iontorrent

Ion Reporter™ Software 5.12

USER GUIDE

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Revision B.0

For Research Use Only. Not for use in diagnostic procedures.

Thermo Fisher
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Revision	Date	Description
B.0	November 7, 2019	• New topics: – Sort read coverage tracks by variant – Adjust IRGV BAM tracks – Change Y axis height in Whole Genome data visualization – Visualization of triploid samples • Updates to the following topics: – Visualize variants in analysis run AmpliSeqHD analysis workflow – Set IRGV preferences – Custom BED file causes an error – Create an analysis workflow that contains a copy number baseline – View the Mutation Load statistics – Locus View versus Allele View of variants – Download detailed analysis logs – Gene symbol filter – GRCh38 and hg19 human reference genomes
A.0	September 7, 2019	• New features: – **Sequence Variant Baseline** creation – Audits of user activities, and changes made to software – Add an electronic signatures to reports – QC Metrics included in Final Report template – Molecular coverage analysis metrics included in QC Reports – Coverage analysis metrics included in QC Reports – Updates to administrative policies for system access password policies – Updates to information about transcript sets – New classification terms for variants – Notes can be added to completed analyses – New sample attributes: Mouse, Population, Sample Collection date and Sample Collection date – New API call: Get current results TSV – Updates to the Oncomine™ Variant Annotator plugin – Ability to upload list of genes for use with filter chains – New predefined filter chain for Extended Oncomine™ results – Tumor Mutational Burden (TMB) improvements – MicroSatelliteInstability (MSI) detection – Immune Repertoire—Haplotype attribute and algorithm updates for Immune Repertoire to allow adverse events to be predicted. New plots added to Immune Repertoire visualizations. – Enhanced ability to add visualized content to Final Reports and Final Report templates – Updates to 16S Metagenomics workflow—allows uploading of custom databases, use of different filter settings and parameters for one or more additional databases

Revision	Date	Description
		- Updates to BRCA workflows and visualizations: LongDel column added to BRCA Report, allows use of alternate transcript annotation for BRCA1 in Exon Deletion, improvements to BRCA graphics and visualizations. - Sections rewritten, enhanced, updated, and improved – Updates to name change from "Ion Reporter™ Software on Thermo Fisher Cloud" to "Ion Reporter™ Software on Connect" – Import and Manage Samples – Manage analysis workflows – Analyses – Visualize analysis results with Ion Reporter™ Software – Create and view reports – Filters and Filter Chains – Appendix added for the Oncomine™ Variant Annotator plugin – Administer Ion Reporter™ Software – Fusions appendix

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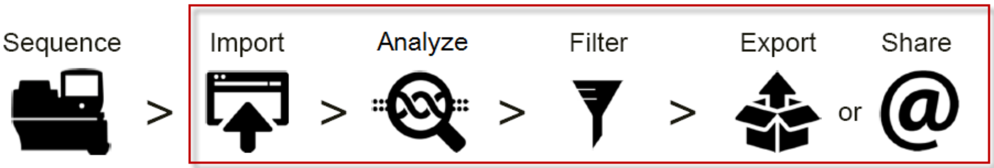
1

Introduction to Ion Reporter™ Software

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.

Admin -

Ion AmpliSeq panel types

AmpliSeq.com offers four design and ordering options for Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels:

- On -Demand Ion AmpliSeq™ research panels of optimized amplicons for germline analysis. Configurable to a specific human disease area, and ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ research panels of DNA and RNA designs for germline or somatic analysis of any genome, ordered in large reaction packs. Made-to-Order panels were formerly known as Custom Panels.
- Ready-to-Use Ion AmpliSeq™ research panels are predesigned DNA and RNA panels for germline and somatic analysis, ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ HD research panels are ultra high-sensitivity DNA and RNA designs for germline or somatic analysis of the human genome, including dual barcoded amplicons, and bidirectional sequencing.

hg19 and GRCh38 human genome references

In Ion Reporter™ Software you can use human genome references hg19 or GRch38 for either pre-defined or custom workflows. You can use the Ion GRCh38 human reference when you create custom analysis workflows. The Ion GRCh38 Reference Genome in 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 one GRCh38 human reference and related target reference (BED) file for GRCh38 experiments. Optionally, you can also convert existing coordinates to GRCh38 by using a publicly available lift-over tool, such as **CrossMap**.

