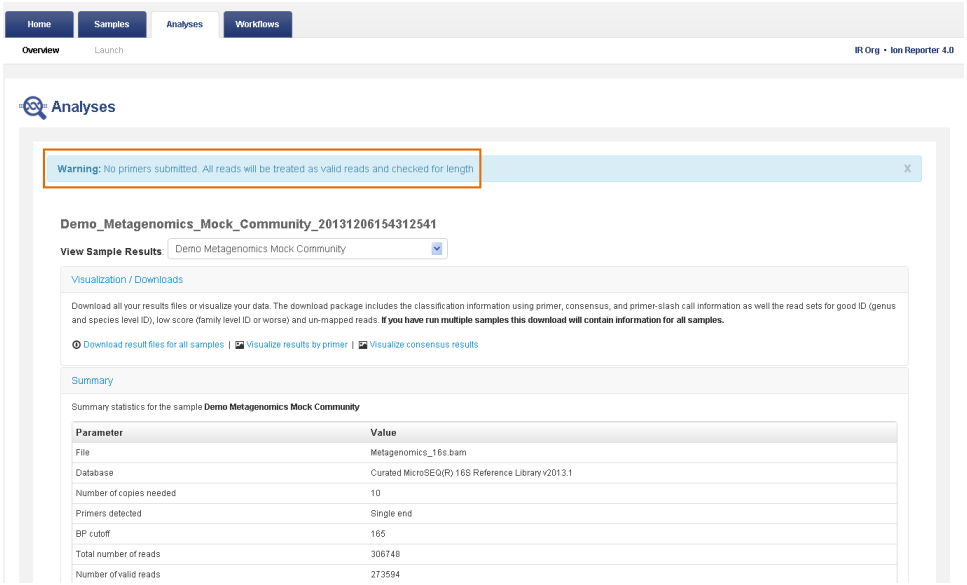
Note: When your metagenomics analysis workflow uses two databases, only reads that do not map to the first database are attempted with the second database. In the **Results** section of the screen, the **DB counters** column shows how many reads matched sequences in each database.



Primer	Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters
V2								35469	30575 : 4894
	Actinobacteria							450	450 : 0
		Actinobacteria						450	450 : 0
			Actinomycetales					450	450 : 0
				Propionibacteriaceae				450	450 : 0
					Propionibacterium			450	450 : 0
						acnes	100 - 100	450	450 : 0
	Bacteroidetes							3053	3014 : 39
		Bacteroidia						3053	3014 : 39
			Bacteroidales					3053	3014 : 39
				Bacteroidaceae				3053	3014 : 39
					Bacteroides			3053	3014 : 39
						(genus level ID only)		13	0 : 13
						vulgatus	99.09 - 100	3040	3014 : 26

For example, in the last row, for the species *vulgatus*, the value 3014:26 means that 3014 reads matched sequences in the MicroSEQ™ database and 26 matched sequences in the Greengenes database.

Note: When primer information is missing in the metagenomics analysis workflow, a warning message appears during analysis review.



Reference output files for Metagenomics

The metagenomics reference FASTA output files may have 2 formats:

- The FASTA line starts with the text ">MG and is followed by `primerset , primerName, direction , count , percentage ,Phylum , Class , Order , Family , Genus , Species separated by "|" .`

  The following line is an example:

  ```
  >MG|1|V2|R|27|97.72|Proteobacteria|Epsilonproteobacteria|
  Campylobacterales|Helicobacteraceae|Helicobacter|pylori|
  ```

- The FASTA line starts with the text ">MG and is followed by `count, percentage , Phylum , Class , Order , Family , Genus , Species separated by "|".`

  The following line is an example:

```
>MG|187|99.1|Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|
Bacteroides|vulgatus|
```

For details about the FASTA file, see the *Metagenomics 16S algorithms overview* white paper at **https://tools.thermofisher.com/content/sfs/brochures/ion-reporter-16s-metagenomics-algorithms-whitepaper.pdf**.

For more information about metagenomics, visit the Ion 16S™ Metagenomics product page on Connect at **https://www.thermofisher.com/us/en/home/life-science/sequencing/dna-sequencing/microbial-sequencing/microbial-identification-ion-torrent-next-generation-sequencing/ion-16s-metagenomics-solution.html**.

Alpha-beta diversity results

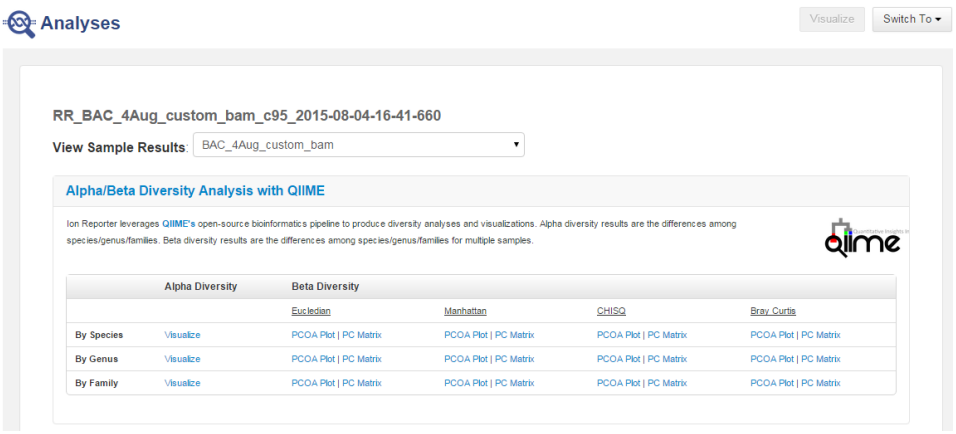
The Metagenomics 16S analysis workflow in Ion Reporter™ Software includes alpha diversity calculations and beta diversity calculations.

Alpha diversity results describe the diversity in a single sample at the species, genus, and family levels. Beta diversity results describe the diversity between multiple samples at the species, genus, and family levels. For results interpretation guidance, see the QIIME online help at **qiime.org**.

Note: If you run only one sample, Ion Reporter™ Software returns only alpha diversity results. If you have two or more samples, you get beta diversity results. However, PCOA plots can be generated only for more than three samples, depending on the quality of data input.

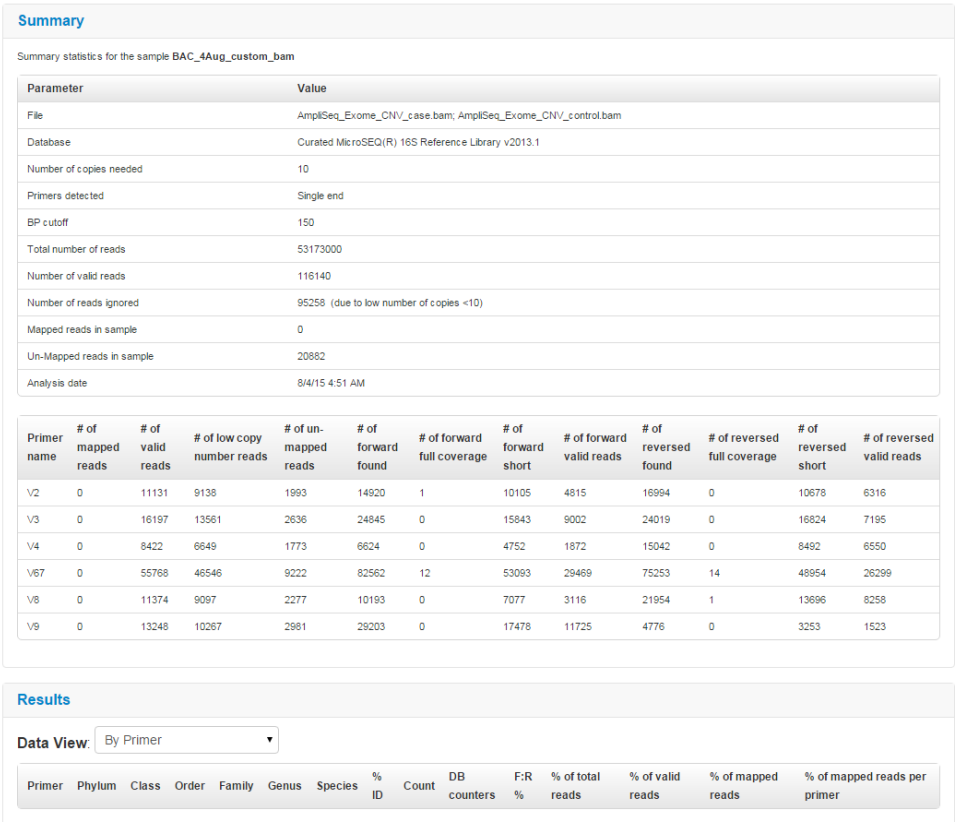
Quantitative Insights Into Microbial Ecology (QIIME) algorithms determine what species, genus, or families are present.

1. In the **Analyses** tab, click **Overview**,
2. In the **Analyses** screen, click the name of your metagenomics analysis. You can filter the list to display only metagenomics analyses.
   An **Analyses** summary opens.



3. Review your results by clicking the Visualize links in the **Alpha Diversity** column or the various plots and matrices links in the **Beta Diversity** column.

4. Scroll down to **Summary** and **Results** sections to see overall statistics.



Summary

Summary statistics for the sample **BAC_4Aug_custom_bam**

Parameter	Value
File	AmpliSeq_Exome_CNV_case.bam; AmpliSeq_Exome_CNV_control.bam
Database	Curated MicroSEQ(R) 16S Reference Library v2013.1
Number of copies needed	10
Primers detected	Single end
BP cutoff	150
Total number of reads	53173000
Number of valid reads	116140
Number of reads ignored	95258 (due to low number of copies <10)
Mapped reads in sample	0
Un-Mapped reads in sample	20882
Analysis date	8/4/15 4:51 AM

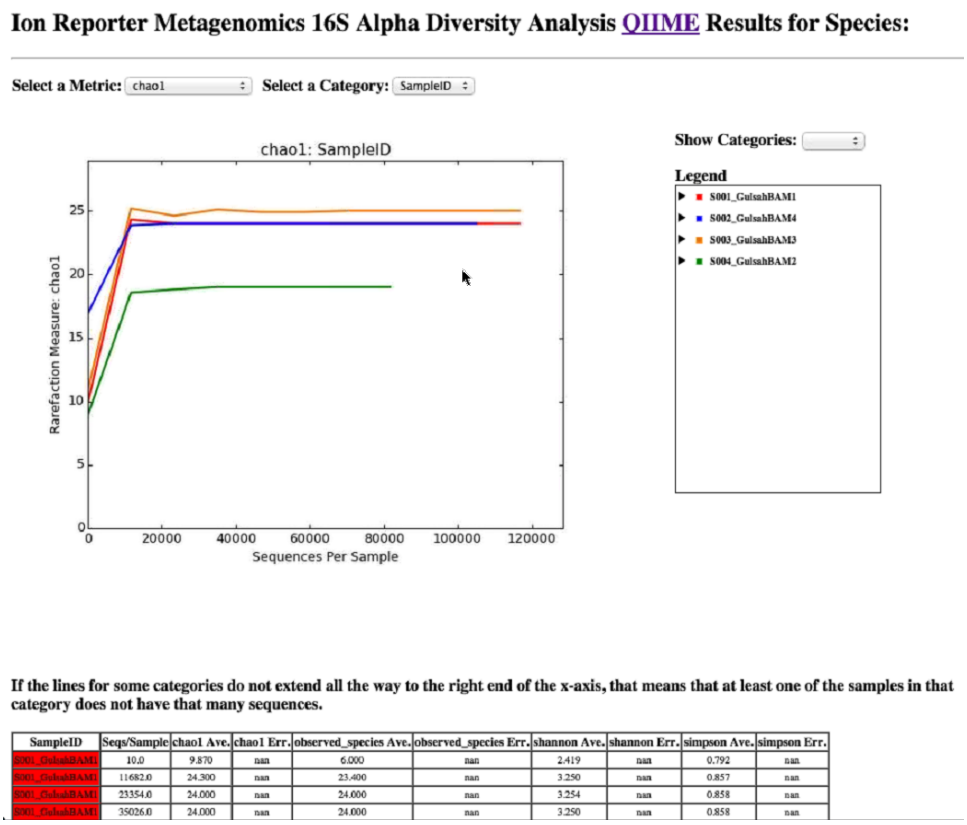
Primer name	# of mapped reads	# of valid reads	# of low copy number reads	# of un-mapped reads	# of forward found	# of forward full coverage	# of forward short	# of forward valid reads	# of reversed found	# of reversed full coverage	# of reversed short	# of reversed valid reads
V2	0	11131	9138	1993	14920	1	10105	4815	16994	0	10678	6316
V3	0	16197	13561	2636	24845	0	15843	9002	24019	0	16824	7195
V4	0	8422	6649	1773	6624	0	4752	1872	15042	0	8492	6550
V67	0	55768	46546	9222	82562	12	53093	29469	75253	14	48954	26299
V8	0	11374	9097	2277	10193	0	7077	3116	21954	1	13696	8258
V9	0	13248	10267	2981	29203	0	17478	11725	4776	0	3253	1523

Results

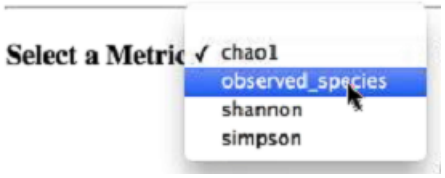
Data View: By Primer

Primer	Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters	F:R %	% of total reads	% of valid reads	% of mapped reads	% of mapped reads per primer

Alpha-beta diversity calculations are based on the information that is gathered from the consensus files that are generated by the Metagenomics 16S analysis workflow. Read counts per sample for species, genus, and family are collected. Operational taxonomic unit (OTU) tables are generated. These tables are used by QIIME to generate alpha-beta diversity results.



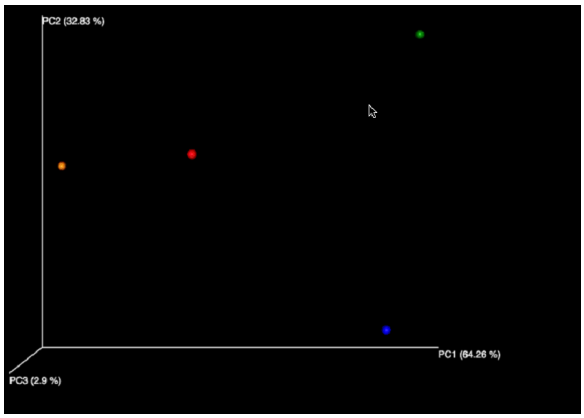
5. In the **By Species** row, in the **Alpha Diversity** column, click **Visualize**, then in the resulting screen, select your metrics.



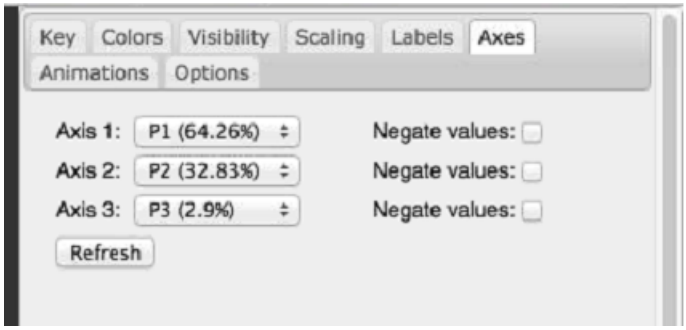
6. Repeat for **By Genus** and **By Family** links in the **Alpha Diversity** column to see the genus and families that are in the sample.

7. In the **By Species** row, in the **Beta Diversity** column click the **PCOA Plot/PC Matrix** link.

8. In the resulting screen, you can view several plot types, including: Eucledian, Manhattan, Chi-Square and Bray Curtis PCOA Plots and PC Matrices.



9. Tools on the right can be used to produce an image.

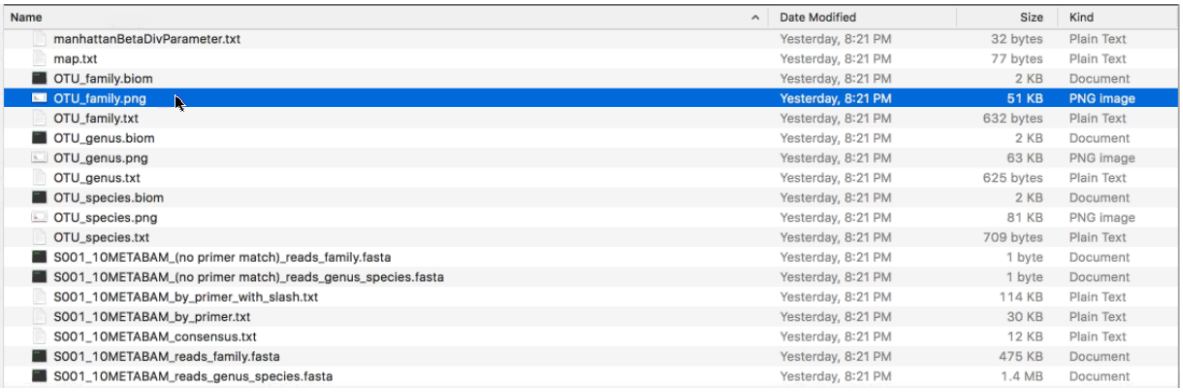


Visualize OTU results with R-scripts graphs

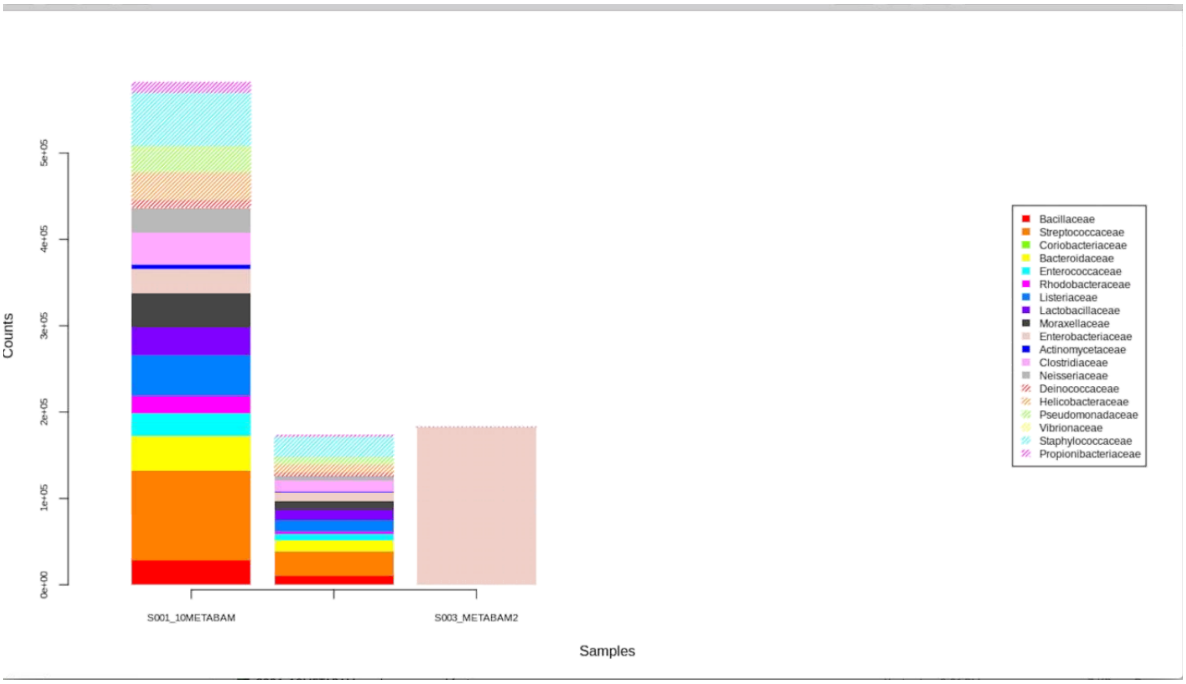
In Ion Reporter™ Software, OTU files are displayed graphically. For instance, in the example below, the `OTU_family.txt` file is rendered graphically in the `OTU_family.png` file.

1. Download your results.

2. Click the PNG file of the results.



The data is displayed visually.



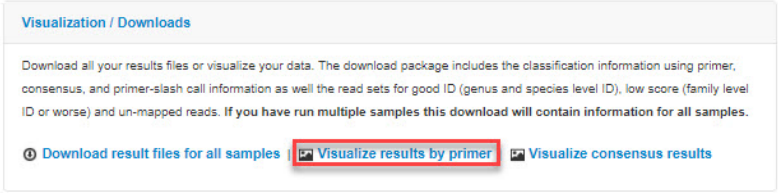
View interactive taxonomy charts for metagenomics analyses

You can use interactive taxonomy views to browse through metagenomics data.

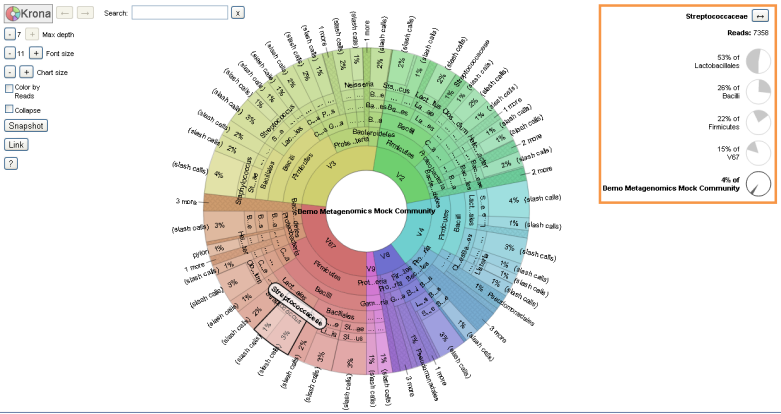
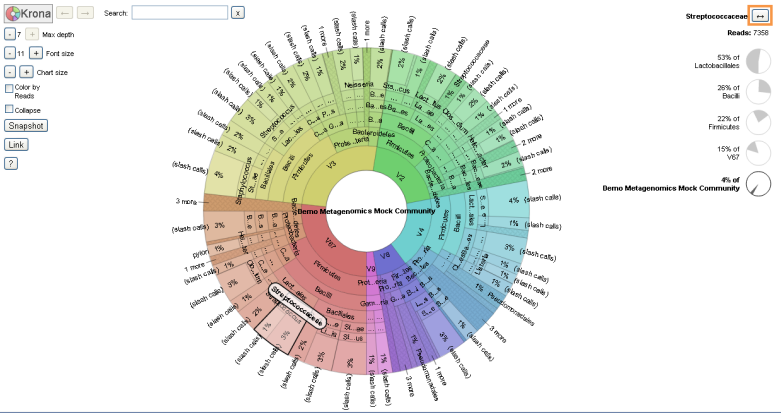
Note: The **Snapshot** button does not operate on the visualization screen for the Metagenomics 16S analysis results in some browsers. You need to instead create a PDF of the visualization.

1. In the **Analyses** tab, click **Overview**.
2. In the **Analyses** screen, click the name of your metagenomics analysis. You can filter the list to display only metagenomics analyses.
3. In the results screen for the selected analysis, in the **Visualization/Downloads** section, click **Visualize results by primer** or **Visualize consensus results** to open an interactive taxonomy view.
4. Click **Download results files for all samples** to download all results files.

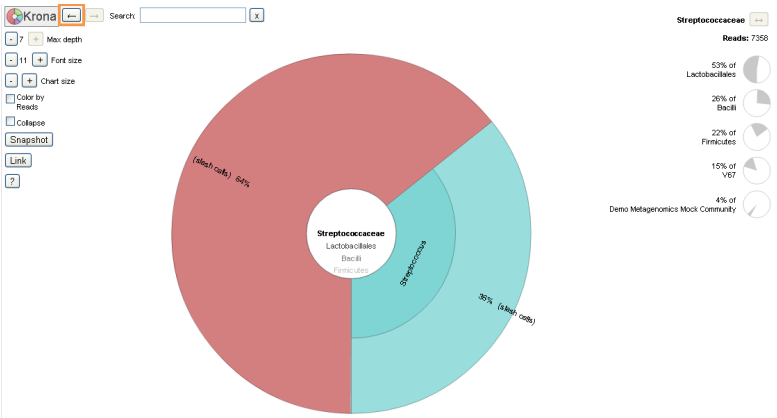
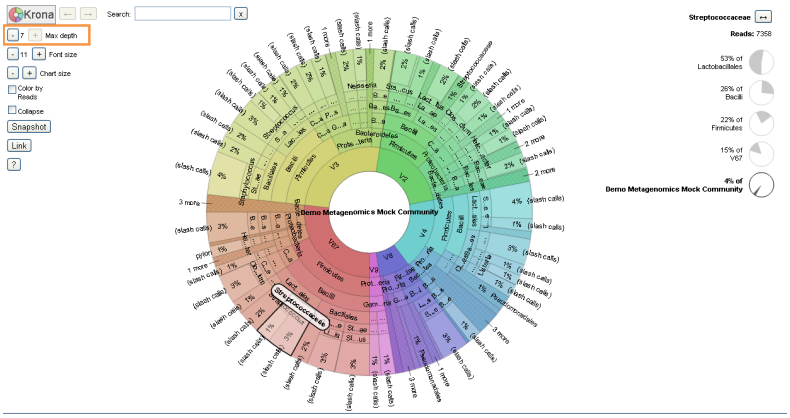
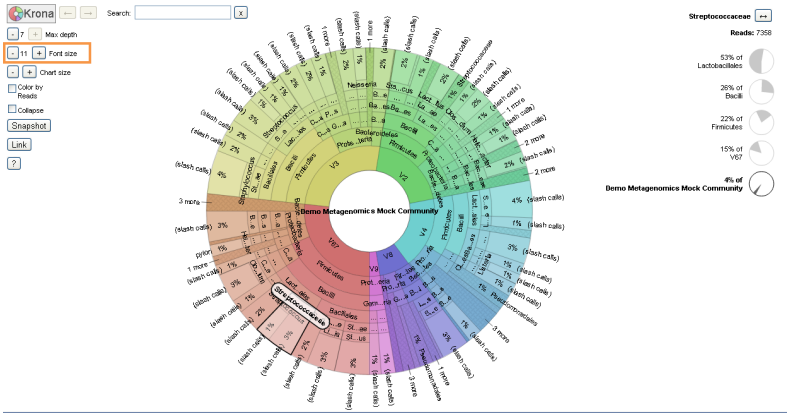
This table describes ways to use the interactive taxonomy views. For more information, see the documentation at this **github site**.

Task	Your action
Open the analysis data in an interactive taxonomy view	Click the **Visualize results by primer** link. 

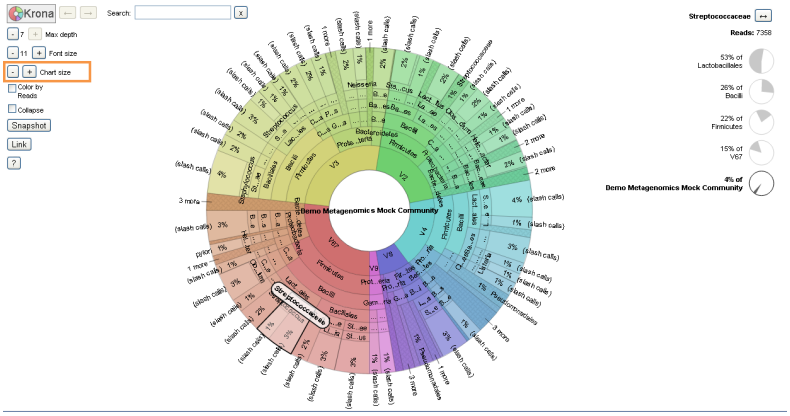
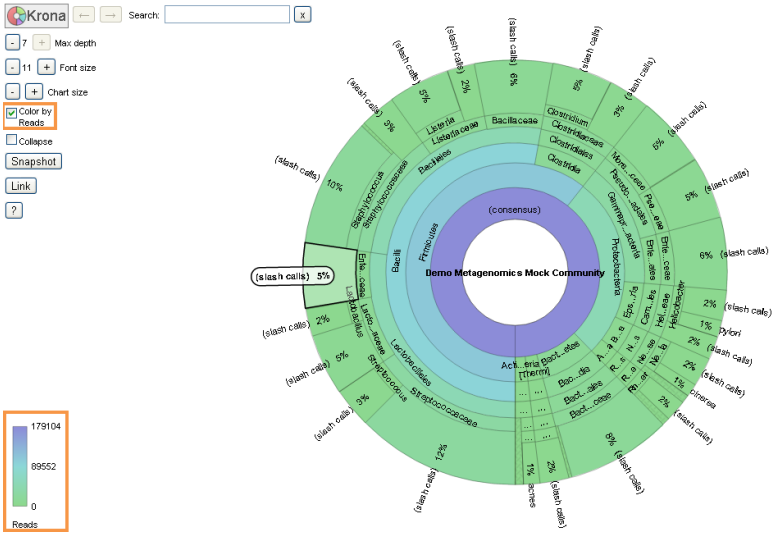
(continued)

Task	Your action
Open interactive taxonomy views on the consensus data	Click the **Visualize consensus results** link. 
Download results files for all samples	Click the **Download results files for all samples** link to download all results files for the metagenomics analysis. 
Display percentages for one section of the chart	Click that section of the chart. The percentages for that section are displayed in the top right of the page. 
Make that section the new focus of the chart	Click the expand box in the top right of the screen. 

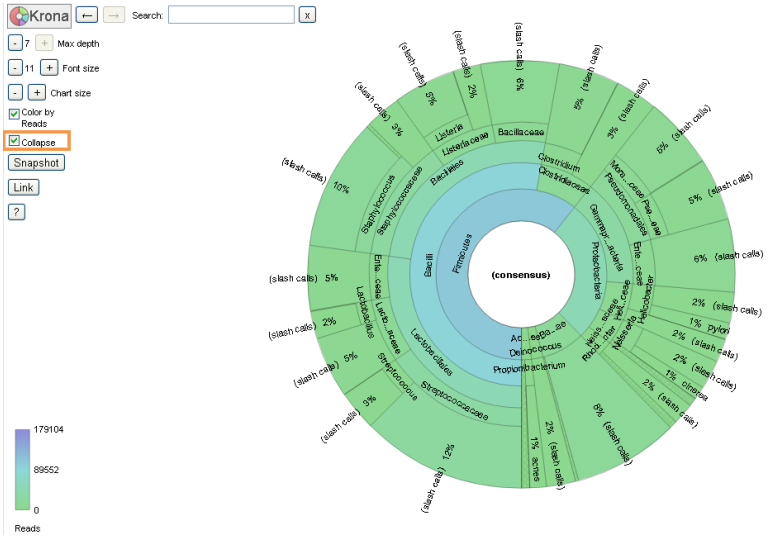
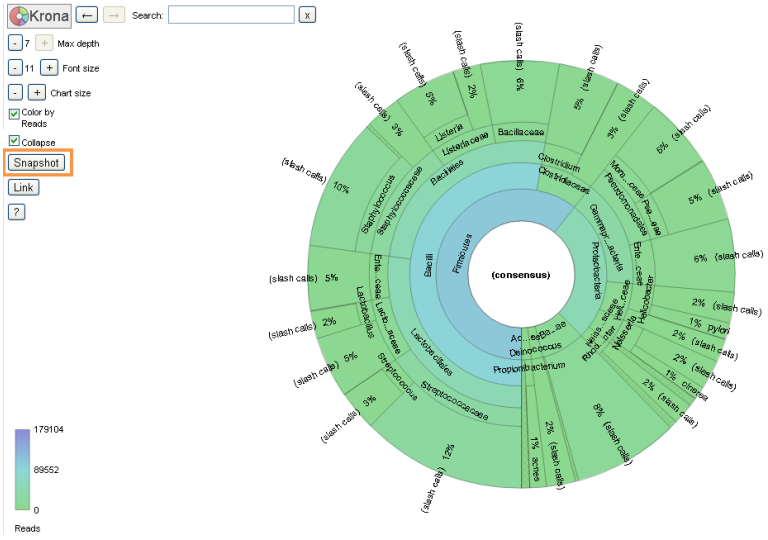
(continued)

Task	Your action
Return to the previous chart focus	Click the back arrow ← at the top left of the screen. You can complete only one go-back action at a time. 
Change the number of circles shown in the chart	Click the minus or plus icons - + near the **Max depth** label. 
Change the font size	Click the minus or plus icons - + near the **Font size** label. 

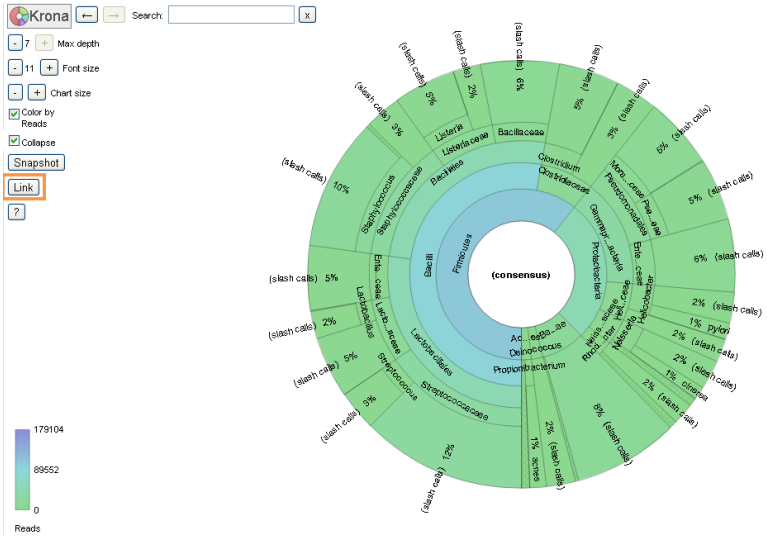
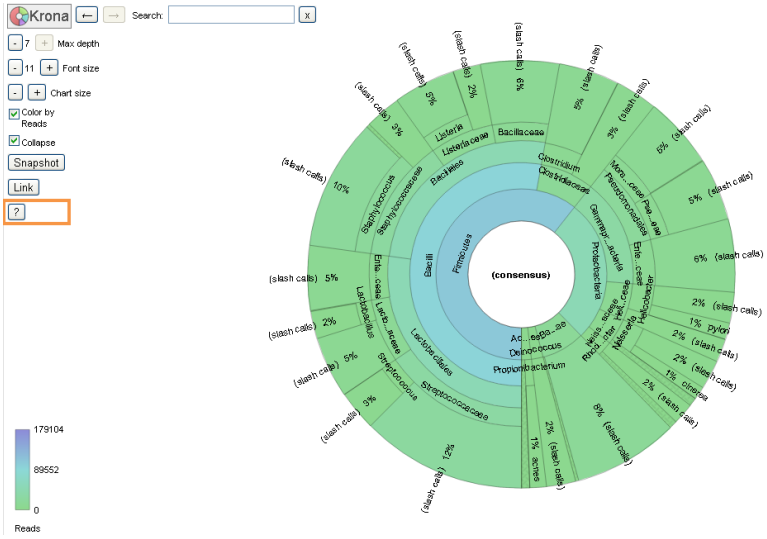
(continued)

Task	Your action
Change the size of the chart	Click the minus or plus icons [-] [+] near the **Chart size** label. 
Show read depth by color	Select the **Color by Reads** checkbox. A color legend appears in the bottom left. (This example shows a consensus chart.) 

(continued)

Task	Your action
Simplify wedges	Select the **Collapse** checkbox. This selection combines redundant wedges (that include only another wedge). 
Create an image of the pie chart	Click **Snapshot**.  Clicking this button creates a SVG (Scalable Vector Graphics) format image file. See the documentation at this **github site** for more information about browser support. (For example, in Chrome™ browser, an SVG file is not created directly. You need to save the screen as a PDF file.)

(continued)

Task	Your action
Create a sharable link to the chart	Click **Link**. 
Open Krona™ documentation	Click the question mark to open the documentation in a new tab. 

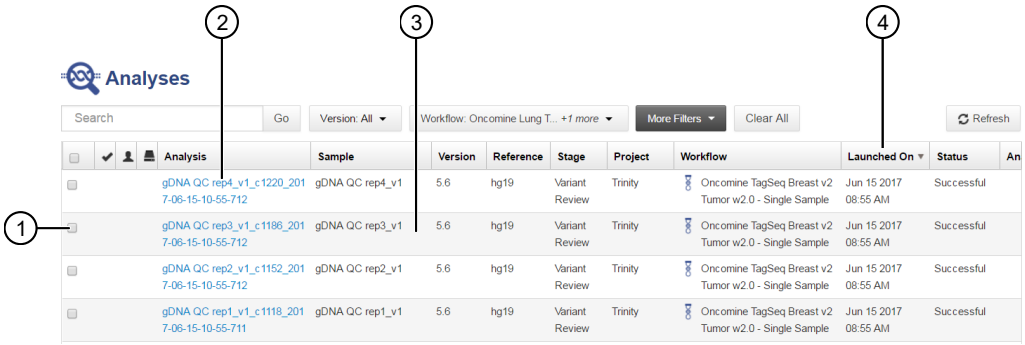
View analysis results for a single sample

Use this procedure to view and manage the extended analysis results of a single sample.

Note: Analyses that are performed as described in this procedure generate a variants table that does not have optimized TagSeq specific filters applied. These filters are applied only when you generate results analysis as described in "Visualize identified variants in an Oncomine™ analysis from a TagSeq analysis workflow" on page 392.

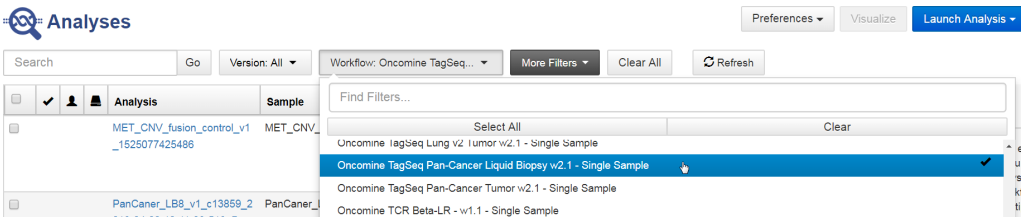
1. Click the **Analyses** tab, then click **Overview**.
2. In the **Analyses** screen you can:

To	Action
Select an analysis	Enable the checkbox.
Open an **Analysis Results** screen.	Click the hyperlink (in the **Analysis** column).
View details	Click anywhere in the analysis' row, except on the hyperlink.
Sort	Click column headings to sort the analyses based on the column contents.



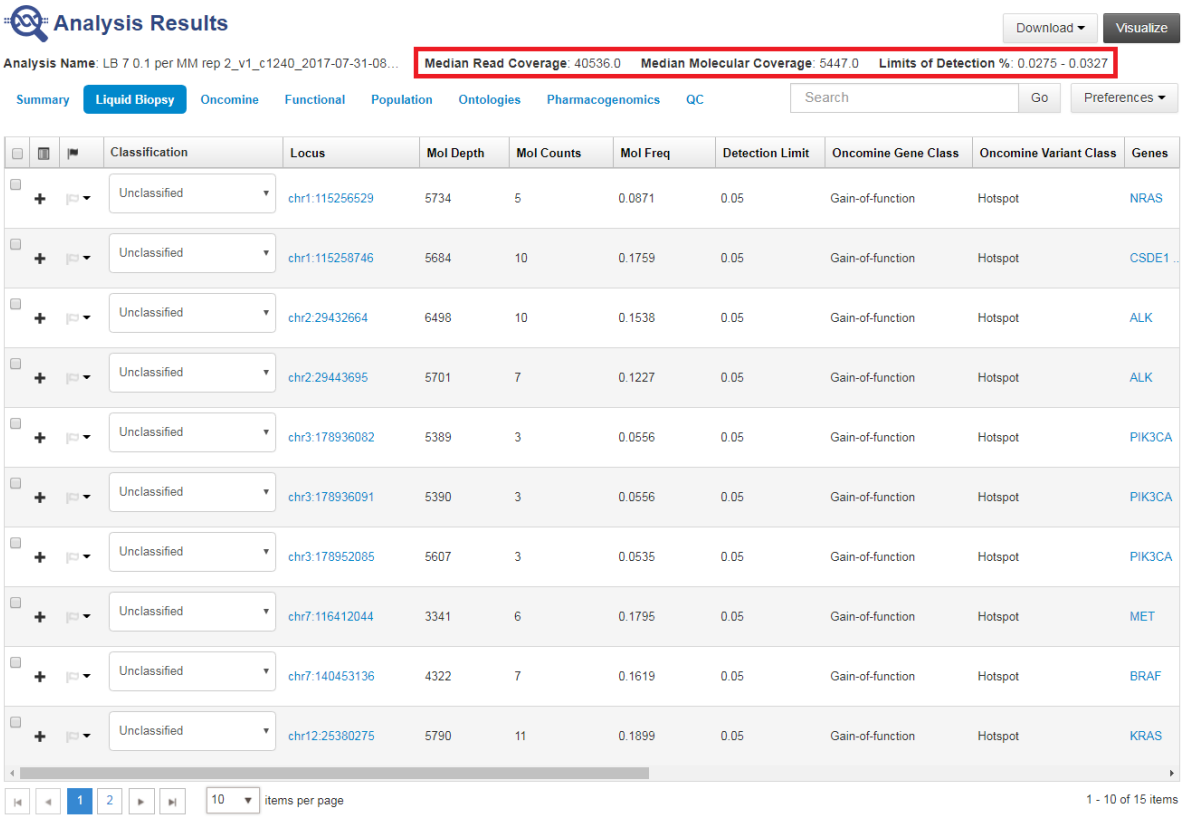
① Select analysis
② Open **Analysis Results** screen
③ View details
④ Sort a column

3. To view and manage the extended Oncomine™ Cell-Free Research Assay results, use the available filters to limit the list of analyses (for example, select your analysis workflow from the **Workflow** list), then click the hyperlink in the **Analysis** column.



4. In the **Analysis Results** screen, sort or filter the data using the Oncomine™-specific annotations. See the software help menu for more options.

5. Review the results in the **Median Read Cov**, **Median Mol Cov**, and **LOD %** columns.



Column	Description
Median Read Coverage	Reports median coverage across targets. Median Molecular Coverage reports median number of individual interrogated DNA molecules across targets.
Median Molecular Coverage	Directly influences the limit of detection in a sample run. We always require two independent molecular families to identify a variant for it to be called. Lower median molecular coverage values result in less sensitive detection of variants at 0.1% frequency, although still sufficient for sensitive detection of variants with higher frequency. For example, Median Molecular Coverage of 700 is sufficient for accurate detection of variants at 0.5% frequency.
LOD %	A segment (e.g., 0.02–0.03) where 0.02 represents the median value across all targets, and 0.03 represents the limit of detection (LOD) for the 80th percentile targets. If both numbers are ≤0.1% then the sequencing run is of acceptable quality for 0.1% LOD.

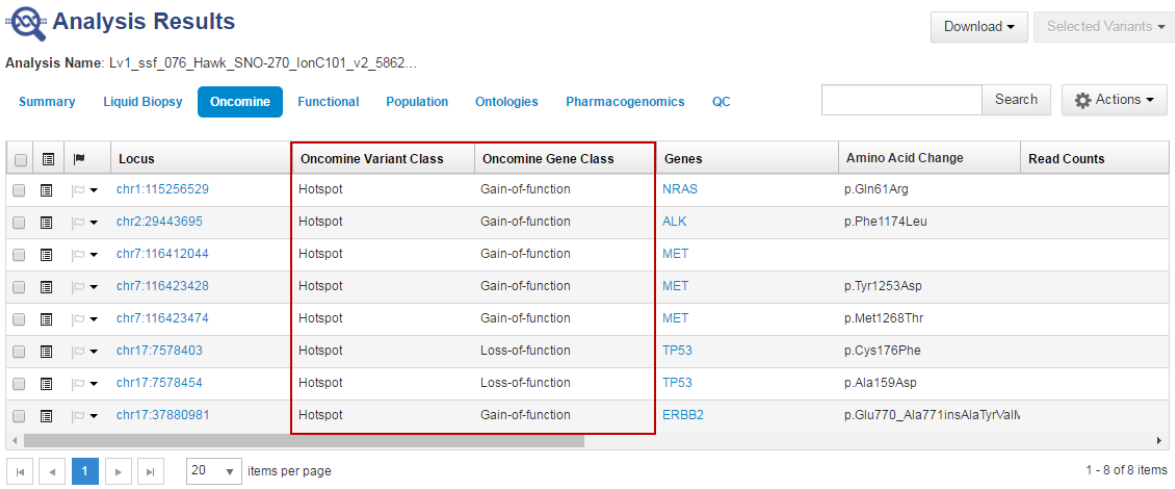
For sensitive variant detection down to 0.1% frequency, we see optimal results when targeting a Median Read Coverage >25,000, Median Molecular Coverage >2,500, and both numbers of the LOD % segment are ≤0.1.

6. In the **Liquid Biopsy** tab, view **Mol Depth**, **Mol Counts**, and other columns.

Note: When you open a visualization that includes REFERENCE calls for hotspot alleles, and consecutive REFERENCE calls are present, some records might have empty values in the **Mol Counts**, **Mol Freq**, and **Detection Limits** columns. This occurs when consecutive REF calls occur. The empty values are equivalent to values reported in the first record of the group of consecutive REF calls that are found in the genome, and shown in the BED file, or as sorted by the software in consecutive rows of the Analysis Results table.

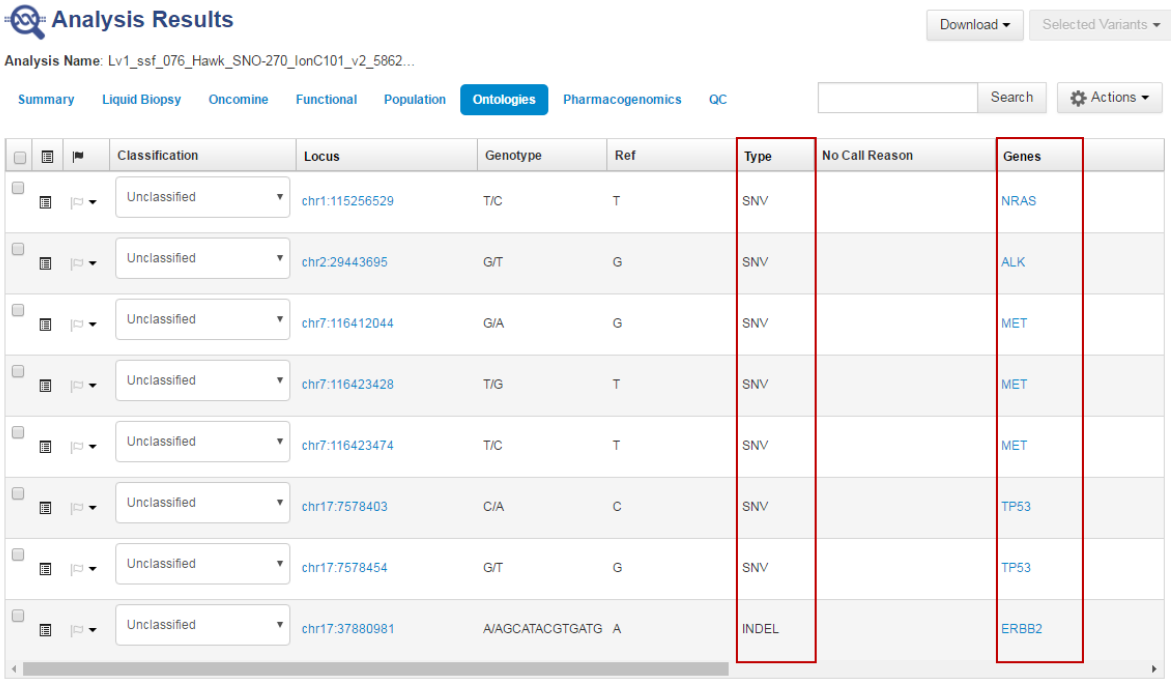
Column	Description
Molecular Depth	Reports number of interrogated DNA molecules containing target. It defines limit of detection at hotspot position in a particular run and sample. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at 0.2%. If molecular depth is ≥2,500, you can have high confidence that no variant is present down to 0.1% LOD. For reference calls, Molecular Depth provides measurable metric that serves as confirmation for variant absence among a large number of interrogated molecules.
Molecular Counts	Reports the number of detected DNA molecules containing variant allele.

7. In the **Oncomine** tab, click the column headings to sort the list of variants by **Oncomine Variant Class** and **Oncomine Gene Class**.



Reference calls display chromosomal position with empty value in the **Amino Acid Change** column.

8. In the **Ontologies** tab, click the column headings to sort the list by variant **Type** or **Genes** to analyze your results.



7 Detailed analysis metrics

Metric	Description
Sample ID	Name of the sequenced sample imported from a sequencing run.
SNV/INDEL	
Gene	HGNC reviewed official gene symbol.
AA Chg	Amino acid change resulting from non-synonymous DNA variant.
Mutant Frequency %	Frequency of mutant allele expressed as a percentage.
Oncomine Variant Class	Variant class annotation that provides a high-level summary of the variant type (hotspot, fusion, amplification, and so on). The annotation is included when the Oncomine™ Variant Annotator plugin is run in Ion Reporter™ Software.
Oncomine Gene Class	Variant gene functional annotation that provides a high-level summary of gene type (Gain-of-Function, Loss-of-Function, or Unclassified). The annotation is included when the Oncomine™ Variant Annotator plugin is run in Ion Reporter™ Software.
Info	HS (targeted hotspot) or PN (potentially novel TP53 variant). *De novo* variant calls available for the breast panel only.
Genotype	Genotype measured associated with a DNA variant call.
Ref Allele	Reference allele as defined in the human genome reference (hg19).
Mut Molecular Cov.	Molecular coverage of the mutant allele.
WT Molecular Cov.	Molecular coverage of the wild type allele from the reference genome.
Depth	Total read coverage across amplicon containing SNV/INDEL hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling. **Note:** This description applies to both, Ion AmpliSeq™ HD and non-Ion AmpliSeq™ HD analyses.
QC Test (LOD) %	Quality control check for SNV/INDEL target regions based on molecular coverage.
Transcript ID	NCBI accession number for the transcript representing the gene target being measured.
Locus	Chromosome and position of detected variant. Click the hyperlink to open the Ion Reporter™ Genomic Viewer to the specified locus.
CNV	
Gene	Gene locus targeted for CNV measurement.
Gain/Loss	Detected copy number gain or loss.

(continued)

Metric	Description
CNV ratio	Ratio of measured CNV gene locus coverage relative to coverage on non-CNV loci.
p-value	Significance of CNV ratio measurement.
Med. Mol Cov. Gene	Median molecular coverage of targeted CNV gene.
Med. Mol Cov. Ref	Median molecular coverage of non-CNV reference loci.
Med. Read Cov. Gene	Median read coverage of targeted CNV gene.
Med. Read Cov. Ref	Median read coverage of non-CNV reference loci.
QC Test	Assay quality control as determined by amplicon coverage uniformity and number of amplicons remaining after outlier removal.
Valid CNV Amplicons	Number of CNV amplicons remaining after outlier removal.
CNV Locus	Chromosomal location of CNV gene being targeted.
Fusion	
Variant (exons)	Name of fusion targeted and respective acceptor and donor exons.
Oncomine Driver Gene	The gene thought to be associated with increased oncogenic properties due to inappropriate activation by the fusion.
COSMIC/NCBI	COSMIC mutation or NCBI accession number.
Mol Cov. Mutant	Median molecular coverage across fusion amplicon.
Read Cov. Mutant	Median read coverage across fusion amplicon.
Detection	Detection status from assay.
QC Test	Assay quality control measured from expression detection of housekeeping genes.
Type	Assay type (For example, Fusion, RNA exon variant (exon skipping), Proc Control).
Locus	Chromosomal locations of targets included in assay.
Ratio To Wild Type	Ratio molecular for exon skipping assay relative to wild type control amplicons.
Norm count Within Gene	Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons. (Lung panel only).

8 Visualize analysis results with Ion Reporter™ Software

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■ Visualization interpretation guidance 397

You can visualize any human DNA or RNA analysis type with Ion Reporter™ Software for research use. You can also visualize multiple analyses in a single browser tab that is useful for comparisons.

Note: The maximum number of analysis results that can be included in a single visualization depends on the size of the analyses results data.

Ion Reporter™ Genomic Viewer (IRGV) is a streamlined version of the Integrative Genomics Viewer (IGV) that is developed by the Broad Institute. IRGV is included with Ion Reporter™ Software. You can set preferences that specify which viewer opens by default and which elements are included in IRGV visualizations. For more information, see "Set IRGV or IGV as the default viewer" on page 311 and "Set IRGV preferences" on page 312.

Genomic segmentation analysis results

The Ion Reporter™ Software can detect heterozygous population SNPs to determine ploidy levels of genomic segments, when some IR analysis workflows are used, such as the Oncomine™ Comprehensive Assay Plus, DNA. The genome is divided into contiguous segments of similar ploidy levels using a circular binary segmentation (CBS) algorithm. Log odd ratios for variant allele frequency of observed population SNPs (by TVC) and copy-number (CN) ratios (by CNV pipeline) for each segment are calculated. Log odd ratio and CN ratios are then used to infer:

- Tumor cellularity percentage (percentage of tumor cells in the sample)
- Loss-of-heterozygosity (LOH) for each genomic segment. Segment level LOH events are intersected with targeted gene boundaries to determine LOH events in selected genes. Segment level LOH events are also aggregated to determine genomic (%LOH).
- Genomic Instability Metric (GIM)

To download the results, see "Download filtered variants" on page 209.

Visualization of genomic segmentation analysis, Allele Specific Copy Number plots

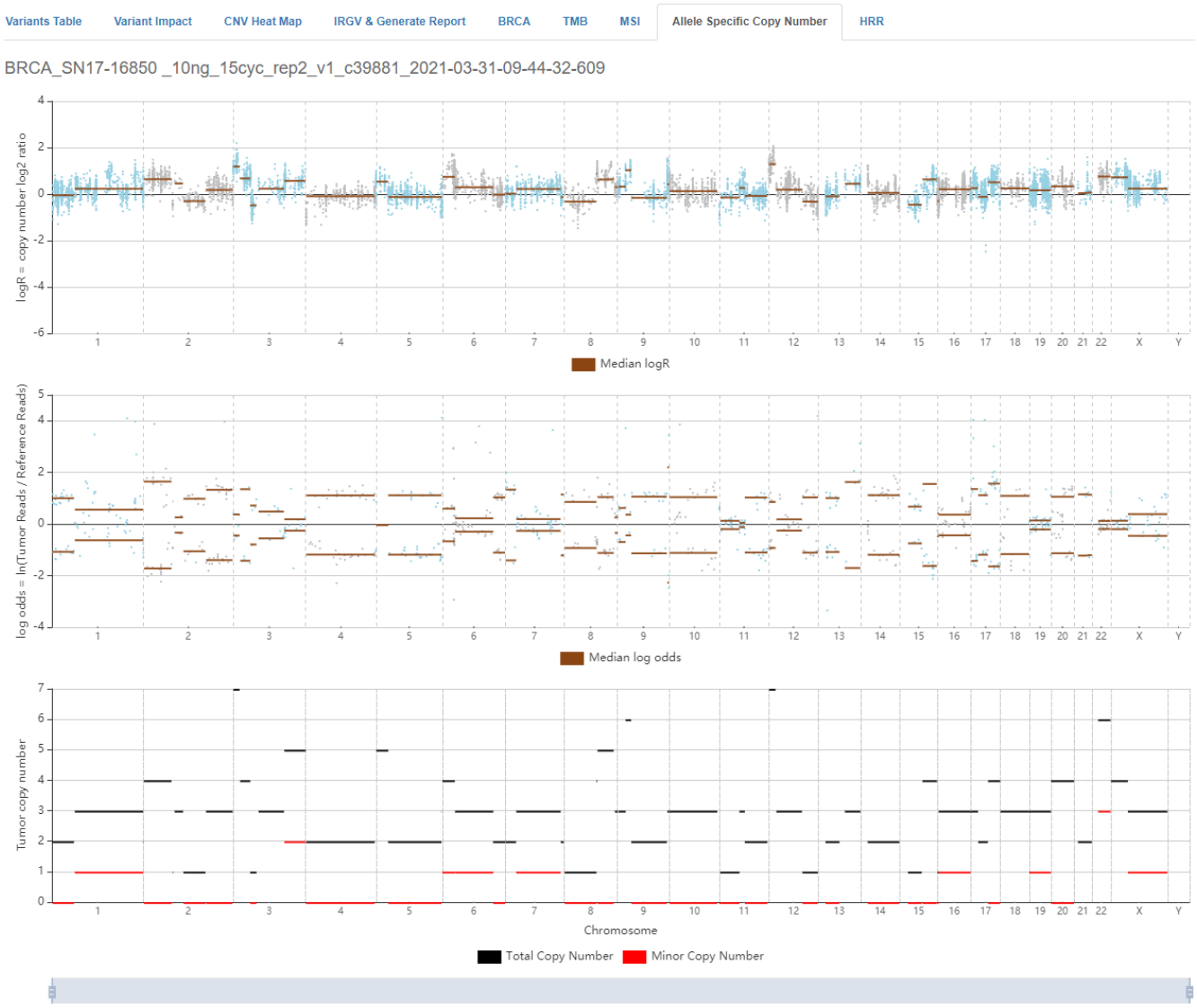
1. Do one of the following to visualize analysis results that used an analysis workflow to detects genomic segmentation and allele specific copy number.

Option	Description
Visualize analysis results from one or more analyses simultaneously from the **Analyses** table.	In the **Analyses** table, select a row for an analysis or select the checkbox next to one or more analyses that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions** ▸ **Visualize**.
Visualize analysis results individually from the **Analysis Results** screen.	In the **Analyses** table, click an analysis hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**.

Note: The **Analysis Visualization** screen opens to the **TMB** tab by default.

2. Click the **Allele Specific Copy Number** tab.

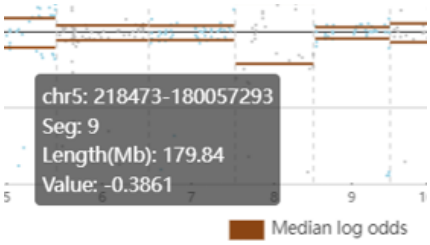
   The **Allele Specific Copy Number** tab displays plots showing copy number variation across the entire genome. If visualizing multiple analyses simultaneously, individual sample results are listed sequentially. This visualization shows identified genomic segments (shown as horizontal lines) using log2 ratios from the Copy Number Variation (CNV) analysis and log odds from the Torrent Variant Caller (TVC) analysis. Segments are identified using the heterozygous population SNPs (shown as dots in the upper two panels) targeted by assay amplicons.



Plot scale (x-axis)	Description
logR = copy number log2 ratio	Log2 ratios (top panel) of the copy-number estimates relative to the baseline copy-number as calculated by the CNV algorithm for each amplicon in the assay. Ratio representative Genome segmentation overlay (horizontal brown bars) shows segments with similar log2 ratios clustered together.
log odds = ln(Tumor Reads / Reference Reads)	Log odds (middle panel) for each heterozygous SNP in the assay. Log odds is calculated as the natural logarithm of the ratio of the sequencing reads with variant allele and reference allele that are obtained using Torrent Variant Caller (TVC). Genome segmentation overlay (horizontal brown bars) shows segments with similar log odds clustered together. Because the variant allele could be major or minor for any SNP and the corresponding log odds could be positive or negative, the long odds are displayed as segments that are mirror images around 0.
Tumor Copy Number	Bottom panel shows the total (black horizontal line) and minor (red horizontal line) copy-number estimates for each of the identified genomic segments (top two panels). Genomic segments where total CN ≥1 and minor CN = 0 are segments with LOH. There may exist genomic segments for which minor copy-number estimates can not be determined.

3. *(Optional)* Select one of the following actions.

   - To see the gene or gene segment information corresponding to the genomic loci, hover over a dot in the plot.



   - To save a PNG image of all three plots, click **Export Image** to save a PNG image of all three plots to a location of your choice.

Visualize HRR analysis results

Cells use the Homologous Recombination Repair (HRR) pathway to repair double-strand breaks generated as by-products of cellular metabolism, DNA replication errors, or exogenous factors.

Homologous Recombination Deficiency (HRD) is the inability of cells to repair double-strand DNA breaks using the HRR pathway.

The HRR visualization table summarizes relevant loss-of-function variants to provide information and enable assessment about likely biallelic variants in that gene, for example, BRCA1 and BRCA2. In addition, the HRR visualization table displays the cause and consequences of HRD by displaying metrics for measuring HRD (genomic loss-of-heterozygosity in the sample (%LOH), Genomic Instability Metric (GIM) score, and Genomic Instability Status).

Gene list for gene-level LOH (Ion Reporter™ Software 5.14): ATM, BRCA1, BRCA2, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, and RAD54L.

1. Select one of the following options.

Option	Description
Visualize analysis results from an individual sample or from multiple samples simultaneously from the **Analyses** table.	In the **Analyses** table, select an individual sample result row, or select the checkbox next to each sample result that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions** ▸ **Visualize**.
Visualize analysis results individually from the **Analysis Results** screen.	In the **Analyses** table, click a sample result hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**.

   The **Analysis Visualization** opens to the **TMB** tab by default.

2. Select the **HRR** tab to review a summary of the analysis results about the genes and genomic alterations in the HRR pathway. For example, you can view information about genomic LOH (sample %LOH), CNVs, SNVs, INDELs and the Genomic Instability Metric (GIM). You can also view any exon-level deletions and duplications that are associated with BRCA1 and BRCA2 genes.

   Note:
   - If variants meeting a category are not detected, **Not Detected** is reported.
   - You may see differences in variant calls reported in the Oncomine Variant tab or default variant table on the **Analyses** page and HRR summary table due to differences in the filter chain used and allele frequencies.
     - Variants in the HRR table are filtered using the TMB (Non-germline Mutations) filter chain. Variants in the default variants table are filtered using the Oncomine™ Extended filter chain (OEFC). OEFC retains pathogenic germline variants reported in ClinVar, whereas TMB (Non-germline Mutations) does not. As a result, the HRR summary table does not contain these variants.
     - Variants reported in HRR summary table have variant allele frequency ≥5%. As OEFC does not apply this criteria, all variant frequencies are reported in the default variant table.

3. Click **Download** ▸ **Current Results TSV** to download a tab-separated list of detected variants in the analysis results. Open the file with compatible software, such as Microsoft™ Excel™.

   For more information about downloading files, see “Download filtered variants” on page 209.

4. Click **Download** ▸ **Filtered Variants** or **Download** ▸ **All Variants** to download a ZIP folder of files that list the variants in the analysis results.

   The file `hrr_summary.tsv` is included in the downloaded results.

5. Select the **BRCA** tab to visualize information about whole exon and multiple exon deletion in the BRCA1 and BRCA2 genes in somatic and germline samples with high sensitivity. For more information, see “View Oncomine™ BRCA analysis results” on page 237.

6. Select the **IRGV & Generate Report** tab, then click **Generate Report** to create and download a visualization report in PDF format, which includes the analysis results with graphs and metrics. For more information, see “Create a visualization report” on page 413.

Mutational Signature profiles

Cancer genomes are subject to diverse mutational processes that generate recognizable mutational signatures. Some processes are driven by defects in specific DNA repair pathways (for example, MMR) whereas others are characteristic of environmental mutagens (for example, ultra-violet light, tobacco smoke). These processes generate unique combinations of mutation types, a “Mutational Signature”.

Somatic SNVs in the sample are filtered to remove germline mutations and are used to construct a normalized single base substitution (SBS) matrix, or mutational signature. The normalized sample mutational signature is then compared with 54 signatures from the COSMIC Mutational Signatures v3.1 and the cosine similarity is calculated. COSMIC signatures with a cosine similarity score ≥ 0.7 to the sample signature are analyzed using the deconstructSigs algorithm to determine the COSMIC mutational signatures with the highest cosine similarity to the sample.

View the Mutational Signature Prediction report

To view the mutational signature prediction report (`signature_identification_<analysis_name>_report.pdf`) you must first download the ZIP folder. For more information, see “Download filtered variants” on page 209. The folder contains the following files.

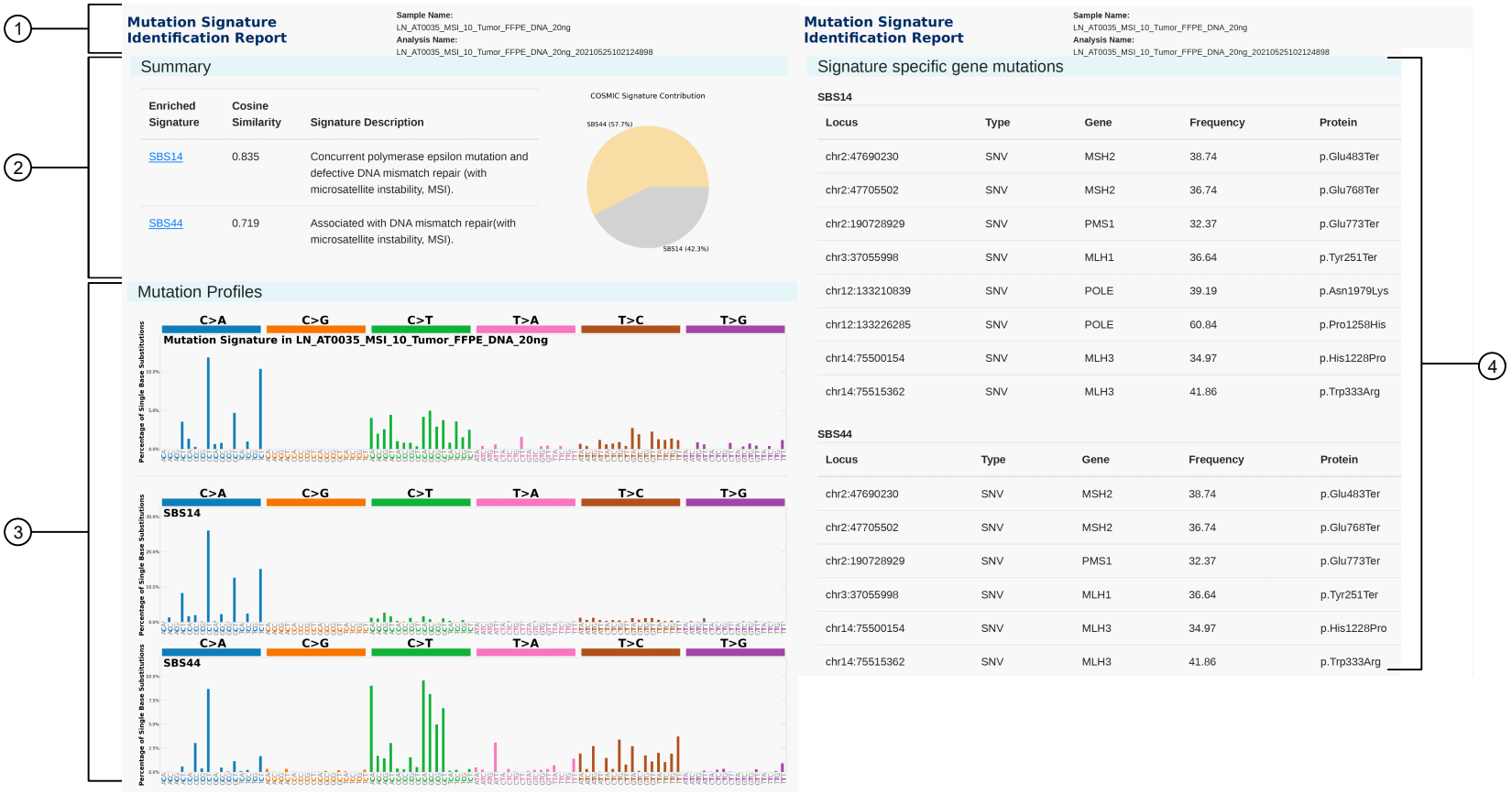
- PDF Report
- Profile of mutational signature of sample (Available only if a mutational signature match is found.)
- Profile of enriched COSMIC signature (One or more, available only if a mutational signature match is found.)
- deconstructSigs Weights Pie Chart (Available only if a mutational signature match is found.)
- Trinucleotide distribution of variants

1. Extract the downloaded ZIP folder to your hard drive.
2. Open the **signature_prediction** folder, then double-click the PDF Report file.

Mutational Signature Prediction report

File	Description
PDF Report	FileName format is “signature_identification_<analysis_name>_report.pdf”. The PDF report consists of 4 sections: **1.** Header • Report Name - "Mutational Signature Prediction Report" • Sample Name • Analysis Name (If a sample is reanalyzed, the Analysis name will change) **2.** Summary • Table **a.** Name of enriched signature, from COSMIC **b.** Cosine similarity between sample mutational profile and COSMIC Signature **c.** Signature Description or Aetiology • COSMIC Signature Weights—pie chart denoting weights from deconstructSigs. This reflects the strength of the evidence for the match between the data and the Cosmic signature. **3.** Mutation Profiles **4.** Signature Specific Gene Mutations
Profile of Mutational signature of Sample	File name format is “<analysis_name>_normalized_sample_profile.png”. The image is present in the PDF report under “Mutation Profile” section.
Profile of COSMIC signature	File name format is “<analysis_name>_<cosmic_signature>.png”. The image is present in the PDF report under “Mutation Profile” section. One image is generated per enriched COSMIC signature. The output will not be generated if any enriched signature is not found.
deconstructSigs Weights Pie Chart	File name format is “<analysis_name>_signatures_pie.png”. The image is present in the PDF report under “Summary” section. The output will not be generated if any enriched signature is not found.
Trinucleotide distribution of Variants	File name format is “<analysis_name>_contextFile.txt”. It contains frequencies of mutations within each of the 96 trinucleotides.

Mutation Signature Prediction report example



① Header
② Summary
③ Mutation Profiles
④ Signature Specific Gene Mutations

Time series visualizations

You can track and visualize variants over time in Ion Reporter™ Software. With time series visualizations, you can view trends and changes for variant alleles over time. For example, you could graph the percent frequency of TP53 c2369C>T at regular intervals from specimens collected from a single individual. Time series graphs show both qualitative (such as no call instances) and quantitative (such as the allele ratio) results for variants. You can remove and restore variants dynamically in the graphs that are shown in the software, and adjust how data are organized in the graph before you generate the chart. Customization of the graphs allows you to visualize the most relevant information.

To visualize results with a time series graph, you must select analyses that use the same analyses workflows. The name of the analysis workflow, the version number, and the revision number, if applicable, must be identical.

Time series graphs are supported for the following variant types:

Type of graph	Supported variants
Fusion	• **Fusion** • **RNAExonVariant**
SNVs/Indel	• **SNV** • **MNV** • **Indel**
CNV	• **CNV**

Create and view a time series graph

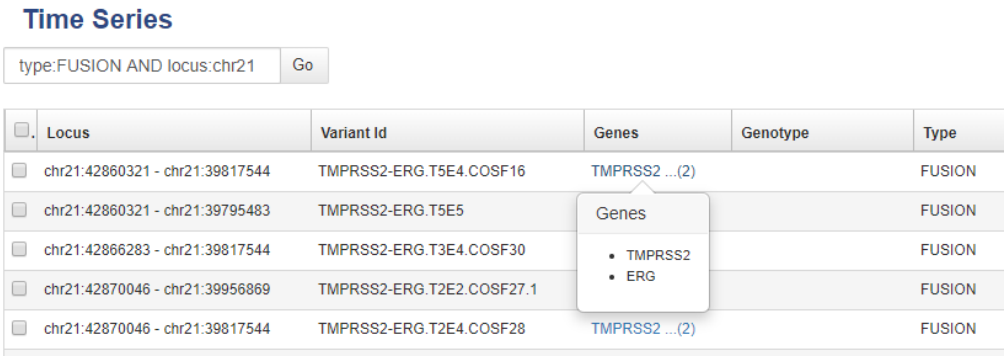
You can create and view time series line graphs for **Fusions**, **SNVs/Indels**, and **CNVs**. **Fusions** time series graphs include **Fusion** and **RNAExonVariant** variants, **SNVs/Indels** graphs include **SNV**, **MNV**, and **Indel** variants, and **CNV** graphs show **CNV** variants only. You can customize the Y-axes values and the X-axis labels. Time series graphs can only be created for analyses that were launched with the same analysis workflow.

In the software, you can select one type of variant and generate a single time series graph or you can select multiple types of variants (such as both **SNVs** and **Fusions**) and generate multiple graphs simultaneously. If you plot multiple types of variants, the graphs appear side-by-side. You can add or remove variants, or add or remove entire graphs to change the appearance of the graphs in the screen. You can also download image files of time series graphs.

1. In the **Analyses** tab, select at least 2 analyses that use the same analysis workflow and contain the data of interest.
2. Click **Actions** ▸ **Time Series** next to **Selected Analyses**.

Note: **Time Series** is only available as an option when 2 or more analyses that use the same workflow are selected.

The **Time Series** screen opens. By default, no filter is applied and all variants from the analyses are displayed. In the **Time Series** screen, you can view details about the variants and filter the list of variants to find the variants that you want to add to a time series graph.



The following columns are included in the **Time Series** table.

Column	Description
☐	Selection box—Use to select the locus in the row that contains the selection box. Select one or more loci to create a chart or charts.
Locus	The location of the variant on a specific chromosome or chromosomes.
Variant ID	The identifier of the variant.
Genes	The affected gene or genes.
Genotype	The genotype sequence.
Type	The type of variant or call.
Ref	The reference sequence.
Observed Allele	The observed allele variation.
Coding	HGVS notation that represents a nucleotide change.
Amino Acid Change	HGVS notation that represents an amino acid change.
Subtype	The variant subtype.

3. *(Optional)* In the **Filter Options** dropdown list, select a filter chain from the **Filter Chains** list to limit variant results.

4. *(Optional)* Use the **Search** field to limit the list of analyses. For example, in the **Search** field, enter ***type:FUSION*** to find fusions. See “Searches on the Analysis Results screen” on page 223 for more information.

5. Select at least one variant in the **Time Series** screen, then click **Generate Chart**.

   The order of the variants in the line graph match sequentially with the order in which you select the variants. For example, to show the changes in allele frequency over time from the oldest to most recent, select the oldest time point first, and continue to select variants in sequential order. The first variant that you select is positioned first (in the left-most position of the graph along the X-axis).

If variants for the analyses are displayed in **Locus View**, a **Select Alleles** window opens. For more information, see "Locus View versus Allele View of variants" on page 197.

a. In the **Called Alleles** column for each locus, in each dropdown list, select the alleles you want to graph.

b. Click **Select**.

The **Chart Preferences** window opens.

6. In the **Chart Preferences** window, select the **X-Axis Label**, the **Y-Axis Value**, and the **Variant Label** to customize the data displayed in the graph. Available options depend on the analyses.

All Charts

Label	Option
X-Axis Label	• **Analyses Name** (default option) • **Sample Name** • **Sample Collection Date** • **Sample Receipt Date** • User-defined sample attributes are also available selections if all selected and analyzed samples contain an entry. If any sample included in the selected analyses does not have an entry for the user-defined sample attribute, that attribute is not listed as a chart option.

SNV/Indel

Label or Value	Option
Y-Axis Value	• **Allele Frequency %** (default option) • **Allele Ratio** • **Mol Freq %**[1]
Variant Label	• **Gene + Coding** (default option) • **Gene + AA Change** • **Locus** • **Coding** • **Amino Acid Change** • **Variant ID**

[1] Option appears in Ion AmpliSeq™ HD and TagSeq analyses only.

CNV

Label or Value	Option
Y-Axis Value	• **CNV Ratio**[1] (default option) • **Copy Number**[2] • **Ploidy (log scale)**
Variant Label	• **Locus** (default option) • **Genes**

[1] Option appears in Ion AmpliSeq™ HD and TagSeq analyses only.
[2] Option appears in Ion AmpliSeq™ analyses only.

Fusions

Label or Value	Option
Y-Axis Value	• **Read Counts Per Million** (default option)
Variant Label	• **Locus** (default option) • **Variant ID** • **Genes**

7. Click **Generate**.
   The **Time Series** graph or graphs displayed.

8. *(Optional)* Click **Download** to download a ZIP file that contains PNG files of the graph or graphs.

9. *(Optional)* Remove or add the variants that appear in the graph.
   - Click a variant label in the color key at the top of a graph to remove the data points for that variant from the graph. Variants that have been removed from a graph are shaded in the key.
   - Click shaded variant labels in the key to add the data points back to the graph.

   For an example, see “Example of dynamic edits to time series graphs” on page 310.

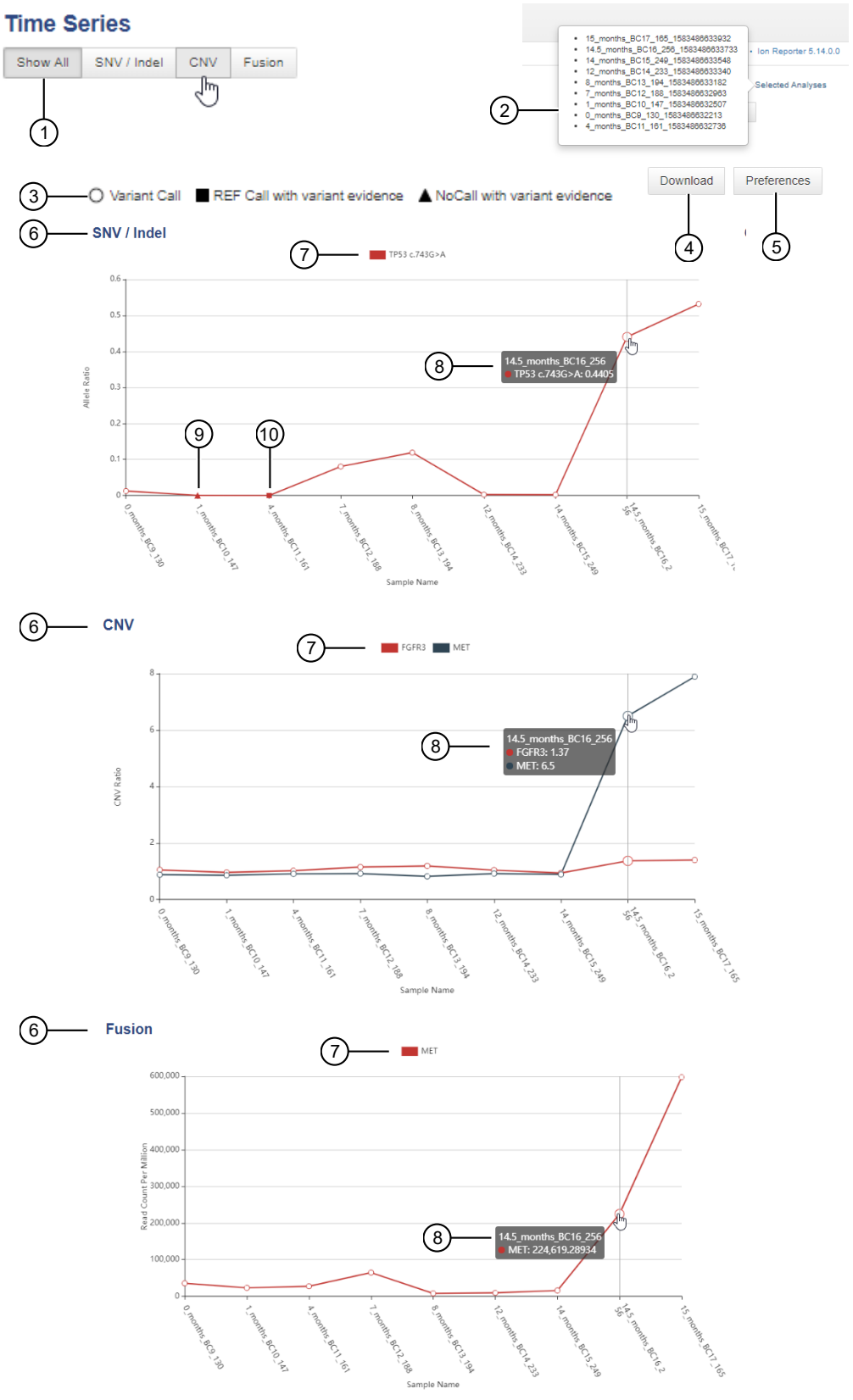
10. *(Optional)* In the table below the graph, deselect current variants or select different variants, then click **Generate Chart** to add or remove selections from the graphs or create time series graphs with new selections.

Example time series graphs

Time series line graphs show quantitative variant changes, such as the allele frequency, over time. In the software, you can generate a single time series graph or you can simultaneously generate multiple graphs with different types of variants, such as both **SNVs** and **Fusions** graphs. In this example, all three variant graphs are shown. The **SNV/Indel** graph shows the allele ratio of c743G>A TP53 mutations over the course of 15 months. The **CNV** graph shows the CNV ratio of FGFR3 and MET and the **Fusions** graph shows read counts per million for MET over 15 months.

The following Variant Label preferences were set for this example.

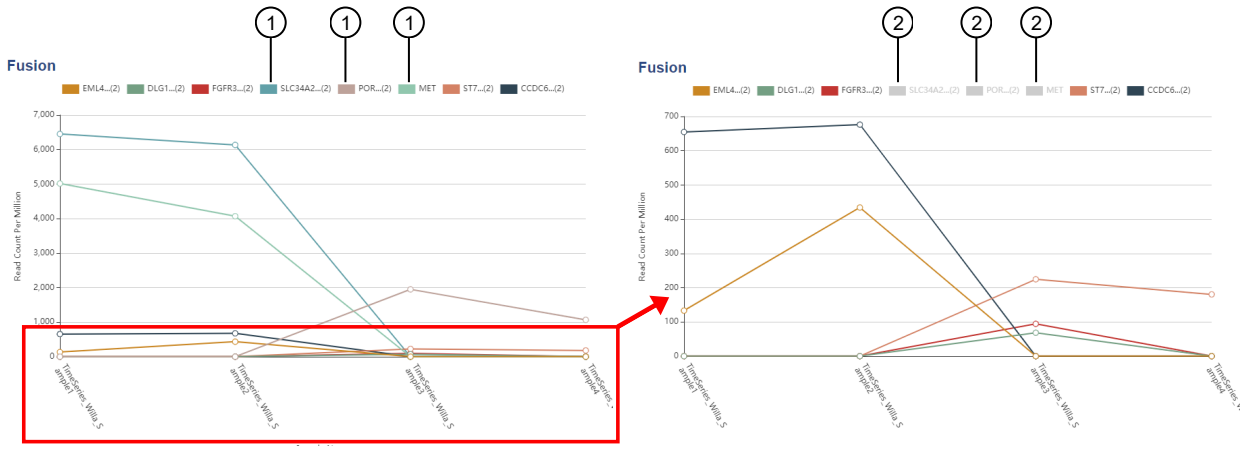
Type of chart	Variant Label choice	Example shown
SNV/Indel	**Gene + Coding**	• TP53 c743G>A
CNV	**Genes**	• FGR3 • MET
Fusions	**Genes**	• MET **Note:** In this example only one gene is shown because an **RNAExonVariant** is selected.



1. Click **Show All**, **SNV / Indel**, **CNV**, or **Fusion** to view all graphs or limit the view to the selected graph.
2. Click **Selected Analyses** to view the list of the analyses that were selected.
3. A general key for graphs. Variant calls are represented by an open circle, hotspot reference alleles are represented by a filled square, and data points with no call are represented by a filled triangle.
4. Click **Download** to download a ZIP file that contains PNG files of the graph or graphs.
5. Apply **Preferences** to customize the graphs. For more information, see "Create and view a time series graph" on page 304.
6. The type of variant graph: **SNV / Indel**, **CNV**, or **Fusion**.
7. A key for each graph that can be customized. Click a variant label in the key to remove all data points for that variant label from the graph.
8. Hover the mouse over a

Example of dynamic edits to time series graphs

You can remove variants from a time series graph. For example, if a graph shows many variants, it can be easier to visualize trends when fewer variants are displayed. For **Fusion** line graphs, the value of the Y-axis adjusts according the values of the variants that are shown in the graph. To pinpoint the trend of a variant, it can be helpful to visualize the graph for that variant within a scale relevant for that variant. The following image shows a **Fusion** time series graph with eight variants selected on the left panel. In the right panel, three variants are removed and the value of the Y-axis is adjusted to the range of the values of the selected five variants.



① Click a variant label to remove the data points for that variant from the graph.

② Variants that have been removed from a graph are shaded. Click a shaded variant label in the key to add the variant back to the graph.

Set IRGV or IGV as the default viewer

You can set a preference for the user account that determines whether Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV) opens by default when you visualize analyses.

IGV is the genomic viewer that is made by the Broad Institute. IRGV is the faster-loading genomics viewer that is built into Ion Reporter™ Software.

1. Click ⚙ **(Settings)** ▸ **Account Preferences**.
2. In the **Account Preferences** dialog box, in the **IGV launch preference** row, select **IGV** or **IRGV**, then click **Save**.
   A visualization opens when you perform the following actions.

Option	Description
A visualization of one or more analyses in IRGV.	1. In the **Analyses** tab, in the **Overview** screen, enable the checkbox in for the analysis you want to visualize, then click **Visualize**. The visualization opens in the **Analysis Visualization** screen in **IRGV**.
A visualization of a gene of interest in a separate browser tab.	1. In the **Analysis Results** screen, click the locus of interest in the **Locus** column. • If the **Account Preference** is set to **IRGV**, IRGV is launched in a separate browser window. • If the **Account Preference** is set to **IGV**, a ZIP file that contains files for upload to the Integrative Genomics Viewer (IGV). Open IGV on your computer. Unzip the files, then open the ***session.xml*** file in IGV. The files that are exported contains only the following information for the selected variant. – For DNA variants – Sliced data are available for download that is based on the variant locus and flanking 200 base pairs. – Required files in the ZIP file includes all files, except for the reference genome FASTA files, which are not included. – For DNA CNV variants – Sliced data are available for download that is based on the CNV start position of - 200 and CNV end position of + 200 base pairs. – Required files in the ZIP file includes all files, except for the reference genome FASTA files, which are not included. – For RNA variants – Sliced data are available for download that is based on the fusion isoform.

Set IRGV preferences

You can modify the elements that are included in Ion Reporter™ Genomic Viewer (IRGV) analysis visualizations with preferences. To set the preferences, you must first visualize an analysis in the viewer.

1. Under the **Analyses** tab, click ***Overview***.
2. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.
3. In the **IRGV & Generate Report** tab, scroll to the bottom of the visualization, then click **IRGV/Export & Preferences ▸ Show IRGV preferences** to expand the **Preferences** section.

   You can set the following IRGV preferences.

Option	Description
Default mapd value	This value is used as default value in the mapd filter.
Default sort order [1]	The preferred sort order for Aneuploidy analyses (NR, CID, EID or BIOPSY) for the summary table and tracks. NR sorts by the analysis nr, CID sorts by couple ID, EID sorts by embryo ID and BIOPSY sorts by biopsy days.
Analysis limit for BAM tracks	Maximum number of analysis that includes BAM tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and a crowded view.
Analysis limit for BED tracks	Maximum number of analysis that includes BED tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and a crowded view.
Limit for coverage data	By default, the maximum limit of 25000 is used. Decrease this number if you do not want to see the coverage data, or you have problems with the browser, such as freezing.
Max number tracks in karyo	By default, 5 tracks are shown. You can set a value of 1 to 10. When you increase the number of tracks, more space is used in the Karyo view.
Max Ploidy in Whole Genome View	Maximum value of the Y-axis (ploidy) in the Whole Genome View graphs.
Display Fixed Ploidy Lines	You can show fixed ploidy lines at copy number=1 and copy number=3 across the Whole Genome View. • **true**—Select this default setting to show fixed ploidy lines at CN=1 and CN=3 across the Whole Genome View. • **false**—Select this setting to show ploidy lines that are not fixed.

(continued)

Option	Description
Whole Genome View Height	You can double the height of the Y-axis when you view whole genome data in IRGV. The **Enlarged** view is useful to review mosaic data, because more data are shown on a single plot and more space is available between data points, so that the mosaic data more pronounced. • **Compressed**—Select this default setting for IRGV images that are 300 pixels high. • **Expanded**—Select this setting for IRGV images that are 600 pixels high.

[1] This preference can be set only when multiple analyses are selected.

4. For each value that you want to change in the **Preferences** section, click **Edit** next to the value that you want to change, then enter a new value into the **Edit Preferences** dialog box, then click **OK**.

5. When the edits are complete, click **Save Preferences**.

   The preferences are reset to the default settings if you click **Reset Preferences**.

   The selected preferences are applied to all analyses that you visualize immediately after you save the changes.

Browse a visualization by amplicon name

You can use the links on the amplicon names that are in the **Amplicon Coverage** table to browse through analysis results that are open in the **IRGV & Generate Report** tab or a visualization that is open in Integrative Genomics Viewer (IGV).

In some web browser versions, this functionality may be blocked for IGV. If you cannot browse by amplicon names in an IGV, use instead IRGV, or use a different web browser.

1. Click a link for an amplicon name in IRGV or IGV for the relevant experimental data, panel files, or genomic annotation sources.

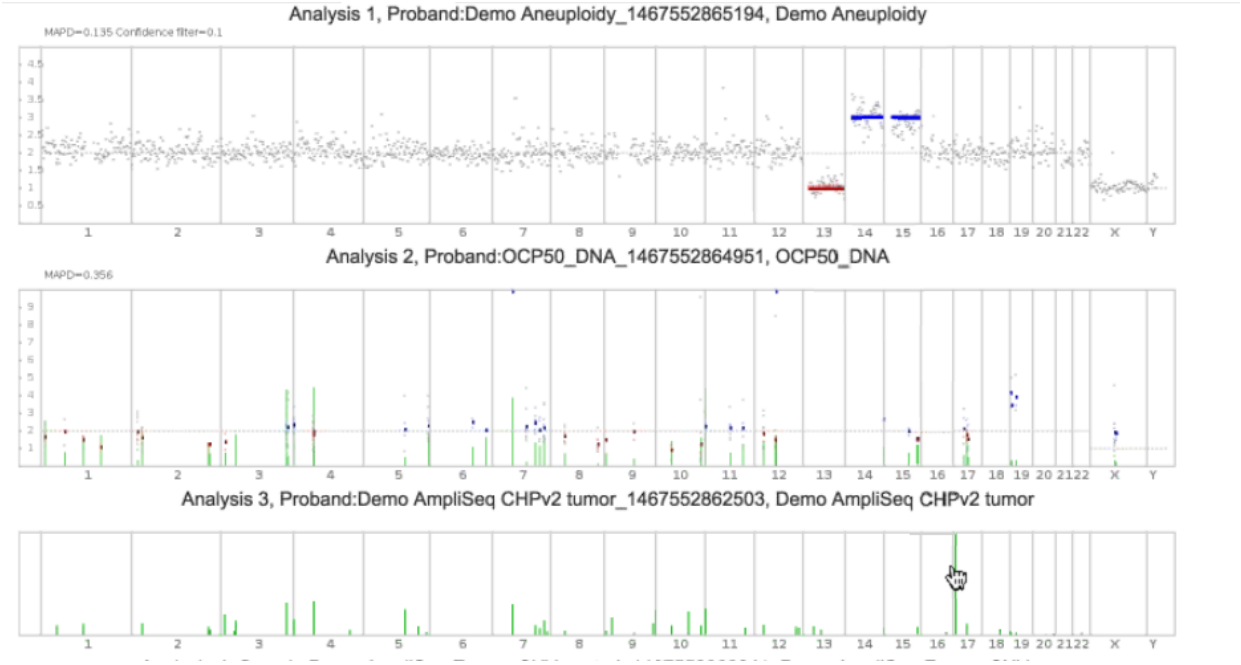
   If you use IRGV, drag, then drop, the newly opened **IRGV & Generate Report** tab to view the tab as a separate window.