Note: If you edit earlier versions of analysis workflows that use the hg19 reference genome, you cannot change the reference genome to Ion GRCh38.

See the following topics for more information about custom analysis workflows:

- "Steps in analysis workflow creation" on page 91
- "Create a custom analysis workflow from an existing analysis workflow" on page 75
- "Create a custom analysis workflow without predefined settings" on page 76
- "Create a copy number baseline workflow preset" on page 136

Requirements for GRCh38 custom BED file

If you create a custom BED file for a custom analysis that uses the GRCh38 reference, consider the following:

Public standard:

- See the BED file specification as described by UCSC.
- Annotation files contain three types of lines: browser lines, track lines and data lines.
- Empty lines and those starting with '#' are ignored.
- A track line begins with the word 'track', followed by one or more key=value pairs.
- There are currently 16 key=value pairs recognized by UCSC, but other key=value pairs are allowed.
- The key db=<UCSC_assembly_name> specifies the reference, but must be a valid UCSC assembly ID (e.g. hg19).

Example:

track name="CHP2_designed" description="Amplicon_Insert_CGP2" visibility=2 db=hg19

track name="CHP2_designed" description="Amplicon_Insert_CGP2" visibility=2 db=hg38

Recommendation:

Include the key=value pair db=<UCSC_assembly_name> as shown in the example.

Define an additional key=value pair in the format reference=hg19 or reference=GRCh38.p2 and include it on the track line.

For hg19, including the key=value pair reference=hg19 is optional.

If a URI is desired, define an additional key=value pair in the format referenceURI=http://www.thermofisher.com/GRCh38.p2.fasta .

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.

2

Get started with Ion Reporter™ Software

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 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 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 24.

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, from the Ion Reporter™ Software dashboard, under **All 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. Above the list of users, 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 24.

Sign in to Ion Reporter™ Software on Connect

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

You must 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 22 or "Join an existing Ion Reporter™ Software organization on Connect" on page 24.

1. Go to **https://ionreporter.thermofisher.com**, then click **Sign In**.

2. Enter your user name and password, then click **Sign In**.

3. If the **Connect Terms of Use** opens, review the terms, then click **Accept** to agree to 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 27.

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 23.

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 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 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 invitation is sent to your email address. Click the link in the email to accept.
3. Your browser opens to a screen that prompts you to accept the invitation for the organization. Select the organization name to accept.
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. 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

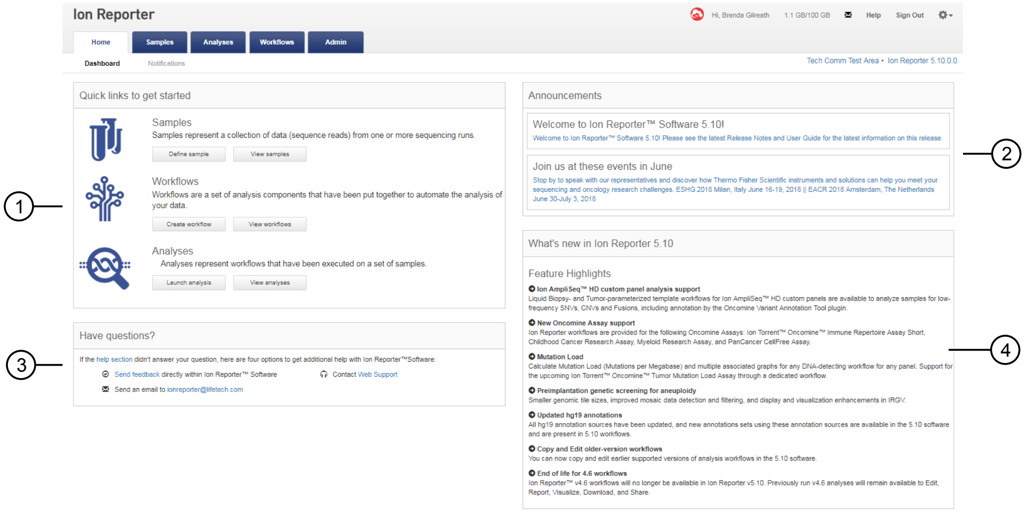
In Ion Reporter™ Software, access is granted to specific functions based on roles that are assigned to users. Typically, an Ion Reporter™ Software organization has different team members performing different functions. Therefore, each user role within Ion Reporter™ Software has access to different areas 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: • Upload data. • View samples. • Define samples. • View the analyses list (but not review results). • Launch analyses. • Access the **Samples** and **Analyses** tabs.

User role	Access
Analyze	The Analyze User is the person in the Ion Reporter™ Software organization who can create analysis workflows and launch analyses. Specific access includes: • 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: • 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.
Admin	The Admin is the person in the Ion Reporter™ Software organization who can manage user access. The Admin user can: • 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, see *Ion Reporter™ Server Upgrade Instructions User Bulletin* (Pub. No. MAN0017841).

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**.



Samples
Samples represent a collection of data (sequence reads) from one or more sequencing runs.

Workflows
Workflows are a set of analysis components that have been put together to automate the analysis of your data.

Analyses
Analyses represent workflows that have been executed on a set of samples.

If the help section didn't answer your question, here are four options to get additional help with Ion Reporter™Software:
Send feedback directly within Ion Reporter™ Software
Contact Web Support
Send an email to ionreporter@lifetech.com

Welcome to Ion Reporter™ Software 5.10!
Welcome to Ion Reporter™ Software 5.10! Please see the latest Release Notes and User Guide for the latest information on this release.

Join us at these events in June
Stop by to speak with our representatives and discover how Thermo Fisher Scientific instruments and solutions can help you meet your sequencing and oncology research challenges. ESHG 2018 Milan, Italy June 16-19, 2018 || EACR 2018 Amsterdam, The Netherlands June 30-July 3, 2018

Ion AmpliSeq™ HD custom panel analysis support
Liquid Biopsy- and Tumor-parameterized template workflows for Ion AmpliSeq™ HD custom panels are available to analyze samples for low-frequency SNVs, CNVs and Fusions, including annotation by the Oncomine Variant Annotation Tool plugin.

New Oncomine Assay support
Ion Reporter workflows are provided for the following Oncomine Assays: Ion Torrent™ Oncomine™ Immune Repertoire Assay Short, Childhood Cancer Research Assay, Myeloid Research Assay, and PanCancer CellFree Assay.

Mutation Load
Calculate Mutation Load (Mutations per Megabase) and multiple associated graphs for any DNA-detecting workflow for any panel. Support for the upcoming Ion Torrent™ Oncomine™ Tumor Mutation Load Assay through a dedicated workflow.

Preimplantation genetic screening for aneuploidy
Smaller genomic tile sizes, improved mosaic data detection and filtering, and display and visualization enhancements in IRGV.

Updated hg19 annotations
All hg19 annotation sources have been updated, and new annotations sets using these annotation sources are available in the 5.10 software and are present in 5.10 workflows.

Copy and Edit older-version workflows
You can now copy and edit earlier supported versions of analysis workflows in the 5.10 software.

End of life for 4.6 workflows
Ion Reporter™ v4.6 workflows will no longer be available in Ion Reporter v5.10. Previously run v4.6 analyses will remain available to Edit, Report, Visualize, Download, and Share.

① Quick links pane
② Announcements pane
③ Support pane
④ Information 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.

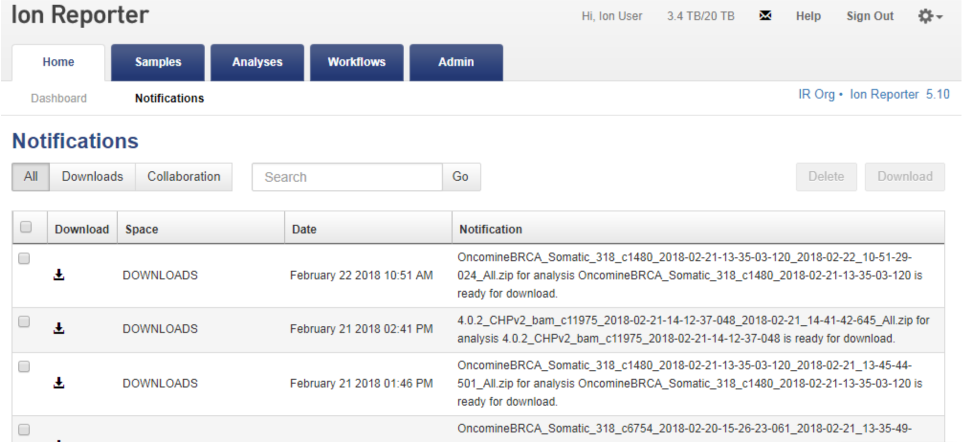
View notifications

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 ✉ (Email) in the menu bar.