2. Click another link for an amplicon name in the **Analysis Results** table to browse to the next amplicon name in the visualization.

Multiple analyses and multiple analysis types in IRGV

You can view multiple analyses in a single Ion Reporter™ Genomic Viewer (IRGV) visualization. The analyses can be different types of analyses. The following image is a whole genome view of an aneuploidy, Oncomine™, and cancer hotspot analyses. Different types of analyses look different in the whole genome view and include different information in the **Summary** table.

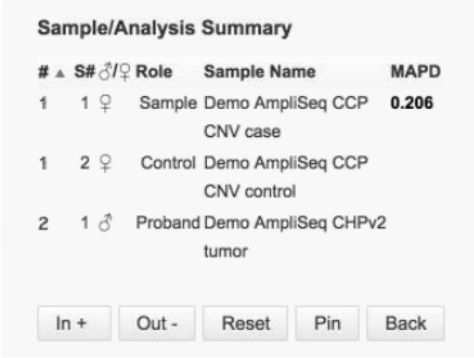
Note: The maximum number of analyses that you can open in a single visualization depends on the size of the analyses results data.



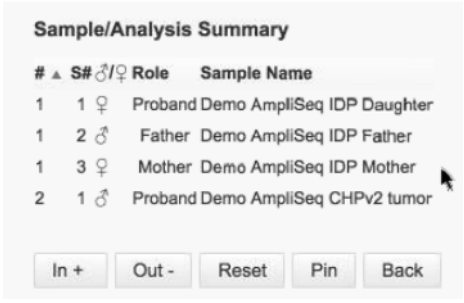
The first analysis on the whole genome view plot is an aneuploidy analysis. The plot displays CNV calls. It also displays **MAPD value**, productive read count of the sample, and the confidence filter used in the default filter chain. Blue and red bars in the whole genome view represent CNV calls. Blue bars represent calls above the expected normal ploidy value (Copy Number Gains/Duplications/Insertions) for that chromosome (chr14 and 15 in this aneuploidy example). The Red bars represent calls below the expected ploidy value for the chromosome (Copy Number Losses/Deletions, chr 13 in this aneuploidy example).

The second whole genome view plot is an Oncomine analysis where somatic CNV calls have been made. Instead of using a confidence filter, these data uses 5% and 95% Confidence intervals that are not shown in this view. The green bars represent a density plot of SNPs, INDELs, and other calls. Click any green density plot and the software zooms in on details.

The various types of analyses also produce different summary tables.

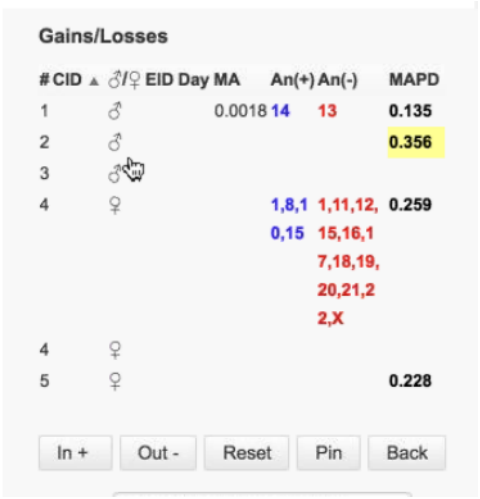


Summary table from a CNV analysis

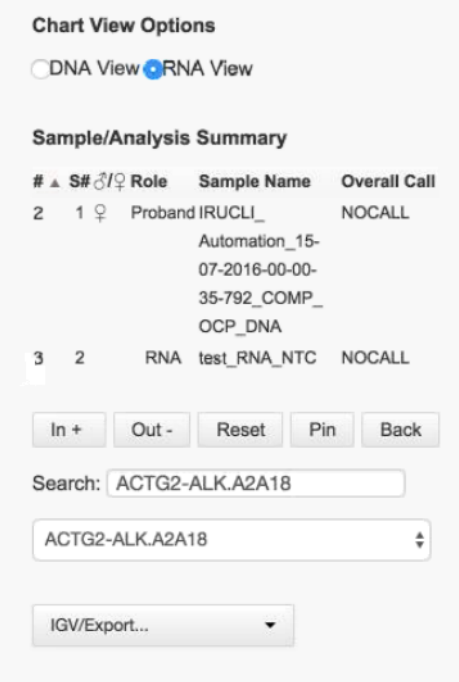


Summary table from a Trio analysis

This summary table is from an aneuploidy analysis:



Summary table from an aneuploidy analysis



Summary table from the RNA View option in a DNA analysis

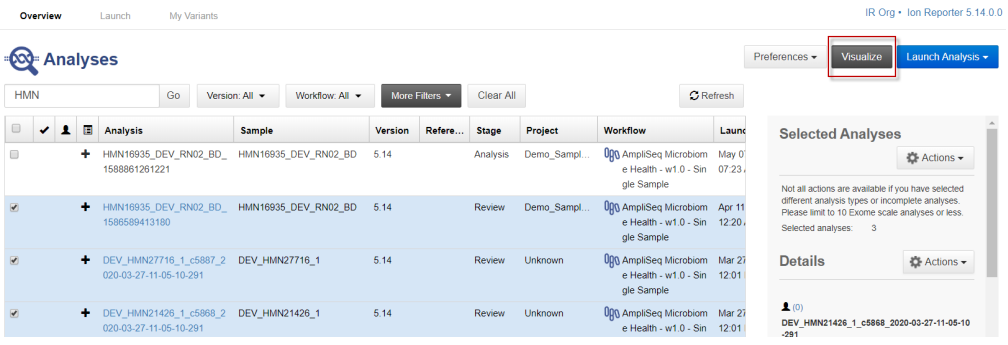
Visualize microbiome health research analysis results

If you use the AmpliSeq Microbiome Health analysis workflow, you can visualize analysis results in Ion Reporter™ Software.

To view the analysis results from a single analysis, see “View microbiome health research analysis results” on page 264.

1. In the **Analysis** tab, click **Overview** tab.
2. Select the check box for each analysis to be used for analysis, then click **Visualize**.

   You must select at least three analyses. A minimum of three analyses are required for statistical analysis.

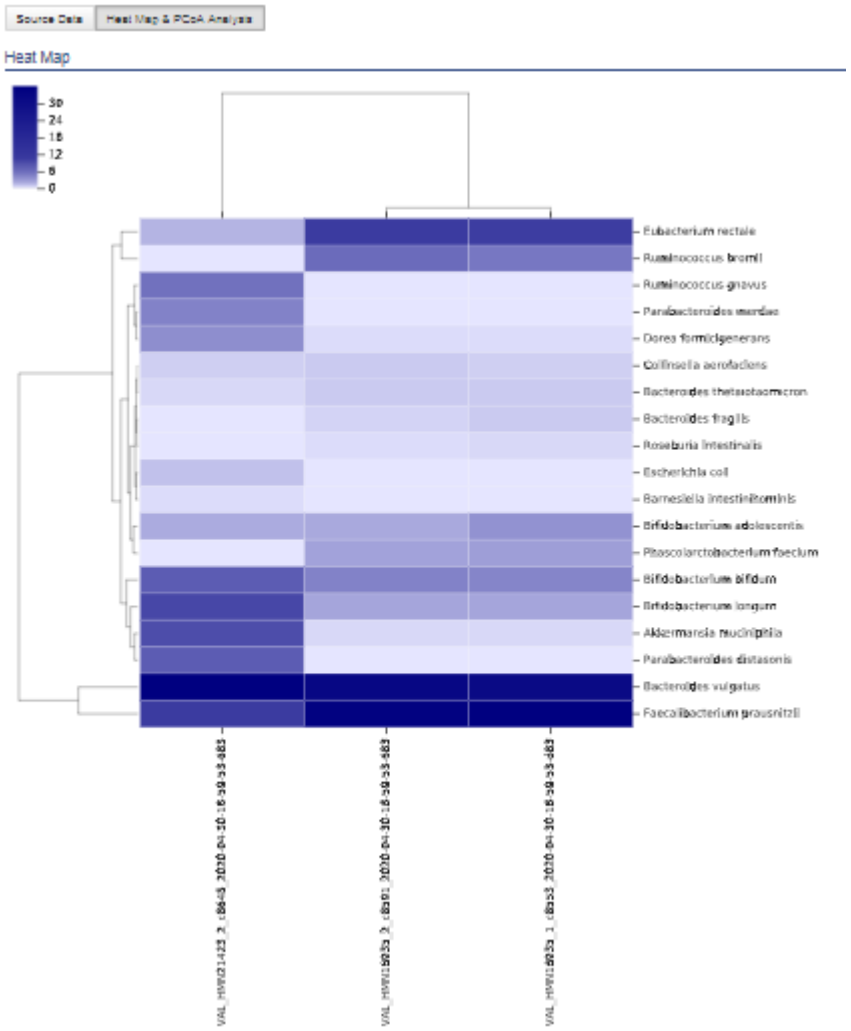


The **Analysis Results** screen opens to the **Summary** tab.

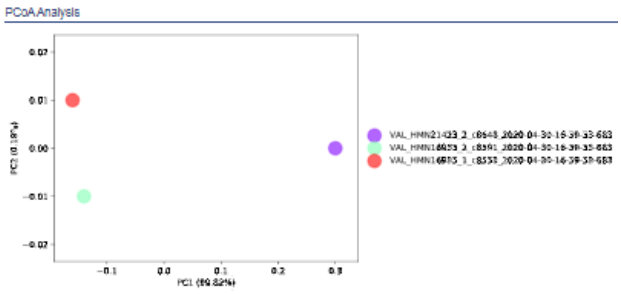
The **Summary** tab lists data for both the target species and the 16S rRNA gene. The **Target Species Markers** lists the total number of detected species. The **16S rRNA Gene Markers** lists taxonomic data that is identified in the sample, and a value for **Alpha Diversity**. The alpha diversity results describe the diversity in a single sample at the species, genus, and family levels, and show the following metrics for each analyses.

- Pool QC—Pool QC is determined to be either PASS or FAIL. If the total number of valid mapped reads is greater than the threshold applied and the mean read length of all the reads from the BAM file is greater than the threshold, pool QC is determined to be PASS, otherwise FAIL. If pool QC is determined to be FAIL, it means that library pool failed and the results are not valid.
- Total Reads—The total number of filtered and trimmed reads independent of length reported in the output BAM file.
- Total Valid Mapped Reads—Total number of reads mapped to the reference sequences with good mapping quality (reads with alignment score greater than the minimum local alignment score threshold).
- Mean read length—The average length of all reads from the BAM file.

3. You can view results for individual samples, or view graphical representations of the selected analyses.
    - To view data for an individual sample, click **View Results** in the **Results** column of the **Target Species Markers** or the **16S rRNA Gene Markers** sections. Click **Back to Summary** to return to the **Summary**.
4. To view target species data, select the **Target Species** tab, then click the **Source Data** or **Heat Map & PCoA Analysis** sub tab.
    - The Ion AmpliSeq™ Microbiome Health Research – Target Species Pool detects 73 bacterial species that are associated with human disease. Click **Download Results** to download a file with a list of the 73 species.
    - Click **Source Data** to view data on the abundance of each species in each of the selected samples.
    - Click **Heat Map & PCoA Analysis** for a heat map of the abundance of the identified target species for each sample and a PCoA analysis plot, which shows Beta diversity results to describe similarities and dissimilarities between samples.



Species distribution heat map



PCoA analysis plot

5. To view taxonomy data, in the **16S rRNA Gene Markers** section, click **View Results** in row of a sample, then select one of the following sub tabs.
   a. To view a summary of the taxonomy data, click **Source Data**.

b. To view a bar chart of the abundance of detected species at the phylum, family and genus levels and a heat map, click **Microbe Distribution**.

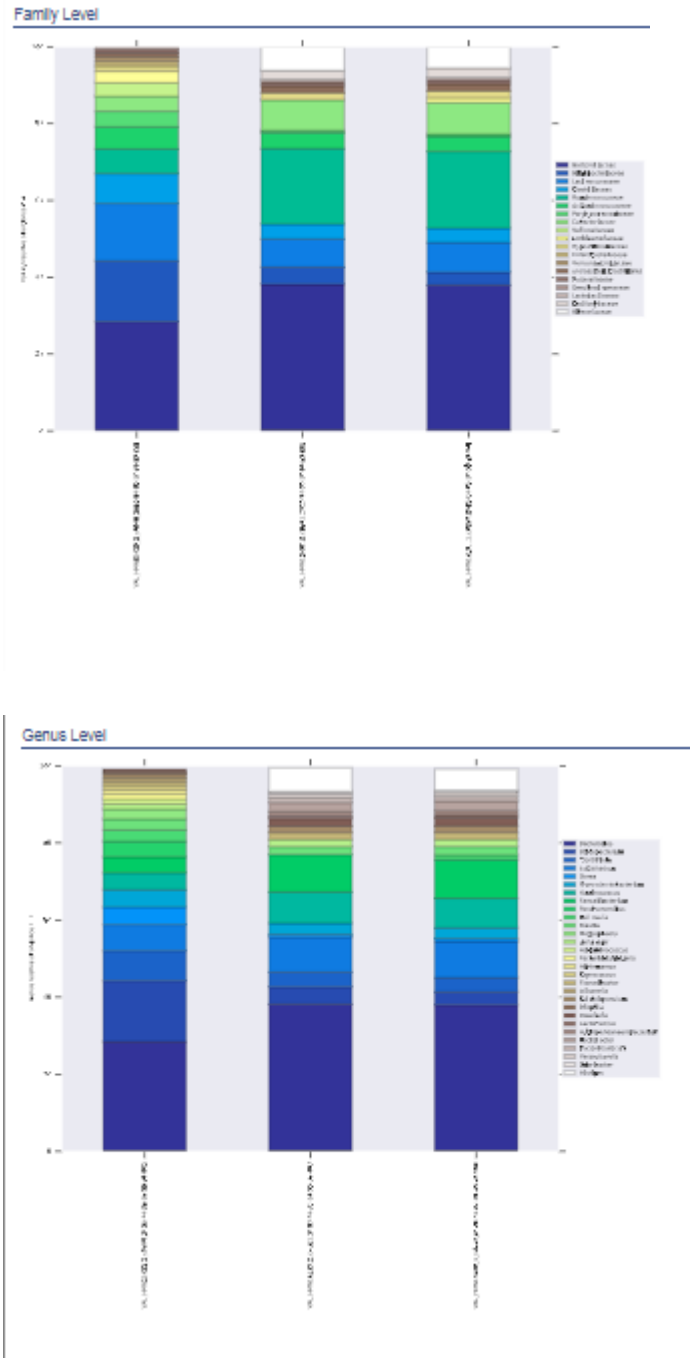
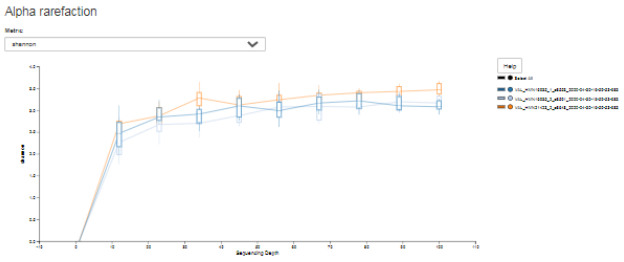


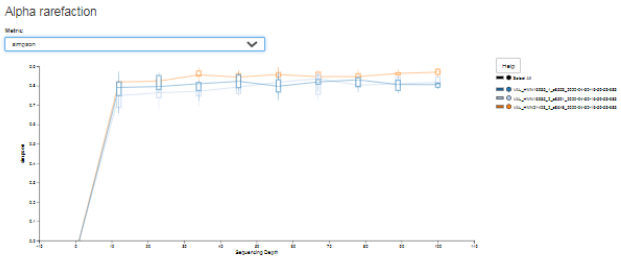
Figure 2 Bar charts for family and genus levels. Family level bar charts are also available.

c. To view the Alpha diversity values for each sample, click **Alpha Diversity Analysis**. Select any of the following options.

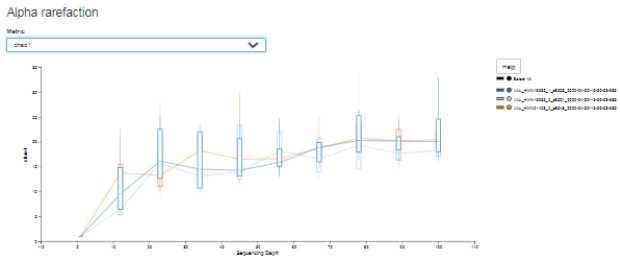
- **shannon**



- **simpson**



- **chao1**



d. To view Beta Diversity analyses of the selected samples, click **Beta Diversity Analysis**, then click a matrix or plot.

Note:

- Matrix files in TSV format are downloaded to your local drive.
- Plots are shown in a new window.
- Click **Download Results** to download a ZIP file that includes a list of the 73 species in the target species panel.

---

6. To download data files, click **Download Results**.

Note: The Alpha and Beta diversity data sets take longer to load. Click to save or open the file, depending on your browser settings.

A ZIP file containing the following files is downloaded to your local drive.

File	File extension	Description
Target Species Panel	XLSX	A list of the 73 bacterial species detected by Ion AmpliSeq™ Microbiome Health Research – Target Species Pool. Not all of the species are detected in a particular sample.
Various files	BIOM, QZA, TXT, SVG, XLSX	Files for the taxonomic data, matrixes, bar plots and heat maps that are available for the analysis results.
Folders for diversity tests	Includes TSV, QZA, JSONP, QZV, HTML, XLXS.	Files for all (alpha and beta) diversity test results.

Visualize aneuploidy analysis results

You can view interactive aneuploidy analysis results and customize visualizations in the Ion Reporter™ Genomic Viewer (IRGV). With this viewer, you can visualize multiple analyzes at once, zoom in on chromosomes of interest, and generate reports of the data that you view.

You must have IRGV set as the default viewer for this procedure. For more information, see “Set IRGV or IGV as the default viewer” on page 311.

Note: When you use aneuploidy analysis workflows, if there are not enough mapped reads to calculate **Called Gender**, the analysis will complete successfully and the sample coverage section in the `analysis.log` file contains information on why the gender calling failed.

1. Under the **Analyses** tab, click **Overview**.
2. Select the **Workflow** filter and enter ***ReproSeq*** to find analyses that used a **ReproSeq** analysis workflow, or select a specific analysis workflow filter, such as **ReproSeq No Gender PGS w.1.1**.
3. Select one or more analyses then click **Visualize**.
   The **Analysis Visualization** screen opens in the **IRGV & Generate Report** tab.

4. Use the interactive view to customize the visualization and generate reports, as needed.

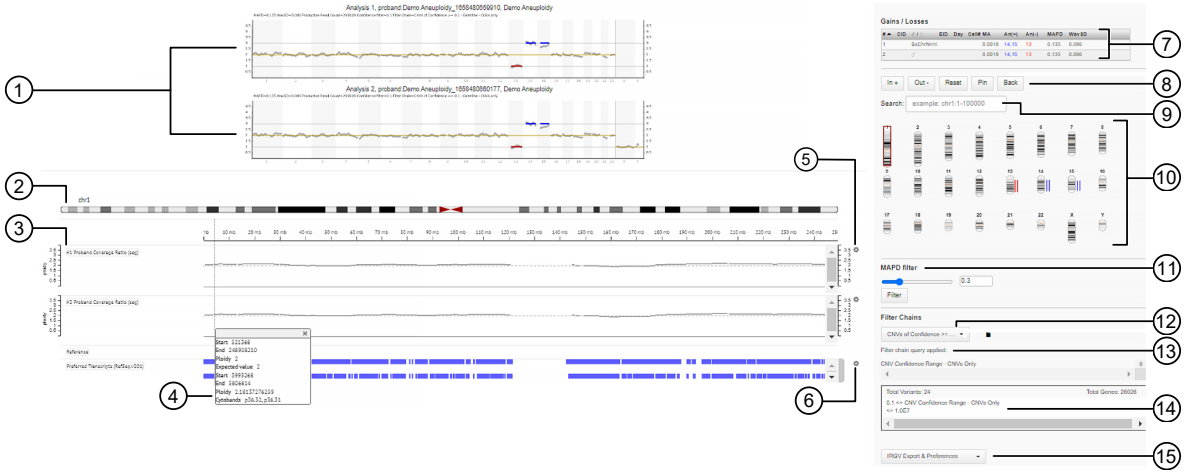


Figure 3 Aneuploidy analysis results

(1) Analysis whole genome view. Provides a genome-wide perspective of the sample data. A chart or graph is shown for each selected analysis. Click on the chart to zoom in on a region of the genomic data, or a chromosome, and view more information about your selection throughout the visualization.

(2) Click on this IRGV track to view details about the selected chromosome and view more information about the chromosome in the tracks below. Or, select data in the whole genome view or Karyogram to view data reflected in each track below.

(3) Click **Shift** and drag across the x-axis of the ploidy scale to zoom in on a region of the scale and change the other data in the visualization to reflect the selection.

(4) Hover over a tile in the visualization to get the following information.

- **Start:** Start coordinate for the copy number call region.
- **End:** End coordinate for the copy number call region.
- **Ploidy:** Called ploidy value. The highest allowable ploidy value calculated by the software is 10.
- **Expected value:** Expected ploidy value for this chromosome. The value is 2 for autosomes and chrX for females. It is 1 for chrX and chrY for males.
- **Start:** Start position of the selected tile.
- **End:** End position of selected tile.
- **Ploidy:** The calculated ploidy value of the tile. The highest value that will be shown by the software is 11.
- **Cytobands:** The cytoband range of the tile.

(5) Adjustable ploidy scale for selected chromosomes or chromosome regions. Change the track name or track height for the histogram, or hide the track. See “Adjust IRGV BAM tracks” on page 331 for more information.

(6) Adjustable **Reference** and **Preferred Transcript** tracks. Change the BAM track name, track height, and track color, or collapse, squish or expand the track. See “Adjust IRGV BAM tracks” on page 331 for more information.

⑦ Click in a row of the **Gains/Losses** table to open a detailed view of a single sample visualization. See "View single sample aneuploidy details" on page 323 for more information.

The **Gains/Losses** table shows the following information.

CID (Couple ID): An identifier for the parents of an embryo.

EID (Embryo ID): An identifier for the embryo biopsied and run through the ReproSeq workflow)

Cell# (Cell Num): The number of cells in the sample, if known.

MA: A calculated result the ratio of Mitochondrial DNA and Autosomal DNA.

An: Aneuploidy, shown as An(+) for a copy number gain, and An(-) for a copy number loss. The affected chromosomes are shown in a comma-separated list.

MAPD (Median Absolute Pairwise Difference): An estimate of coverage variability between adjacent amplicons.

WaveSD (Waviness Standard Deviation): Standard deviation of all log2 read count ratios after subtraction of the contribution of pairwise differences.

⑧ Zoom in and out of the chromosome Karyogram, and click **Reset** to return to the default chromosome view.

⑨ Enter a set of chromosomal coordinates in the **Search** field to zoom into chromosome data, or click on a chromosome in the Karyogram to view its chromosomal coordinates. Or, view the chromosomal coordinates when you change the selections in the whole genome chart, IRGV, or Karyogram.

⑩ Chromosome Karyogram.

⑪ Adjust the MAPD filter.

⑫ Select a filter chain to apply filters to the data. If you select multiple analyses generated from analysis workflows with different filter chains that use different confidence settings, the software will apply the filter chain with the lowest confidence setting to all selected analyses.

⑬ View the filter change, or filter chain query that is applied to the visualization.

⑭ View details about the filter chain, or filter chain query, the total number of genes, and the variants that were filtered in.

⑮ Export the visualization, or change the IRGV preferences. See "Set IRGV preferences" on page 312 for more information.

View single sample aneuploidy details

You can view an interactive view of details from a single sample aneuploidy analysis in the Ion Reporter™ Genomic Viewer (IRGV). This visualization provides a view of the genomic data that you can zoom in on to view individual chromosomal data, and a table of events that were called. You can adjust the list of events to show only events that were filtered in for the analysis by the applied filter chain.

You must have IRGV set as the default viewer for this procedure. For more information, see "Set IRGV or IGV as the default viewer" on page 311.

1. In the **Analyses** tab, click **Overview**.
2. Select the **Workflow** filter and enter ***ReproSeq*** to find analyses that used a **ReproSeq** analysis workflow, or select a specific analysis workflow filter, such as **ReproSeq No Gender PGS w.1.1**.
3. Select one or more analyses then click **Visualize**.
   The **Analysis Visualization** screen opens in the **IRGV & Generate Report** tab.
4. Click in a row of the **Gains/Losses** table.
   The **IRGV Details** screen opens in a new browser tab.

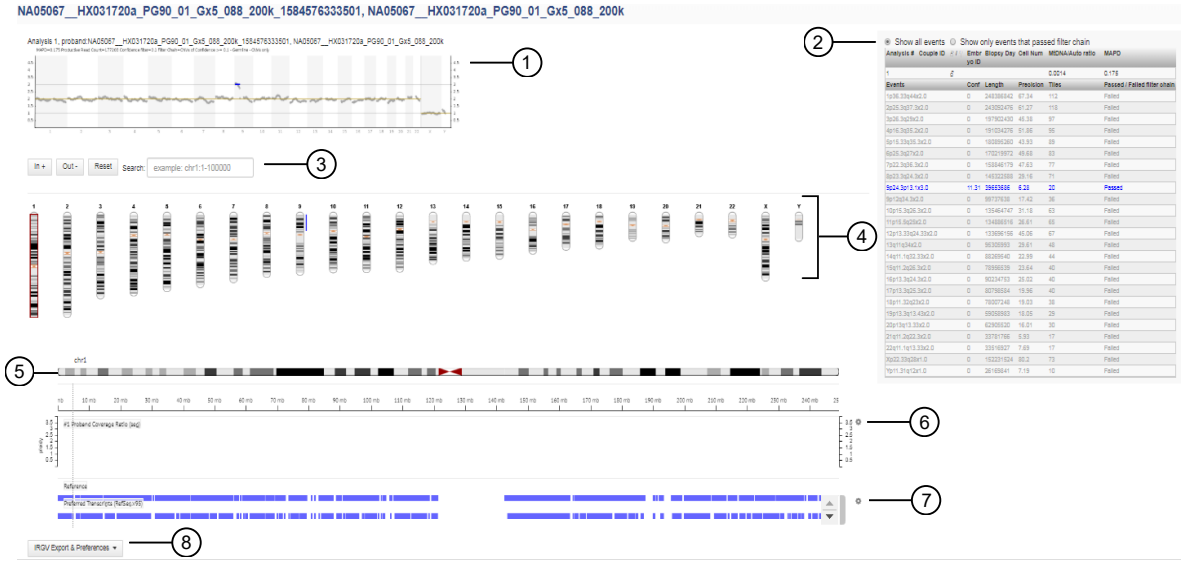


Figure 4 IRGV Details screen

(1) Analysis Whole Genome View. Provides a genome-wide perspective of the data. Metrics above the view are:

- (a) Productive Read Count The sum of mapped reads from a sample that align to the autosomes and chrX in females or to the autosomes and chrX + chrY in males. If the CNV finding parameter ***Remove Duplicates*** is set to ***True***, then the **Productive Read Count** metric excludes duplicates. Productive reads are the specific reads that are used to establish the relative coverage of tiles across the sample.
- (b) Confidence
- (c) Filter Chain The filter chain that was applied to the analysis.

(2) Click in a row of the **Gains/Losses** table to open a visualization with the data charts and a table of the events in a separate tab. Select an option in the table to see all events, or only events that passed the applied filter chain.

(3) Enter a chromosome coordinate in the **Search** field to zoom into chromosome data, or click on a chromosome in the Karyogram to view its chromosome coordinate. Zoom in and out of the Karyogram, and click **Reset** to return to the default chromosome view.

(4) Select a chromosome on the Karyogram to the chromosome coordinate, narrow the focus of the whole genome view, and update the IRGV tracks to reflect the selection.

(5) IRGV tracks. Each track updates dynamically as you select data in the whole genome view or the Karyogram view.

(6) Adjustable ploidy scale for selected chromosomes or chromosome regions. Change the track name or track height for the histogram, or hide the track. See “Adjust IRGV BAM tracks” on page 331 for more information.

(7) Adjustable reference and preferred transcript tracks. Change the BAM track name, track height, and track color, or collapse, squish or expand the track. See “Adjust IRGV BAM tracks” on page 331 for more information.

(8) Export the visualization, or change the IRGV preferences. See “Set IRGV preferences” on page 312 for more information.

Change Y-axis height in Whole Genome aneuploidy visualization

You can expand the height of the Y-axis when you view whole genome aneuploidy analysis results in Ion Reporter™ Genomic Viewer (IRGV). By default the image of the data is 300 pixels high. IRGV includes the option to double this height to 600 pixels. The expanded view is useful when you review mosaic data, because more data is shown on a single plot and more space is available between data points, making the mosaic data more pronounced.

1. Under the **Analyses** tab, click ***Overview***.
2. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.
3. In the **IRGV & Generate Report** tab, scroll to the bottom of the visualization, then click **IRGV/Export & Preferences ▸ Show IRGV preferences** to expand the **Preferences** section.

Option	Description
Expanded	Select this option to increase the height of the Whole Genome view to 600 pixels.
Compressed	This is the default setting. Select this option to set the height of the Whole Genome view to 300 pixels.

Smoothing, no gender, and mosaicism results in IRGV

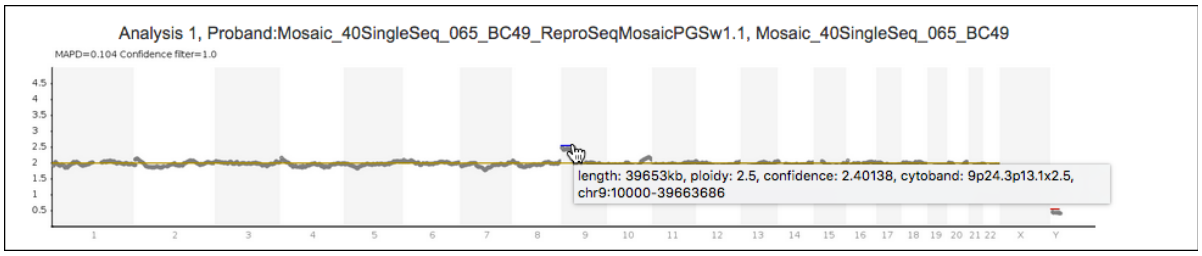


Figure 5 Example of a mosaic sample

The following Ion Reporter™ Genomic Viewer (IRGV) plot shows non-integer ploidy for the short arm of chromosome 9, indicating that the sample is mosaic.

This image shows five analyses run with various Ion Reporter™ Software aneuploidy analysis workflows that show how smoothing, no gender, and mosaicism appear in IRGV visualizations. Visualization of aneuploidy detection is enhanced by tile-by-tile data points that appear as easier-to-see circles in the whole-genome views. All results from IRGV aneuploidy analysis workflows use this enhanced view.

Note: Analyses 2 through 5 have smoothing of the discrete tile data turned on by default. Smoothing of the discrete data have no effect on the calls that are made by the aneuploidy pipeline in the software. Smoothing can be turned on or off in any analysis workflow.



Figure 6 Examples of smoothing, No Gender and mosaicism

① An analysis launched with the Low Pass Whole-Genome Aneuploidy analysis workflow and the Demo Aneuploidy sample that is available in the software, with a loss of one copy of chr13 and single copy gains of chrs 14 and 15. This analysis was run with the earlier version of an Ion Reporter™ Software analysis workflow version that does not include smoothing of the discrete tile data.

② An analysis launched with the default ReproSeq analysis workflow and the Demo Aneuploidy sample in Ion Reporter™ Software.

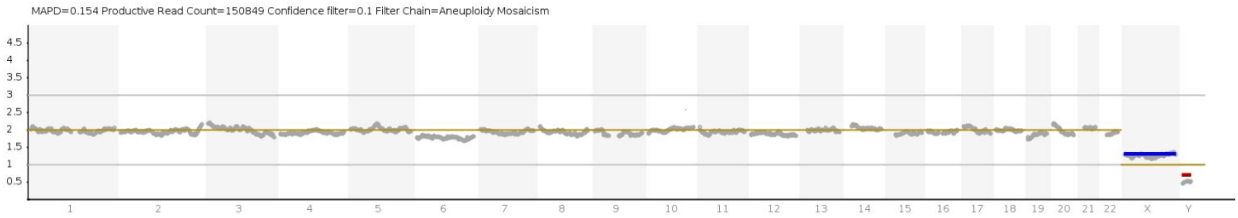
③ An analysis launched with the ReproSeq No Gender analysis workflow and the Demo Aneuploidy sample. Use of the no gender analysis workflow generates analysis results that do not record or show the called gender of the sample in the software or in data files. Instead, a called gender value of SxChrNrml is given when the sample is either a normal XY male or a normal XX female with no sex chromosome aberrations. A gender call is SxChrAbnrml when the sex chromosomes deviate from normal male or normal female by either whole-chromosome or subchromosomal aneuploid events. Aneuploidies on autosomes do not affect the called gender value, and can be present in samples with normal sex chromosomes.

④ An analysis launched with the ReproSeq Mosaic analysis workflow and the Demo Aneuploidy sample. Some chromosomes can look like they were called aneuploid if the copy number is called as CN loss = ploidy 1.95, or CN Gain = ploidy 2.05 (as shown in this example on chr1), and on the Y chromosome as a slight ploidy gain. Gain or Loss events with ploidy very near expected normal ploidy can be a result of slight differences in normalization instead of true biological ploidy changes.

⑤ An analysis of a spiked-in sample that was mixed to have a chr9p event of relative copy number ploidy of 2.5 and analyzed through the ReproSeq Mosaic analysis workflow. The two mixed samples consisted of one male and one female, one of which had an integer copy number gain of ploidy=3 for chr 9, although the other sample was normal ploidy=2 for chr 9. The mixing of two samples of different genders can be seen in the observed ploidies of the X and Y chromosomes.

Visualization of triploid samples

Mosaic workflows call triploid male biopsies (69, XXY). Mosaic events on chrX and chrY are called in 69, XXY samples since three copies of autosomes will be normalized to 2N at a ratio of 1.5 (3/2 = 1.5), thus 2N chrX should be called 1.33 (2/1.5 = 1.33) and 1N chrY should be called 0.66 (1/1.5 = 0.66).



Example genome view plot and table from a 69, XXY sample with chrX called ploidy 1.3 and chrY called ploidy 0.7

Visualize the BRCA report

Some Oncomine™ assays used with Ion Reporter™ Software include BRCA1 and BRCA2 amplicons that enable detection and visualization of whole exon and multiple exon deletion in BRCA1 and BRCA2 genes in somatic and germline samples with high sensitivity.

1. In the **Analysis Results** screen, click **Visualize**, then select the **BRCA** tab.

   When you open a visualization for a panel that contains amplicons that target the BRCA1 and BRCA2 genes, you can view a **BRCA Report** that shows a boxplot of read counts of each BRCA1 and BRCA2 exon that are normalized to the Oncomine BRCA DNA Baseline. The **Post-Corrected** view is shown by default. Click **Pre-Corrected** to view the uncorrected raw data.

   Note: For brevity, only genes from the HRR pathway are shown. Consequently, the figures cannot be used to interpret gain-of-function or loss-of-function from these genes.

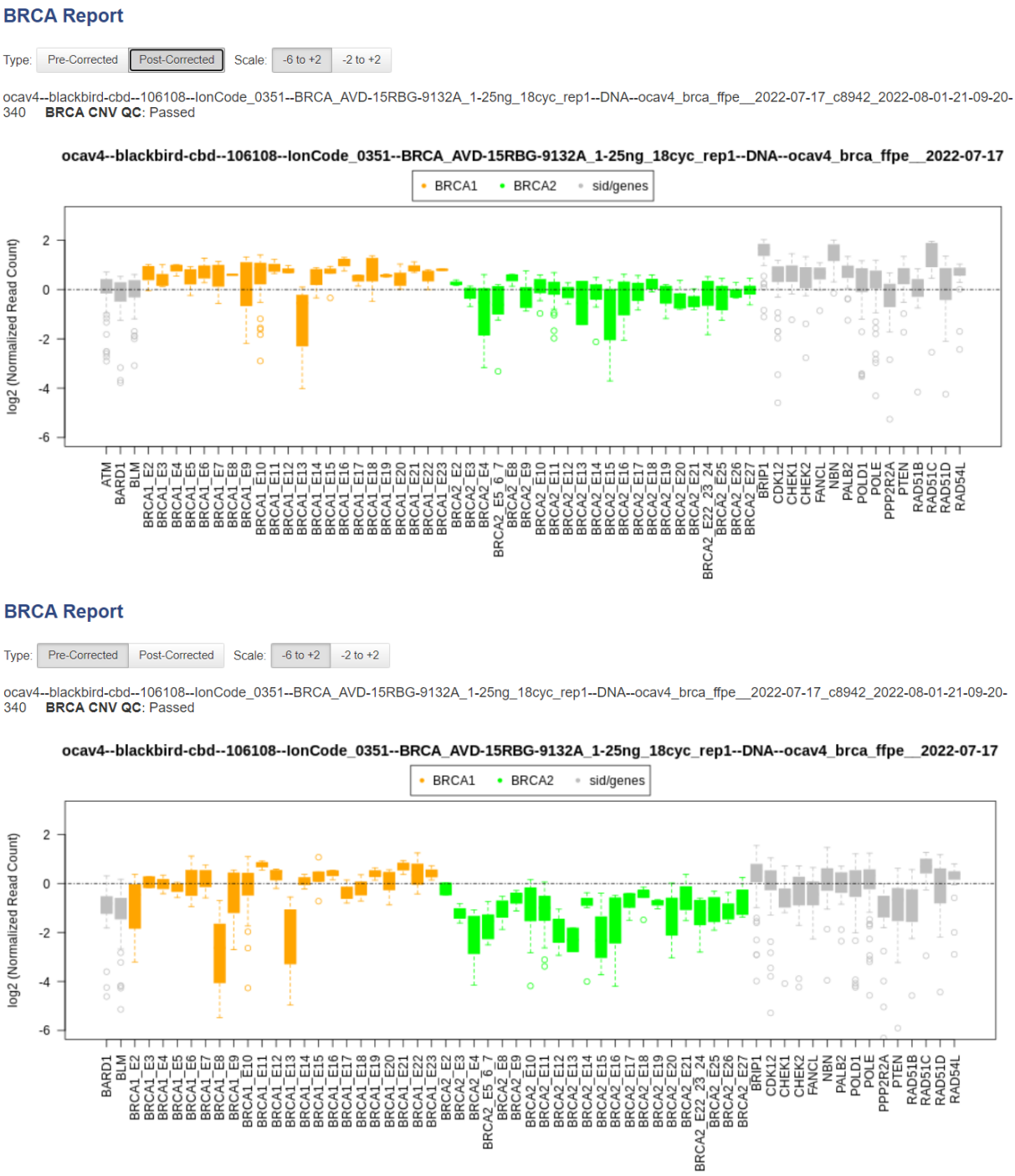
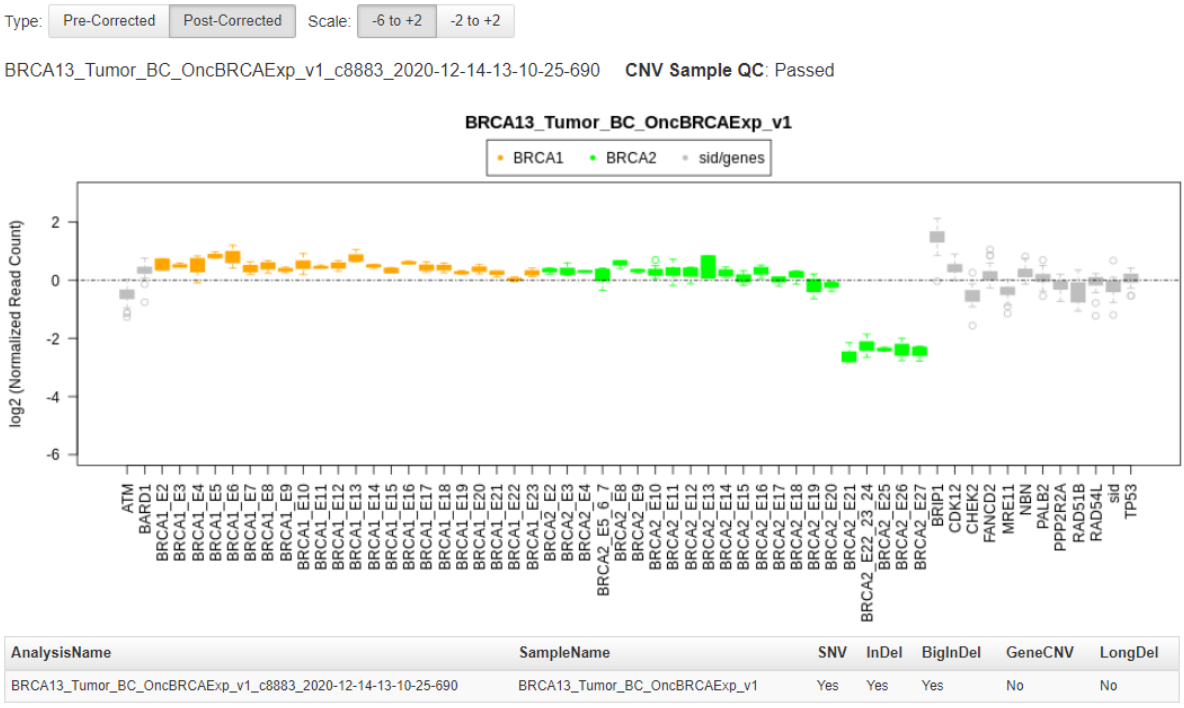


Figure 7 Normal sample, no BRCA whole-gene or exon deletion



AnalysisName	SampleName	SNV	InDel	BigInDel	GeneCNV	LongDel
BRCA13_Tumor_BC_OncBRCAExp_v1_c8883_2020-12-14-13-10-25-690	BRCA13_Tumor_BC_OncBRCAExp_v1	Yes	Yes	Yes	No	No

Figure 8 Tumor sample with BRCA exon deletion

2. (Optional) To download the figures as PNG files, click **Download Results**.
   A ZIP file containing the PNG files for the graphs is downloaded.

3. Click the **IRGV & Generate Report** tab to view exon deletions or duplications on chromosomes 13 and 17.



① Click to open the **Sample Analysis Summary** in a separate browser window

② Zoom in and zoom out for the selected chromosome

③ Click to select and view a chromosome

④ Slide the **MAPD filter** to adjust the threshold value. This filter applies to the entire sample. If the reported MAPD value is higher than the selected **MAPD filter** value, no CNVs are shown in the BRCA Report.

⑤ Scroll down in this section to see more of the alignments or hotspots.

⑥ Oncomine BRCA Expanded designed BED file alignment. This **IRGV** track shows the alignment of the amplicons to the reference sequence.

4. Click **IRGV Export & Preferences** to open the visualization in IGV, export the whole genome view or the IRGV tracks to a PNG file, or show IRGV preferences.

Adjust IRGV BAM tracks

You can adjust the BAM read coverage track in an Ion Reporter™ Genomic Viewer (IRGV) visualization to make it easier to view the data. When you adjust the BAM read coverage track, it changes the amount of data that is shown in the plot. For example, you can see more BAM tracks when you use the **Squish** setting.

Use the **Expanded** option to view hover help with details about variants and base calls in each read coverage track.

Note: A maximum of 500 aligned reads are shown in each BAM track in Ion Reporter™ Genomic Viewer (IRGV). To see more than 500 aligned reads, use Integrative Genomics Viewer (IGV).

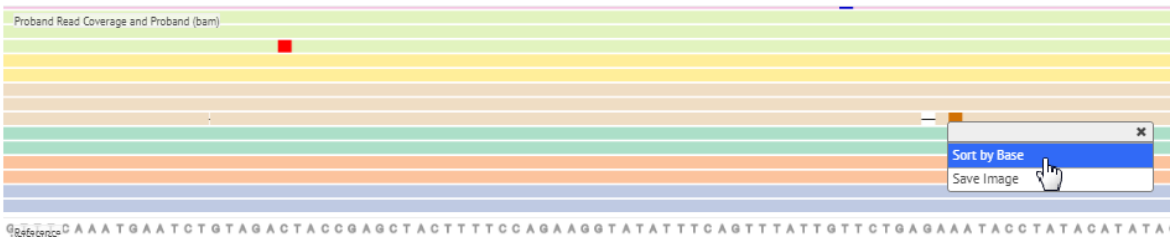
In an open **Analysis Visualization**, in the **IRGV & Generate Report** tab, click ⚙ **Actions** next to the read coverage track, then select an option to adjust the view of the track.

Option	Description
Expanded	Select this option to view the read coverage track with the maximum visible height for each BAM track.
Squish	Select this option to view reads in the coverage track in a single line that has minimal height for each BAM track.
Collapse	Select this option to view no BAM tracks and view only the coverage density at the top of the BAM track.

Sort read coverage tracks by variant

The read coverage tracks reports read counts, molecular counts, insertions and deletions. In an analyses that were run with Ion AmpliSeq™ HD or TagSeq analysis workflows, you can sort the coverage tracks by variants. This type of sort, which groups the visualized variants together as the top of the coverage chart, is useful if the read coverage is high.

1. In the **Analyses** tab, click **Overview**.
   The **Analyses** table lists all the available analyses.
2. Click a link for an analysis to open the **Analysis Results** screen.
3. Click the link for a locus that includes variants that you want to visualize.
4. In the coverage track, place the cursor at the position of the variant, then right-click and select **Sort by Base**.



The read coverage track is reordered to show the families and reads that contain variants at the top.

CNV segment data in chromosome view

In IRGV, chromosome segments that exhibit CNV gain or loss are indicated by a blue line. Place the cursor over the blue line to display context-sensitive information. Some CNV segment analyses, such as those with overlapping segments, include two start, two end, and two ploidy values, as shown in this example.



The Start, End, and Ploidy values that are listed at the top of the context-sensitive information box represent the start, end, and ploidy information for the entire gene segment that is selected in the software. These values are static and do not change.

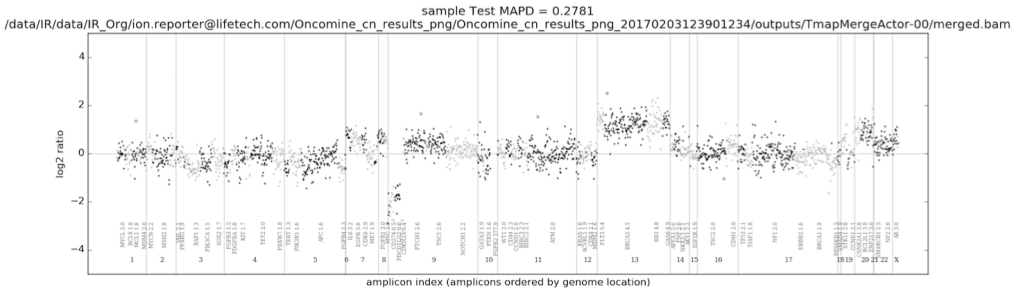
The **Start**, **End**, and **Ploidy** information that is listed at the bottom of the context-sensitive information box represent the represent the start, end, and ploidy information for the amplicon that is closest to the cursor.

Visualize an analysis workflow that contains a CNV baseline

If you add a CNV baseline to an analysis workflow, you can you can download and view the analysis results in the `cn_results.png`. In this example, a Variability Correction Informatics Baseline (VCIB) baseline was used.

These example analysis results shows a plot with log2 ratios across the genome and highlights panel CNV IDs, in this case gene symbols, to help with reviews of calls made by the CNV detection algorithm. The alternating gray and black dot color is used to distinguish between adjacent amplicons in the CNV IDs. The outliers in the data are drawn as small circles. The X axis shows the chromosomes numbers. The CNV ID names and the mean copy number call for each CNV ID are indicated above the

chromosomes at their approximate location. This example has copy number gains on chromosomes 13 and 20, and deletion of a portion of chromosome 9, and possibly other subchromosomal events. The MAPD value of 0.2781 for the sample is displayed at the top of the plot and is a QC metric measuring the noisiness of the sample. A low MAPD is good. MAPD at, or greater than, 0.5 is considered to fail QC. Log2 ratios of 0 are equivalent to a copy number call of 2 (normal for autosomes and female X). If the sample is for a male, you would expect to see the log ratio equivalent to a copy number of 1 on chromosome X.



Visualize tumor mutational burden analysis results

To visualize tumor mutational burden analysis results in Ion Reporter™ Software, the analysis workflow must be any DNA-single sample, or DNA and Fusions-single sample analysis workflow that has tumor mutational burden enabled.

1. Do one of the following to open tumor mutational burden analysis results:

Option	Description
Visualize analysis results from an individual sample or from multiple samples simultaneously from the **Analyses** table.	In the **Analyses** table, select an individual sample result row, or select the checkbox next to each sample result that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions** ▸ **Visualize**.
Visualize analysis results individually from the **Analysis Results** screen.	In the **Analyses** table, click a sample result hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**.

2. Click **Download Report** to download the **Tumor Mutational Burden** visualization report in PDF format, which includes the results with graphs and metrics.

3. Scroll down to view graphical representations of the analysis results and QC metrics, and download additional files.

Note: Multiple sample results are listed sequentially.

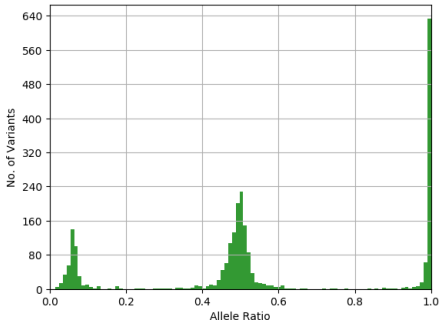
4. In the lower right corner of the screen:
    - Click **Download Variant Details TSV** to download a tab-separated list of detected variants that contributed to the tumor mutational burden count. Open the file with compatible software, such as Microsoft™ Excel™.
    - Click **Download the TMB statistic.txt file** to download the tumor mutational burden output file. The file is named `<analysis name>_statistic.txt`. For example, `NCI-1395_c3495_2020-03-03-10-58-54-007_statistic.txt`.

Sample results

Tumor mutation burden results are represented graphically in the **TMB** tab when you visualize analysis results with Ion Reporter™ Software.

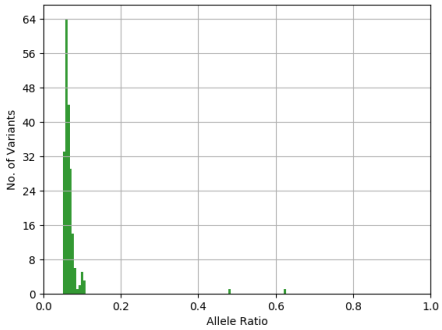
Germline and Somatic Variants

This histogram shows the frequency distribution of allele ratio for total called germline and somatic variants. Listed below the figure is the combined total of called germline and somatic variants. The value is reported in the `statistic.txt` file as **Variant count (Germline + somatic)**. For more information, see Ion Reporter™ Software help system.



Only Somatic Variants

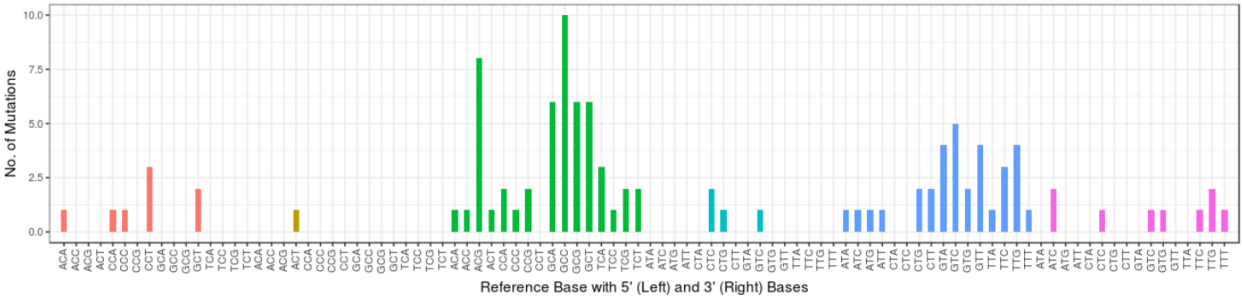
This histogram shows the frequency distribution of allele ratio for only somatic mutations as determined by the selected TMB filter chain. Listed below the figure are:



- The number of **Total Somatic Variants** , reported in the `statistic.txt` file as **Total Somatic Filtered Variants Count (numerator for TMB calculation)**.
- Of the **Total Somatic Variants** , the number of SNVs that are determined to be nonsynonymous (detrimental) and synonymous (non-detrimental) as annotated by Ion Reporter™ Software. Values are reported by different Ion Reporter™ annotation types in the `statistic.txt` file.
- The number of detected somatic variants found in the COSMIC database. The value is reported in the `statistic.txt` file as **COSMIC Annotated Somatic Variants**.

For more information, see “Visualize tumor mutational burden analysis results” on page 333..

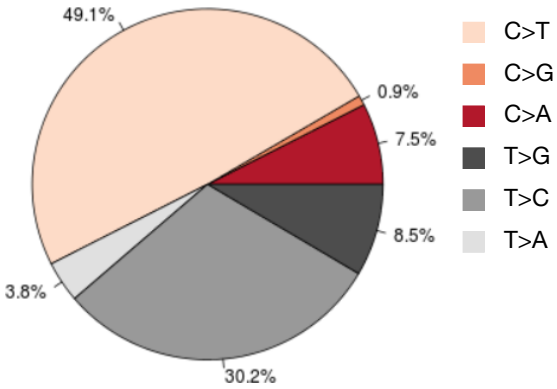
Substitution Type and Context of Somatic Mutations



Somatic mutations can be divided into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type. After incorporating information on the bases immediately 5′ and 3′ to each mutated base, 96 possible mutation types are in this classification. These 96 mutation types are represented on the x-axis, and variant frequency for mutation type on the y-axis. Bars for each substitution class are grouped and displayed with different color. A summary TXT file of these results is also available, see “Download filtered variants” on page 209.

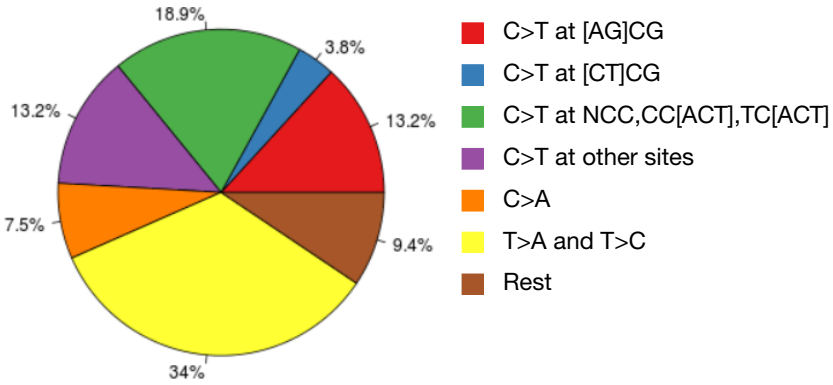
Substitution Type of Somatic Mutations

A pie chart dividing somatic mutations into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type.



Signature Pattern of Somatic Mutations

A pie chart dividing somatic mutations in groups consistent with specific mechanisms.



In the pie chart, a small fraction of multiple signature types can be observed in the sample. However, significant dominance of a single signature pattern often correlates to the respective tumor type. For example, 56.7% of the variants detected (sum of blue, green, and yellow) are an observed UV damage signature in this sample.

Note: Underlined bases represent the reference base being substituted (for example, CpG in the first bullet is same as to [ACGT]CG).

- High C>T at CpC, CpC, TpC, T>A, and T>C is consistent with UV damage. (Blue + Green + Yellow)
  For more information, see **https://www.nature.com/articles/nature22071**
- High C>T at CpG is consistent with spontaneous deamination of 5-methylcytosine. (Red + Blue).
  For more information, see **https://www.nature.com/articles/nature12477**
- High C>A is consistent with smoking damage.(Orange)
  For more information, see **https://science.sciencemag.org/content/354/6312/618.full**
- High C>T (site independent) is consistent with FFPE processing. (Green + Purple)
  For more information, see **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4032349/**

Note: Samples of low quality that exhibit deamination (high C>T) can be analyzed if you increase the minimum allele frequency in an assay. For more information, see Ion Reporter™ Software help system.

QC metrics for tumor mutational burden

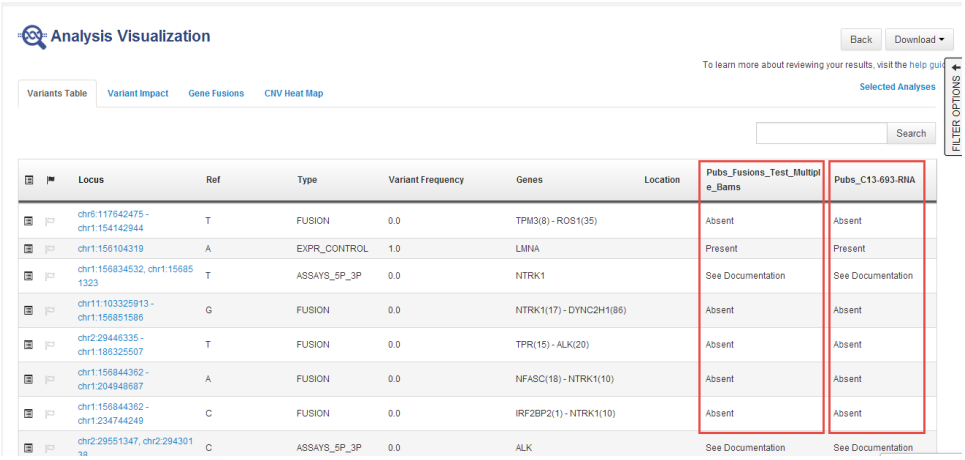
QC metrics for tumor mutational burden are listed at the top of **Analysis Visualization** in the **TMB** tab when you visualize analysis results with Ion Reporter™ Software.

QC metric	Definition
Average Coverage	The following formula is used to calculate average coverage: $\frac{\text{Total base coverage across the genomic positions defined by the assay BED file}}{\text{Number of bases in the DNA BED file}}$ If the average coverage is below 150, the analysis completes successfully but Tumor Mutational Burden is reported as `-1`. **Note:** This setting cannot be changed in Ion Reporter™ Software.
Number of bases used in calculating TMB	The number of bases used as a denominator for the tumor mutational burden calculation. Only bases with sufficient base coverage are used in the calculation, as defined in the workflow parameters. In the parameters, you can also select only the genomic regions covered by the panel to be used instead of all exonic regions. See the Ion Reporter™ Software help system for more information.
Number of variant calls	The number of somatic variants that are identified in the sample. This value is reported in the `statistic.txt` file as **Total Somatic Filtered Variants Count (numerator for TMB calculation)** and **Variant Count**. For more information, see Ion Reporter™ Software help system.
Deamination score	Deamination score was previously reported as the estimated SNP proportion consistent with deamination (mainly FFPE). The deamination score can be used to determine the quality of the FFPE sample. For more information on how to minimize the impact of high deamination on a tumor mutational burden score, see Ion Reporter™ Software help system. A deamination QC status of ***PASS/FAIL*** is also reported. The threshold for this QC can be adjusted in the analysis workflow parameter settings. For more information, see Ion Reporter™ Software help system.

Compare results of single or multiple analyses

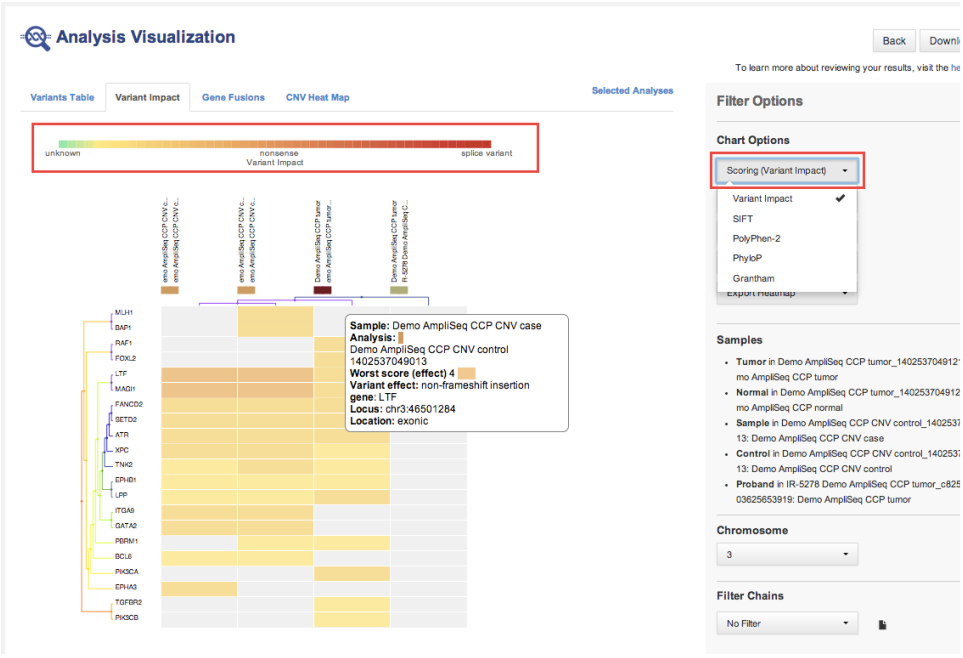
You can review results for single analyses, or compare the results of multiple analyses in the **Analysis Visualization** screen. Visualization for multiple analyses supports the following views, depending on analysis workflow type.

- In the **Variants Table** tab, the table view shows side-by-side columns to compare variant calls in different analyses:

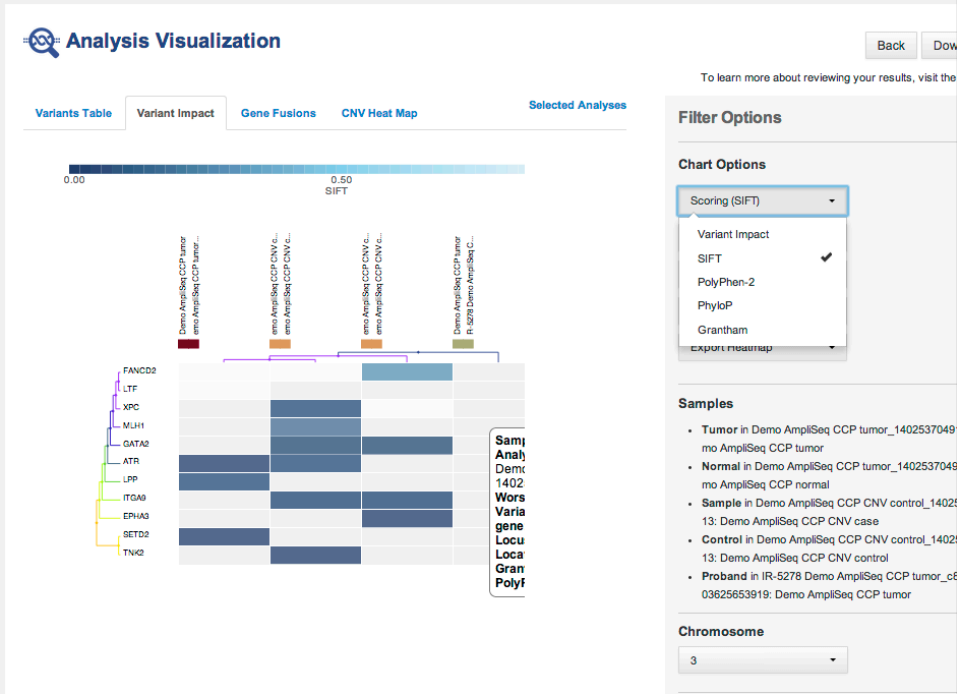


- In the **Variant Impact** tab, the variant impact heat map that displays is based on the predicted variant impact:

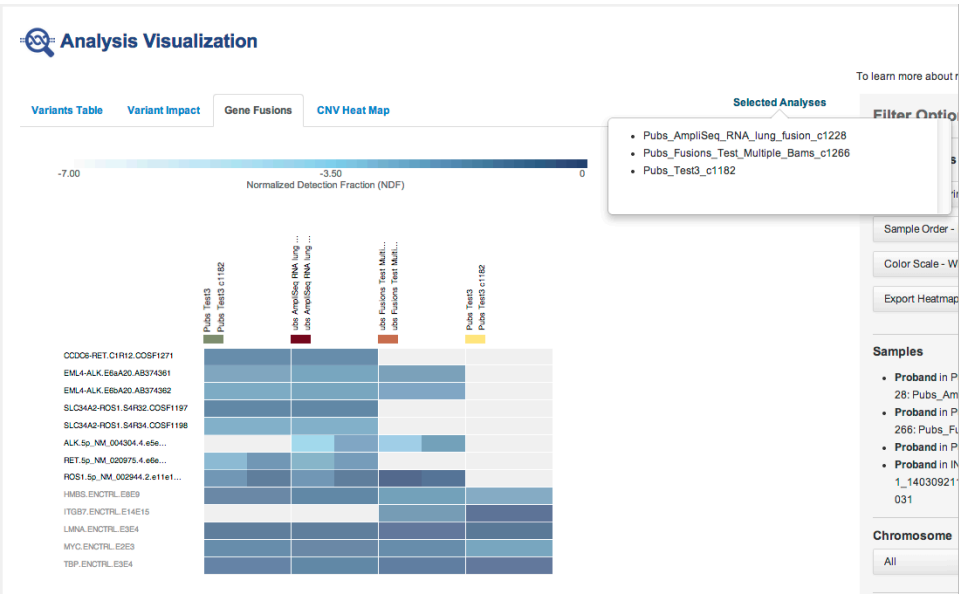
  The following is a variant impact heat map that is based on the predicted variant impact.



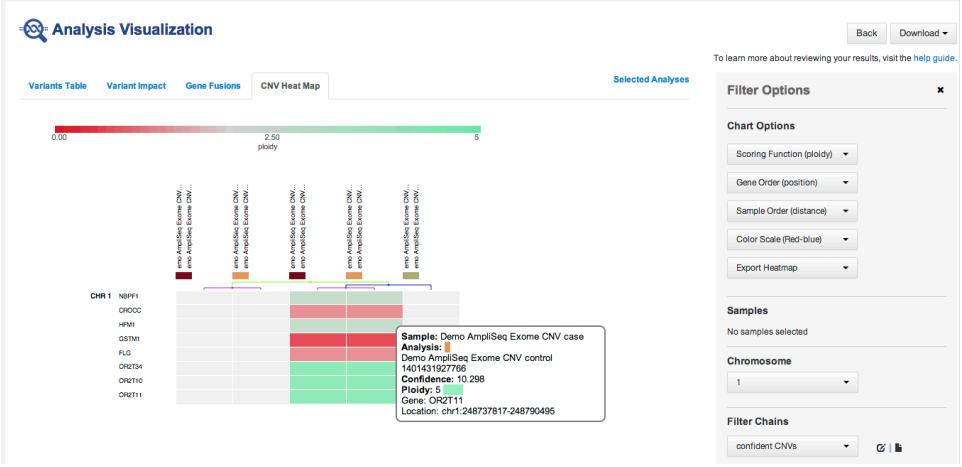
The following is a variant impact heat map that is based on other scoring.



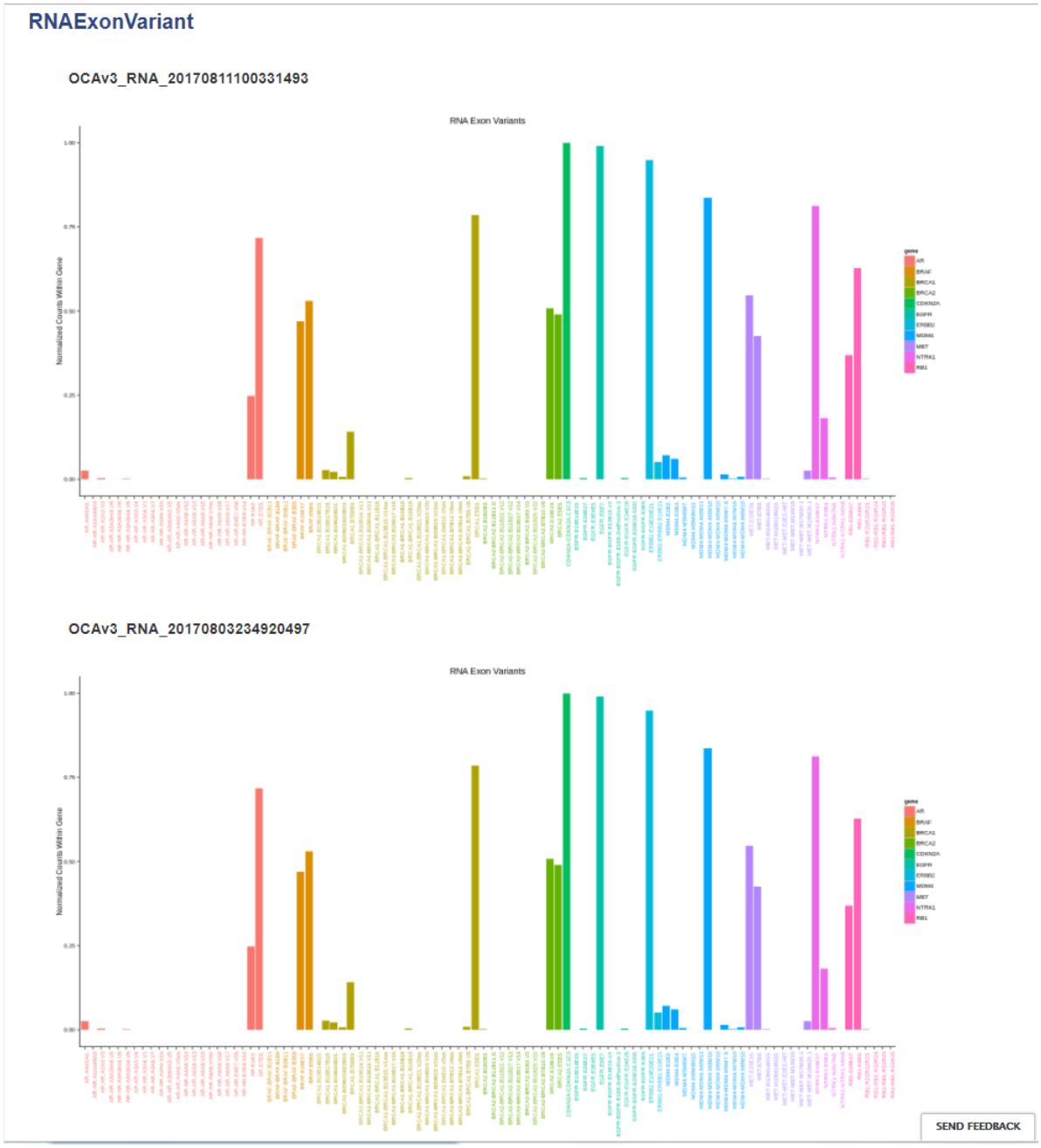
The following is a gene fusions heat map.



The following is a CNV heat map.

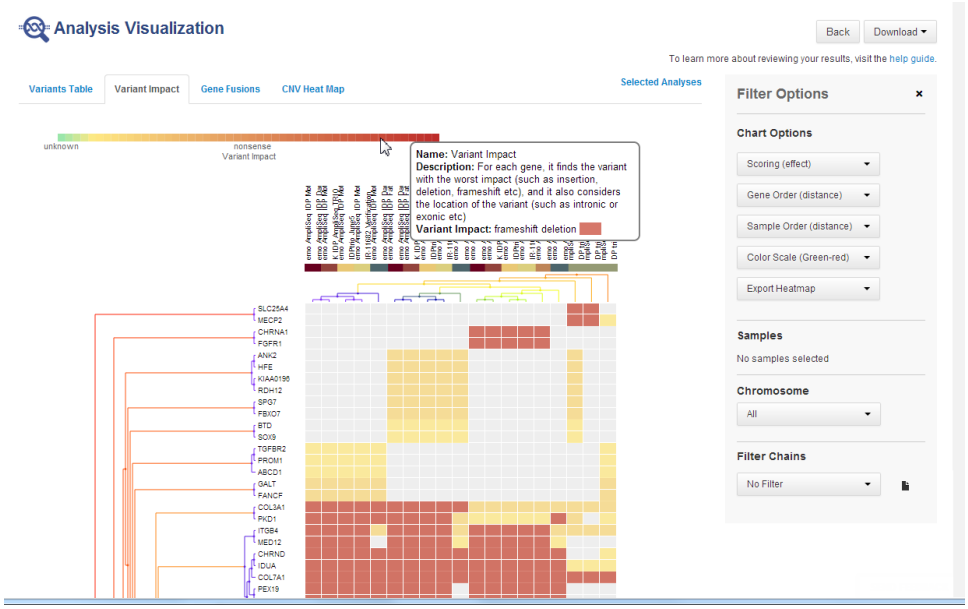

Analysis Visualization
Back
Download
To learn more about reviewing your results, visit the help guide.
Variants Table
Variant Impact
Gene Fusions
CNV Heat Map
Selected Analyses
0.00
2.50
ploidy
5
CHR 1
NBPF1
OR2C1
HFM1
GSTM1
FLG
OR2T34
OR2T10
OR2T11
Sample: Demo AmpliSeq Exome CNV case
Analysis:
Demo AmpliSeq Exome CNV control
1401431927766
Confidence: 10.298
Ploidy: 5
Gene: OR2T11
Location: chr1:248737817-248790495
Filter Options
Chart Options
Scoring Function (ploidy)
Gene Order (position)
Sample Order (distance)
Color Scale (Red-blue)
Export Heatmap
Samples
No samples selected
Chromosome
1
Filter Chains
confident CNVs

- RNA Exon Variants:

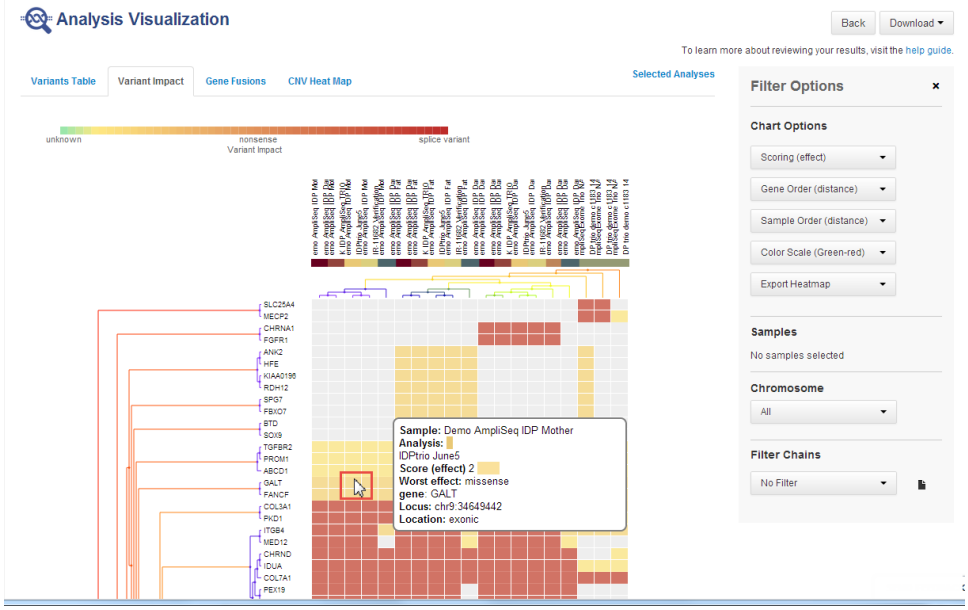


Note:

- In most cases, similar analyses are compared. However, Ion Reporter™ Software does not restrict comparisons to only similar analyses.
- You can compare one trio or one paired analysis without selecting multiple analyses. Each trio or paired analysis contains more than one set of variants.
- Place the pointer over the legend of a heat map:



Hover over the legend of a cell to see more information:



Track order for non-aneuploidy analyses

Note: In the GRCh38 genome reference, there exists alternate contigs. Ion Reporter™ Software includes the contig that contains the GSTT1 gene and reports on amplifications or deletions at the GSTT1 gene if they occur.

After the Whole Genome View of each non-aneuploidy analysis, the various data tracks are loaded in this order by default:



1. Selected chromosome ideogram
2. Copy Number segment (.seg) file
3. VCF file
4. BAM track for each sample.

   Note: A maximum of 500 aligned reads are shown in each BAM track in Ion Reporter™ Genomic Viewer (IRGV). To see more than 500 aligned reads, use Integrative Genomics Viewer (IGV).
5. Genome reference track
6. Design BED files and hotspot files
7. Annotation tracks (COSMIC, ClinVar, and preferred transcript annotation tracks)

Compare multiple analyses and download a TSV file

You can compare multiple analyses, filter the variants that appear in the visualizations of the compared analyses, and download the collective results of the multiple analyses in a TSV file. Comparisons can be used to analyze results and identify common or unique hotspots of interest across analyses. TSV files that contain the results of multiple analyses can be downloaded separately or in a batch download that also includes VCF result files for each analysis. To download a TSV file from the **IRGV & Generate Report** tab, **RNA Exon Variant** tab, or **TMB** tab, you need to download the TSV file in a folder of files. For more information, see “Variants file downloads” on page 208.

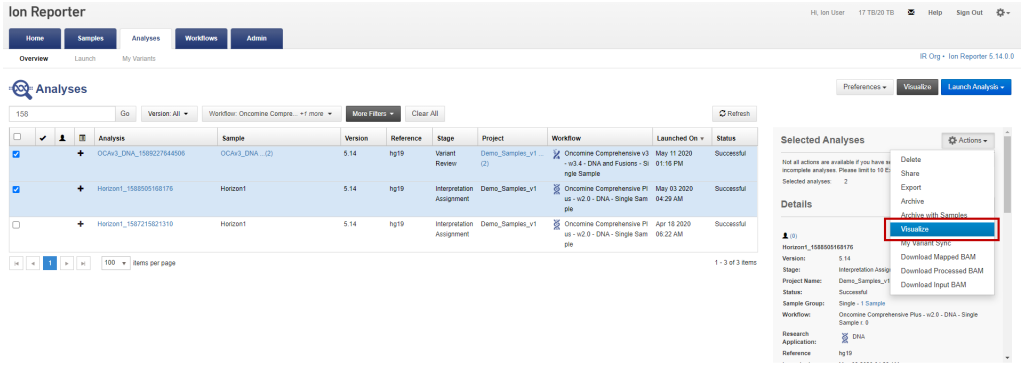
When you compare analysis results from one, 2, or 3 similar analyses, the software generates a Venn diagram to show the sets of variants that are common between the analysis results. The sets of variants that are not common between the selected analyses. You can use the Venn diagram to select the unique or common sets of variants to narrow and refine the list of variants that are displayed in the **Variant Table**.

Venn diagrams are generated in Ion Reporter™ Software for 2 or 3 sets of analysis results. For more information, see “Venn diagram generation” on page 346. Venn diagrams are not generated for Ion AmpliSeq™ HD, Tagseq, immune repertoire, metagenomics, and microbiome analysis workflows, although you can make comparisons across similar analysis results with these analysis workflows.

1. In the **Analysis** tab, click **Overview**, then select multiple analyses.

   Note: You can use the filters above the **Analyses** table to view only analyses of interest. For more information, see “Find analyses” on page 163.

2. Click **Actions** ▸ **Visualize** in the **Selected Analyses** section.

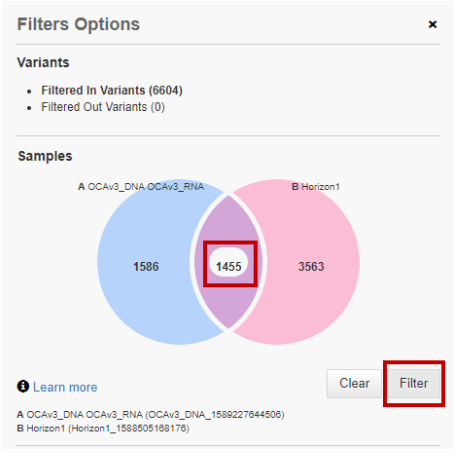


   The **Analysis Visualization** screen opens to the **TMB** tab.

3. Click the **Variants Table** tab.
   The table view shows side-by-side columns that list the variants for the analyses.

Genes	Location	OCAv3_DNA1:BON1059_...	OCAv4_139-1395_IonXpr...
BRCA2		13q13.1(32890491-32972932)x.	13q13.1(32890491-32972932)x2
BRCA2	BRCA2:utr_5:NM_000059.3	G/A	G/A
BRCA2	BRCA2:splicesite_5:NM_000059	G/G	G/G
BRCA2	BRCA2:splicesite_3:NM_000059	A/A	A/A
BRCA2	BRCA2:splicesite_3:NM_000059	A/A	A/A
BRCA2	BRCA2:exonic:NM_000059.3	G/G	A/G
BRCA2			T/C
BRCA2	BRCA2:exonic:NM_000059.3	G/G	G/G
BRCA2	BRCA2:exonic:NM_000059.3	C/T	C/T
BRCA2	BRCA2:exonic:NM_000059.3	C/C	C/C
BRCA2	BRCA2:splicesite_3:NM_000059	A/A	A/A
BRCA2	BRCA2:exonic:NM_000059.3	G/G	A/G
BRCA2	BRCA2:exonic:NM_000059.3	C/C	C/C
BRCA2	BRCA2:splicesite_5:NM_000059	G/G	G/G
BRCA2	BRCA2:splicesite_3:NM_000059	G/G	G/G
BRCA2	BRCA2:intronic:NM_000059.3	T/C	T/C

4. Refine the list of variants in the **Filter Options** section:
   a. Click the number in the overlapping set of the Venn diagram or other set of interest.
   b. Click **Filter** to update the table to include only the selected variants.
   c. *(Optional)* Click **Clear** to deselect all variants and restore the list to include all variants.



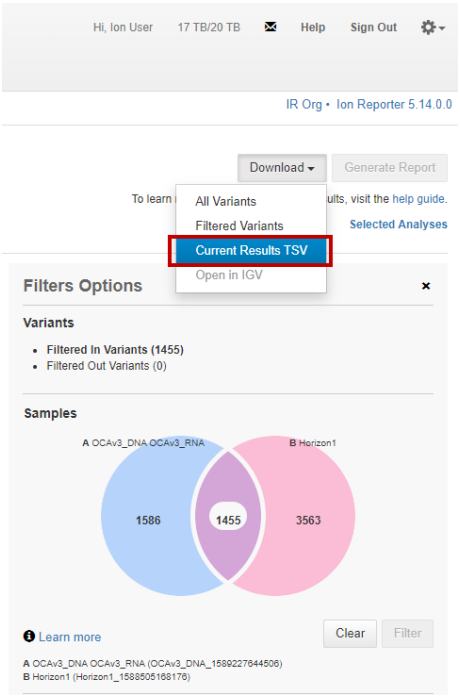
   Note: You can place the pointer over a sample name in the Venn diagram to see the full sample name and the analysis to which the sample belongs. The analysis name is listed in parentheses.

5. *(Optional)* View the filtered results in other visualization screens. For example, click **CNV Heat Map** to view a visual representation of copy number variation for the refined variant list.

6. To download the refined multianalysis results in a TSV file:
   a. Click **Download** ▸ **Current Results TSV**.
   b. Click the **Home** tab, then click **Notifications**.
   c. Find the download file in the list, then click **(Download)**.

   The TSV file that contains the refined list of variants is downloaded. The TSV file is a tab-separated list of the variants. It contains the data and columns presented in the same order shown in the **Variants Table**.

7. *(Optional)* Click **Clear** to deselect all variants and restore the **Variants Table** to include all variants.



Note: Under rare circumstances, the TSV file results can be duplicated in the Variants Table results in the Amino acid and Coding columns. This happens when the variant is Ref in one analysis, and a NOCALL in the other analysis.

Clustering in CNV heat map For CNV heat maps, ploidy is used as the score for every gene-sample pair to generate the heat map. After that hierarchical clustering is conducted for clustering genes and samples in the heat map.

Venn diagram generation

Ion Reporter™ Software generates Venn diagrams to display the number of variants that are unique and common between analyses. You can use Venn diagrams to limit visualizations to subsets of variants. For more information, see “Samples Venn diagram in Filter Options” on page 363.

Venn diagrams are generated for up to three similar analyses. Venn diagram generation in the software is analysis workflow, research application, and sample group dependent.

Venn diagrams are generated with following single analysis visualizations.

- One **DNA Paired** analysis.
- One **DNA Trio** analysis.

Venn diagrams are generated with multi-analysis visualizations of any of the analyses combinations.

- One **DNA Single** analysis and one **DNA Tumor–Normal** analysis.
- One **DNA Single** analysis and one **DNA and Fusions** analysis.
- One **DNA Single** analysis and one **DNA Paired** analysis.
- One **DNA and Fusions** analysis and one **Fusions** analysis.
- One **DNA Tumor–Normal** analysis and one **DNA Paired** analysis.
- Two **DNA Single** analyses.
- Two **DNA and Fusions** analyses.
- Two **DNA Tumor–Normal** analyses.

- Two **Fusions** analyses.
- Two **Annotate Variants** analyses.

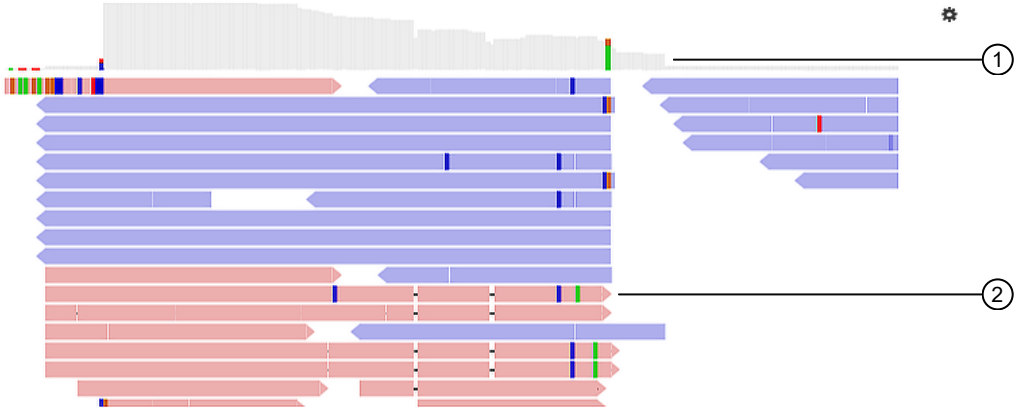
Venn diagrams are generated with multi-analysis visualizations of any of the analyses combinations.

- One **DNA Single** analysis and two **DNA Tumor–Normal** analyses.
- One **DNA Tumor–Normal** analysis and two **DNA Single** analyses.
- Three **DNA Single** analyses.
- Three **DNA and Fusions** analyses.
- Three **DNA Tumor–Normal** analyses.
- Three **Fusions** analyses.
- Three **Annotate Variants** analyses.

Venn diagrams are not generated for Ion AmpliSeq™ HD, TagSeq, immune repertoire, metagenomics, and microbiome analysis workflows,

Coverage histogram

When you zoom in on the variant pileup view, you see the coverage histogram and reads from the .bam track. The histogram in gray shows read depth at that location and reads from the .bam track show read direction and location of variants.



① Read depth
② Read location and direction of variants

Evaluate analysis results

You can evaluate analysis results in Ion Reporter™ Software by comparing multiple analyses. For example, you might compare new analysis results to known accurate analysis results.

Note: Comparison and visualization are not supported for Metagenomics analyses, or for analyses with a status of **Failed** or **In-Progress**. Tumor-normal analyses, which has only one set of variants for the tumor sample, and does not report variants for the normal sample, also cannot be visualized.

Visualization of a single trio or paired analysis is supported, because trio and paired analyses each contain more than one set of variants.

1. In the **Analyses** tab, click **Overview**.
2. Select multiple analyses, then click **Actions** ▸ **Visualize**.

You can review the results in various ways. For more information, see "Compare results of single or multiple analyses" on page 198.

Variants Table tab

The **Variants Table** lists the variant information for the analyses with Ion AmpliSeq™ workflows. If you selected more than one analysis, the Variants Table displays a side by side comparison of the variant calls for each analysis.

Note: To review variant information for analyses with Ion AmpliSeq™ HD workflows, see "Variant Matrix tab" on page 348.

Use the menus in the **Filter Options** pane to limit the data that is displayed in the Variants Table.

- For more information about the **Samples** Venn diagram, see "Samples Venn diagram in Filter Options" on page 363.
- For more information about using the **Chromosome** filter, see "Chromosome filter in the Filter Options" on page 363.
- For more information about using the **Filter Chains** filter, see "Filter Chains filter in the Filter Options" on page 363.

Variant Matrix tab

The Variant Matrix tab lists the variant information for the analyses with Ion AmpliSeq™ HD workflows. You can select one analysis or multiple analyses for side-by-side comparison. The Variant Matrix tab provides a summary of all variants that were called in the selected analyses, as well as detailed analysis metrics for each variant (see Figure 9 and Figure 10). For more information, see "Visualize variants in an analysis run with an Ion AmpliSeq™ HD analysis workflow" on page 388.

Note: To review variant information for analyses with Ion AmpliSeq™ workflows, see "Variants Table tab" on page 348.