   The **Notifications** screen appears.

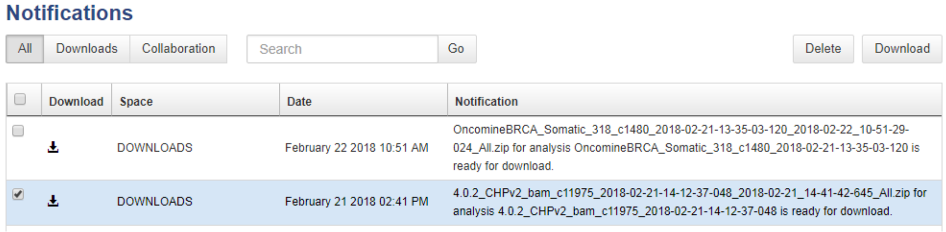


☐	Download	Space	Date	Notification
☐	⤓	DOWNLOADS	February 22 2018 10:51 AM	OncomineBRCA_Somatic_318_c1480_2018-02-21-13-35-03-120_2018-02-22_10-51-29-024_All.zip for analysis OncomineBRCA_Somatic_318_c1480_2018-02-21-13-35-03-120 is ready for download.
☐	⤓	DOWNLOADS	February 21 2018 02:41 PM	4.0.2_CHPv2_bam_c11975_2018-02-21-14-12-37-048_2018-02-21_14-41-42-645_All.zip for analysis 4.0.2_CHPv2_bam_c11975_2018-02-21-14-12-37-048 is ready for download.
☐	⤓	DOWNLOADS	February 21 2018 01:46 PM	OncomineBRCA_Somatic_318_c1480_2018-02-21-13-35-03-120_2018-02-21_13-45-44-501_All.zip for analysis OncomineBRCA_Somatic_318_c1480_2018-02-21-13-35-03-120 is ready for download.
☐				OncomineBRCA_Somatic_318_c6754_2018-02-20-15-26-23-061_2018-02-21_13-35-49-

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.



☐	Download	Space	Date	Notification
☐	⤓	DOWNLOADS	February 22 2018 10:51 AM	OncomineBRCA_Somatic_318_c1480_2018-02-21-13-35-03-120_2018-02-22_10-51-29-024_All.zip for analysis OncomineBRCA_Somatic_318_c1480_2018-02-21-13-35-03-120 is ready for download.
☑	⤓	DOWNLOADS	February 21 2018 02:41 PM	4.0.2_CHPv2_bam_c11975_2018-02-21-14-12-37-048_2018-02-21_14-41-42-645_All.zip for analysis 4.0.2_CHPv2_bam_c11975_2018-02-21-14-12-37-048 is ready for download.

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.

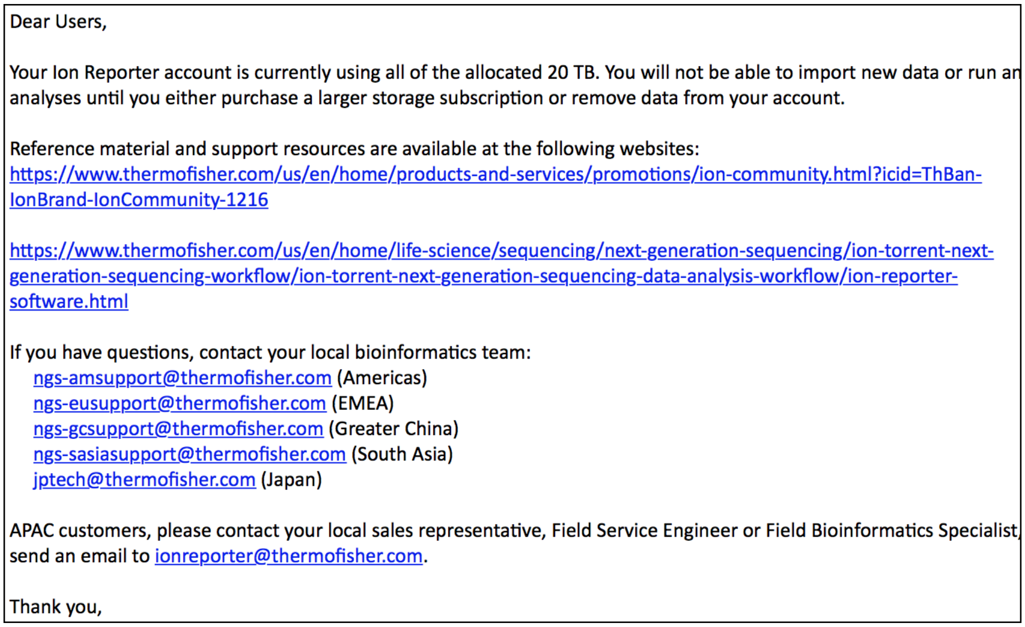
Status alert emails

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: For Ion Reporter™ Software 5.10 or later, a single email will be sent if multiple samples or barcodes are transferred from a single Torrent Suite™ Software run report. Additional emails will be 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

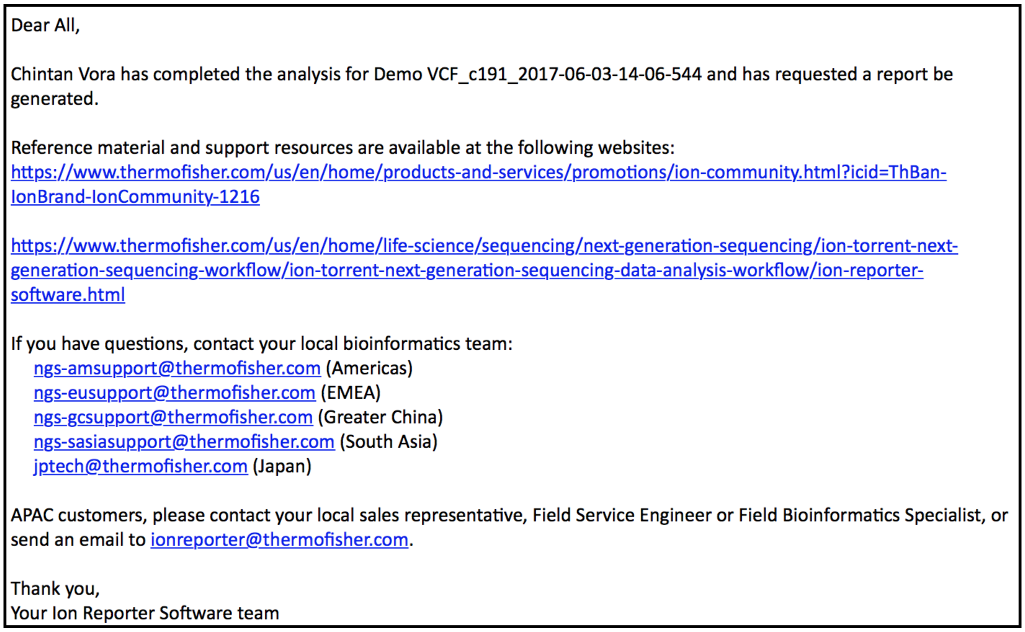
https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html

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

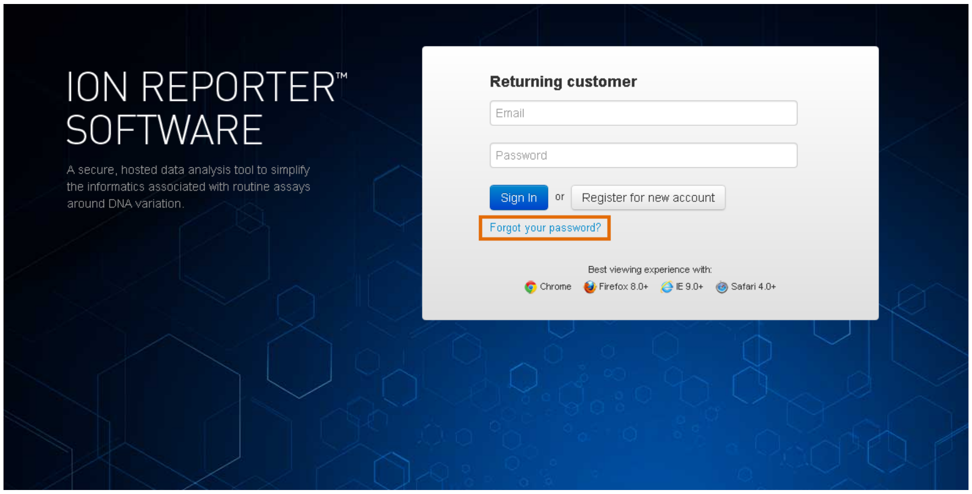
Example of a message that is generated when an analysis is complete and a request is sent to the person in the report role to generate a report.