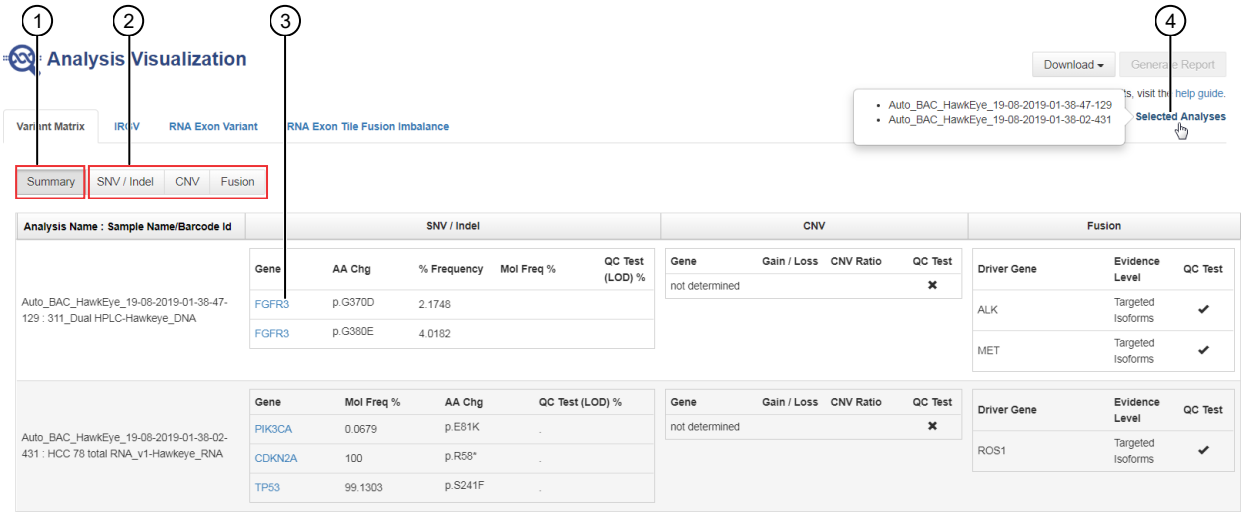


Figure 9 Representative Summary screen in the Variant Matrix tab

① The **Summary** screen displays all called SNV / Indel, CNV, and Fusion variants and a subset of analysis metrics for each variant. If you selected more than one analysis, the summary of called variants for each analysis is displayed side by side.

② Click **SNV / Indel**, **CNV**, or **Fusion** to view detailed analysis metrics for each variant (see Figure 10).

③ In the **Gene** column, click the gene symbol to access the HGNC report for that gene.

④ Click **Selected Analyses** to view the list of the analyses that were selected for visualization.

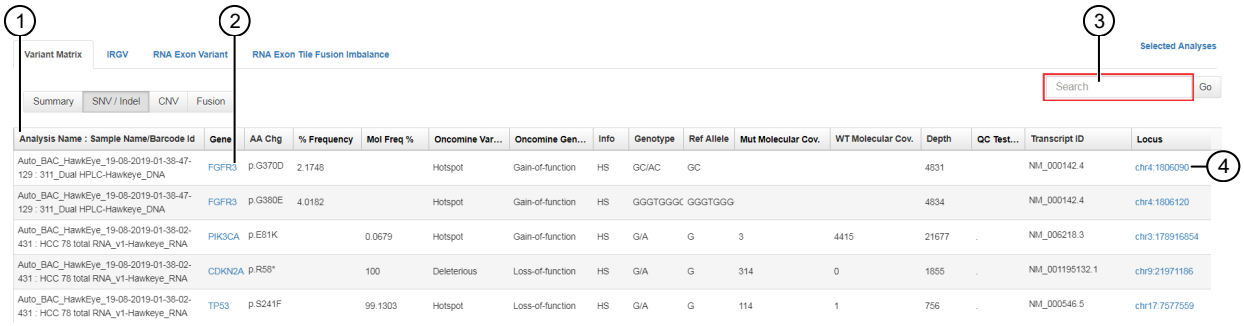


Figure 10 Representative detailed analysis metrics for SNV / Indel variants

① If you selected more than one analysis for visualization, all analyses and their corresponding variants will be listed in the same table.

② In the **Gene** column, click the gene symbol to access the HGNC report for that gene.

③ Use the **Search** field to search the variants list for a specific gene or locus.

④ In the **Locus** column, click the chromosome location to open the IRGV report for that locus.

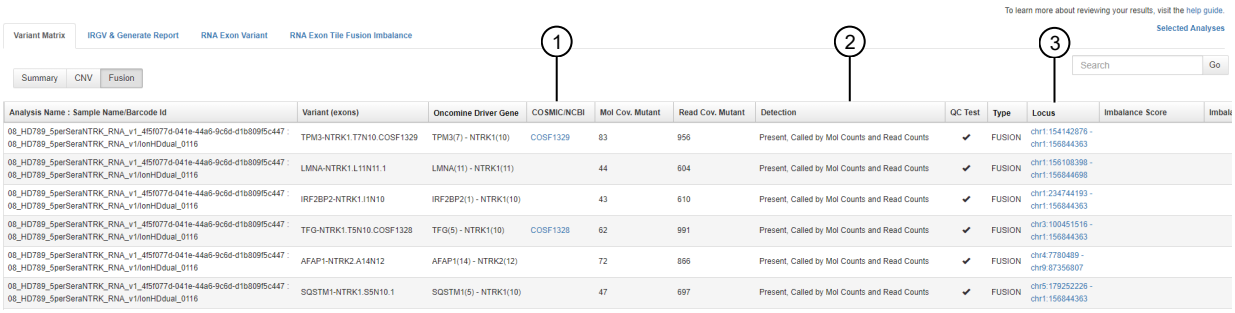


Figure 11 Representative detailed analysis metrics for Fusion variants

① In the **COSMIC/NCBI** column, you can click the COSMIC ID or NCBI GenBank ID to access the detailed report for the gene in these public web sites.

② In the **Detection** column, you can view if the variant is **Present** or **Absent**, and the reason for the call. In this example, all of the fusion variants shown are present on the basis of molecular counts and read counts (**Called by Mol Counts and Read Counts**).

③ In the **Locus** column, click the chromosome location to open the IRGV report for that locus.

IRGV & Generate Report tab

You can view visualized analysis results in the **IRGV & Generate Report** tab.



Use **In+** and **Out-** to zoom in and out on the selected chromosome.

1. Analysis Whole Genome View—Provides a genome-wide perspective of the data.
2. Selected Chromosome bar—To see the variant location on the selected chromosome, click the Whole Genome View image.
3. Proband Coverage Ratio—Type in the chromosome coordinates, or click the Karyo view to see discrete data for Copy Number calls.
4. Reference—Lists the genomic reference that is used for the analysis.
5. Shows annotation source results. To rearrange the annotation source tracks, click **(Settings)**.
6. Chart View Options—Toggle between a **DNA View** or **RNA View**. Select **RNA View** for fusion analyses to get a menu to search for individual fusions in the panel.
7. Sample/Analysis Summary—Sort by Sample number and rearrange the analysis rows on the left.

⑧ Search—Enter chromosome coordinates or gene names to find data.

⑨ Filter chain—Apply a filter chain and immediately view its results. You cannot save the results of the applied filter chain to the visualization. You can also create a new filter chain. For more information, see Chapter 10, "Filters and filter chains".

⑩ MAPD filter—An analysis sample-wide metric of noise. You can adjust it to determine when a particular sample has met the threshold that was set for the MAPD filter.

⑪ IGV/Export—Use multiple export options, including an export to IGV, and access to preferences. For more information, see "Visualize variants with IGV" on page 394 and "Set IRGV preferences" on page 312.

Variant Impact tab

The **Variant Impact** tab provides visual representation of the impact that the detected variants have on the sequence of the affected genes and transcripts, as well as the sequence, structure, and function of the affected proteins. The data is presented in the form of a heat map.

Use the menus in the **Filter Options** pane to limit the data that is displayed in the heat map.

- For more information on the **Samples** Venn diagram, see "Samples Venn diagram in Filter Options" on page 363.
- For more information on using the **Chromosome** filter, see "Chromosome filter in the Filter Options" on page 363.
- For more information on using the **Filter Chains** filter, see "Filter Chains filter in the Filter Options" on page 363.

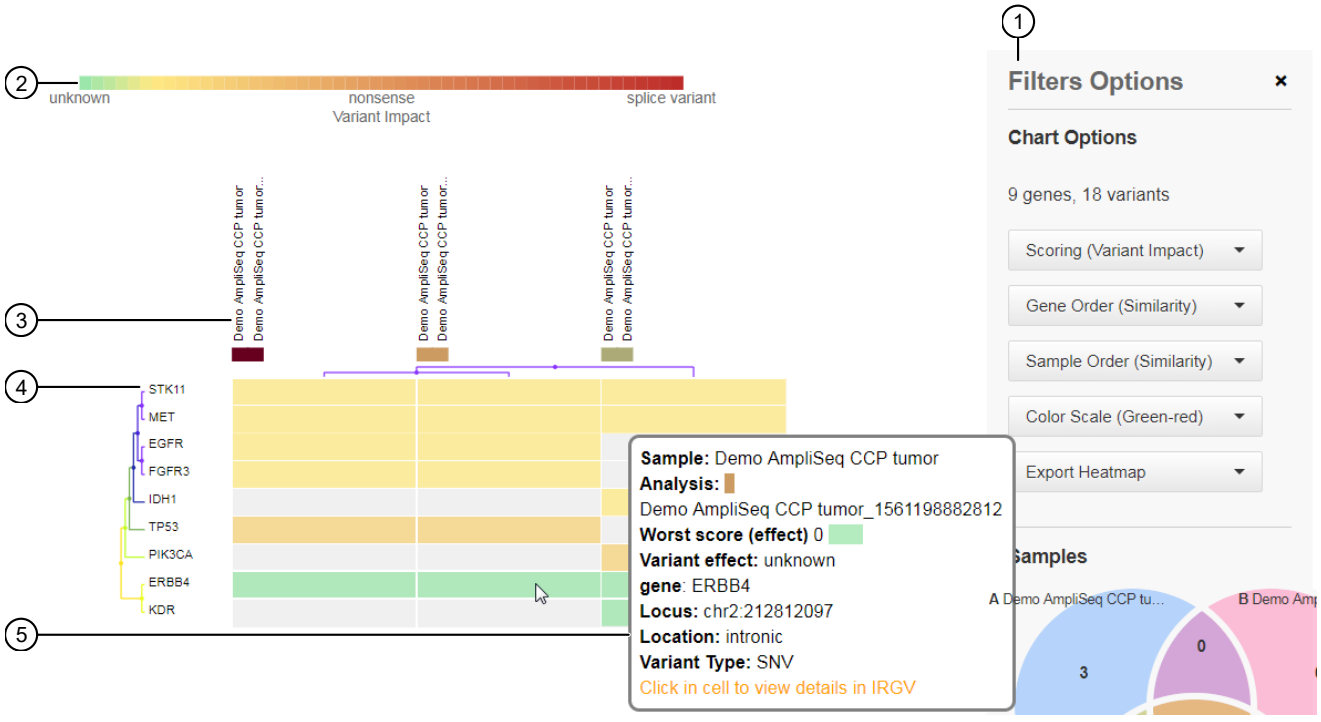


Figure 12 Example heat map

① Heat map **Filter Options**.

② Heat map legend. The legend changes, depending on the scoring that is option that is selected in the **Chart Options**.

③ Samples and analyses: hover over sample and analysis names to view the sample and analysis details.

④ Genes: hover over a gene ID to view the sum of scores for that gene or click on a gene ID to view the details for all analyses in IRGV.

⑤ Variants: hover over a variant to view the details pane for that variant, or click in the cell to view the details for that variant in IRGV.

Use the following **Chart Options** to display the heat maps of choice and customize the heat map view.

Chart Option	Description
Scoring – The heat map for each option are color coded based on the selected scoring option.	
Variant Impact	For each gene, the variant impact is scored based on the severity of the impact of the variant. The heat map is color coded using the following impact score values, where higher values are associated with mutations that have more significant, or worse, impact. • Unknown: 0 • Synonymous: 1 • Missense: 2 • Non-frameshift block substitution: 3 • Non-frameshift insertion: 4 • Non-frameshift deletion: 4 • Nonsense: 5 • Stop-loss: 6 • Frameshift block substitution: 7 • Frameshift insertion: 7 • Frameshift deletion: 7 • Splice variant: 8
SIFT	A SIFT score predicts whether an amino acid substitution affects protein function. The SIFT score ranges from 0 to 1, where the score of 0 represents the most deleterious amino acid substitution and the score of 1 represents the most tolerated amino acid substitution.
PolyPhen-2	The PolyPhen-2 score predicts the possible impact of an amino acid substitution on the structure and function of a human protein. The score represents the probability that a substitution is damaging. The reported score range is 0–2, with the following classifications for the impact of an amino acid substitution. • Benign: 0–0.99 • Borderline: 1–1.24 • Potentially damaging: 1.25–1.49 • Possibly damaging: 1.5–1.99 • Damaging: ≥2
PhyloP	The PhyloP score measures the evolutionary conservation at individual alignment sites. Positive scores measure conservation, or slower than expected evolution, and represent sites that are predicted to be conserved. Negative scores measure acceleration, or faster than expected evolution, and represent sites that are predicted to be fast-evolving.
Grantham	The Grantham score provides a prediction for the evolutionary distance between the amino acids. A lower score predicts a smaller evolutionary distance. A higher score predicts a greater evolutionary distance.
Order – Organize the displayed results based on gene or sample order.	
Gene Order	Select one of following options from the **Gene Order** dropdown list to arrange the order in which the genes are listed in the heat map. • **Name** • **Similarity** • **Similarity (left/right reversed)**

(continued)

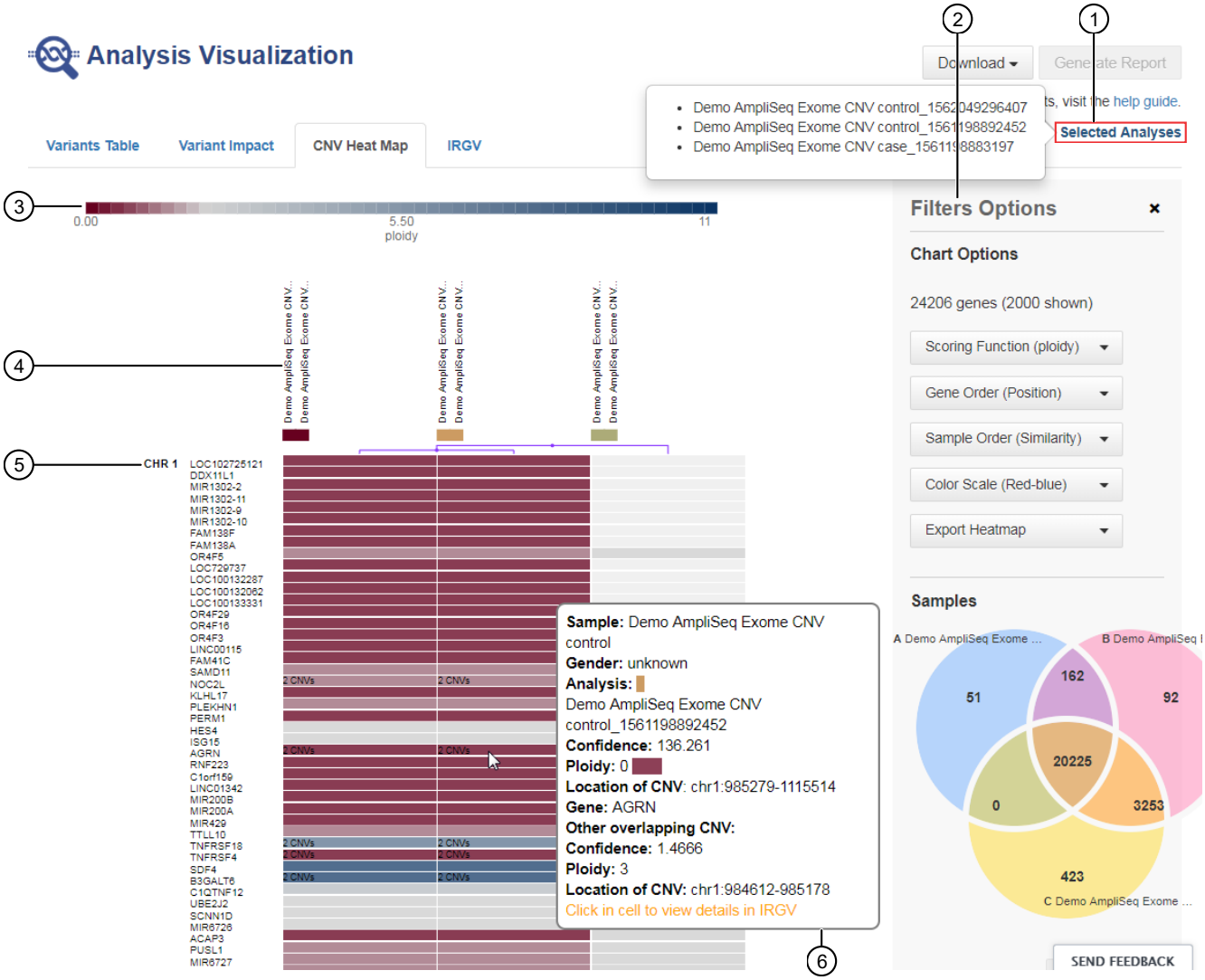
Chart Option	Description
Sample Order	Select one of following options from the **Sample Order** dropdown list to arrange the order in which the genes are listed in the heat map. • **Name**: Samples are listed alphabetically by name from left to right. • **Similarity**: Samples are listed by how similar they are in the fusion calls made, presented from fewest fusions to most fusions from left to right. • **Similarity (left/right reversed)**: Samples are listed by how similar they are in the fusion calls made, presented from fewest fusions to most fusions from right to left. • **Analysis**: Samples are listed alphabetically by analysis name from left to right.
Other	
Color Scale	Select a color scale to customize the colors that are displayed in the heat map.
Export Heatmap	You can export and save your heat map in PNG, SVG, and CSV file formats. The file is automatically downloaded once you make your selection from the **Export Heatmap** dropdown list.

CNV Heat Map tab

The **CNV Heat Map** tab provides a visual representation of copy number variation (CNV) data in the form of a heat map. You can select multiple analyses to compare the CNV data for multiple samples side by side. Ploidy values are used to quantify and compare call number variations. The ploidy value of 2 is expected for wild type samples where no genetic amplification or deletion was detected. A ploidy value of >2 indicates amplification. A ploidy value of <2 indicates deletion. In the heat map, a color scale is used to visualize ploidy, where the expected value of 2 is colored gray. For X and Y chromosome, the color that is applied to the plot considers the expected value given the gender. For example, for a male, the expected value for Y is 1, therefore, the cell for that variant appears as gray.

Use the menus in the **Filter Options** pane to limit the data that is displayed in the heat map. For a large data set, the data for the first 2,000 genes are retrieved.

- For more information about the **Samples** Venn diagram, see “Samples Venn diagram in Filter Options” on page 363.
- For more information about using the **Chromosome** filter, see “Chromosome filter in the Filter Options” on page 363.
- For more information about using the **Filter Chains** filter, see “Filter Chains filter in the Filter Options” on page 363.



① Click **Selected Analyses** to view the list of the analyses that are visualized.

② Heat map **Filter Options**.

③ Heat map legend: ploidy. Place the pointer over the legend the view the relationship between the ploidy value and the colors used in the heat map.

④ Samples and analyses: place the pointer over sample and analysis names to view the sample and analysis details.

⑤ Genes: place the pointer over a gene ID to view the details for that gene or click the gene ID to view the details for all analyses in IRGV. Gene order is determined by the chromosomal position of the gene. The chromosome number is displayed to the left of the gene list.

⑥ Variants: place the pointer over a variant to view the details pane for that variant, or click the cell to view the details for that variant in IRGV. If >1 CNVs are detected for the same gene, the number of CNVs is listed in the cell.

Use the following **Chart Options** to show the heat maps of choice and customize the heat map view.

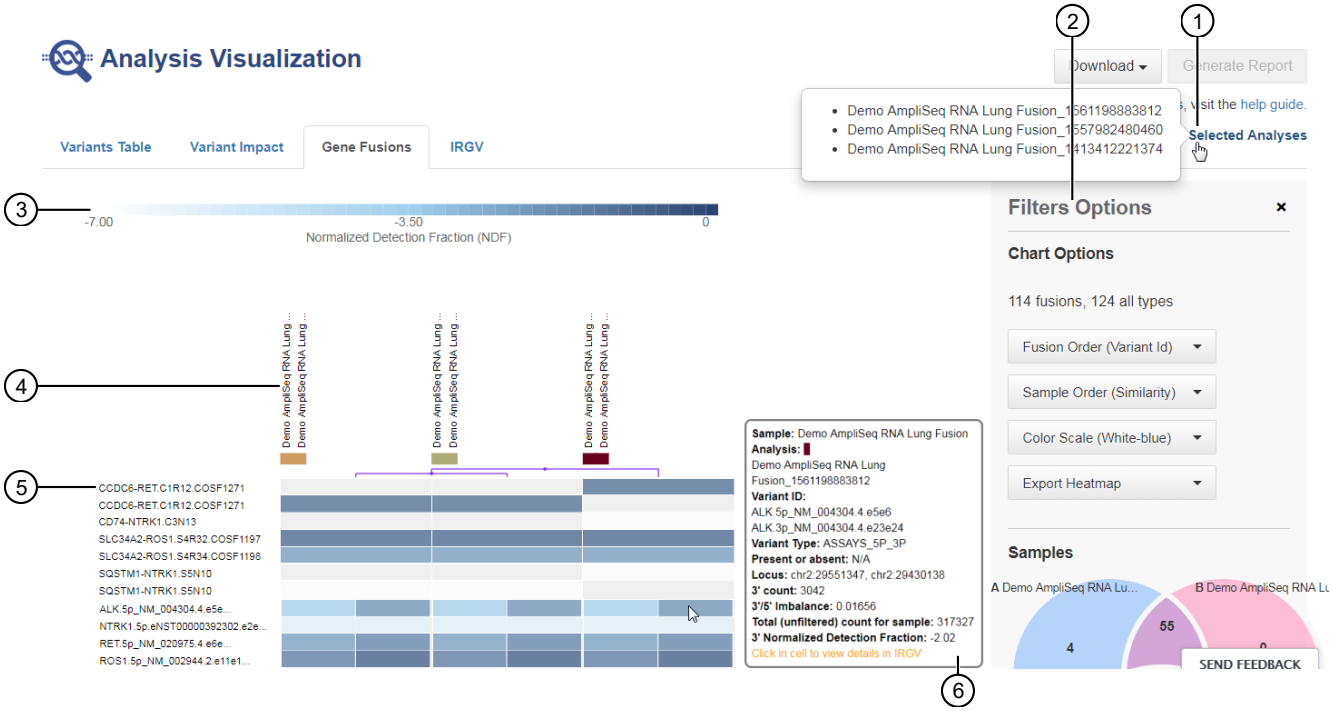
Chart Option	Description
Scoring Function	The CNV heat map uses ploidy values to score copy number variants.
Gene Order	The CNV heat map orders the list of genes based on their position on the chromosome.
Sample Order	Select one of following options from the **Sample Order** dropdown list to arrange the order in which the genes are listed in the heat map. • **Name**: Samples are listed alphabetically by name from left to right. • **Similarity**: Samples are listed by how similar they are in the CNV calls made, presented from fewest to most CNV calls in common from left to right. • **Similarity (left/right reversed)**: Samples are listed by how similar they are in the fusion calls made, presented from fewest to most CNV calls in common from right to left. • **Analysis**: Samples are listed alphabetically by analysis name from left to right.
Color Scale	Select a color scale to customize the colors that are displayed in the heat map.
Export Heatmap	You can export and save the heat map in PNG, SVG, and CSV file formats. The file is downloaded after you make a choice from the **Export Heatmap** dropdown list.

Gene Fusions tab

The **Gene Fusions** tab provides visual representation of the detected gene fusion variants. The data is quantified using the Normalized Detection Fraction (NDF) score and presented in the form of a heat map. NDF is a logarithm of the read count divided by the total number of reads per sample. The NDF score ranges from –7.0 to 0, where values closer to 0 = higher number of detected fusion events and values closer to –7 = lower number of detected fusion events.

Use the menus in the **Filter Options** pane to limit the data that is displayed in the heat map.

- For more information on the **Samples** Venn diagram, see “Samples Venn diagram in Filter Options” on page 363.
- For more information on using the **Chromosome** filter, see “Chromosome filter in the Filter Options” on page 363.
- For more information on using the **Filter Chains** filter, see “Filter Chains filter in the Filter Options” on page 363.



① Click **Selected Analyses** to view the list of the analyses that are visualized.

② Heat map **Filter Options**.

③ Heat map legend: Normalized Detection Fraction (NDF). Hover over the legend the view the relationship between the NDF score and the colors used in the heat map.

④ Samples and analyses: hover over sample and analysis names to view the sample and analysis details.

⑤ Gene fusions: hover over the gene fusion variant ID to view the details for that gene fusion or click on the variant ID to view the details for that variant in IRGV.

⑥ Variants: hover over a variant to view the details pane for that variant, or click in the cell to view the details for that variant in IRGV.

Use the following **Chart Options** to customize the heat map view.

Chart Option	Description
Fusion Clustering	From the **Fusion Clustering** dropdown list, select one of the following options to arrange the order in which the fusion variants are listed in the heat map. • **Variant Id**: Variants are listed alphabetically by their Variant ID. • **5′ name**: Variants are listed alphabetically by the 5′ gene name. • **3′ name**: Variants are listed alphabetically by the 3′ gene name.
Sample Order	Select one of following options from the **Sample Order** dropdown list to arrange the order in which the genes are listed in the heat map. • **Name**: Samples are listed alphabetically by name from left to right. • **Similarity**: Samples are listed by how similar they are in the fusion calls made, presented from fewest fusions to most fusions from left to right. • **Similarity (left/right reversed)**: Samples are listed by how similar they are in the fusion calls made, presented from fewest fusions to most fusions from right to left. • **Analysis**: Samples are listed alphabetically by analysis name from left to right.

(continued)

Chart Option	Description
Color Scale	Select a color scale to customize the colors that are displayed in the heat map.
Export Heatmap	You can export and save your heat map in PNG, SVG, and CSV file formats. The file is automatically downloaded once you make your selection from the **Export Heatmap** dropdown list.

RNA Exon Variants tab

The **RNA Exon Variant** tab displays a bar graph summary of intragenic exon rearrangements or fusions for each selected analysis. If you selected more than one analysis, the data for each sample is summarized in a separate bar graph. The RNA exon variants that are displayed in the tab are defined in the BED file. The **RNA Exon Variant** tab is available for all analyses that use RNA and Fusion workflows.

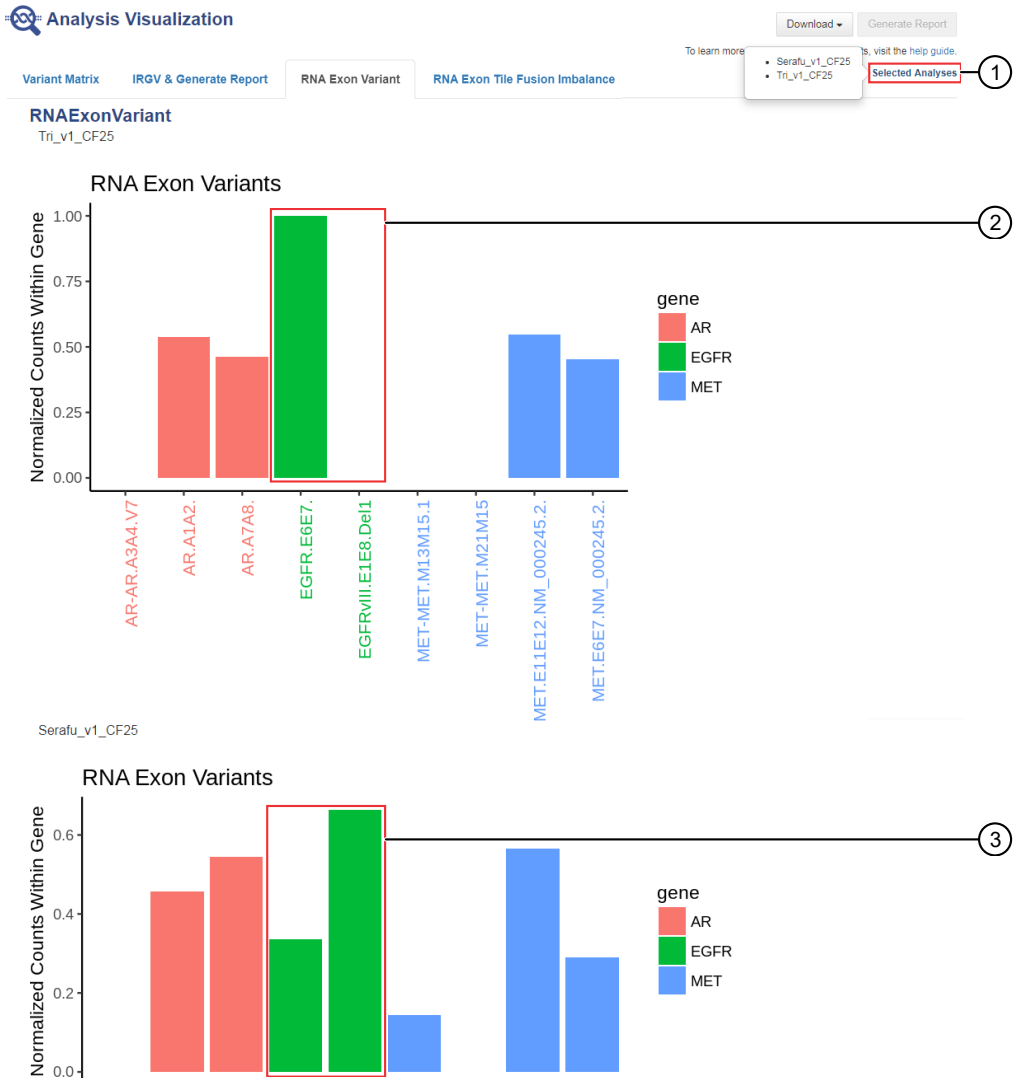
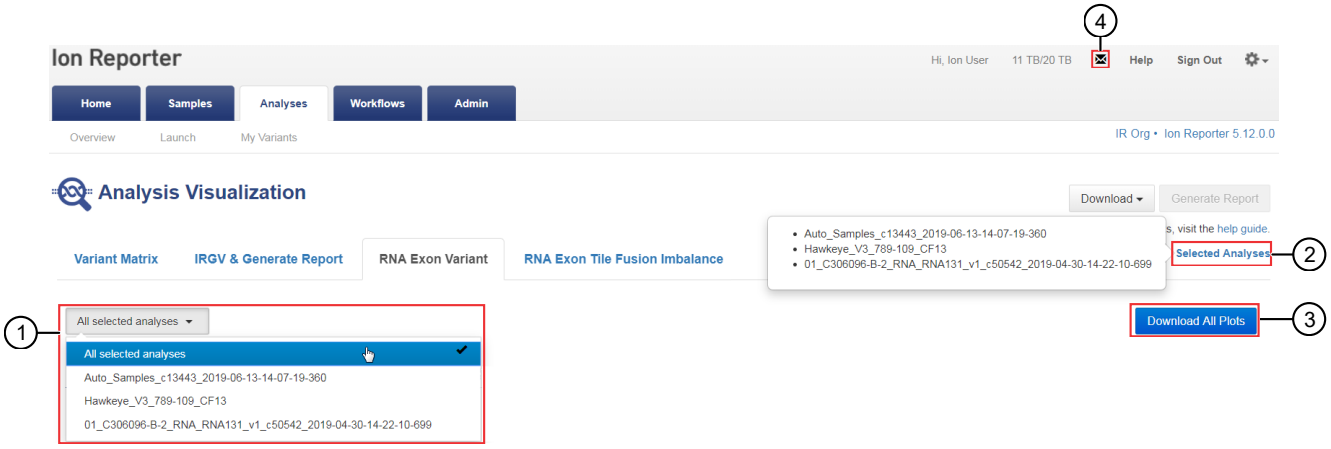


Figure 13 Representative RNA Exon Variants bar graphs The X-axis represents specific exon variants, where each variant is labeled with a gene ID followed by a sequence of adjacent exons. The Y-axis measures the read counts for each variant, normalized to the wild type.

1. Click **Selected Analyses** to view a list of the analyses that you selected for visualization.
2. Example analysis where only the wild type EFGR (EFGR.E6E7) was detected.
3. Example analysis where RNA exon 2–7 deletion occurred in the EFGR gene. The deletion of exons 2–7 resulted in an increase of normalized read counts for the EFGR variant that contains the intragenic fusion of exon 1 and exon 8 (EFGR.E1E8.Del1) and a decrease of normalized read counts for the wild type EFGR (EFGR.E6E7).

RNA Exon Tile Fusion Imbalance tab

The **RNA Exon Tile Fusion Imbalance** tab provides visual representation of the RNA fusion imbalance analyses. If you selected two or more analyses (or samples) for visualization, the **RNA Exon Tile Fusion Imbalance** tab displays multi-analysis view of the data. You can select to view each individual analysis separately, or compare the selected analyses side by side. Each gene in each sample is normalized to baseline and read coverage, therefore, you can compare multiple samples from different chips, tissues, and so on.



① From the dropdown list, select a single analysis or select all analyses to compare side by side.

② To view a list of analyses that are selected and displayed in the current **RNA Exon Tile Fusion Imbalance** tab, click **Selected Analyses**.

③ To download a batch ZIP file that contains all plots, click **Download All Plots**. The ZIP file is available for download in the **Notifications** screen.

④ To access the list of **Notifications**, click ✉ **(Notifications)**.

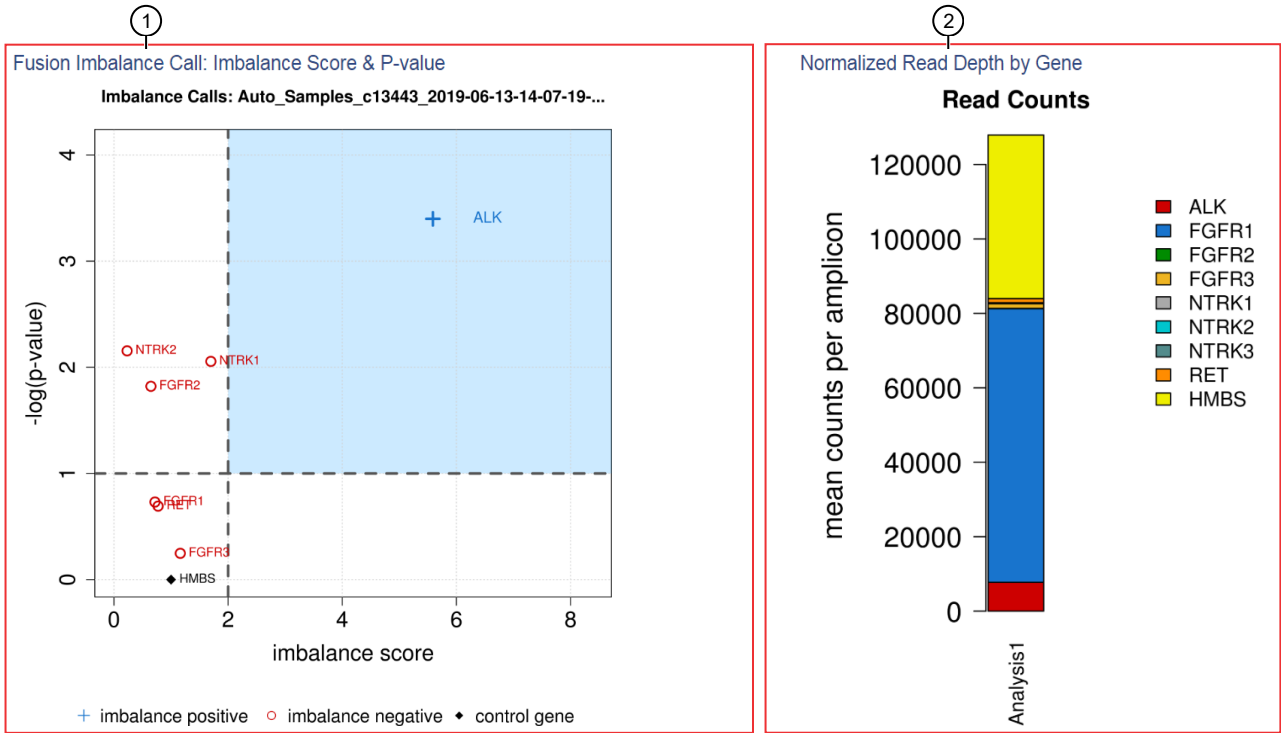


Figure 14 Single-sample analysis visualization

① The **Fusion Imbalance Call: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in the selected sample. The dashed gray lines mark the threshold for an imbalance call, which is applied to all genes across all samples. Points that fall within the blue shaded area of the plot represent fusion-positive genes (+). All other points that are outside of the blue shaded area represent fusion-negative genes (◯). Control genes are marked with ◆.

② The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip for the selected sample. For each gene, the read counts are normalized to the number of amplicons.

③ The **Sample Coverage for Imbalance Analysis** plots show the expression profile for each exon-exon tiling amplicon for each gene. The y-axis represents the corrected molecular counts. The x-axis represents individual exon-exon junctions, which are listed from 5′ to 3′. The **imbalance score** and **p-value** are listed in the panel of each gene that was called positive for fusion.

④ Baseline (a cluster of gray lines), generated from a fusion-negative sample.

⑤ Test sample corrected read coverage (blue line), normalized to the baseline. Each point on the line represents a unique exon-exon junction that was covered by the assay and normalized to the baseline.

⑥ Predicted range for the fusion break point for a fusion-positive gene (dashed red line).

⑦ Sample coverage profile for the control gene (orange line).

⑧ If the collected data are insufficient to determine an imbalance score, the **INVALID SAMPLE** message appears in the panel for that gene.

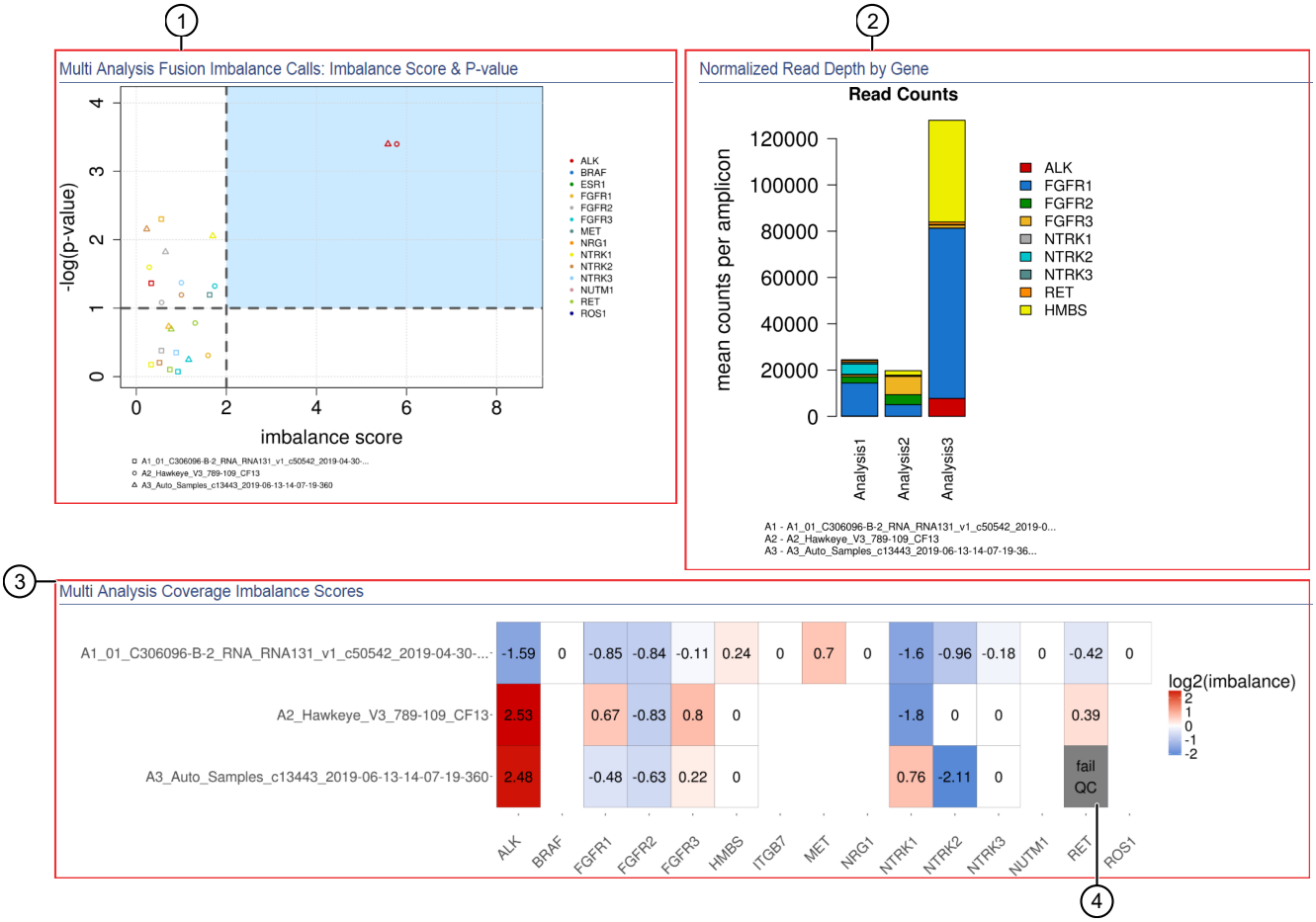


Figure 15 Multi-sample analyses visualization

① The **Multi Analysis Fusion Imbalance Calls: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in all the selected samples. Each data point corresponds to a gene-sample pair. Points that have the same color represent the same gene and are defined in the legend on the right. Points that have the same shape represent genes from the same sample and are defined in the legend on the bottom. The dashed lines mark the threshold for an imbalance call, which is applied to all genes across all samples. Points that fall within the blue shaded area of the plot represent fusion-positive genes.

② The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip. For each gene, the read counts are normalized to the number of amplicons. For multi-analysis visualization, the data are displayed side by side. The number of bars corresponds to the number of samples.

③ The **Coverage Imbalance Scores** plot shows a heatmap of imbalance scores for all selected analyses. Each row corresponds to a specific sample and each column corresponds to a specific gene. The **Coverage Imbalance Scores** plot is generated for multi-analysis comparison only, and does not take into account the p-values. To determine the significance of the imbalance score, review the **Fusion Imbalance Call: Imbalance Score & P-value** plot and **Sample Coverage for Imbalance Analysis** plot for each individual sample (see Figure 14, callout 1 and callout 3).

④ If the collected data are insufficient to determine an imbalance score for a gene, the gene is marked with **fail QC** in the **Coverage Imbalance Scores** plot and **INVALID SAMPLE** message appears in the **Sample Coverage for Imbalance Analysis** plot in the panel for that gene (see Figure 14, callout 8).

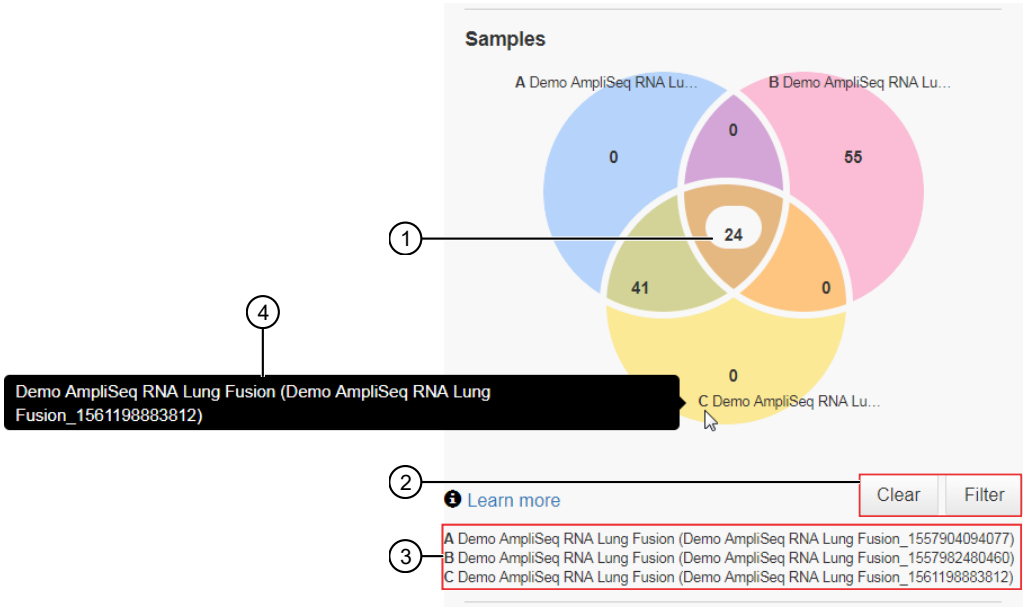
Filter Options

Use the menus in the **Filter Options** pane to limit the data that is displayed in the analysis results.

Samples Venn diagram in Filter Options

Use the **Samples** Venn diagram to limit the visualization of the selected analyses to a subset of variants. The numbers in the overlapping areas of the diagram represent variants that are common in the corresponding samples. The numbers in the non-overlapping areas represent the variants that are unique to that sample.

IMPORTANT! The **Samples** Venn diagram is available only when you select to compare two or three analyses side by side.



① Click the numbers in the Venn diagram to select the variants for inclusion on the heat map.

② Click **Filter** to update the heat map view to include only the selected variants. Click **Clear** to deselect all variants and restore the heat map to include all variants.

③ Sample and Analysis legend. The analysis name is listed in the parentheses.

④ Hover over a sample name to see the full sample name and the analysis to which the sample belongs. The analysis name is listed in the parentheses.

Chromosome filter in the Filter Options

In the **Filter Options** pane, you can use the **Chromosome** dropdown list to limit the visualization of the data to one chromosome. From the **Chromosome** dropdown list, select a chromosome number to visualize only the variants that are mapped to that chromosome. Select **All** to visualize all variants.

Filter Chains filter in the Filter Options

In the **Filter Options** pane, you can use the **Filter Chains** dropdown list to limit data visualization to a subset of variants. You can select an existing filter chain, or click to create a new filter chain. For more information, see Chapter 10, “Filters and filter chains”.

Novel fusion detection using expression imbalance

There are two methods of novel fusion detection in Ion Reporter™ Software. The method that is used depends on which analysis workflow is applied when the analysis is launched.

One method is the exon tiling imbalance method. Exon tiling is a partner agnostic fusion detection method that enables the discovery of novel fusions and predicts the fusion breakpoint within a range of exons of key driver genes. With this method, the software identifies novel fusions in assays that include exon tiling amplicons. For more information, see “Fusion detection methods” on page 539 and “View RNA Exon Tile Fusion Imbalance plots” on page 364.

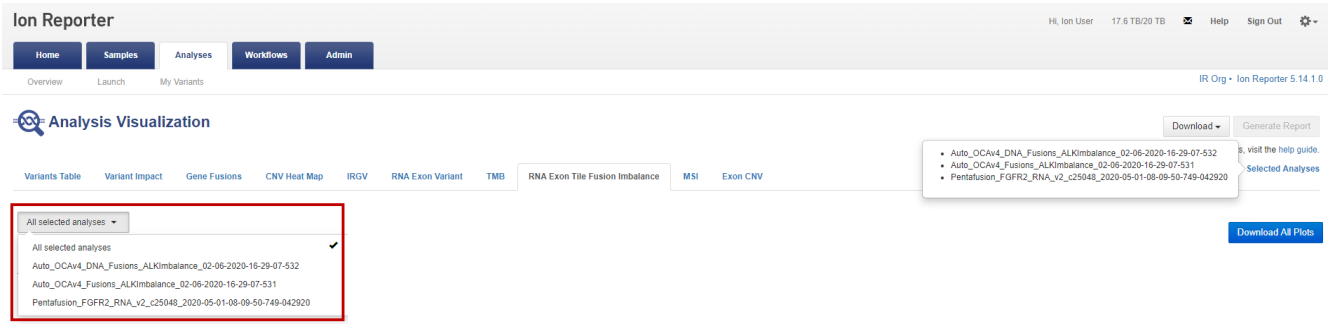
In the other method, the software generates a 3'/5' imbalance value that you can evaluate to detect novel fusions. This method only applies to panels that include 3' and 5' primers. The 3'/5' imbalance values are generated with analyses launched with the Oncomine™ Focus Assay, Oncomine™ Comprehensive Assay v1, analysis workflows applied to the Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel, and some custom panels from **AmpliSeq.com** that include 3'/5' amplicons. For more information, see “3'/5' Imbalance scores for assay calls” on page 370.

View RNA Exon Tile Fusion Imbalance plots

You can identify novel fusions with predicted breakpoints of select driver genes in Ion Reporter™ Software with **RNA Exon Tile Fusion Imbalance** analysis results and plots.

You can view **RNA Exon Tile Fusion Imbalance** analysis results and plots from a single analysis or from multiple analyses simultaneously. If multiple analyses are compared, analysis results for the selected analyses are displayed side by side. Because each gene in each sample is normalized to baseline and read coverage, you can compare multiple samples from different chips, tissues, and so on.

1. In the **Analyses** tab, click **Overview**.
2. Select one or multiple analyses, then click **Visualize**.
3. In the **Analysis Visualization** screen, click **RNA Exon Tile Fusion Imbalance**.
   The graphs and plots that are visual representations of RNA fusion imbalance analysis results are shown.
4. To change the analysis or analyses that are displayed, select a single analysis or select **All selected analyses** from the dropdown list.



RNA Exon Tile Fusion Imbalance plots are shown for a single analysis or all selected analyses. For example plots, see the following sections:

- "Example RNA Exon Tile Fusion Imbalance plots for a single analysis" on page 365.
- "Example RNA Exon Tile Fusion Imbalance read count plot for a single analysis" on page 367.
- "Example sample coverage plot for a single analysis positive for a fusion imbalance in NTRK1" on page 367.
- "Example RNA Exon Tile Fusion Imbalance call plot for multiple analyses" on page 368.
- "Example Normalized Read Depth by Gene RNA Exon Tile Fusion Imbalance plot for multiple analyses" on page 369.
- "Example Coverage Imbalance Scores plot" on page 370.

5. *(Optional)* Click **Download All Plots** to download a ZIP file that contains all of the plots for all of the analyses shown in the **RNA Exon Tile Fusion Imbalance** visualization. For more information about file downloads, see "View notifications" on page 27.

Example RNA Exon Tile Fusion Imbalance plots for a single analysis

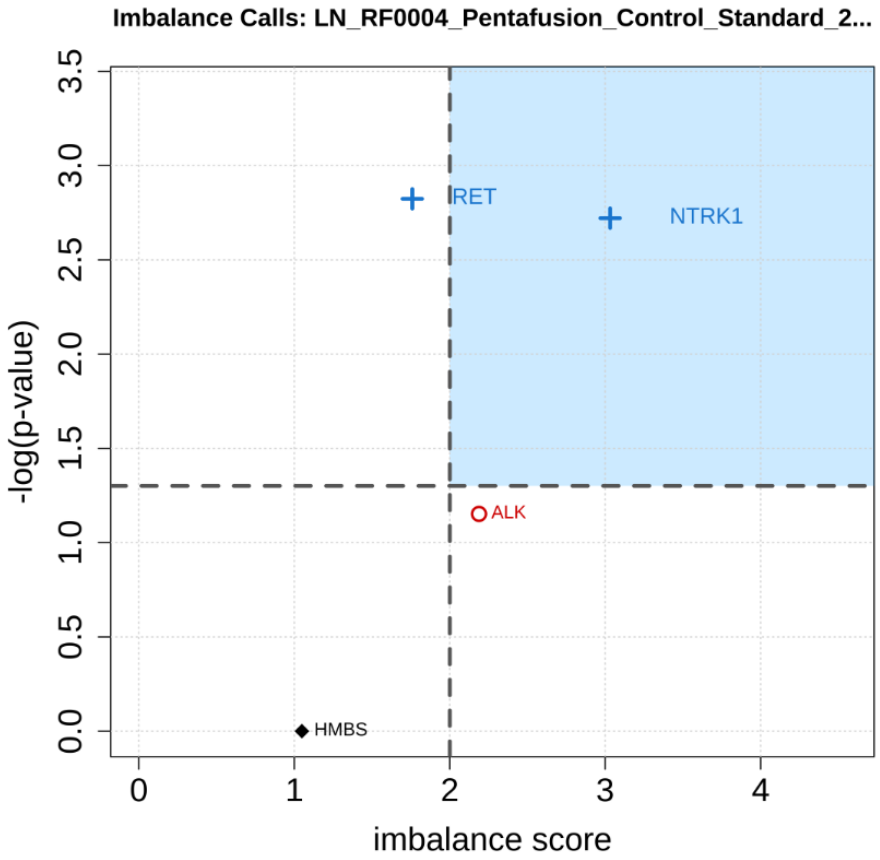
RNA Exon Tile Fusion Imbalance plots provide a visual representation of the RNA fusion imbalance results for the detection of novel fusions in a partner agnostic manner. This plot shows the imbalance scores and p-values for genes in the selected analysis result, including the control gene, genes that are imbalance-positive, and genes that are imbalance-negative.

In the plot, dashed gray lines mark the threshold for an imbalance call. The blue quadrant is at a fixed position, with the **imbalance score** set at 2 and the **–log(p-value)** set at 1.3. The plot provides a visual estimate for positive imbalance calls. This image shows that NTRK1 is an exon tile fusion imbalance positive gene in the selected analysis. The plotted position of some fusion positive genes do not appear within the blue (positive) quadrant. Genes denoted with + are exon tile fusion imbalance positive, such as RET, although RET is not plotted within the blue quadrant. This is because the fusion imbalance threshold for RET is lower than the fixed quadrant that is displayed. The blue quadrant is a guideline. Refer to the symbol displayed in the plot for the fusion imbalance call.

Graph symbols

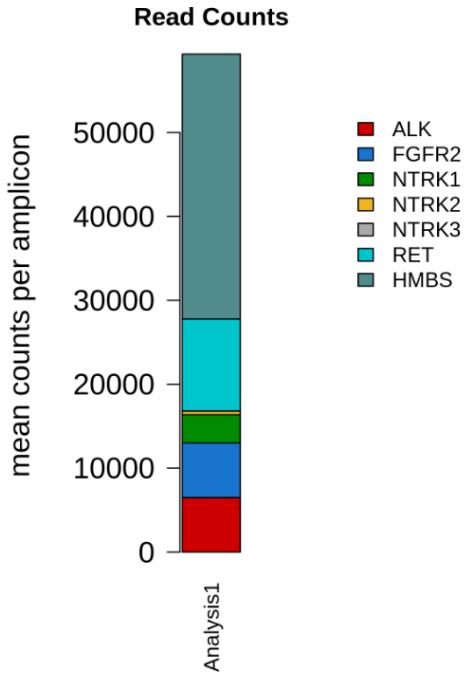
Symbol	Description
+	Fusion-positive genes
○	Fusion-negative genes
◆	Control genes

Fusion Imbalance Call: Imbalance Score & P-value



Example RNA Exon Tile Fusion Imbalance read count plot for a single analysis

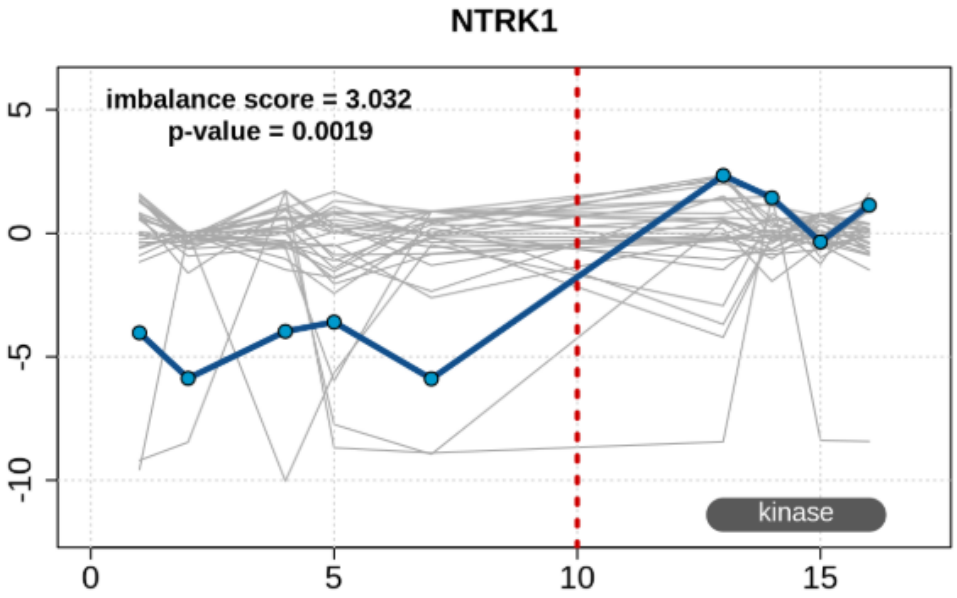
RNA Exon Tile Fusion Imbalance plots provide a visual representation of the RNA fusion imbalance analysis results for the detection of novel fusions in a partner agnostic manner. The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip for the selected sample. For each gene, the read counts are normalized to the number of amplicons.



Example sample coverage plot for a single analysis positive for a fusion imbalance in NTRK1

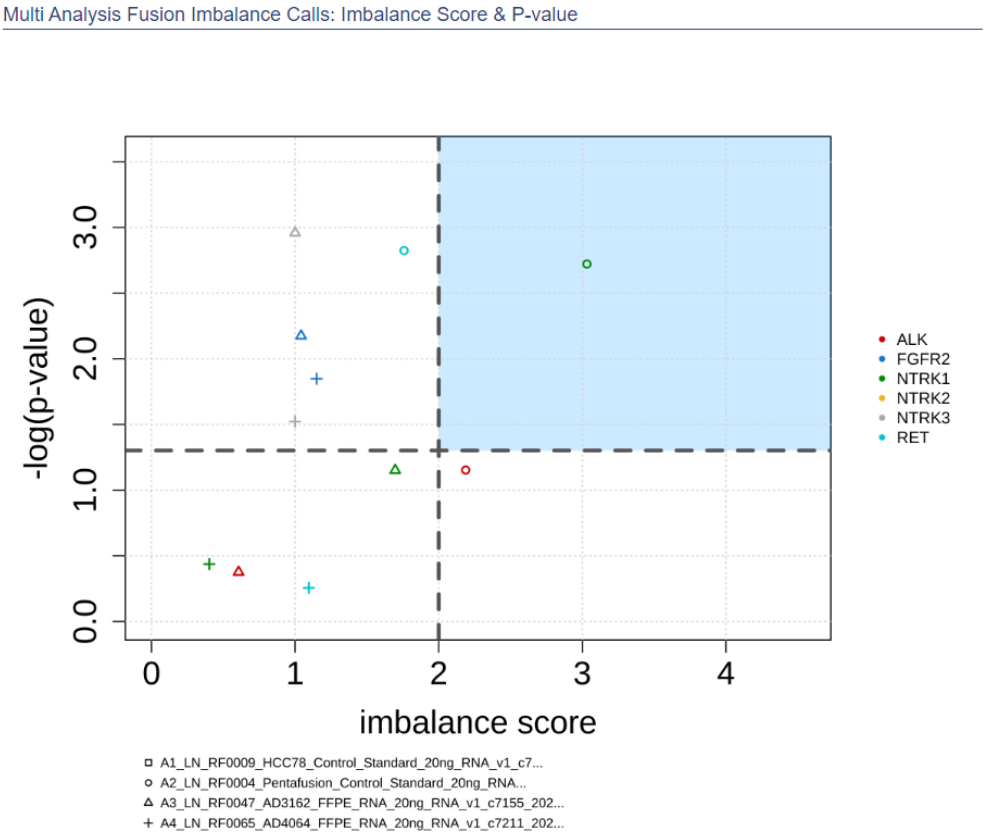
RNA Exon Tile Fusion Imbalance plots provide a visual representation of the RNA fusion imbalance analysis results for the detection of novel fusions in a partner agnostic manner. **Sample Coverage for Imbalance Analysis** plots show the expression profile for each exon-exon tiling amplicon for each gene.

The blue line indicates the expression level across NTRK1 and indicates a significant imbalance in this example. The collection of fusion-negative samples is used to construct the baseline and each fusion-negative sample is represented by a grey line in the plot. The distinction between NTRK1 and the baseline shows a significant difference with a **p-value** of 0.0019. The location of the predicted breakpoint is denoted with the dashed red line.



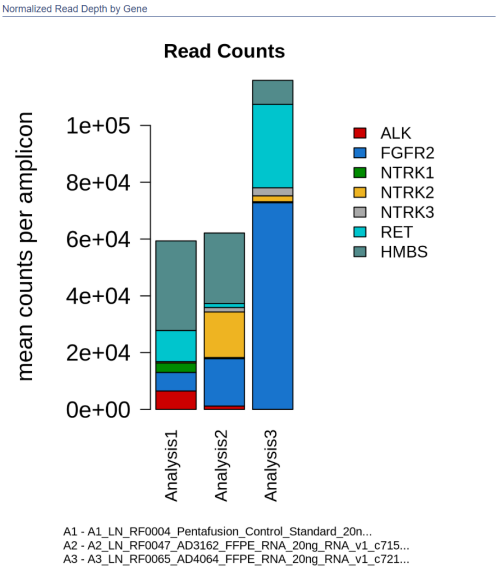
Example RNA Exon Tile Fusion Imbalance call plot for multiple analyses

RNA Exon Tile Fusion Imbalance plots provide a visual representation of the RNA fusion imbalance analysis results for the detection of novel fusions in a partner agnostic manner. The **Multi Analysis Fusion Imbalance Calls: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in all the selected analyses. Each data point corresponds to a gene-sample pair. Points that have the same color represent the same gene and are defined in the legend on the right. Points that have the same shape represent genes from the same sample and are defined in the legend on the bottom. This plot provides an overview of the relative confidence and strength of the fusion imbalance call only. To confirm the fusion imbalance call for any given gene, review and verify the imbalance score and P value for any given gene in the visualizations for a single analysis. For more information, see “Example RNA Exon Tile Fusion Imbalance plots for a single analysis” on page 365 and “Example sample coverage plot for a single analysis positive for a fusion imbalance in NTRK1” on page 367.



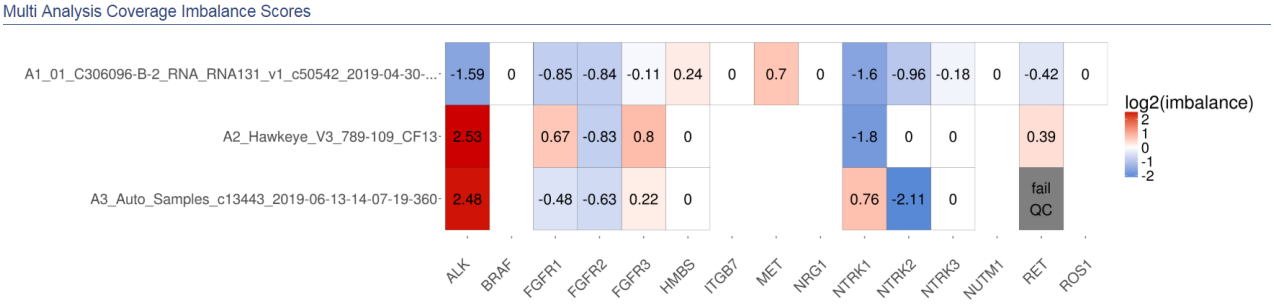
Example Normalized Read Depth by Gene RNA Exon Tile Fusion Imbalance plot for multiple analyses

RNA Exon Tile Fusion Imbalance plots provide a visual representation of the RNA fusion imbalance analysis results for the detection of novel fusions in a partner agnostic manner. The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip. For each gene, the read counts are normalized to the number of amplicons. For multi-analysis visualization, the data are displayed side by side. The number of bars corresponds to the number of analyses.



Example Coverage Imbalance Scores plot

RNA Exon Tile Fusion Imbalance plots provide a visual representation of the RNA fusion imbalance analysis results for the detection of novel fusions in a partner agnostic manner. The **Coverage Imbalance Scores** plot shows a heatmap of imbalance scores for all selected analyses. Each row corresponds to a specific sample and each column corresponds to a specific gene. The **Coverage Imbalance Scores** plot is generated for multi-analysis comparison only, and does not take into account the p-values. To determine the significance of the imbalance score, review the **Fusion Imbalance Call: Imbalance Score & P-value** plot and **Sample Coverage for Imbalance Analysis** plot for each individual sample. For more information, see “Example RNA Exon Tile Fusion Imbalance plots for a single analysis” on page 365 and “Example sample coverage plot for a single analysis positive for a fusion imbalance in NTRK1” on page 367.



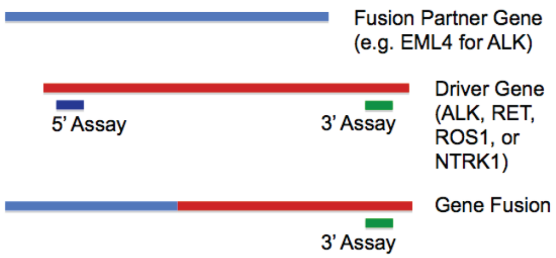
3'/5' Imbalance scores for assay calls

Panels that include 3'/5' amplicons generate 3'5 imbalance values. The imbalance values report the difference in expression between the 5' assay and the 3' assay of each driver gene. Samples that do not contain a fusion are expected to have similar expression of the 5' assay compared to the 3' assay of the driver gene. Samples that contain a fusion are often expected to have elevated expression of the 3' assay compared to the 5' assay.

The 3'/5' Imbalance values provide a measurement of the strength of fusion calls that involve ALK, RET, or ROS1 driver genes, whether included or not included in the Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel.

The 3'/5' Imbalance score is therefore included for two alternate purposes:

- To confirm presence of a fusion from the ALK, RET, or ROS1 driver genes included in the panel, as shown in this figure:



- If none of the fusions targeted by the panel is detected, to provide evidence of a fusion other than those targeted by the panel but still including ALK, RET, or ROS1 driver genes.

The following approaches interpret the 3'/5' Imbalance values for each gene and are reliable only under the conditions that are described in the following sections:

- “Read number impact on calculation sensitivity” on page 379
- “Sensitivity” on page 379
- “High 5' expression” on page 380

Most samples that are tested for the 3'/5' Imbalance assay have been lung tumor tissue. Threshold settings for other sample types can be sensitive to varying expression of fusion gene and expression control genes.

View 3'/5' Imbalance scores

You can detect novel fusions of select driver genes in Ion Reporter™ Software with **3'/5' Imbalance scores** that are shown in analysis results.

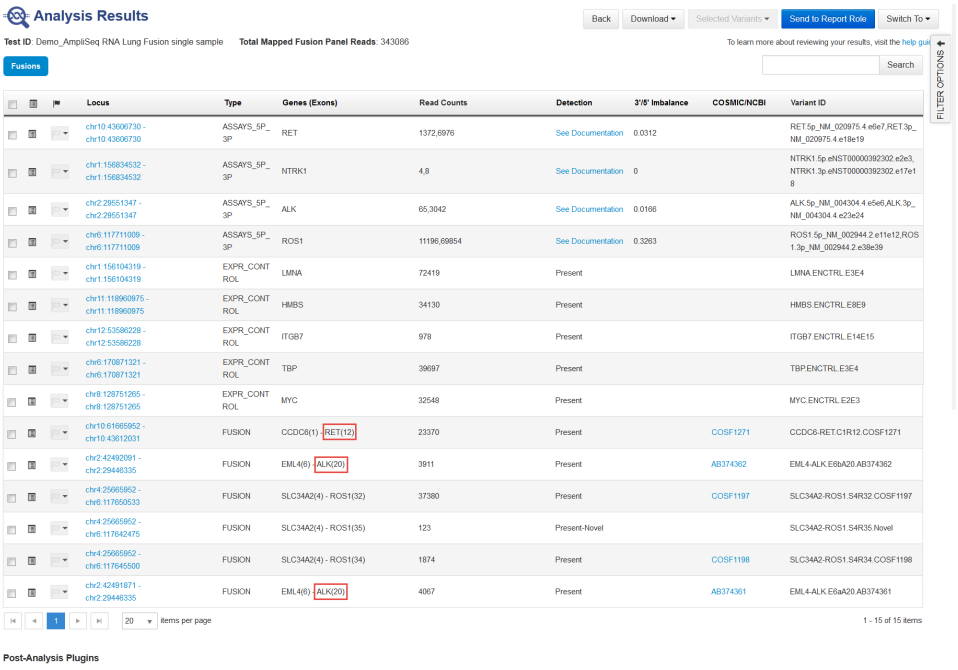
1. In the **Analyses** tab, click **Overview**.
2. Click an analysis name.
   The **Analysis Results** screen opens to the **Fusions** table.

The 3'/5' imbalance values are listed in the **3'/5' Imbalance** column of the **Fusions** table.

Interpret 3'/5' Imbalance scores for assay calls

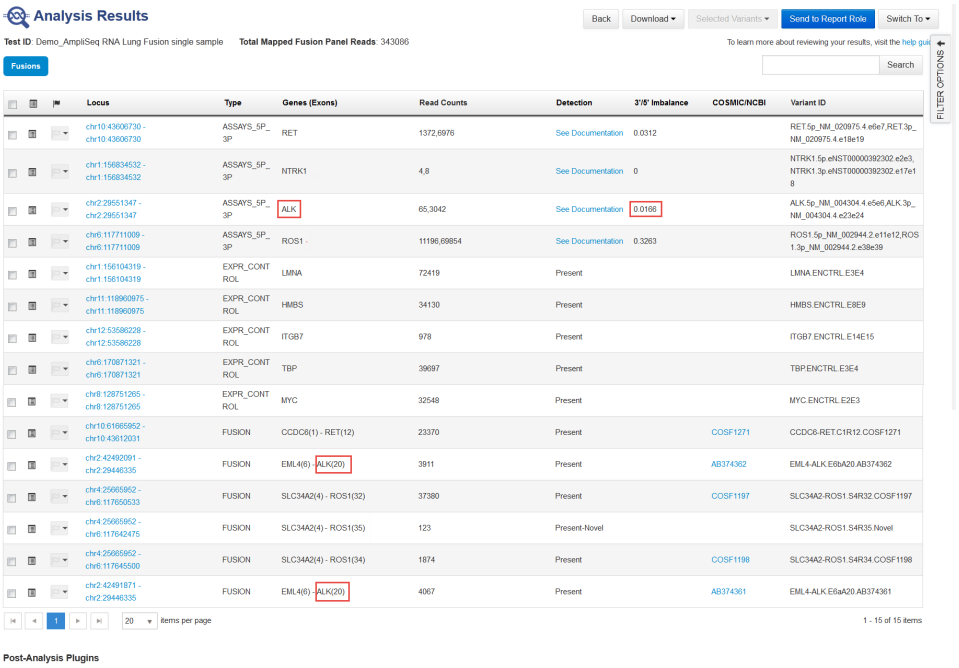
3'/5' Imbalance values are reported in the **3'/5' Imbalance** column. The 3'/5' Imbalance score for an assay applies to all fusion calls with the driver gene that is reported in the **Genes (Exons)** column.

1. Find the driver gene for the fusion, which is the second gene listed in the **Genes (Exons)** column.



2. Find the ASSAYS_5P_3P entry that reports that driver gene in its **Genes (Exons)** field.

3. Use the Imbalance value for that ASSAYS_5P_3P entry to interpret the fusion calls for all fusions whose driver gene matches the ASSAYS_5P_3P's **Genes (Exons)** field.



An Imbalance value is calculated by subtracting the number of 5' reads from the number of 3' reads, and dividing the result by the sum of all EXPR_CONTROL reads:

- (3' reads - 5' reads) / (sum of all EXPR_CONTROL reads)

Note: The column heading **3'/5' Imbalance** in the **Analysis Results** table implies that the imbalance value is a direct ratio of the 3' and 5' reads. The value is not a direct ratio of 3' and 5' reads.

- Imbalance values are interpreted as follows:
  - **Higher Imbalance values** (0.025 or greater) indicate a greater likelihood that the fusion *is* present in your sample.
  - **Lower Imbalance values** (either close to zero or negative) indicate a greater likelihood that the fusion *is not* present in your sample.

Note: Imbalance values provide information that is supplementary to the detection calls of **Present** or **Absent**. If a fusion call is **Absent**, then it is recommended that you check the imbalance value to see if there is supplemental evidence of the presence of a fusion.

- For the Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel and similar panels from **AmpliSeq.com**, the following table lists the imbalance score thresholds.

Gene	No evidence of a fusion	Uncertain	Strong evidence of a fusion
ALK	≤0.001	0.001—0.015	≥0.015
RET	0.03	0.03—0.55	≥0.55
ROS1	≤0.2	0.2—0.5	≥0.5

- For the Oncomine™ Focus Assay, the following table lists the imbalance score thresholds:

Gene	No evidence of a fusion	Uncertain	Strong evidence of a fusion
ALK	≤0.001	0.001—0.0015	≥0.0015
RET	0.3	0.3—0.55	≥0.55
ROS1	≤2.1	2.1—2.1	≥2.1

Imbalance value calculation and predictions

Note: The label 3'/5' Imbalance in the **Analysis Results** table relays that the imbalance value is a direct ratio of the 3' and 5' reads.

A 3'/5' Imbalance value is calculated by subtracting the number of 5' reads from the number of 3' reads, and dividing the result by the sum of all EXPR_CONTROL reads:

(3' reads - 5' reads)/(sum of all EXPRE_CONTROL reads)

We find that imbalance values are a good predictor of the presence of a fusion:

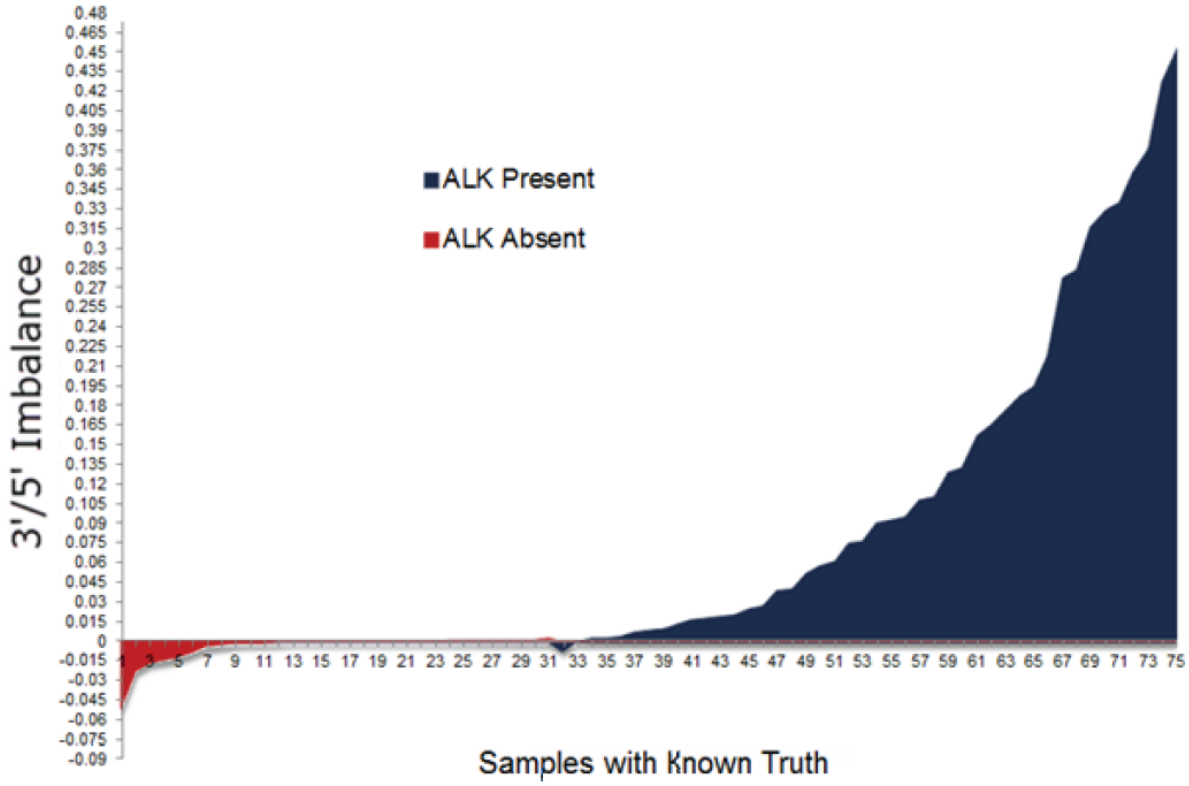
- In normal samples (without a fusion), imbalance values are very low (either close to zero or negative).
- In samples that contain a fusion, imbalance values are higher, with gene-specific thresholds shown in the above table.

In some cases, lower imbalance values are also good predictors of the presence of fusions, as shown in the plots linked below.

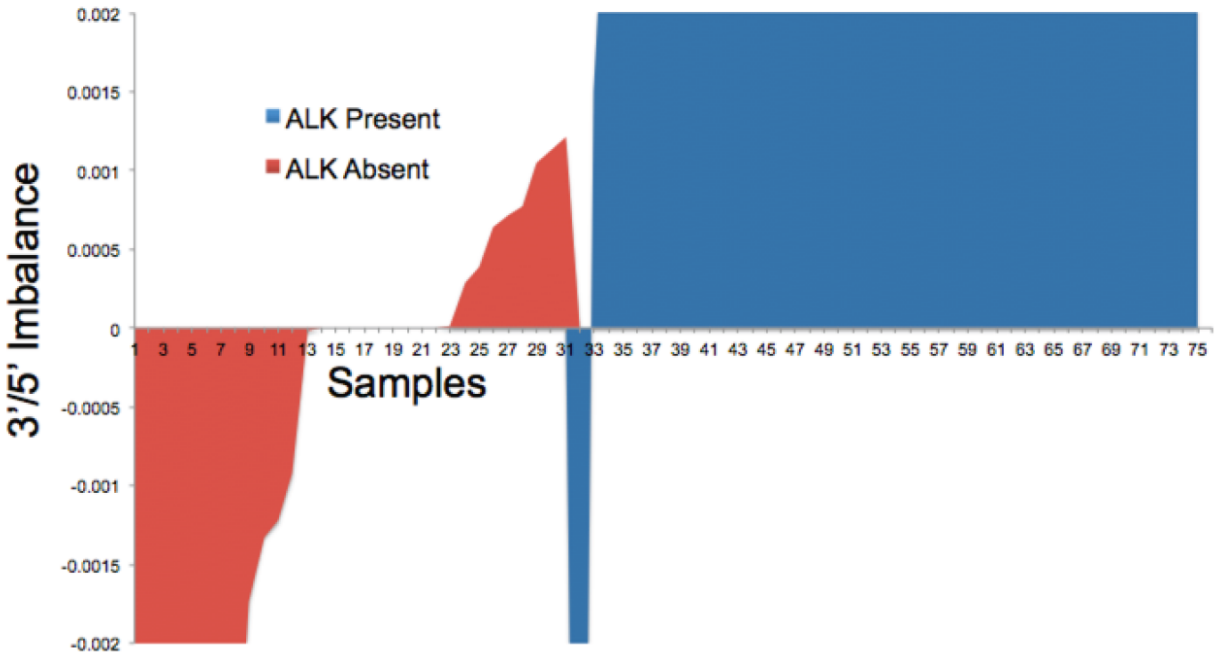
Note: These guidelines apply to samples that meet the criteria described in the sections: Minimum number of reads, Sensitivity, and High 5' expression. The values in the plots below have been based on results obtained from 75 FFPE lung cancer research samples previously tested with other technologies like FISH, IHC, and RT-PCR.

Example plot for ALK driver gene

This example plot for the ALK driver gene shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that contain a fusion (in blue) all contain higher imbalance values. For more information on novel fusion detection in Ion Reporter™ Software, see “Novel fusion detection using expression imbalance” on page 364.



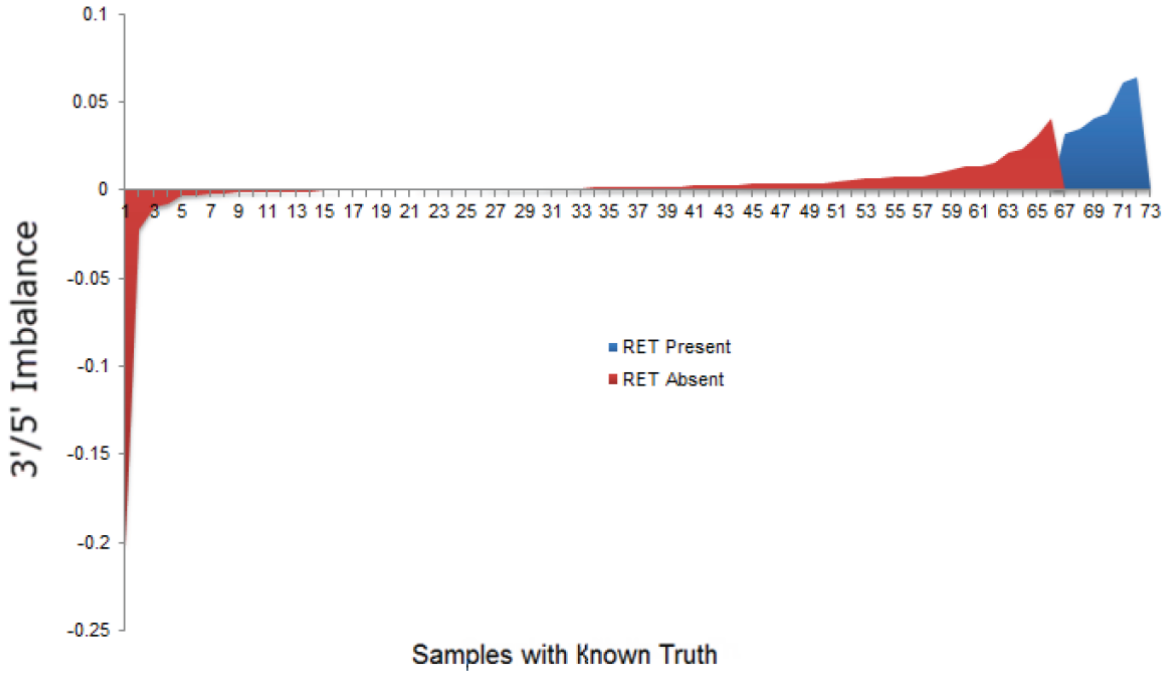
Zoomed in view:



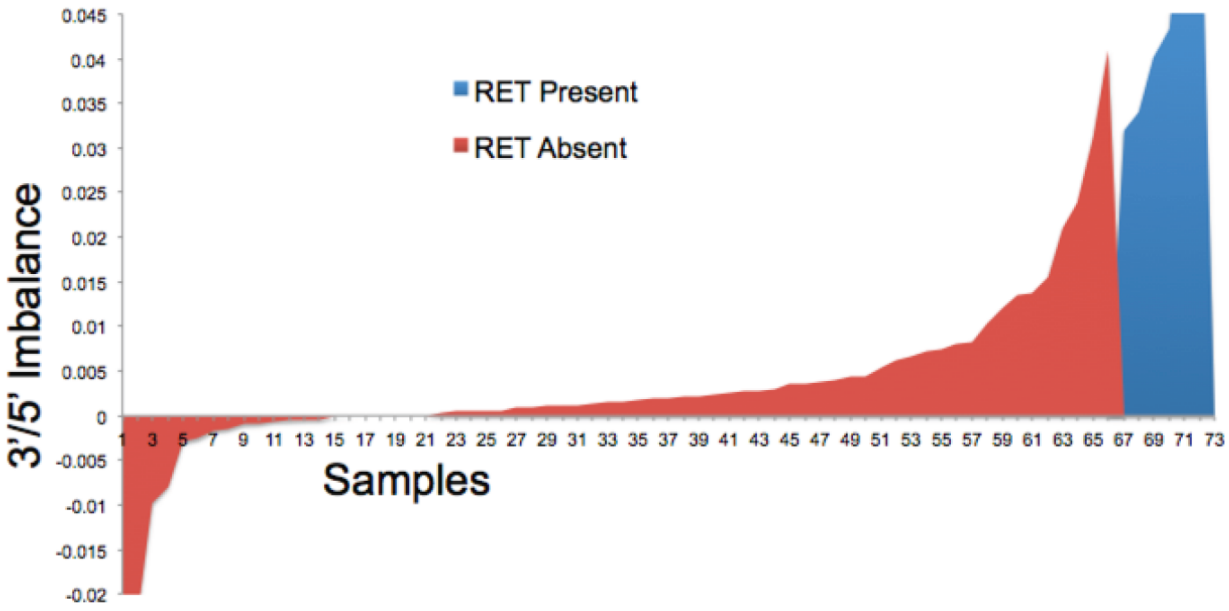
- A 3'/5' Imbalance value of 0.025 or greater indicates the likely presence of an ALK fusion.
- Below 0.001, there is no evidence that an ALK fusion is present.
- The range of 3'/5' Imbalance values between 0.001 and 0.025 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a fusion is present.

Example plot for RET driver gene

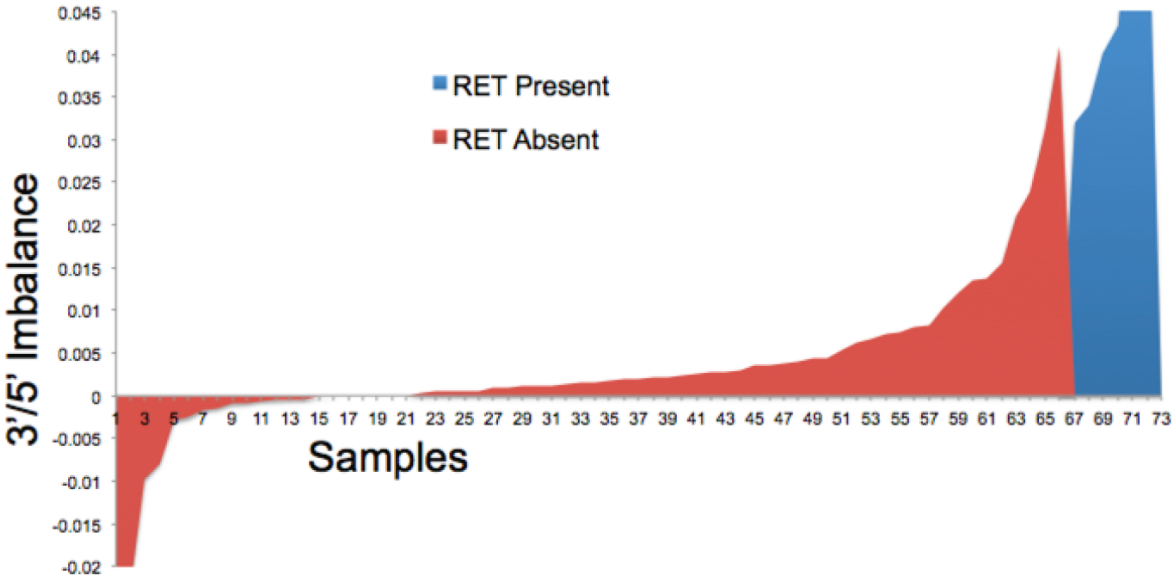
This example plot for the RET driver gene shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that contain a fusion (in blue) all contain higher imbalance values. For more information on novel fusion detection in Ion Reporter™ Software, see “Novel fusion detection using expression imbalance” on page 364.



Zoomed in view:



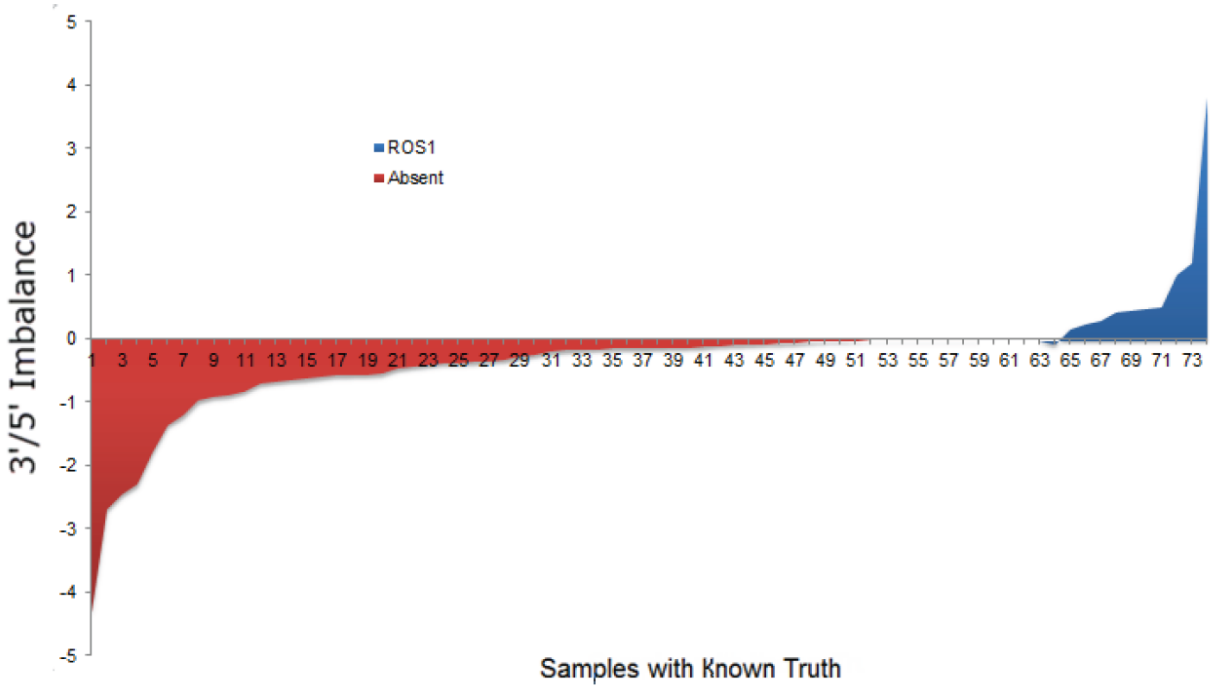
- A 3'/5' Imbalance value of 0.045 or greater indicates the likely presence of a RET fusion.