Troubleshoot sign ins and provide feedback for Ion Reporter™ Software

This section addresses questions that are related to how to troubleshoot sign-ins, provide feedback 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 will need to 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 415.
- If you are a cloud user, click the **Forgot your password?** link on the sign in form.

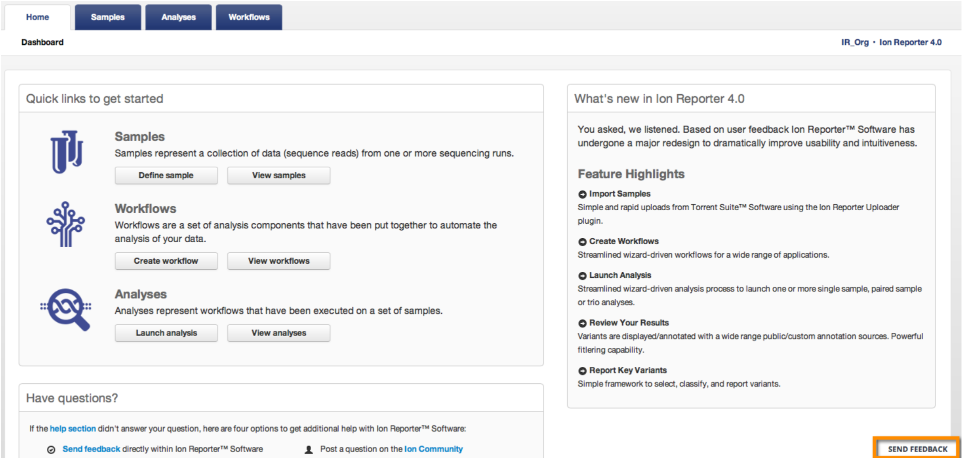


A secure, hosted data analysis tool to simplify the informatics associated with routine assays around DNA variation.

How can I provide feedback?

Feedback on your experience or any recommendations is always welcomed. Providing feedback in Ion Reporter™ Software is very easy:

1. Navigate to the page you would like to provide feedback on. In the bottom-right corner of the page you see a **Send Feedback** button.



Samples
Samples represent a collection of data (sequence reads) from one or more sequencing runs.

Workflows
Workflows are a set of analysis components that have been put together to automate the analysis of your data.

Analyses
Analyses represent workflows that have been executed on a set of samples.

If the help section didn't answer your question, here are four options to get additional help with Ion Reporter™ Software:

Send feedback directly within Ion Reporter™ Software

Post a question on the Ion Community

You asked, we listened. Based on user feedback Ion Reporter™ Software has undergone a major redesign to dramatically improve usability and intuitiveness.

Import Samples
Simple and rapid uploads from Torrent Suite™ Software using the Ion Reporter Uploader plugin.

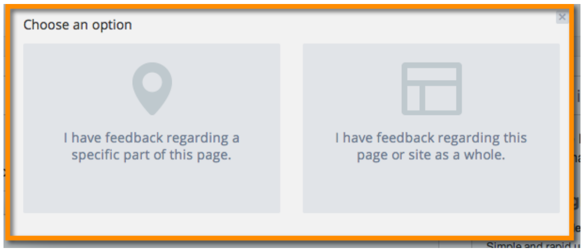
Create Workflows
Streamlined wizard-driven workflows for a wide range of applications.

Launch Analysis
Streamlined wizard-driven analysis process to launch one or more single sample, paired sample or trio analyses.

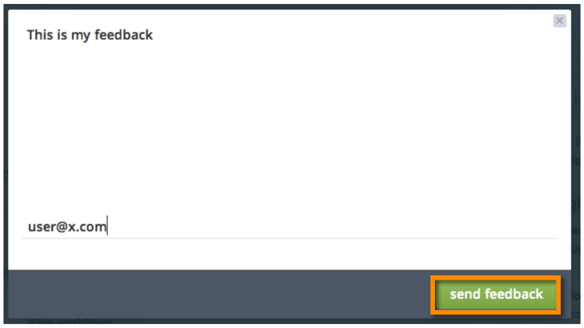
Review Your Results
Variants are displayed/annotated with a wide range public/custom annotation sources. Powerful fitlering capability.