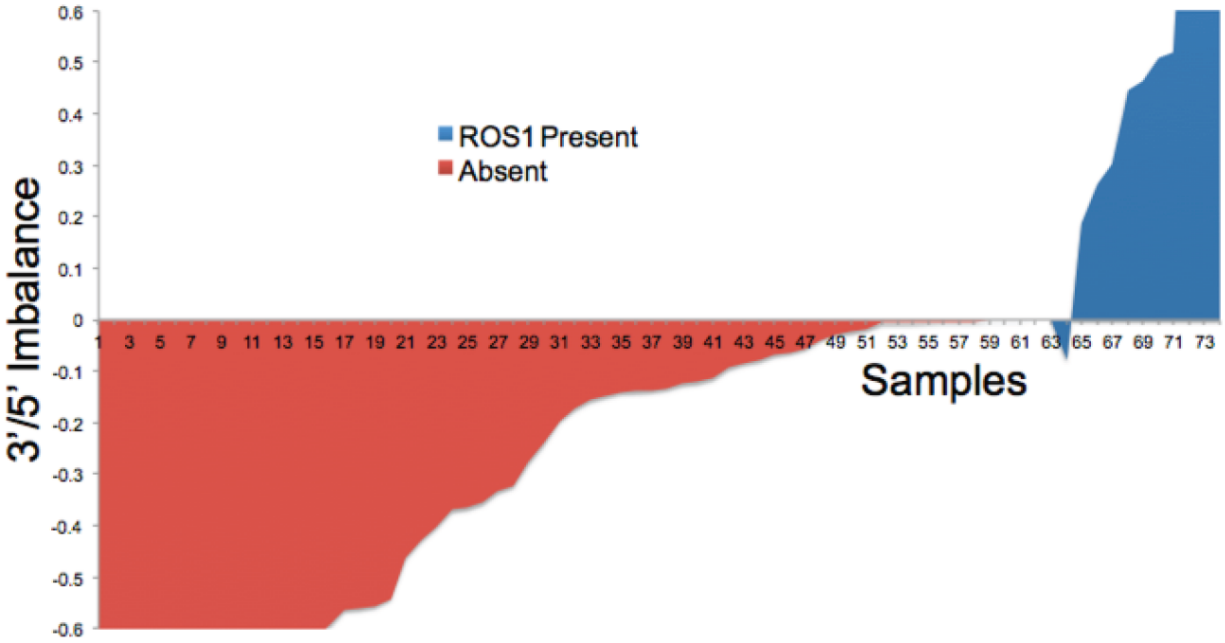
- With a 3'/5' Imbalance value of 0.03, there is no evidence that a RET fusion is present.
- The range of 3'/5' Imbalance values between 0.045 and 0.03 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a RET fusion is present.

Example plot for ROS1 driver gene

This example plot for the ROS1 driver gene shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that do contain a fusion (in blue) all contain higher imbalance values. For more information on novel fusion detection in Ion Reporter™ Software, see “Novel fusion detection using expression imbalance” on page 364.



Zoomed in view:



- A 3'/5' Imbalance value of 0.5 or greater indicates the likely presence of a ROS1 fusion.
- With a 3'/5' Imbalance value of 0.2, there is no evidence that a ROS1 fusion is present.
- The range of 3'/5' Imbalance values between 0.2 and 0.5 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance value, the more evidence there is that a ROS1 fusion is present.

Read number impact on calculation sensitivity

- At above 150,000 mapped reads, the analysis workflow is very sensitive.
- Between 20,000 and 150,000 mapped reads, the more reads, the more sensitive the analysis workflow is.
- With 20,000 or few mapped reads, we recommend that you rerun the sequencing experiment, if possible. At around 20,000 or fewer reads, the potential for false negatives (missing a real fusion) increases.

Sensitivity

The analysis workflow is very sensitive for fusion detection and can possibly pick up noise if there are problems with, for instance, the sample or library preparation.

Check for High 5' expression and also check for the minimum number of reads before assuming that a fusion call of 'Present' is confirmed.

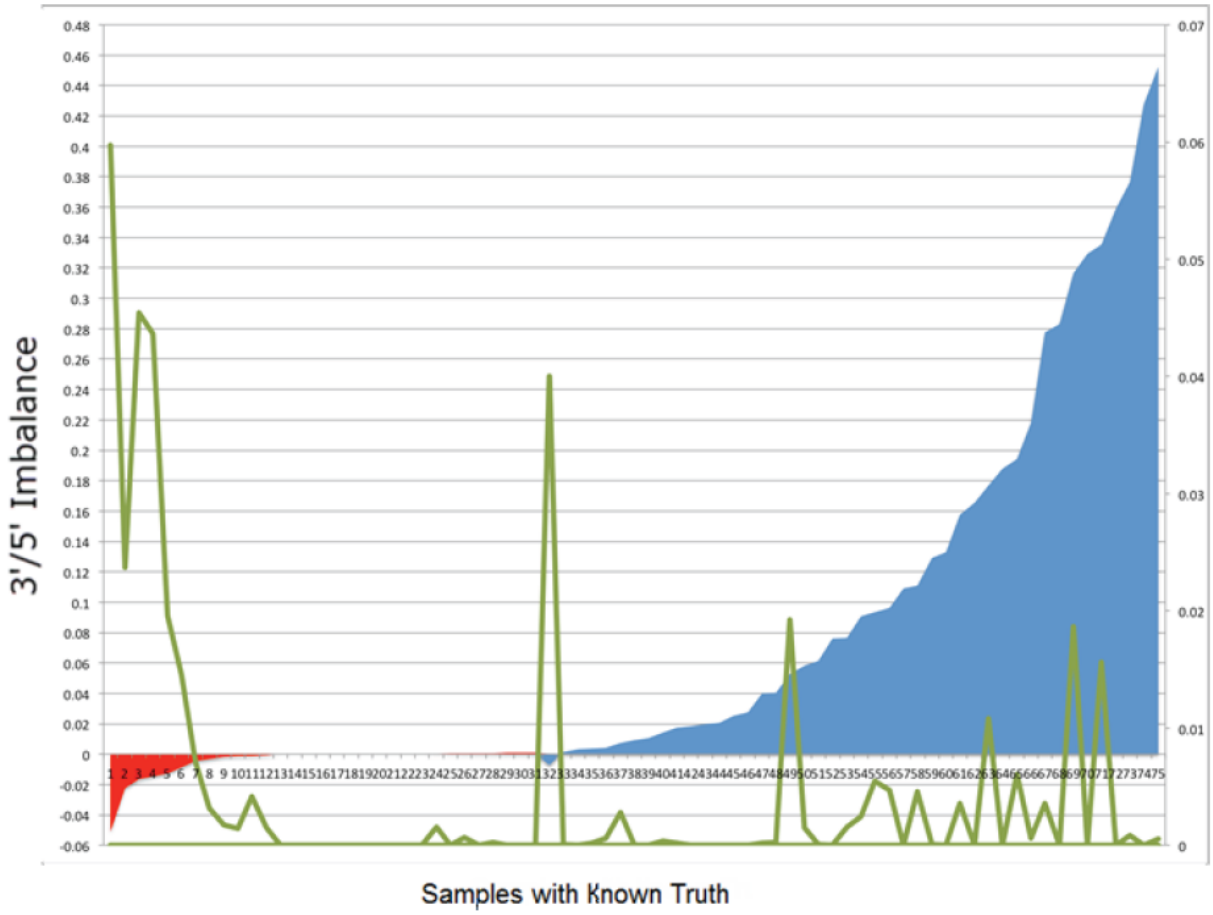
High 5' expression

If the 5' read count is very high compared to the EXPR_CONTROL read count, the 3'/5' Imbalance value is not a reliable predictor. With high 5' read counts, the potential for false negatives (missing a real fusion) increases.

This example plot shows a false negative in sample 32. The green line tracks the 5' expression as 5' read counts divided by EXPR_CONTROL read counts. Note the spike of high 5' expression at the false negative for sample 21.

We have not seen a high 5' expression to be associated with a false positive.

Note: For more information on novel fusion detection in Ion Reporter™ Software, see “Novel fusion detection using expression imbalance” on page 364
.



Normalized Detection Fractions (NDF)

Normalized Detection Fractions provide the color-coding of calls in heatmaps.

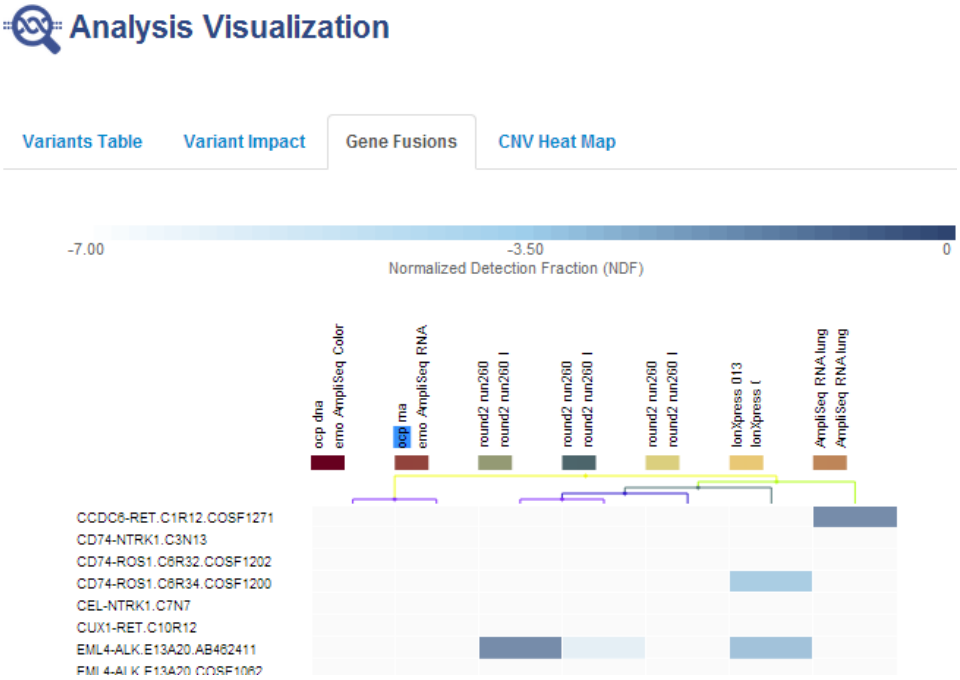


Normalized Detection Fractions are interpreted as follows:

- **Higher values** indicate a greater likelihood that the fusion call is correct and that the fusion *is* present in your sample.
- **Lower values** indicate a greater likelihood that the fusion *is not* present in your sample.

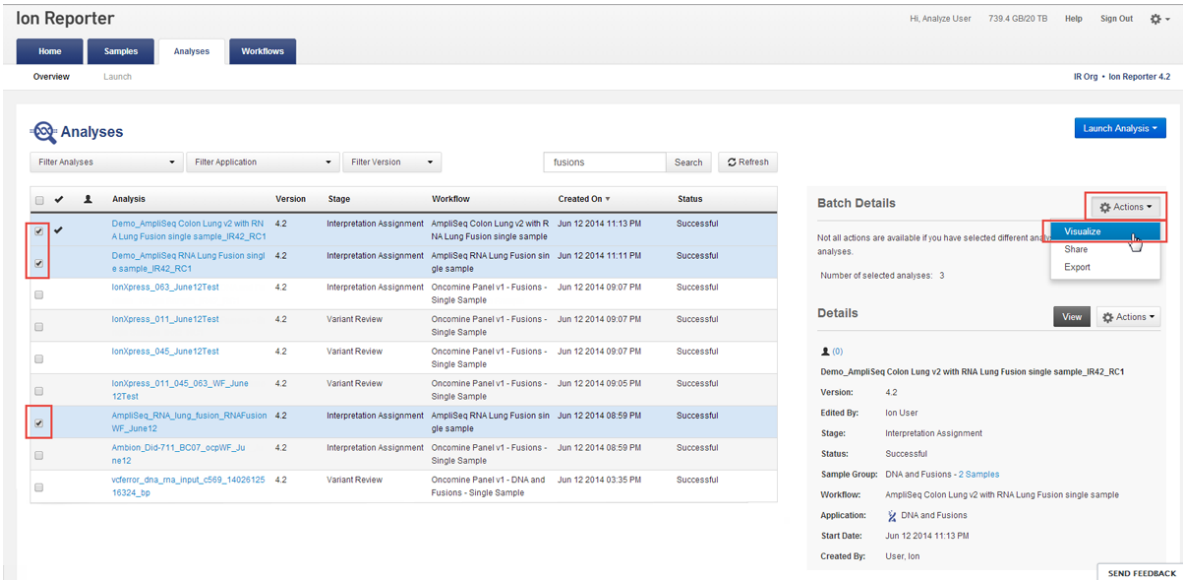
Visualize and compare fusion results of multiple analyses with heatmaps

With heatmap visualizations, you can see the presence or absence of fusions in your samples, and you can compare the fusion calls across multiple analyses.



Follow these steps to visualize analysis results of multiple fusion analyses:

1. In the **Analyses Overview** screen, enable the checkboxes of the analyses that contain the results you want to visualize.



2. In the Action menu on the right, click the **Visualize** option.

Note: The Visualize menu option is only available when you select more than one analysis.

3. In the Analysis Visualization page, click the **Gene Fusions** tab.

Notes about heatmap visualizations:

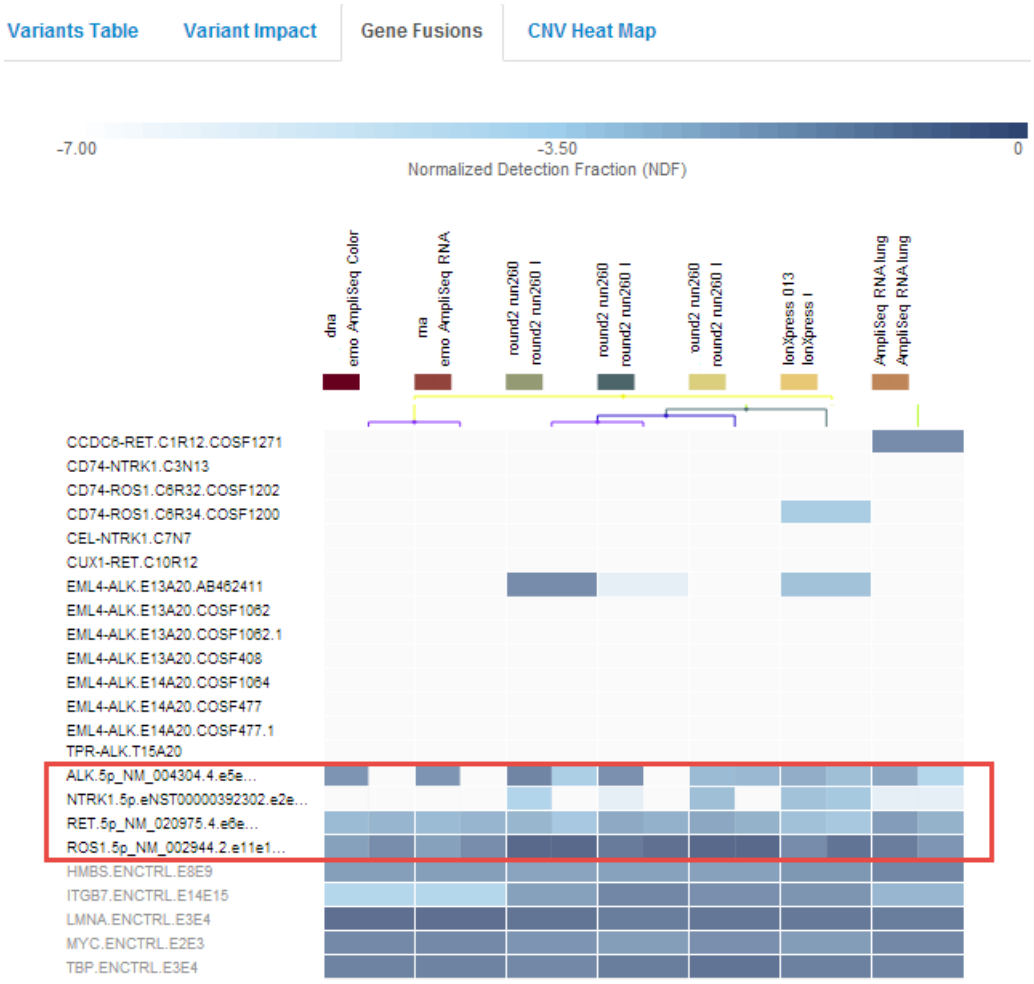
- Interpret each cell according to the color scale above the chart. The strongest fusion calls are indicated with the darker color as shown in the color scale.



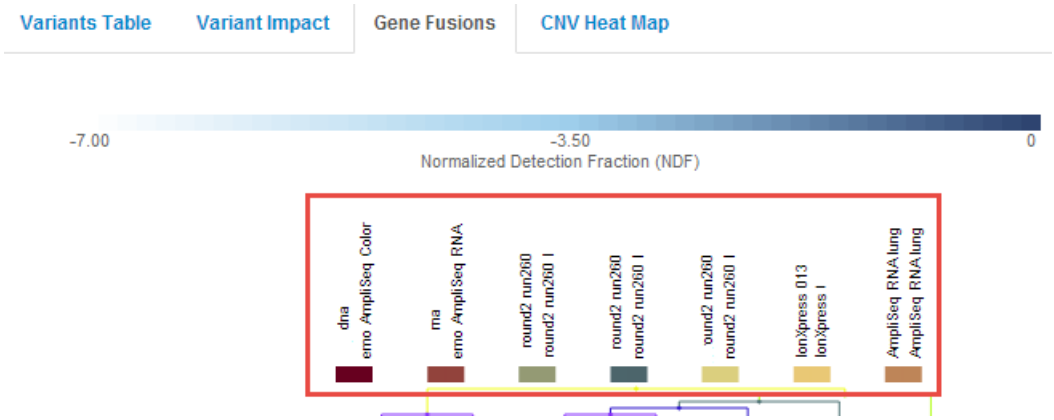
- Normalized Detection Fraction (NDF) values are calculated as follows (for a fusion F in sample S):

```
log10((read count of F) / (total read count in S))
```

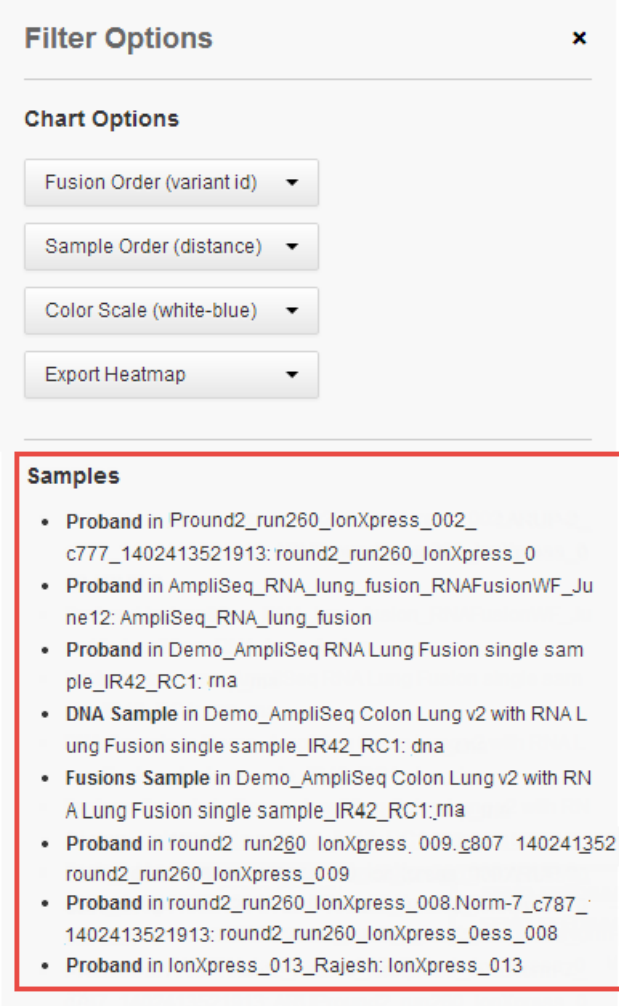
- Each fusion isoforms that is included in the Ion AmpliSeq™ Panel is listed in the **Details** pane.
- For fusions heat maps, NDF is used as the score for every fusion-sample pair to generate the heat map. Thereafter, hierarchical clustering is conducted for clustering fusions and samples within the heat map.
- By default, expression control and ASSAY_5P_3P calls are listed close to the bottom of the chart.



- Click the **Filter Options** menu to change the order of the fusions. You can order the chart by:
  - Fusion ID
  - 5' name
  - 3' name
- Each sample is listed at the top of the **Gene Fusions** tab.



- Click the **Sample Order** menu to change the order of the fusions. You can order the chart by:
  - **Name**—Samples are listed alphabetically by name.
  - **Similarity**—Sorts by similarity of the samples that are in the fusion calls made, in order from the fewest fusions to the most numerous fusions.
  - **Similarity (reversed)**—Sorts by similarity of the samples that are in the fusion calls made, in order the most numerous fusions to the fewest fusions.
  - **Analysis**—The list of analysis is sorted alphabetically by analysis name.
- Samples are also listed below the **Chart Options**.



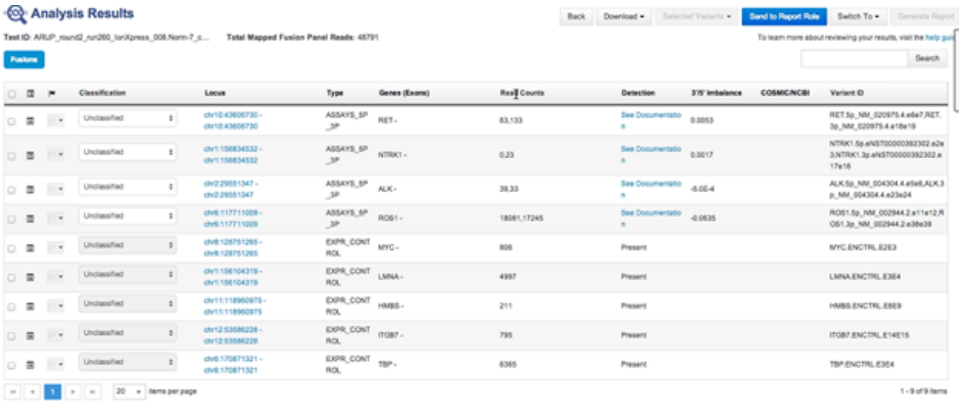
- Click **Selected Analyses** to see the list of analyses in the heatmap.
- You can change the order of the color scheme used in the chart with the **Color Scale** menu. White or blank cells indicate no reads (in the default color scheme). Many cells in the example heatmap show no reads. With red-green and red-blue color schemes, red indicates a no-reads cell.
- Click **Export Heatmap** to export a heatmap as an image or as a comma-separated text file.

Supported export formats are:

- PNG—Portable Network Graphics format
- SVG—Scalable Vector graphics format
- CSV—A comma-separated text file, readable by spreadsheet programs or as a text file

Example of an analysis with no fusions called

This image shows an example of the **Analysis Results** table for a run in which no fusions are called.



Note: When you sort on the **Gene** column, the software sorts on both of the genes involved in the fusion. As a result, the lexicographically smaller gene is used for an ascending sort, while the lexicographically larger gene is used for a descending sort.

Visualize RNA exon variants

You can view intragenic exon rearrangements, such as exon deletions, exon skipping events, and alternate splice transcripts graphically in Ion Reporter™ Software. You can view **RNA Exon Variant** graphs from a single analysis or from multiple analyses simultaneously. If multiple analyses are compared, the data for each sample is summarized in a separate bar graph. For more information, see “Compare results of single or multiple analyses” on page 198. **RNA Exon Variant** graphs are available for all analyses that use RNA or Fusion analysis workflows.

RNA Exon Variant graphs show variant read counts, normalized to the wild type, for specific exon variants.

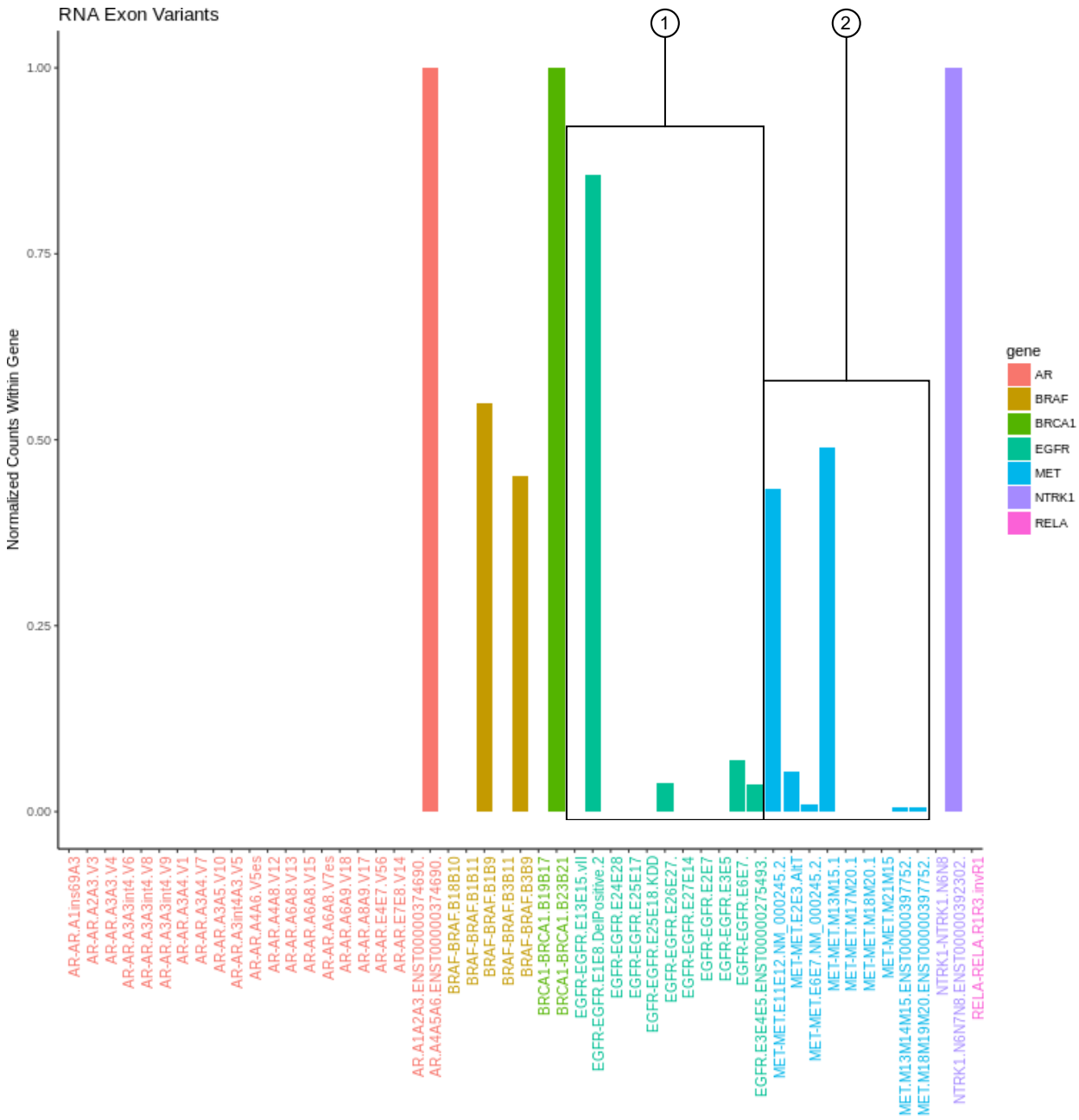
View RNA exon variants

You can visualize intragenic exon rearrangements with RNA exon variants graphs.

1. In the **Analyses** tab, click **Overview**.
2. Select the checkbox in the row of the analysis that you want to visualize, then click **Visualize**.
3. Click the **RNA Exon Variant** tab.
   The RNA Exon Variant graph is displayed.

Example RNA Exon Variant graph

You can view bar graphs that illustrate intragenic exon rearrangements in Ion Reporter™ Software. **RNA Exon Variant** graphs show exon variant read counts normalized to the wild-type. Specific exons and exon variants are shown along the X-axis. In wild-type assays shown along the x-axis, entries are labeled with a gene ID followed by a sequence of adjacent exons. For example, wild-type exons six and seven of the epidermal growth factor receptor gene is represented as EFGR.E6E7. The Y-axis represents the read counts for each variant, normalized to the wild type.



① Example analysis where an RNA exon 2–7 deletion occurred in the EFGR gene. Normalized read counts for the EFGR variant that contains the intragenic fusion (EGFR-EGFR.E1E8.DelPositive.2) are higher relative to the normalized read counts for a wild type EFGR assay (EFGR.E6E7).

② Example analysis where exon-skipping (i.e., exon deletion) of exon 14 in the MET gene was detected. Normalized read counts for the variant representative of the exon-skip event (MET-MET.M13M15.1) are higher relative to the MET wild-type assays (for example, MET.M13M14M15.ENST00000397752.WT and MET.M17M18M19M20.ENST00000397752.WT).

Visualize variants in an analysis run with an Ion AmpliSeq™ HD analysis workflow

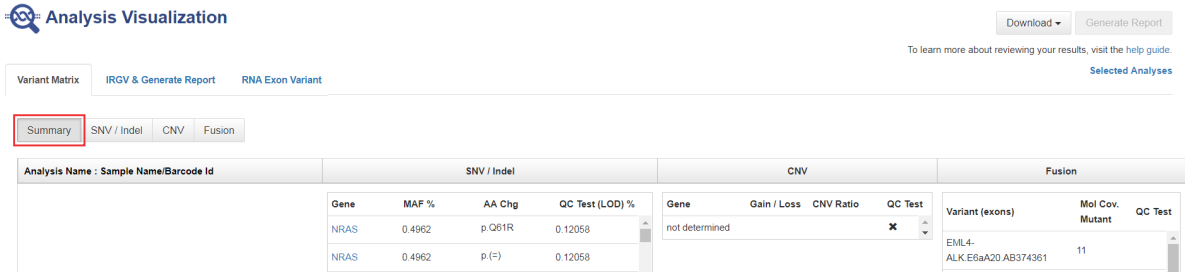
You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants.

1. In the **Analyses** tab, click **Overview**.
   The **Analyses** table lists all the available analyses results.

2. Click the column headings to sort the results. Alternatively, use the available filters or the **Search** field to limit the list of analyses.

3. Select the checkbox in the row of the analysis that you want to visualize or select 2 or more analyses to visualize a side-by-side comparison of multiple results.

4. Click **Visualize**.
   The **Analysis Visualization** screen opens to the **Variant Matrix** tab, displaying the **Summary** screen that shows all the identified SNVs/INDELs, CNVs, and Fusions.



Note: A **none detected** result indicates that down to the displayed limit of detection (LOD), no variants were observed in the sample within or above the LOD range.

5. Review detailed variant data.
   - In the **Variant Matrix** tab, in the **Summary** screen, click the gene name in the **Gene** column to access the HGNC report for that gene.
   - *(Fusion analyses only)* In the **RNA Exon Variant** tab, visualize normalized counts within gene for each exon variant.
   - Click **SNV/INDEL**, **CNV**, or **Fusion** to view detailed analysis metrics. For a description of each metric, see Chapter 7, “Detailed analysis metrics”.

- In the **SNV/INDEL**, **CNV**, or **Fusion** screen, click the link in the **Locus** column to view specific variants in the Ion Reporter™ Genomic Viewer (IRGV) in a separate window.



Figure 16 Example SNV/Indel visualization in IRGV

① Variant density overview, shown as copy number (Y-axis) at a specific position on the chromosome (X-axis)
② Displayed chromosomal region; use the search field to view a different region
③ Proband variant position on the displayed chromosomal region
④ Proband read coverage tracks

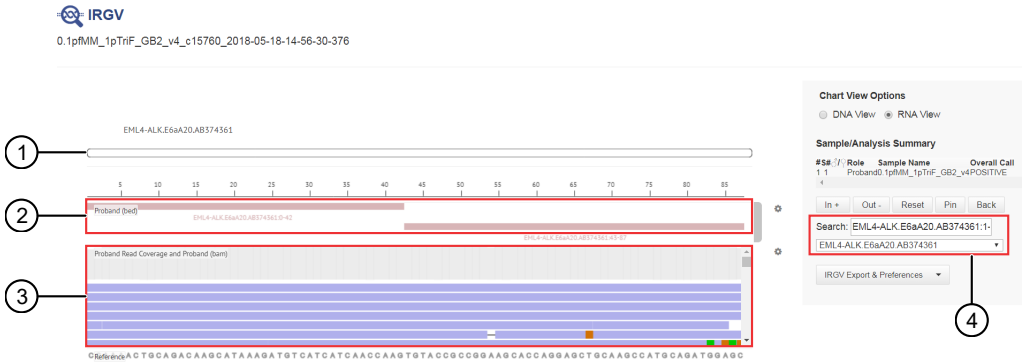


Figure 17 Example fusion visualization in IRGV

① Fusion target track
② 5′ (top) and 3′ (bottom) gene track
③ Read coverage track
④ Use the dropdown list or search for another fusion variant

6. Review proband read coverage tracks.

   Ion AmpliSeq™ HD analyses group consensus reads into families. A family is a group of reads that are associated with the same DNA molecule before library amplification. Each family is identified using the molecular tags, and consensus reads with the same molecular tags are grouped into the same family. In IRGV data view, the color of the consensus reads is used to indicate a family. Side-by-side consensus reads with the same color belong to the same family.

Within each read track, each nucleotide variant is indicated by a different color. T, A, C, and G are red, green, blue, and orange, respectively. An "I" denotes insertion, and white color with a dash indicates deletion.

You can sort, adjust, and view details about variants and base calls that are visualized in each read coverage track.

Option	Description
Sort read coverage tracks by variant	In the coverage track, place the pointer at the position of the variant, then right-click and select **Sort by Base**.
Adjust (IRGV) BAM tracks	Click ⚙ **(Actions)** next to the read coverage track, then select an option to adjust the view of the track. For more information, see “Adjust IRGV BAM tracks” on page 331.
Review detailed data about a BAM read	Click the read track to get information such as the mapping quality, the strand and the read base.
Review distribution of base calls at a selected position	Click the density plot (the gray bar at the top of the read coverage tracks) to view information about the: • total count • total reads • total number of molecules • distribution of single nucleotides at that position • number of insertions and deletions.

Note: The number of molecules in the (IRGV) coverage track can be slightly different from what is reported in the VCF output. The values seen in IRGV are based on initial estimates made by the variant caller, whereas read or molecular counts in VCF output are based on calculations that can include more processing by the variant detection pipeline.

7. Generate **Visualization Report**.
   a. In the **IRGV & Generate Report** tab, click **Generate Report**.
   b. In the **Select Visualization Report Template**, select an existing template from the dropdown list or select **Create New Report Template**, then click **OK**.
   c. If needed, change the report configuration, then proceed to the **Preview** step.
   d. Click **Actions** ▸ **Download PDF** to download the PDF report.

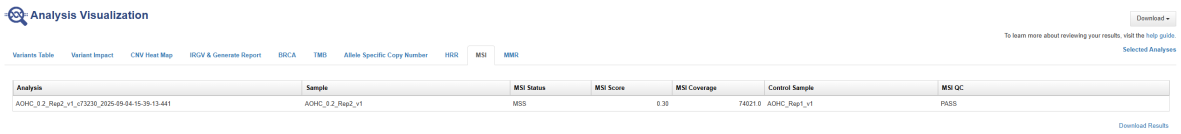
Visualize MSI analysis results

1. There are 2 ways to visualize MSI analysis results in Ion Reporter™ Software:

Option	Description
Visualize MSI results from one or more analyses simultaneously from the **Analyses** table.	In the **Analyses** table, select a row for an analysis or select the checkbox next to one or more analyses that you want to visualize simultaneously, then click **Visualize**. Alternatively, click **Actions** ▸ **Visualize**.
Visualize analysis results individually from the **Analysis Results** screen.	In the **Analyses** table, click an analysis hyperlink in the **Analysis** column to open the **Analysis Results**, then click **Visualize**.

   The **Analysis Visualization** screen opens to the **TMB** tab.

2. Click the **MSI** tab.
   A table with **MSI Status**, **MSI Score**, **MSI Coverage**, and **MSI QC** is shown.



Item	Description
MSI Status	A sample is assigned an MSI status that is based on the MSI Score. The MSI status can be one of the following: • MSI-High—MSI score is greater than the threshold value set in the MSI-High Threshold parameter. • MSS—MSI score is less than the threshold value set in the MSS Threshold parameter. • No Call—MSI score is equal to or greater than the value entered in the **MSS Threshold** parameter AND less than or equal to the value entered in the **MSI-ThresholdHigh** parameter. • QCFail—Indicates determination of MSI status was not reliable due to the MSI baseline.
MSI Score	A sample-level MSI score that is calculated with individual MSI marker scores. The overall score is used to determine the MSI status of the sample.
MSI QC	Alert messages for quality control of the MSI analysis results. Messages include the following. • Number of Forward markers with expected coverage is low. • Number of Reverse markers with expected coverage is low. • Tumor Fraction: Cannot calculate MSI with TF. No TF information was provided. • No warning
MSI Coverage	A combined sample-level coverage that is calculated with the individual MSI marker-level coverage.
Control Sample	Specifies the name of the control sample used as MSI on-chip control for the analysis. **Control Sample** is available only for analysis results from sequencing runs that use Oncomine™ Comprehensive Assay Plus analysis workflows on the Ion 560™ Chip. For results from other sequencing runs on the Ion 550™ Chip, the value "NA" is displayed.

3. Click **Download Results** to download a report of the MSI results. The report is downloaded to the folder that is used for downloads, depending on the browser settings.

Visualize identified variants in an Oncomine™ analysis from a TagSeq analysis workflow

You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants in Ion Reporter™ Software.

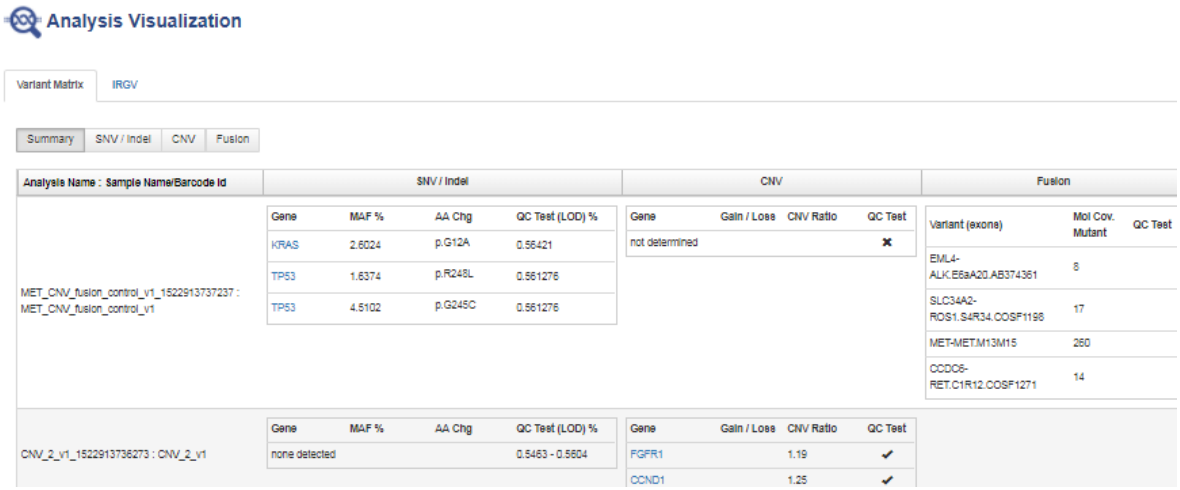
1. Under the **Analyses** tab, click **Overview**.
2. Click the column headings to sort the results, or use the available filters to limit the list of analyses.

3. Select the checkbox adjacent to each analysis of interest; select two or more analyses to visualize a side-by-side comparison of multiple results. Click **Visualize**.

   Note: Alternatively, select the analyses, then click **Actions** ▸ **Visualize** next to **Selected Analyses**.

4. The **Analysis Visualization** screen opens to the **Variant Matrix** tab with a summary of all of the identified SNVs, CNVs, and Fusions (Lung only).

   Click a hyperlinked **Gene** name to be redirected to the HGNC report for that gene.

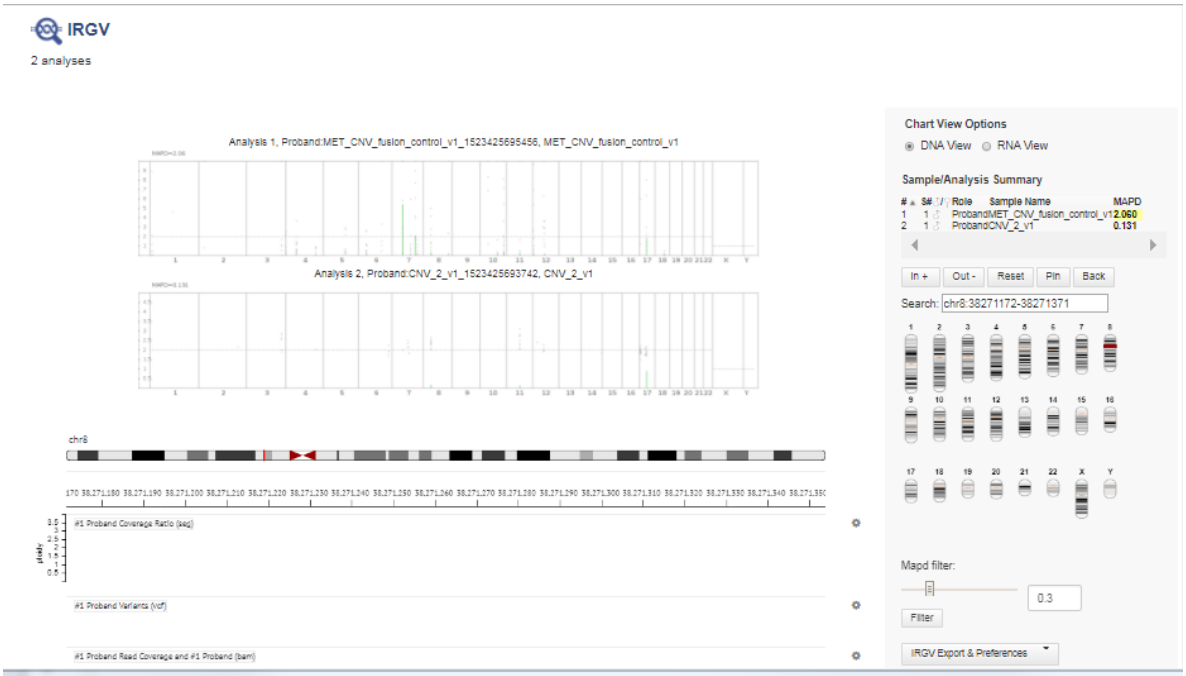


   Example visualization of an Oncomine™ assay used with a TagSeq analysis workflow.

   A none detected result indicates that down to the displayed limit of detection (LOD), no variants were observed in the sample within or above the LOD range.

5. Click **SNV/INDEL**, **CNV**, or **Fusion** (Lung only) to view detailed analysis metrics.

6. In the **SNV/INDEL**, **CNV**, or **Fusion** detailed view, click the link in the **Locus** column to view specific variants in the Ion Reporter™ Genomic Viewer (IRGV).



Example analysis results for CNV variants in the Ion Reporter™ Genomic Viewer (IRGV).

- The IRGV viewer displays CNVs as ploidy assuming 100% tumor cellularity, whereas we report CNVs as fold difference.

Visualize variants with IGV

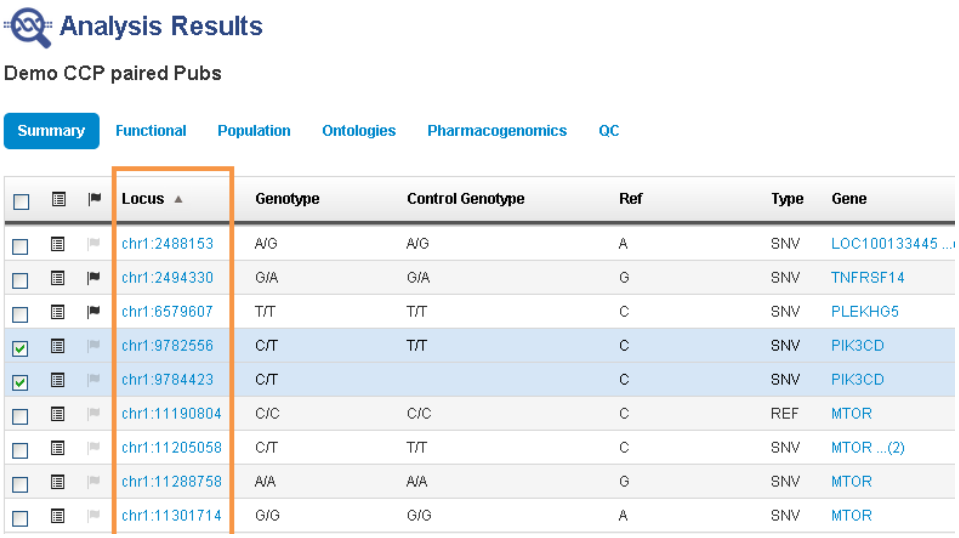
You must have IGV set as the default viewer in Ion Reporter™ Software before you start this procedure. For more information, see “Set IRGV or IGV as the default viewer” on page 311.

You can visualize data from Ion Reporter™ Software with the Broad Institute Integrative Genomics Viewer (IGV). The viewer is available at the Broad Institute website: **http://software.broadinstitute.org/software/igv/home**.

If you visualize different analyses in IGV that use the same samples or the same panel files (but different algorithm versions, for example), the reads, read counts, BED file, and other tracks load into the browser only one time. In this case, if the software detects exact duplicate tracks, all unique tracks load separately if the software detects any differences in results data or input tracks between multiple analyses for visualizations of the requested analyses.

Note: In an IGV visualization, hotspots files that are in shown in a track in can include IGV annotations for hotspots that were not used to call bases in the data.

1. In the **Analysis** tab, click the name for the analysis of interest to open analysis results.
2. In the **Analysis Results** screen, click the locus for a variant of interest, and then complete the following steps.



A ZIP file that contains files for upload to the Integrative Genomics Viewer (IGV).

   a. Open IGV on your computer.

   b. Unzip the files, then open the ***session.xml*** file in IGV.

The files that are exported contains only the following information for the selected variant.

- For DNA variants
  - Sliced data are available for download that is based on the variant locus and flanking 200 base pairs.
  - Required files in the ZIP file includes all files, except for the reference genome FASTA files, which are not included.
- For DNA CNV variants
  - Sliced data are available for download that is based on the CNV start position of -200 and CNV end position of + 200 base pairs.
  - Required files in the ZIP file includes all files, except for the reference genome FASTA files, which are not included.
- For RNA variants
  - Sliced data are available for download that is based on the fusion isoform.

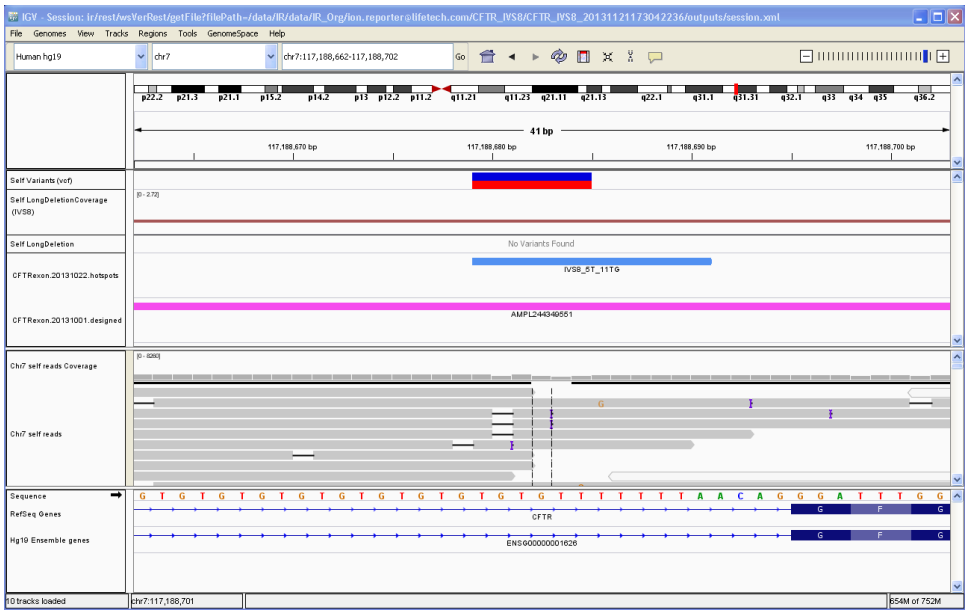


Figure 18 Example of an IGV visualization of a variant

Unidentified developer error in IGV on Macintosh computers

Observation	Possible cause	Recommended action
The following error message is seen on Macintosh™ computers when you try to open Integrative Genomics Viewer (IGV) on a Macintosh™ computer: `"igv.jnlp" cannot be opened because it is from an unidentified developer.`	This error occurs based on security settings for application downloads.	Change your Macintosh™ security preferences to allow applications from unidentified developers. Change your Macintosh™ browser to allow applications from unidentified developers.

Unable to parse header in IGV

Observation	Possible cause	Recommended action
The following error is seen when you attempt to view one of the variants in Integrative Genomics Viewer (IGV): `Unable to parse header: Your input file has a malformed header: unknown column name 'CHR'; it does not match a legal column header name.`	IGV does not load variants and displays this error message when a VCF file uses ***CHR*** instead of ***CHROM*** for a column header name.	To avoid this error, use the ***CHROM*** column header name in input VCF files. For example, ***#CHROM POS ID REF***.

Visualization interpretation guidance

Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay

Metric	Description
Copy Number Variation	
CNV Ratio	Should be interpreted as the fold amplification (gain) as detected by the assay. CNV specific amplicon (MET) coverage levels are compared to non-CNV amplicon coverage.
P-value	Significance of CNV Ratio measurement based on amplicon coverage variability (MAPD level) and magnitude of the pairwise coverage differences between the CNV and non-CNV amplicons. High coverage variability will result in less significant p-values.
Fusion detection	
Nomenclature	Each reported fusion target follows a specific naming convention such that the 5'- and 3'-genes are reported along with donor and acceptor exon numbers. Lastly, a COSMIC ID or NCBI transcript accession number is added to the end of each target name. For example, EML4-ALK.E13A20.COSF463 identifies the EML4-ALK fusion variant with exon 13 of EML4 fused to exon 20 of ALK.
Fusion QC genes	Two non-fused process control genes (HMBS and TBP) that have been shown to be consistently detected in cell-free nucleic acid extracts are included in the assay to inform quality of fusion variant calls.
Analysis detail	• Fusion targets are reported as **FUSION** in the **Type** column. • Fusion QC genes are reported as **ProcControl** in the **Type** column.
MET Exon 14 Skipping Assay	
Nomenclature	• One assay is specific to the exon 14 skipping detection in the MET gene called MET-MET.M13M15. • Two additional wild type assays are provided to inform the quality of a MET exon 14 skipping variant call. These are named MET.E6E7.WT and MET.E11E12.WT.

(continued)

Metric	Description
Analysis detail	• MET exon 14 targets are reported as **RNAExonVariant** in the **Type** column.

Oncomine™ Breast cfDNA Research Assay v2

Metric	Description
Copy Number Variation	
CNV Ratio	Should be interpreted as the fold amplification (gain) as detected by the assay. CNV specific amplicon (CCND1, ERBB2, FGFR1) coverage levels are compared to non-CNV amplicon coverage.
P-value	Significance of CNV Ratio measurement based on amplicon coverage variability (MAPD level) and magnitude of the pairwise coverage differences between the CNV and non-CNV amplicons. High coverage variability will result in less significant p-values.
***De novo* (non-hotspot) variant calling in TP53**	
Analysis detail	• Panel includes approximately 80% coverage of the TP53 gene. • These variants are reported as **PN** (potentially novel) in the **Info** column. If the variant is reported as **HS** in the **Info** column, this variant is a hotspot specifically targeted by the breast panel. • These variant calls must be at a frequency of ≥0.5% to be reported in the analysis visualization. To view *de novo* TP53 variants at lower frequencies, download a VCF file from the visualization pages.

9 Create and view reports

- Final reports 400
- Visualization reports 412
- Amplicon coverage reports 415
- Quality Control (QC) reports 417
- Available report sections 421
- Audit logs 427

Ion Reporter™ Software provides several types of reports for various uses in sequencing, quality control, and audits of user activities. To save or distribute a report, you can download the report and the files that are related to the report.

- Final reports - You can create final reports directly from a single analysis. You can customize the layout and contents of a final report while you work, or you can apply a final report template. System-installed templates are available, or you can create and apply a custom template. If you want to ensure that the same report is always available when the analysis is viewed or launched, you can lock and publish a final report for that analysis.
- Visualization reports - When you open a visualization you can generate a PDF report for one or more analyses that contain graphics and data from each analysis results set. When you generate a visualization report, the analyses are not locked. You can generate a new and different visualization report every time you open one or more unlocked analyses in the visualization area.
- Amplicon coverage reports - Amplicon coverage reports list amplicons, SNPs, and bases in the amplicons. When you view the amplicon coverage report, you can adjust the amplicon coverage threshold to focus the report on only the amplicons that met the threshold that you set. You can view, filter, and download these reports from an open analysis or analysis results.
- Quality Control reports - QC reports provide a high-level summary of key metrics that you can use to evaluate the quality of the data for analysis and samples, or amplicon coverage reports. Each report is specific to an individual analysis and the samples used for the sequencing run. You can view and download these reports from an open analysis or analysis results.

You must have the Report role to view and create reports. Otherwise, you can send completed analyses to someone who has the Report role. For more information, see “Send an analysis to the Report role” on page 184.

Final reports

You can create final reports directly from analysis results in Ion Reporter™ Software. You can customize the reports to include information about variants of your choice, and report sections that you can reorder, add and remove dynamically. Each section contains details about an analysis, so you can include information to meet requirement needs.

For example, reports can record information about the analysis workflow used, the annotations and filter chains that were applied to the analysis, the report generation date, and the name of the person who launched the analysis.

The reports can include sections for the samples used in the analysis, the genomic variants, and annotations for each variant. For example, the reports reflect the notes and classifications, quality control metrics, and background information for the analysis. Other sections that you can add are disclaimers, comments, electronic signatures, and images.

You can use system templates or create custom templates for these reports, and add a report template to an Ion Reporter™ Software analysis workflow to reduce report setup time.

You can create a final report in different ways:

- Design and create a final report directly from a completed analysis. Customize the content that you include in the report and its organization, and make changes dynamically in a report preview. For more information, see “Create a final report” on page 404.
- Predesign a final report template that you can attach to an analysis workflow. For more information, see “Create a final report template from analysis results” on page 407.
- Predesign a final report template as an Ion Reporter™ Software workflow preset. The resulting report is not as customizable as a final report template that is created from an open analysis. For more information, see “Create a final report template workflow preset” on page 135.

View a published final report

You can view a published final report of a completed analysis. A published final report includes details such as location, type, coverage, and annotations. You can download published final reports as PDF files.

A published final report of analysis results cannot be edited. That is, you cannot add annotations, notes, My Variants, or classifications to the analysis. However, you can generate another report if you reanalyze the results.

1. In the **Analyses** tab, click **Overview**.
2. In the **Stage** column, look for an analysis that is marked as **Report Published**.
3. Select the checkbox for the analysis of interest.
4. Click ⚙ **(Actions)View Final Report**.

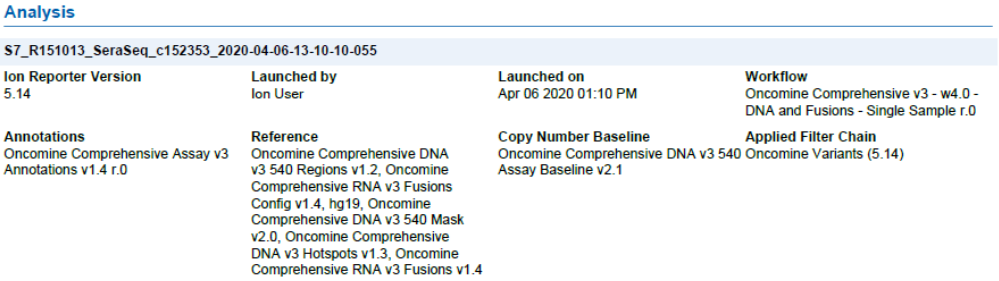
5. In the report preview, review the report.

   You can:

   - Click **Download** to download a PDF copy of the report.
   - Click **Switch To** to view a QC report, an audit log, or view analysis results. For more information, see “View a Quality Control (QC) report” on page 418, “View and download audit logs” on page 427 and “Display different views of analysis results” on page 193.

These images show sections of a final report.

Analysis



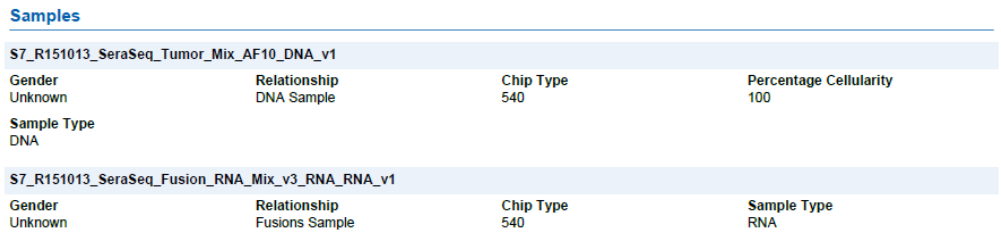
Analysis

S7_R151013_SeraSeq_c152353_2020-04-06-13-10-10-055

Ion Reporter Version	Launched by	Launched on	Workflow
5.14	Ion User	Apr 06 2020 01:10 PM	Oncomine Comprehensive v3 - w4.0 - DNA and Fusions - Single Sample r.0

Annotations	Reference	Copy Number Baseline	Applied Filter Chain
Oncomine Comprehensive Assay v3 Annotations v1.4 r.0	Oncomine Comprehensive DNA v3 540 Regions v1.2, Oncomine Comprehensive RNA v3 Fusions Config v1.4, hg19, Oncomine Comprehensive DNA v3 540 Mask v2.0, Oncomine Comprehensive DNA v3 Hotspots v1.3, Oncomine Comprehensive RNA v3 Fusions v1.4	Oncomine Comprehensive DNA v3 540 Assay Baseline v2.1	Oncomine Variants (5.14)

Samples



Samples

S7_R151013_SeraSeq_Tumor_Mix_AF10_DNA_v1

Gender	Relationship	Chip Type	Percentage Cellularity
Unknown	DNA Sample	540	100

Sample Type
DNA

S7_R151013_SeraSeq_Fusion_RNA_Mix_v3_RNA_RNA_v1

Gender	Relationship	Chip Type	Sample Type
Unknown	Fusions Sample	540	RNA

QC Metrics for a DNA Sample

QC Metrics

Metric	S7_R151013_SeraSeq_Tumor_Mix_AF10_DNA_v1 (DNA Sample)
% BED region > threshold	99.978
% amplicons > threshold	99.9736
Coverage Threshold	0
Total number of Reads	9968983
Total number of Bases(Mbp)	1124.7174
Total number of Bases(AQ20)(Mbp)	993.2010
Mean Coverage Depth(fold)	3104.6608
Coverage within Target Region	99.9780
Mean Read Length(AQ20)	107.3015
Mean Read Length(AQ30)	98.5890
Number of Homozygous SNVs	56
Number of Homozygous INDELs	1
Number of Heterozygous SNVs	151
Number of Homozygous MNVs	1
Number of Heterozygous MNVs	0
Number of Heterozygous INDELs	11

Metric	S7_R151013_SeraSeq_Tumor_Mix_AF10_DNA_v1 (DNA Sample)
Ti/Tv Ratio (SNPs)	2.833
dbSNP concordance	0.995
Heterozygotes/Homozygotes	2.793
Indels/Total	0.009
Indels/kb	0.034
SNPs/kb	0.593
CNV/Total	0.110
LongDels/Total	0.000
Number of CNVs	146
Number of LongDels	0
MAPD	0.23695508166886592

QC Metrics for a Fusions Sample

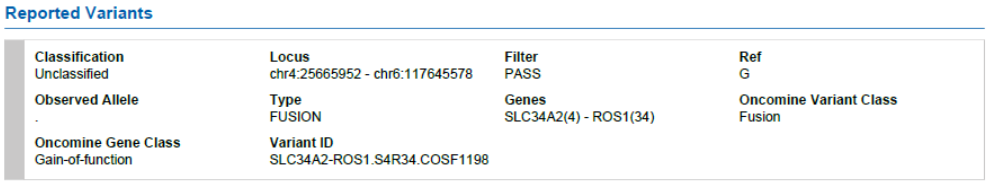
Metric	S7_R151013_SeraSeq_Fusion_RNA_Mix_v3_RNA_RNA_v1 (Fusions Sample)
Fusions/Total	0.021
Number of Fusions	18
Total Mapped Fusion Panel Reads	1666758
Fusion Sample QC	PASS,[TotalMappedFusionPanelReads>500000;MeanReadLength>60]
Fusion Overall Call	POSITIVE,[DriverGene=NTRK1,IsoformsDetected=LMNA-NTRK1.L2N11,TPM3-NTRK1.T7N12,TPM3-NTRK1.T7N13,TPM3-NTRK1.T7N10.COSF1329][DriverGene=RET,IsoformsDetected=NCOA4-RET.N7R12.COSF1491,NCOA4-RET.N6R12.COSF1340,KIF5B-RET.K24R11.COSF1262][DriverGene=ALK,IsoformsDetected=EML4-ALK.E13A20.COSF408.1][DriverGene=NTRK3,IsoformsDetected=ETV6-NTRK3.E5N15.COSF571.1][DriverGene=PPARG,IsoformsDetected=PAX8-PPARG.P9P2.COSF1217][DriverGene=BRAF,IsoformsDetected=SLC45A3-BRAF.S1B8.COSF871][DriverGene=ERG,IsoformsDetected=TMPRSS2-ERG.T1E2.COSF23.1,TMPRSS2-ERG.T1E4.COSF25][DriverGene=ROS1,IsoformsDetected=SLC34A2-ROS1.S4R34.COSF1198,CD74-ROS1.C6R34.COSF1200][DriverGene=FGFR3,IsoformsDetected=FGFR3-BAIAP2L1.F17B2.COSF1346,FGFR3-TACC3.F17T11.COSF1348][DriverGene=MET,IsoformsDetected=MET-MET.M13M15][DriverGene=EGFR,IsoformsDetected=EGFR-EGFR.E1E8.DelPositive.2,EGFR-SEPT14.E24S10]
Total Unmapped Reads	434706
Average Read Length	129
POOL-1 Mapped Fusion Reads	945739
POOL-2 Mapped Fusion Reads	617747
POOL-1,2 Mapped Fusion Reads	103272
Expression Controls Total Reads	518106
POOL-1 Expression Control Total Reads	253860
POOL-2 Expression Control Total Reads	264246

Coverage Metrics

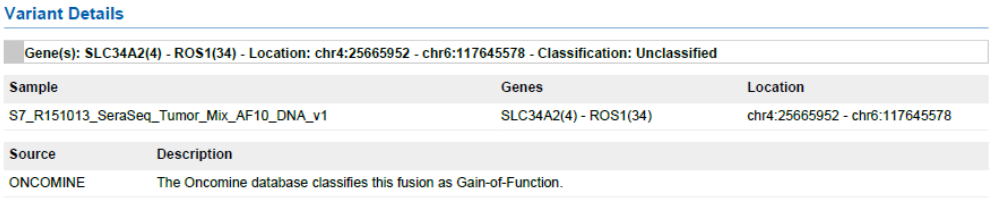
Coverage Metrics

Sample Name	BarCode	Mapped Reads	On Target	Mean Depth	Uniformity
S7_R151013_SeraSeq_Fusion_RNA_Mix_v3_R	IonCode_0115	2302471	99.59%		
S7_R151013_SeraSeq_Tumor_Mix_AF10_DNA_v1	IonCode_0131	10453084	95.38%	3105	97.79%

Reported Variants



Variant Details



Create a final report

You can create final reports directly from analysis results in Ion Reporter™ Software. You can customize the reports with variants that you select, and report sections that you can reorder, add and remove. The report layout and content are visible as a preview in the software that you can review and change as you create the report. When you are satisfied with the changes, you can publish a final report or save the report as a final report template.

Only one final report for each analysis can be published for later use. After a report has been published, you cannot change flags for MyVariants, classifications, or notes that are associated with the analysis results. A final report that is published to PDF file format cannot be edited. To create a different report for the analysis, you need to reanalyze the sample using the same analysis workflow, then create and publish the final report.

You can add a section for one or more images in PNG, GIF, JPG, or JPEG file formats. Each image must include a title, and can include an optional description, which appears next to the image. There is no limit on the number of images that can be added to a report, but all images are in a single section in the published final report.

You need to have the Report role to generate final reports.

1. In the **Analyses** tab, click **Overview**.
2. Click an analysis link to open the **Analysis Results** screen.
3. Select the variants to be included in the report by selecting the checkbox next to each variant. You can also select the checkbox at the top of the column to select all variants to be included in the report.

4. *(Optional)* Update the notes, flags, or classifications for each variant.
   a. In the **(Flag)** column, expand **(Flag list)**, then select the appropriate flag.

Option	Description
Important	**Important** is used to track variants that are relevant to the research. When selected, the flag icon is orange in the row of the variant and the variant is added to the MyVariants database.
Ignore	**Ignore** is often used to indicate known false positives or false negatives, or variants that are not associated with the research area. When selected, the flag icon is gray in the row of the variant and the variant is added to the MyVariants database.
None	All variants are marked **None** by default. Select **None** to remove an **Important** or **Ignore** flag. Variant flags that are changed to **None** are removed from the MyVariants database.

   b. In the **(Note)** column, click **(Add)** , click the **Notes** tab, then enter a note for the variant. When you are finished, click **Add Note**

   c. In the **Classification** column, select a classification for each variant. Each classification has a color associated with it in the report.
      - Unclassified (Grey)
      - Unknown (Black)
      - Benign (Green)
      - Suspected Benign (Yellow)
      - Likely Benign (Yellow)
      - Deleterious (Red)
      - Suspected Deleterious (Orange)
      - Pathogenic (Red)
      - Likely Pathogenic (Orange)
      - VUS (Black)
      - Uncertain Significance (Black)
      - Technical Artifact (White)

   d. Click **Selected Variants** to complete one or more of the options in the dropdown list. For more information, see “Create a final report template from analysis results” on page 407.

5. Click **Generate Report**.

6. *(Optional)* If a final report template is associated with the analysis workflow, update the **Select Final Report Template** dialog box.
   - Select a template, then click **OK**.
   - Click **Create Report Template** to create a new report template. For more information, see “Create a final report template from analysis results” on page 407.

   If a report template is not associated with the analysis workflow, go to the next step.

7. Enter information into the report sections.

   For more information, see “Available report sections” on page 421.

8. To add one or more images to the final report, add an **Image** section and the image.

   a. Click **Add Image Section**, then scroll to the **Images** section that is added to the report preview.

   b. To name the images section, enter a title for the section in the **Images** field. If you do not enter a title, the section is named **Images**.

   c. Enter an optional description for the image.

   d. Click **Upload Image**, then click **Select file** in the **Add Image** dialog box and browse to and select the image.

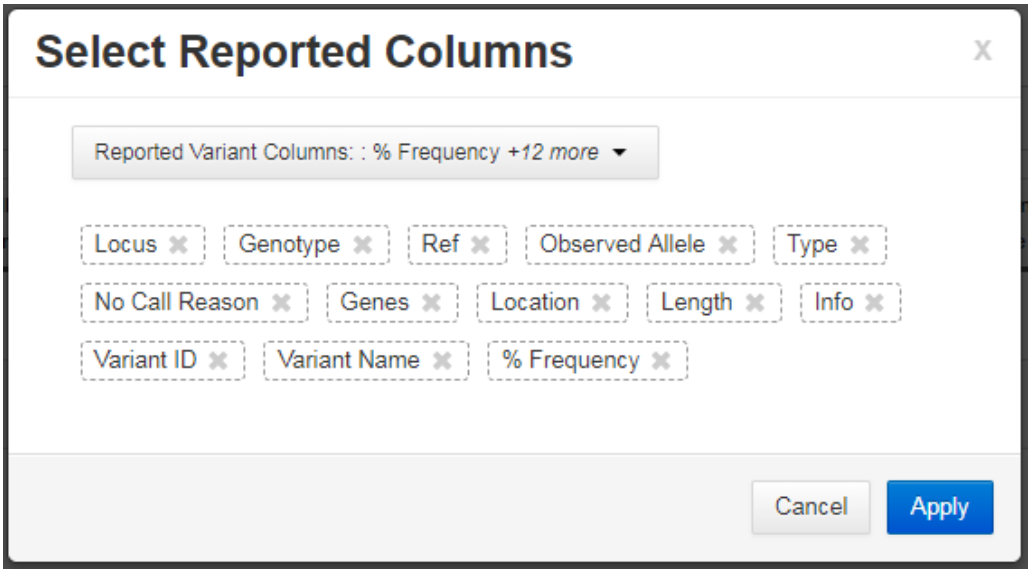
   e. Ensure that the correct image is selected, then click **Upload**. To select another image, click **Change** or **Remove**, then browse to the image.

      The image is added to the **Generate Report** screen and, after you save the new report template, is included in reports that use the template.

9. *(Optional)* Scroll to the **Reported Variants** section, then double-click **Select Columns**.

   Use the **Select Reported Columns** dialog box to change the view.

   - Delete columns—Click the **x** in the column name to remove that column from the report.
   - Change column order—Rearrange the column names, for example, to reflect the position that you want the date to appear in the report, then click **Apply**.



10. In the **Sign-Off** section, enter the name and title for a handwritten signature, then click **Add**. If needed, repeat this step to add information for additional required signatures, until all designated signers are added.

11. *(Optional)* In the **Footer Section**, remove the Thermo Fisher Scientific logo from the report footer, then add a custom logo. Acceptable image file formats are PNG, GIF, and JPG.
    a. Click **Remove** to remove the Thermo Fisher Scientific logo from the report footer.
    b. Click **Upload Logo**, then click **Select File** to browse to a custom logo file.
    c. Select the file, then click **Open** to include the logo in the report footer.
12. When you are satisfied with the report design, click **Next**.
    A preview of the PDF report opens. Click **Configuration** to go back to the previous screen to change the report sections before publishing.
13. When you are satisfied with the report sections, click **Lock and Publish**, then review the PDF preview. If edits are needed, click **Cancel**, then make the edits.

    IMPORTANT! After a report is published, the analysis that is used for the report cannot be edited. That is, you can no longer edit flags, classifications, or notes that are associated with the analysis results.
14. Click **Publish** to create the final report.
15. Click **Download** to download the published report.

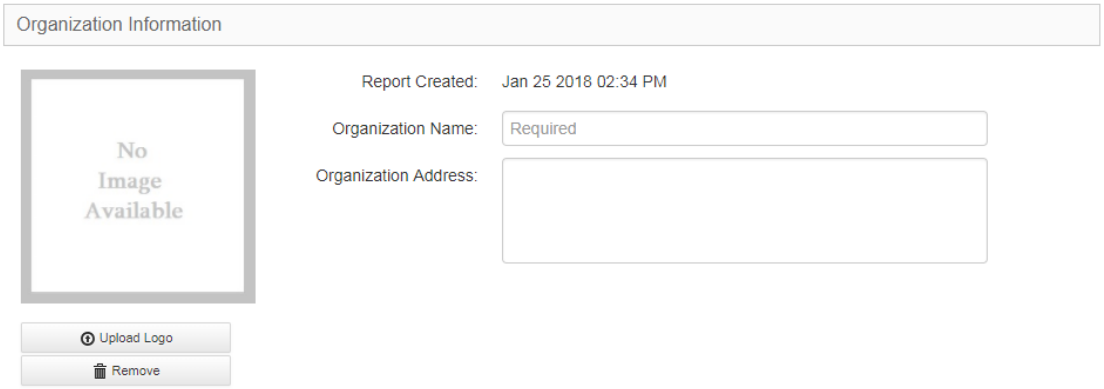
Create a final report template from analysis results

You can create a new final report template and save it for future analysis reports. As you create the report template, you can reorder sections, and add or remove sections.

You must have the Report role to create final report templates.

1. In the **Analyses** tab, click **Overview**.
2. Click an analysis link to open the **Analysis Results** screen.
3. In the **Analysis Results** screen, click **Generate Report**.

4. In the **Generate Report** screen, in the **Configuration** step, configure the report.
   a. In the **Organization Information** section,
      - Enter an organization name.
      - *(Optional)* To use a logo on the report, click **Upload Logo**, then in the **Upload Header Logo** dialog box, click **Select File** to browse to the logo file. Acceptable formats are PNG, GIF, and JPG.
      - *(Optional)* Enter the organization address.

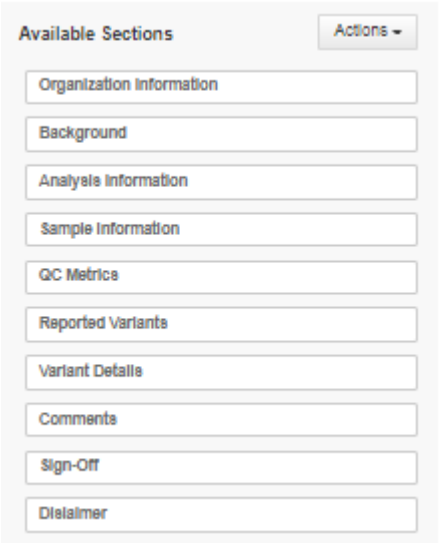


   b. *(Optional)* Enter other standard information for the **Background**, **Comments**, **Sign-Off**, and **Disclaimer** sections.

5. *(Optional)* Remove the Thermo Fisher Scientific logo from the report footer and add a custom logo. Acceptable formats are PNG, GIF, and JPG.
   a. Click **Remove** to remove the Thermo Fisher Scientific logo from the report footer.
   b. Click **Upload Logo**, then click **Select File** to browse to a custom logo file.
   c. Select the file, then click **Open** to include the logo in the report footer.

6. In each section, use the up, down, and X icons to move or delete sections from the template.

   Or, click **Actions**, then select one of the following to view a list of the sections that you can drag-and-drop into the report preview:

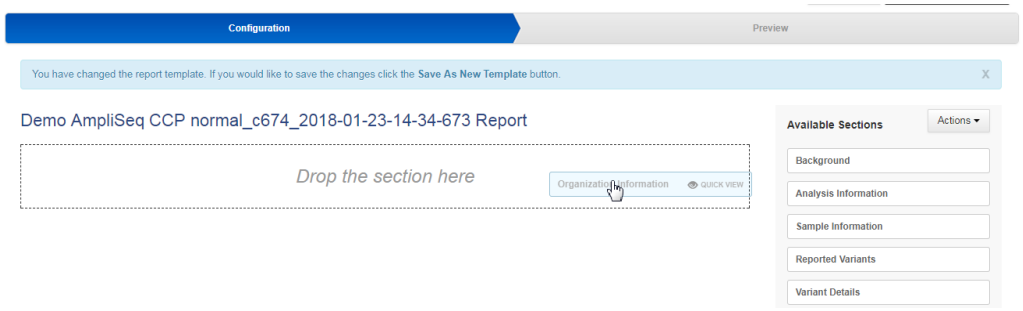


Option	Description
Add all	Add all of the available report template sections to the customized template or report.
Remove all	Remove all of the report template sections from the customized template or report.
Reset	Return to the template to the default section configuration.

The sections that you can add to the report template are listed under **Available Sections**.

For example, to select a limited set of sections, click **Remove all**, then drag and drop any of the **Available Sections** back into the report template.

For more information about the sections that you can add to the report template, see "Available report sections" on page 421.



7. To add one or more images to the report, add an **Image** section and the image.
   a. Click **Add Image Section**, then scroll to the **Images** section that is added to the report preview.
   b. To name the images section, enter a title for the section in the **Images** field. If you do not enter a title, the section is named **Images**.
   c. Enter an optional description for the image.
   d. Double-click **Upload Image**, then click **Select file** in the **Add Image** dialog box and browse to and select the image.
   e. Confirm that the correct image is selected, then click **Upload**. To select another image, click **Change** or **Remove**, then browse to the image.

      The image is added to the **Generate Report** screen and, after you save the new report template, is included in reports that use the template.
8. Click **Save As New Template**.
9. Enter a template name, then click **Save**.

The report template is available for use. To use the report template in future analyses, you must add it to an analysis workflow. For more information, see "Add a report template to an analysis workflow" on page 410.

Search for a final report template

You can review an existing custom and predesigned report templates.

1. In the **Workflows** tab, click **Presets**.
2. In the first dropdown list, select **Final Report Templates**, then, select a template type.

Option	Description
System-installed report templates	System-installed report templates are labeled ion and cannot be altered. However, you can review the components of the factory templates in the **Details** pane.
Custom templates	Custom templates and can be added to analysis workflows, deleted, edited, or locked.

Add a report template to an analysis workflow

If you know which information that you want to include in a final report from sequencing results that are shown in the **Analysis Results** table, you can add a report template to an Ion Reporter™ Software analysis workflow. Reports published from the analysis results that use the analysis workflow are based on the final report template that is associated with an analysis workflow.

1. Create a custom report template or select a system template.
   For more information, see “Create a final report template from analysis results” on page 407 or “Create a final report template workflow preset” on page 135.
2. In the **Workflows** tab, click **Create**.
3. Make the appropriate selections in the **Research Application**, **Reference**, **Annotation**, **Filters**, **Copy Number**, and **Plugins** steps.
   For more information, see “Create a new analysis workflow” on page 79.
4. In the **Final Report** step, under **Final Report Template**, select the report template that you want to associate with the analysis workflow, then click **Next**.
5. Make appropriate selections in the **Parameters** step, then enter a **Workflow** name and optional description in the **Confirm** step, then click **Save Workflow**.
   The report template is now associated with the analysis workflow. When you view the analysis results, you can publish a final report that uses the report template. You can also publish a final report for other analyses that use the analysis workflow with which the report template is associated.

Edit a final report template

You can edit custom final report templates that exist in the user account.

1. In the **Workflows** tab, click **Presets**.
2. Select **Final Report Templates** from the list.
3. Select the row of the final report template that you want to edit, then click ⚙ **(Actions)** ▸ **Edit**.
4. You can change the name and the description, and select sections to include or exclude, then drag and drop Reported Variants details.
5. In the **Footer Section**, you can remove the Thermo Fisher Scientific logo from the report footer and add a custom logo. Acceptable formats are PNG, GIF, and JPG.
   a. Click **Remove** to remove the Thermo Fisher Scientific logo from the report footer.
   b. Click **Upload Logo**, then click **Select File** to browse to a custom logo file.
   c. Select the file, then click **Open** to include the logo in the report footer.
6. Click **Save**.
   If the template is included in an Ion Reporter™ Software analysis workflow, it is now available for use when you review analysis results. If it was not, you must add the template to an analysis workflow. For more information, see “Add a report template to an analysis workflow” on page 410.

Lock a report template

You can lock custom final report templates so that they cannot be altered.

1. In the **Workflows** tab, click **Presets**.
2. Select **Final Report Templates** from the list.
3. Select the row of the final report template that you want to lock, then click ⚙ **(Actions)** ▸ **Lock**.
   A lock icon appears the row of the final report template in the **Workflow Presets** screen. You can add this report template to an analysis workflow. For more information, see “Add a report template to an analysis workflow” on page 410.

Delete a final report template

You can delete unwanted custom final report templates.

1. In the **Workflows** tab, click **Presets**.
2. Select **Final Report Templates** from the list.
3. Select the row of the final report template that you want to delete, then click ⚙ **(Actions)** ▸ **Delete**.
   The final report template is now removed from the list of available templates in the **Workflow Presets** screen and can no longer be added to an Ion Reporter™ Software analysis workflow.

Generate a final report and approve with an electronic signature

If a final report uses a template that requires an electronic signature, a user with the Report role generates and electronically signs the report. After a final report is published and signed, it cannot be altered.

An analysis that has already been published, indicated by **Report Published** in the **Stage** column, cannot be published or signed.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Analyses** tab, click **Overview**.
2. Select the checkbox for the analysis of interest, then click ⚙ **(Actions)** ▸ **Edit Analysis Results**. Alternatively, in the **Analysis** column, click the link for the analysis.
3. In the **Analysis Results** screen, select some variants to include in the report.
4. *(Optional)* Click ＋ **(Add)** in the **Analysis Results** screen to add a note for a variant.
5. Click **Generate Report**.
   A report preview is created.
6. In the **Generate Report** dialog box, name the organization, then scroll to review the various sections of the report.
7. Scroll to the bottom of the report, then click **Next**.
8. In the **Lock and Publish** dialog box, enter the user email address and password, enter any comments, then click **Publish**.
   The report is downloaded in PDF file format.
9. Open the downloaded PDF file.
   The electronic signature and comment appears only on the first page in the footer of the final report.

Visualization reports

When you visualize analysis results in Ion Reporter™ Software, you can generate a PDF report for one or more analyses that contain graphics and data from each analysis results set. Visualization reports can be generated when you open a visualization that includes data in the **IRGV & Generate Report** tab of the **Analysis Visualization** screen. When you generate a visualization report, the analysis, or analyses, are not locked.

Note: You cannot create visualization reports when Ion Reporter™ Genomic Viewer (IRGV) is launched from the **Locus** link in analysis results.

Items that are available from each visualization depend on the type of analyses that are run. The following sections are examples of sections can be added from a visualization:

- A summary for the samples or analysis
- The **Chromosome View** that is selected at the time the report is created
- **CNV Heat Map**
- **Karyo View**
- Details from the samples or the analysis
- A summary of Microsatellite Instability (MSI) results

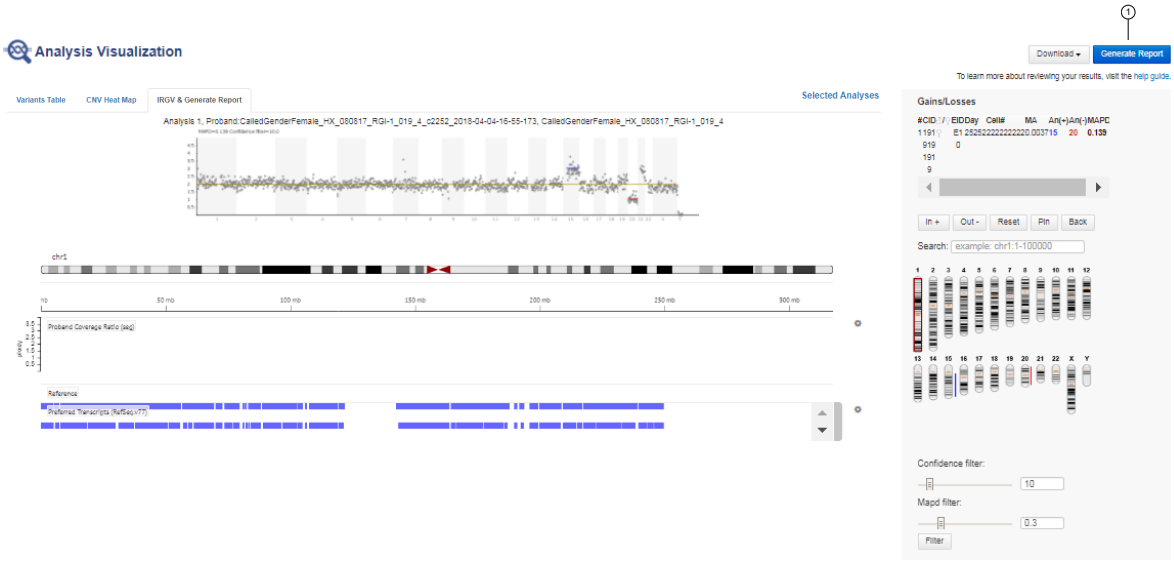
For more information about other sections that can be added to the report, see “Available report sections” on page 421.

Create a visualization report

You can create a report that includes visualization images and data. When you open a visualization, you can generate the report with an existing report template, or from a new report template that you create in the **Analysis Visualization** screen.

1. On the **Analyses** tab, click **Overview**, then select one or more analyses that you want to visualize in Ion Reporter™ Genomic Viewer.

2. Click **Visualize**.
   The visualization of the data are viewable in the **IRGV & Generate Report** tab of the **Analysis Visualization** screen. For example, a visualization can include a copy number histogram for each analysis, ploidy maps for selected chromosomes or chromosome regions, and karyograms that show copy number gains and losses.



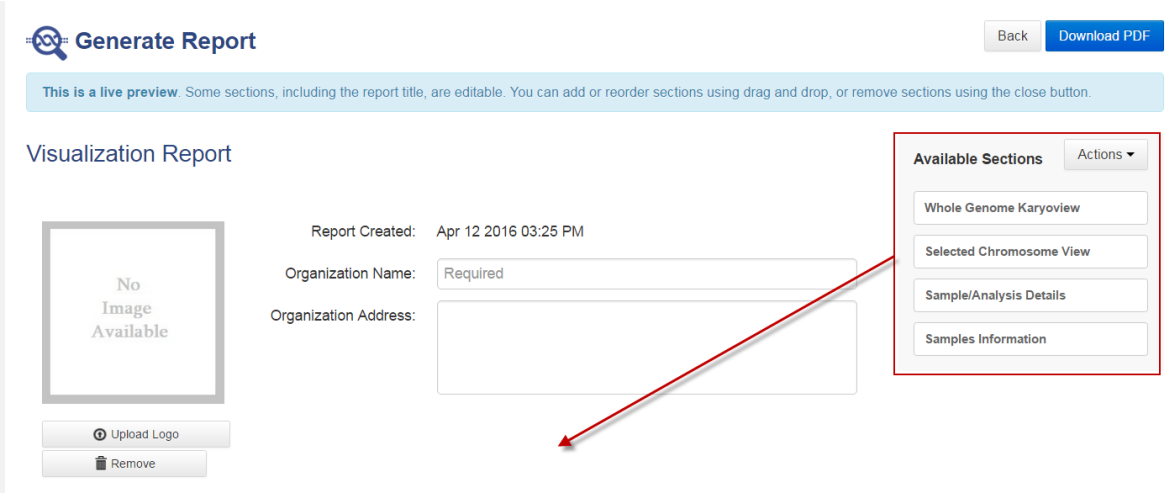
① Generate Report for the visualization

   For six or more analyses results, the whole genome **Karyo View** cannot show Gain and Loss data segments.

3. Click **Generate Report**.

4. In the **Select Visualization Report Template** dialog box, select an existing report template in the drop-down menu or click **Create New Report Template**, then click **OK**.
   A preview of the report is shown. You can customize the report in the **Configuration** step.

5. Drag the title of the graphics and data that you want to include in the report from the **Available Sections** into the **Visualization Report** preview.



6. Enter an organization name for the visualization report. Enter any other relevant information.

7. In the **Sign-Off** section, enter the name and title for a handwritten signature, then click **Add**. Repeat this step to add the information for all additional required signatures, until all designated signers are added.

8. In the **Footer Section**, you can remove the Thermo Fisher Scientific logo from the report footer and add a custom logo. Acceptable formats are PNG, GIF, and JPG.
   a. Click **Remove** to remove the Thermo Fisher Scientific logo from the report footer.
   b. Click **Upload Logo**, then click **Select File** to browse to a custom logo file.
   c. Select the file, then click **Open** to include the logo in the report footer.

9. When you are satisfied with the design of the visualization report, click **Next**.
   A preview of the PDF report opens. Click **Configuration** to go back to the previous screen to change the report sections before publishing.

10. Click **(Actions)** ▸ **Download PDF** to download the published report.

The report is downloaded to the location used for downloads from the browser. This location depends on the browser settings.

Note: When you generate a visualization report, the analysis is not locked.

Amplicon coverage reports

Amplicon coverage reports list amplicons, SNPs, and bases in the amplicons. When you view the amplicon coverage report, you can adjust the amplicon coverage threshold to focus your report on only the amplicons that met the threshold that you set.

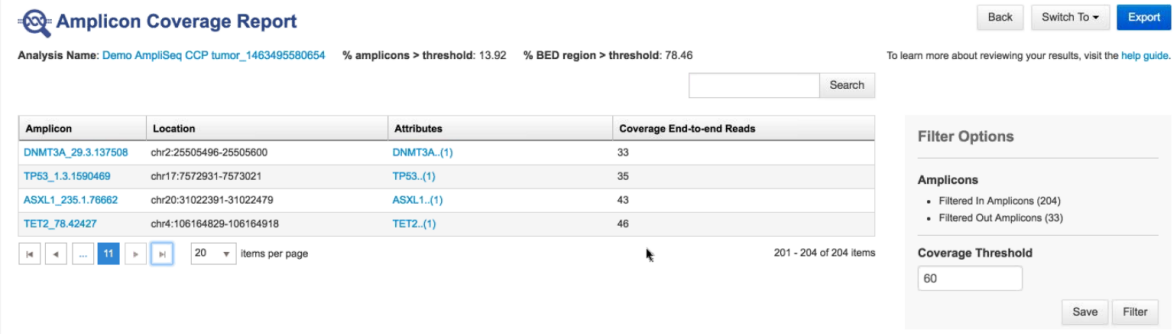
View and filter an amplicon coverage report

You can view an amplicon coverage report in Ion Reporter™ Software that shows which amplicons in an analysis *do not* have coverage. The report covers all bases to a user-defined coverage depth threshold. You can filter the list of amplicons that are shown in the report. The list is based on the threshold that you select and can adjust.

1. In the **Analyses** tab, click **Overview**, then select row for the analysis of interest.
2. Click **(Actions)** ▸ **View Coverage Report**.
   Alternatively, you can click the analysis of interest to open the **Analysis Results** table, then click **Switch To** ▸ **Coverage Report**.
   The **Amplicon Coverage Report** screen opens. The default value for the threshold is ***0***, so by default no amplicons are included in the list.
3. In the **Filter Options** section, enter a **Coverage Threshold** value, for example `60`, then click **Filter**. To save this threshold as the default for this amplicon coverage report, click **Save**.
   The following metrics appear in the **Amplicon Coverage Report** screen.

Coverage Quality Control (QC) metric	
(% amplicons > threshold)	Percentage of the total number of amplicons in the panel that have coverage at all bases at a level greater than the **Coverage Threshold** value.
(% BED region > threshold)	Percentage of the total bases that are defined in the panel regions BED file with coverage that is greater than the **Coverage Threshold** value .

If the filter option was previously saved, the **% amplicons > threshold** and **% BED region > threshold** values change to reflect the **Coverage Threshold** value.



4. Click **Export** to generate a tab-separated CSV file that contains amplicon metrics details such as chromosome location, and start and end positions on forward and reverse strands.
   The downloaded file is named `ampliconmetrics.csv`.