Report Key Variants
Simple framework to select, classify, and report variants.

2. Click on the **Send Feedback** button and then select whether you have general feedback on the page or if your feedback is specific to a certain component.



3. If you select to provide feedback on a specific part of the page, you then are prompted to select that part.
4. Then just enter your feedback and your email address.
5. After you enter your feedback and email, simply click the **Send Feedback** button.


This is my feedback

user@x.com

How do I contact support if I have additional questions?

There are three ways that you can get help with Ion Reporter™ Software:

- Send feedback by following the steps outlined in "How can I provide feedback? " on page 32.
- 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**.

Are there any naming restrictions?

- Most names in Ion Reporter™ Software, including those used to name analyses, samples, custom analysis workflows, and users, require at least three characters. The name field appears in red with a bold outline if fewer characters are entered.
- Ion Reporter™ Software does not support either a user first name or user last name of only two characters. If a user name has less than 3 characters, you can append the name with an underscore (_) or number.

What special characters are allowed in names?

- Most names in Ion Reporter™ Software, including names used for analyses, samples, custom analysis workflows, users, and other text fields, allow these characters:
-
  - Alphanumeric characters: A-Z a-z 0-9
  - Underscore: **_**
  - Space: **' '**
  - Dash: **-**
  - Period: **.**

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 define samples, based on the transferred files. The IonReporterUploader command-line utility uses your login ID to transfer run data from Torrent Suite™ Software to Ion Reporter™ Software. The utility supports these transfer scenarios:

- Transfer a single BAM or VCF file.
  The name of a BAM file that is uploaded to Ion Reporter™ Software cannot exceed 200 characters.
- Transfer all results files for a Torrent Suite™ Software analysis.
- Transfer results files that are in a single flat folder.
- Transfer 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 your Torrent Server, or can be run on any standard (and current) computer that runs Linux™, Mac OS™, 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.

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

Download IonReporterUploader command-line utility

The IonReporterUploader command-line utility is an 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/**. For information on IonReporterUploader plugin, see the Torrent Suite™ Software Help, or the *Torrent Suite™ Software User Guide*.

This procedure explains how to download and install the IonReporterUploader command-line utility from Ion Reporter™ Software. The procedure can vary, based on the operating system of the target computer. In general, decompress the downloaded directory on your target machine, then copy the directory `IonReporterUploader-cli` to a convenient location.

Ideally, download the IonReporterUploader command-line utility onto the computer where it is to be run. At a minimum, you must use a computer with the same operating system.

IMPORTANT! Use only the decompression utility available on your local computer. Do not decompress files on a different operating system and copy those files to a computer that uses a different operating system.

---

1. Click **Settings (⚙)** ▸ **Download Ion Reporter Uploader**.
2. Click the filename `IonReporterUploader-cli.zip`, then download the file to the target computer.
3. Extract the downloaded `IonReporterUploader-cli.zip` file, then copy the `IonReporterUploader-cli` directory 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 are downloaded with the utility. For more information, see the *Torrent Suite™ Software User Guide*.

3 Import and manage samples

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.

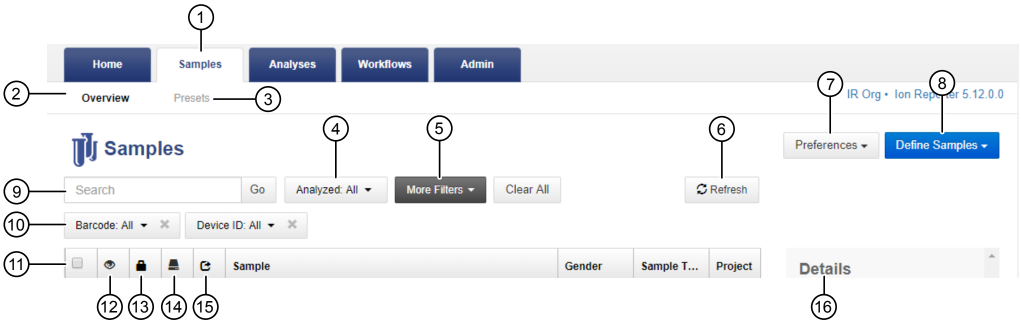
Samples can be automatically transferred from the sequencer through Torrent Suite™ Software to Ion Reporter™ Software using the IonReporterUploader plugin, or imported manually as individual BAM or VCF files. For more information see, “Sample import options“ on page 45.