Download an amplicon coverage report

You can download an amplicon coverage report in Ion Reporter™ Software. Amplicon coverage reports contain details such as amplicon coverage, chromosome location, and start and end positions.

1. In the **Analysis** tab, click **Overview**.
2. Select the row of the analysis that you want to report on.
3. Click ⚙ **(Actions)** ▸ **View Coverage Report**.
4. Click **Export** to generate a tab-separated CSV file that contains details such as chromosome location, and start and end positions on forward and reverse strands.
   The downloaded file is named `ampliconmetrics.csv`.

Amplicon coverage report metrics

The amplicon coverage report includes the following metrics:

Metric	Description
Region	The amplicon name as defined in the BED file for the amplicon region.
Chr	Chromosome
Start	The start coordinate of the amplicon.
End	the end coordinate of the amplicon.
Attributes	The attribute in the BED file for the amplicon.
TotalE2E	The number of assigned reads from end to end for the amplicon region.
FwdE2E	The number of assigned forward strand reads from end to end for the amplicon region.
RevE2E	The number of assigned reverse strand reads from end to end for the amplicon region.
Total	The total number of reads assigned to this amplicon. This value is equal to Fwd + Rev.

(continued)

Metric	Description
Fwd	The number of forward strand reads assigned to this amplicon. This value may differ from the reads for end to end.
Rev	The number of reverse strand reads assigned to this amplicon. This value may differ from the reads for end to end.

Visualize an amplicon coverage report

To view an amplicon visually, click an amplicon link in the **Amplicon Coverage Report** screen to view the results in the Ion Reporter™ Genomic Viewer (IRGV) or the Integrative Genomics Viewer (IGV), depending on the viewer settings. For more information, see “Set IRGV or IGV as the default viewer” on page 311.



Figure 19 Amplicon visualization in IRGV

Quality Control (QC) reports

QC reports provide a high-level summary of key metrics that you can use to evaluate the quality of the data for analysis and samples, or amplicon coverage reports. Each report is specific to an individual analysis and the samples used for the sequencing run.

View a Quality Control (QC) report

You can view a Quality Control (QC) report for a completed analysis in Ion Reporter™ Software. The QC report provides a high-level summary of key metrics that you can use to evaluate the quality of the data. You can also download a PDF version of the QC report.

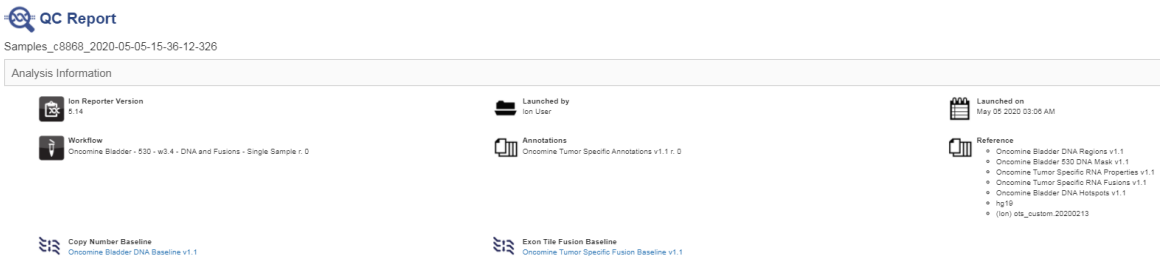
1. In the **Analysis** tab, click **Overview**.
2. Select the row for the report of interest, then click **(Actions)** ▸ **View QC Report**.

   Alternatively, you can click the analysis link to open the **Analysis Results** screen, then click **Switch To:** ▸ **QC Report**.

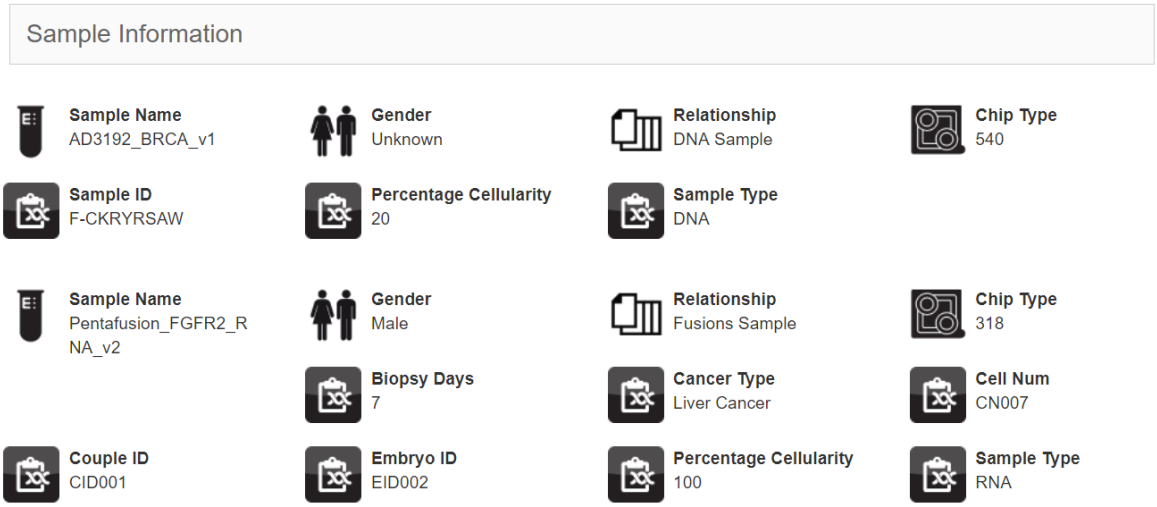
   The QC Report opens.

   These images show sections of the QC Report.

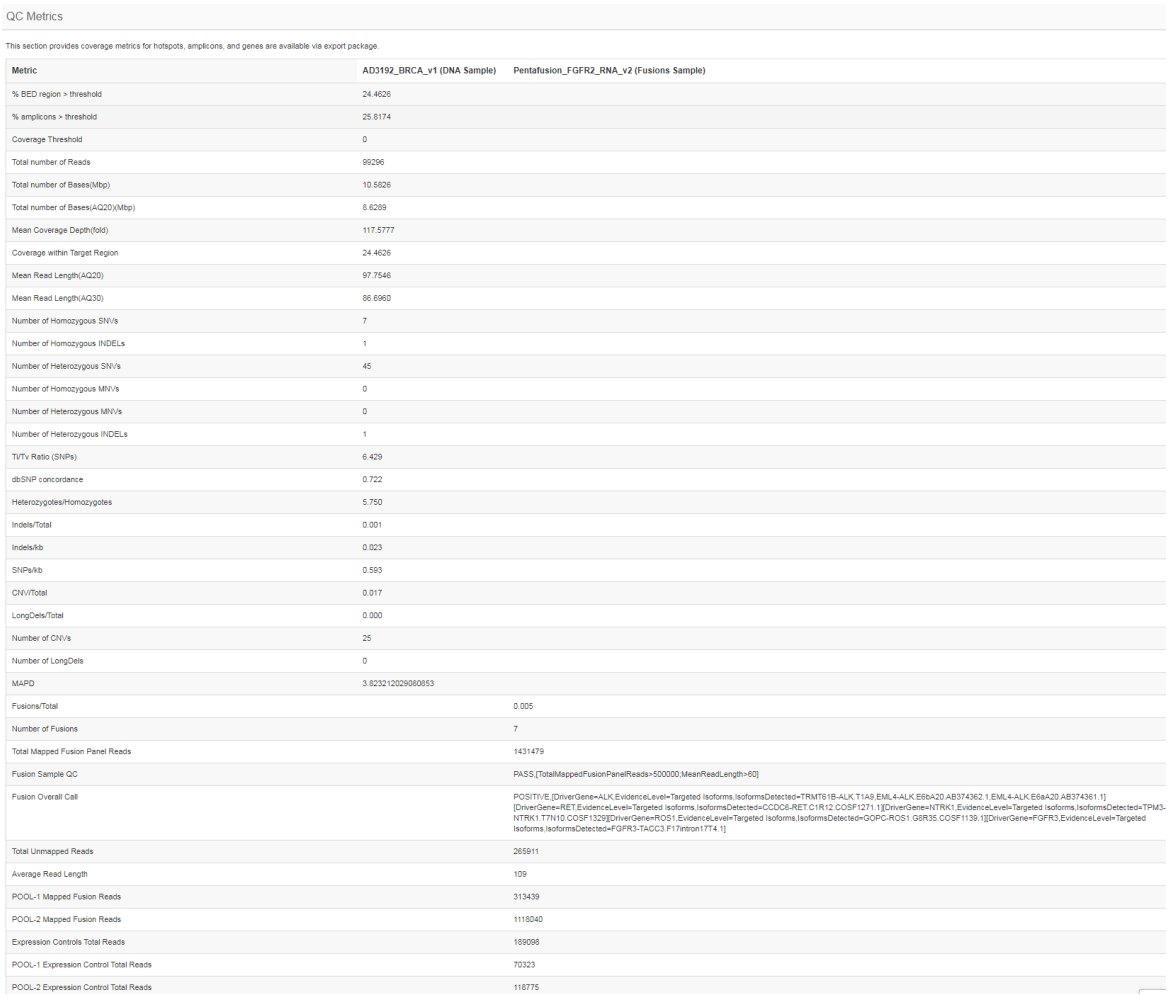
Analysis Information



Sample Information



QC Metrics



QC Metrics

This section provides coverage metrics for hotspots, amplicons, and genes are available via export package.

Metric	AD3192_BRCA_v1 (DNA Sample)	Pentafusion_FGFR2_RNA_v2 (Fusions Sample)
% BED region > threshold	24.4626	
% amplicons > threshold	25.8174	
Coverage Threshold	0	
Total number of Reads	99296	
Total number of Bases(Mbp)	10.5826	
Total number of Bases(AQ20)(Mbp)	8.6289	
Mean Coverage Depth(fold)	117.5777	
Coverage within Target Region	24.4626	
Mean Read Length(AQ20)	97.7546	
Mean Read Length(AQ30)	86.6960	
Number of Homozygous SNVs	7	
Number of Homozygous INDELs	1	
Number of Heterozygous SNVs	45	
Number of Homozygous MNVs	0	
Number of Heterozygous MNVs	0	
Number of Heterozygous INDELs	1	
Ti/Tv Ratio (SNPs)	6.429	
dbSNP concordance	0.722	
Heterozygotes/Homozygotes	5.750	
Indels/Total	0.001	
Indels/kb	0.023	
SNPs/kb	0.593	
CNV/Total	0.017	
LongDels/Total	0.000	
Number of CNVs	25	
Number of LongDels	0	
MAPD	3.823212029080853	
Fusions/Total		0.005
Number of Fusions		7
Total Mapped Fusion Panel Reads		1431479
Fusion Sample QC		PASS,[TotalMappedFusionPanelReads>500000;MeanReadLength>60]
Fusion Overall Call		POSITIVE,[DriverGene=ALK,EvidenceLevel=Targeted Isoforms,IsoformsDetected=TRMT61B-ALK.T1A9,EML4-ALK.E6bA20.AB374362.1,EML4-ALK.E6aA20.AB374361.1] [DriverGene=RET,EvidenceLevel=Targeted Isoforms,IsoformsDetected=CCDC6-RET.C1R12.COSF1271.1][DriverGene=NTRK1,EvidenceLevel=Targeted Isoforms,IsoformsDetected=TPM3-NTRK1.T7N10.COSF1329][DriverGene=ROS1,EvidenceLevel=Targeted Isoforms,IsoformsDetected=GOPC-ROS1.G6R35.COSF1139.1][DriverGene=FGFR3,EvidenceLevel=Targeted Isoforms,IsoformsDetected=FGFR3-TACC3.F17intron17T4.1]
Total Unmapped Reads		265911
Average Read Length		109
POOL-1 Mapped Fusion Reads		313439
POOL-2 Mapped Fusion Reads		1118040
Expression Controls Total Reads		189096
POOL-1 Expression Control Total Reads		70323
POOL-2 Expression Control Total Reads		118775

Coverage Metrics



Coverage Metrics

This section provides the sample name, barcode and coverage report information.

Sample Name	BarCode	Mapped Reads	On Target	Mean Depth	Uniformity	Coverage Report
AD3192_BRCA_v1	IonDual_D8_0160	899506	56.72%	550.4	84.92%	Download

3. To download a PDF of the QC report:
   a. Click **Export** ▸ **PDF**.
   b. Download the report from the **Notifications** screen. The file name for the PDF is `id_QC.pdf`, where `id` is a system-generated analysis identifier.

   You can also view the QC report in your browser, or find the PDF file in the folder that you use for downloads, depending on the browser settings.

4. To download a ZIP file that contains the QC Report PDF, a tab-separated file of the QC metrics, and individual text files of coverage statistics by amplicon, analysis, and gene:
   a. Click **Export** ▸ **QC Package**.

b. Download the ZIP file from the **Notifications** screen. The file name is `qc-report-id.zip`, where `id` is a system-generated analysis identifier.

You can also view the QC report in the browser, or find the PDF in the folder that you use for downloads, depending on the browser settings.

QC package coverage columns

Quality Control (QC) statistics files report coverage distribution. The QC files that contains the statistics can be downloaded. QC report settings can be customized when you create a custom analysis workflow or edit an analysis workflow.

QC package coverage distribution is calculated for each

region and reported in the **No**, **Low**, **Medium**, and **High** columns in the ***_coverage_stastics.txt** files.

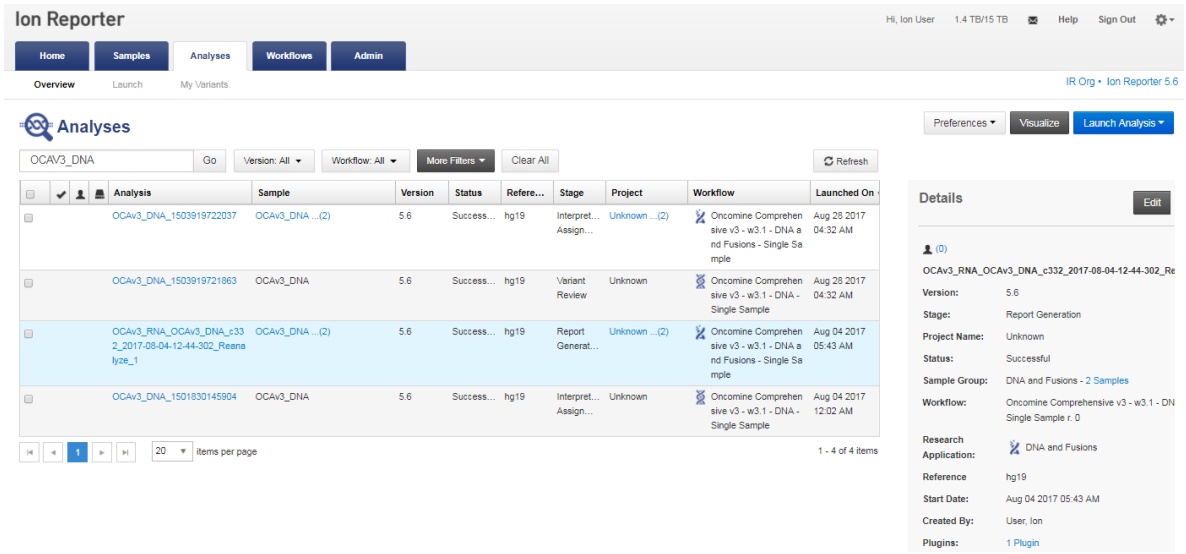
Coverage	Definition
No	0 to 0 coverage range
Low	1 to Maximum Low Coverage
Medium	Max Low Coverage +1 to Max Medium Coverage
High	Maximum Medium Coverage +1 to Maximum Coverage

Download Quality Control (QC) files

You can download files from a Quality Control (QC) report that you open in Ion Reporter™ Software. The Quality Control (QC) file download contains a compressed directory with the following files:

- `id_QC.pdf`: A copy of the QC Report PDF file. The QC report PDF file for a completed analysis contains a high-level summary of QC metrics. For more information, see “Quality control (QC) metrics” on page 423.
- `QC/sample_name/AnnotatorActor/variome-stats.csv`: A tab-separated file of variants statistics for this sample. These statistics are also listed in the QC report PDF file.
- `QC/sample_name/StatsActor/amplicons_low_no_coverage_statistics.txt`: Coverage statistics by amplicon.
- `QC/sample_name/StatsActor/analysis_low_no_coverage_statistics.txt` : Coverage statistics for the analysis.
- `QC/sample_name/StatsActor/genes_low_no_coverage_statistics.txt` : Coverage statistics by gene.

1. In the **Analysis** tab, click **Overview**.
2. Find the analysis of interest, then select the row that lists the analysis to generate a QC report.
   Alternatively, you can select the checkbox in the row to generate the QC report.



3. Click **(Actions)** ▸ **View QC Report**.
   A view of the QC Report opens.
4. Click **Export** ▸ **QC Package** to download the QC Package to Ion Reporter™ Software.
   - If you use Ion Reporter™ Software on Connect, open the **Home** tab, then click **Notifications** to open the notifications list, then find the analysis of interest and click **(Download)** to download the QC package.
   - If you use Ion Reporter™ Server, download the file through the browser that you use for Ion Reporter™ Software. Then, find the QC package in the folder that is used for downloads.

   The downloaded file name is `qc-report-id.zip`, where `id` is a system-generated analysis identifier.

Available report sections

The following sections are available to add to report templates, final reports, or visualization reports.

More sections might be available in a visualization report. For more information, see “Create a visualization report” on page 413.

Section	Description
Organizational Information	• **Required**: Organization name. • Upload a logo and optionally enter the organization address.
Background	Enter background information.

(continued)

Section	Description
Analysis Information	Generates a list of details for the analysis. • Software version number • Report generation date • Name of person who launched the report • Analysis workflow • Name of person who launched the analysis • The annotation set that was used in the analysis • The filter chain that was applied to the analysis at the time that the report was generated • Reference information • Baselines that were used for the analysis, if applicable
Samples Overview	Includes all sample attributes that are included in the Samples table when the data are viewed in Ion Reporter™ Software.
QC Metrics	Generates a table coverage metrics for hotspots, amplicons and genes. For a complete list of the metrics that are included, see “Quality control (QC) metrics” on page 423. You can also view and download these metrics in a separate report. For more information, see “Create a final report template from analysis results” on page 407
Reported Variants	Generates a table of reported variants and includes columns of variant details.
Variant Details	Includes name and description of variants, and which samples they came from. In addition, it lists Annotations and Notes for each variant.
Molecular Coverage Analysis	Metrics that describe the level of sequence coverage for target genomic regions. The **Coverage Metrics** section is available if the coverageAnalysis plugin was run in Torrent Suite™ Software, before data was transferred to and analyzed in Ion Reporter™ Software. See “Molecular Coverage Analysis metrics” on page 426 for more information.
Coverage Analysis Metrics	Metrics for molecular coverage over the targeted regions of the reference genome. The **Molecular Coverage Analysis** section is available if the molecularCoverage Analysis plugin was run in Torrent Suite™ Software, before data was transferred to and analyzed in Ion Reporter™ Software. See “Coverage analysis metrics” on page 425 for more information.
Comments	Enter comments about the report or report contents.
Sign–Off	This section provides lines with the name and qualifications of people who are required to provide a handwritten signature for the report. Enter the name and title for a handwritten signature, then click **Add**. Continue to add the information for all required signatures, until all designated signers are added.
Disclaimer	Enter a custom legal disclaimer.

(continued)

Section	Description
Image	You can add an **Image** section to a final report or final report template. If included, the **Image** section can be used to upload one or more images in PNG, GIF, JPG, or JPEG formats into the report. There is no limit to the number of **Image** sections that can be added.
Footer	You can remove the Thermo Fisher Scientific logo from the report footer and add a custom logo. Acceptable formats are PNG, GIF, and JPG.

Quality control (QC) metrics

Quality control (QC) metrics can be viewed in QC reports and in final reports. The metrics are based on data that are included in an analysis. For example, metrics that describe fusions are available only for analyses that include fusions data. See “Create a final report” on page 404.

For more information about QC reports and how to add QC metric to final reports, see “Create a final report template from analysis results” on page 407.

QC metrics	Description
% BED region > threshold	Percentage of the total bases that is defined by the threshold included in the target regions BED file for the panel, with coverage that is greater than the **Coverage Threshold** value.
% amplicons > threshold	Percentage of the total number of amplicons in the panel that have coverage at all bases at a level greater than the **Coverage Threshold** value.
Coverage Threshold	A coverage adequacy setting.
Total Number of Reads	The total number of reads.
Total Number of Bases (Mbp)	The total number of bases, in million base pairs.
Total Number of Bases (AQ20)(Mbp)	The total number of bases at AQ20 (1% error rate) accuracy, in million base pairs.
MAPD	Median Absolute Pairwise Difference, a metric for noise in copy number data.
Mean Coverage Depth (fold)	The mean depth of coverage.
Coverage within Target Region	The percentage of positions in the target regions file for the panel that have coverage >1.
Mean Read Length (AQ20)	The mean read length at AQ20 (1% error rate) accuracy.
Mean Read Length (AQ30)	The mean read length at AQ30 (0.1% error rate) accuracy.
Number of Homozygous SNVs	The number of homozygous SNV calls.
Number of Homozygous INDELs	The number of homozygous INDEL calls.

(continued)

QC metrics	Description
Number of Heterozygous SNVs	The number of heterozygous SNV calls.
Number of Homozygous MNVs	The number of homozygous MNV calls.
Number of Heterozygous MNVs	The number of heterozygous MNV calls.
Number of Heterozygous INDELs	The number of heterozygous INDEL calls.
Ti/Tv Ratio (SNPs)	The ratio of transition to transversion substitutions.
dbSNP concordance	The overall dbSNP concordance, which is a ratio of the number of SNP and INDEL calls that appear in dbSNP with hit levels of "locus" or "allele", to the total number of SNP and INDEL calls.
Heterozygotes/Homozygotes	The ratio of heterozygotes to homozygotes. This ratio is calculated from SNP and INDEL variants only. MNVs are not considered.
INDELs/Total	The ratio of INDEL calls to all variant calls.
INDELs/kb	The number of INDEL calls per 1,000 bases. For an analysis that uses a regions of interest BED file, the number of bases in those regions is used.
SNPs/kb	The number of SNP calls per 1,000 bases.
CNV/Total	CNVs divided by total variant count minus no calls.
LongDels/Total	LongDels divided by total variant count minus no calls.
Number of CNVs	The number of CNV variants.
Number of LongDels	The number of LongDel variants.
Total Mapped Fusion Panel Reads	The sum of all reads for fusion variants and for the markers ASSAYS_5P_3P, and EXPRESSION_CONTROL on a fusion panel.
Expression Controls Total Reads	The number of all reads that are assigned to the expression control targets in the sample. If panels contain multiple pools, this metric is the aggregate sum from all expression control targets in all pools.
POOL-n Expression Control Total Reads	The sum of expression control reads for individual reads are also reported separately as POOL-1 ExpressionControlTotalReads and POOL-2 **ExpressionControlTotalReads**, and so on.

The following information is included in each of the `*_coverage_statistics.txt` files.

Column	Description
#Id	The amplicon ID or gene name (if any).
Region	Usually the chromosome that contains the amplicon, gene, or hotspot region.
Start	The initial position of the amplicon, gene, or hotspot region.
End	The end position of the amplicon, gene, or hotspot region.
No	The number of reads with zero coverage.
Low	The number of reads with low coverage.
Medium	The number of reads with medium coverage.
High	The number of reads with high coverage.
TargetSize	The region size (in bases) of the amplicon, gene, or hotspot region.
Min	The minimum coverage of any base in the amplicon, gene, or hotspot region.
Max	The maximum coverage in the amplicon, gene, or hotspot region.
Avg	The average coverage in the amplicon, gene, or hotspot region.
1XBases	The number of bases with at least 1X coverage in the amplicon, gene, or hotspot region.
AvgBQ	The average base quality value.

Coverage analysis metrics

Coverage analysis metrics are included in a Quality Control report and can be added to a final report template if the coverageAnalysis plugin was run in Torrent Suite™ Software, before data was transferred to and analyzed in Ion Reporter™ Software. These metrics describe the level of sequence coverage for target genomic regions.

To download a report that contains the metrics, click the **Download** link in the **Coverage Report** column of an open Quality Control report. To create a final report template, see “Create a final report template from analysis results” on page 407.

Coverage analysis metric	Description
Sample Name	The name of the sample, as it was entered into the Planned Run for Torrent Suite™ Software.
Barcode	The name of the specific barcode in the barcode kit that was used for the sequencing run.
Mapped Reads	The total number of reads that were mapped to the reference in Torrent Suite™ Software. Reads that are not uniquely mapped can have equally well-aligned reads that are mapped to multiple locations. Such reads are typically mapped randomly to one location.

(continued)

Coverage analysis metric	Description
On Target	The total number of reads that were mapped in the target regions file to any targeted region of the reference in Torrent Suite™ Software. A read is *on target* only if at least one aligned base overlaps with a target region. For example, a read is not counted if the read overlaps a targeted region on a region that includes a flanking sequence that is aligned. In this case the read is not counted due to poor matching of 5' bases.
Mean Depth	The summary statistics for reads that are assigned to specific amplicons. Each sequence read is assigned to exactly one of the amplicons specified by the targets file. Reads are assigned to particular amplicon targets based on whether the (5') mapping location is sufficiently near the end of the amplicon region, taking into account the read direction (mapping strand).
Uniformity	The percentage of bases in all targeted regions (or whole-genome) that is covered by at least 20% of the average base coverage depth reads. Cumulative coverage is linearly interpolated between nearest integer base read depths.

Molecular Coverage Analysis metrics

Molecular Coverage Analysis metrics are included in a Quality Control report. These metrics can be added to a final report template if the molecular Coverage Analysis plugin was run in Torrent Suite™ Software, before data was transferred to and analyzed in Ion Reporter™ Software. These metrics report molecular coverage over targeted regions of the reference genome.

To download a report that contains the metrics, click the **Download** link in the **Molecular coverage analysis report** column. To create a final report template, see “Create a final report template from analysis results” on page 407.

Molecular Coverage Analysis metric	Description
Sample Name	The name of the sample, as it was entered into the Planned Run for Torrent Suite™ Software that is used to generate the BAM file that was uploaded to Ion Reporter™ Software.
Barcode	The name of the barcode, as it was entered into the Planed Run for Torrent Suite™ Software that is used to generate the BAM file that was uploaded to Ion Reporter™ Software.
Molecular Uniformity	Percentage of amplicons having molecular coverage between 0.5x and 2x of the median molecular coverage.
Median Reads per Functional Molecule	For each amplicon, the number of reads supporting each functional molecule is averaged across all functional molecules for that amplicon to determine the number of reads per functional molecule at the amplicon level. The median is calculated across all amplicons.

(continued)

Molecular Coverage Analysis metric	Description
Median Perc Functional Reads	For each amplicon, the percentage of reads supporting functional molecules is (Number of Reads Supporting Functional Molecules) / (Number of Reads). The median is calculated across all amplicons.
Median Molecular Coverage	The number of molecules that satisfies size criteria in the parameters file. The median is calculated across all amplicons.

Audit logs

Audit logs record user activities, such as who launches analyses, the start and end time of analyses, report creation and publication, and edits of analyses, including the addition of annotations, MyVariants flags, classifications, and notes.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

View and download audit logs

You can view and download logs for samples and analyses activities in Ion Reporter™ Software. A sample audit log records which users access a specific sample and any activity that is associated with the sample. Analysis audit logs contain information regarding who launched or edited analyses.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

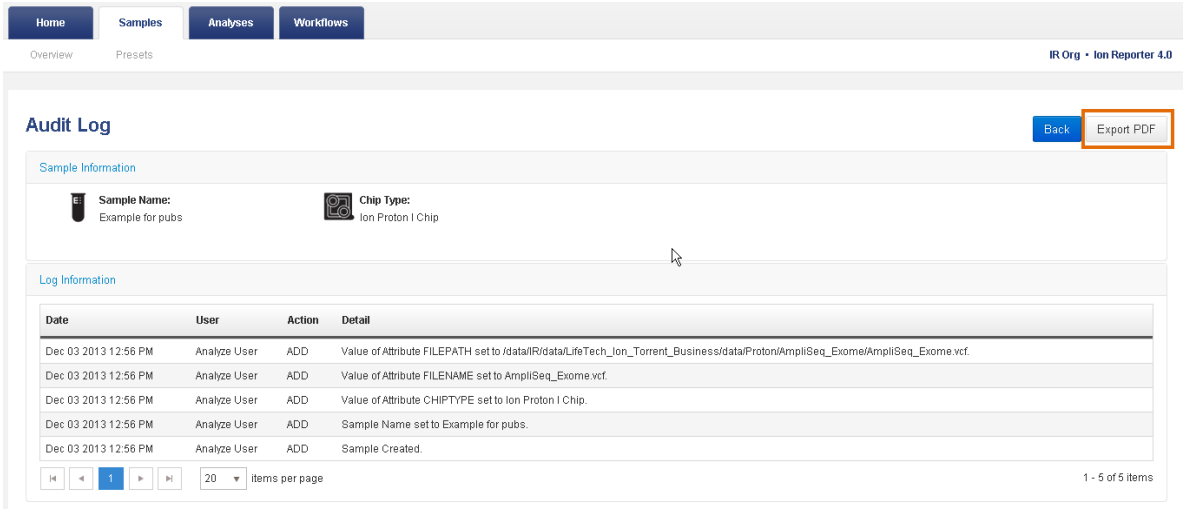
View an audit log for a sample

You can view an audit log for samples in Ion Reporter™ Software. The sample audit log allows you to see who has accessed a specific sample and changes that are associated with the sample. Sample audit logs are not available for the preinstalled demo data samples.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Samples** tab, click **Overview**.
2. Select the row of the sample that you want to report on.
3. Click ⚙ **(Actions)** ▸ **Audit Log**.
   The audit log opens.

4. (*Optional*) Click **Export PDF**. The downloaded filename is `sample_name.pdf`, where `sample_name` is the name of the sample.



View an audit log for a completed analysis

You can view an audit log for a completed analysis in Ion Reporter™ Software. Information is recorded for activities that change the state of an analysis. The analysis states are:

- Analysis
- Variant Review
- Interpretation Assignment
- Pending Report
- Variant Classification
- Report Generation
- Report Publishing
- Report Published

Audit logs also record information about analyses, samples, and logs.

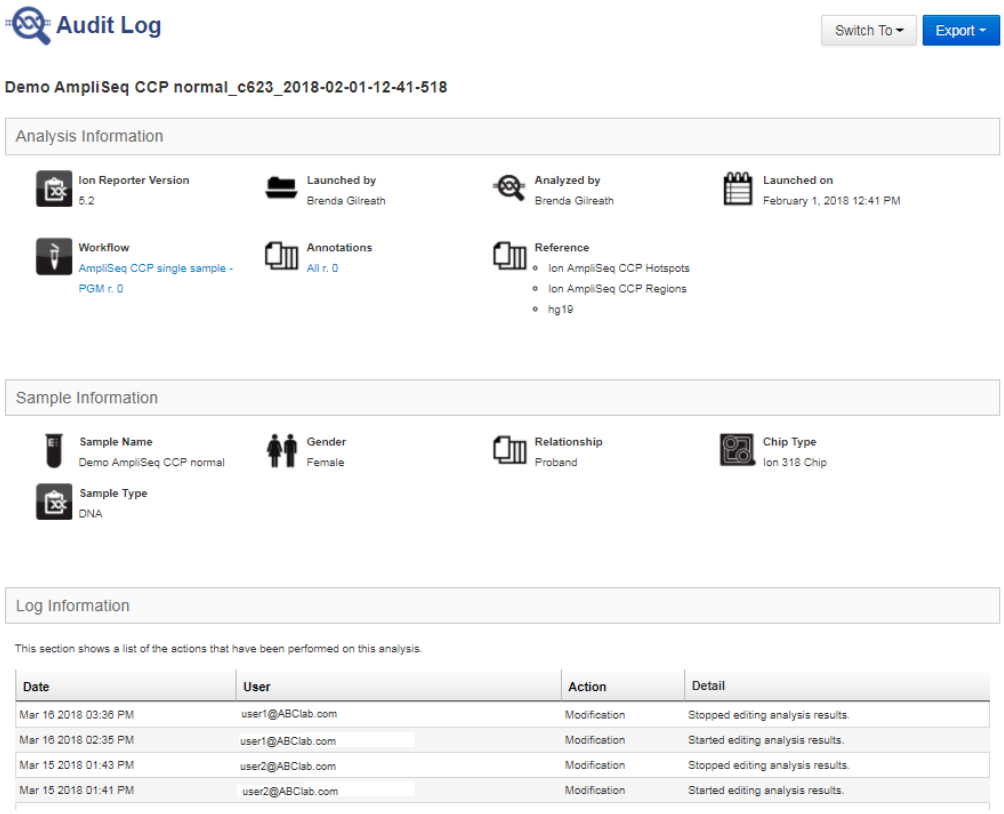
This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

Section	Description
Analysis information	Information on the analysis that was performed, such as: software version, analysis workflow, annotations, reference, launched by, and so on.
Sample information	Information on the samples that are part of the analysis, such as: gender, relationship, type, and so on.
Log information	The individual actions that have been recorded for this analysis including: date, time, user, action, and details on the action performed.

1. In the **Analysis** tab, click **Overview**.
2. Select the row of the analysis that you want to view.
3. Click **(Actions)** ▸ **Audit Log**.

   Alternatively, you can click the analysis link to open the **Analysis Results** screen, then click **Switch To** ▸ **Audit Log**.

   The **Audit Log** opens:



4. (*Optional*) Click **Export** ▸ **PDF**.

Download an audit log

You can download the audit logs that record user activities for samples, analyses and workflow presets in Ion Reporter™ Software.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Samples** or **Analyses** tabs, click **Overview**.
2. Select the row of the Sample or Analysis that you want to audit.

3. Click **Actions** ▸ **Audit Log**.
   The audit log opens.

4. Click **Export** ▸ **PDF**.
   The downloaded filename is `sample_name.pdf` or `analysis_name.pdf`.

10 Filters and filter chains

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About filters and filter chains

A filter chain is a set of filters that Ion Reporter™ Software uses to narrow the list of variants that are included in analysis results and visualizations. Filter chains are also available in analysis workflows.

You can select a filter chain when you view analysis results or visualizations to change the variants that are included. If you save the filter chain to an analysis result or visualization, the variants that are included reflect the filtered results when the analysis results or visualization is later opened. If you apply the filter chain temporarily, you can review the results before you decide whether to save the updated analysis results, or discard the changes. In either case, you can download a VCF file of the variants that are shown in the analysis results.

Ion Reporter™ Software includes predefined filter chains that you can apply to analysis workflows, analysis results, or visualizations. Most predefined analysis workflows include default filter chains, which you can change. If you add a filter chain to an analysis workflow, or change the default filter chain in an analysis workflow, the list of variants is filtered when the analysis workflow is launched. For more information, see “Change the default filter chain for an analysis workflow” on page 129.

You can also create user-defined filter chains that are built from predefined filter chains, or one or more filters. The filters are based on public and proprietary annotation sources and data types that are included in Ion Reporter™ Software. For more information, see “Annotation source filters” on page 455 and “Data type filters” on page 461. After you create a user-defined filter chain, you can add it to an analysis workflow, or apply to analysis results or visualizations.

Note: The `Tumor Mutational Burden Filter Chain` is a unique parameter that can be added to an analysis through the parameters that you add to an analysis workflow.

View filter chain details

You can view the details of predefined and user-defined filter chains in Ion Reporter™ Software. For example, you can check whether the genome reference version is suited to your research. If it is not, you can build a new filter chain. User-defined filter chains are created by combining filters to make a filter chain that meets specific variant filtering needs.

- When you plan your analysis workflow or create a workflow preset, you can view filter chain details from the **Workflows Presets** screen.
  a. Click **Workflows**, then click **Presets**.
  b. Select **Filter Chains** in the search filter category.
  c. Select the row that contains the filter chain of interest.
  d. In the **Details** pane, view the details.

Option	Description
Version	The software version when the filter was released.
Reference	The genome reference that is used in the filter chain.
Last Modified By	The person who last set or changed the filter chain settings.
Last Modified On	The date that the filter chain was last modified.
Created By	The person who created the filter chain.
Created On	The date that the filter chain was created.
Filter Chains	The list of other filter chains included in this filter chain and the order in which they are applied.
Filter chain query applied	The list of query settings, or filter chain logic, applied to the filter chain.

- When you view analysis results, you can review filter chain details from the **Analysis Results** screen.
  a. Click **Analyses**, then click **Overview**.
  b. Select the link for the analysis of interest.
  c. In the **Analysis Results** screen, view the details of the filter chain that is applied to an analysis.

Option	Description
Filter Chain	The name of filter chain applied. A filter chain from the list may be applied when the analysis workflow is launched, or applied to completed analysis results.
Total Variants	The total number of variants that are detected in the analysis.
Total Genes	The total number of genes that are detected in the analysis.
Filter chain details	A description of the applied filters and their condition settings.
Variants	The number of variants that are filtered in or filtered out.
Genes	The number of genes that are filtered in or filtered out.

Create a user-defined filter chain

You can create a user-defined filter chain to refine analysis results in Ion Reporter™ Software. A filter chain is a set of filters that can be applied to the variants identified in an analysis that are used to narrow the analysis results to only the variants of interest. After you create a user-defined filter chain, you can associate the filter chain with an Ion Reporter™ Software analysis workflow when you create or edit the analysis workflow. You can also apply the filter chain to analysis results.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

1. To create a user-defined filter chain, use one of the following procedures to get started.

Option	Description
Workflow Presets	a. In the **Workflows** tab, click **Presets**. b. Click **Create Preset** ▸ **Filter Chain**.
Analysis Results	a. In the **Analysis** tab, click **Overview**. b. Click the link of an analysis of interest. The **Analysis Results** screen appears. c. In the right pane, in the **Filter Chains** section, click **(New)**.

2. In the **Create Filter Chain** dialog box, enter a name and, optionally, a description.
3. In **Reference**, select the reference type to use for this filter chain.
4. Apply filters to the filter chain.

   The available filters depend on the selected reference type.

   a. From the **Choose Filter** list, select one or more filters to add.

      For more information, see “Annotation source filters” on page 455 and “Data type filters” on page 461.

   b. If more settings are available, set the appropriate conditions for the selected filter.

      For more information, see “Annotation source filters” on page 455.

c. Click **Add** to add the filter to the **Selected Filters** list.

d. Repeat to add more filters.

   The filter count is incremented (for example, --2) as you add more filters, and the selected filters are added to the **FilterChain Query** in order.

5. *(Optional)* Use **Update** to add a second instance of an existing filter, or to configure and replace the last filter instance in the list without changing the filter count.

6. Click **Save**.

The filter chain is available to be associated as the default filter chain when an analysis workflow is created, or to be used to change an existing default filter chain. For more information, see "Add a filter chain to an analysis workflow" on page 124 and "Change the default filter chain for an analysis workflow" on page 129.

The filter chain can also be applied to analysis results or to a visualization. For more information, see "Apply a filter chain to analysis results" on page 195 and "Use filter chains to change analysis results" on page 236.

Create a gene symbol filter

To filter variants in analysis results by many gene symbols, you can create a filter that is based on gene symbols, then add the filter to a filter chain.

If you have a small number of gene symbols to that you want to filter in an analysis, see instead "Create a gene symbol filter chain" on page 438.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

Before you complete this procedure, create a text file that contains one gene symbol on each line. For example:

Gene symbols must use the nomenclature standard that is published by the HUGO Gene Nomenclature Committee (HGNC). Gene symbols that are used in the file must match the nomenclature, however, the letters are not case-sensitive.

Gene Symbol Example.txt

```
1  AKT
2  AKT3
3  ALK
4  AR
5  AXL
6  BRAF
7  CCND1
```

1. In the **Workflows** tab, click **Presets**.

2. Click **Create Preset** ▸ **Filter Chain**.

3. Name, then upload the text file that contains the gene symbols.

   a. In the **Create Filter Chain** dialog box, enter a name and an optional description.

   b. In the **Choose Filter** list, select **Gene Symbol**.

c. Click **Select file** , browse to your gene symbol list TXT file, then click **Upload**.
The gene names from the text file that are valid are listed in the **Selected** list. Any gene symbol names or abbreviations that are not correct are listed in the **Rejected** list.

d. If you have gene names in the **Rejected** list, correct the text file, then upload the file again to add the corrected gene symbols.

4. Click **Save**.

The gene symbol filter is available to be added to a new filter chain or to an existing filter chain. Then, the filter chain can be added to an analysis workflow. For more information, see “Add a filter chain to an analysis workflow” on page 124.

You can also add the filter chain to analysis results or to a visualization. For more information, see “Apply a filter chain to analysis results” on page 195 and “Use filter chains to change analysis results” on page 236.

Edit a filter chain

You can edit a user-defined filter chain to change the conditions or remove filters in Ion Reporter™ Software. You cannot edit a predefined filter chain.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

1. To edit a user-defined filter chain, use one of the following procedures to get started.

Option	Description
Workflow Presets	a. In the **Workflows** tab, click **Presets**. b. Select **Filter Chains** from the list. c. Select the row of the filter chain that you want to modify, then click **Edit**.
Analysis Results	a. In the **Analyses** tab, click **Overview**. b. Click the link of an analysis of interest. The **Analysis Results** screen appears. c. In the right pane, in the **Filter Chains** section, select the user-defined filter chain that you want to edit, then click **(Edit)**.
Visualize	a. In the **Analyses** tab, click **Overview**. b. Click the link of an analysis of interest. The **Analysis Results** screen appears. c. Click **Visualize**, then select the **IRGV & Generate Report** tab. d. In the right pane, in the **Filter Chains** section, select the user-defined filter chain that you want to edit, then click **(Edit)**.

2. Update the **Edit Filter Chain** dialog box.

   If you edit a filter chain, and want to cancel the edits, refresh the screen to clear all edits before you click **Cancel**. Otherwise, some changes that were made in the **Edit Filter Chain** dialog box, can persist in the dialog box.

Option	Description
Change name	In **Name**, change the filter chain name.
Change description	In **Description**, change the filter chain description.
Change reference	Under **Reference**, select **GRCh38** or **hg 19**.
Modify FilterChain Query	In the **Choose Filter** list, select a filter. In the **FilterChain Query** field, cut-and-paste the filter names to change the order in which filters are applied.
Modify filter conditions	In the **Choose Filter** list, select a filter that is included in the filter chain. Change the conditions of the filter, then click **Add**. *(Optional)* Use **Update** to define and replace the last filter in the list without changing the filter count.
Delete filters	To remove a filter that is included in a filter chain, select the filter, then click **(Delete)**.

3. Click **Save**.

The edited filter chain is available to be associated as the default filter chain when an analysis workflow is created, or to be used to change an existing default filter chain. For more information, see “Add a filter chain to an analysis workflow” on page 124 and “Change the default filter chain for an analysis workflow” on page 129.

It can also be applied to analysis results or a visualization. For more information, see “Apply a filter chain to analysis results” on page 195 and “Use filter chains to change analysis results” on page 236.

Lock a user-defined filter chain in workflow presets

After you create a user-defined filter chain in Ion Reporter™ Software, you can lock it. A locked filter chain cannot be edited or unlocked.

1. In the **Workflows** tab, click **Presets**.
2. In the search filter category, select **Filter Chains**.
3. In the row of the filter chain that you want to lock, click **(Actions)** ▸ **Lock**.
4. In the **Lock Filter Chain** dialog box, click **Yes** to lock the filter chain.

The filter chain preset has a lock symbol in its row on the **Workflow Presets** screen.

Delete a user-defined filter chain in workflow presets

You can delete a user-defined filter chain from the available filter chains list in Ion Reporter™ Software. You cannot delete a predefined filter chain.

User-defined filter chains that are in use by an analysis workflow cannot be deleted.

1. In the **Workflows** tab, click **Presets**.
2. Select **Filter Chains**.
3. In the row of the filter chain that you want to delete, click **(Actions)** ▸ **Delete**.
4. In the **Confirm Delete** dialog box, click **Yes** to delete the filter chain.

The filter chain is removed from the **Workflow Presets** table and is no longer available in the **Filters** step when creating an analysis workflow. It is also no longer available from the **Filter Chains** lists in the **Analysis Results** and **Visualization** screens.

Filter chain examples

You can create different types of user-defined filter chains, depending on your research requirements.

Create an Expected Normal Ploidy Buffer filter chain

Mosaic analysis workflows can increase the frequency of false positives around normal ploidy, which is 2N for autosomes and the X chromosome in females, or 1N for sex chromosomes in males. You can create an Expected Normal Ploidy Buffer (ENPB) filter chain and add it to an analysis workflow to filter false positives in these analyses.

When you create the filter chain, you can adjust the range values when to customize the amount of buffer. For more information about the filter, see “Expected Normal Ploidy Buffer filter” on page 465.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

---

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Filter Chain**.
3. In the **Create Filter Chain** dialog box, select the dropdown list of filter chains, and enter ***Expected*** to find, then select the **Expected Normal Ploidy Buffer** filter chain.

4. Set the filter chain values and settings.

Option	Description
Expected Normal Ploidy Buffer filter	By default, the ENPB filter filters out all copy number variant segments of gain or loss within 0.2 ploidy value of expected normal. With this setting, you can set the filter to filter ploidy changes for the following ranges: • between 2.2 and 1.8 on autosomes and female X chromosomes • between 1.2 and 0.8 on male X and Y chromosomes
Confidence	A log ratio that is between the called ploidy state likelihood of the region and the expected ploidy state likelihood. Large confidence values increase the likelihood that the software results indicate that the ploidy state differs from what is expected. **Recommended values:** > .1 for non-mosaic ploidy calling and for high-sensitivity detection, and > 1 for mosaic ploidy calling.

5. Enter a name and, optionally, a description.

6. Click **Save**.

The ENPB filter chain can now be added to a mosaic analysis workflow. When an analysis is launched with the analysis workflow, the ENPB filter chain filters for false positives. For more information, see “Add a filter chain to an analysis workflow” on page 124, “Create a user-defined analysis workflow for mosaicism, No Gender, or different tile sizes” on page 90, and “Launch an analysis” on page 165.

Create a gene symbol filter chain

You can create a user-defined filter chain that is based on gene symbols. This filter chain can be used to narrow analysis results in Ion Reporter™ Software to only specific genes. If you have numerous gene symbols to filter, you can use a text file to create the gene symbol filter chain. For more information, see “Create a gene symbol filter” on page 434.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

---

1. In the **Workflows** tab, click **Presets**.

2. Click **Create Preset** ▸ **Filter Chain**.

3. In the **Create Filter Chain** dialog box, enter a name and an optional description.

4. Select a **Reference**.

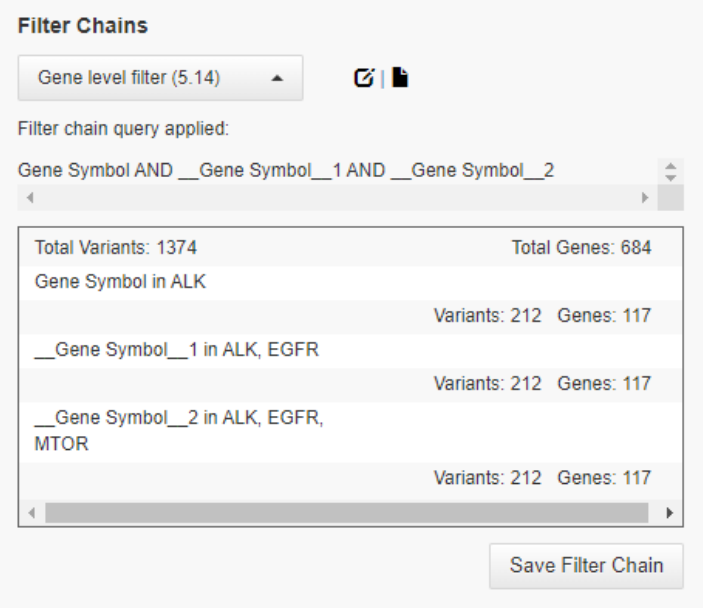
5. In the **Choose Filter** list, select **Gene Symbol**.

6. Add genes of interest in the list.
   - Select a checkbox for a gene symbol in the list.
   - To find gene symbols in the list, enter the gene symbol into the search field, then click **Go**.

7. After you select the gene symbols that you want to include in the list, click **Add**.
   Your genes appear in the **Selected Filters** list in the **Create Filter Chain** dialog box.
8. Repeat to add more genes.
9. *(Optional)* Use **Update** to configure and replace the last filter in the list without changing the filter count.
10. Click **Save**.

The gene symbol filter chain is available be added to an analysis workflow, or applied to analysis results or a visualization. For more information, see “Add a filter chain to an analysis workflow” on page 124, “Apply a filter chain to analysis results” on page 195, and “Use filter chains to change analysis results” on page 236.

Example of a gene symbol filter chain that is applied to analysis results.



Create a filter chain that specifies the order in which filters are applied

You can create user-defined filter chains that combine filters and specify the order in which they are applied in Ion Reporter™ Software. The modifiers are AND and OR and brackets ( ) are used to set the order. The modifiers AND and OR must be in all caps.

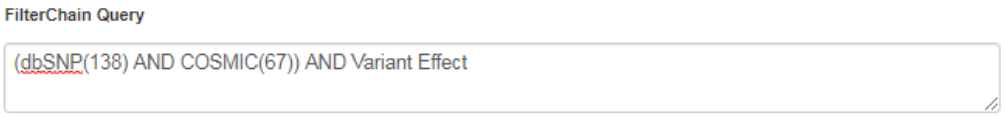
IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

---

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Filter Chain**.

3. In the **Create Filter Chain** dialog box, add the filters to create a chain.
   a. Enter a name and, optionally, a description.
   b. Select the first filter, set its value to **In**, then click **Add**.
      The filter name appears on the right side of the **Create Filter Chain** dialog box.
   c. Add another filter, set its value to **In**, then click **Add**.
      The second filter name appears on the right side of the **Create Filter Chain** dialog box.
   d. Add a third filter, set its value to **In**, then click **Add**.
      The third filter name appears on the right side of the **Create Filter Chain** dialog box.
   e. *(Optional)* Use **Update** to configure and replace the last filter in the list without changing the filter count.
4. In **FilterChain Query**, your selected filters are listed by name. You can change the order in which your filters are applied.
   - Enter brackets ( ) around the filter names that you want the software to apply first. All other filters are applied in order, that is, left to right.

   In this example, the dbSNP and COSMIC filters are applied first, and then the Variant Effect filter is applied.

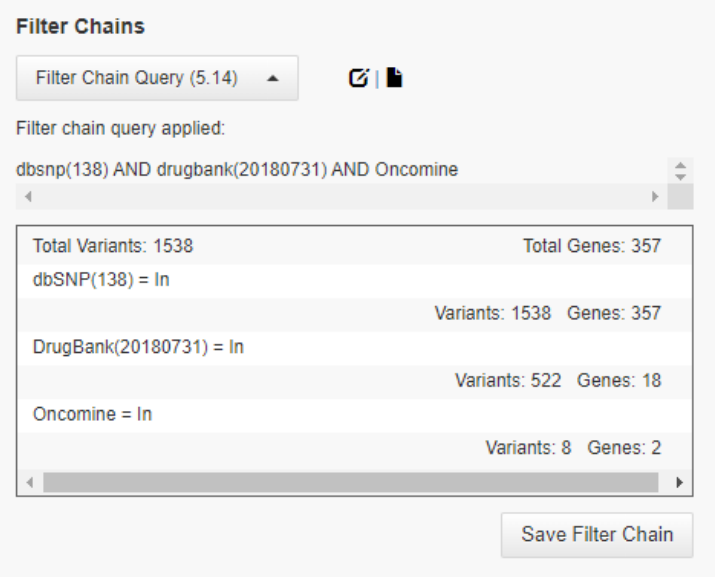
   - Enter or edit the modifiers. Enter AND to include all filters that are specified, or enter OR to specify that another filter be run if the first filter did not detect any variants.

   In this example, the dbSNP and COSMIC filters are applied, if no variants were detected, then the Variant Effect filter is applied.
5. Click **Save**.

The filter chain can now be added to an analysis workflow, or applied to analysis results or a visualization. For more information, see “Add a filter chain to an analysis workflow” on page 124, “Apply a filter chain to analysis results” on page 195, and “Use filter chains to change analysis results” on page 236.

Example of a filter chain query applied to analysis results.



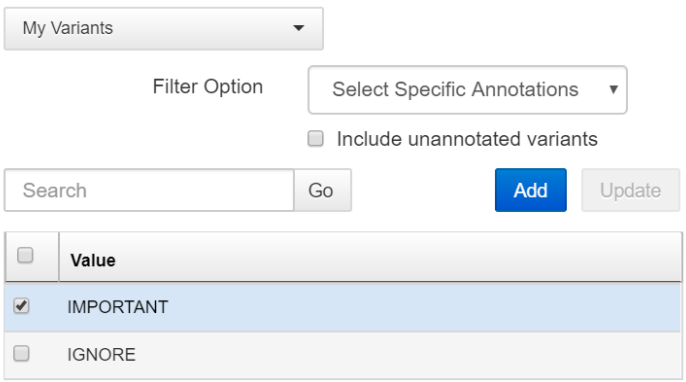
Create a MyVariants and Variant Classification filter chain

You can create a user-defined filter chain to narrow the analysis results to include only the variants that are flagged and classified by members of your Ion Reporter™ Software organization. This example shows how to set up a filter chain for MyVariants that are flagged as **Important** and classified as **Deleterious** in Ion Reporter™ Software.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

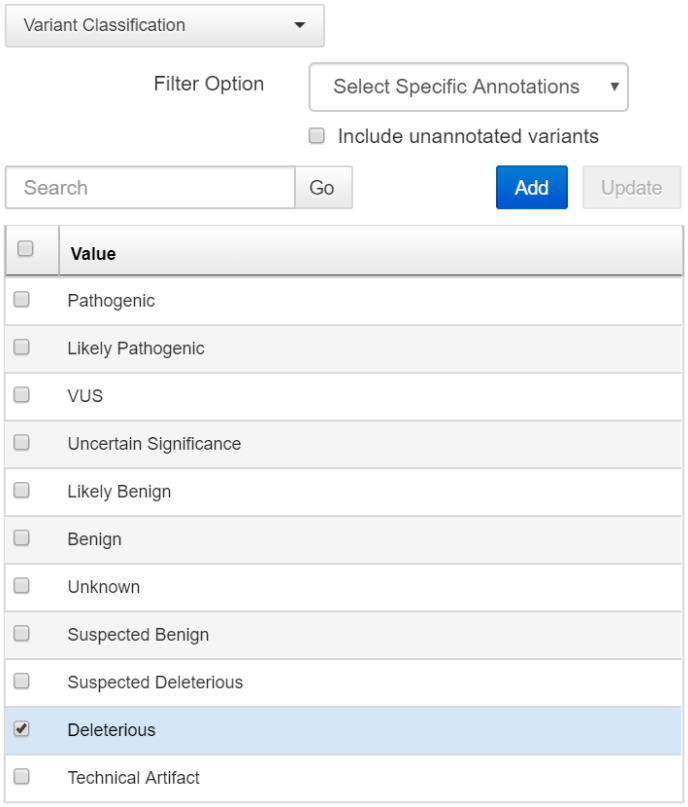
---

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Filter Chain**.
3. In the **Create Filter Chain** dialog box, enter a name and optional description.
4. In the **Choose Filter** list, select **MyVariants**.
   a. Select **Important**.



b. Click **Set**.

5. In the **Choose Filter** list, select **Variant Classification**.
   a. Select **Deleterious**.

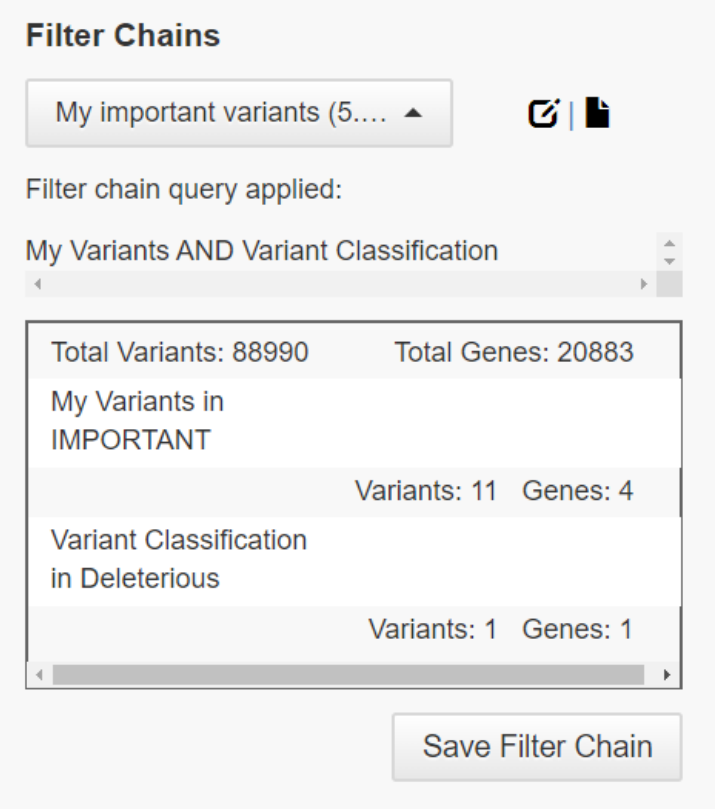


   b. Click **Set**.

6. Click **Save**.

The user-defined filter chain is available to be added to an analysis workflow, or applied to analysis results or a visualization. For more information, see “Add a filter chain to an analysis workflow” on page 124, “Apply a filter chain to analysis results” on page 195, and “Use filter chains to change analysis results” on page 236.

Example of a MyVariants and Variant Classification filter chain applied to analysis results.



Create a COSMIC, ClinVar, MAF, and Variant Effect filter chain

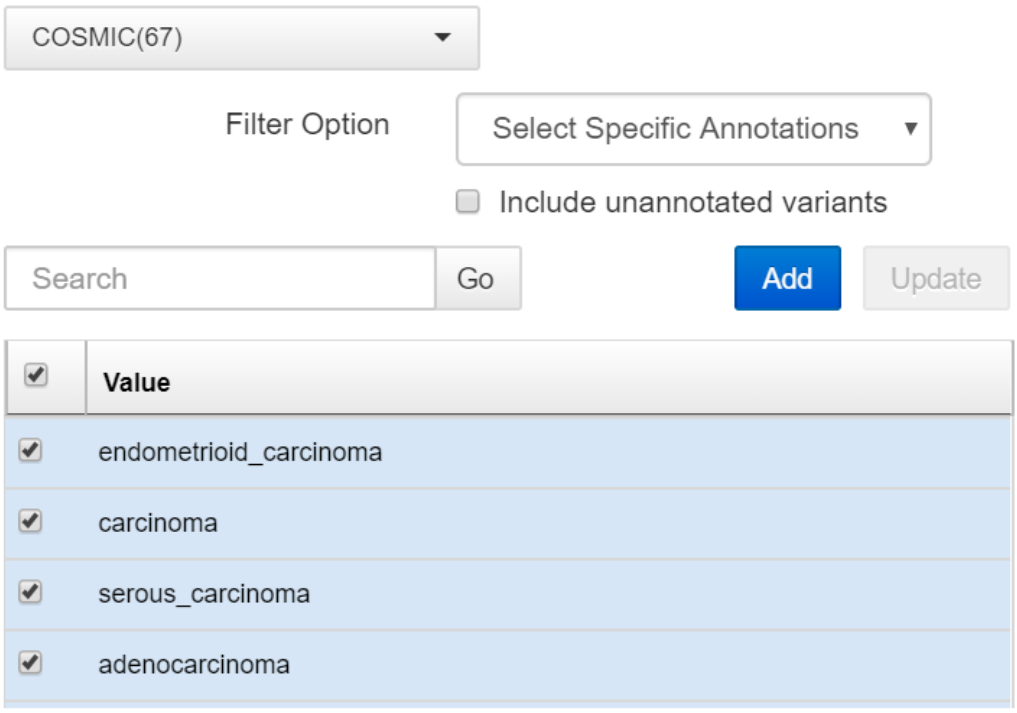
You can create a IR 5.22: user-defined filter chain that includes both annotation source filters and Ion Reporter™ Software variant data type filters. This procedure shows how to create a COSMIC, ClinVar, Minor Allele Frequency (MAF), and Variant Effect filter chain.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

---

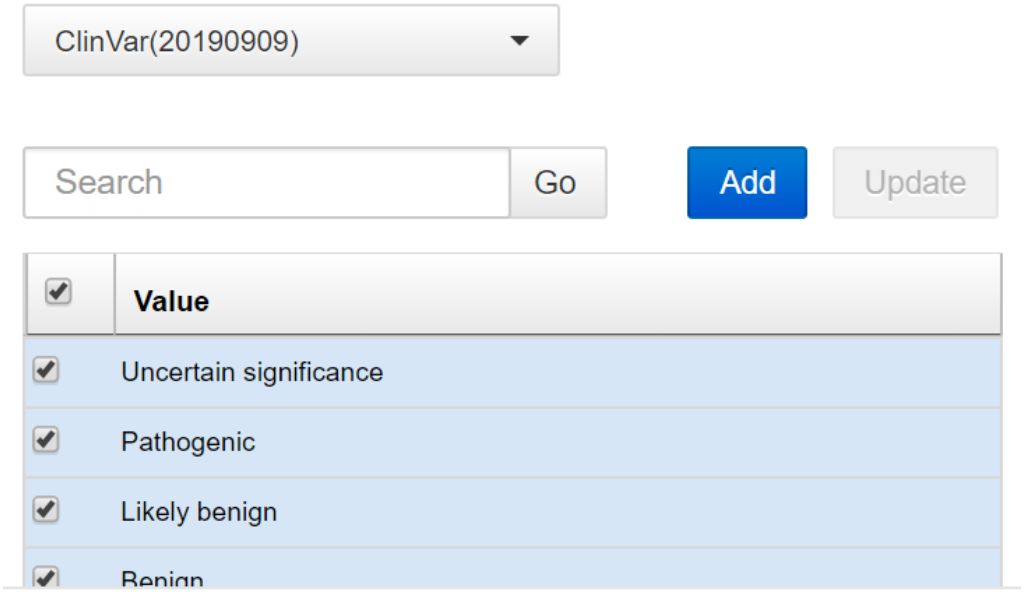
1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Filter Chain**.
3. In the **Create Filter Chain** dialog box, enter a name and, optionally, a description.

4. In the **Choose Filter** list, select **COSMIC**.
   a. Select the top checkbox to select all values.



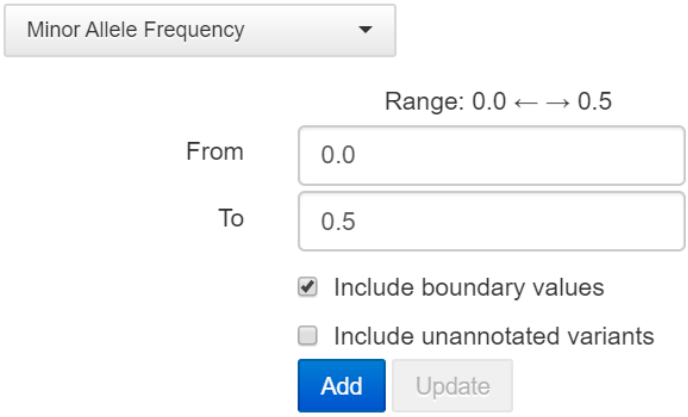
   b. Click **Set**.

5. In the **Choose Filter** list, select **ClinVar**.
   a. Select the top checkbox to select all values.



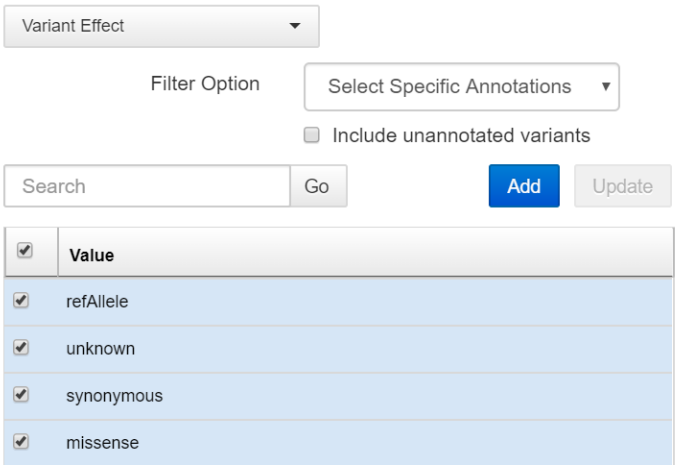
   b. Click **Set**.

6. In the **Choose Filter** list, select **Minor Allele Frequency**.
   a. Enter a range value, then select **Include boundary values**.



   b. Click **Set**.

7. In the **Choose Filter** list, select **Variant Effect**.
   a. Select the top checkbox to select all values.

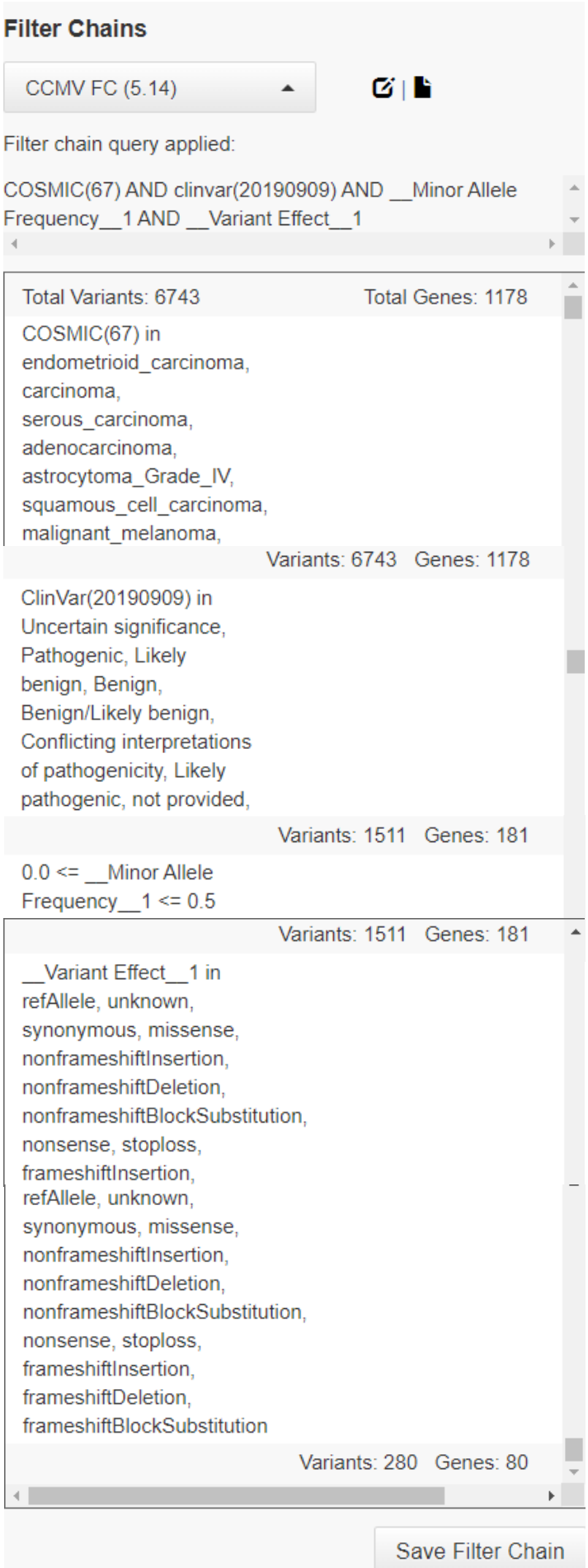


   b. Click **Set**.

8. Click **Save**.

Your filter chain can now be added to an analysis workflow, or applied to analysis results or a visualization. For more information, see “Add a filter chain to an analysis workflow” on page 124, “Apply a filter chain to analysis results” on page 195, and “Use filter chains to change analysis results” on page 236.

Example of a COSMIC, ClinVar, MAF, and Variant Effect filter chains applied to analysis results.


Filter Chains
CCMV FC (5.14)
Filter chain query applied:
COSMIC(67) AND clinvar(20190909) AND __Minor Allele Frequency__1 AND __Variant Effect__1
Total Variants: 6743
Total Genes: 1178
COSMIC(67) in endometrioid_carcinoma, carcinoma, serous_carcinoma, adenocarcinoma, astrocytoma_Grade_IV, squamous_cell_carcinoma, malignant_melanoma,
Variants: 6743 Genes: 1178
ClinVar(20190909) in Uncertain significance, Pathogenic, Likely benign, Benign, Benign/Likely benign, Conflicting interpretations of pathogenicity, Likely pathogenic, not provided,
Variants: 1511 Genes: 181
0.0 <= __Minor Allele Frequency__1 <= 0.5
Variants: 1511 Genes: 181
__Variant Effect__1 in refAllele, unknown, synonymous, missense, nonframeshiftInsertion, nonframeshiftDeletion, nonframeshiftBlockSubstitution, nonsense, stoploss, frameshiftInsertion, refAllele, unknown, synonymous, missense, nonframeshiftInsertion, nonframeshiftDeletion, nonframeshiftBlockSubstitution, nonsense, stoploss, frameshiftInsertion, frameshiftDeletion, frameshiftBlockSubstitution
Variants: 280 Genes: 80
Save Filter Chain

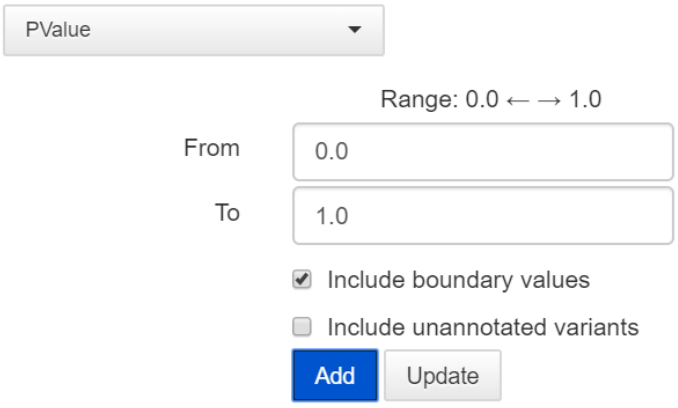
Create a PValue, dbSNP, and Variant Type filter chain

You can create a user-defined filter chain with multiple filters in Ion Reporter™ Software. This example shows how to create a filter chain with PValue, dbSNP, and Variant Type filters.

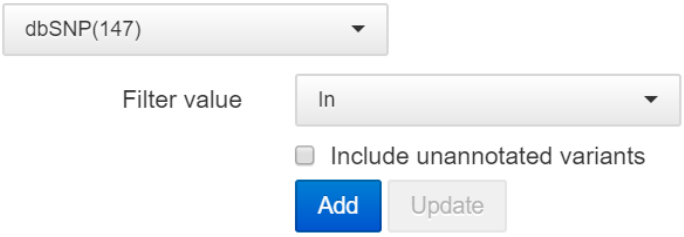
IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

---

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Filter Chain**.
3. In the **Create Filter Chain** dialog box, enter a name and an optional (*optional*) description.
4. In the **Choose Filter** list, select **PValue**.
   a. Enter a range, then select **Include boundary values**.

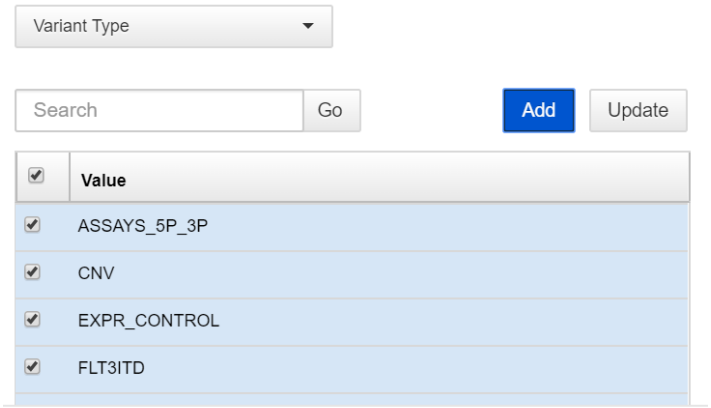


   b. Click **Set**.
5. In the **Choose Filter** list, select **dbSNP**.
   a. Set **Filter value** to **In**.



   b. Click **Set**.

6. In the **Choose Filter** list, select **Variant Type**.
   a. Select the checkbox to select all values.

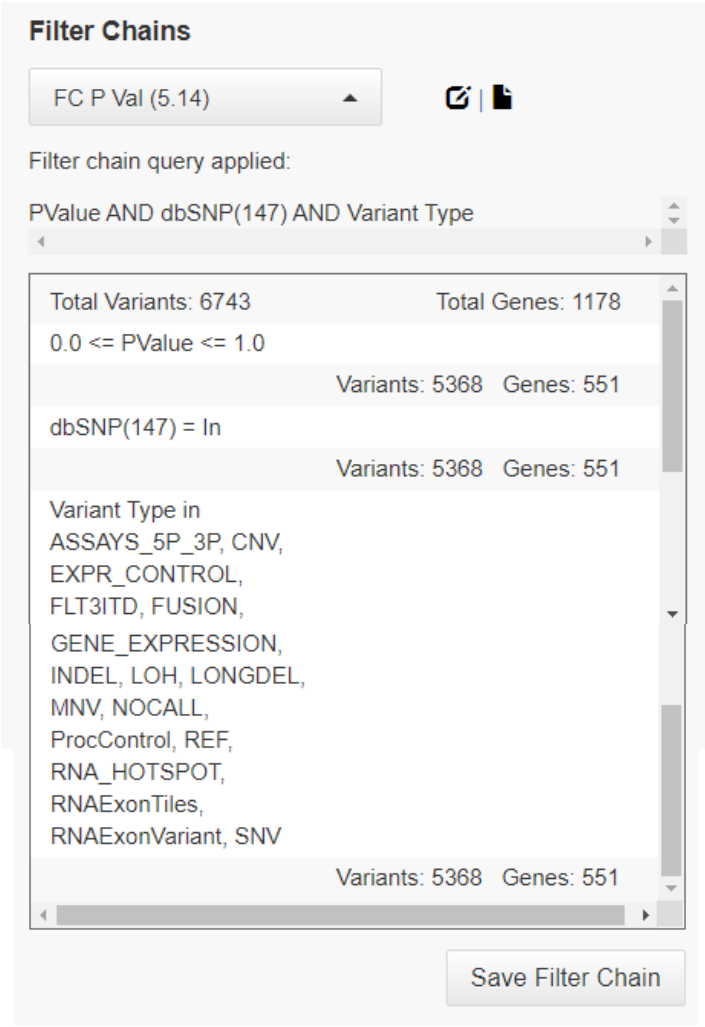


   b. Click **Set**.

7. Click **Save**.

Your filter chain is available to be added to an analysis workflow, or applied to analysis results or a visualization. For more information, see “Add a filter chain to an analysis workflow” on page 124, “Apply a filter chain to analysis results” on page 195, and “Use filter chains to change analysis results” on page 236.

Example of a PValue, dbSNP, and Variant Type filter applied to analysis results.



Predefined filter chains

Ion Reporter™ Software includes predefined filter chains that you can apply to analysis workflows, analysis results, or visualizations. Filter chains are sets of filters that Ion Reporter™ Software uses to narrow the list of variants that are included in analysis results. The filter chains are based on public and proprietary annotation sources and data types that are included in Ion Reporter™ Software. For more information, see “Annotation source filters” on page 455 and “Data type filters” on page 461.

You cannot edit predefined filter chains, but you can create user-defined filter chains that are built from predefined filter chains, or from one or more filters. For more information, see “Create a user-defined filter chain” on page 433.

The available filter chain version depends on the versions of the analysis workflows that are installed in the software. It is recommended that you use the filter chain version that is compatible with the analysis workflow that you use. Some predefined filter chains are selected by default as the compatible filter chain when you create or edit an analysis workflow, or view analysis results.

Details, including the version and the logic that is used by each filter chain, can be viewed in the software. For more information, see "View filter chain details" on page 432.

Predefined filter chain name	Available versions	Reference genome	Description
AmpliSeq Exome Tumor Normal v1	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain detects all CNVs, confident somatic variants with allele ratios between 0.1 and 1.0, allele read counts between 4 and 1,000,000, and PValue between 0 and 5.0E-6. This is the default filter chain for Ion AmpliSeq™ Exome tumor-normal pair analysis workflows. The confidence range is 10.0 to 1.0E7.
Aneuploidy Mosaicism	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain detects mosaicism by allowing decimal-level copy number gain or loss calls with a confidence score of at least 0.1, while filtering out false positive calls near expected normal copy number. This filter chain is turned on by default in the ReproSeq Mosaic PGS w1.1 analysis workflow, and is not selected by default in other predefined aneuploidy analysis workflows.
Called Hotspot Variants and Controls	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain reports all hotspot variants that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, and RNA Exon Tiles.
Called Variants and Controls	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain reports all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3-ITD, RNA Exon Tiles.
CNVs of Confidence ≥0.1–Germline–CNVs only	• 5.16 • 5.18 • 5.20 • 5.22	hg19	For germline analyses, this filter chain narrows the analysis results to copy number variants with a confidence value of ≥0.1.
Confident Germline CNVs–CNVs Only	• 5.16 • 5.18 • 5.20 • 5.22	hg19	For germline analyses, this filter chain narrows the analysis results to copy number variants with a confidence value of ≥10. Not a default filter.
Confident Somatic CNVs–CNVs Only	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain includes 5% confidence interval range and 95% confidence interval range.

(continued)

Predefined filter chain name	Available versions	Reference genome	Description
Default CarrierSeq View	5.16	GRCh38	This is the default filter chain for CarrierSeq analysis workflows. It filters out all of the reference calls and displays the others that are relevant to genotypes and CNVs. For more information, see *Ion Torrent™ Ion CarrierSeq™ ECS Kits User Guide*, MAN0018483.
Default DNA and Fusions View	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This is the default filter chain for the Ion AmpliSeq™ Colon Lung v2 with RNA Lung Fusion single-sample analysis workflow. Either fusion detection is present, or the variant type is not fusion. Results include: • FUSIONS variants detected as **Present** • All EXPR_CONTROL markers • All ASSAYS_5P_3P markers • DNA variants of all types
Default Fusions View	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This is the default filter chain for the Ion AmpliSeq™ RNA Lung fusion single-sample analysis workflow. Either fusion detection is present, or the variant type is: • EXPR_CONTROL • ASSAYS_5P_3P • RNA_HOTSPOT • GENE_EXPRESSION
Default Germline Variants view	5.22	hg19	This is the default filter chain of the Ion AmpliSeq™ germline custom CNV workflow or assay to refine their copy number variation (CNV) analysis results. It returns CNVs based on the specific filter conditions and all “Present” SNV/INDEL and fusion variants. To pass this filter, CNVs must meet specific confidence thresholds. You can enable or disable the default filter chain during assay creation and in the CNV results table.
Default Germline Variants view GRCh38	5.22	GRCh38	This is the default filter chain of the Ion AmpliSeq™ germline custom CNV workflow or assay to refine their copy number variation (CNV) analysis results. It returns CNVs based on the specific filter conditions and all “Present” SNV/INDEL and fusion variants. To pass this filter, CNVs must meet specific confidence thresholds. You can enable or disable the default filter chain during assay creation and in the CNV results table.

(continued)

Predefined filter chain name	Available versions	Reference genome	Description
Default Somatic Variants view	5.22	hg19	This is the default filter chain of the Ion AmpliSeq™ somatic custom CNV workflow or assay to refine copy number variation (CNV) analysis results. This filter chain defines criteria for both copy number amplification (gain) and depletion (loss). Only targets that meet these criteria are reported in the results table when the filter chain is applied. You can enable or disable the default filter chain during assay creation and in the CNV results table.
Default Somatic Variants view GRCh38	5.22	GRCh38	This is the default filter chain of the Ion AmpliSeq™ somatic custom CNV workflow or assay to refine copy number variation (CNV) analysis results. This filter chain defines criteria for both copy number amplification (gain) and depletion (loss). Only targets that meet these criteria are reported in the results table when the filter chain is applied. You can enable or disable the default filter chain during assay creation and in the CNV results table.
Default Variant View	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain narrows the analysis results to confident variant types, which are not CNVs, but can include SNV, INDEL, MNV, REF, NOCALL, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3-ITD.
Genetic Disease Variants	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This filter chain narrows the analysis results to genetically-relevant variant types: IsNewlyHomozygousNonRef, HasDeNovoNonRefAllele, HasUnknownX, InTransPhaseCompoundHeterozygote, and HasMaleMaternalX. This is the default filter chain for the Ion AmpliSeq™ Exome trio and Ion AmpliSeq™ IDP trio analysis workflows.
Oncomine™ BRCA	• 5.16 • 5.18 • 5.20 • 5.22	hg19	This is the default filter chain for Oncomine™ BRCA analysis workflows. It removes any variants in the sample ID amplicons that are not in the BRCA1 and BRCA2 genes.

(continued)

Predefined filter chain name	Available versions	Reference genome	Description
Oncomine™ Extended	• 5.22 • 5.18 • 5.20	hg19	This filter chain includes all Oncomine™-annotated variants and variants that may be relevant to cancer due to their inclusion in one or more of the following classes: • Targeted and non-targeted fusions. • CNV variants with FILTER value of GAIN or LOSS. • Likely somatic mutations based on dbSNP, 5000Exomes, gnomAD (ExAC in versions 5.18 and 5.20), and UCSC Common SNPs annotation source databases. The minor allele frequencies range lies between 0.0 and 1.0E-6. Mutations must also be nonsynonymous and occur in exonic or splice-site regions. • Variants with ClinVar annotations of pathogenic or likely pathogenic.
Oncomine Extended GRCh38	5.22	GRCh38	This filter chain includes all Oncomine™-annotated variants and variants that may be relevant to cancer due to their inclusion in one or more of the following classes: • Targeted and non-targeted fusions. • CNV variants with FILTER value of GAIN or LOSS. • Likely somatic mutations based on dbSNP, 5000Exomes, gnomAD (ExAC in versions 5.18 and 5.20), and UCSC Common SNPs annotation source databases. The minor allele frequencies range lies between 0.0 and 1.0E-6. Mutations must also be nonsynonymous and occur in exonic or splice-site regions. • Variants with ClinVar annotations of pathogenic or likely pathogenic.
Oncomine™ Variants	• 5.18 • 5.20 • 5.22	hg19	This filter chain includes all Oncomine™-annotated variants.
Oncomine™ Variants, 5% CI CNV ploidy ≥gain of 2 over normal	• 5.20 • 5.22	hg19	This filter chain restricts copy number variants to gains of > 2 based on the 5% confidence interval level. It also returns Oncomine™-annotated variants.

(continued)

Predefined filter chain name	Available versions	Reference genome	Description
TMB (Non-germline Mutations)	• 5.18 • 5.20 • 5.22	hg19, GRCh38	This filter chain returns results for somatic variants based on dbSNP, 5000Exomes, gnomAD (ExAC in versions 5.18 and 5.20), and UCSC Common SNPs annotation source databases. The minor allele frequencies range is between 0.0 and 1.0E-6. This filter chain also filters out variants of homopolymer lengths >7. The **Tumor Mutational Burden Filter Chain** parameter for this filter chain must be enabled for Ion Reporter™ Software to generate tumor mutational burden analysis results. By default, tumor mutational burden calculation is disabled. You must also copy and edit either a DNA-Single Sample or a DNA and Fusions-Single Sample analysis workflow to enable tumor mutational burden calculations on DNA samples. Unlike other Ion Reporter™ Software filter chains, the TMB (Non-germline Mutations) filter chain generates final analysis results, and cannot be used to change the variants that are included in the analysis results. That is, tumor mutational burden results that are generated by the use of this filter chain cannot be changed after an analysis is complete. The filter chain is applied before the parameter application. The filtered variant file is passed to the TML script that further applies the user parameters.
TMB GRCh38 (Non-germline Mutations)	• 5.18 • 5.20 • 5.22	GRCh38	This filter chain returns results for somatic variants based on dbSNP, 5000Exomes, gnomAD (ExAC in versions 5.18 and 5.20), and UCSC Common SNPs annotation source databases. The minor allele frequencies range is between 0.0 and 1.0E-6. This filter chain also filters out variants of homopolymer lengths >7. The **Tumor Mutational Burden Filter Chain** parameter for this filter chain must be enabled for Ion Reporter™ Software to generate tumor mutational burden analysis results. By default, tumor mutational burden calculation is disabled. You must also copy and edit either a DNA-Single Sample or a DNA and Fusions-Single Sample analysis workflow to enable tumor mutational burden calculations on DNA samples. Unlike other Ion Reporter™ Software filter chains, the TMB (Non-germline Mutations) filter chain generates final analysis results, and cannot be used to change the variants that are included in the analysis results. That is, tumor mutational burden results that are generated by the use of this filter chain cannot be changed after an analysis is complete. The filter chain is applied before the parameter application. The filtered variant file is passed to the TML script that further applies the user parameters.

(continued)

Predefined filter chain name	Available versions	Reference genome	Description
Variant Matrix Summary	• 5.16 • 5.18 • 5.20 • 5.22	hg19	Select this filter chain for analysis results that include the same set of variants that are included in the Variant Matrix Analysis visualization for Ion AmpliSeq™ HD analysis results. The **Variant Matrix Summary** filter chain filters in: • Positive hotspot calls • Positive gene-level CNV, GAIN, or LOSS calls • FLT3-ITD variants • Non-wild-type intragenic structural variations • For TagSeq Breast and TagSeq Pan-Cancer assays: non-hotspot variants in TP53 with QUAL >50 and Allele Frequency > 1.0 (Breast) or 0.5 (Pan Cancer) This filter chain allows analysis results to be exported. You can then import the results into another software application for further analysis. Filter variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants. If you use the Oncomine™ Myeloid MRD analysis workflow, a FLT3-ITD variant subtype named **Combined** can be reported in analysis results. The **Combined** subtype is a value for the sum of all reported FLT3-ITD variant allele frequencies.

Predefined filters

Ion Reporter™ Software includes predefined filters that you can apply to analysis workflows, analysis results or visualizations. The filters are categorized as annotation source filters and data type filters.

Annotation source filters

You can create filter chains that identify variants based on the annotations associated with those variants. The software uses annotation sources from public and proprietary genomic databases to apply these annotations to variants during analysis.

Filters that are made from annotation sources are available in the software and you can use these filters to build filter chains that narrow the list of variants that appear in the analysis results.

5000Exomes filters

The 5000Exomes filters can be added to filter chains to detect minor allele frequencies (MAF) for specific population groups in the software. The filters are derived from the 5000Exomes annotation database. The specific filters are as follows.

- 5000Exomes AfricanAmerican MAF(20161108)
- 5000Exomes EuropeanAmerican MAF(20161108)
- 5000Exomes Global MAF(20161108)

Filter conditions include the ability to set minor allele frequency ranges from 0.0 to 0.5. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns minor allele frequency results in samples that align with the 5000Exomes minor allele frequency database.

Clinvar filter

This filter can be added to filter chains in the software to evaluate the impact of variants observed in samples that match those in the National Center for Biotechnology Information (NCBI) ClinVar database. The specific filters are as follows.

- Clinvar (20190909)
- Clinvar (20170404
- Clinvar (20200329)
- Clinvar (20230311)
- Clinvar (20180729)
- Clinvar (20160802)
- Clinvar (20201121)
- Clinvar (20220709)

The impact values that can be included in the filter chain are: Pathogenic, Likely benign, Benign, other, Likely pathogenic, Uncertain significance, not provided, and drug response.

The filter returns all variants with the selected impact values that match those in the NCBI ClinVar database.

COSMIC filter

This filter can be added to filter chains in the software to compare variants to the catalog of somatic mutations in tumor tissue as compiled by the COSMIC database. The specific databases are as follows.

- COSMIC(85)
- COSMIC(89)

Filter conditions include the ability to select all COSMIC values, to select specific annotation values, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants that match those in the COSMIC database.

dbSNP filter

This filter can be added to filter chains in the software to compare single nucleotide polymorphism variants in samples against the dbSNP database. The specific filters are as follows.

- dbSNP(151)
- dbSNP(153)

Filter conditions include the ability to select all dbSNP values (In), or to exclude all dbSNP values (Not In).

The filter detects SNP variants that match those in the dbSNP database, which can be included in or excluded from filtered analysis results.

DGV filter

This filter can be added to filter chains in the software to detect human genomic structural variants that match those in the Database of Genomic Variants (DGV). The specific filters are as follows.

- DGV filter(20130723)
- DGV filter(20160515)

Filter conditions include the ability to include all DGV variants (In), or to exclude all DGV variants (Not In) in filtered analysis results.

The filter detects human genomic structural variants as defined by DGV, which can be included in or excluded from filtered analysis results.

DrugBank filter

This filter can be added to filter chains in the software to detect variants that are correlated with drugs and drug targets listed DrugBank database. The specific filters are as follows.

- GRch38 reference genome:
  - DrugBank (20190111)
  - DrugBank (20190723)
  - DrugBank (20200619)
  - DrugBank (20201029)
  - DrugBank (20210727)
- hg19 reference genome:
  - DrugBank (20187031)
  - DrugBank (20190723)
  - DrugBank (20200619)
  - DrugBank (20201029)
  - DrugBank (20210727)

Filter conditions include the ability to select specific values, to include all DrugBank values (In), or to exclude all DrugBank values (Not In). In addition, unannotated variants can be included or excluded in filtered analysis results.

This filter detects variants that are correlated with drugs and drug targets in DrugBank, which can be included in or excluded from filtered analysis results.

ExAC filters

If you use ExAC filters, it is recommended that you set the **Variant View** parameter to **Allele View**. For more information, see “Locus View versus Allele View of variants” on page 197.

These filters can be added to filter chains to detect rare gene variants for specific population groups in the software. The filters are derived from the Exome Aggregation Consortium (ExAC) database. The specific filters are:

- ExAC AAF(01)
- ExAC EAAF(01)
- ExAC EFAF(01)
- ExAC ENFAF(01)
- ExAC GAF(01)
- ExAC LAF(01)
- ExAC OAF(01)
- ExAC SAAF(01)

Filter conditions include the ability to set a range from 0.0 to 1.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter detects rare gene variant results that align with the ExAC database, which can be included or excluded from filtered analysis results.

FATHMM Scores filter

This filter can be added to filter chains in the software to calculate Functional Analysis through Hidden Markov Models (FATHMM) Scores for coding variants, nonsynonymous single-nucleotide variants (nsSNVs), and noncoding variants. The specific filters are as follows.

FATHMM Scores(85)

FATHMM Scores(89)

Filter conditions include the ability to set a range from 0.0 to 1.0 for the score. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter calculates FATHMM scores for coding variants, nsSNVs, and noncoding variants.

Gene Ontology (GO) filter

This filter can be added to filter chains in the software to detect various gene ontologies (functions of specific genes). The specific filters are as follows.

- GRch38 reference genome:
  - Gene Ontology (20151216)
  - Gene Ontology (20190930)
  - Gene Ontology (20200511)
  - Gene Ontology (20201118)
  - Gene Ontology (20210830)

- hg19 reference genome:
  - Gene Ontology (20190930)
  - Gene Ontology (20200511)
  - Gene Ontology (20201118)
  - Gene Ontology (20210830)
  - Gene Ontology (20230314)

Filter conditions include the ability to select all (In), none (Not In), or to select specific annotations, and to include or exclude unannotated variants in filtered analysis results.

This filter returns gene ontologies of samples based on the selections made.

Gene Symbol filter

This filter can be added to filter chains in the software to report on specific genes.

Filter conditions include the ability to select all (In), none (Not In), or specific gene symbols from the list, and to include or exclude unannotated variants in filtered analysis results.

This filter returns the gene symbols of genes that are found in your samples.

gnomAD filters

These filters can be added to filter chains to detect rare gene variants for specific population groups in the software. The filters are derived from the genome aggregation database (gnomAD) database. The specific filters are as follows.

- gnomAD AAF (African Population Allele Frequency) (2)
- gnomAD AJAF (Ashkenazi Jewish Population Allele Frequency) (2)
- gnomAD EAAF (East Asian Population Allele Frequency (2)
- gnomAD EFAF (European Finnish Population Allele Frequency) (2)
- gnomAD FEM (Female Population Allele Frequency) (2)
- gnomAD FNFAF (European Non-Finnish Population Allele Frequency) (2)
- gnomAD GAF(African Population Allele Frequency) (2)
- gnomAD LAF (Latino Population Allele Frequency) (2)
- gnomAD MAF (male Population Allele Frequency) (2)
- gnomAD OAF (Other Population Allele Frequency) (2)
- gnomAD SAAF (South Asian Population Allele Frequency) (2)

Filter conditions include the ability to set a range from 0.0 to 1.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter detects rare gene variant results that align with the gnomAD database, which can be included or excluded from filtered analysis results.

Minor Allele Frequency filter

This filter can be added to filter chains in the software to detect variants with minor allele frequencies that match those in the dbSNP database from the 1000 genomes project.

Filter conditions include the ability to set a range from 0.0 to 0.5 for minor allele frequencies. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns variants in samples that match those in the dbSNP database from the 1000 genomes project.

OMIM filter

This filter can be added to filter chains in the software to detect variants that match those in the Online Mendelian Inheritance in Man® (OMIM) database. The specific filters are as follows.

- GRch38 reference genome:
  - OMIM(20180823)
  - OMIM(20191113)
  - OMIM(20200609)
  - OMIM(20201202)
  - OMIM(20210625)
- hg19 reference genome:
  - OMIM(20191001)
  - OMIM(20200508)
  - OMIM(20201202)
  - OMIM(20210625)
  - OMIM(20230303)

Filter conditions include the ability to search for all (In), none (Not In), or specific annotations, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in samples that match those in the OMIM database.

Pfam filter

This filter can be added to filter chains in the software to detect protein domain families in the coded proteins as defined by the Pfam database. The specific filters are as follows.

Pfam (31)

Pfam (32)

Filter conditions include the ability to include all (In), none (Not In), or specific annotations, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in the samples that match the variants in the Pfam database.

PhyloP Scores filter

This filter can be added to filter chains in the software to measure the conservation of protein across a wide range of organisms in 16S metagenomics analyses. The specific filters are as follows.

- PhyloP Scores(20160919)

Filter conditions include the ability to set a score range from –14.0 to 3.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

This filter returns PyloP scores for 16S metagenomics samples.

UCSC Common SNPs filter

This filter can be added to filter chains in the software to detect variants that match those in the UCSC Genome Browser Common SNPs database.

Filter conditions include the ability to include (In) or exclude (Not In) variants that match the UCSC Common SNPs in filtered results.

The filter returns variants in samples that match the UCSC Common SNPs database.

Data type filters

You can create filter chains that identify variants based on variant data types in the software, such as allele frequency and allele ratio.

Filter chains that include data type filters narrow the list of variants that appear in the analysis results, when applied to an analysis workflow, or when used to change the variants shown when you view or visualize analysis results.

Allele frequency filter

The Allele frequency filter can be added to filter chains in the software to report the frequency of alleles observed in raw data.

Filter conditions include the ability to set allele frequency ranges from 0.0 to 1.0, to include or exclude boundary values in the range, and to include or exclude unannotated variants in filter analysis results.

The filter returns allele frequencies of alleles observed in raw data.

Allele Ratio filter

This filter can be added to filter chains in the software to narrow analysis results to nonreference allele frequencies.

Filter conditions include the ability to set allele ratio ranges from 0.0 to 1.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns all variants that have at least one nonreference allele reported with a frequency in the selected filter range.

Allele Read-Count filter

This filter can be added to filter chains in the software to set the minimum count for genotype alleles.

Filter conditions include the ability to set allele read-count ranges from 0 to 100,000. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter removes variant candidates that do not have the required numbers of supporting reads from analysis results.

Alternate Allele Count filter

This filter can be added to filter chains in the software to set the minimum count for alternate alleles.

Filter conditions include the ability to set alternate allele read-count ranges from 0 to 100,000. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter removes variant candidates that do not have the required numbers of reads from analysis results.

CNV Confidence Range filter

This filter can be added to filter chains in the software to return copy number variants (CNV) with confidence levels between 10 and 10,000,000 and other variants.

Filter conditions include the ability to set the CNV confidence range from 10 to 1.0E7. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns copy number variants with high confidence levels and other variants.

CNV Confidence Range—CNVs Only filter

This filter can be added to filter chains in the software to return copy number variants (CNV) with confidence levels between 10 and 10,000,000.

Filter conditions include the ability to set the CNV confidence range from 10 to 1.0E7. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns only copy number variants with high confidence levels.

CNV p-value filter

This filter can be added to the Default Somatic Variants view filter chain in the software to detect copy number depletion (loss) when using the AmpliSeq™ somatic custom CNV analysis workflow or assay.

Filter conditions include the ability to set the p-value of detected copy number at specific value. Stringent p-values settings (that is, settings with low p-values cutoffs) can help return CNVs with greater statistical significance. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns copy number losses that have low p-values with high statistical significance, and other variants.

CNV Somatic Confidence—CNVs Only filter

This filter can be added to filter chains in the software to make ploidy estimates with lower and upper confidence levels.

Filter conditions include the ability to enable and set the range for Minimum Ploidy Gain (5% CI) over expected, to enable and set the Minimum Ploidy Loss (95% CI) under expected ploidy values. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns only somatic copy number variants within the lower and upper limits of the set confidence ranges.

CNV Somatic Confidence Range filter

This filter can be added to filter chains in the software to make ploidy estimates with lower and upper confidence values on somatic samples. The filter detects extra copies over the expected normal ploidy value (2 for autosomes, 2 for X chromosomes in females, and 1 for X chromosomes in males). The 5% lower confidence bound value is the ploidy estimate where there is a 5% chance that the true ploidy is below that value. The 95% upper confidence bound is the ploidy estimate where it is 95% certain that the true ploidy is below that value. The lower bound is the most important for gains. The upper bound is most important for losses.

By default, the filter is set to detect gains and losses using the confidence interval values of 5% confidence interval for Minimum Ploidy Gain over the expected value and 95% confidence interval for Minimum Ploidy Loss under the expected value. The default boundary values are set to 0.0.

If you set the **Minimum Ploidy Gain (5% CI) over expected** to **2.0** (2+2=4) and the **Minimum Ploidy Loss (95% CI) under expected** to **0.0**, you can expect the following example CNV call CI data:

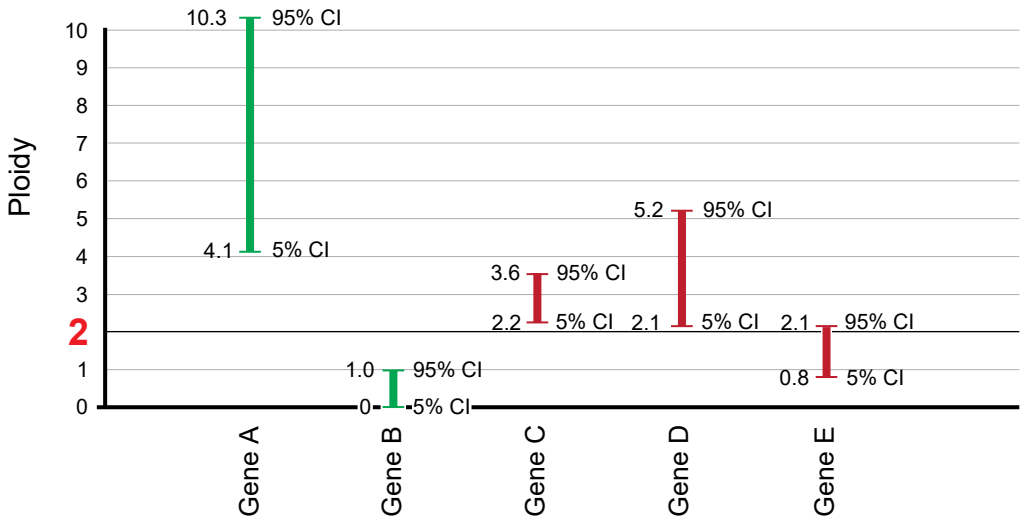
Gene A—A gene with suspected gain of 5% CI = 4.1 and 95% CI = 10.3 filtered in.

Gene B—A gene with suspected loss of 5% CI = 0 and 95% CI = 1.0 filtered in.

Gene C—A gene with suspected gain of 5% CI = 2.2 and 95% CI = 3.6 filtered out (2.2 is less than 4).

Gene D—A gene with suspected gain of 5% CI = 2.1 and 95% CI = 5.2 filtered out (2.1 is less than 4).

Gene E—A gene with 5% CI = 0.8 and 95% CI = 2.1 filtered out (0.8 is less than 4 and 2.1 is greater than 2).



Confident Somatic Variants filter

This filter detects confident somatic variants in tumor-normal analyses. The filter compares tumor samples to matched normal samples. It does not apply to single sample analyses.

Filter conditions include the ability to include the confident somatic variants (In), or to exclude the confident somatic variants (Not In) in filtered analysis results.

Deamination filter

This filter can be added to filter chains in the software to filter C:G>T:A SNVs that are detected at all allele frequencies. Filter conditions include the ability to exclude (Not In) or include (In) these variants in filtered analysis results.

Default DNA and Fusions View filter

This filter can be added to filter chains in the software to detect all of the fusion variants, expression control markers, assay 5′/3′ markers, and all DNA variants that the predefined Default DNA and Fusions View filter chain detects.

Filter conditions include the ability to select specific annotations (Present or Absent), to include all default DNA and Fusions view variants (In), or to exclude all Default DNA and Fusions view variants (Not In) in filtered analysis results. In addition, unannotated variants can be included or excluded in filtered analysis results.

The filter detects the fusion variants, expression control markers, assay 5′/3′ markers, and all DNA variants, which can be included or excluded in filtered analysis results.

Default Fusions View filter

This filter can be added to filter chains in the software to detect all of the fusion variants, expression control markers, and assay 5′/3′ markers that the predefined Default Fusions View filter chain detects.

Filter conditions include the ability to select specific annotations (Present or Absent), to include all Default Fusions View variants (In), or to exclude all Default Fusions View variants (Not In) in filter analysis results. In addition, unannotated variants can be included or excluded in filtered analysis results.

The filter detects fusion variants, expression control markers, and assays 5′/3′ markers, which can be included or excluded in filtered analysis results.

Disease Research Area (DRA) filter

This filter can be added to filter chains in the software to annotate disease research categories as defined by the Disease Research Area (DRA) annotation source database. The specific filters are:

- Disease Research Area (DRA)(20170112)
- Disease Research Area (DRA)(20170914)

Filter conditions include the ability to select specific annotations, to include all Disease Research Area values (In), or to exclude all Disease Research Area values (Not In) in filtered analysis results. In addition, unannotated variants can be included or excluded in filtered analysis results.

This filter annotates disease research areas that match the DRA database, and can be included or excluded.

Expected Normal Ploidy Buffer filter

The Expected normal ploidy buffer filter can be added to filter chains in the software to detect mosaic chromosomal aneuploidies in research samples and to set a buffer for normal ploidy values (2 for autosomes, 2 for X chromosomes in females, and 1 for X and Y chromosomes in males) so as to identify the most likely aneuploidy samples. Ploidy calls do not have to be integers because mosaicism detection allows decimal-level Copy Number Gain or Loss calls.

By default, the filter is set to filter out all copy number variant segments of gain or loss within 0.2 ploidy value of expected normal. Gains must be above ploidy 2.2 and below ploidy 1.8 to remain filtered in on autosome and female X, and ploidy 1.2 and ploidy 0.8 on male Y. Filter conditions include the ability to change the amount of buffer by adjusting the From and To values. This Expected Normal Ploidy Buffer overrides any Confidence filtering that may be in effect within the buffer zone.

If default settings are applied, ploidy values of 1.8 to 2.2 are filtered out as normal for autosomes and X chromosomes in females, and 0.8 to 1.2 are filtered out as normal for X and Y chromosomes in males. Values outside this range are detected as chromosomal ploidy samples.

Filtered Coverage filter

This filter can be added to filter chains in the software to review results that meet a specific coverage threshold. By default, the filter reports all results with a coverage threshold greater than 100. Or, you can use the filter settings to change the threshold to include or exclude boundary values for the threshold, and to include or exclude unannotated variants in filtered analysis results. The default threshold setting is 100.

Filter conditions include the ability to set a minimum and a maximum value for minor allele frequency for the coverage threshold. Ranges from 0.0 to 0.0045 can be selected for the minimum in the **From** field and in the **To** field for the maximum. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

Fold difference filter

This filter can be added to Default Somatic Variants view filter chains in the software to detect criteria for both copy number depletion (loss) when using the Ion AmpliSeq™ somatic custom CNV analysis workflow or assay.

Filter conditions include the ability to set the CNV fold difference range from 0 to 0.85. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns copy number variants with stringent fold differences and other variants.

Functional Scores filter

This filter can be added to filter chains in the software to provide functional scores based on SIFT, PolyPhen, and Grantham scores. SIFT and PolyPhen scores are predictions of the functional effect of a variant on a protein. A Grantham score attempts to predict the distance between two amino acids, in an evolutionary sense (lower is less distance, higher is greater distance).

Filter conditions include the ability to include or exclude SIFT, PolyPhen, and Gratham scores, and to set ranges for those scores. SIFT and PolyPhen ranges can be set from 0.0 to 1.0, and Grantham can be set from 0.0 to 215.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns a functional score based on the three (or fewer) scores.

Fusion Read Counts filter

This filter can be added to filter chains in the software to produce counts for fusion reads.

Filter conditions include the ability to set a minimum count of 0 or higher, to include the boundary value for the count, and to include or exclude unannotated variants in filtered analysis results.

This filter returns results that meet the fusion read minimum count value.

Genetic Category Type filter

This filter can be included in a filter chain in the software to detect variants of specific genetic category types.

Filter conditions include the ability to select all, none, or specific genetic category type annotations, and to include or exclude unannotated variants in filtered analysis results.

Genetic category types include:

- HasDeNovoNonRefAllele
- HasDeNovoRefAllele
- IsNewlyHomzygousNonRef
- IsNewlyHomozygousRef
- InCompoundHeterozygote
- InTransPhaseCompoundHeterozygote
- HasMaleMaternalX
- HasUnknownX

- InconsistentWithFather
- InconsistentWithMother
- InconsistentWithParents

This filter returns genetic category type annotations for the variants included in the samples.

Genomic Coordinates filter

This filter can be added to filter chains in the software to narrow analysis results to specific genomic regions. The filter can also be used to identify regions that are targeted by an amplicon or amplicons in a user-defined assay.

The filter value is one chromosome region, or multiple regions that are separated by OR operators or commas.

The Genomic Coordinates filter returns variants in samples that are in the range for the genomic coordinates that you set.

IMPORTANT! Do not use AND, OR, or NOT when adding values to the Genomic Coordinates filter.

Homopolymer Length filter

This filter can be used in a filter chain in the software to detect INDELs of specific homopolymer lengths.

Filter conditions include the ability to set a range from 0 to 1,000 for homopolymer length. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

This filter returns INDEL variants in samples that meet the specified homopolymer length.

Hotspot Genotype Allele filter

This filter can be added to filter chains in the software to detect variants that overlap hotspots.

Filter conditions include the ability to include (In) or exclude (Not In) hotspot position overlaps in filtered analysis results.

The filter returns variants that overlap hotspots that are defined in an uploaded hotspots file.

Hostpot Position Overlap filter

This filter can be added to filter chains in the software to detect hotspot position overlaps.

Filter conditions include the ability to include (In) or exclude (Not In) hotspot position overlaps in filtered analysis results.

The filter returns variants that overlap hotspots that are defined in an uploaded hotspots file.

Location filter

This filter can be added to filter chains in the software to detect variants in specific positions, such as exonic.

Filter conditions include the ability to select all locations or specific locations in filtered analysis results. Locations include:

- unknown
- intergenic
- intronic
- exonic
- utr_5
- utr_3
- splicesite_5
- splicesite_3
- upstream
- downstream
- exonic_nc
- intronic_nc
- ncRNA
- nonCoding

The filter returns variants in samples that match the selected filter locations.

Minimum Limit of Detection at Genomic Location filter

The Minimum Limit of Detection at Genomic Location filter can be added to filter chains to filter for a minimum limit of detection (LOD) at chromosomal locations within a selected range.

This LOD calculation depends on the depth of coverage at a given chromosomal location and the probability that is defined by `Minimum Callable Probability` parameter.

This filter is for use with Ion AmpliSeq HD and TagSeq analysis results and analysis workflows only. For more information, see “Variant Finding parameters” on page 113.

Filter conditions include the ability to set a minimum and a maximum value for minor allele frequency. Ranges from 0.0 to 0.0045 can be selected for the minimum in the **From** field and in the **To** field for the maximum. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

Minor Allele Frequency filter

This filter can be added to filter chains in the software to detect variants with minor allele frequencies that match those in the dbSNP database from the 1000 genomes project.

Filter conditions include the ability to set a range from 0.0 to 0.5 for minor allele frequencies. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns variants in samples that match those in the dbSNP database from the 1000 genomes project.

MyVariants filter

This filter can be included in a filter chain in the software to report on MyVariants annotations as set by users.

Filter conditions include the ability to select Important, Ignore, or None for specific MyVariants annotations, and to include or exclude variants that are not flagged in filtered analysis results. Possible combinations include:

Option	Description
Important	Select **Important** to filter for MyVariants that are flagged "Important".
Ignore	Select **Ignore** to filter for MyVariants that are flagged "Ignore".
Important and Ignore	In the **Filter Option** list, select **In** to include all flagged MyVariants.
Ignore and None	1. Select **Include unannotated variants** to include all unflagged variants. 2. Select **Ignore** to include MyVariants that are flagged "Ignore".
Important and None	1. Select **Include unannotated variants** to include all unflagged variants. 2. Select **Important** to include MyVariants that are flagged "Important".
None	In the **Filter Option** list: 1. Select **Not In** to not include any flagged MyVariants. 2. Select **Include unannotated variants** to include all unflagged variants.

The filter returns variants in samples that have the specified MyVariants annotations.

Named Variants filter

This filter can be added to filter chains in the software to detect known variants in the Ion AmpliSeq™ Community Panel for the cystic fibrosis transmembrane regulator (CFTR) gene, for example: c.2054delA.

Filter conditions include the ability to select all named variants, to select specific named variants, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in samples that match the CFTR variants that are specified in the filter.

IMPORTANT! The **Named Variants** filter option is not shown in the dropdown list when you create a filter chain. To create a filter chain that uses the **Named Variants** filter, you need to first select an annotation set that includes the **Named Variants** annotation source. After you select the annotation set, the **Named Variants** option is shown. To determine if the annotation set contains the **Named Variants** annotation source, you can view the details for the annotation set in the **Workflow Presets** screen. For more information, see “View custom annotation sources” on page 135.

Oncomine™ filter

This filter is the default filter for most Oncomine™ assays. It presents the user with Oncomine™ driver variants that are based on their Oncomine™ Gene Class and Oncomine™ Variant Class annotations. It can be added to filter chains in the software to detect Oncomine™-annotated variants in specific Oncomine™ panels.

Analysis workflows for Oncomine™ assays and analysis workflow templates for Ion AmpliSeq™ HD panels in Ion Reporter™ Software include the Oncomine™ Variant Annotator plugin. The plugin integrates into analysis results data from more than 24,000 exomes across solid tumor and hematological cancer types, and annotates variants relevant to cancer with Oncomine™ Gene class and Oncomine™ Variant class information. For more information and a full list of annotation rules for each Oncomine™ assay, see Appendix D, "Oncomine™ Variant Annotator plugin criteria", or contact your local support representative, Field Bioinformatics Specialist (FBS), or Clinical Account Consultant (CAC).

The Oncomine™ filter (Oncomine = In) returns variants in the samples that have Oncomine™ Gene class and Oncomine™ Variant class annotation.

This filter can be used only if an Oncomine™ analysis workflow was used for the analysis. You can apply filter chains that include the Oncomine™ filter only if the analysis workflow that was used for the analysis includes the Oncomine™ Variant Annotator plugin. Examples of filter chains that include this filter are the Oncomine™ Variants and Oncomine™ Extended filter chains.

Phred QUAL Score filter

This filter can be added to filter chains in the software to filter the by the Phred QUAL score. Phred QUAL scores are defined as a property which is logarithmically related to the base-calling error probabilities.

The filter returns Phred Qual scores for variants calls in the samples and is calculated as follows.

- For variants, the score is computed by the posterior probability that the sample variant allele frequency is greater than the minimum allele-frequency that is specified for the variant type.
- For reference calls, the score is computed by posterior probability that the variant allele frequency is below this threshold.

Posterior probability computed conditional on the reads observed, includes sampling variability.

Filter conditions include the ability to set a range from 0.0 to 1000000.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

Possible Polyploidy Alleles filter

The Possible Polyploidy Alleles filter can be added to filter chains to detect searches alleles which have passed all variant calling filters used by the Torrent Variant Caller (TVC) module but missed by genotype. The **Variant Finding** parameter **PPA** must be set to **True** for this filter to function. For more information, see "Customize tuning parameters" on page 94.

The Possible Polyploidy Alleles filter is useful for identification of variants that are beyond diploid genomes, that is, cancer cells or polyploidy species.

Filter conditions include the ability to select **YES** to find alleles that have passed all filters, **NO**, to find alleles that have *not* passed all filters in addition to genotype, or both **YES** and **NO**.

PValue filter

This filter can be added to filter chains in the software to determine the *p*-value of variants. The *p*-value is a statistical method for the detection of variant calls from next-generation sequencers.

Filter conditions include the ability to set a range from 0.0 to 1.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded in filtered analysis results.

The filter returns *p*-values for variants calls in the samples.

Variant Classification filter

This filter can be added to filter chains in the software to narrow results to the following user-set variant classifications:

- Unclassified
- Unknown
- Benign
- Suspected Benign
- Likely Benign
- Suspected Deleterious
- Deleterious
- Pathogenic
- Likely Pathogenic
- VUS
- Uncertain Significance
- Technical Artifact

Filter conditions include the ability to include all (Filter Option set to In), none (Filter Option set to Not In), or to select specific classifications in filtered analysis results.

The filter returns variants that are classified by software users.

Variant Effect filter

This filter can be added to filter chains in the software to detect the effect of variants on coding sequences. Specific values include:

- refAllele
- unknown
- synonymous
- missense
- nonframeshiftInsertion
- nonframeshiftDeletion
- nonframshiftBlockSubstitution
- nonsense
- stoploss

- frameshiftInsertion
- frameshiftDeletion
- frameshiftBlockSubstitution

Filter conditions include the ability to select all (Filter Option set to In), none (Filter Option set to Not In), or to select specific values, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in samples that match the selected variant effects.

Variant Subtype filter

This filter can be added to filter chains in the software to detect variant subtypes, including:

- BigDel
- BigDup
- GeneCNV
- REF
- NOCALL

Filter conditions include the ability to select all or specific variant subtypes in filtered analysis results.

The filter returns variants that match the selected specific variant subtypes.

Variant Type filter

This filter can be added to filter chains in the software to detect variants that match the following variant types:

- SNV
- INDEL
- MNV
- REF
- NOCALL
- CNV
- LONGDEL
- FUSION
- EXPR_CONTROL
- ASSAYS_5P_3P
- RNA_HOTSPOT
- GENE_EXPRESSION
- RNAExonVariant
- ProcControl
- FLT3ITD

Note: The FLT3ITD variant type is detected only in Oncomine™ Myeloid Assay analysis workflows.

Filter conditions include the ability to select all variant types or specific variant types in filtered analysis results.

The filter returns variants in analyses that match the selected specific variant types.

Zygosity filter

This filter can be added to filter chains in the software to detect variants that are homozygous or heterozygous.

Filter conditions include the ability to search for both homozygous and heterozygous variants, or either of them, in filtered analysis results.

The filter returns variants in analyses that are homozygous or heterozygous.

11 Annotation sources

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Ion Reporter™ Software provides a variety of annotation sources from a wide range of public resources. You also have the option to import your own annotations sources if you want to add custom annotation data. Both types of annotation sources are for use directly in Ion Reporter™ Software.

Annotations available in Ion Reporter™ Software

Ion Reporter™ Software provides several annotation sources are derived from public and private annotation databases for hg19 and GRCh38. Ion Reporter™ Software also provides annotation sets. An annotation set is a collection of annotation sources that you can use to annotate variants in the analyses. You can use annotation sets that are predefined in the software, or create custom annotation sets.

Each analysis workflow uses a different version of the annotation source. The versions for each analysis workflow are shown in the **Presets** tab, and in the TSV file for the analysis results. Every analysis workflow also has an associated annotation set that lists all annotation sources that are used, along with the versions.

To add custom annotation sources to an annotation set, see “Import a custom annotation source” on page 479.

The following annotation are included in Ion Reporter™ Software.

Annotations	Description	Source[1]
5000 exomes	Population frequency information from the 5000 exomes project	**NHLBI ESP**
Allele coverage	Number of reads supporting the called allele	Ion Reporter™ Software
Allele frequency	Frequency of the allele observed from the raw data	Ion Reporter™ Software
Amino acid change	HGVS notation that represents an amino acid change	Ion Reporter™ Software
Genetic category	Unique genetic category that is used by Ion Reporter™ Software This annotation is for use only with trio analysis workflows.	Ion Reporter™ Software
ClinVar	Assessment of the impact of the variant observed from NCBI ClinVar database	**ClinVar**
Coding	HGVS notation that represents a nucleotide change	Ion Reporter™ Software
Copy number	The copy number ploidy state	Ion Reporter™ Software
COSMIC	Catalog of somatic mutations in tumor tissue	**COSMIC**
Coverage	Total coverage for a variant	Ion Reporter™ Software
Custom	See “Import a custom annotation source” on page 479for more information	Ion Reporter™ Software
Cytogenetic band	The cytogenetic band where the CNV was detected This annotation is for use only with aneuploidy analysis workflows.	Ion Reporter™ Software
DRA	Disease Research Area	Ion Reporter™ Software
dbSNP	Single Nucleotide Polymorphism database. The dbSNP annotation source in the software contains a flag carried by a subset of its SNPs that have been curated by UCSC to be "UCSC Common SNPs". In order for a variant to be annotated as a UCSC Common SNP, the variant is first annotated as being present in dbSNP, and it might also be classified as a UCSC Common SNP.	**dbSNP**
DGV	Database of Genomic Variants: A curated database of human genomic structural variation	**DGV**

(continued)

Annotations	Description	Source[1]
DrugBank	List of drugs known to target the gene or genes affected by the variant	**DrugBank** **Note:** When you create an hg19 annotation set, do not use the annotation source DrugBank version 20150107. Use instead annotation source DrugBank version 1 or DrugBank version 20161212 or 20180731, which are the latest versions available in Ion Reporter™ Software for hg19. If you use the DrugBank version 20150107 in an hg19 annotation set, cannot create a filter chain of DrugBank for any analysis that uses the annotation set.
ExAC	Exome Aggregation Consortium—Database catalog of variant frequencies	**ExAC**
Father genotype	Genotype of father. This annotation is for use only with trio analysis workflows.	Ion Reporter™ Software
FATHMM scores	Prediction of the functional consequences of a variant. In Ion Reporter™ Software, FATHMM scores are available for COSMIC version 77 and later. These scores are populated when "COSMIC" is chosen as a part of the annotation preset that is used.	**COSMIC**
Gene models	Set of genes that overlap with the variant	**RefSeq** **Ensembl**
Gene panel	Filter variants based on specific amplicon in an AmpliSeq panel. For example, Ion AmpliSeq CCP.	Ion Reporter™ Software
Gene set	Focus on variants found in a specific set of genes	Ion Reporter™ Software
Genetic category	The genetic category. For example, compound heterozygote of the variant identified. This annotation is for use only with trio analysis workflows.	Ion Reporter™ Software

(continued)

Annotations	Description	Source[1]
Genotype	Genotype of the sample in each position	Ion Reporter™ Software
Gene Ontology	Standardized ontology for gene and gene products. For example, functional role or localization.	**GO**
gnomAD	The gnomAD v2.1.1 data set (Exomes). In Ion Reporter™ Software, GRCh38 gnomAD annotation source is added to the **All_GRCh38** and **All** annotation sets. ExAC annotation source has been removed from the **All** annotation set.	**GnomAD**
Grantham score	A measure of evolutionary distance. See “Grantham score” on page 501.	Ion Reporter™ Software
Hotspot information	Indicates whether a variant overlaps a hotspot file	Ion Reporter™ Software
Location	Position of the variant. For example, exonic.	Ion Reporter™ Software
Locus	Position of the variant.	Ion Reporter™ Software
MAF	Population frequency information from the 1000 genomes project. MAF numbers are provided by the dbSNP in Ion Reporter™ Software, which gets the MAF numbers from 1000 genomes. Therefore, the version of dbSNP annotation sources used in the Ion Reporter™ analysis may impact these MAF values.	**1000 Genomes**
Mother genotype	Genotype of mother This annotation is for use only with trio analysis workflows.	Ion Reporter™ Software
MyVariants	A personal knowledge base of genomic variants (formerly, VariantKB database)	Ion Reporter™ Software
Named Variants	A list of known variants in the CFTR gene panel	Ion Reporter™ Software
OMIM	Online Mendelian Inheritance in Man®	**OMIM**
p-value	*p*-value of the variant call	Ion Reporter™ Software
Pfam	Protein domain families in the coded protein	**Pfam**
PhyloP	Measure of conservation of the protein across a wide range of organisms	**Cornell University**
PolyPhen-2	Prediction of the functional effect of a variant on a protein	**Harvard University**
Reference	The reference allele (hg19)	Ion Reporter™ Software

(continued)

Annotations	Description	Source[1]
Reference / variant coverage	Individual strand coverage information for the reference and variant calls	Ion Reporter™ Software
SIFT	Prediction of the functional effect of a variant on a protein	JCVI
Size	Size of the variant	Ion Reporter™ Software
Transcript set	Preferred transcripts used to determine coding regions of genes. If you include a transcript file, only transcripts that are present in the selection of canonical transcripts are reported. Other transcripts are filtered out.	RefSeq canonical; Ensembl canonical
Type	Type of variant. For example, SNP, INDEL, CNV, and so on.	Ion Reporter™ Software
UCSC common SNPs	The dbSNP annotation source in the software contains a flag carried by a subset of its SNPs that have been curated by UCSC to be "UCSC Common SNPs". In order for a variant to be annotated as a UCSC Common SNP, the variant is first annotated as being present in dbSNP, and it might also be classified as a UCSC Common SNP.	Ion Reporter™ Software
Variant effect	The effect of the variant on the coding sequence. For example, missense or stoploss.	Ion Reporter™ Software

[1] In this table, Ion Reporter™ Software refers to either a calculation or functional annotation that is performed by the software.

Transcripts in REFSEQ and ENSEMBL canonical transcripts file

In UCSC curated canonical transcripts set, there are some discrepancies when multiple transcripts are used for a given gene. The shorter transcripts have been removed and the transcript with the `appris_principal_1` tag is retained in the Ion Reporter™ Software REFSEQ canonical transcript set. This tag is used by gencode to mark the primary transcript of a specific gene. If the `appris_principal_1` tag is missing, the longest transcript is used so that there is just one canonical transcript for a given gene. If two transcripts are the same length, Ion Reporter™ Software includes both genes and lists them in alphabetical order.

The UCSC transcripts for the following genes are replaced with the transcripts in the New transcript column in Ion Reporter™ Software:

Gene	Current transcript	New transcript
FGFR2	NM_022970.3	NM_000141.5
FGFR3	NM_001163213.1	NM_000142.5
ABL1	NM_007313.2	NM_005157.6
ARAF	NM_001256196.1	NM_001654.5
CHEK2	NM_001005735.1	NM_007194.4

(continued)

Gene	Current transcript	New transcript
GNAS	NM_080425.2	NM_000516.7
TP53	NM_001276760.1	NM_000546.6

Contigs annotated in the GRCh38 reference genome

Ion Reporter™ Software annotates only variants that map to the following contigs as provided in the `GRCh38.fasta` and `GRCh38.fasta.fai` files.

The following contigs (sequences) are supported by Ion Reporter™ Software.

Contig Name	Length (bps)
chr1	248956422
chr2	242193529
chr3	198295559
chr4	190214555
chr5	181538259
chr6	170805979
chr7	159345973
chr8	145138636
chr9	138394717
chr10	133797422
chr11	135086622
chr12	133275309
chr13	114364328
chr14	107043718
chr15	101991189
chr16	90338345
chr17	83257441
chr18	80373285
chr19	58617616
chr20	64444167
chr21	46709983

(continued)

Contig Name	Length (bps)
chr22	50818468
chrX	156040895
chrY	57227415
chrM	16569
chr22_KI270879v1_alt	304135

Two kinds of variants are not supported:

1. Variants that map to a contig we do not support
2. Variants that map to a masked region

In particular, chr22_KI270879v1_alt is completely masked except for 269,814 - 279,355, which contains only the GSTT1 gene. No amino acid changes are provided for the masked regions. However, gene and transcript information is provided.

hg19 and GRCh38 dbsnp

The hg19 and GRCh38 dbsnp versions in Ion Reporter™ Software are both dbsnp versions 151.

Import a custom annotation source

You can use custom annotation source files in Ion Reporter™ Software. If you have annotation data that you want to use, you need to first create a new annotation set for the custom annotation source. Then, you can import the files into the new annotation set.

IMPORTANT! Annotation files that have been edited and uploaded from a computer running Microsoft™ Windows OS to an Ion Reporter™ Server running a Linux OS, can have extra characters added at the end of new lines. Use the ***dos2unix*** command to avoid potential errors with the software service caused by the characters.

There are 4 types of annotation sources that you can import into Ion Reporter™ Software. The types of annotation sources and the file formats required for each annotation source are as follows.

Note: Only one version of an annotation source can be used in an annotation set.

Annotation source type	Description	File format for import
Genomic region	A set of regions	BED
Gene set	A set of genes	TXT
Preferred transcript set	A set of transcripts	TXT
VariantDB	A set of variants	VCF

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Annotation Set**.
3. Enter a descriptive **Name** for the custom annotation set.
4. (Optional) Enter a description for the custom annotation set.
5. Select a custom annotation type from the dropdown list:
   - Genomic Region (Custom)
   - Gene Set (Custom)
   - Preferred transcript set (Custom)
   - VariantDB (Custom)
6. Click the **Create New** tab.
7. Enter a descriptive **Name** and **Version** for the custom annotation source.

   IMPORTANT! When naming custom annotation sources and annotation versions:

   - Do not use capitalized AND, NOT, or OR. You can use lower case *and*, *not*, or *or*.
   - You cannot use two consecutive underscores (__).

   You cannot save the object using these characters.
8. (Optional) Enter a description for the custom annotation source.
9. Click **Select File**, then find and select the custom annotation source file, then click **Open**.
10. Click **Save**.
11. The new custom annotation source is added to the custom annotation set.

Genomic region

You can provide custom annotation information for specific regions of interest in the genome when you import a genomic region annotation source file.

- The genomic region annotation source file must be a `.txt` format file with four, tab-separated columns. Columns must list a chromosome number, start value, end value, and annotation values.
- Enter custom annotations in the **Annotation value** column of the file, as key-value pairs separated by semi-colons.

The information in your input genomic region annotation source file is used in the following ways in your analysis results:

- In the Analysis Results screen. In the downloaded TSV variant files, information from the **Annotation values** column is added to matching variants in the analysis results.
- In the **Analysis Results** screen, you can create a filter based on the first key-value pair that is shown in the **Annotations** column. Only the first key-value pair can be used as a filter chain.

In this example file, `fld1=abc` is the first key-value pair.

```
#CHROM    Start    End    Annotation values
chr1    000000    000001    fld1=abc;fld2=123;fld3=this
chr1    000001    000002    fld1=abd;fld2=124;fld3=that
chr1    000002    000003    fld1=abe;fld2=125;fld3=this
chr1    000003    000004    fld1=abf;fld2=126;fld3=that
chr1    000004    000005    fld1=abg;fld2=127;fld3=this
chr1    000005    000006    fld1=abh;fld2=128;fld3=that
```

The hit level for a genomic region annotation source is not configurable and is always set to overlap.

To import a genomic region custom annotation, see “Import a custom annotation source” on page 479

Gene set

You can provide annotation information for specific genes of interest when you import a gene set annotation source file. The file lists gene names and categories for those genes.

- The gene set annotation source file must be a TXT format file with two, tab-separated columns with the gene name in the first column and a corresponding category for the gene in the second column. Entries for the columns must be comma-separated gene symbols, followed by a tab character, and any annotation string to describe the gene category.
- The file can include an optional heading name for the columns. The second column name is used as the annotation key name. If the heading line is not provided, the default annotation key name of Name is used.

The information in the gene set annotation source file is used in the following ways in the analysis results:

- Information from the second column of the gene set file is added to matching variants in the analysis results. The category name that is assigned to the gene is added to the **Analysis Results** screen and to downloaded TSV variant files.
- In the **Analysis Results** screen, you can create a filter that is based on the gene category name. You can filter the results on any of the values that appear in the second column.

Here is an example file:

```
#Geneset5    Disease
Gene1,Gene2  disease1
Gene3        disease2
Gene4,Gene5,Gene6    disease3
Gene7,Gene8  disease4
```

To import a gene set annotation source file, see “Import a custom annotation source” on page 479.

Transcript set

You can use a transcript set in Ion Reporter™ Software to provide annotation information for specific transcripts of interest. A transcript set limits the annotations that are applied to your variant calls to annotations that match your list of preferred transcripts.

Use the following guidelines when you use or create a transcript set:

- Ensure that the transcript set includes a header line that indicates the human genome on which it is based. If this reference header information is not included, the software processes the file as if it is based on the hg19 human genome reference. The options for the header line are:
  - ***##reference=GRCh38***
  - ***##reference=hg19***
- The contents of the file for the transcript set are in the format: ***GENE_NAME transcript_accession_id1, transcript_accession_id2, ...***, with a tab character between the gene name and the first transcript id.
- Gene names must be specified as HGNC gene symbols.
- A tab character is required after the gene name.
- A comma and a space character are required between transcripts.
- If gene name is missing in the file, all transcript annotations for that gene are preserved.
- Transcripts can be specified as RefSeq or Ensembl® accession ids that include a version. However, if the accession ID contains no decimal point version, then all versions of the transcript are matched. For example, Gene1 NM_0000006 is matched with all versions of this transcript. This annotation source uses a TXT format with tab characters.

Here is an example file with a header to indicate that the transcript set is based on the GRCh38 human genome reference:

```
##reference=GRCh38
GENE REFSEQ_AND_ENSEMBL_TRANSCRIPTS
Gene1 NM_00000006.2, NM_000000005.3, ENST000000000007.3
Gene2 NM_00000001.1, NM_000000007.1, NM_0000000004.5,
NM_000000008.3, ENST000000000006.4
GENE REFSEQ_AND_ENSEMBL_TRANSCRIPTS
Gene1 NM_00000006.2, NM_000000005.3, ENST000000000007.3
Gene2 NM_00000001.1, NM_000000007.1, NM_0000000004.5, NM_000000008.3,
ENST000000000006.4
```

RefGene GeneModel and Ensembl GeneModel transcript versions

You can view the details about the annotations that Ion Reporter™ Software supports for a specific gene model transcript in the list of **Variant Details**, including the versions for the RefGene GeneModel and Ensembl GeneModel transcripts.

1. In the **Analysis** tab, click **Overview**.
2. Click an analysis name to open the **Analysis Results** screen.

3. In the **Analysis Results** screen, click **(Variant Details)** to open the **Variant Details** screen. The transcript version is listed in the **Annotation Value** column.



Variant Details: chr20:54961541

Variant Details | Notes

Annotation Source	Annotation Value
Coding	c.91T>A
Codon	ATT
Coverage	290
DrugBank	1-[5-(2-(1-methyl-1H-pyrazol-3-ylaminodipyrimidin-7-ylaminoethyl)-1,3-thiazol-2-yl)-3,3-(trilluoromethyl)phenylurea …(17)
EMAF	0.2169
ExAC AAF	1.414E-1 (pos.54961541)
ExAC EAAF	6.784E-1 (pos.54961541)
ExAC EFAF	2.705E-1 (pos.54961541)
ExAC ENAF	2.172E-1 (pos.54961541)
ExAC GAF	2.735E-1 (pos.54961541)
ExAC LAF	3.687E-1 (pos.54961541)
ExAC OAF	2.941E-1 (pos.54961541)
ExAC SAAF	3.112E-1 (pos.54961541)
Exon	3
Filter	PASS
GMAF	0.1882
Gene Ontology	ATP binding …(61)
Genes	AURKA
Genotype	A/T
Grantham	21.0
Homopolymer Length	3
Info	HS
Length	1
Linear-scaled quality score	0.00022
Location	AURKA:exonic:NM_003600.4
Locus	chr20:54961541
MAF	0.31
Observed Allele	T
Oncomine Gene Class	Gain-of-Function

4. Alternatively, open the TSV variants file in the `Variants/sample_name` subdirectory, to see the transcript versions.



```
#chr  pos   type  ref   length      genotype    pvalue      coverage    allele_coverage   maf   iscn  confidence  precision   gene  transcript  location
      function    codon exon  protein     coding      sift  polyphen    grantham    5000Exomes  Oncomine    clinvar     cosmic      dbsnp drugbank    go    omim
      pfam  phylopMyVariants
1     324100      SNV   T     1     A/A                                                   LOC100132287|LOC100132062|LOC100133331    NR_028322.1|NR_028325.1|NR_028327.1
      intronic_nc|intronic_nc|intronic_nc ||    ||    ||    ||    ||    ||    ||    ||
1     985955      SNV   G     1     G/C                                                   AGRN  NM_198576.3 exonic      missense    CGG   29    p.Gly1709Arg
      c.5125G>C   0.0   1.0   125.0             pathogenic        rs199476396       clustering of voltage-gated sodium channels:axon guidance:extracellular
space:neurotransmitter receptor metabolic process:structural constituent of cytoskeleton:cell surface:laminin binding:extracellular region:G-protein coupled
acetylcholine receptor signaling pathway:synapse organization:signal transduction:receptor clustering:extracellular matrix:protein binding:plasma membrane
organization:positive regulation of neuron apoptosis:acetylcholine receptor regulator activity:synapse assembly:positive regulation of transcription from RNA
polymerase II promoter:regulation of synaptic growth at neuromuscular junction:synapse:basal lamina   Agrin EGF-like domain:SEA domain:Laminin G domain:Kazal-type
serine protease inhibitor domain:Laminin EGF-like (Domains III and V):Agrin NtA domain    2.04
1     2488068     CNV         118059986                                 1p36.32p12(2488068-120548054)x2     0     143.56      LOC100133445|TTC34|FLJ42875|MIR4251
|MIR551A|TP73-AS1|LOC388588|LOC100133612|LOC728716|LOC284661|MIR4417|MIR4689|MIR4252|PLEKHG5|ENO1-AS1|MIR34A|C1orf200|MTOR-AS1|CLCN6|NPPA-AS1|MIR4632
|SNORA59B|SNORA59A|PRAMEF3|PRAMEF15|PRAMEF9|LRRC38|C1orf126|FLJ37453|CLCNKB|CROCCP3|MIR3675|NBPF1|CROCCP2|MST1P2|ESPNP|MST1P9|MIR4695|AKR7L|LOC100506730|LOC339505
|LOC100506801|LINC00339|MIR4684|MIR4253|LOC729059|MIR3115|C1orf213|MDS2|LOC100506963|MIR378F|PNRC2|LOC284632|RCAN3AS|LOC646471|MIR3917|TRIM63|MIR1976|NUDC|SLC9A1
|LOC644961|SCARNA1|SNHG3|SNHG12|SNORD99|SNORA61|SNORA44|SNORA16A|RNU11|LOC100129196|MIR4420|SNORD103A|SNORD103B|SNORD85|PRO0611|LOC149086|LOC284551|MIR4254
|MTMR9LP|LOC100128071|MIR3605|LOC402779|LOC653160|MIR4255|LOC728431|MIR5581|SNIP1|CDCA8|MIR3659|LOC339442|GJA9-MYCBP|PPIEL|SNORA55|ZMPSTE24|LOC100130557
|MIR30E|MIR30C1|CITED4|LOC100507178|LOC100129924|FLJ32224|LOC100132774|SLC6A9|MIR5584|SNORD55|SNORD46|SNORD38A|SNORD38B|EIF2B3|LOC400752|CCDC163P|RPS15AP10
|LOC729041|LOC100507423|LOC100130197|CYP4Z2P|PDZK1IP1-AS1|MGC12982|LOC388630|SKINTL|MIR761|TXNDC12|LOC100507564|SLC25A3P1|MIR4781|HEATR8-TTC4|LOC100507634|MIR4422
|LOC100131060|HSD52|MIR4711|LINC00466|DLEU2L|MIR4794|MIR3671|MIR101-1|DNAJC6|MIR3117|GNG12-AS1|MIR1262|PIN1P1|ZRANB2-AS1|MIR186|ZRANB2-AS2|NEGR1-IT1
|SNORD45C|SNORD45A|SNORD45B|MGC27382|MIR548AP|UOX|C1orf180|MIR4423|LOC646626|CLCA3P|LOC339524|LOC100505768|GBP1P1|FLJ27354|GEMIN8P4|HSP90B3P|SNORD21|SNORA66
|LOC100131564|BCAR3|LOC100129046|MIR760|SLC44A3|LOC729970|FLJ31662|DPYD-AS1|MIR137HG|MIR2682|MIR137|LOC729987|LOC100129620|MIR548D1|MIR548AA1|MIR553|LOC100128787
|EXTL2|RNU6-6|DNAJA1P5|ACTG1P4|LOC100129138|VAV3-AS1|SLC25A24|NBPF6|AKNAD1|SRG7|SCARNA2|MIR197|AMPD2|LOC440600|CYMP|RP11-165H20.1|CHIA|PGCP1|LOC100129269
|LOC100506343|MIR4256|AKR7A2P1|LOC643441|LOC100287722|ATP1A1OS|MIR320B1|MIR942|HSD3BP4|LOC644242

1     2615740     SNV,MNV     GGTCG 4     GAACC/GGTTG                                                 TTC34 NM_001242672.1    intronic
                                          rs71490543:rs80062728:rs76497889:rs76926500          binding          Tetratricopeptide repeat      0.5,-1.76,-
2.75,0.5,-1.85
1     2706398     NOCALL      C           ./.                                                   TTC34 NM_001242672.1    upstream
                                          rs113477241       binding                 0.73
1     3334486     SNV   C     1     T/T                                                         PRDM16      NM_022114.3 exonic      missense    CTC   11    p.Pro929Leu
      c.2786C>T   0.0   0.002 98.0  AMAF=0.001:EMAF=0.0:GMAF=3.0E-4           untested    malignant_melanoma      rs145632008       tongue development:zinc ion
binding:somatic stem cell maintenance:positive regulation of transcription, DNA-dependent:transcriptional repressor complex:metal ion binding:transcription
coactivator activity:palate development:negative regulation of transcription from RNA polymerase II promoter:negative regulation of granulocyte
differentiation:regulation of cellular respiration:protein binding:neurogenesis:sequence-specific DNA binding:white fat cell differentiation:brown fat cell
differentiation:SMAD binding:negative regulation of transcription, DNA-dependent:intracellular:negative regulation of transforming growth factor beta receptor
signaling pathway:nucleus     PR domain-containing protein 16     Zinc finger 2.29
1     7527892     SNV   C     1     G/A                                                         CAMTA1      NM_015215.2 exonic      missense|nonsense TGG|TGA     6
      p.Cys147Trp|p.Cys147*     c.441C>G|c.441C>A 0.0|  0.968|      215.0|                                                 calmodulin binding:regulation of transcription,
DNA-dependent:cytoplasm:nucleus     Calmodulin-binding transcription activator 1    IPT/TIG domain:CG-1 domain    2.25
1     7804961     INDEL T     2     TAA/TAA                                                           CAMTA1      NM_015215.2 exonic      nonsense    TAA   17    p.Phe1417*
      c.4249_4250insAA                                                  calmodulin binding:regulation of transcription, DNA-dependent:cytoplasm:nucleus
      Calmodulin-binding transcription activator 1    IPT/TIG domain:CG-1 domain    2.06
1     7826549     INDEL T     1     T/TC                                                        CAMTA1      NM_015215.2 exonic      stoploss    TCG   23    p.*1674Ser  c.5020
_5021insC                                       calmodulin binding:regulation of transcription, DNA-dependent:cytoplasm:nucleus     Calmodulin-
```

5. Use these version numbers with the transcript IDs in the preferred transcript file.

VariantDB files

You can provide custom annotation information for specific variants of interest when you import a VariantDB file.

Note: Custom variantDBs must have unique names. For example, name and version number can be combined to create unique names.

```
1  Name: variantdbA_v1     Version: v1
2  Name: variantdbA_v2     Version: v2
3  Name: variantdbA_v3     Version: v3
```

A tab-delimited file with a header line is required.

```
##fileformat=VCFv4.1
                #CHROM     POS     ID     REF     ALT     QUAL     FILTER     INFO
                chr1     124535436    COSM00001    TG   AA   .    .
AMPID=AMPL495041;TEMP_ID=0
                chr1     124535494    COSM00002    G    T    .    .
AMPID=AMPL495041;TEMP_ID=1
                chr1     128808434    COSM00003    T    A    .    .
AMPID=AMPL30014;TEMP_ID=2
                chr1     124597624    .            T    G    .    .
AMPID=AMPL30014;TEMP_ID=3
                chr1     136671158    .            TT   CA   .    .
AMPID=AMPL30014;TEMP_ID=5
```

```
                chr1    141128903   COSM00006   TTG CTT .   .
AMPID=AMPL30014;TEMP_ID=6
```

We recommend that the custom input file provided to VariantDB be left-aligned. Left alignment is used to normalize the positions of ambiguous INDELs that can be placed at multiple positions.

The information in the input VariantDB file is used in the following ways in the analysis results:

- In a downloaded variants TSV file, the content in the ID, REF, ALT, and INFO fields are added to the variant.
- In the Analysis Results screen, the content in the ID and INFO fields are added to the variant.
- In the Analysis Results screen, you can create a filter that is based on the content in the ID field. If the content of the ID field does not include a value (contains only a period), then the first key-value pair of the INFO field is used.

More information about VCF format:

- **Official specification of VCF (Variant Call Format) version 4.1:**
  http://www.1000genomes.org/wiki/Analysis/Variant%20Call%20Format/vcf-variant-call-format-version-41
  In VCF format files, missing values are represented by dots. The content must be tab-separated. Ensure that no extra or hidden characters are added to the VCF files, which can occur when they are opened in programs like Excel or Word, or when emailed as an unzipped attachment.
- **Mandatory headers required when creating a VariantDB file:** The following three headers must be present in the first three lines of the VCF file (FORMAT and Sample columns are optional in VCF files):

```
##fileformat=VCFv4.1

##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
                        #CHROM    POS    ID    REF    ALT    QUAL
FILTER    INFO    FORMAT    Sample
```

- **Hit-level information in a VariantDB file:** You can adjust the hit level of each VariantDB file individually by including this information in the header. The following hit-level parameters can be included in the VCF header.
  - **##HITLEVEL=overlap** matches all annotations whose loci overlap with variant.
  - **##HITLEVEL=locus** matches all annotations whose loci start at the variant locus.
  - **##HITLEVEL=allele** matches all annotations that are 'locus' matches plus have at least one allele in common with variant.
  - **##HITLEVEL=genotype** matches all annotations that are 'allele' matches where the genotypes also match.
  - **##HITLEVEL=auto** matches the most specific hit level possible, which can be any of the hit levels listed above.

- **Mandatory columns required in the VCF file when creating a VariantDB:** Providing FORMAT and SAMPLE fields is not required according to the official VCF specification. However, to perform a "genotype" hit level match in Ion Reporter™ Software, you need to specify a GT (genotype) for the variant in the FORMAT column.

  An example of a variant with a GT field of 0/1 in the FORMAT field of a VCF file is given below:

```
chr1    141128903   COSM00006   TTG CTT .   .
AMPID=AMPL30014;TEMP_ID=6    GT    0/1
```

  If only an "overlap" or "locus" or "allele" match is needed, you do not need to specify a GT field. However, the missing values must be represented by dots in the appropriate columns. For example:

```
chr1    141128903   COSM00006   TTG CTT .   .
AMPID=AMPL30014;TEMP_ID=6...
```

  If the "auto" hit level match is chosen, Ion Reporter™ Software attempts to find the most specific hit level match possible. However, if no GT value is supplied, the most specific hit level possible is an allele match, as there is no GT value to do an allele or genotype level match.

- **How to filter on VariantDB:**
  - **Option 1:**

    Ion Reporter™ Software automatically exposes a filter on the first INFO key of the VariantDB VCF file if such a key is specified and if the ID field of the VCF file is absent.
  - Consider the example below:

```
##fileformat=VCFv4.1

##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
                                    #CHROM    POS    ID    REF    ALT
QUAL    FILTER    INFO    FORMAT    Sample
                                    chr1    124535436   .    TG
AA  .   .   AMPID=AMPL495041;TEMP_ID=0...
                                    chr1    128808434   .    T
A   .   .   AMPID=AMPL30014;TEMP_ID=2...
```

    If the above VCF file with two variants is used to make a VariantDB in Ion Reporter™ Software, you can filter on the AMPID field, because the AMPID key is the first INFO key present in the INFO field of the VCF file and the ID fields are absent (represented by dots).
  - **Option 2:**

    If the INFO field is not populated, filtering is automatically enabled on the ID column.

    Consider the example below, in which the INFO field is absent and represented with a dot:

```
##fileformat=VCFv4.1

##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
                                    #CHROM    POS    ID    REF    ALT
QUAL    FILTER    INFO    FORMAT    Sample
                                    chr1    124535436   COSM00001   TG
AA  .   .   ....
```

```
                                    chr1    141128903    COSM00006    TTG
CTT .    .    ....
```

If the VCF file above with two variants is used to make a VariantDB in Ion Reporter™ Software, you can filter on the ID field, because the INFO field of the VCF file is not populated.

For more information about marking and tracking of variant annotation in addition to the VariantDB annotation presets in analysis workflows, see “MyVariants” on page 172.

Delete custom annotation sources

An administrator can view and delete custom annotation sources that were created by users. Custom annotation sets created by users are displayed in the **Workflow Presets** screen.

Predefined annotation sources cannot be deleted. If a custom annotation source is used in an analysis workflow, it cannot be deleted.

1. In the **Workflows** tab, click **Presets**.
2. From the presets list, click **Annotation Sets**, then select **Annotation Source**.
   A table opens that lists the custom annotation sources. It shows information about the annotation, such as: name, version, source version, reference, created by, created on, and source type.
3. In the **Workflow Presets** list, click the custom annotation source that you want to delete, then click **Actions (⚙) ▸ Delete**.

   The annotation source is removed from the **Workflow Presets** list.

Change amino acid code

Ion Reporter™ Software gives you the option to annotate amino acid changes with the single-letter IUPAC amino acid codes, or the three-letter codes, based on your preference. By default, the three-letter code is used. To use the one-letter IUPAC code, you can change a parameter when you edit an analysis workflow. Then, for example, **Val600Glu** would be used in the annotation and displayed in the analysis results as **V600E**.

1. In the **Workflows** tab, select the analysis workflow of interest, then click **Edit**.
2. Click **Parameters** in the workflow bar.
3. In the **Annotation** section, under **Use IUPAC Single Letter Code for Amino Acid**, select the option that you want to use for amino acid changes:

Option	Description
True	Select to use the single-letter IUPAC code
False	Select to use the three-letter amino acid code

4. Click **Next**.
5. Review your selected options for the edited analysis workflow, then click **Confirm**.

Variant identifiers for Oncomine™ panels

Hotspots BED files

The BED file specification (**http://genome.ucsc.edu/FAQ/FAQformat.html#format1.7**) indicates that the fourth column is the name of the BED line. The values in this column are used to label the variant region in the UCSC genome browser or the Integrative Genomics Viewer (IGV). The labels are used in Ion Reporter™ Software to populate the ID field in the output VCF files and the Variant ID columns.

Oncomine™ panel hotspots files include genomic representations that correspond to somatic variants that have often been observed in cancer samples and therefore are likely to be relevant to the cancer phenotype. These files also include variants that are observed less often, or are implicated in literature reports as functionally relevant, such as activating or inactivating variants.

When possible, variants in the hotspots files are assigned an identifier consistent with a publicly available data source, preferentially COSMIC (**https://cancer.sanger.ac.uk/cosmic**) but also including dbSNP (**https://www.ncbi.nlm.nih.gov/snp/**) and ClinVar (**https://www.ncbi.nlm.nih.gov/clinvar/**). If a variant cannot be found in one of these databases, the variant can receive an arbitrarily assigned identifier to help with variant calling interpretation and troubleshooting. Such identifiers, for example, BT144, OM3324, OMINDEL700, MAN103, are designed to be consistent across Oncomine™ panels. When hotspots files are updated, Thermo Fisher Scientific reviews the COSMIC database to determine whether variants with such identifiers have been assigned COSMIC IDs and sometimes replaces them with the more meaningful COSMIC IDs.

COSMIC ID changes due to database updates

The COSMIC database updates approximately twice a year, and these updates involve the addition of newly curated variants, removal of a smaller number of variants, and changes in genomic representations to a very small number of variants. Therefore, there is a chance that the variant identifiers in the hotspots files are not synchronized with – or are no longer found on – the COSMIC website. Additionally, before COSMIC version 90, the same normalized genomic variant (see **https://pubmed.ncbi.nlm.nih.gov/25701572/**) can have had multiple redundant legacy COSMIC variant identifiers (COSM); since COSMIC version 90, these identifiers have been replaced with one consistent COSV identifier, to which multiple legacy COSMIC IDs can map.

Fusions files

A comprehensive, universally recognized database of oncogenic gene fusion breakpoints does not exist. Therefore, gene fusion isoform identifiers are generated by concatenating the two gene symbols with a hyphen, and then combining the first letter of the 5’ partner with the last retained 5’ exon along with the first letter of the 3’ partner with the first retained 3’ exon. For example, EML4-ALK.E6A17 involves a fusion between the sixth exon of EML4 with the 17th exon of ALK. Fusion isoforms involving junctions between incomplete exons, or involving intronic insertions can include “ins” or “del” modifiers followed by the number of nucleotides removed or added. Additionally, assay names can include additional identifiers such as COSMIC COSF ids or GenBank accession numbers.

Preferred transcripts in default analysis workflows

The predefined analysis workflows that are provided in Ion Reporter™ Software include an annotations set with a preferred transcript set, the RefGene canonical transcripts. The default annotation set that includes the default RefSeq canonical transcripts is named **All**.

Default analysis workflows that use the **All** annotation set report only transcripts that are included in the RefGene canonical transcripts. By default, the following RefGene canonical transcripts are included in the **All** annotation set:

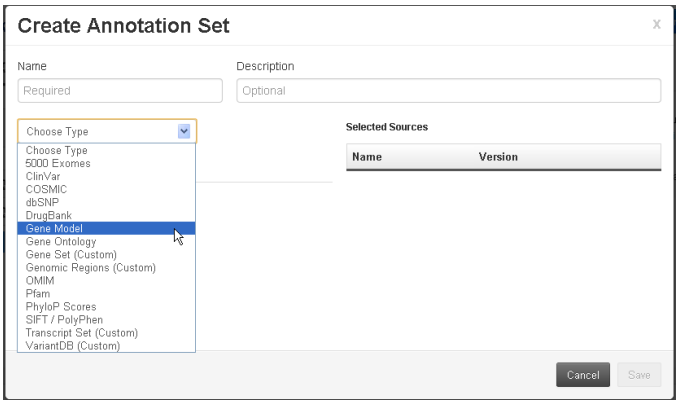
- RefGene
- Functional
- Canonical
- Transcript Scores

All other transcripts are filtered out and not reported.

To instead have additional transcripts reported in your analyses, see “Create annotation set without preferred transcript sets” on page 490.

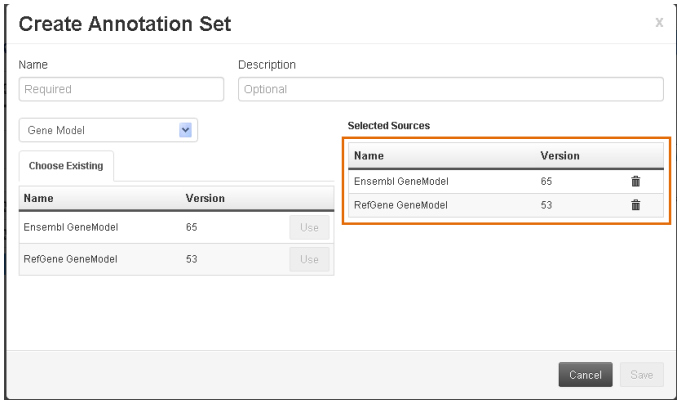
Create annotation set without preferred transcript sets

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Annotation Set**.
   a. In the **Reference** drop-down menu, select **Gene Model** as the type of annotation source.



b. In the **Choose Existing** tab, click **Use** for either the RefSeq Gene Model or the Ensembl® Gene Model.

Note: Only one gene model can be added to each annotation set.



3. Enter a descriptive name for the custom annotation set.
4. (Optional) Enter a description for the custom annotation set.
5. Click **Save**.
   The new annotation source is listed in the **Workflow Presets** screen.

Annotation sources and scores

This section provides more detail about select annotation sources.

P-value scores

The P-value represents the probability that the variant call is incorrect. The range is from 0.00001 to 0.99999, with numbers approaching 1 being the least confident (that the call is incorrect), and numbers close to zero being the most confident (that the call is incorrect).

P-values closer to 0.0 represent more confidence that the variant call is correct. P-values closer to 1.0 represent less confidence that the variant call is correct.

The p-value reported by Ion Reporter™ Software is a logarithmic transformation of the Phred quality score value made by the VariantCaller. For example, a VariantCaller quality score of 20 is associated with a p-value of 0.01. A VariantCaller quality score of 30 is associated with a p-value is 0.001.

Pfam annotations

Ion Reporter™ Software has multiple Pfam versions. For more information on Pfam, see **https://www.ebi.ac.uk/interpro/**.

Pfam consist of parts A and B. Ion Reporter™ Software uses Pfam-A. "A is curated and contains well-characterized protein domain families with high quality alignments, which are maintained by using manually checked seed alignments and HMMs to find and align all members", according to this site:

Pfam: a comprehensive database of protein domain families based on seed alignments

See also the following link for information about Pfam:

The Pfam protein families database:: R.D. Finn, J. Mistry, J. Tate, P. Coggill, A. Heger, J.E. Pollington, O.L. Gavin, P. Gunesekaran, G. Ceric, K. Forslund, L. Holm, E.L. Sonnhammer, S.R. Eddy, A. Bateman. Nucleic Acids Research (2010) Database Issue 38:D211-222

Note: Pfam annotations for all domains of the gene's protein are added for any variant.

IMPORTANT! The interpretations listed here are the recommendations from the referenced web sites.

Annotation versions and calculations

Details about the annotation scores that used in Ion Reporter™ Software and how these scores are calculated are explained in this section.

SIFT

- **SIFT version**—5.1.1
- **Protein database**—UniprotTrEMBL. Updated January 2023.
- **BLAST version**—2.2.25. Updated to 2.2.26.
- **BLIMPS version**—3.9

Polyphen-2

- **Version**—PolyPhen-2 v2.1.0r367, using the stand-alone default installation instructions and usage
- **BLAST version**—2.2.25
- **Protein database**—Uniref100, downloaded January 2023 from ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/uniref/uniref100/uniref100.fasta.gz

Codon differences in chromosome M

Chromosome M has its own genome that is different in 4 codons from the other chromosomes.

Ion Reporter™ Software uses the Homo sapiens mitochondrion, complete genome NCBI Reference Sequence: NC_012920.1.

The new rCRS chrM assembly has 16569 bases. The previous version had 16571 bases.

The 2 coding tables, one for standard chromosomes and one for chromosome M, are listed below. These tables are from the following NCBI site:

http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi#SG2

The Standard Code (transl_table=1) for chromosomes 1-22, X and Y (NUCLEAR)

By default all transl_table in GenBank flat files are equal to id 1, and this is not shown. When transl_table is not equal to id 1, it is shown as a qualifier on the CDS feature.

Note: In these tables, an asterisk represents a STOP codon.

```
TTT F Phe       TCT S Ser       TAT Y Tyr       TGT C Cys
TTC F Phe       TCC S Ser       TAC Y Tyr       TGC C Cys
TTA L Leu       TCA S Ser       TAA * Ter       TGA * Ter
TTG L Leu i     TCG S Ser       TAG * Ter       TGG W Trp
```

```
CTT L Leu       CCT P Pro       CAT H His       CGT R Arg
CTC L Leu       CCC P Pro       CAC H His       CGC R Arg
CTA L Leu       CCA P Pro       CAA Q Gln       CGA R Arg
CTG L Leu i     CCG P Pro       CAG Q Gln       CGG R Arg
```

```
ATT I Ile       ACT T Thr       AAT N Asn       AGT S Ser
ATC I Ile       ACC T Thr       AAC N Asn       AGC S Ser
ATA I Ile       ACA T Thr       AAA K Lys       AGA R Arg
ATG M Met i     ACG T Thr       AAG K Lys       AGG R Arg
```

```
GTT V Val       GCT A Ala       GAT D Asp       GGT G Gly
GTC V Val       GCC A Ala       GAC D Asp       GGC G Gly
GTA V Val       GCA A Ala       GAA E Glu       GGA G Gly
GTG V Val       GCG A Ala       GAG E Glu       GGG G Gly
```

The Vertebrate Mitochondrial Code (transl_table=2) for chromosome M.

```
TTT F Phe       TCT S Ser       TAT Y Tyr       TGT C Cys
TTC F Phe       TCC S Ser       TAC Y Tyr       TGC C Cys
TTA L Leu       TCA S Ser       TAA * Ter       TGA W Trp
TTG L Leu       TCG S Ser       TAG * Ter       TGG W Trp
```

```
CTT L Leu       CCT P Pro       CAT H His       CGT R Arg
CTC L Leu       CCC P Pro       CAC H His       CGC R Arg
CTA L Leu       CCA P Pro       CAA Q Gln       CGA R Arg
CTG L Leu       CCG P Pro       CAG Q Gln       CGG R Arg
```

```
ATT I Ile i    ACT T  Thr      AAT N Asn      AGT S  Ser
ATC I Ile i    ACC T Thr      AAC N Asn      AGC S Ser
ATA M Met i    ACA T Thr      AAA K Lys      AGA * Ter
ATG M Met i    ACG T Thr      AAG K Lys      AGG * Ter
```

```
GTT V Val      GCT A Ala      GAT D Asp      GGT G Gly
GTC V Val      GCC A Ala      GAC D Asp      GGC G Gly
GTA V Val      GCA A Ala      GAA E Glu      GGA G Gly
GTG V Val i    GCG A Ala      GAG E Glu      GGG G Gly
```

The differences from the Standard Code are:

	Code 2	Standard
AGA	Ter *	Arg R
AGG	Ter *	Arg R
AUA	Met M	Ile I
UGA	Trp W	Ter *

Background information

The UCSC website has this note about chrM:

“Note on chrM:

Since the release of the UCSC hg19 assembly, the Homo sapiens mitochondrion sequence (represented as "chrM" in the Genome Browser) has been replaced in GenBank with the record NC_012920. We have **not** replaced the original sequence, NC_001807, in the hg19 Genome Browser. We plan to use the Revised Cambridge Reference Sequence (rCRS) in the next human assembly release.”

The **IGSR: The International Genome Sample Resource** has this entry:

Which reference assembly do you use?

The reference assembly the 1000 genomes project has mapped sequence data that has changed over the course of the project.

For the pilot phase we mapped data to NCBI36. A copy of our reference FASTA file can be found on the **ftp site**.

For the phase1 and phase 3 analysis we mapped to GRCh37. Our FASTA file which can be found **here** called human_g1k_v37.fasta.gz, it contains the autosomes, X, Y and MT but no haplotype sequence or EBV.

Other links related to chrM:

Original sequence, NC_001807:

http://www.ncbi.nlm.nih.gov/nuccore/NC_001807.4?report=genbank

The updated Mitochondrial sequence, NC_012920 can be found here:

http://www.ncbi.nlm.nih.gov/nuccore/NC_012920

Effect of Ensembl and RefSeq sources on Polyphen and SIFT scores

You can create a custom annotation set with Polyphen and SIFT scores. When you use the scores in an annotation set, ensure that you use the corresponding RefSeq or Ensembl® gene models.

- Functional scores v21 is for the gene model GRCh38 Ensembl® 108.
- Functional scores v20 is for the gene model GRCh38 RefSeq 210.
- Functional scores v19 is for the gene model hg19 Ensembl® 108.
- Functional scores v18 is for the gene model hg19 RefSeq 210.
- Functional scores v17 is for the gene model GRCh38 Ensembl® 103.
- Functional scores v16 is for the gene model hg19 Ensembl® 103.
- Functional scores v15 is for the gene model versions RefSeq 201 and Ensembl® 100.
- Functional scores v14 is for the gene model versions RefSeq 201 and Ensembl® 100.
- Functional scores v13 is for the gene model versions RefSeq 99 and Ensembl® 99.
- Functional scores v12 is for the gene model versions RefSeq 98 and Ensembl® 99.
- Functional scores v11 is for the gene model versions RefSeq 95 and Ensembl® 97.
- Functional scores v10 is for the gene model versions RefSeq 95 and Ensembl® 97.
- Functional scores v9 is for the gene model versions RefSeq 89 and Ensembl® 92.
- Functional scores v8 is for the gene model versions RefSeq 89 and Ensembl® 92.
- Functional scores v7 is for the gene model versions RefSeq 83 and Ensembl® 90.
- Functional Scores v6 is for the gene model versions RefSeq 77 and Ensembl® 84.
- Functional Scores v5 is for the gene model versions RefSeq 72 and Ensembl® 79.
- Functional Scores v4 is for the gene model versions RefSeq 63 and Ensembl® 74.
- Functional scores v3 is for the gene model versions RefSeq 53 and Ensembl® 65.

For more information, see “Create annotation set workflow presets” on page 133.

Genetic Category Type variants

This help section provides information about the variant categories that are defined by trio analysis workflows. During these analysis workflows, each variant called in the proband sample is assigned all categories that apply. A variant might be assigned one, none, or many categories.

During your review of analysis results, the Genetic Category Type filter applies to these categories.

HasDeNovoNonRefAllele

An allele of a proband variant is *de novo* if it does not appear in the genotype of either of the parents of the proband at this variant position.

The category HasDeNovoNonRefAllele is assigned to a proband variant if the variant meets all following criteria.

- The variant has an allele that:
  - Is not the same as the reference allele
  - Does not appear in the genotype of the father
  - Does not appear in the genotype of the mother
- The coverage of the genotype of the father at this variant position is at least denovo_nonref_allele_min_father_coverage.
- The coverage of the genotype of the mother at this variant position is at least denovo_nonref_allele_min_mother_coverage.

Note: The coverage parameters help avoid false-positive calls that might occur because the sequencing coverage is inadequate to call an accurate genotype. Without coverage information, the absence of a variant call for a parent is only absence of evidence, and does not exactly determine that the allele for the proband is *de novo*. To establish evidence of absence, the software requires that the parents be covered well enough that variants are called if actually present. Therefore the software requires that the position in both parents meets a coverage threshold.

HasDeNovoRefAllele

A proband variant is assigned the category HasDeNovoRefAllele if all of the following are true:

- The variant has an allele that:
  - Is the same as the reference allele.
  - Does not appear in the father's genotype.
  - Does not appear in the mother's genotype.
- The coverage of the father's genotype at this variant position is at least denovo_ref_allele_min_father_coverage.
- The coverage of the mother's genotype at this variant position is at least denovo_ref_allele_min_mother_coverage.

See also “HasDeNovoNonRefAllele” on page 496.

IsNewlyHomozygousNonRef

A proband variant is newly homozygous if it is homozygous but genotypes of both of the parents are heterozygous.

A proband variant is assigned the category IsNewlyHomozygousNonRef if all of the following are true:

- The variant is homozygous.
- The variant's allele is not the same as the reference.
- The father's genotype is heterozygous at this variant position.
- The mother's genotype is heterozygous at this variant position.

- The variant's functional annotations include at least one specified by newly_homozygous_nonref_functional_types.
- The frequency of the allele in the population is at most newly_homozygous_nonref_max_population_allele_frequency.

This situation can indicate a recessive disease or condition, in which the parents carry the same deleterious mutation but are unaffected because they are heterozygous. The trio analysis later examines the variant's functional annotations to help determine if the variant is deleterious.

IsNewlyHomozygousRef

A proband variant is newly homozygous if it is homozygous but genotypes of both of the parents are heterozygous.

A proband variant is assigned the category IsNewlyHomozygousRef if all of the following are true:

- The variant is homozygous.
- The variant's allele is the same as the reference.
- The father's genotype is heterozygous at this variant position.
- The mother's genotype is heterozygous at this variant position.
- The frequency of the allele in the population is at most newly_homozygous_nonref_max_population_allele_frequency.

IsNewlyHeterozygousNonRef

A proband variant is heterozygous non-ref if it is heterozygous and neither allele is the reference allele. The variant is *newly* heterozygous non-ref if the genotypes of both parents contain the reference allele.

A proband variant is assigned the category IsNewlyHeterozygousNonRef if all of the following are true:

- The variant is heterozygous.
- Neither of the variant's alleles is the same as the reference.
- The father's genotype contains the reference allele at this variant position.
- The mother's genotype contains the reference allele at this variant position.
- The variant's functional annotations include at least one specified by newly_homozygous_nonref_functional_types.
- The frequency in the population of at least one of its alleles is at most newly_homozygous_nonref_max_population_allele_frequency.

This situation can indicate a recessive trait or condition, in which the parents carry the same deleterious mutations but are unaffected because they are heterozygous-ref.

Note: The parameters newly_homozygous_nonref_functional_types and newly_homozygous_nonref_max_population_allele_frequency are used in both the IsNewlyHomozygousNonRef and IsNewlyHeterozygousNonRef categories.

InCompoundHeterozygote

A compound heterozygote is a pair of putatively deleterious variants on the same gene.

A proband variant is assigned the category InCompoundHeterozygote if both of the following are true:

- The variant's functional annotations include at least one of the types in compound_heterozygote_functional_types.
- The variant is in the same gene as another such variant.

The variants can be either homozygous or heterozygous. The trio analysis later determines whether a variant is putatively deleterious by examining the existing functional annotations associated with the variant.

InTransPhaseCompoundHeterozygote

A trans-phase compound heterozygote is a compound heterozygote in which one variant has a non-reference allele on the paternal homolog, and the other variant has a non-reference allele on the maternal homolog.

A trans-phase heterozygote is a pair of deleterious proband variants in a gene where:

- One of the two variants has a non-reference allele that is present in the father's genotype, and whose other allele is present in the mother's genotype, *and*
- The other variant has a non-reference allele that is present in the mother's genotype, and whose other allele is present in the father's genotype.

To determine the InTransPhaseCompoundHeterozygote category, for each gene, the trio analysis creates two lists of proband variants, *fromFather* and *fromMother*.

Both of the following apply to each variant in the *fromFather* list:

- Its functional annotations include at least one in compound_heterozygote_functional_types.
- It has a non-reference allele that is present in the father's genotype, and whose other allele is present in the mother's genotype at this variant's position.

Similarly, both of the following apply to each variant in the *fromMother* list:

- Its functional annotations include at least one in compound_heterozygote_functional_types.
- It has a non-reference allele that is present in the mother's genotype, and whose other allele is present in the father's genotype at this variant's position.

Any variant in the *fromFather* list paired with any variant in the *fromMother* list forms a trans-phase heterozygote pair, and any trans-phase heterozygous pair of variants has one variant in the *fromFather* list and the other in the *fromMother* list.

A proband variant is assigned the category InTransPhaseCompoundHeterozygote if either of the following are true:

- The variant appears in the *fromFather* list and there is at least one other variant in the *fromMother* list, or
- The variant appears in the *fromMother* list and there is at least one other variant in the *fromFather* list.

HasMaleMaternalX

A proband variant is assigned the category HasMaleMaternalX if all of the following are true:

- The proband is male.
- The variant is on the X chromosome.
- The variant is hemizygous (non-reference).
- The father's genotype at this variant's position is hemizygous reference.
- The mother's genotype at this variant's position is heterozygous.
- The mother's genotype includes the proband variant's allele.
- The variant's functional annotations include at least one type in maternal_x_functional_types.
- The frequency of the proband's allele is less than maternal_x_max_population_allele_frequency.

The following explain why these conditions apply to this category:

- This category detects possible causative variants inherited from the mother on the single X-chromosome of a male proband (conditions 1 and 2 above).
- The father is not affected, so when a variant is causative, the father, with only one X-chromosome, cannot also have that variant at this position (condition 4).
- If the variant is *de novo*, it is assigned the categoryHasDeNovoNofRefAllele. For the category HasMaleMaternalX, a variant allele is inherited from the unaffected mother (conditions 5 and 6).
- The variant is detrimental (condition 7).

HasUnknownX

A proband variant is assigned the category HasUnknownX if all of the following are true:

- The proband is male.
- The variant is on the X chromosome.
- The variant is hemizygous (non-reference).
- The father's genotype at this variant's position is hemizygous reference.
- The mother's genotype does not include the proband variant's allele.
- The variant's functional annotations include at least one in unknown_x_functional_types.
- The frequency of the proband's minor allele is less than unknown_x_max_population_allele_frequency.

InconsistentWithFather

A proband variant is assigned the category InconsistentWithFather if all of the following are true:

- Neither of the variant's alleles appear in the father's genotype at this variant position.
- The variant has coverage at least consistencyMinCoverage.
- The father's genotype has coverage at least consistencyMinCoverage at this variant position.

InconsistentWithMother

A proband variant is assigned the category InconsistentWithMother if all of the following are true:

- Neither of the variant's alleles appear in the mother's genotype at this variant position.
- The variant has coverage at least consistencyMinCoverage.
- The mother's genotype has coverage at least consistencyMinCoverage at this variant position.

InconsistentWithParents

A proband variant is consistent with its parents if one of its alleles is present in the genotype of the father at this variant position and its other allele is present in the genotype of the mother at this variant position.

A proband variant is assigned the category InconsistentWithParents if all of the following are true:

- The variant has coverage of at least consistencyMinCoverage.
- The father's genotype has coverage of at least consistencyMinCoverage at this variant position.
- The mother's genotype has coverage of at least consistencyMinCoverage at this variant position.
- The variant is not consistent with variants of parents.

Gene Category Type and Variant Effect filter types can be applied to the same variants

The Gene Category Type (GCT) has a functional type parameter similar to ‘Variant Effect’ which you can set when creating a custom trio analysis workflow. Both this functional type selection and Variant Effect will be applied if used together in a filter chain. For example, if a variant has a GCT functional type of INDEL, it may have a Variant Effect type of frameshift or non-frameshift. The functional annotation types differ for these two filter types.

The GCT functional filter types are as follows:

- SNP
- INDEL
- synonymous
- missense
- stoploss
- stopgain
- splicejunction
- utr

The Variant Effect filter types are as follows:

- unknown
- synonymous
- missense
- nonframeshiftInsertion
- nonframeshiftDeletion
- nonframeshiftBlockSubstitution

- nonsense
- stoploss
- frameshiftBlockSubstitution

Genetic Category Type and MAF filter

If a variant is not a minor allele, MAF filter will not be effective, because MAF filter only applies if the variant is a minor allele. Users may have a threshold setting for MAF filter which will not be effective if variant is not a minor allele.

Grantham score

The Grantham score attempts to predict the distance between two amino acids, in an evolutionary sense. A lower Grantham score reflects less evolutionary distance. A higher Grantham score reflects a greater evolutionary distance. Higher Grantham scores are considered more deleterious:

- The more distant two amino acids are, the less likely the amino acids are to be substituted with one another.
- The more distant two amino acids are, the more damaging is their substitution.

The distance scores published by Grantham range from 5 to 215. A substitution of isoleucine for leucine, or of leucine for isoleucine, has a score of 5 (and is predicted to be tolerated). A substitution cysteine for tryptophan, or of tryptophan for cysteine, has a score of 215. Any variation involving cysteine has a high or very high Grantham score (and is predicted to be deleterious).

For more information on Grantham scores, see the following sites:

- Abstract for **Amino acid difference formula to help explain protein evolution**.
- Article for Grantham R. "**Amino acid difference formula to help explain protein evolution**", Science. 1974 Sep 6;185(4154):862-4.

HGVS genomic codes

Genomic codes in the Analysis Results table use the Human Genome Variation Society (HGVS) sequence variant nomenclature.

HGVS c. notations for intronic, upstream, and downstream variants in genes are shown on the **Functional** tab in the list of variants in the **Analysis Results** screen. HGVS c. notations are not shown for intergenic variants.

	CHR	POS	REF	ALT	GT	Genomic	Coding	Protein
Homozygous SNP	3	124535434	G	A	1/1	chr1:124535434**G>A**	c.1531G>A	p.Ala511Thr
Heterozygous SNP	4	90545103	A	T	0/1	chr2:90545103**A>T**;[=]	c.1638A>T	p.Lys546Asn
Insertion	4	Insertion of bases “AAT” between the positions 61310513 and 613105134	G	GAAT	0/1	chr7:61310513_ 61310514ins**AAT**;[=]	c.1663_1664insAAT	NA
Deletion	17	Deletion of bases “GGT” in positions 61917157, 61917158,61917159	AGGT	A	0/1	chr7:61917157_61917159del**GGT**;[=]	c.737_739delGGT	NA
MNV	Y	Replacing bases “CC” in positions 50367679 and 50367680 with bases “TT”	CC	TT	0/1	chr7:50367679_50367680delCCinsTT;[=]	c.235_236delCCinsTT	p.Pro79Leu
Homozygous reference call	22	50833853	A	.	0/0	chr11:50833853A	c.=	p.Tyr505Tyr
No call	5	38335801	G	.	./.	chr16:38335801G>?	NA	NA

For more information about the HGVS nomenclature, go to the Human Genome Variation Society website at **https://varnomen.hgvs.org/**.

Ion Reporter™ Software references

- **hg19: This human reference is based on the GRCh37.p5 version of the human genome assembly. The GRCh37.p5 version is described at this web site: https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh37.p5.**
- **GRCh38: This human reference is based on the GRCh38.p2 version of the human genome assembly. The GRCh38.p2 version is described at this web site: https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh38.p2.**

Ambiguity codes for three positions on chromosome 3

Three positions on chromosome 3 are marked with `'N'` in the UCSC version of the genome. These positions have IUPAC ambiguity codes in our version.

Position	IUPAC ambiguity code in Ion reference	Hard masked character in UCSC hg19
60830534	M	N
60830763	R	N
60830764	R	N

Hard masked PAR regions in chromosome Y

The chromosome Y sequence has the pseudoautosomal regions (PAR) hard masked. This practice is consistent with the 1000 Genome Consortium's decision to hard mask these regions in chromosome Y in order to prevent mis-mapping of reads and issues in variant calling on the gender chromosomes.

The masked Y pseudoautosomal regions are chrY:10001-2649520 and chrY:59034050-59363566. (A related file can be downloaded from **ftp://ftp.ensembl.org/pub/release-56/fasta/homo_sapiens/dna/Homo_sapiens.GRCh37.56.dna.chromosome.Y.fa.gz**.)

The following background information is from the UCSC site **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**

"The Y chromosome in this assembly contains two pseudoautosomal regions (PARs) that were taken from the corresponding regions in the X chromosome and are exact duplicates:

chrY:10001-2649520 and chrY:59034050-59363566?

chrX:60001-2699520 and chrX:154931044-155260560"

Chromosome M

Ion Reporter™ Software uses the Cambridge Reference Sequence (rCRS) for chromosome M with the GenBank accession number NC_012920. Ion Reporter™ Software uses the UCSC curated RefSeq genes which currently do not contain chrM genes. To view gene annotation on chrM use the ENSEMBL gene model, which does have chrM genes.

More information is available at: **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**.

SIFT score

A SIFT score predicts whether an amino acid substitution affects protein function.

The SIFT score ranges from 0.0 (deleterious) to 1.0 (tolerated). The score can be interpreted as follows:

- 0.0 to 0.05 -- Variants with scores in this range are considered deleterious. Variants with scores closer to 0.0 are more confidently predicted to be deleterious.
- 0.05 to 1.0-- Variants with scores in this range are predicted to be tolerated (benign). Variants with scores very close to 1.0 are more confidently predicted to be tolerated.

Note: PolyPhen-2 and SIFT scores use the same range, 0.0 to 1.0, but with opposite meanings. A variant with a PolyPhen score of 0.0 is predicted to be benign. A variant with a SIFT score of 1.0 is predicted to be benign.

---

phyloP score

phyloP scores measure evolutionary conservation at individual alignment sites. Interpretations of the scores are compared to the evolution that is expected under neutral drift.

- Positive scores — Measure conservation, which is slower evolution than expected, at sites that are predicted to be conserved.
- Negative scores — Measure acceleration, which is faster evolution than expected, at sites that are predicted to be fast-evolving.

phyloP scores are useful to evaluate the signatures of selection at particular nucleotides or classes of nucleotides. For example, as with third codon positions, or first positions of miRNA target sites.

The absolute values of phyloP scores represent –log p-values under a null hypothesis of neutral evolution.

The following range of scores is used in the Ion Reporter™ Software phyloP filter. See “phyloP” on page 505 for an explanation of how this range is determined.

- Min — –20
- Max — 30

For more information on phyloP, see the following website.

http://compgen.bscb.cornell.edu/phast/background.php

To find the latest UCSC Genome Browser database search for "UCSC Genome Browser update" in the PubMed website: **https://www.ncbi.nlm.nih.gov/pubmed/**.

PolyPhen-2 score

The PolyPhen-2 score predicts the possible impact of an amino acid substitution on the structure and function of a human protein. This score represents the probability that a substitution is damaging. Ion Reporter™ Software reports the pph2-prob PolyPhen-2 score.

The PolyPhen-2 score ranges from 0.0 (tolerated) to 1.0 (deleterious). Variants with scores of 0.0 are predicted to be benign. Values closer to 1.0 are more confidently predicted to be deleterious. The score can be interpreted as follows:

- 0.0 to 0.15 -- Variants with scores in this range are predicted to be benign.
- 0.15 to 1.0 -- Variants with scores in this range are possibly damaging.
- 0.85 to 1.0 -- Variants with scores in this range are more confidently predicted to be damaging.

PolyPhen-2 and SIFT scores use the same range, 0.0 to 1.0, but with opposite meanings. A variant with a PolyPhen-2 score of 0.0 is predicted to be benign. A variant with a SIFT score of 1.0 is predicted to be benign.

phyloP

The phyloP filter range that is used in Ion Reporter™ Software corresponds to the values given in the following UCSC Table Browser website:

`http://genome.ucsc.edu/cgi-bin/hgTables?command=start`

The following table shows the Table Browser settings and values that are used to retrieve the phyloP values:

Setting	Value
clade	Mammal
genome	Human
assembly	Feb. 2009 (GRCh37/hg19)
group	All Tables
database	hg19
table	phyloP46wayPlacental
region	genome
output format	data points
file type returned	plain text
button	summary/statistics

The following minimum and maximum ranges among all chromosomes that the Table Browser returned, with the rounded values used in the Ion Reporter™ Software phyloP filter:

	UCSC Table Browser	Ion Reporter™ Software phyloP filter
Min	-13.796	-14
Max	2.941	3

12 Administer Ion Reporter™ Software

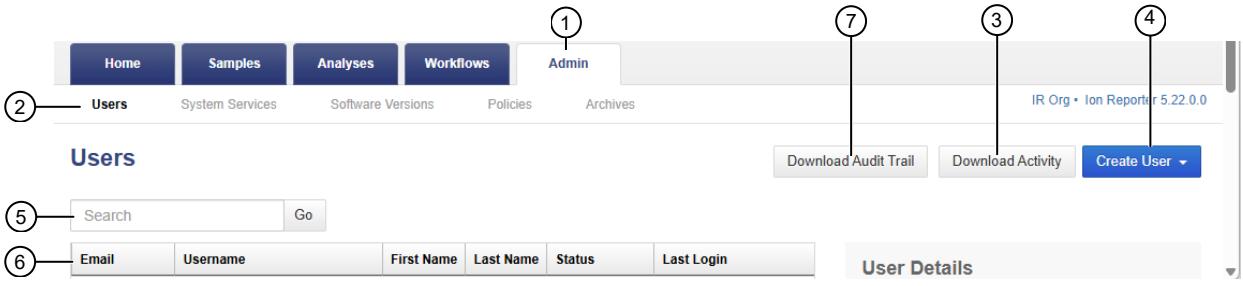
- Admin tab overview 507
- Manage users 508
- Manage system services 514
- View software version information 515
- Manage archives 515
- Manage policies 518
- Manage audit records 520
- Reset the API token for Ion Reporter™ Software on Connect 522
- Reset the API token on Ion Reporter™ Server 524

This section provides an overview of basic administrative functions for Ion Reporter™ Software. Most procedures and functions that are described in this section require administrative permissions for an organization that you administer.

Unless otherwise indicated the procedures in this section apply to both Ion Reporter™ Software that is installed on local servers or to Ion Reporter™ Software on Connect.

Admin tab overview

An administrator can access administration screens in the **Admin** tab.



1. **Admin** tab.
2. The **Users** screen for managing user accounts. For more information, see “Manage users” on page 508.
   The **System Services** screen for managing system functions. For more information, see “Manage system services” on page 514.
   The **Software Versions** screen for viewing software package version information. For more information, see “View software version information” on page 515.
   The **Policies** screen for managing e-signatures and password rules. For more information, see “Manage policies” on page 518.

③ **Download Activity** enables you to download logs of recent activity. For more information, see "Download an audit log of system activities" on page 521.
④ **Create User** enables you to create user accounts.
⑤ In the **Search** box, you can enter key words or text strings to find users.
⑥ Table columns for the **Users** table.
⑦ **Download Audit Trail** enables you to download information about user activities for a selected activity type and time period.

Manage users

An administrator is responsible for managing Ion Reporter™ Software users.

Create user accounts on Ion Reporter™ Server

An administrator can create new user accounts on Ion Reporter™ Server. You can add individual user accounts or add multiple user accounts in a batch with a spreadsheet.

- Create a single new user account.
  a. Click the **Admin** tab.
  b. On the **Users** screen, click **Create User** ▸ **Manual**.
  c. In the **Create User** dialog box, enter the user information, then assign one or more roles to the user account. For more information, see "User roles and permissions" on page 25.
  d. *(Optional)* Select **Requires private folder** to give the user the authority to restrict all visibility to their data. For details, see "Enable or disable private folders on Ion Reporter™ Server" on page 511.
  e. Click **Save**.

  The new user receives an email with a link to activate their account and reset the password.

- Create multiple new user accounts in a batch.
  a. Click the **Admin** tab.
  b. On the **Users** screen, click **Create User** ▸ **Batch**.
  c. On the **Import Users** screen, click **download example** to obtain a blank spreadsheet.
  d. Open the `UserDefinitionTemplate.csv` file that is downloaded through the browser.
  Complete this spreadsheet and upload this file in Ion Reporter™ Software to create new users in a batch.

e. Enter information into the spreadsheet columns for each new user that you want to create using the spreadsheet.

Column name	Description
First Name	First name of the account user. Use a minimum of two letters for first names in user accounts.
Last Name	Last name of the account user. Use a minimum of two letters for last names in user accounts.
EmailId	Email address of the account user. The email address will be used as the user ID when the user signs in. For example, user@institute.com.
Import Role	Enter 1 to assign the Import role.
Analyze Role	Enter 1 to assign the Analyze role.
Report Role	Enter 1 to assign the Report role. The Report role is required in order to add an electronic signature to a report.
Administrator	Enter 1 to assign the Admin role.

f. Save the spreadsheet to a file directory on your hard drive or in a location that you can get back to.

g. In the **Import Users** screen, click **Select File**, navigate to the directory containing the spreadsheet, select the file, then click **Open**.

h. Click **Upload**.

The information in the spreadsheet is uploaded to the software. The new users appear on the **Users** screen.

The new users receive an email with a link to activate their user ID and reset their password.

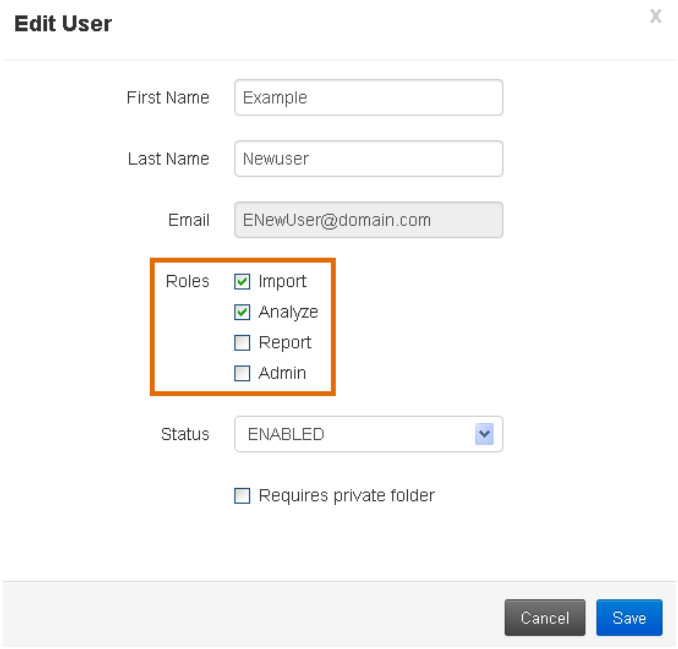
Modify the permissions of a user on Ion Reporter™ Server

An administrator can modify the permissions of an Ion Reporter™ Software user.

1. Click the **Admin** tab, then click the user account in the **Users** list (without clicking on the hyperlink for the account).

2. In **User Details**, click **Edit**.

3. In the **Edit User** dialog box, in the **Roles** area, modify the user permissions.



4. *(Optional)* Select **Requires private folder** if you want to give the user the authority to restrict all visibility to their data. For details, see “Enable or disable private folders on Ion Reporter™ Server” on page 511.

5. Click **Save**.

Enable or disable private folders on Ion Reporter™ Server

An administrator can enable or disable a private folder in Ion Reporter™ Software for individual user accounts. Private folders allow a user to restrict all visibility to their data.

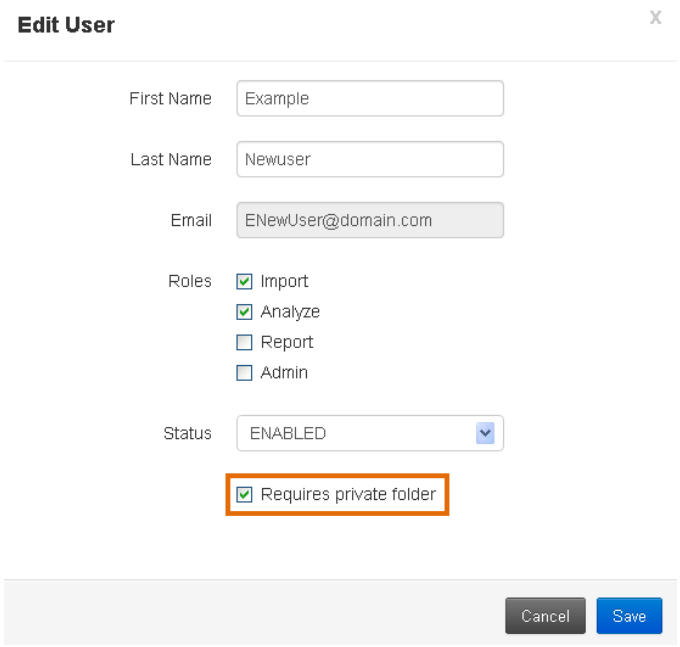
After a private folder is enabled, the data, analysis, and analysis workflows created by that user and generated in that folder are visible only to the user who owns the private folder, and to the administrator who set up the private folder.

This functionality is available only for the Ion Reporter™ Server. It is not available for Ion Reporter™ Software on Connect.

IMPORTANT! If a private folder is disabled any time after it is enabled, all data that was generated in that folder can no longer be accessed. If a new private folder is created by reselecting **Requires private folder**, then all data generated in the previous private folder remains inaccessible.

---

1. Click the **Admin** tab.
2. In the **Users** list, select the row for the user, then click **Edit** in the **User Details**.
3. In the **Edit User** window, select or unselect **Requires private folder**, then click **Save**.



Set or reset the password on a user account on Ion Reporter™ Server

An administrator can set a new password or reset a locked password on a user account.

A user who enters an incorrect password is locked out of Ion Reporter™ Software if they exceed the maximum number of attempts that is set in the password policies. By default, the maximum number of sign-in attempts is five. When a user gets locked out, an administrator must reset the password to unlock the account.

After a password is reset, the administrator must notify the user about the new or reset password.

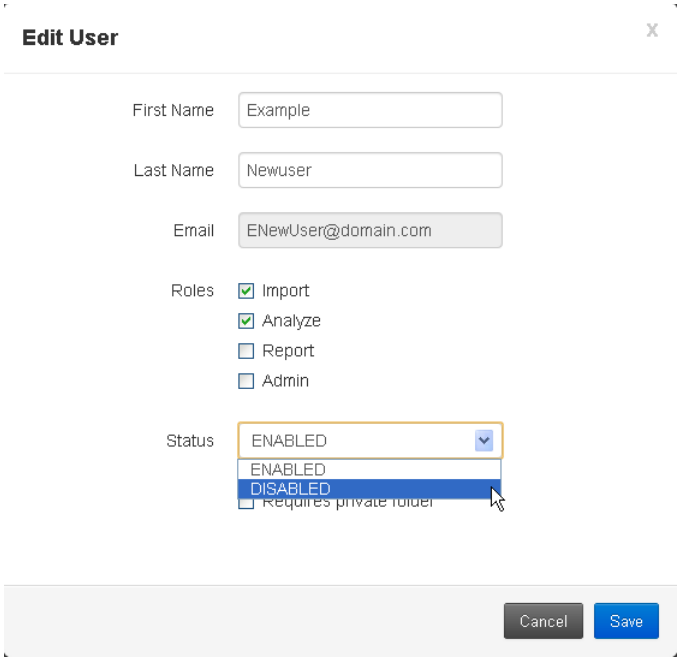
1. On the Ion Reporter™ Software server, sign in to Ion Reporter™ Software, then click the **Admin** tab.
2. In the **Users** screen, select the user account (without clicking the hyperlink for the account), then in **User Details** select **Actions** ▸ **Reset Password**.
3. In the **Reset User Password** dialog box, enter the new password, then retype the password.
4. If you are certain that you want to change the password, click **Confirm**.
5. Notify the user that the password has been set and give the user the new password.

Disable a user account on Ion Reporter™ Server

An administrator can disable an Ion Reporter™ Software user account. A disabled user account can be enabled at a later time.

When a user account is disabled, the email address is not available to be reused for a different user account. If you want to reuse the email address, you must delete the user from the software.

1. Click the **Admin** tab.
2. In the **Users** screen, click the hyperlinked email address of the user.
3. In the **Edit User** dialog box, in the **Status** list, select **Disabled**, then click **Save**.



The user account is disabled. You can enable the account again at any time.

Delete a user account on Ion Reporter™ Server

IMPORTANT! This action can not be undone.

An administrator can delete an Ion Reporter™ Software user account . When you delete a user account, the email address for that user can be reused for a different user account.

1. Click the **Admin** tab.
2. In the **Users** screen, select the user account in the table (without clicking the hyperlink for the account), then in **User Details** click **Actions** ▸ **Delete**.
3. If you are certain that you want to delete the user account, click **Yes** to confirm this deletion.

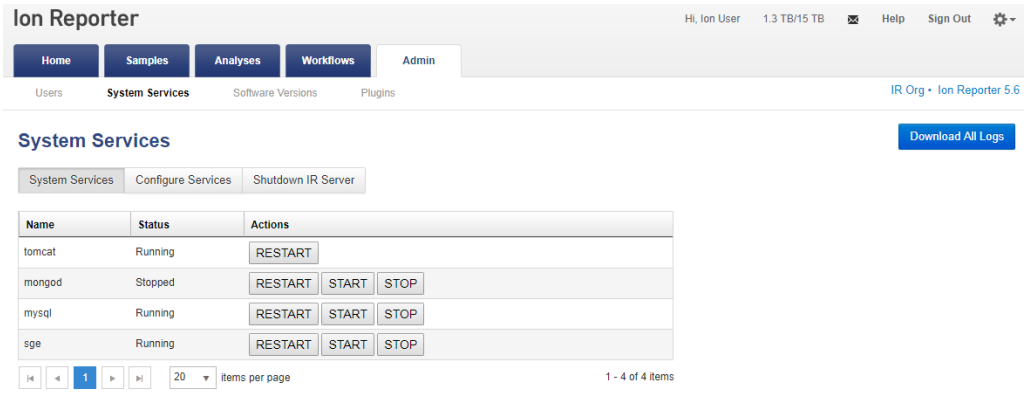
The user account is deleted.

Manage system services

An administrator can manage system services.

This procedure applies only to system services that are related to Ion Reporter™ Server.

1. In the **Admin** tab, click **System Services**.



2. Click the **System Services** tab to see information about the servers that are connected to the Ion Reporter™ Software. The following information is displayed.

Item	Description
Name	Identifies the services that available in the software.
Status	Indicates whether the service is running or stopped.
Actions	Use any of the following buttons to manage a service: • **Restart**: restart a service. • **Start**: start a service. • **Stop**: stop a service.

3. Click the **Configure Services** tab, then enter the number of concurrent analyses that can be run or change the session timeout period.

4. If you need to power off the Ion Reporter™ Server, click the **Shutdown IR Server**, then click **Shutdown IR Server**.

IMPORTANT! All processes are stopped as a result.

5. Click **Download All Logs** to create ZIP files of application logs that you can download to your desktop.

   Completed files can be retrieved from the **Notifications** screen on the **Home** tab, then downloaded to your desktop.

View software version information

An administrator can view version information about the software packages that are installed and available to users of the Ion Reporter™ Software. This information is helpful to determine whether a software package is current or whether the package needs to be updated.

1. In the **Admin** tab, click **Software Versions**.
2. In the **Software Versions** screen, review the information.
3. Click **Package Version** to view the software package installations.
4. Click **Jar Versions** to view the JAR installations.

Manage archives

Archive analyses to an external storage device

An administrator can archive analyses and the samples that are associated with the analyses to an external storage device.

This procedure applies only to Ion Reporter™ Server.

IMPORTANT! Before you start this procedure, ensure that all users are signed out of Ion Reporter™ Software and that no processes are running on the Ion Reporter™ Server. Ion Reporter™ Server is not available to users during the archive process.

You cannot archive analyses or samples that are currently in use. That is, an analysis must have a status of **Successful** and actions such as downloading VCF files, or uploading samples to a analysis must not be in progress. Analyses that are shared with another member of the Ion Reporter™ organization also cannot be archived.

If an analysis is shared with an organization, the analysis can be archived only in the source organization. Samples in the analysis will be not be archived because samples remain common between the source and target organization. To archive samples, first unshare the analysis.

Before you begin this procedure, the paths to the server directory or external device and the directory to which the archives must be configured.

- You must have an external storage device that is mounted to your Ion Reporter™ Server to archive analyses to the device.
- If you have a new Ion Reporter™ Server or upgrade from a previous version, you must specify a storage location for archiving samples and analyses in Ion Reporter™ Server.

  IMPORTANT! Change `archivalMountPath=/tmp/` to `archivalMountPath=/storage IP address/` in the `ionreportermanager/server/server.properties` file, then restart the Tomcat server.

  To check the data that you archived, go to the storage location of the archived data, which is set up in the `/share/apps/IR/ionreportermanager/server/server.properties` file as `archivalMountPath`.
  If you have questions about the Tomcat server, contact your Field Support Engineer or Field Bioinformatics Support representative.

- You can only archive samples if no processes are active on the server. For example, if an analysis is running, or new sample uploads are in progress.
- After the archive process starts, users cannot use the Ion Reporter™ Server until the archive process is complete.

IMPORTANT! Before you start this procedure, ensure that all users are signed off. Ion Reporter™ Server is not available to users during the archive process.

1. In the **Analyses** tab, select one or more analyses that you want to archive.
2. Click **Actions**, then select one of the following choices:

Option	Description
Archive	Archive the analysis alone.
Archive with Samples	Archive the analysis and all samples in the analysis. **Note:** You can only archive the analysis and the samples that were used by that analysis, if no other analyses used those samples.

3. Click **Yes** in the **Confirm Batch Archive** dialog box to begin the archive process for the selected samples. If any processes are running on the server, you will receive a message that contains information about the number of processes that are in progress. Wait until the processes are completed before you proceed.
   The **Archival Manager** screen shows the progress of the archive.
4. (Optional) To stop the archive process, click **Abort**.

   To prevent a loss of data, if you archive multiple analysis you can only abort the archive process if none of the archival processes are complete. For example, if three archives are in progress and two archives are completed but the third archive remains in progress, the abort will not occur.

When the archival process is successful, there is a message in the **Confirm Batch Archive** dialog box to indicate that the process is complete. The analyses and samples are listed in the **Archives** screen.

A successful archive has a **Status** of **Restored**. The table shows the name, type, size, status and any error message for the restoration.

Restore archived analyses and samples

You can restore the Ion Reporter™ Software analyses and samples that you have archived. If you restore data from Ion Reporter™ Software, you can keep or delete the archives.

This information applies only to Ion Reporter™ Server.

IMPORTANT! Before you start this procedure, ensure that all users are signed out of Ion Reporter™ Software and that no processes are running on the Ion Reporter™ Server. Ion Reporter™ Software is not available to users until the restoration process is complete.

You can also restore individual samples or batches of samples that are archived with, or associated with, an analysis. For more information, see "Restore archived samples from an external device to Ion Reporter™ Server" on page 65.

To restore only samples, see "Restore archived samples from an external device to Ion Reporter™ Server" on page 65

- The external storage device that contains archived analyses must be mounted to Ion Reporter™ Server.
- If you have a new Ion Reporter™ Server or upgrade from a previous version, you need to specify a storage location for archiving analyses and samples in Ion Reporter™ Server.

  IMPORTANT! Change `archivalMountPath=/tmp/` to `archivalMountPath=/storage IP address/` in the `ionreportermanager/server/server.properties` file, then restart the Tomcat server.

  To check the data that you archived, go to the storage location of the archived data, which is set up in the `/share/apps/IR/ionreportermanager/server/server.properties` file as `archivalMountPath`.
  If you have questions about the Tomcat server, contact your Field Support Engineer or Field Bioinformatics Support representative.

- You can only archive samples if no processes are active on the server. For example, if an analysis is running, or new sample uploads are in progress.
- After the archive process starts, users cannot use the Ion Reporter™ Server until the archive process is complete.

IMPORTANT! Before you start this procedure, ensure that all users are signed off. Ion Reporter™ Server is not available to users during the restoration process.

---

1. In the **Admin** tab, click **Archives**.
   Archived analyses and samples are listed in the **Archives** screen.

2. Select the analyses or samples that you want to restore, then click **Restore**.

3. In the **Restore Manager** dialog box, indicate whether the archives should be retained on the server or deleted .
   - To delete the samples after the archival process is complete, select the option **do you want to delete the data from path post archive?**.
   - To keep the samples after the archival process is complete, deselect the option **do you want to delete the data from path post archive?**.
4. Click **Yes** to begin the restoration.
   The **Archival Manager** screen shows the progress of the restoration process.
5. (Optional) To stop the archive process, click **Abort**.
   To prevent a loss of data, if you archive multiple analysis you can only abort the archive process if none of the archival processes are complete. For example, if there are 3 archives that are in progress, and 2 archives are completed, but the third item is in progress, the abort does not occur.

After the restoration is complete, the restored analyses is listed in the **Analyses** screen and restored samples are listed in the **Samples** screen. The table shows the name, type, size, status. If any failures occur during the restoral process, any applicable error messages are shown for the restoration.

Manage policies

An administrator is responsible for managing certain policies in the Ion Reporter™ Software. These policies include allowing users who have the Report role to sign reports electronically, and defining password policies for all users.

Enable electronic signatures for final reports

An administrator can allow electronic approval of final reports for completed analyses. When electronic signatures are allowed, a user that is assigned the Report role who generates a final report must also electronically sign the final report. The electronic signature includes the first and last name of the user, a time stamp, and sign-off comments for the signature in the final report footer.

A user can also generate a report with an electronic signature for visualization reports.

Electronic signatures are allowed by default.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Admin** tab, click **Policies**.
2. In the **E-Signature** section, select **Enable E-Signature** to allow users to include electronic signatures in final reports and final report templates.
3. Click **Save**.

Set password policies

An administrator is responsible for setting password policies for all Ion Reporter™ Software users. The software allows for strong password authentication (combination of numbers and letters and special characters), and requires password rotation every 90 days.

This feature is available in Ion Reporter™ Server and not available in Ion Reporter™ Software on Connect.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Admin** tab, click **Policies**.
2. In the **Password Policy** section, specify the password policy values and selections for all Ion Reporter™ Software users.

Password policy	Description	Default value
Password Length	The password must contain a minimum of 6 characters and must not exceed 50 characters.	6 characters
Password Age (Day(s))	The number of days that a password is valid. The value used for this policy must not exceed 365 days.	90 days
Number of Passwords Remembered	A password must not be identical to any of the previous passwords that is set with this policy. By default, the password cannot be identical to any of the previous 5 passwords.	5
User Suspension Period (Minutes)	The amount of time that elapses after the configured **Number of failed login attempts**. The suspension period must be a non-zero value.	1 minute
Number of failed login attempts	The number of failed sign-in attempts must be in the range of 1 to 5.	5
Within (Minutes)	The number of minutes within which multiple failed sign-in attempts will result in a lockout of the account. The value for this time must be within the range of 1 to 30 minutes.	5 minutes
Characters allowed in password	Requirement for passwords to include a variety of types of characters. Select one or more of the following options: • **At least one number** • **At least one uppercase character** • **At least one lowercase number** • **At least one special character**	All 4 options are selected
Password Expiry Notification (Day(s))	The number of days ahead of password expiration that users will be notified about an upcoming password expiration. This value must be within the range of 1 day to 90 days. Users will be notified of an upcoming password expiration with an email notification, and with an alert message displayed in the software.	8 days

(continued)

Password policy	Description	Default value
Repeated characters restriction	Requirements for passwords that avoid the use of repeated characters. Select one of the following options: • **The same characters can be repeated in a password** • **The same character cannot be repeated in a password** • **The same character cannot be used consecutively in a password**	**The same characters can be repeated in a password**

3. Click **Save**.

Manage audit records

An administrator can manage and audit system activities in Ion Reporter™ Software. Knowing when and by whom changes were made in the system helps detect fraud. The audit trail feature also allows governing authorities to audit the system and see proof of activities that were performed.

The audit trail feature:

- Tracks which user did what action and when
- Tracks when records are created, modified, deleted, or changed. The system maintains all entered data, and does not obscure original data when changes are made.
- Records all events with the exact username, date, and time. The system records the identity of the individual who made a change, and requires the user to record the reason for the change.
- Tracks when users log in and records any lock-outs.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

Download an audit trail of user activities

An administrator can track the activities of individual users within the organization in Ion Reporter™ Software. Using this feature, you can select a screen and a time period to audit, then review a log file of the activities performed by users during that time period. For example, you can see who created analyses and who modified samples.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Admin** tab, click **Users**.
2. Click **Download Audit Trail**.
3. Complete the **Download Audit Trail** dialog box.
   a. In **Screen Name**, select a screen of interest from the list.

b. In **Date Range**, select a date range, or specify from and to dates using the calendar.

c. Click **Download**.
A CSV file is created.

4. Open the CSV file, then review user activities performed on the selected screen and date.

Column	Description
Name	Name of the analysis, analysis workflow, sample, or other item created or edited by the selected user.
Type	Type of item created or edited, such as analysis, MyVariants, and so on.
Created By	First and last name of the user who created an event.
Created By Email	Email address of the user who created an event.
Updated By	First and last name of the user who updated an event.
Updated By Email	Email address of the user who updated an event.
Updated By Organization	Name of the organization that updated an analysis or other element.
Screen	The name of the screen that was modified.
Values	The values that were changed on the modified screen.

Download an audit log of system activities

An administrator can track system activities occurring within the organization in Ion Reporter™ Software. For example, you can see who created analyses and who modified samples. Using this feature, you can select a time period to audit.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

For audit logs that list access or changes to samples or analyses, see “View and download audit logs” on page 427.

1. In the **Admin** tab, click **Users**.
2. Click **Download Activity**.
3. In the **Download Activity** dialog box, select a date range, then click **Download**.
A CSV file is created. The file name reflects the screen name selected.
4. Open the CSV file, then review the activities that users performed on the selected screen during the selected date range.

Download an audit log for administrative policies

An administrator can download an audit log that records changes that are made to administrative policies in Ion Reporter™ Software.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the **Admin** tab, click **Users**.
2. Click **Download Audit Trail**.
3. Complete the **Download Audit Trail** dialog box.
   a. In **Screen Name**, select **Admin Policies** from the list.
   b. In **Date Range**, select a date range, or specify from and to dates using the calendar.
   c. (Optional) In the audit report for the selected time period, click **Filter** to refine the results.
   d. Click **Download**.
      A CSV file is created.
4. Click **Download**.

The audit log is downloaded through the browser. Click to save or open the file, depending on your browser settings.

Reset the API token for Ion Reporter™ Software on Connect

These procedure can be performed by either an administrative user or a regular user. To reset the API token for Ion Reporter™ Software on Connect, see instead “Reset the API token on Ion Reporter™ Server” on page 524.

The API token for Ion Reporter™ Software on Connect is used for:

- Configuration of the IonReporterUploader plugin in in Torrent Suite™ Software.
- The access of data from the Web services API.

IMPORTANT! The API token is like a password. When you generate a token, it is similar to resetting a password, and existing users become locked out. Under most circumstances, you should not generate a new API token if one already exists.

Two functions of the IonReporterUploader plugin do not work when you regenerate the API token. The functions and the steps to recover functionality are as follows:

- IonReporterUploader plugins that are configured with your Ion Reporter™ Software on Connect account.
- Scripts or code that access the Ion Reporter™ Software on Connect Web services API with your previous token. To recover, use your new token with those scripts.

You must have an access code to complete this procedure if you use Ion Reporter™ Software on Connect.
If you use Ion Reporter™ Software on Connect and do not already have an access code, regenerate an access code and access the API token. The access code must contain at least 6 characters. The maximum length of the access code is 50 characters.

1. Sign into Ion Reporter™ Software on Connect.
2. Click ⚙ **(Settings)** ▸ **Manage Tokens**.
3. Click **Set New Access Code**, then enter an access code in the **New Access code** field.
4. Select an expiration time in the **Access code Age** dropdown menu, then click **Save and Generate**.
5. Click **Copy** to copy the API token to the clipboard.

Note:

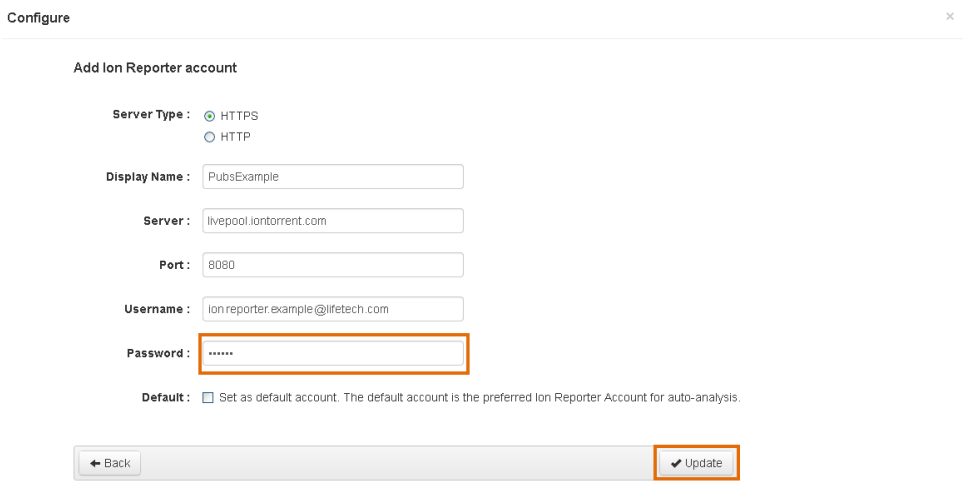
The IRU token is for use with the IonReporterUploader command-line utility and is not required for this procedure.

A new API token is generated.

- Use the new API token to reactivate the account that is configured for use with the IonReporterUploader plugin in Torrent Suite™ Software.

  In Torrent Suite™ Software when you set up an account for use with the IonReporterUploader plugin, you must enter the password in the **Password** field, then click **Update**.

  When you set up an account for use with the IonReporterUploader plugin, enter the access code in the **Password** field, then click **Update**.



- Use the new API token with scripts or code that access the Ion Reporter™ Software on Connect Web services API.

Reset the API token on Ion Reporter™ Server

These procedure can be performed by either an administrative user or a regular user of Ion Reporter™ Software on Connect. To reset the API token for Ion Reporter™ Software on Connect, see instead “Reset the API token for Ion Reporter™ Software on Connect” on page 522.

The API token for Ion Reporter™ Server is used for:

- Configuration of the IonReporterUploader plugin in in Torrent Suite™ Software.
- The access of data from the Web services API.

IMPORTANT! The API token is like a password. When you generate a token, it is similar to resetting a password, and existing users become locked out. Under most circumstances, you should not generate a new API token if one already exists.

Two functions of the IonReporterUploader plugin do not work when you regenerate the API token. The functions and the steps to recover functionality are as follows:

- IonReporterUploader plugins that are configured with your Ion Reporter™ Software account.
- Scripts or code that access the Ion Reporter™ Software Web services API with your previous token. To recover, use your new token with those scripts.

You must have an access code to complete this procedure if you use Ion Reporter™ Software on Connect.
Use the following steps to reset the API token on Ion Reporter™ Server.

1. Click ⚙ **(Settings)** ▸ **Manage API Token**
2. Click **Generate** to generate a new token.
3. Click **Copy** to use the token for the Ion Reporter™ Server Web Services API.

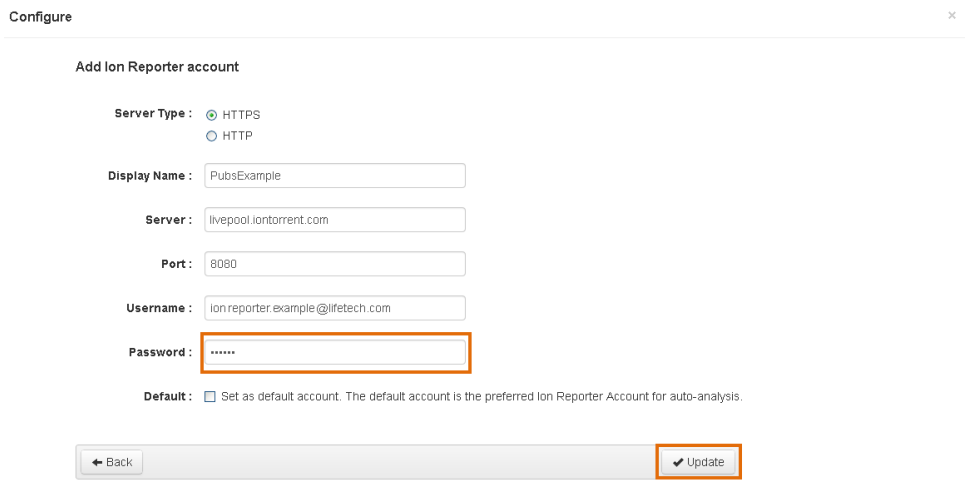
Note:

The IRU token is for use with the IonReporterUploader command-line utility and is not required for this procedure.

A new API token is generated.

- Use the new API token to reactivate the account that is configured for use with the IonReporterUploader plugin in Torrent Suite™ Software.

  In Torrent Suite™ Software when you set up an account for use with the IonReporterUploader plugin, you must enter the password in the **Password** field, then click **Update**.



- Use the new API token with scripts or code that access the Ion Reporter™ Software Web services API.

13 Troubleshooting

- Software is unresponsive and cannot be rebooted 526
- Custom BED file causes an error 526

This section provides basic information on how to troubleshoot any issues you may be having with Ion Reporter™ Software. If you do not find the answer to your question here, please contact Support.

Software is unresponsive and cannot be rebooted

Observation	Possible cause	Recommended action
Software screen freezes or is unresponsive **Details:** This applies to Ion Reporter™ Server only. When using a local Ion Reporter™ Server, the Ion Reporter™ Software screen freezes or becomes unresponsive, and you are unable to open the software, and/or unable to reboot the software.	Disk crash on Ion Reporter™ Server. **IMPORTANT!** If there are backups available for the Ion Reporter™ Server, recovery of data to the last backup might be possible. If there are no backups, then the data might be lost.	Work with your Field Support Engineer (FSE) or Technical Support to back up data.

Custom BED file causes an error

If you use import a custom BED file into Ion Reporter™ Software, the file is validated. The following errors indicate that there is a problem with the BED file. To resolve an error, click the link in the error message to download validation error logs, then review the BED file, and edit the BED file if necessary. Contact your Field Bioinformatics Specialist (FBS) for further assistance with troubleshooting BED files.

Observation	Possible cause	Recommended action
Error parsing track line key=value pairs	There is an error for in the key values that are used in the track line. Example key value pairs are: `track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" version="Ampliseq.com1.0"` type="bedDetail".	Review the BED file and ensure that the value pairs are correct.
Error parsing track line, no closing quotes	The closing quotes are missing from the key value pair in the first line (track line) of the BED file. For example: `track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" version="Ampliseq.com1.0" type=bedDetail.`	Review the track line and add closing quotes.

Observation	Possible cause	Recommended action
ionVersion track key is only valid with type=bedDetail	A BED file that includes an `IonVersion` field must also have a `type=bedDetail` field.	Review the first line (track line) of the BED file and ensure that the `type=bedDetail` and the `ionVerison` field are included.
Field OBS cannot be the same as REF	The strings used in the OBS and REF fields cannot be the same. Both fields cannot be `ATC`, for example.	Change `OBS=string` so that it is not the same as the `REF=string`.
Inconsistent number of columns	Every line in the BED file must include the same number of columns.	Review the BED file and ensure that every line in the file has the same number of columns.
BED track line only allowed in the first line: All lines ignored after this one	BED files allow only one track line in the first line of the header. An example track line is: `track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" version="Ampliseq.com1.0" type=bedDetail`.	Remove the redundant track line.
Line length exceeds 64K	A line in the BED file exceeds 64K characters.	Review the BED file and reduce the line length so that it is 64K or less.
Unknown chromosome name	A chromosome name in the BED file is not included in the FASTA file.	Review the BED file and provide the correct chromosome name.
Region start not in a valid range	For hotspot BED file, the region start value is not in a targeted BED region.	Remove this line.
Region end not in a valid range	For hotspots BED file, the region end value is not in a target bed region.	Remove this line.
Region start and end in reverse order	The region end has a smaller value than the value for the start region.	Ensure that the value for the region start has a smaller value than the value for the end region.
Detail BED file with type=bedDetail must have between 5 and 14 columns	The numbers of columns in a Detail BED file that includes `type=bedDetail` must have between 5 and 14 columns	Review the number of columns in the BED file and ensure that there are between 5 and 14 columns.
Hotspots BED file must have format type=bed	Information is missing from the header of the BED file.	Add `format type=bedDetail` to the file header of the hotspots BED file.
BED file without type=bedDetail must have between 3 and 12 columns	The number of columns for a BED file without `type=bedDetail` has the incorrect number of columns	Review the number of columns in the BED file and ensure that there are between 3 and 12 columns.
BED file contains no usable regions	The BED file might be empty, or every line has at least one error.	Ensure that you are using a BED file that is valid and correctly formatted.
REF field contains characters other than ATCGatcg	The alpha-numeric characters ATCG, or atcg, are not used in the REF field.	Find the REF field in the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.

Observation	Possible cause	Recommended action
OBS field contains characters other than ATCGatcg	The alpha-numeric characters ATCG, or atcg, are not used in the OBS field.	Find the OBS field in the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.
ANCHOR field contains characters other than ATCGatcg	The alpha-numeric characters ATCG, or atcg, are not used in the ANCHOR field.	Find the ANCHOR field in the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.
REF field does not match content of reference fasta file	The string for the REF sequence does not match the reference file.	Look at the REF=`string` and ensure that string is the as the one at that position of the reference sequence from the FASTA file
Mandatory OBS field not found	A field is missing from the custom BED file.	Add an OBS field to the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.

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Fusion analyses

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- Data types for gene fusions analyses 535
- Fusion sample QC metrics 537
- Fusion detection methods 539
- Confirm the presence of fusion calls in Ion Reporter™ Software 544
- Filter chains for fusion panels 544

This appendix applies to analyses that are based on the following Ion AmpliSeq™ and Oncomine™ fusion panels:

- Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2 and Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel.
- Custom Ion AmpliSeq™ and Ion AmpliSeq™ HD fusion panels designed by Ion Torrent White Glove team and Ion AmpliSeq™ Designer.
- Oncomine™ fusion panels for solid tumor, liquid biopsy, heme oncology, and immuno-oncology research applications.

For information on importing fusion panels, see “Import panel files from AmpliSeq.com” on page 125.

Fusion calls in analysis results

Fusion calls describe a translocation of genetic material. The Genes (Exons) column reports the donor gene and the partner gene. The exon number for each gene is reported in parentheses. For the donor gene (the first one in the pair), exon number specifies the exon *before* the fusion. For the partner gene (the second one in the pair), exon number specifies the exon *after* the fusion.

For example, in the second row, the Genes (Exons) column reports EML4(6) - ALK(20):

- EML4(6) refers to exon number 6 on the EML4 gene (the donor gene in this example). Exon number 6 in the donor gene indicates that the fusion starts after exon 6.
- ALK(20) refers to exon number 20 in the ALK gene (the partner gene in this example). Exon number 20 in the partner gene indicates that the fusion starts before exon 20.

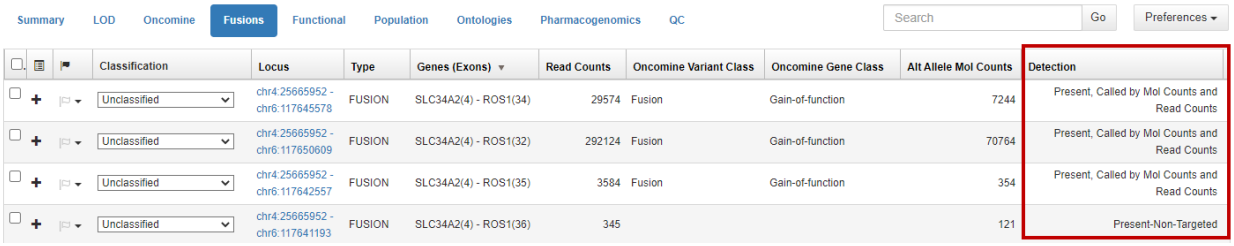
The Detection column reports Present for calls that are supported by read evidence. The default values for the thresholds can be changed and the threshold for read evidence support varies by call type.

- **Fusions**: >20 supporting reads are required to report Present.
- **Expression controls**: >15 supporting reads are required to report Present.

For example, if the threshold of the Detection column is set to >20 reads for fusions and >250 reads for non-targeted fusions, then the following occurs:

- **Absent**: Either no evidence for the fusion is found or <21 reads support the fusion.
- **Present**: >20 reads provide evidence for the fusion.
- **Present-Non-Targeted**: >250 reads provide evidence for a fusion that is not explicitly included in the Ion AmpliSeq™ panel. A non-targeted fusion is a fusion for which the panel did not include a specific primer pair, but it was picked up by a combination of 2 of the primers used for 2 different targeted isoforms. The fusion was not among the targeted isoforms and was not explicitly targeted by the panel. If there are a small number of reads providing evidence for such fusions (for example, less than a few hundred reads), these may not be important. If the evidence for non-tarted fusion is strong, you might want to follow up with more detailed study. A literature search or a search of COSMIC or other databases can be helpful in determining whether such fusions have ever previously been observed, and can provide guidance in interpretation.
- **No Call**: There is not sufficient evidence to determine whether a fusion is present.

For TagSeq and Ion AmpliSeq™ HD analysis workflows: To make **PRESENT/ABSENT** calls, minimum molecular count threshold (default value : >=3) is applied for each target along with the minimum read count threshold (default value > =21). For these analysis workflows, the reason for the call, whether by molecular counts, read counts, or both, is listed in the **Detection** column in the **Fusions** table:



In Ion Reporter™ Software 5.16 and later, fusion calling in analysis workflows for Ion AmpliSeq™ HD and TagSeq panels and assays can be done by using either molecular (family) counts or read counts, and is based on the thresholds for each type of count for a specific fusion. When this parameter is set to **True** in the analysis workflow, the software calculates molecular counts and makes a **Present** call if the threshold value is met. If the threshold is not met, the software then calculates read counts to determine if the **Present** call can be made. Optionally, you can require the threshold value of both molecular count and read count metrics to be met to make a **Present** call, which is the case when the parameter is set to **False**. The parameter which controls the way a call is made is named “Use either family counts only or read counts only to make calls”. The type or types of counts used to make the call is displayed in the **Detection** column of the **Analysis Results** screen. The affect of this parameter, when turned on, is the potential for increased fusion call sensitivity. This parameter has no affect on **NoCalls**.

Other information that is reported for fusions includes the following:

- The Locus column reports the start and end positions of the fusion transcript.
- The Read Counts column reports the number of reads that provide evidence for the fusion call. For ASSAYS_5P_3P, read counts are displayed in the order 5', 3'.
- The COSMIC/NCBI column provides links at which the fusion is described in these public Websites.
- The variant ID column reports our identifier for the fusion variant (or assay or expression control). Each target in the Ion AmpliSeq™ panel has a unique variant ID.

An identifier contains information that is available in other fields in the results table.

Information	Description, example
Partner gene name	ALK
Gene and exon number	A13, for exon number 13 in the ALK gene
COSMIC ID, NCBI GenBank ID	COSF1198, AB374361
Non-Targeted	Appended to the Variant ID for fusion calls that are not targeted by the Ion AmpliSeq™ panel. Example: SLC34A2-ROS1.S4R35.Non-Targeted

Examples of Variant IDs for fusion calls (these examples are from demonstration data):

- **EML4-ALK.E6bA20.AB374362**:
  - **EML4**: Donor gene
  - **ALK**: Partner gene
  - **E6**: Exon number 6 in the ELM gene ("E" for "ELM")
  - **b**: The second fusion (ordered by locus) with the same value in the Genes (Exons) column
  - **A20**: Exon number 20 in the ALK gene ("A" for "ALK")
  - **AB374362**: GenBank ID
- **SLC34A2-ROS1.S4R35.**: Non-Targeted
  - **SLC34A2**: Donor gene
  - **ROS1**: Partner gene
  - **S4**: Exon number 4 in the SLC34A2 gene ("S" for "SLC34A2")
  - **R35**: Exon number 35 in the ROS1 gene ("R" for "ROS1")
  - **Non-Targeted**: Not included in the Ion AmpliSeq™ panel

Analysis configuration file for gene fusion analysis

You can set individual assay-specific thresholds for editable properties, as applicable for the type of that individual assay, in an analysis configuration file that is available in Ion Reporter™ Software.

The analysis configuration file is a readable and editable tab-separated file that is specific to a fusion panel. For a given panel, the file contains all of the targets in the panel as different rows and has eight columns. The first two columns are the name and type of the target in that row. The assay name is the unique identifier and provides mapping between the analysis configuration file and the reference files that are used by the analysis. You can use the other columns to set the six different properties at individual target level.

Edit the gene fusion analysis configuration file

For Oncomine™ fusion analysis workflows, a default analysis configuration file is provided. You can change the file to set custom thresholds. For more information, see “Analysis configuration file for gene fusion analysis” on page 531.

1. In the **Workflows** tab, click **Overview**.
2. In the **Workflows** table, select the Oncomine™ fusion analysis workflow that contains the configuration file that you want to edit, then click **Actions** ▸ **Copy and Edit** for predefined analysis workflows, or **Actions Edit** for user-defined an analysis workflows.
3. Select a **Research Application** and **Sample Group** in the **Edit** workflow bar, then click **Next**.
4. Click **Parameters** in the workflow bar, then select **Fusions**, then click the **Advanced** tab and scroll to the **Analysis Configuration File** section.
5. Click **Download** to download the default analysis configuration file for the analysis workflow. The file name is appended with `properties.txt`.
6. Save the file to a local directory, then open the file and make edits to any of the values for the following properties.
   - Minimum read count
   - Minimum normalized read count
   - Make calls
   - Minimum wild type ratio
   - Do not report
   - Maximum read count negative
   - Minimum molecular count (for use with AmpliSeq HD analyses)
   - Minimum Imbalance Score, for targets of type RNAExon Tile
   - Minimum Imbalance P value, for targets of type RNAExon Tile
   - Minimum average read count for Exon Tiling QC, for targets of type RNAExon Tile
   - Minimum wild type QC RNAExonVariants
   - Percent homology
   - Percent exact matches
   - Maximum weak amplicons
   - Minimum number of flanking amplicons for predicted RNATile breakpoint

   For more information, see “Editable parameters in the analysis configuration file” on page 533
7. Save the file.
8. In the **Analysis Configuration File** section of the **Parameters** workflow bar, click **Upload**.
9. Browse to the updated file and select the file, then click **Upload** in the dialog.

The updated configuration file is added to top of the dropdown list in the **Analysis Configuration File** section of the analysis workflow parameters.

Editable parameters in the analysis configuration file

Parameter	Description
`Min Read Count`	This column allows users to set custom minimum read count thresholds for different targets. If the value is specified as ***Use Global Value*** for a target (the value in the row) in this column, then the global minimum read count threshold is used. (For example: 20 reads or 40 reads for Fusions as set using the minimum read count for Fusions parameter.) If a user changes the value for any target from ***Use Global Value*** to an allowed integer value (for example: 50), then that value overrides the global threshold. The new threshold (50 in this example) is used to call that target as Present/Absent. All other targets will still use the global threshold. This column overrides these global parameters at the individual target level (minimum read count for Fusions, minimum read count for Controls, minimum read count for Gene Expression, minimum read count for Non-Targeted fusions). This property is not applicable to 5p3pAssay type targets.
`Min Normalized read count`	This column allows users to set custom minimum normalized read count thresholds for different targets. If the value is specified as ***Use Global Value*** for a target (the value in the row) in this column, then the global minimum read count threshold is used. (For example : 0.0 or 0.0015 reads for Fusions.) If a user changes the value for any target from ***Use Global Value*** to an allowed value (for example : 0.1) , then it overrides the global threshold and the new threshold (0.1 in this example) is used to call the target as Present/Absent. This column can be used to override the minimum normalized read count global parameters at individual target. This property is not applicable to 5p3pAssay type targets.
`Make Calls`	This column allows users to enable making Present/Absent call for RNAExonVariants at individual target level. This property is not applicable to any other type. By default the analysis workflow parameters are set to not make calls for all the RNAExonVariants. If the value for any RNAExonVariant target (the value in the row) is changed from ***Use Global value*** to ***True***, the target will be called Present/Absent based on the read count and wild type ratio.
`Min WT Ratio`	This column allows users to set custom threshold for minimum wild type ratio for RNAExonVariants at individual target level.
`Do Not Report`	By changing the value to ***True*** for any target, that target is not displayed in the output files and reports.

(continued)

Parameter	Description
`Max Read Count Negative`	This column allows users to set nocall range for any fusion target. The value specified in this column should be always less than or equal to the value for minimum read count as set by global parameter threshold or custom threshold for that target. For example, if a global threshold for minimum read count for fusion is 40, and `Max Read Count Negative` is set to 20 for a target (T1)_ , if the count of target T1 is between 20 and 40, it will be called as nocall. If the count is <20, it is called as absent; if it is >40 it is called as present. This is not applicable to 5p3pAssay type targets. If the value for `Max Read count Negative` is equal to the min read count for any target, then the target is called Absent; if the read count is less than that value, it is called as Present.
`Minimum Imbalance Score for the RNAExon Tile Assays`	Minimum imbalance score for calling ***imbalance positive*** from RNA exon tiling assays in a driver gene. Positive calls also depend on p-value for imbalance.
`Maximum p-value for Imbalance for the RNAExon Tile Assays`	Maximum statistical significance p-value for calling ***imbalance positive*** from RNA exon tiling in a driver gene. Positive calls also depend on imbalance scores.
`Minimum average read counts from all the Exon Tiling assays in a Driver Gene to proceed with Imbalance`	Mean coverage of a driver gene with RNA exon tiling assays. Measured per gene, as the total valid mapped reads counts from all exon-tiling assays divided by the number of exon-tiling assays.
`Max Weak Amplicons`	Maximum number of failed amplicons that are allowed beyond breakpoint. The RNA Exon Tiling amplicons beyond the breakpoint are required to have sufficient expression. The parameter defines an upper bound to allowed number of Exon Tiling amplicons that fail to reach a coverage threshold. (Coverage that is below than 20x coverage the maximal amplicon in the gene.)
`Min Flank Amplicons`	Minimum number of flanking amplicons for predicted RNAExonTile breakpoint. The breakpoint predicted by the RNA Exon Tiling algorithm should be flanked by a minimal number of amplicons – defined by the parameter – on both sides. This setting will exclude breakpoint prediction occurring at the 3’ or 5’ end of the genes.

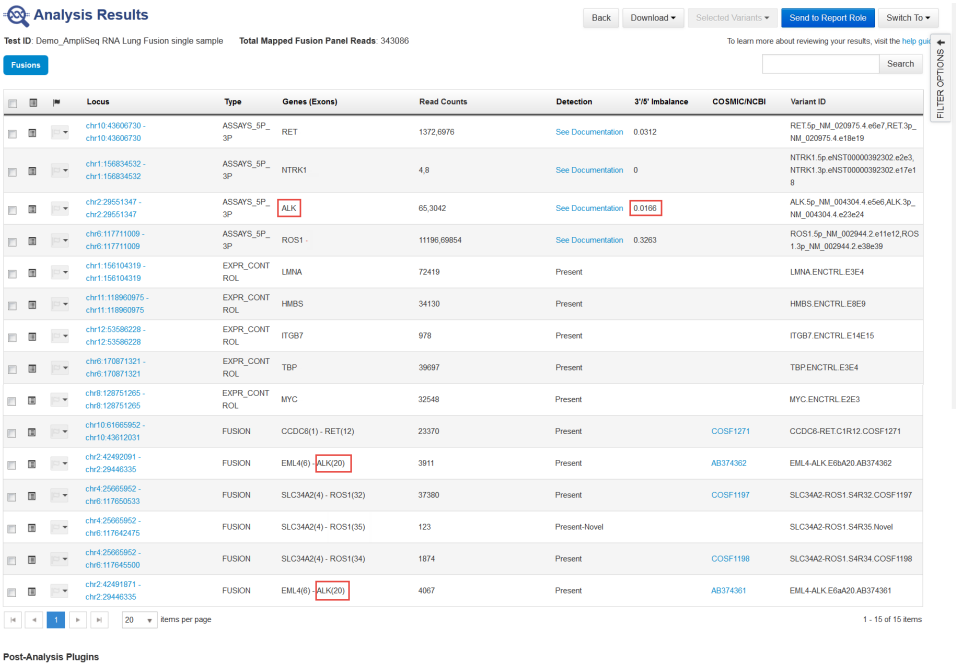
Data types for gene fusions analyses

Use the following guidelines to interpret the fusion calls and other information that is includes in analysis results. In addition to fusion type assays, the analysis results and visualization include information on assays of other types:

- ASSAYS_5P_3P—5' and 3' assays provide confirmation for the fusion calls. This score is reported in the **3'/5' Imbalance** column.

  Four assays are built into the Ion AmpliSeq™ RNA fusion panels. Each assay corresponds to one of the acceptor genes in the panel, and that corresponding acceptor gene is reported in the **Genes (Exons)** column.

  For example, the assay reported in the third column names ALK in the **Genes (Exons)** column. For example, in rows 10 through 15 in this example, the 3'/5' Imbalance score for this assay reflects the strength of the calls for all fusions with the ALK driver gene.



  The **Genes (Exons)** column does not report exon numbers for assays rows.

- EXPR_CONTROL—Few expression control assays are built into the panel to confirm the success of the sequencing run that the analysis is based on.

  A limited number of expression control genes are built into the Ion AmpliSeq™ panel for quality checking purposes. If multiple EXPR_CONTROL calls are reported as Absent in the **Detection** column, check the Torrent Suite™ Software run report for the sequencing run, as this result could indicate a low-quality run or failed amplification.

- Fusion— Fusion assays are built into the panels to confirm the presence of the targeted fusion isoforms and non-targeted fusion isoforms in a sample. For more information, see “Fusion detection methods” on page 539 and “Fusion calls in analysis results” on page 529.

- ProcessControl—A few control assays are built into the TagSeq and Ion AmpliSeq™ HD panels to help determine if the sample is valid or not, similar to the Expression control assays

- GENE_EXPRESSION—These assays provide confirmation for the Gene expression amplicons that are built into the panel and their level of expression.
- RNA_HOTSPOT—These assays provide confirmation for SNPs or INDELs at a particular position (hot spot).
- RNAExonVariant—These assays provide measurement of expression for intragenic events such as Exon Deletions, Exon skipping events, Alternate Splice transcripts and Wild type transcripts. For these assays, two additional metrics are reported.
  - **Ratio to Wild Type**
    - For all the RNAExonVariant assays that belong to a Gene with at-least one RNAExonVariant type assay. It is designed to amplify the wild type transcript of that gene and report this value.
    - Ratio to Wild type of a RNAExonVariant Vi = (Read count of Vi) / (Mean read count of all Wild Type RNAExonVariants for that gene)
  - **Norm Count within Gene**

    For all the RNAExonVariant type assays, this normalized count is reported.

    Norm count within Gene for RNAExonVariant Vi = (Read Count of Vi) / (Sum of read counts of all RNAExonVariants of the same Gene).

    Detection column for RNAExonVariant will have " N/A " as the value for all the RNAExonVariants.
- RNAExonTile—RNAExonTile assays are designed across a few exon-exon junctions in each fusion driver gene. The measurement of expressions from these assays per each gene can be used to confirm the presence of a fusion in the sample. For more information, see “Fusion detection methods” on page 539.

Note: You can view the NORM_COUNT_TO_HK metric in the variants VCF file. The normalized expression to housekeeping value is the read count of a target normalized by the average expression of housekeeping genes rather than by the total number of mapped reads. The value is calculated from the read count:

- log2(read count of target + 1) – ([Sum of log2(housekeeping read count + 1) / number of housekeeping targets)]) + log2(10^6)

Process controls for TagSeq and Ion AmpliSeq™ HD research panels

For TagSeq and Ion AmpliSeq™ HD panels only, sample QC is also dependent on the expression of the process controls. Depending on the number of process control (PC) assays that are detected to be present in the sample, the sample QC is either qualified as PASS, FAIL, or WARN.

If the number of PC assays present is greater than the value specified by the `Minimum Number Of PC Amplicons Required To Pass QC` parameter, the sampleQC is PASS.

If the number of PC assays present is less than the value specified by the `Minimum Number Of PC Amplicons Required To Pass QC` parameter, but greater than the value specified by the `Maximum Number Of PC Amplicons Required To Fail QC` parameter, the sampleQC is WARN.

If the number of PC assays present is less than the value specified by the `Maximum Number Of PC Amplicons Required To Fail QC` parameter, the sampleQC is FAIL.

Multiple fusions in the same gene

Occasionally, when a true positive fusion in a gene pair is detected, a second fusion in the same gene pair may also be reported. Generally, a single fusion has occurred in this pair of genes in this sample, and all the read evidence that covers any exon is reported, and occasionally appears as a second fusion that usually describes a different exon in one of the genes. This second fusion may be described as one of the following:

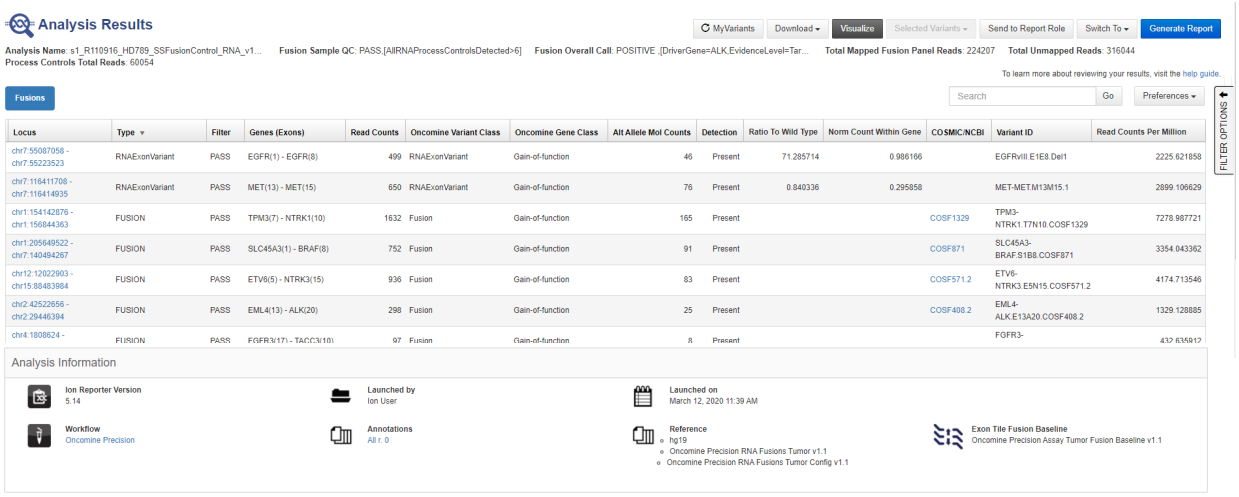
- A targeted fusion, that is, the panel has a specific assay designed to detect this fusion, so is designated as Present in the Detection column.
- A non-targeted fusion. That is, the fusion is detected, but the panel has no specific assay pair designed for this fusion, so it is designated as Present-Non-targeted in the Detection column.

Biologically, both of these calls are likely from the same underlying fusion in the gene pair.

Fusion sample QC metrics

Fusion Sample QC, Fusion Overall Call, Total Mapped Fusion Panel Reads, Total Unmapped Reads, and Process Controls Total Reads are included in Ion Reporter™ Software.

These metrics are available in the **Analysis Results** screen for analyses that include fusions.



Note: In the **Analysis Results** screen, you can close the **Filter Options** on the right, to expand the table.

FusionSampleOverallCall

The FusionSampleOverallCall allows the user to confirm whether a sample is a No Call, Positive, or Negative. The following logic is applied for the panels that have both targeted fusion isoforms and 5'/3' Imbalance Assays:

Fusion Isoforms	RET/ROS1/ALK Imbalance Score	Final Call
Positive	Positive	Positive
Positive	No Call/Negative	Positive
Negative	Positive	Positive
Negative	Positive	Positive
Negative	No Call	No Call
Negative	Negative	Negative

Total Mapped Fusion Panel Reads

The Total Mapped Fusion Panel Reads is a QC metric that is reported for gene fusion analyses. This metric reports the sum of all reads that are valid and are assigned to any assay in the panel. Unmapped reads and poorly aligned reads are excluded from this total.

Minimum Mean Read length

Mean Read Length is a QC metric available for the gene fusion analyses that indicates whether the mean read length computed from all the reads in the sample is less than the specified threshold. If the mean read length is below the threshold, the sample does not pass the quality control.

A 50bp minimum mean read length is recommended to avoid the possibility of missing real fusions (false negatives) and reporting false positive fusions.

Minimum total mapped reads per pool

If the panel contains multiple primer pools, it is important to compute QC metrics per each pool separately. Minimum number of total valid mapped reads per pool is an additional QC metric for RNA Fusion panels with multiple primer pools. Total valid mapped reads per pool is the count of all reads assigned to any target in each pool. If any of the pools have total valid mapped reads less than the specified threshold, that pool does not pass QC.

Note: We recommend that you set a total mapped reads for the fusion assay depending upon the expected number of total reads per barcode which is based on the sequencing platform, chip type and number of barcodes per run.

For example : For a sample sequenced using PGM 318 chip multiplexed using 8 barcodes, we expect more than 100000 reads per sample. Our recommended threshold in this case is 20000 minimum total valid mapped reads.

A 20,000 minimum threshold is recommended to avoid the possibility of missing a real fusion (a false negative). 20,000 mapped reads provide acceptably sensitive fusion detection. At that coverage, fusions calls are reliable. However, a real fusion at low abundance may be missed.

Below 20,000 mapped reads, the assay may lack sensitivity, and we recommend repeating the experiment if possible (if sufficient original sample is available). In addition, the 3'/5' Imbalance number is less reliable for very low mapped reads.

The assay is highly sensitive, and if a gene fusion isoform is detected, it is highly likely to be truly present in the sequencing reads. If a fusion is detected in a sample with a low number of total mapped reads, it is highly likely to be a true positive. However, if a sample has a low number of mapped reads, a real fusion at low abundance in the sample relative to the expression control genes may be missed. The limit of detection is lower with larger numbers of mapped reads.

Minimum number of Valid pools

For multi-pool RNA pools, you can specify the minimum number of pools in a Sample that are required to pass QC to qualify the Sample as Valid, then proceed with the Analysis. If the number of valid pools in any sample is less than the specified threshold, that Sample does not pass QC. Valid pools are determined by the total valid mapped reads per pool.

Example: If a panel has two pools, use `value=2` to specify that both pools need to have a sufficient number of reads in order to qualify that sample. Similarly use `value=1` to proceed with the analysis even if one of the pools failed.

Fusion detection methods

For some panels, Ion Reporter™ Software uses the targeted panel design with software algorithms to detect known and novel gene fusions. The assay uses the following methods to detect fusions.

Targeted fusion detection method

In the targeted fusion detection method, panel primers are designed to target specific exon-exon junctions of fusions where the driver gene, the partner gene, and the breakpoint between the driver and the partner gene are known. The sequencing reads are mapped to a reference file that contains only the known gene fusions.

Non-targeted fusion detection method

In the non-targeted fusion detection method, the panel primers are used to detect fusions between novel combinations of known driver and partner genes. The sequencing reads are mapped to a broader reference, such as the whole-exome. Mapping the reads to a broader reference allows for the detection of multiple configurations of driver and partner genes as well as detection of novel breakpoints between the known partner and driver genes.

Low confidence non-targeted fusions are identified in VCF output files with key value pairs as follows.

Key value pairs for non-targeted fusions

Type of non-targeted fusion identified	Definition	Key value pair
Out-of-frame sequence	The expected non-targeted sequence from the amplicon reference file is used to check whether the sequence is in frame. If the non-targeted sequence is out of frame (compared to the original partner or driver genes), the key-value pair `NT_FUSION_IN_FRAME=FALSE` is included in the VCF file.	`NT_FUSION_IN_FRAME`[1] Values • – `FALSE` – `TRUE`
Partner-partner fusion	Non-targeted fusions are identified if one of the genes in an isoform is not a driver gene. The input reference BED file includes annotations for driver genes, such as, in the case of EML4-ALK.E6A19.COSF1296.2, the DRIVER_GENE=ALK. By default, the software uses a 3p-partner of a targeted fusion isoform as the driver gene if that targeted isoform BED entry does not include the `DRIVER_GENE` parameter, as is the case with earlier panel files and also a few targets in later panel files. In this case, the software will check if at least one of the two genes involved in non-targeted fusion is a 3p gene.	`NT_FUSION_DRIVER_INVOLVED`[2]. Values • – `FALSE` – `TRUE`
Secondary isoform fusion	In some samples, a non-targeted fusion isoform might be detected in a sample while additional targeted isoforms with the same gene pair are also detected (that is, isoforms that are defined in the panel). In this case, the non-targeted fusion isoform should be identified as `NT_FUSION_SECONDARY=TRUE`, if its read count coverage is below ***<x%*** of the reads of any of the other isoforms with the same gene pair. By default, the threshold for secondary isoforms is ***<5%*** of another targeted fusion isoform.	`NT_FUSION_SECONDARY`[3] Values • – `FALSE` – `TRUE`

[1] If the key value pair is not included then `NT_FUSION_IN_FRAME` is assumed to be `True`, indicating that an out-of-frame sequence is not found.

[2] If the key value pair is not included then `NT_FUSION_DRIVER_INVOLVED` is assumed to be `True`, indicating that a partner-partner fusion is not found.

[3] If the key value pair is not included then `NT_FUSION_SECONDARY` is assumed to be `False`, indicating that a secondary isoform fusion is not found.

Additional attributes that indicate whether the non-targeted fusion is out of frame, involves partner-partner fusion, or is a secondary isoform, are added to the VCF files. This information is used by the Oncomine™ Variant Annotator plugin to annotate variants in Ion Reporter™ Software for each Oncomine™ assay and the Ion AmpliSeq™ HD Library Kit.

Exon tiling fusion detection method

The exon tiling method is a partner agnostic fusion detection method that enables the discovery of novel fusions and breakpoints. In this method, the primers are designed to target several exon-exon junction of key driver genes. Each driver gene that is surveyed is analyzed individually. After the sequencing reads undergo normalization and baseline correction, the software measures the intragenic 3′ to 5′ expression ratio for each gene and compares the ratio to the baseline (computed from a cohort of normal samples). Genes that do not undergo a fusion event are expected to have a 3′ to 5′ expression ratio similar to the baseline. Genes that undergo a fusion event typically have a 3′ to 5′ expression ratio greater than the baseline. The imbalance score measures the magnitude of change in 3′ to 5′ expression ratio relative to the baseline. For each driver gene in which fusion was detected, the software also predicts the most likely position of fusion breakpoint. This allows for discovery of novel fusion breakpoints.

Imbalance score = Observed imbalance (test sample) ÷ Expected imbalance (normal sample)

For example, if the observed (test sample) 3′ to 5′ expression ratio is 3, while the expected 3′ to 5′ imbalance for a wild type transcript is 1.5, the imbalance score is 2. Typically, an imbalance score of ≥1.75–2 is indicative of a gene fusion event.

The significance of the expression imbalance is measured by the imbalance p-value. The p-value measures the significance of the imbalance at the predicted breakpoint compared to the negative control gene in the sample. Both the p-value and the imbalance score are used to determine the occurrence of a fusion event.

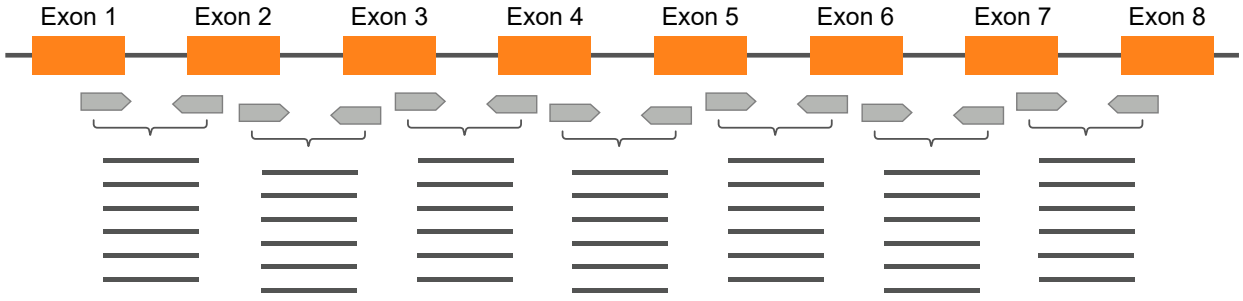


Figure 20 Representative primer design for an exon tiling assay

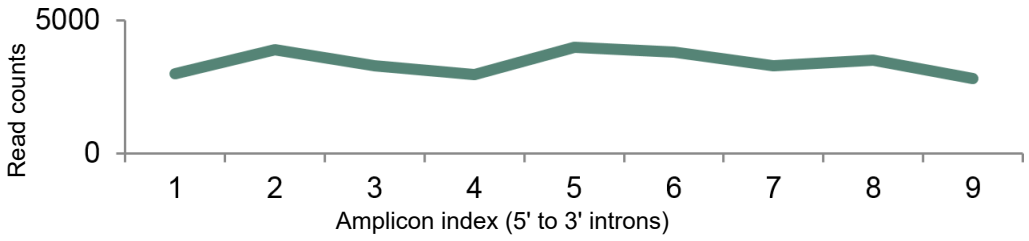


Figure 21 Example coverage profile for a sample with no fusion present In this example, no fusion is present in the sample. The wild type transcript has uniform coverage of 3′ and 5′ introns.

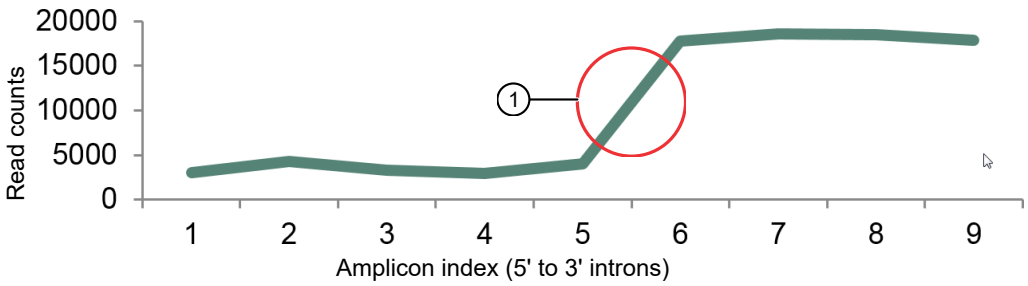


Figure 22 Example coverage profile for a sample with fusion present In this example, a mixture of wild type and fusion transcript is present in the sample. The presence of the fusion transcript accounts for the elevated expression of the 3′ gene region.

① Predicted fusion breakpoint

Barcode CrossTalk QC

This module analyzes the reads from all the barcodes on a chip from one run and determines if there are any reads in a particular barcode that could belong to any of the barcodes.

For example, if barcode manufacturers provide us a QC threshold of 0.5%, i.e., there is a chance that up-to 0.5% of the reads in any one barcode (say barcode7) could be mislabeled as that barcode (barcode 7) where in reality those reads are from a different barcode (say barcode 8). Usually for many applications this is not important. But this is very important for applications such as fusions which require high sensitivity.

For example, if barcode 8 is positive for a fusion involving ROS1 gene with read_count = 100000.

If barcode 7 also has ROS1 read_count = 30. (30 is less than 0.5% of 100000)

Current fusions algorithm will call both barcode 7 and barcode 8 as positive, because the read count in both these samples is >20 (our default threshold).

Barcode 7 is false positive and barcode 8 is true positive.

Barcode crosstalk is not the only source of the contamination, these types of reads could also be seen due to sample-level contaminations as well.

In order to identify these reads, this module needs to look at the reads from all barcodes. The maximum estimated percentage of crosstalk is by default 0.5%, but there is a parameter exposed in user interface that users can change.

This module generates as qcInfo file per barcode and summary file for the entire chip. These files are generated before launching fusions calling module on any one of the barcodes and are passed to the fusions module.

Exon deletion thresholds for Oncomine™ Fusion panels

Oncomine™ Fusion Panel	Exon Deletion Assay	Type	Present/ absent threshold	Instrument/chip	Ion Reporter™ Software analysis workflow
Oncomine™ Comprehensive Fusion Panel	EGFRvIII	Fusion	120	Ion PGM™ System/Ion 318™ Chip	Oncomine™ Comprehensive - w2.2 - Fusions
					Oncomine™ Comprehensive - w2.2 - DNA and Fusions
Oncomine™ Focus Fusion Panel	MET exon 14	Fusion	120	Ion PGM™ System/Ion 318™ Chip	Oncomine™ Focus - w2.2 - Fusions
	EGFRvIII	Fusion	120		Oncomine™ Focus - w2.2 - DNA and Fusions
Oncomine™ Focus Fusion Panel for Ion 520™ Chip	MET exon 14	Fusion	120	Ion S5™ System/Ion 520™ Chip	Oncomine™ Focus - 520 -w2.2 - Fusions
	EGFRvIII	Fusion	120		Oncomine™ Focus - 520 - w2.2 - DNA and Fusions
Oncomine™ Comprehensive & Focus Fusion panel for Ion 540™ Chip	MET exon 14	Fusion	1,000	Ion S5™ System/Ion 540™ Chip	Oncomine™ Comprehensive v1 - 540 - w2.2 - DNA and Fusions
	EGFRvIII	Fusion	1,000		Oncomine™ Comprehensive v2 - 540 - w2.2 - DNA and Fusions - Single Sample
Oncomine™ Comprehensive v3 Panel	MET exon 14	RNAExon Variant	1,000	Ion S5™ System/Ion 540™ Chip	Oncomine™ Comprehensive v3 - w3.0 - DNA and Fusions - Single Sample
	EGFRvIII	RNAExon Variant	1,000		Oncomine™ Comprehensive v3 - w3.0 -Fusions - Single Sample
	Others (9 other genes have similar assays)	RNAExon Variant	Present/ absent calls are not made		

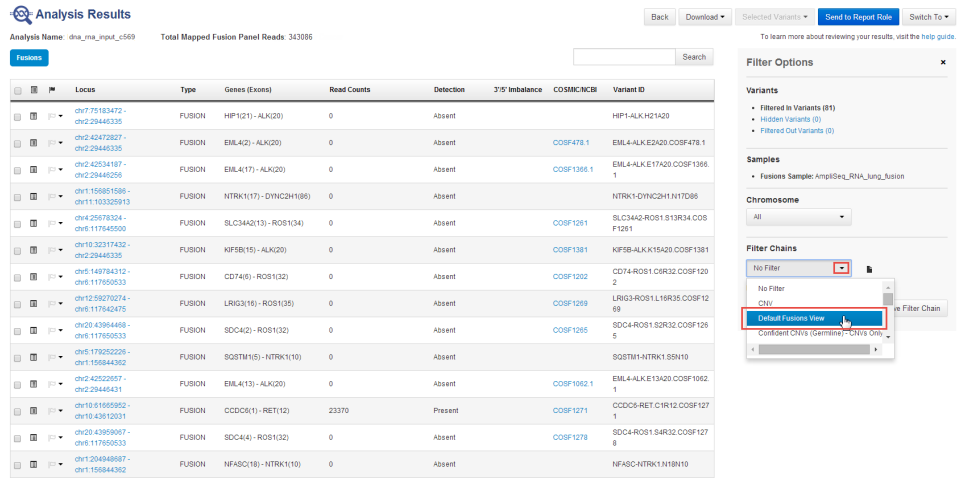
Confirm the presence of fusion calls in Ion Reporter™ Software

Analysis results in Ion Reporter™ Software provide several ways to confirm a fusion call, including Imbalance values, visualization, and Normalized Detection Fractions. For more information, see “Novel fusion detection using expression imbalance” on page 364, “View RNA Exon Tile Fusion Imbalance plots” on page 364, and “3'/5' Imbalance scores for assay calls” on page 370.

Filter chains for fusion panels

For Oncomine™ fusion panels, the **Oncomine Variants** filter chain is applied by default. There are many other filter chains for use with all other fusion panels and filters for non-Oncomine™ Fusion panels. You can use the **Default Fusion View** filter chain for other fusion panels, including custom panels from **AmpliSeq.com**.

1. For analysis results that include only **Present** fusion calls, click the **Filter** menu.



2. Select the **Default Fusions View** filter.

Note: The following data types along with present fusions calls will always be displayed as **Filtered In** variants in the **Type** column, whether the **Default Fusions View** filter is set to **Present** or **Absent**:

- **EXPR_CONTROL**
- **ASSAYS_5P_3P**
- **GENE_EXPRESSION**

B

Ion Reporter™ Software web services API

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Ion Reporter™ Software has a web services API that allows you to automate returns to retrieve key information from the system.

This section provides examples that demonstrate how to leverage the API.

- These examples are used with the hosted Ion Reporter™ Software instance. If you are running a local version on your own Ion Reporter™ Server, then replace the hosted URL

  ```
  https://ionreporter.thermofisher.com/
  ```

  with the URL root of your local instance.
- API tokens that are included in examples are invalid and cannot be used for authentication.
- If the JSON download process converts `@` symbols to `%40`, do not change the text. Instead, copy the JSON files as is into the browser.

Check if analysis is running

isAnalysisRunning	
URL	`/api/v1/isAnalysisRunning`
Description	Checks the status of the analysis. Returns JSON with true and message if any analysis is running in that user account.
Method	`POST`
Request query parameters	`userId` String. *Mandatory*. UserId
Example request	`https://ionreporter.thermofisher.com/api/v1/isAnalysisRunning`
Request headers	`Content-Type`: application/x-www-form-urlencoded `Authorization:API Token`
Returns	`{ "status":true, "message":"success"}`

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/isAnalysisRunning -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NjMyZmUwODMzOTY3YzVhYzA1OGE5M2I4ZjMyZWY0ZmY1NWQ0YzgyYzhjOTJmZT
BjZjZkZTg2N2QxZjA3MGZlMA" -d "userId=100"
```

Check if a sample exists

isSampleExists	
URL	`/api/v1/isSampleExists`
Description	Checks whether there is an existing sample with the sample name that is entered in the call.
Method	`POST`
Request query parameters	`sampleName` String. *Mandatory*. Valid sample name.
Example request	`https://user1.XYZ.com/api/v1/isSampleExists`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`
Returns	`true` if sample with the sample name that is entered already exists in the organization based on the API token for the user is in header. `false` if the sample does not exist.

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/isSampleExists -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NjMyZmUwODMzOTY3YzVhYzA1OGE5M2I4ZjMyZWY0ZmY1NWQ0YzgyYzhjOTJmZT
BjZjZkZTg2N2QxZjA3MGZlMA" -d "sampleName=IR-57220"
```

Download a BAM file

downloadBAM	
URL	`/api/v1/downloadBAM`
Description	Download a BAM file for the sample or analysis in the filepath entered. This procedure applies to Ion Reporter™ Server only.
Method	`GET`
Request query parameters	• `filePath` String. *Mandatory*. Filepath of bam file that was returned using getAssociatedBamfiles API. • String. *Optional*. Valid values are "pdf" or "" (by default creates a ZIP file and downloads it.)
Example request	`https://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ-Labs/data/IRU_Uploads/20180302_01_06_30/v1/CFTR_15KB_deletion/1_AmpCFTR_contig7_15KBDeletions.bam"`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`
Returns	Downloads the BAM file in the filepath entered. User can replace "download.bam" in curl command with the BAM file name to change the name of the BAM file downloaded.

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -o download.bam -L -k -X GET "https://<server name>/api/v1/downloadBAM?
filePath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/
Demo_AmpliSeq_CHPv2_tumor/Demo_AmpliSeq_CHPv2_tumor_20180319063625805/
outputs/VariantCallerActor-00/merged.bam.ptrim.bam" -H "Content-
Type:application/x-www-form-urlencoded" -H "auth:
ZWJjODU2ODIwYWY4OTk2Yjg2MzI5NTU1MzIxZTA0NGVlYTZlNjIyYTJlMGJlNDMwMGI3OWM4ZGNhZ
TNlNzg1Mw" -H "Connection: close"
```

Download a file

download	
URL	`/api/v1/download`
Description	Gets the file, creates a compressed archive file in ZIP file format and downloads it.
Method	`GET`
Request query parameters	• `filePath` String. *Mandatory*. Valid filePath. • `type` String. *Optional*. Valid values are "pdf" or "" (by Default creates a zip file and downloads it.)

Example request	`https://user1.XYZ.com/api/v1/download?type=pdf&`
	`filePath=/data/IR/data/IR_Org/download/pdf/3bf2ffb3-c55e-4da8-819e-f51a6957ad66/`
	`IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_oct1.pdf"`

Request headers	`Content-Type`: application/x-www-form-urlencoded `Authorization:API Token`
Returns	Downloads the file that is located at the file path that is specified.

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -O -v -k -X GET "https://<server name>/api/v1/download?filePath=/
data/IR/data/IR_Org/data/IRU_Uploads/2016-7-26_23_8_42/v2/report.pdf" -H
"Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Download QC and final reports

To retrieve one Quality Control (QC) report in JSON format, enter the following.

```
curl --request GET -k -H
"Authorization:ZDMxNDUwZGYyNjM4NGRlZjY0NjAyNTc1YTE1ZjU2MzFkNjVmYzQxYjJhOTQwNT
YyYWM3OTJmZDY2YTU5NGQwNA"  -O "https://<server name>/api/v1/download?
filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/new_CFTR/
new_CFTR_20151106002127527/new_CFTR_1446798039543.zip"
```

The output in JSON format resembles this:

```
[
    {
        "reports": {
            "qc": {
                "link": "https://
<server name>/api/v1/download?filepath=/data/IR/data/IR_Org/download/pdf/
335a1f5e-19f1-4ff4-b80c-af620f1dfe1f/ff80818150dbc2430150dbe2d9650072_QC.pdf"
            },
            "final": {}
        },
        "report_published": "",
        "workflow": "AmpliSeq CFTR single sample",
        "data_links": {
            "filtered_variants": "https://<server name>/api/v1/
download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/new_CFTR/
new_CFTR_20151106002127527/new_CFTR_1446798039543.zip",
            "unfiltered_variants": "https://<server name>/api/v1/
download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/new_CFTR/
new_CFTR_20151106002127527/new_CFTR_1446798039543.zip"
        },
        "variants_saved": "",
        "samples": {
```

```
            "PROBAND": "new_CFTR"
        },
        "shared_with": [],
        "stage": "Review Variants",
        "flagged": false,
        "name": "new_CFTR_1446798039543",
        "id": "ff80818150dbc2430150dbe2d9650072",
        "ion_reporter_version": "5.0",
        "started_by": "Ion User",
        "start_date": "November 06, 2015",
        "status": "SUCCESSFUL"
    }
]
```

To retrieve all of the QC reports in JSON format, enter:

```
curl --location "https://<server name>/api/v1/qcreport?format=json" \
--header "Authorization:
OTAzNzY1ZjMzY2U4YmE5NzhmN2RhYTFkYTJmMzI2NTE1OGI2YWMzMjRkMGM5\OTI1MDkzNWMxMjFi
OWRjYWI4MA" \
--header "Content-Type: application/x-www-form-urlencoded"
```

To retrieve one final report in JSON format, enter:

```
curl --location "https://<server name>/api/v1/finalreport?
format=json&name=Demo%20Aneuploidy_c23157_2025-05-1010-03-46-508" \
--header "Authorization:
OTAzNzY1ZjMzY2U4YmE5NzhmN2RhYTFkYTJmMzI2NTE1OGI2YWMzMjRkMGM5OTI1MDkzNWMxMjFiO
WRjYWI4MA" \
--header "Content-Type: application/x-www-form-urlencoded"
```

To retrieve all of the final reports in JSON format, enter:

```
curl --location "https://<server name>/api/v1/analysis?
format=json&name=Demo%20AmpliSeq%20CCP%20tumor_prod" \
--header "Authorization:
OTAzNzY1ZjMzY2U4YmE5NzhmN2RhYTFkYTJmMzI2NTE1OGI2YWMzMjRkMGM5OTI1MDkzNWMxMjFiO
WRjYWI4MA" \
--header 'Content-Type: application/x-www-form-urlencoded'
```

Get analysis details

analysis	
URL	`/api/v1/analysis`
Description	Gets the analysis details and links to download a Quality Control (QC) report, final report, and compressed archive (ZIP format) of unfiltered and filtered variants files. Returns a JSON object that contains the download information.
Method	`GET`

(continued)

analysis	
Request query parameters	• `format` String, *Optional*. Response type, current version supports only json. • `type`. String. *Optional*. Valid values are "sample" or "analysis". If the type parameter is not provided, "analysis" is used as the default value. • `name` String. *Mandatory*. Name of sample or analysis. • `id` String. *Optional*. Id of an analysis. If type is sample, this corresponds to the sample id. • `start_date` String. *Optional*. Parameter to filter the results by date boundary. Use date format: `yyyy-MM-dd`. • `end_date`. String. *Optional*. Parameter to filter the results by date boundary. Use date format: `yyyy-MM-dd`. • `duration`. String. *Optional*. Parameter to filter the results by days/months Valid value starts with "-" followed by numeric value and ends with "d" or "m". • `exclude` String. *Optional*. Parameter to filter the response. Valid values are any single or comma-separated combination of values "reports, unfilteredvariants, filteredvariants". • `view` String. *Optional*. Valid values are "summary" or "." If the value is empty, reports are not generated.
Example request	`https://user1.XYZ.com/api/v1/analysis?format=json&name=xyzsampletest&exclude=reports,filteredvariants,unfilteredvariants&start_date=2016-01-01&end_date=2016-02-01`

(continued)

analysis	
Request headers	`Content-Type`: application/x-www-form-urlencoded `Authorization:API Token`

Returns	[
	{
	"reports": {
	"qc": {
	"link": "http://user1.XYZ.com/api/v1/download?type=pdf&
	filePath=/data/IR/data/IR_Org/download/pdf/
	30cf8ff3-fe3d-4cac-8f7d-ce45aa7d34e1/
	ff80818153ed40a401540716785a081a_QC.pdf"
	},
	"final": {}
	},
	"report_published": "",
	"workflow": "Annotate variants single sample",
	"data_links": {
	"filtered_variants": "http://user1.XYZ.com/api/v1/download?
	filePath=/data/IR/data/IR_Org/user1.XYZ.com/
	Demo_AmpliSeq_Exome_VCF/
	Demo_AmpliSeq_Exome_VCF_20160411134952720/
	Demo_AmpliSeq_Exome_VCF_1460407585676.zip",
	"unfiltered_variants": "http://user1.XYZ.com/api/v1/download?
	filePath=/data/IR/data/IR_Org/user1.XYZ.com/
	Demo_AmpliSeq_Exome_VCF/
	Demo_AmpliSeq_Exome_VCF_20160411134952720/
	Demo_AmpliSeq_Exome_VCF_1460407585676.zip"
	},
	"variants_saved": "",
	"samples": {
	"PROBAND": "Demo AmpliSeq Exome VCF"
	},
	"shared_with": [],
	"stage": "Review Variants"
	"flagged": false,
	"name": "Demo AmpliSeq Exome VCF_1460407585676",
	"id": "ff80818153ed40a401540716785a081a",
	"ion_reporter_version": "5.22",
	"started_by": "User1",
	"start_date": "April 11, 2025",
	"status": "SUCCESSFUL"
	}
	]

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<server name>/api/v1/analysis?
format=json&name=xyzsampletest
&id=ff8081819807726c0198119763d8009a&exclude=reports,filteredvariants,unfilte
redvariants
&start_date=2025-07-03
```

```
&end_date=2025-07-15" -H "Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NjMyZmUwODMzOTY3YzVhYzA1OGE5M2I4ZjMyZWY0ZmY1NWQ0YzgyYzhjOTJmZT
BjZjZkZTg2N2QxZjA3MGZlMA"
```

Download all analyses with one call

To retrieve all analyses in JSON format:

```
curl --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFmZjk3NjExNDA0OD
VmYjYwMjA4YzQ5MGEwNDEzZg" https://<server name>/api/v1/analysis?format=json
```

Download analysis information

This example uses curl commands to download information about a specific analysis. The output of the first curl command gives us URLs to use in later curl requests.

This curl command requests analysis information for an analysis named IR50_Rc10_Ane_BC18_DeganCases_1014_np:

```
curl --request GET -k -H
"Authorization:ZTRhNWYyNjkzZjhlYjMxMTdmM2FkZGM1ZmQxYmRkNzU2ZTIxODk5OGRjZWM5ZD
Y2MjMzMTBiOGIwZWE5NmNlNg" "https://<server name>/api/v1/analysis?
format=json&name=IR50_Rc10_Ane_BC18_DeganCases_1014_np"
```

The output is in JSON format:

```
[
    {
        "reports": {
            "qc": {
                "link": "https://
<server name>/api/v1/download?filepath=/data/IR/data/IR_Org/download/pdf/
98ccb759-010f-4c92-acba-2da2d8aa9f4d/ff8081815067ca42015068f99193000d_QC.pdf"
            },
            "final": {}
        },
        "report_published": "",
        "workflow": "Low-pass whole-genome aneuploidy",
        "data_links": {
            "filtered_variants": "https://<server name>/api/v1/
download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/
Ane_BC18_DeganCases/Ane_BC18_DeganCases_20151014174956618/
IR50_Rc10_Ane_BC18_DeganCases_1014_np___4f776c35-4a9f-4a0f-
a787-6ee28ebb8c7c.zip",
            "unfiltered_variants": "https://baseline.itw/api/v1/
download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/
Ane_BC18_DeganCases/Ane_BC18_DeganCases_20151014174956618/
IR50_Rc10_Ane_BC18_DeganCases_1014_np.zip"
        },
        "variants_saved": "",
        "samples": {
            "PROBAND": "Ane_BC18_DeganCases"
        },
        "shared_with": [],
        "stage": "Send for Report Generation",
        "flagged": false,
```

```
        "name": "IR50_Rc10_Ane_BC18_DeganCases_1014_np",
        "id": "ff8081815067ca42015068f99193000d",
        "ion_reporter_version": "5.0",
        "started_by": "Ion User",
        "start_date": "October 14, 2015",
        "status": "SUCCESSFUL"
    }
]
```

This output contains links to three downloads about this analysis (backslashes have been removed):

- qc: "https://<server name>/api/v1/download?filepath=/data/IR/data/IR_Org/download/pdf/98ccb759-010f-4c92-acba-2da2d8aa9f4d/ff8081815067ca42015068f99193000d_QC.pdf"
- unfiltered_variants:" https://<server name>/api/v1/download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/Ane_BC18_DeganCases/Ane_BC18_DeganCases_20151014174956618/IR50_Rc10_Ane_BC18_DeganCases_1014_np.zip"
- filtered_variants: "https://<server name>/api/v1/download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/Ane_BC18_DeganCases/Ane_BC18_DeganCases_20151014174956618/IR50_Rc10_Ane_BC18_DeganCases_1014_np___4f776c35-4a9f-4a0f-a787-6ee28ebb8c7c.zip"

Take one of these links, remove the backslashes ('\'), and send this URL as the second curl command. Redirect the output to a file. For example, this command downloads the zipped filtered variants file:

```
curl --location 'https://<servername>/api/v1/analysis?
format=json&name=Demo%20AmpliSeq%20CCP%20tumor_prod' \
--header 'Authorization:
OTAzNzY1ZjMzY2U4YmE5NzhmN2RhYTFkYTJmMzI2NTE1OGI2YWMzMjRkMGM5OTI1MDkzNWMxMjFiO
WRjYWI4MA' \
--header 'Content-Type: application/x-www-form-urlencoded'
```

Get associated BAM files

getAssociatedBamfiles	
URL	`/api/v1/getAssociatedBamfiles`
Description	For use with the Ion Reporter™ Server only. Gets the BAM file or files that are associated with a single sample or analysis, or multiple samples or analyses, and downloads the BAM files using the `/downloadBAM` API call. Returns a JSON response object that contains the BAM file links. After BAM files are displayed as output as JSON, users can download the BAM file using the `/api/v1/downloadBAM` API call.
Method	`GET`

(continued)

getAssociatedBamfiles	
Request query parameters	• `name` String, *Mandatory*. Single/multiple names of either sample or analysis. • `type` String. *Conditional*. Valid values are "sample" or "analysis". If the type parameter is not provided, "analysis" is used as the default value.
Sample request	`https://user1.XYZ.com/api/v1/getAssociatedBamfiles?name=multi bam test,Sample_1_v2,test62,1194954B_Bladder_ManLib,Demo%AmpliSeq Exome VCF,invalid_sample,Demo AmpliSeq IDP Mother&type=sample`
Analysis request	`http://user1.XYZ.com/api/v1/getAssociatedBamfiles?name=Demo AmpliSeq IDP Daughter_1518065163079,invalid_name,trio_c1078_2018-03-01-20-35-30-948`
Request headers	`Content-Type`: application/x-www-form-urlencoded `Authorization:API Token`

(continued)

getAssociatedBamfiles	
Returns	Sample Response


```
[

    {
       "sample": "1194954B_Bladder_ManLib",
        "bam_links": [
            "http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/
Zyklus/data/IRU_Uploads/20170727_08_45_03/v1/1194954B_Bladder_ManLib/
1_IonXpress_030_R_2016_12_20_13_27_19_user_S5-00105-348-
C656s2_OCAv3.4_CNVbaseline_ManLibs_Pool6_chip6_Auto_user_S5-00105-348-
C656s2_OCAv3.4_CNVbaseline_ManLibs_Pool6_chip6_1074.bam"
        ]
    },
    {
        "sample": "invalid_sapmle1",
        "bam_links": [
            "Sample or Bam file could not be found for the specified name."
        ]
    },
    {
        "sample": "multi bam test",
        "bam_links": [
            "http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ/
data/IRU_Uploads/2017-12-29_1_51_48/v1/e5272-wfa-l165_v3/rawlib.bam",
            "http://user1.XYZ.com.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ/
data/IRU_Uploads/20171229_14_54_21/v1/sample_test16/1_I-9_rawlib12.bam"
        ]
    },
    {
        "sample": "Demo AmpliSeq Exome VCF",
        "bam_links": [
            "Sample or Bam file could not be found for the specified name."
        ]
    }
]
```

(continued)

getAssociatedBamfiles	
Returns	Analysis Response


```
[
    {
        "analysis": "trio_c1078_2018-03-01-20-35-30-948",
        "version": "IR510",
        "analysisStatus": "SUCCESSFUL",
        "sampleDetails": [
            {
                "sampleName": "Demo AmpliSeq IDP Daughter",
                "sampleRole": "proband",
                "inputBam": [
                    "http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ-
Labs/demodata/PGM/AmpliSeq_IDP/AmpliSeq_IDP_daughter.bam"
                ],
                "processedBam": [

"http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ-Labs/user1@XYZ.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_Mother/
Demo_AmpliSeq_IDP_Daughter_20180301150555171/outputs/VariantCallerActorProband-00/
merged.bam.ptrim.bam"
                ]
            },
            {
                "sampleName": "Demo AmpliSeq IDP Father",
                "sampleRole": "father",
                "inputBam": [
                    "http://user1.XYZ.com.com:80/api/v1/downloadBAM?filePath=/shared/data/
XYZ-Labs/demodata/PGM/AmpliSeq_IDP/AmpliSeq_IDP_father.bam"
                ],
                "processedBam": [

"http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ-Labs/user1@XYZ.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_Mother/
Demo_AmpliSeq_IDP_Daughter_20180301150555171/outputs/VariantCallerActorFather-00/
merged.bam.ptrim.bam"
                ]
            },
            {
                "sampleName": "Demo AmpliSeq IDP Mother",
                "sampleRole": "mother",
                "inputBam": [
                    "http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ-
Labs/demodata/PGM/AmpliSeq_IDP/AmpliSeq_IDP_mother.bam"
                ],
                "processedBam": [

"http://user1.XYZ.com:80/api/v1/downloadBAM?filePath=/shared/data/XYZ-Labs/user1@XYZ.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_Mother/
Demo_AmpliSeq_IDP_Daughter_20180301150555171/outputs/VariantCallerActorMother-00/
merged.bam.ptrim.bam"

                ]
            }
        ]
    },
    {
        "analysis": "invalid_name",
        "message": "Analysis could not be found for the specified name."
    }
]
```

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl commands

```
curl -X GET -k "http://<server name>/api/v1/getAssociatedBamfiles?name=multi
bam test,Sample_1_v2,
test62,1194954B_Bladder_ManLib,Demo AmpliSeq Exome VCF,invalid_sapmle1,Demo
AmpliSeq IDP Mother&type=sample" -H
"Content-Type:application/x-www-form-urlencoded" -H
"Authorization:ODA5YTVlZDcyNjkyNDg5Mjg2YmZiNjI3ODExZWFmM2I3NzhkNzg0MDU5M2U
0YmIxOGU2YjQ2OTRmYmIxZjAxNw"
```

```
curl -X GET -k "http://<server name>/api/v1/getAssociatedBamfiles?name=Demo
AmpliSeq IDP
Daughter_1518065163079,invalid_name,trio_c1078_2018-03-01-20-35-30-948" -H
"Content-Type:application/x-www-form-urlencoded" -H
"Authorization:ODA5YTVlZDcyNjkyNDg5Mjg2YmZiNjI3ODExZWFmM2I3NzhkNzg0MDU5M2U0Ym
IxOGU2YjQ2OTRmYmIxZjAxNw
```

Get available cancer types

getAvailableCancerType	
URL	`/api/v1/getAvailableCancerType`
Description	Returns the list of all available cancer types defined in attribute-controlled vocabulary.
Method	`POST`
Example request	`https://user1.XYZ.com/api/v1/getAvailableCancerType`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`
Returns	`["Bladder Cancer","Breast Cancer","Colorectal Cancer","Endometrial Cancer", "Esophageal Cancer","Gastric Cancer","Gastrointestinal Stromal Tumor","Glioblastoma" ,"Head and Neck Cancer","Kidney Cancer","Liver Cancer","Melanoma","Mesothelioma", "Non-Small Cell Lung Cancer","Osteosarcoma","Ovarian Cancer","Pancreatic Cancer", "Prostate Cancer","Skin Basal Cell Carcinoma","Small Cell Lung Cancer", "Soft Tissue Sarcoma","Testicular Cancer","Thyroid Cancer","Unknown"]`

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/getAvailableCancerType -H
"Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get available storage space

getAvailableStorageSpace	
URL	`/api/v1/getAvailableStorageSpace`
Description	Returns the available storage space in bytes for the organization that is associated with the API token in headers.
Method	`POST`
Example request	`https://user1.XYZ.com/api/v1/getAvailableStorageSpace`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`
Returns	`20452031596466`

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/getAvailableStorageSpace -H
"Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get current results TSV file

getcurrentResultTsv	
URL	`api/v1/currentResultTsv`
Description	Returns the Current Results TSV file path in the same column order as shown in the analysis results in Ion Reporter™ Software. The results are based on the table preference and the filter chain that were last saved for the analysis results.
Method	`GET`
Request parameters	• `format`. String, *Optional*. Response type, current version supports only json. `format=json` • `type` String, *Optional*. Valid values are "sample" or "analysis". If the type parameter is not provided, "analysis" is used as the default value. • `name` String, *Mandatory*. If `type` is specified as sample, then only the sample name is considered. If `type` is specified as analysis or not included, then the analysis name is used for name. If there are two analyses with the same name for two different versions of Ion Reporter™ Software, then the request returns two file paths for the TSV files. If the analysis is not found, the software returns an error with an explanation that the analysis was not found.
Example request	`http://user1.XYZ.com/api/v1/currentResultTsv?format=json&name=xyzsampletest`

(continued)

getcurrentResultTsv	
Request headers	`Content-Type`: application/x-www-form-urlencoded `Authorization:API Token`

Returns	[
	{
	"report_published": "",
	"workflow": "Low-pass whole-genome aneuploidy w1.0",
	"data_links": {
	"current_result_tsv": "https://
	<server name>/api/v1/download?filePath=/data/IR/data/IR_Org/
	user1@XYZ.com/Demo_Aneuploidy/Demo_Aneuploidy_20180419122112036/analysis_downloads/currentTsv/
	Demo_Aneuploidy_c1531_2018-04-19-12-26-20-867-2018-07-10-16-04-13-869.tsv"
	},
	"samples": {
	"PROBAND": "Demo Aneuploidy"
	},
	"variants_saved": "",
	"shared_with": [],
	"stage": "Send for Report Generation",
	"flagged": false,
	"name": "Demo Aneuploidy_c1531_2018-04-19-12-26-20-867",
	"id": "ff80818162dca67d0162dcabdd6b0001",
	"ion_reporter_version": "5.20",
	"started_by": "Ion User",
	"start_date": "April 19, 2018",
	"status": "SUCCESSFUL"
	}
	]

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<server name>/api/v1/currentResultTsv?
format=json&name=BRCA_Plus_RR_jul29" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get data upload path with authentication

getDataUploadPathWithAuth	
URL	`/api/v1/getDataUploadPathWithAuth`
Description	Used by the IonReporterUploader plugin to return upload path for the current data of the organization that is associated with the API token for the user, based on the configuration of the `server.properties` file.
Method	`POST`
Request query parameters	`auth` String, *Mandatory*. Valid API token.
Example request	`https://user1.XYZ.com/api/v1/getDataUploadPathWithAuth`
Request headers	`Content-Type`: application/x-www-form-urlencoded
Returns	`/data/IR/data/IR_Org/data/IRU_Uploads`

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -d
"auth=NjMyZmUwODMzOTY3YzVhYzA1OGE5M2I4ZjMyZWY0ZmY1NWQ0YzgyYzhjOTJmZTBjZjZkZTg
2N2QxZjA3MGZlMA" --header
"Content-Type: application/x-www-form-urlencoded" -k https://<server name>
/api/v1/getDataUploadPathWithAuth"
```

Get link to a final report

finalreport	
URL	`/api/v1/finalreport`
Description	Gets the analysis details and returns links to the final report. Returns a JSON object that contains download information.
Method	`GET`
Request query parameters	• `format`. String. *Optional*. Response type, current version supports only json • `type` String, *Optional*. Valid values are "sample" or "analysis". If the type parameter is not provided, "analysis" is used as the default value. • `name` String, *Mandatory*. Name of sample or analysis. • `id` String. *Optional*. Id of an analysis. • `start_date` String, *Optional*. Parameter to filter the results by date boundary. Date format should be in `yyyy-MM-dd` format. • `end_date` String, *Optional*. Parameter to filter the results by date boundary. Date format should be in `yyyy-MM-dd`. format.
Example request	`https://user1.XYZ.com/api/v1/finalreport?fomat=json&type=analysis&name=IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_oct1&start_date=2014-01-30&end_date=2016-04-12`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`

(continued)

finalreport	
Authorization	API Token

Returns	[
	  {
	  "summary": "",
	  "final_report_template": "Default Final Report Template",
	  "report_pdf": "http://user1.XYZ.com/api/v1/download?type=pdf&
	  filePath=/data/IR/data/IR_Org/download/pdf/
	  3bf2ffb3-c55e-4da8-819e-f51a6957ad66/
	  IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_oct1.pdf",
	  "created_on": "2016-03-24T03:11:16.000-07:00",
	  "name": "IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_oct1",
	  "id": "ff80818148c803530148cdb524020071",
	  "variants": [
	  {
	  "{\"_id\":[\"com.mongodb.BasicDBObject\",
	  {\"c\":1,\"p\":115252204}],\"GT\":\"0/0\"}": {
	  "comments": "",
	  "gene": "NRAS",
	  "annotations": "",
	  "locus": "chr1:115252204",
	  "classification": "UNCLASSIFIED",
	  "exon": "4",
	  "sample": [
	  {
	  "Auto_user_PG1-10-140127__ColonLung_v3_test_627_279_IonXpress_003_v1": {
	  "mutation": "",
	  "protein": ""
	  }
	  }
	  ]
	  }
	  }
	  ],
	  "analysis": "http://user1.XYZ.com/api/v1/analysis?id=
	  ff80818148c803530148cdb524020071",
	  "ion_reporter_version": "4.2",
	  "created_by": "Report User"
	  }
	]

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<server name>/api/v1/finalreport?
format=json&name=BRCA_Plus_RR_jul29&type=analysis&start_date=2016-07-25&end_d
ate=2016-07-31" -H "Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get QC report

qcreport	
URL	`/api/v1/qcreport`
Description	Gets the Quality Control (QC) Report. Returns a JSON object that contains download information.
Method	`GET`
Request query parameters	• `name` String, *Mandatory*. Name of sample or analysis. • `format`. String. *Optional*. Response type, current version supports only json. • `type` String, *Optional*. Valid values are "sample" or "analysis". If the type parameter is not provided, "analysis" is used as the default value. • `id` String. *Optional*. ID of an analysis. • `start_date`. String, *Optional*. Parameter to filter the results by date boundary. Date format should be in `yyyy-MM-dd` format. • `end_date` String, *Optional*. Parameter to filter the results by date boundary. Date format should be in `yyyy-MM-dd`. format. • `view` String,*Optional*. Valid values are "summary" or "" .
Example request	`https://user1.XYZ.com/api/v1/qcreport?fomat=json&type=analysis&name=Demo AmpliSeq Exome VCF_1460407585676&start_date=2016-01-30&end_date=2016-04-12`

(continued)

qcreport	
Request headers	`Content-Type`: application/x-www-form-urlencoded `Authorization:API Token`

Returns	[
	{
	"stage": "VARIANT_REVIEW",
	"flagged": false,
	"name": "Demo AmpliSeq Exome VCF_1460407585676",
	"id": "ff80818153ed40a401540716785a081a",
	"qc_metrics": {
	"Demo AmpliSeq Exome VCF": {
	"Number of Heterozygous SNVs": " 32451",
	"CNV/Total": " 0.000",
	"Number of Homozygous INDELs": " 1089",
	"Ti/Tv Ratio (SNPs)": " 2.308",
	"MAPD": "N/A",
	"LongDels/Total": " 0.000",
	"Number of Heterozygous INDELs": " 2113",
	"Heterozygotes/Homozygotes": " 1.737",
	"Indels/kb": " 0.001",
	"Number of Heterozygous MNVs": " 0",
	"Number of LongDels": " 0",
	"Number of Homozygous MNVs": " 0",
	"Indels/Total": " 0.059",
	"SNPs/kb": " 0.017",
	"Number of Homozygous SNVs": " 18814",
	"Number of CNVs": " 0",
	"dbSNP concordance": " 0.934"
	}
	},
	"started_by": "Ion User",
	"ion_reporter_version": "5.22",
	"samples": {
	"PROBAND": "Demo AmpliSeq Exome VCF"
	},
	"qc_report_pdf": "http://user1.XYZ.com/api/v1/download?type=pdf&
	filePath=/data/IR/data/IR_Org/download/pdf/
	890e4268-46dc-442b-a4ed-aa87bb28f71a/
	ff80818153ed40a401540716785a081a_QC.pdf",
	"start_date": "2016-04-11 13:49:53.0",
	"status": "SUCCESSFUL",
	"qc_package": "http://user1.XYZ.com/api/v1/download?
	filePath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/
	Demo_AmpliSeq_Exome_VCF/Demo_AmpliSeq_Exome_VCF_20160411134952720/
	analysis_downloads/QC_Bundle/
	qc-report-Demo_AmpliSeq_Exome_VCF_1460407585676_2025-04-12 04-33.zip"
	}
	]

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<server name>/api/v1/qcreport?
format=json&name=BRCA_Plus_RR_jul29&type=analysis&start_date=2016-07-25&end_d
```

```
ate=2016-07-31" -H "Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get user details

getUserDetails	
URL	`/api/v1/getUserDetails`
Description	Used by IonReporterUploader plugin to get user details based on username and password. Returns the user details.
Method	`POST`
Request query parameters	• `userName` String, *Mandatory*. Valid username. • `password` String, *Mandatory*. Valid password.
Example request	`https://user1.XYZ.com/api/v1/getUserDetails`
Request headers	`Content-Type`: application/x-www-form-urlencoded

Returns	{
	"tokendate": "2013-12-08 15:39:37.0",
	"firstname": "Ion",
	"eulaAccepted": "true",
	"userStatus": "ENABLED",
	"orgname": "IR Org",
	"exporttokendate": "2015-11-05 03:25:12.0",
	"exporttoken": "49bbe49a8307ba61ef28ee9a9122a98347576488c975c857b7e1c82978f3818c",
	"lastname": "User",
	"token": "wVcoTeYGfKxItiaWo2lngsV/r0jukG2pLKbZBkAFnlPbjKfPTXLbIhPb47YA9u78"
	}

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/getUserDetails -H "Content-
Type:application/x-www-form-urlencoded" -d "userName=ion.reporter@lifetech
.com&password=IonPass123"
```

Get user details with authentication

getUserDetailsWithAuth	
URL	`/api/v1/getUserDetailsWithAuth`
Description	Used by the IonReporterUploader plugin to return user details based on the API token.
Method	`POST`
Request query parameters	String. *Mandatory*. Valid user API token.
Example request	`https://user1.XYZ.com/api/v1/getUserDetailsWithAuth`
Request headers	`Content-Type:` application/x-www-form-urlencoded

Returns	{
	"tokendate": "2013-12-08 15:39:37.0",
	"firstname": "Ion",
	"eulaAccepted": "true",
	"userStatus": "ENABLED",
	"orgname": "IR Org",
	"exporttokendate": "2015-11-05 03:25:12.0",
	"exporttoken": "49bbe49a8307ba61ef28ee9a9122a98347576488c975c857b7e1c82978f3818c",
	"lastname": "User",
	"token": "wVcoTeYGfKxItiaWo2lngsV/r0jukG2pLKbZBkAFnlPbjKfPTXLbIhPb47YA9u78"
	}

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/getUserDetailsWithAuth -H
"Content-Type:application/x-www-form-urlencoded" -d
"auth=NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjODU3YjdlMWM
4Mjk3OGYzODE4Yw"
```

Get VCF files

getvcf	
URL	`/api/v1/getvcf`
Description	Gets the links to download VCF files. Returns a JSON object that contains the download information.
Method	`GET`
Request query parameters	• `format` String. *Optional*. Response type, current version supports only json. • `type` String. *Optional*. Valid values are "sample" or "analysis". • If the type parameter is not provided, "analysis" is used as the default value. • `name` String. *Mandatory*. Name of sample or analysis. • `id` String. *Optional*. Id of an analysis. • `start_date` String. *Optional*. Parameter to filter the results by date boundary. Date format should be in `yyyy-MM-dd`. • `end_date` String. *Optional*. Parameter to filter the results by date boundary. Date format should be in `yyyy-MM-dd`. • `duration` String. *Optional*. Parameter to filter the results by days/months. Valid value starts with "-" followed by numeric value and ends with "d" or "m" • `exclude` String. *Optional*. Parameter to filter the response. Valid values are `unfilteredvariants` or `filteredvariants`.
Example request	`https://ionreporter.thermofisher.com/api/v1/getvcf?format=json&name=xyzsampletest&start_date=2016-01-01&end_date=2016-02-01`

(continued)

getvcf	
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`

Returns	[
	{
	"data_links": "http://user1.XYZ.com/api/v1/download?
	filePath=/data/IR/data/IR_Org/user1@XYZ.com/JohnSmithSample
	/JohnSmithSample_20160429014705727/JohnSmithSample_c150_2016-04-29-14-16-534.zip",
	"name": "JohnSmithSample_c150_2016-04-29-14-16-534",
	"id": "ff808181545d907901546133336be0008"
	}
	]

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<server name>/api/v1/getvcf?
format=json&name=BRCA_Plus_RR_jul29&start_date=2016-07-25&end_date=2016-07-31
" -H "Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get whole genome view PNG

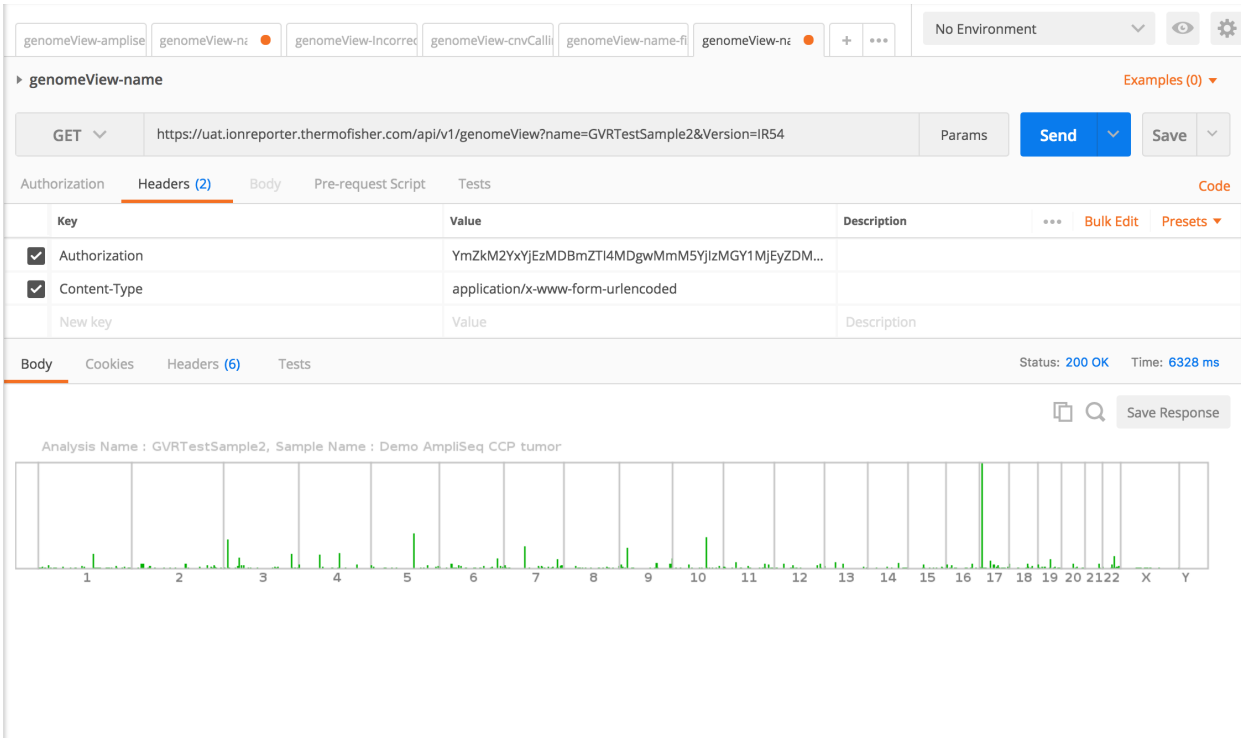
genomeView	
URL	`/api/v1/genomeView`
Description	Gets the whole genome view PNG image file of the sample or analysis.
Method	`GET`

(continued)

genomeView	
Request query parameters	• `name` String. *Mandatory*. Name of a sample or analysis. – If both `id` and `name` are given as input, then the genome-view PNG is displayed for `id`, and `name` is not be considered. – Whole Genome View image will be displayed as part of the response, along with analysis and sample name as the header. – If no PNG file is available in the `/outputs` folder, then the response is 200 with the following message: "Genome View PNG not found." [with analysis name and sample name] • `id` String. *Optional*. Id of an analysis. • `version` String. *Mandatory*. Application version on which the analysis is launched. For and analysis with older application versions, provide "Version=<IR version>" as a parameter, along with name.
Example request	`https://user1.XYZ.com/api/v1/genomeView?name=Demo Aneuploidy_1752920408115&Version=IR522&id=ff808181982214ca01982235013c05e9`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`

Returns

Returns the PNG image file for a whole genome view of the analysis.



Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<server name>/api/v1/genomeView?
name=GVRTestSample2&Version=IR54"-H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:YmZkM2YxYjEzMDBmZTI4MDgwMmM5YjIzMGY1MjEyZDMyMGI3ZTU5MmQyZGY4Zm
IxZWI5M2ViYzIxZWNhOWU3NQ"
```

Get analysis workflow names

getWorkflowNamesWithApplicationTypeWithAuth	
URL	`/api/v1/getWorkflowNamesWithApplicationTypeWithAuth`
Description	Used by IonReporterUploader plugin to get a list of analysis workflows that are tagged with IRU. Returns the list of analysis workflow details for the version that is specified in the header. If there are no workflows that are tagged with IRU, all workflows for a specified version are returned.
Method	`POST`
Example request	`https://ionreporter.thermofisher.com/api/v1/getWorkflowNamesWithApplicationTypeWithAuth`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token` `Version:` IR522
Returns	[{ "tag_Oncomine": "true", "tag_FusionCovImbalance": "true", "tag_isFactoryProvidedWorkflow": "true", "tag_TF_CALL_REQUIRED": "true", "wfl_plugin_OncomineV34": "true", "Workflow": "Oncomine Comprehensive Plus - 560 - w3.2 - DNA and Fusions - Single Sample", "tag_RNAExonVariant": "true", "irVersion": "522", "irReference": "hg19", "tag_BRCA-PLUS": "true", "ApplicationType": "Oncomine_DNA_RNA_Fusion", "tag_HRR": "true", "CELLULARITY_PCT_REQUIRED": "1", "tag_GRANDPRIX": "true", "tag_MMR": "true" }]

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/
getWorkflowNamesWithApplicationTypeWithAuth -H "Content-Type:application/x-
www-form-urlencoded" -H
"Authorization:NjMyZmUwODMzOTY3YzVhYzA1OGE5M2I4ZjMyZWY0ZmY1NWQ0YzgyYzhjOTJmZT
BjZjZkZTg2N2QxZjA3MGZlMA" -H "Version:522"
```

Start an analysis

startAnalysis	
URL	`/api/v1/startAnalysis`
Description	Used by the IonReporterUploader plugin to start an analysis for the `setId` that is entered. Returns a JSON object with status and message.
Method	`POST`
Request query parameters	• `setId` String. *Mandatory*. `setId` column value of the sample in the MG_SPECIMENUPLOADERDETAIL table when the sample is uploaded through the IonReporterUploader plugin. • `containerName` String. *Mandatory*. User-specified unique name for the analysis that is shown in Ion Reporter™ Software.
Example request	`https://user1.XYZ.com/api/v1/startAnalysis`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`
Returns	`{ "status":true, "message":"success"}`

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/startAnalysis -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5NzVjOD
U3YjdlMWM4Mjk3OGYzODE4Yw" -d "setId=4__f535ccde-5c6a-490d-
a1d2-7b81352e6830_2016-6-17_1_13_24&containerName=New_Analysis_Name"
```

Query by sample name API call

You can query by sample name using this API call:

```
/api/v1/analysis?format=json&name=[samplename]&type=sample
```

This call returns a list of analysis names performed on the sample, ordered by start date.

Unshare analyses

unsharedAnalyses	
URL	`/api/v1/unsharedAnalyses`
Description	Unshares all analyses which have been shared with the user whose `userId` is entered. Returns a JSON file with status and message.
Method	`POST`
Request query parameters	`userId` String. *Mandatory*. UserId for email.
Example request	`https://user1.XYZ.com/api/v1/unsharedAnalyses`
Request headers	`Content-Type:` application/x-www-form-urlencoded `Authorization:API Token`
Returns	`{ "status":true, "message":"success"}`

Response fields

Status code	Response message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k "https://<server name>/api/v1/unsharedAnalyses" -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NjMyZmUwODMzOTY3YzVhYzA1OGE5M2I4ZjMyZWY0ZmY1NWQ0YzgyYzhjOTJmZT
BjZjZkZTg2N2QxZjA3MGZlMA" -d "userId=user123@xyz.com"
```

View a list of analyses

To get a list of all analyses that a user has access to without the links to the actual analyses, enter the following.

```
view=summary
```

Example:

```
webservices_42/rest/api/analysis?format=json&view=summary
```

This approach saves time over the original calling API.

Programming guidelines for Ion Reporter™ Software plugins

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Guidelines to develop Ion Reporter™ Software plugins

A short description for the plugin can be included after the plugin class declaration as shown in the line 4 in the code sample. If you do not include a short description, you will receive a warning message.

The version attribute is mandatory in the plugin script. Define the version in the plugin as given in line 5 in the code example.

The Python class that inherits from the IonPlugin base class should call the main as shown in the sample code in lines 9 and 10.

MyIonPlugin.py

```
1 #!/usr/bin/env python
 2 from ion.plugin import *
 3 class MyIonPlugin(IonPlugin):
 4     '''This is a sample plugin'''
 5     version = '5.4.0.1'
 6     def launch(self):
 7         print "This is a python-based plugin
 8
 9 if __name__ == "__main__":
10     PluginCLI()
```

The above mentioned class is considered as a wrapper class to the plugin script. This above class is used for the IR plugin framework to validate. We would recommend developer to maintain this format and write another class called MyIonPlugin_plugin.py which contains all the main processing. Calling <plugin_name>_plugin.py from <plugin_name>.py is recommended way of structuring.

Example <plugin_name>.py which acts as a wrapper class and which in-turn calls <plugin_name> _plugin.py:

```
1  #!/usr/bin/python
2  # Copyright (C) 2013 Ion Torrent Systems, Inc. All Rights Reserved
3
4  import os
5  import sys
6  from subprocess import *
7  from ion.plugin import *
8
9  class ampliSeqRNA(IonPlugin):
10   '''Whole Transciptome AmpliSeq-RNA Analysis. (Ion supprted)'''
11   version = '5.4.0.6'
12   major_block = True
13   runtypes = [ RunType.FULLCHIP, RunType.THUMB, RunType.COMPOSITE ]
14   runlevels = [ RunLevel.DEFAULT ]
15
16   def launch(self,data=None):
17     plugin = Popen([
18         '%s/ampliSeqRNA_plugin.py' % os.environ['DIRNAME'], '-V', self.version, '-d',
19         'startplugin.json', 'barcodes.json'
20       ], stdout=PIPE, shell=False )
21     plugin.communicate()
22     sys.exit(plugin.poll())
23
24
25 if __name__ == "__main__":
26   PluginCLI()
27
```

At the time of plugin run, Plugin is provided with the `startplugin.json` and `barcodes.json` in its running directory which allows the plugin to access these json files as if they are available in their current working directory.

In the code above, lines 18 and 19 show the way how to access these files.

More information on these json files are provided in the sections below.

Naming convention for the plugin zip

- IR plugin framework has some validations against how the zip file should be named.
- After compressing the plugin contents into the zip, the zip name should be given as <plugin_name>_<version>.zip.
- For example: From the above code snippet, the plugin zip file name should look like **MyIonPlugin_5.4.0.1.zip.**

How to create the plugin zip

1. Create a folder with pluginname_version.
2. Keep the files/folders in it.

3. Below are the files that every plugin should contain for IR to validate it.
    - **Instance_ir.html**
        - This html file is used by plugin framework to show the plugin configuration page in the plugins tab at the analysis launch time.
        - This html file should contain the parameters that should be configured at the time of launching the plugin.
        - User can modify/configure parameters during workflow creation/analysis launch. When user clicks on the configure icon then it shows instance_ir.html which get the default parameters from database and user can modify those. Pefer the instance_ir.html in ampliSeqRNA plugin for more information.
    - **Parameters.json**
        - Plugin framework expects the file called parameters.json in the plugin zip.
        - At the time of installation, plugin framework will save the default values required for the plugin in the database. These default values for those keys to be given in parameters.json.
        - At run time, IR will display plugin's instance_ir.html to configure plugin parameters at the analysis launch time. Once configured, plugin framework will provide these values for the plugin usage under key called "pluginconfig" in the `startplugin json` file. The `startplugin.json` file is provided under the plugin results directory. The results directory can be accessed through the environment variable called "RUNINFO_RESULTS_DIR".
        - The `parameters.json` file should contain all the keys and default values for all these keys. These keys in this file should match the parameters to be configured using instance_ir.html.
        - The `parameters.json` file should be specific to plugin. ampliSeqRNA plugin parameters.json is different from RNAseq plugin parameters.json.
        - Please refer the default `paramaters.json` file for more information.
    - **`MyIonPlugin.py` file**
        - The `MyIonPlugin.py` file name should be the same name as the ZIP file name (`MyIonPlugin_5.4.0.1.zip`), but with no version in it.
        - This python class should implement IonPlugin base class provided by the plugin framework.
        - This IonPlugin base class expects the plugin to have mandatory attribute version, have a method called PluginCLI() method inside __main__, and also to have a method definition for launch(self).

- Sample contents in ampliSeqRNA plugin:

```
ionadmin@liverpool:/data/IR/data/IR_Org/apps/IR56/ampliSeqRNA_5.4.0.6$ ls -lts
total 136
 4 drwxr-sr-x 2 iruser irgrp  4096 May 15 22:53 templates
 4 drwxr-sr-x 2 iruser irgrp  4096 May 15 22:53 scripts
20 -rwxr-xr-x 1 iruser irgrp 16616 May 15 22:53 instance.html
12 -rwxr-xr-x 1 iruser irgrp 11610 May 15 22:53 instance_ir.html
 4 drwxr-sr-x 3 iruser irgrp  4096 May 15 22:53 lifechart
 4 -rwxr-xr-x 1 iruser irgrp   288 May 15 22:53 parameters.json
 4 -rwxr-xr-x 1 iruser irgrp  3325 May 15 22:53 plan.html
 4 -rwxr-xr-x 1 iruser irgrp   334 May 15 22:53 README
16 -rwxr-xr-x 1 iruser irgrp 13174 May 15 22:53 run_ampliseqrna.sh
 4 drwxr-sr-x 5 iruser irgrp  4096 May 15 22:53 flot
 0 drwxr-sr-x 2 iruser irgrp   113 May 15 22:53 bed
 4 -rwxr-xr-x 1 iruser irgrp  1025 May 15 22:53 CMakeLists.txt
52 -rwxr-xr-x 1 iruser irgrp 52484 May 15 22:53 ampliSeqRNA_plugin.py
 4 -rwxr-xr-x 1 iruser irgrp   714 May 15 22:53 ampliSeqRNA.py
```

4. Select the folder and create the ZIP file by right-clicking.

Plugin APIs

Plugin APIs are used to fetch/update the plugin parameters. These APIs are part of `instance_ir.html`; refer to `instance_ir.html` for more info.

Following are the available APIs:

- To get the reference genome:
  - URL: /plugin-api/v1/referencegenome
- To fetch the already saved parameters:
  - URL: '/plugin-api/v1/results?pluginId=' + <<PLUGINID>>+ '&workflowId=' + <<WORKFLOWID>>
- To fetch the target region's based on reference genome:
  - /plugin-api/v1/targetRegions?genome= <<REFERENCE-GENOME>>
- T
  o fetch the target region's based on reference genome and target region:
  - URL: /plugin-api/v1/targetRegions?genome=<<REF-GENOME>> &targetRegion= <<TARGET-REGION_NAME>>

Validation

After the plugin is finalized, it needs to be compressed into the ZIP file. The Imbalance file name should be the same name given to the file <plugin_name>.py. Ion Reporter™ Software validates this ZIP file to check whether this plugin has valid version attributes and whether this plugin has implemented IonPlugin class.

Below are the validations performed during the plugin installation:

1. Plugin ZIP file should have the at least one python file.
2. Valid python file should import the ion.plugin package.
3. Valid python file should have class name.
4. Valid python file should have version attribute.

5. pluginname_version is used to check the uniqueness. So newly installed plugin should not have the plugin name and version, which is already installed.
6. Plugin ZIP file should have parameters.json inside it with default values otherwise plugin will not generate the proper result.

HTML's for visualization

After the plugin analysis is launched and successful, for the developer to view the plugin results using IR visualization:

- Plugin should generate <plugin_name>.html and <any_name>_block.html (optional) files in the plugin output folder. path: <analysis_root_dir>/outputs/TsPluginActor-00/<Plugin_name>/
- If the _block.html is generated then IR software would show the results in a block like frame as a mini view.
- The frame will have a button called "View Summary" that contains the link to open <plugin_name> .html file in a new tab.

Plugin results

For the plugin developer to view the results in the backend, the results are available at the following path: <analysis_root_dir>/outputs/TsPluginActor-00/<Plugin_name>/

Download logs

For the plugin developer to view the plugin logs in the backend, the logs are available at the following path: <analysis_root_dir> /log/TsPluginActor-00/ <Plugin_name>/

If the <plugin_name>.log file is generated, user can download logs. Ion Reporter™ Software would show the "Download Log" in a block header.

Scratch directory

- For the plugin developer to save any intermediate generated files from that plugin for re-use in subsequent plugin runs, these could be saved in pluginScratch directory.
- Developer should be able to access the environment variable called TSP_PLUGIN_SCRATCH and can create a folder under this scratch directory with your plugin name <plugin_name> to save the results.

Sample and Environment details

At run time, the developer is provided with two JSON files, `startplugin.json` and `barcodes.json`, in the plugin results directory.

The developer can access these JSON files in the MyIonPlugin.py script in the `barcodes.json` file.

- The `barcodes.json` file contains the sample information that has been selected at the analysis launch time.
- The `barcodes.json` file is mainly used to read the bam_file_path, target_regions_file_path, genome_reference_name, genome_reference_file_path, and barcode_name.
- Below is an example `barcodes.json` file generated by the plugin framework:

```
{
  "bc1" : {
    "genome_urlpath" : "",
    "nucleotide_type" : "",
    "control_sequence_type" : "",
    "barcode_name" : "bc1",
```

```
    "sample_id" : "",
    "barcode_type" : "",
    "barcode_annotation" : "",
    "sample" : "SampleCustom",
    "reference_fullpath" : "/data/IR/data/.reference/hg19/hg19.fasta",
    "target_region_filepath" : "",
    "reference" : "hg19",
    "filtered" : "",
    "barcode_sequence" : "",
    "hotspot_filepath" : "",
    "barcode_index" : "",
    "bam_file" : "1_1_IonXpress_009_rawlib.bam",
    "barcode_adapter" : "",
    "barcode_description" : "",
    "bam_filepath" : "/data/IR/data/IR_Org/data/IRU_Uploads/
20170306_02_12_48/v1/new_ocp_rna/1_1_IonXpress_009_rawlib.bam",
    "aligned" : "",
    "control_type" : "",
    "read_count" : ""
  }
}
```


- If the sample being used in the analysis was uploaded through the IonReporterUploader plugin, then the barcodes json might contain more information, for example the "read_count", "barcode_adapter", "nucleotide_type", "barcode_sequence", etc.

  .
- Below is the sample barcodes json if the sample is uploaded through IonReporterUploader plugin:


```
{
  "IonXpress_005" : {
    "read_count" : 1836237,
    "sse_filepath" : "",
    "reference" : "hg19",
    "genome_urlpath" : "",
    "barcode_adapter" : "GAT",
    "sample" : "Dw2-4hr",
    "sample_id" : "",
    "barcode_type" : "",
    "hotspot_filepath" : "",
    "barcode_description" : "",
    "control_type" : "",
    "target_region_filepath" : "",
    "control_sequence_type" : "",
    "bam_filepath" : "/data/IR/data/IR_Org/data/IRU_Uploads/
2017-3-20_11_10_49/v1/Dw2-4hr_RNA_v1/IonXpress_005_rawlib.bam",
    "filtered" : false,
    "barcode_index" : 5,
    "bam_file" : "IonXpress_005_rawlib.bam",
    "aligned" : true,
    "barcode_name" : "IonXpress_005",
    "barcode_annotation" : "",
    "nucleotide_type" : "RNA",
    "barcode_sequence" : "CAGAAGGAAC",
    "reference_fullpath" : "/data/IR/data/.reference/hg19/hg19.fasta"
  }
}
```


The `startplugin.json` file

At the analysis launch time, in the user interface, the user could configure his/her own plugin according to their requirements. These configured parameters will be provided in the `startplugin.json` file under the key called "pluginconfig".

For example, if the user has selected the reference as hg19 in the plugin user interface (`instance_ir.html` using plugin APIs to get the values from IR DB (see below plugin APIs section for more information), then these values would be provided to the plugin in the `startplugin.json` file as below:

```
"pluginconfig" : {
 "reference" : "/data/IR/data/.reference/hg19/hg19.fasta"
 }
```

Therefore, the plugin developer could read the above path to access the reference file that has been selected in the user interface.

The `startplugin.json` file contains some Ion Reporter™ Software and plugin metadata, such as plugin_dir (plugin zip contents), results_dir (where plugin output should be written).

If the sample being used in Ion Reporter™ Software was uploaded using IonReporterUploader plugin in Torrent Suite™ Software, then the `startplugin.json` file will contain extra metadata, such as chipType, library, barcodeName, system_type and so on. Given the example `startplugin.json` as below, if the sample has been uploaded using the IonReporterUploader plugin:

```
{
  "expmeta" : {
    "sample" : "HBR",
    "runid" : "QRIBT",
    "run_name" : "R_2016_08_05_13_17_55_user_S5-00111-181-
LifeLab_DW_08052016_B",
    "run_flows" : 500,
    "output_file_name_stem" : "R_2016_08_05_13_17_55_user_S5-00111-181-
LifeLab_DW_08052016_B_Auto_user_S5-00111-181-LifeLab_DW_08052016_B_30836",
    "run_date" : "2016-08-05T20:19:39Z",
    "chiptype" : "530",
    "flowOrder" : "TACGTACGTCTGAGCATCGATCGATGTACAGC",
    "barcodeId" : "IonXpress",
    "analysis_date" : "2016-08-05",
    "chipBarcode" : "DBCD02486",
    "project" : "LifeLab",
    "instrument" : "S5-00111",
    "results_name" : "Auto_user_S5-00111-181-LifeLab_DW_08052016_B_30836",
    "notes" : ""
  },
  "pluginconfig" : {
    "reference" : "/data/IR/data/.reference/hg19/hg19.fasta",
    "genome" : "hg19"
  },
  "runinfo" : {
    "testfrag_key" : "ATCG",
    "url_root" : "",
    "chipType" : "530",
    "library" : "hg19",
    "sigproc_dir" : "",
    "pluginresult" : 881041,
    "api_url" : "",
    "barcodeId" : "IonXpress",
    "results_dir" : "/data/IR/data/IR_Org/
```

```
ion.reporter@lifetech.com/Dw2-4hr_RNA_v1/Dw2-4hr_RNA_v1_20170321131007199/
outputs/TsPluginActor-00/RNASeqAnalysis",
    "platform" : "s5",
    "pk" : 61178,
    "alignment_dir" : "",
    "api_key" : "",
    "basecaller_dir" : "",
    "net_location" : "",
    "username" : "",
    "library_key" : "TCAG",
    "systemType" : "S5",
    "plugin_name" : "RNASeqAnalysis",
    "tmap_version" : "",
    "report_root_dir" : "/data/IR/data/IR_Org/ion.reporter@lifetech.com/
Dw2-4hr_RNA_v1/Dw2-4hr_RNA_v1_20170321131007199",
    "chipDescription" : "530",
    "plugin_dir" : "/share/apps/IR/ionreporter54/apps/IR_Org/RNASeqAnalysis",
    "raw_data_dir" : "",
    "analysis_dir" : "/data/IR/data/IR_Org/ion.reporter@lifetech.com/
Dw2-4hr_RNA_v1/Dw2-4hr_RNA_v1_20170321131007199"
  },
  "plan" : {
    "planName" : "LifeLab_DW_08052016_B",
    "username" : "",
    "sequencekitname" : "Ion S5 Sequencing Kit",
    "librarykitname" : "Ion AmpliSeq RNA Library Kit",
    "sampleGrouping" : null,
    "sseBedFile" : "",
    "barcodeId" : "IonXpress",
    "controlSequencekitname" : "",
    "regionfile" : "",
    "runMode" : "single",
    "bedfile" : "",
    "templatingKitName" : "Ion Chef S530 V1",
    "samplePrepKitName" : "",
    "runType" : "AMPS_RNA",
    "runTypeDescription" : "AmpliSeq RNA",
    "reverse_primer" : "",
    "threePrimeAdapter" : "ATCACCGACTGCCCATAGAGAGGCTGAGAC",
    "sampleTubeLabel" : ""
  }
}
```

D Oncomine™ Variant Annotator plugin criteria

- Oncomine™ BRCA Research Assay 587
- Oncomine™ Breast cfDNA Assay 588
- Oncomine™ Breast cfDNA Research Assay v2 588
- Oncomine™ Childhood Cancer Research Assay 589
- Oncomine™ Colon cfDNA Assay 592
- Oncomine™ Comprehensive Assay v3 593
- Oncomine™ Comprehensive Assay Plus 595
- Oncomine™ Focus Assay 599
- Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay 601
- Oncomine™ Lung cfDNA Assay 603
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- Oncomine™ Myeloid MRD (RUO) Assays 607
- Oncomine™ Pan-Cancer Cell-Free Assay 609
- Oncomine™ tumor specific panels 611
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- Custom panels 618
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This appendix contains information that is relevant to the Oncomine™ analysis workflows in Ion Reporter™ Software. Each table in the appendix summarizes the criteria that is used by the Oncomine™ Variant Annotator plugin to find and annotate variants. The information shown in the **Annotation Criteria** columns is provided in VCF files. The variants are annotated for each listed variant type only if all of the conditions in the corresponding **Annotation Criteria** column are satisfied.

Oncomine™ Variant Annotator plugin annotates variants in Ion Reporter™ Software for each Oncomine™ assay and the Ion AmpliSeq™ HD Library Kit.

Oncomine™ BRCA Research Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number deletion	**Loss-of-Function**	**Deletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • SUBTYPE = "GeneCNV"
Copy number exon deletion	**Loss-of-Function**	**ExonDeletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • SUBTYPE = "BigDel"
Copy number exon duplication	**Loss-of-Function**	**ExonDuplication**	• SVTYPE = "CNV" • FILTER = "GAIN" • SUBTYPE = "BigDup"
Long deletion	**Loss-of-Function**	**Truncating**	• Positive mutation call • SVTYPE = "LongDel"
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a designated loss-of-function gene
Missense hotspot mutation	**Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
Splice site hotspot mutation	**Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list

Oncomine™ Breast cfDNA Assay

Variant type	Oncomine™ gene class	Oncomine™ variant class	Annotation criteria
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

Oncomine™ Breast cfDNA Research Assay v2

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a de-novo calling gene • Meets de-novo thresholds
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

Oncomine™ Childhood Cancer Research Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	**Loss-of-Function** **Unclassified**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function or unclassified gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
CEBPA-bZIP[1]	**Loss-of-Function**	**CEBPA-bZIP**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 282–345 of CEBPA[2]
CEBPA-N-terminal[1]	**Loss-of-Function**	**CEBPA-N-terminal**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution, frameshift deletion, frameshift insertion, or frameshift block substitution • INDEL impacts codons 1–120 of CEBPA[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
JAK2 exon 12 INDEL[1]	**Gain-of-Function**	**JAK2Exon12Indel**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 536-547 of JAK2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.
[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Colon cfDNA Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622
[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Comprehensive Assay v3

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Long deletion	**Loss-of-Function**	**Truncating**	• Positive mutation call • SVTYPE = "LongDel"
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744-761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762-775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770-783 of ERBB2[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 550–580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 550–580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 Skipping confirmed list
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 841–847 of PDGFRA[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.
[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Comprehensive Assay Plus

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Copy number deletion	**Loss-of-Function**	**Deletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • Occurs in a designated copy-loss gene

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number exon deletion	**Loss-of-Function**	**ExonDeletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • SUBTYPE = "BigDel"
Copy number exon duplication	**Loss-of-Function**	**ExonDuplication**	• SVTYPE = "CNV" • FILTER = "GAIN" • SUBTYPE = "BigDup"
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Expression imbalance	**Gain-of-Function**	**ExpressionImbalance**	• SVTYPE = "RNAExonTiles" • FILTER = "PASS" • Record meets Targeted Isoforms Detected Requirement
Long deletion	**Loss-of-Function**	**Truncating**	• Positive mutation call • SVTYPE = "LongDel"
Loss-of-function truncating de novo mutation	**Loss-of-Function** **Unclassified**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function or unclassified gene

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Synonymous hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is synonymous • Transcript and coding syntax occur in predefined synonymous hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744-761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762-775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 770-783 of ERBB2[2]
JAK2 exon 12 INDEL[1]	**Loss-of-Function**	**JAK2Exon12Indel**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 536-547 of JAK2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 Skipping confirmed list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.
[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Focus Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Missense hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Splice site hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
MNV hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 skipping confirmed list
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.

[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Synonymous hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is synonymous • Transcript and coding syntax occur in predefined synonymous hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4 bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 skipping confirmed list

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.

[2] In some cases the assay design may not cover the entire codon range.

Function, transcript, and coding syntax occur in predefined truncating hotspot list

Oncomine™ Lung cfDNA Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Synonymous hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is synonymous • Transcript and coding syntax occur in predefined synonymous hotspot list
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 skipping confirmed list

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.

[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Myeloid Research Assays

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
CEBPA-bZIP[1]	**Loss-of-Function**	**CEBPA-bZIP**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 282–345 of CEBPA[2]
CEBPA-N-terminal[1]	**Loss-of-Function**	**CEBPA-N-terminal**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution, frameshift deletion, frameshift insertion, or frameshift block substitution • INDEL impacts codons 1–120 of CEBPA[2]
JAK2 exon 12 indel[1]	**Gain-of-Function**	**JAK2Exon12Indel**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution • INDEL impacts codons 536–547 of JAK2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 550–580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 550–580 of KIT[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
FLT3 internal tandem duplication	**Gain-of-Function**	**FLT3ITD**	• Positive mutation call • Functional impact is a non-frameshift insertion in exon 14/15 of FLT3 or SVTYPE = "FLT3ITD"

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.
[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Myeloid MRD (RUO) Assays

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a de novo calling gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
CEBPA-bZIP[1]	**Loss-of-Function**	**CEBPA-bZIP**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 282–345 of CEBPA[2]
CEBPA-N-terminal[1]	**Loss-of-Function**	**CEBPA-N-terminal**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution, frameshift deletion, frameshift insertion, or frameshift block substitution • INDEL impacts codons 1–120 of CEBPA[2]
FLT3 internal tandem duplication	**Gain-of-Function**	**FLT3ITD**	• SVTYPE = "FLT3ITD" • SUBTYPE is not "Combined"

[1] For more information, see “Ion Torrent™ class-based variants” on page 622.

[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Pan-Cancer Cell-Free Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a de-novo calling gene • Meets de-novo thresholds
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 skipping confirmed list
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.

[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ tumor specific panels

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Copy number deletion	**Loss-of-Function**	**Deletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • Occurs in a designated copy-loss gene
Copy number exon deletion	**Loss-of-Function**	**ExonDeletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • SUBTYPE = "BigDel"
Copy number exon duplication	**Loss-of-Function**	**ExonDuplication**	• SVTYPE = "CNV" • FILTER = "GAIN" • SUBTYPE = "BigDup"

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Expression imbalance	**Gain-of-Function**	**ExpressionImbalance**	• SVTYPE = "RNAExonTiles" • FILTER = "PASS" • Record meets Targeted Isoforms Detected Requirement
Long deletion	**Loss-of-Function**	**Truncating**	• Positive mutation call • SVTYPE = "LongDel"
Loss-of-function truncating de novo mutation	**Loss-of-Function** **Unclassified**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function or unclassified gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Synonymous hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Functional impact is synonymous • Transcript and coding syntax occur in predefined synonymous hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in predefined MNV hotspot list
CEBPA-bZIP[1]	**Loss-of-Function**	**CEBPA-bZIP**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 282–345 of CEBPA[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
CEBPA-N-terminal[1]	**Loss-of-Function**	**CEBPA-N-terminal**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution, frameshift deletion, frameshift insertion, or frameshift block substitution • INDEL impacts codons 1–120 of CEBPA[2]
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
JAK2 exon 12 indel[1]	**Loss-of-Function**	**JAK2Exon12Indel**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • Indel impacts codons 536-547 of JAK2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 skipping confirmed list
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]
FLT3 internal tandem duplication	**Gain-of-Function**	**FLT3ITD**	• Positive mutation call • Functional impact is a non-frameshift insertion in exon 14/15 of FLT3 or SVTYPE = "FLT3ITD"

[1] For more information, see "Ion Torrent™ class-based variants".

[2] In some cases the assay design may not cover the entire codon range.

Oncomine™ Tumor Mutation Load Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
CEBPA-bZIP[1]	**Loss-of-Function**	**CEBPA-bZIP**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 282–345 of CEBPA[2]
CEBPA-N-terminal[1]	**Loss-of-Function**	**CEBPA-N-terminal**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution, frameshift deletion, frameshift insertion, or frameshift block substitution • INDEL impacts codons 1–120 of CEBPA[2]
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
JAK2 exon 12 indel[1]	**Gain-of-Function**	**JAK2Exon12Indel**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 536-547 of JAK2[2]
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET exon 14 skipping confirmed list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]

[1] For more information, see "Ion Torrent™ class-based variants" on page 622.

[2] In some cases the assay design may not cover the entire codon range.

Custom panels

The Oncomine™ Variant Annotator plugin applies universal annotation rules for user-defined analysis workflows for use with custom Ion AmpliSeq™ and Ion AmpliSeq™ HD panels from **AmpliSeq.com**. To apply the rules, the **Apply Oncomine Variant Annotator plugin** option must be selected when an analysis workflow is created. For more information, see "Steps in analysis workflow creation" on page 120.

IMPORTANT! Because these annotations are generic, the annotations can be different from annotations that are generated with a system-installed analysis workflow.

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Copy number deletion	**Loss-of-Function**	**Deletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • Occurs in a designated copy-loss gene
Copy number exon deletion	**Loss-of-Function**	**ExonDeletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • SUBTYPE = "BigDel"
Copy number exon duplication	**Loss-of-Function**	**ExonDuplication**	• SVTYPE = "CNV" • FILTER = "GAIN" • SUBTYPE = "BigDup"

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Expression imbalance	**Gain-of-Function**	**ExpressionImbalance**	• SVTYPE = "RNAExonTiles" • FILTER = "PASS" • Record meets Targeted Isoforms Detected Requirement
Long deletion	**Loss-of-Function**	**Truncating**	• Positive mutation call • SVTYPE = "LongDel"
Loss-of-function truncating de novo mutation	**Loss-of-Function** **Unclassified**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function or unclassified gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript and ID occur in predefined intronic hotspot list
Synonymous hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Functional impact is synonymous • Transcript and coding syntax occur in predefined synonymous hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function** **Unclassified**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in predefined MNV hotspot list
CEBPA-bZIP[1]	**Loss-of-Function**	**CEBPA-bZIP**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 282–345 of CEBPA[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
CEBPA-N-terminal[1]	**Loss-of-Function**	**CEBPA-N-terminal**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, nonframeshift block substitution, frameshift deletion, frameshift insertion, or frameshift block substitution • INDEL impacts codons 1–120 of CEBPA[2]
EGFR exon 19 deletion	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR[2]
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list[2]
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2[2]
JAK2 exon 12 indel[1]	**Loss-of-Function**	**JAK2Exon12Indel**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift insertion, or nonframeshift block substitution • INDEL impacts codons 536-547 of JAK2[2]

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 550-580 of KIT[2] • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, or nonframeshift block substitution • Insertion impacts codons 550-580 of KIT[2]
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, or nonframeshift block substitution • Deletion impacts codons 841-847 of PDGFRA[2]
FLT3 internal tandem duplication	**Gain-of-Function**	**FLT3ITD**	• Positive mutation call • Functional impact is a non-frameshift insertion in exon 14/15 of FLT3 or SVTYPE = "FLT3ITD"

[1] For more information, see “Ion Torrent™ class-based variants”.
[2] In some cases the assay design may not cover the entire codon range.

Ion Torrent™ class-based variants

Ion Torrent™ Class-Based Variants are a set of logic-based rules that the Oncomine™ Variant Annotator plugin uses to computationally identify and annotate novel mutations in important genomic variant classes. The annotation logic is based on scientific literature evidence. Details about the logic follow.

CEBPA-N-terminal and CEBPA-bZIP mutations

- **Background**—The CEBPA gene encodes the enhancer binding protein alpha, a member of the basic region leucine zipper family of transcription factors that recognizes the CCAAT promoter [PMID:19706798]. CEBPA is an intron-less gene located on chromosome 19q13.1 [PMID:24056881] and it produces two protein isoforms—p42 and a shorter p30 that lacks the N-terminal transcriptional activation domain (TAD) of p42 [PMID: 17671234]. Both isoforms have the bZIP domain, which is involved in hetero or homo-dimerization with other CEBP family members and is required for DNA binding [PMID: 19706798]. Mutations in CEBPA are reported in 6–18% of all acute myeloid leukemia (AML) cases [PMID: 24071849, PMID: 18752591, PMID: 29861846, PMID: 20038735]. In AML, CEBPA mutations are observed to occur as either monoallelic (single mutant) or biallelic (double mutant) [PMID: 20439648, PMID: 20038735, PMID: 28179278]. Biallelic CEBPA mutations are heterozygous and observed in 52–78% of CEBPA mutated AML patients [PMID:20038735, PMID: 34448807, PMID: 31477806, PMID: 28250006]. Within these individuals, 85–100% of biallelic combinations involve an N-terminal frameshift on one allele and a C-terminal in-frame mutation on the other allele (referred to as an N/C mutant) [PMID: 17934489, PMID: 28179278, PMID:20038735, PMID: 34448807, PMID: 31477806, PMID: 28250006]. C-terminal in-frame mutations disrupt the bZIP domain which interferes with DNA binding and hetero/homo-dimerization. Monoallelic mutations in the bZIP region of CEBPA and biallelic CEBPA mutations are recognized as diagnostic entities for AML by the World Health Organization (WHO) [PMID: 35732831]. In-frame bZIP region mutations in CEBPA, whether they occur as monoallelic or biallelic mutations, are associated with a favorable prognosis in AML [PMID: 35797463].
- **CBV Criteria**—The boundaries defined to capture the CEBPA N-terminal mutations and the CEBPA-bZIP mutations as class-based variants are summarized in the following two rules.
  a. CEBPA N-terminal mutation boundaries include frameshift mutations and in-frame INDELS within codon 1 and codon 120
  b. CEBPA-bZIP mutation boundaries include in-frame INDELS that start at codon 282 and end at codon 345.

EGFR exon 19 deletion

- **Background**—Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein made up of 1,210 amino acids and located on chromosome 7p11.2. EGFR has 31 exons—exons 2-4 and 8-12 encode the ligand binding domains, exons 5-7 and 13-16 encode the cysteine rich domain, exons 18–24 encode the tyrosine kinase domain (TKD), and exons 25-28 encode the autophosphorylation region [PMID: 17318210]. Mutations in EGFR often target the TKD which make up the ATP-binding pocket of the enzyme [PMID: 17318210]. Exon 19 deletions are INDELS that range between 15-18 bp in length and usually occur within codons 746-756 [PMID: 23768755, PMID: 22190593, PMID: 26933124, PMID: 24163741]. In non-small cell lung cancer (NSCLC), EGFR exon 19 deletions, located within codons 746-750, represent 45-50% of somatic mutations [PMID: 26933124, PMID: 24163741]. Exon 19 deletions result in constitutive activation of the receptor tyrosine kinase and hyperactivation of downstream signaling pathways [PMID: 29455648].
- **CBV Criteria**—The boundaries defined to capture EGFR exon 19 deletions as a class-based variant start at codon 744 and end at codon 761, which encompass common and rare INDELS [PMID: 30473385].

EGFR exon 20 insertion

- **Background**—Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein made up of 1,210 amino acids located on chromosome 7p11.2. EGFR has 31 exons—exons 2-4 and 8-12 encode the ligand binding domains, exons 5-7 and 13-16 encode the cysteine rich domain, exons 18–24 encode the tyrosine kinase domain (TKD), and exons 25-28 encode the autophosphorylation region [PMID: 17318210]. Mutations in EGFR often target the TKD which make up the ATP-binding pocket of the enzyme [PMID: 17318210]. In-frame insertions within exon 20 of EGFR are the third most common type of mutation found in NSCLC, representing 4-12% of all EGFR mutations in NSCLC [PMID: 31208370]. EGFR exon 20 encompasses codons 762 to 823. Exon 20 insertions commonly involve codons 762 to 774 wherein codons 762-766 make up the c-helix and codons 767-774 make up the activation loop [PMID: 31208370, PMID: 30854234]. EGFR exon 20 insertions do not alter the receptor binding affinity; however, it is suggested that the location of exon 20 insertions stabilizes its active confirmation [PMID: 30854234, PMID: 27843613].
- **CBV Criteria**—The boundaries defined to capture EGFR exon 20 insertions as a class-based variant start at codon 762 and end at codon 775. Additionally, known EGFR exon 20 insertions p.A763_Y764insFQEA and p.A763_Y764insLQEA, represented as c.2284–6_2284–5insTCCAGGAAGCCT and c.2284–9_2284–8insCCCTCCAGGAAG due to left alignment into the intronic region, are included in this rule.

  The **Splice Site Size** annotation parameter value can be modified from its default value of 2 to a value of 6, so that the variant is annotated as expected. The current setting affects the annotation for EGFR p.A763_Y764insFQEA.

  IMPORTANT! Changes to the Splice Site Size parameter will also impact the Oncomine Variant Annotator annotations for all variants that are close to the splice site.

ERBB2 exon 20 insertion

- **Background**—Erb-b2 receptor tyrosine kinase 2 (ERBB2) is a transmembrane glycoprotein located on chromosome 17q12 [PMID: 25276427]. ERBB2 (also known as HER2) has 27 exons—exons 2-4 and 9-12 encode the extracellular receptor L domains, exons 5-8 encode the furin like domain, exons 13-16 encode the growth factor receptor domain IV, exon 17 encodes the transmembrane domain, and exons 18–24 encode the tyrosine kinase domain (TKD) [PMID: 22761469, PMID: 29420467]. In lung cancer, the most recurrent ERBB2 activating mutations include in-frame exon 20 insertions [PMID: 30425522]. ERBB2 exon 20 involves codons 770 to 831 and majority of exon 20 insertions occur between codons 775 and 781 within the kinase domain [PMID: 29686424, PMID:22761469]. Insertions at the C-terminal end of ERBB2 exon 20 induce a change in conformation of the α-C helix leading to a constitutively active formation which affects the drug-binding pocket [PMID: 29686424].
- **CBV Criteria**— The boundaries defined to capture ERBB2 exon 20 insertions as a class-based variant start at codon 770 and end at codon 783.

JAK2 exon 12 INDEL

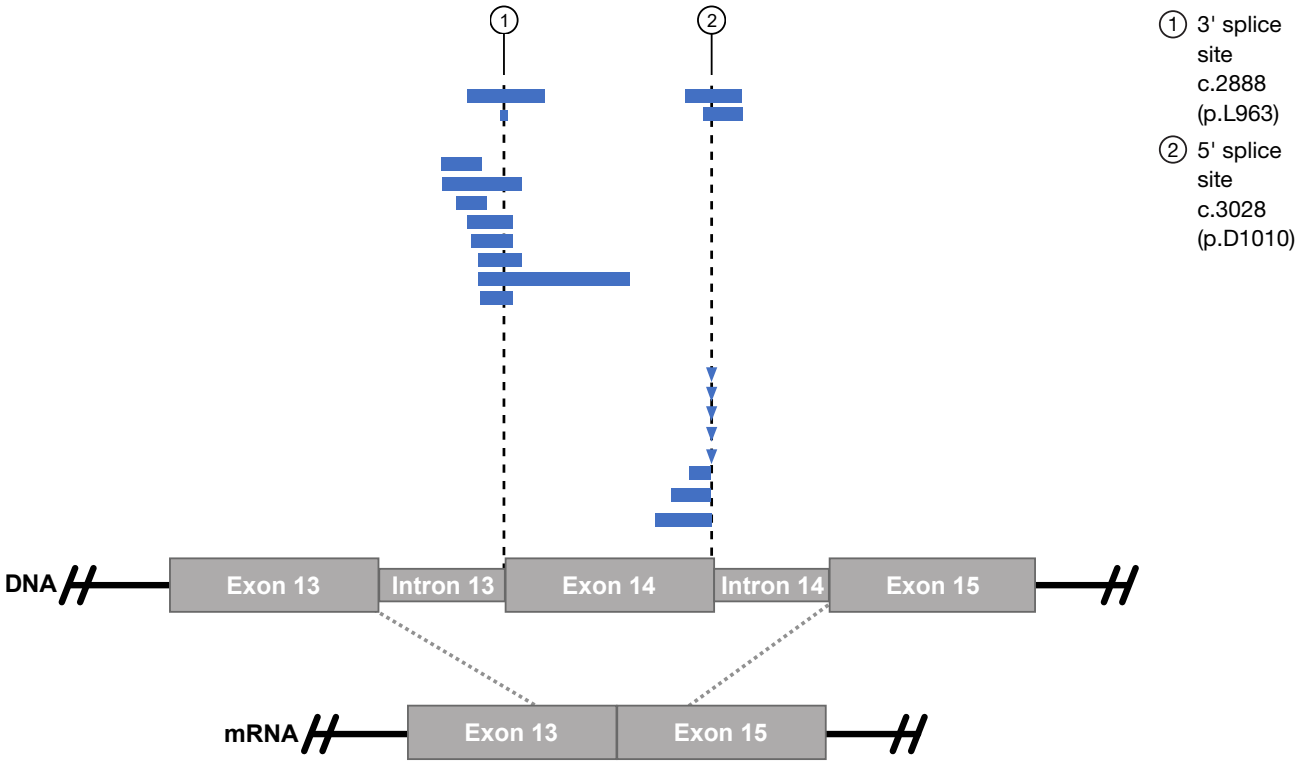
- **Background**—Janus kinase 2 (JAK2) is a non-receptor membrane associated protein tyrosine kinase (PTK) made up of 1,132 amino acids and located on chromosome 9p24. JAK2 belongs to the Janus kinase (JAK) family and has 25 exons—exons 3–7 encode the FERM domain, exons 9–11 encode the SH2-pseudokinase domain, exons 12–19 encode the kinase-like or pseudokinase domain (JH2), and exons 19–25 encode the C-terminal tyrosine kinase domain (JH1) [PMID: 23592968; PMID: 17639043; PMID: 29379470]. In addition, codons 523–544 in exon 12 encode the SH2-pseudokinase linker region and codons 817–839 in exon 19 encode the linker between the JH1 and JH2 protein kinase regions [PMID: 23592968, PMID: 17639043]. Mutations in JAK2 often target exon 12 including the region adjacent to the start of the pseudo-kinase domain [PMID: 21674578]. JAK2 exon 12 mutations (missense and in-frame deletions) are prevalent in myeloproliferative neoplasms including polycythemia vera [PMID: 21674578].
- **CBV Criteria**—The boundaries defined to capture JAK2 exon 12 in-frame insertions and deletions (INDELS) as a class-based variant start at codon 536 and end at codon 547. These boundaries capture the most common JAK2 exon 12 INDELS, including N542_E543del, E543_D544del, F537_K539delinsL and R541_E543delinsK mutations.

KIT exon 11 insertion and KIT exon 11 deletion

- **Background**—KIT, also known as CD117, is a proto-oncogene receptor tyrosine kinase made up of 976 amino acids located on chromosome 4q12 [PMID: 16689459]. KIT is a member of the PDGFR type III receptor tyrosine kinase family [PMID: 26579483; PMID: 26309392]. KIT has 21 exons—exons 1–9 encode the extracellular ligand-binding region containing five Ig-like domains, exon 10 encodes the transmembrane (TM) domain, and exon 11 encodes a cytoplasmic juxtamembrane region. The intracellular region of KIT is encoded by exons 12–21, wherein, exons 12–14 encode the tyrosine kinase domain 1, exons 15–16 encode the kinase insert (KI) region, and exons 17–19 encode the tyrosine kinase domain 2 [PMID: 24449920, PMID: 27536065, PMID: 31378162, PMID: 15342366, PMID: 17555444]. Recurrent somatic KIT alterations are observed in both solid and hematological cancers and include activating mutations such as single nucleotide variants (SNVs), small duplications, and complex in-frame insertions or deletions (INDELS). Mutations in KIT exons 8, 9, 11, and 17 disrupt the auto-inhibitory mechanisms and lead to constitutive activity [PMID: 27536065]. While mutations in exons 8 and 17 are common in myeloid cancers, 60–70% of somatic mutations (including SNVs and INDELS) in gastrointestinal stromal tumor (GIST) occur in exon 11, which encodes the juxtamembrane domain [PMID: 27536065, PMID: 22588877, PMID: 23678293; NCCN-GIST].
- **CBV Criteria**—The boundaries defined to capture KIT exon 11 insertions and deletions as a class-based variant start at codon 550 and end at codon 580.

MET exon 14 skipping

- **Background**—The MET proto-oncogene is a receptor tyrosine kinase made up of 1,390 amino acids and is located on chromosome 7q31 [PMID: 15735036]. MET has 21 exons containing three main structural domains—an extracellular sema domain in exon 2, a juxtamembrane domain in exon 14, and a tyrosine kinase domain in exons 15-21 [PMID: 28376232, PMID: 9380410]. Splice site mutations flanking exon 14 are observed in 3–4% of non-small cell lung cancer (NSCLC). These mutations include canonical splice site mutations affecting exon 14 and deletions that extend into the splicing motifs within intron 13 [PMID: 25971938, PMID: 27343443]. Such mutations disrupt splicing leading to the formation of an alternative transcript that joins exon 13 directly to exon 15 and skips exon 14 entirely. The MET exon 14 skipping transcript lacks the juxtamembrane domain that contains the recognition motif for ubiquitin-dependent proteolysis and thus leads to a marked increase in steady-state level of the MET protein [PMID: 28164087].
- **CBV Criteria**—The boundaries defined to capture MET exon 14 skipping as a class-based variant can be summarized into four individual rules.
  a. Has transcript = NM_000245.4, exon = 14, and location equal to splicesite_3 (at c.2888) or splicesite_5 (at c.3028),
  b. Is an intronic deletion ≥4 bp impacting 30 nucleotides preceding exon 14
  c. Is a missense variant at c.3028 [PMID: 27343443, PMID: 26729443, PMID: 25971938, PMID: 25898962, PMID: 25898965, PMID: 31472177],
  d. Is one of the following variants with confirmed skipping defined as c.3028delG [PMID: 25971938, PMID: 27343443], c.3012_3027delAGCTACTTTTCCAGAA, c.3021_3028delTCCAGAAG [PMID: 31472177], or c.3026_3027delAA [PMID: 26729443].



PDGFRA exon 18 deletion

- **Background**—Platelet derived growth factor receptor alpha (PDGFRA) is a cell surface tyrosine kinase receptor made up of 1089 amino acids and located on chromosome 4q12. PDGFRA contains 23 exons –exons 1-2 encode the signaling peptide, exons 3-10 encode the extracellular domain, exon 11 encodes the transmembrane domain, exon 12 encodes the juxtamembrane domain, and exons 13-23 encode the kinase domain [PMID: 33449152]. Mutations in PDGFRA often target the activation loop located within exon 18 and, to a lesser extent, target the juxtamembrane domain and the N-lobe of the kinase domain found within exons 12 and 14 [PMID: 30778083, PMID: 33449152, PMID: 15146165]. Activating PDGFRA exon 18 mutations such as V561D, N659K, D842V, and in-frame deletions are observed in 30-40% of KIT-negative gastrointestinal stromal tumors (GISTs) [PMID: 17193822, PMID: 15928335, PMID: 12522257, PMID: 23970477]. Specifically, PDGFRA in-frame deletions in codons 841-847 make up 23% of all exon 18 mutations [PMID: 15146165].
- **CBV Criteria**—The boundaries defined to capture PDGFRA exon 18 deletions as a class-based variant start at codon 841 and end at codon 847.

Documentation and support

Related documentation

Record	Publication number	Description
Torrent Suite™ Software 5.22 User Guide	MAN1000552	Describes the Torrent Suite™ Software and provides procedures for common tasks.
Oncomine™ Childhood Cancer Research Assay User Guide	MAN0017117	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Childhood Cancer Research Assay.
Oncomine™ Human Immune Repertoire User Guide	MAN0017438	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ TCR Beta Assay and Oncomine™ BCR IGH Assay.
Oncomine™ Comprehensive Assay v3 User Guide	MAN0015885	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Comprehensive Assay.
Oncomine™ Comprehensive Assay Plus User Guide	MAN0018490	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Comprehensive Assay Plus.
Oncomine™ Focus Assay, Part III: Variant Analysis User Guide	MAN0015821	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Focus Assay.
Oncomine™ cfDNA Assays, Part III: Variant Analysis User Guide	MAN0015874	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Lung cfDNA Assay, Oncomine™ Colon cfDNA Assay, and the Oncomine™ Breast cfDNA Assay.
Ion ReproSeq™ PGS View Kits User Guide	MAN0016158	Describes how to use Ion Reporter™ Software for data analysis of the Ion ReproSeq™ assay.
Oncomine™ Reporter User Guide	MAN0018068	Describes how to generate reports in Oncomine™ Reporter Software.
Ion AmpliSeq™ Library Kit 2.0 User Guide	MAN0006735	Describes sequencing data analysis for libraries prepared using Ion AmpliSeq™ panels.
Ion AmpliSeq™ HD Library Kit User Guide	MAN0017392	Describes data analysis for libraries sequenced with Ion AmpliSeq™ HD analysis workflows.

(continued)

Record	Publication number	Description
Oncomine™ BRCA Research Assay User Guide	MAN0014634	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ BRCA Research Assay.
Oncomine™ Tumor Mutation Load Assay User Guide	MAN0017042	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Tumor Mutation Load Assay.
Oncomine™ Pan-Cancer Cell-Free Assay User Guide	MAN0017064	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Pan-Cancer Cell-Free Assay.
IonReporterUploader Command-Line Utility User Guide	MAN0017648	Describes how to use command-line utility to set up samples and transfer analysis results files to Ion Reporter™ Software.
Ion Torrent™ Ion CarrierSeq™ ECS Kits User Guide	MAN0018483	Describes how to use Ion Reporter™ Software for data analysis of Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel analysis results.

Advanced algorithm documentation

Ion Reporter™ Software contains many advanced algorithms that have been optimized specifically for Ion Torrent™ semiconductor Sequencing technology. Below are application notes and white papers that provide more background and are available at the Thermo Fisher Scientific website.

- *Variant filtering and prioritization using the Ion AmpliSeq™ Exome trio workflow in Ion Reporter™ Software*, available at **http://tools.thermofisher.com/content/sfs/brochures/Ion-AmpliSeq-Exome-Application-Note.pdf**
- *Metagenomics 16S Algorithm Overview*, available at **http://tools.thermofisher.com/content/sfs/brochures/ion-reporter-16s-metagenomics-algorithms-whitepaper.pdf**.
- *Application Note: CNV detection by Ion semiconductor sequencing*, available at **http://tools.thermofisher.com/content/sfs/brochures/CNV-Detection-by-Ion.pdf**.
- *Application Note: Detection of aneuploidy in a single cell using the Ion ReproSeq PGS Kit*, available at **https://tools.thermofisher.com/CONTENT/SFS/BROCHURES/ANEUPLOIDY-APP-NOTE.PDF**.
- *Cancer genomics and transcriptomics research*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=5**.
- *Low-frequency mutations from cell-free DNA*, available at **http://tools.thermofisher.com/content/sfs/brochures/ngs-analysis-mutations-cfdna-app-note.pdf**.
- *Reproductive genomics and inherited disease research*, available at **http://tools.thermofisher.com/content/sfs/brochures/Ion-S5-S5XL-App-Note-Inherited-Disease.pdf**.
- *Rapid sequencing of microorganisms*, available at **http://tools.thermofisher.com/content/sfs/brochures/Ion-S5-S5XL-App-Note-Infectious-Disease.pdf**.

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Glossary

aligned reads

The number of bases covered by reads aligned to the reference sequence.

aligned read length

The aligned length metric of a read at a given accuracy threshold is defined as the greatest position in the read at which the accuracy in the bases up to and including the position meets the accuracy threshold.

allele view

Variants per allele view in Ion Reporter™ Software. See also *locus view*.

alpha-beta diversity

Alpha diversity results describe the diversity in a single sample at the Species, Genus, and Family levels. Beta diversity results describe the diversity between multiple samples at the Species, Genus, and Family levels. Used with Metagenomics 16S analysis workflows in Ion Reporter™ Software. See also *metagenomics*.

amplicon coverage

An amplicon is a piece of DNA or RNA that is the source or product of a natural or artificial amplification or replication event. Coverage refers to the number of times the amplicon is amplified or replicated.

Analyze role

Person in the Ion Reporter™ Software organization who can create analysis workflows and launch analyses. See also *Import role* and *Report role*.

annotation-only analysis workflow

This predefined analysis workflow adds annotations when a VCF file is uploaded to Ion Reporter™ Software; there is no further analysis of the data, and no variants are called in Ion Reporter™ Software with this analysis workflow.

annotation set preset

Set of annotation sources to apply to variants for selection in the **Annotation** step of creating an analysis workflow.

annotation source

Ion Reporter™ Software provides several annotation sources that are derived from public and private annotation databases for hg19. See also *annotation set preset*.

API token

Unique identifier of an API (application programming interface) requesting access to your service, similar to a username-password authentication. See also Ion Reporter™ Software web services API.

average base coverage depth

The average number of reads of all targeted reference bases.

average base read depth

The average number of reads of all targeted reference bases that were read at least once.

BAM file

A BAM (binary alignment map) file (.bam) is the binary version of a SAM (sequence alignment map) file (.sam). A SAM file is a tab-delimited text file that contains sequence alignment data. A BAM file contains aligned reads sorted by reference location.

Bamstats

A software tool built on the Picard Java API(2) that can calculate and graphically display various metrics derived from SAM or BAM files.

barcode

A barcode is a machine-readable code in the form of numbers and a pattern of parallel lines of varying widths, printed on and identifying a product.

There are several applications for barcodes. Libraries can be molecularly barcoded with unique nucleic acid sequence identifiers. Library barcodes are used during data analysis to sort the sequencing results from sequencing reactions that contain combined libraries. Chips and sample tubes also contain unique numeric barcodes that aid in the setup of the experimental analysis workflow.

barcode crosstalk

Reads from a particular barcode that show up in a neighboring barcode. This can be a source of contamination in fusions results.

basecalling input file

Signal processing input files are converted to a single condensed basecalling input file that represents the processed signal. Basecalling input files are required files for basecalling.

base substitution classes

Somatic mutations can be divided into six base substitution classes: C>A, C>G, C>T, T>A, T>C, and T>G.

BED file

Browser Extensible Data file—BED file—defines chromosome positions or regions.

Boolean

A binary value, having two possible values called "true" and "false".

bp

Abbreviation for "base pair(s)".

cellularity (%)

The percentage of tumor cells in a given sample.

CDR

Complementarity-determining regions are components of the variable chains in antibodies and T-cell receptors that are generated by B-cells and T-cells.

cluster

A gene cluster is a group of two or more genes found within a sample's DNA that are similar in makeup.

CNV

Copy number variation (CNV) is the variation in copy number of any given gene between two samples. CNV is a phenomenon in which sections of the genome are repeated and the number of repeats in the genome varies between individuals in the human population.

CNV baseline preset

Set of control samples that are used to create a baseline for detecting CNVs. The baselines are accessible in the Copy Number step when you create an analysis workflow.

codon

A sequence of three nucleotides that form a genetic code in a DNA or RNA molecule.

control sequence

Control nucleic acid sequences can be added to DNA or RNA samples to facilitate post-sequencing data analysis. Two types of control sequences can be used during sample preparation. ERCC RNA Spike-In Mix is used with RNA samples to achieve a standard measure for data comparison across gene expression experiments. Ion AmpliSeq™ Sample ID Panel, comprised of nine specially designed primers, can be added prior to template amplification to generate a unique ID for each sample during post-sequencing analysis.

copy number gain

Greater than expected copy number for a gene or chromosome in a karyotype. See also *copy number loss*.

copy number loss

Less than expected copy number for a gene or chromosome in a karyotype. See also *copy number gain*.

coverage

The average number of reads representing a given nucleotide in the reconstructed sequence. Enables you to estimate the percentage of the genome covered by reads. High coverage overcomes errors in base-calling and assembly. The typical desired coverage of a genome is 30x.

coverage histogram

A graphical representation of coverage in Ion Reporter™ Genomic Viewer (IRGV).

CSV file

A comma-separated values (CSV) file is a delimited text file in which each line represents a data record with information fields separated by a comma. A CSV file stores tabular data (numbers and text) in plain text. Each line of the file is a data record.

CSV files are easily opened using spreadsheet software, such as Microsoft™ Excel™ or Apache® OpenOffice™ Calc, where each comma-separated field is listed in a separate column.

de novo assembly

Nucleic acid sequence data that is assembled from sequencing reads without the aid of a reference genome library sequence.

exon

DNA bases that are translated into mRNA.

FASTA file

A FASTA file is a text-based format for representing either nucleotide sequences or peptide sequences, in which base pairs or amino acids are represented using single-letter codes. A sequence in FASTA format begins with a single-line description, followed by lines of sequence data.

FASTQ file

A FASTQ file is a text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores. Both the sequence letter and quality score are each encoded with a single ASCII character for brevity.

FD (flow disruptiveness)

A data filtering parameter that is used instead of INDEL, SNP, and MNP.

filter chain preset

Set of filters to apply to variants in the Filter step of creating an analysis workflow.

final report template preset

Final report templates that are accessible for selection in the **Final Report** step of creating an analysis workflow.

flow order

The order in which a chip is exposed to each particular dNTP. The default Samba flow order consists of a 32-base sequence, repeated. This flow order resists phase errors by providing opportunities for out-of-phase molecules to catch up and is designed to sample all dimer (nucleotide pair) sequences efficiently. Samba is the default flow order because it improves sequencing accuracy for longer reads by resisting phase errors.

frameshift insertion or deletion

Insertion or deletion of the number of nucleotide bases that are not divisible by 3, hence change in reading frame, the grouping of codons, and completely different protein translation from the original.

functional score

A filter in Ion Reporter™ Software that provides functional scores based on SIFT, PolyPhen, and Grantham scores. See also *SIFT score*, *PolyPhen score*, and *Grantham score*.

fusions

A targeted sequencing technique used for detection and annotation of gene fusions (or translocation of genetic material) in samples.

genomic coordinates

Where variants are located on chromosomes or genes.

germline

Germ-cell lineage.

Genomic Instability Metric (GIM)

Genomic Instability Metric (GIM) is a proprietary measurement in Ion Reporter™ Software that quantifies genomic scarring associated with Homologous Recombination Deficiency (HRD). It summarizes unbalanced copy number changes in autosomes that are determined using genomic segmentation, and generates a value between 0-100. Higher GIM values correlate with the observation of more genomic instability in the sample.

Grantham score

A measure of evolutionary distance. Used in Metagenomics 16S analyses in Ion Reporter™ Software.

GRCh38 human reference

Genome based on the latest Genome Reference Consortium (GRC) human reference assembly. See also *Library Reference*.

hotspots file

A BED or a VCF file that defines variants. Specifying a hotspots file to use in a run enables the Torrent Variant Caller (TVC) module to identify variants that may be present in sample DNA. A hotspots file instructs the Torrent Variant Caller (TVC) module to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspots file does not affect other parts of the analysis pipeline.

IGV

Acronym for the Integrative Genomics Viewer developed by the Broad Institute for visualizing analysis results. See also *IRGV*.

Import role

Person in the Ion Reporter™ Software organization who can import and define samples and launch analyses. See also *Analyze role* and *Report role*.

INDEL

INDEL is an abbreviation used to designate an insertion or deletion of bases in the genome of an organism.

intron

DNA bases found in between exons.

IRGV

Acronym for Ion Reporter™ Genomic Viewer, which is used to visualize analysis results.

ISPs

Ion Sphere™ Particles (ISPs) are particles that contain bound copies of a single (ideally) DNA fragment amplified during template preparation.

IUPAC

Acronym for International Union of Pure and Applied Chemistry. Ion Reporter™ Software uses IUPAC codes for amino acids.

JSON file

JavaScript Object Notation file. Used in Ion Reporter™ Software to import parameters from Torrent Suite™ Software.

key signal

Average 1-mer signal in the library key.

Krona

Visualization package used to display Metagenomics 16S results in Ion Reporter™ Software.

library ISPs

Live ISPs that have a key signal identical to the library key signal.

library key

A short known sequence of bases used to distinguish a library fragment from a test fragment (for example, "TCAG").

Locus view

Locus-centric view of variants in Ion Reporter™ Software. See also *Allele view*.

LOD

Acronym for limit of detection. LOD is the lowest quantity of a substance that can be determined.

LONGDEL

Long deletion.

MAF

Minor allele frequency (MAF) annotation source of population frequency information from the 1000 genomes project.

MAPD

MAPD is one metric that is used to determine whether the panel data are useful for copy number variation (CNV) run results.

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between log2 ratios of each tile for a run. Tiles roughly correspond to amplicons in an Ion AmpliSeq™ assay. Each pair is defined as adjacent in terms of genomic distance. Tiles corresponding to copy number amplicons and other amplicons are being treated equally as no differences in variability are seen between these types. Then, any two log2 ratios that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every log2 ratio belongs to two pairs.

Formally, if `xi` is the log2 ratio at position `i`, with `i` ordered by genomic position:

```
MAPD = median ( | x(i - 1) - x(i) | )
```

MAPD is an estimate of copy number variability in each sequencing run that is similar to standard deviation (SD). If one assumes the log2 ratios are distributed normally with mean 0 and a constant SD, then MAPD/0.95 is approximately equal to SD. However, unlike SD, using MAPD is robust against high biological variability in log2 ratios induced by known conditions such as cancer.

Regardless of the source of the variability, increased variability decreases the quality of CNV calls.

Mbp

Million base pairs.

metagenomics

Population diversity in polymicrobial research samples.

missense SNV

A point mutation that changes the amino acid of the respective protein. SNV is an acronym for single nucleotide variation, which means at one base there is a difference.

MNP

Multiple nucleotide polymorphism (MNP) is a genetic mutation in an allele that differs from the reference allele of the same length by >1 nucleotide.

mosaicism

Decimal-level copy number gain or loss calls.

non-frameshift insertion or deletion

Insertion or deletion of the number of nucleotide base that are divisible by 3, hence, the inclusion or exclusion of amino acid in the protein translation from the original.

non-PAR

Non-Pseudoautosomal Regions (PAR 1 and PAR 2) of the human X and Y chromosomes pair and recombine during meiosis. Therefore, genes in this region are not inherited in a strictly sex-linked fashion.

no template control

Sample that has no cDNA or gDNA content.

nonsense SNV

A point mutation that changes one of the 20 amino acids into a stop codon, hence a shorter or unfinished protein product. SNV is an acronym for single nucleotide variation, which means at one base there is a difference.

OTU

Operational taxonomic unit (OTU) tables used by QIIME to generate alpha-beta diversity results in metagenomics analyses.

paired sample

Control or normal sample paired with a tumor sample.

partner gene

Used in fusions to describe the second gene involved in a translocation of genetic material. Donor gene is the first.

phyloP score

Measure of conservation of protein across a wide range of organisms in metagenomics analyses.

polyclonal ISP

An ISP that carries clones from two or more library sequences.

PolyPhen score

Prediction of the functional effect of a variant on a protein.

primer dimer ISP

An ISP that carries an insert length of less than 8 base pairs.

proband

A person or a sample that is serving as a starting point for the genetic study. Denoting the proband aids in establishing relationships within a group. In medical genetics, the proband is the first affected family member who seeks medical attention for a genetic disorder.

p-value

Probability value. A statistical method for the detection of variant calls from next-generation sequencers.

In Ion Reporter™ Software, p-values in Analysis Results in the column named **p-value** are rounded to 5 decimal places (between 0.00001-0.99999) when shown in the software screen. Very small p-values that are less than 0.00001 are rounded to 0.00001 by default when shown in the software screen. Very large p-values that are greater than 0.99999 are rounded to 0.99999 by default when shown in the software screen.

Q score

Phred quality score (Q score) is used to measure the accuracy of the nucleotide sequence generated by the sequencing instrument. The Q score represents the probability that a given base is called incorrectly by the sequencer.

read mapping

Alignment of sequencing reads to a reference genome.

reference library

A consensus nucleotide sequence that represents the genome of a particular species. The results from a sequencing run are compared to the reference library to identify sequence variants.

relationship group

Defines related samples within a sample set. Related samples are designated by the same relationship group number.

Report role

Person in the Ion Reporter™ Software organization who can generate reports. See also *Analyze role* and *Import role*.

sample

Genetic material from one source (for example, DNA from one individual).

sample pair

Can be a sample from normal tissue and tumor tissue, control sample and test sample.

SIFT score

SIFT stands for Sorting Intolerant from Tolerant and is an algorithm for predicting whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids.

smoothing

Ion Reporter™ Software includes a smoothing algorithm to smooth discrete data points in aneuploidy detection visualization.

SNP

Single nucleotide polymorphism (SNP) is a genetic mutation in an allele that differs from the reference allele of the same length by one nucleotide.

somatic

Cells from the body of an organism.

splice site

A genetic mutation that inserts, deletes, or changes a number of nucleotides at a specific location.

structural variants

Genetic mutations that cause a change in the organism's chromosome structure, such as insertions, deletions, copy number variations, duplications, inversions, and translocations.

target regions file

test fragment ISPs

Live ISPs with a key signal that is identical to the test fragment key signal.

transcripts

Gene transcripts as determined by public annotation sources.

trio

Father, mother and child (proband) samples.

TSV file

A tab-separated values (TSV) file is a tab-delimited file that is used with spreadsheet software. TSV files are essentially text files, and the raw data can be viewed by text editors, though they are often used when moving raw data between spreadsheets. See also *VCF file*.

tumor mutational burden

A calculation of nonsynonomous variants (missense and nonsense single nucleotide variants (SNVs)) plus insertion and deletion variants (INDELs) detected per megabase (Mb) of exonic sequence.

tumor-normal pair

Samples from tumor and normal healthy tissue.

unaligned reads

Nucleotide bases covered by reads that are not aligned to the reference.

VCF file

A variant call format (VCF) file specifies a variant of interest and its location. This file stores the differences between the BAM file and the reference file.

VCIB

Variability Correction Informatics Baseline is a CNV baseline available in Ion Reporter™ Software. Users can start with this CNV baseline and add their samples to it when building CNV baseline analysis workflows.

WavSD

Acronym for Waviness Standard Deviation. WavSD is the standard deviation of all log2 read count ratios after subtraction of the contribution of pairwise differences.
A slow changing low frequency noise that can accumulate along the geometric locations and is independent of pairwise noise is sometimes observed when MAPD is measured. The WavSD metric is designed to measure such low frequency noise. The actual calculation in the software is an approximation based on the assumption of slow changing of the waviness effect.

Ion Reporter™ Software web services API

The Ion Reporter™ Software web services API (application programming interface) can be used to automate returns and retrieve key information from the system. Ion Reporter™ Software APIs are compliant with REST (Representational State Transfer) architectural constraints.