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 179 and Chapter 7, “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 361.

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 43.

④ The **Analyzed: All** filter is the default filter that is set on the **Samples** tab. For more information, see "Filter samples" on page 58.

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 59.

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 50.

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 57.

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

⑪ Table columns with information about the sample. For more information, see "Sample attributes" on page 38.

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

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

⑭ ⏏ **(Archived Ion Reporter™ Server only)** Indicates that the sample is archived.
📥 **(Archive in process Ion Reporter™ Server only)** Indicates that archive of the sample is in progress.

⑮ ⇗ **(Transferred sample)** Indicates that the sample was transferred to a user in another organization

⑯ **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 54, “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 58 and “Launch an analysis“ on page 157.

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

Custom 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 additional 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 59. 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 57.

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 54.

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. Neither the sample attributes nor the files that make up the sample can be edited after a sample is locked.
🖴	Archived (Ion Reporter™ Server only)—Indicates that the sample was transferred to the archive server.
📥	Archive in process (Ion Reporter™ Server only)—Indicates that the sample is in the process of being transferred to the archive server.

Attribute	Description
↗	Transferred sample—Indicates that the sample was transferred to a user in another organization.
Sample	The name of the sample.
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**
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.	
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 biopsy was taken from an embryo.
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.
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.

Attribute	Description
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 custom analysis workflow. Available options are based on the most common mouse strains in the IRGT database.
Percentage Cellularity	The percentage 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.
Plan Name	The name of the Planned Run used in Torrent Suite™ Software. This attribute cannot be changed.

Attribute	Description
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—TCRß 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 217 **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 get a haplotype assignment. 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**.

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. The software displays "FRAGMENT" for Single End and "PE" 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.
User defined—Custom attributes defined by the user.	
*****	Custom attributes are denoted with an asterisk (*****) in the available columns list. Each custom 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 51 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 custom sample attributes, which are known as sample presets, to describe samples that are used in your 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 custom sample attribute

Custom 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 custom 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	1. Enter a **Name** and **Description** for the sample preset. 2. *(Optional)* If you want the internal storage for the attribute to be encrypted, select **Yes** in the **Encrypted** list.
Controlled Vocabulary	1. Enter a **Name** and **Description** for the sample preset. 2. *(Optional)* If you want the internal storage for the attribute to be encrypted, select **Yes** in the **Encrypted** list. 3. 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 custom 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 51.

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

Sample data are imported into Ion Reporter™ Software in four ways.

- 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 46.
- 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 must 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 46 and "Upload a VCF file to create a sample" on page 48.
- You can also import sample data directly into Ion Reporter™ Software if you use one of the following:
  - A CSV file. For more information, see "Define samples with a CSV file" on page 54.
  - An Ion PGM™ Dx System. For more information, see "Import samples from Torrent Suite™ Dx Software" on page 50.
  - The IonReporterUploader command-line utility. For more information, see "IonReporterUploader command-line utility" on page 49.
  - An external data file. For more information or assistance 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 50 and "Manually launch an analysis" on page 160.
- 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 50 "Launch an analysis" on page 157.

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: You can set the **Tmap Mapped Files Enable Re-map** parameter to ***True*** if you want mapped BAM files that you upload to be remapped. For more information, see "Customize tuning parameters" on page 99.

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 (.), hyphens (-), 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.	1. Click the field in the column of the attribute heading. 2. Enter a value or select a value from a list.
Add an attribute and set its value.	1. Click **Add Attribute**, then select an attribute from the list. 2. Click the field in the column of the attribute heading. 3. Enter a value or select a value from a list.
Add a new attribute.	For more information, see "Create a custom sample attribute" on page 44.

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 157.

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 contain 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 VCF files that conform to the 4.1 VCF standard only.
- 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 resulting 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***.

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 a existing attribute.	1. Click the field in the column of the attribute heading. 2. Enter a value or select a value from a list.
Add an attribute and set its value.	1. Click **Add Attribute** and select an attribute from the list. 2. Click the column of the attribute heading. 3. Enter a value or select a value from a list.
Add a new attribute.	Use this option to add a new custom sample attribute. For more information, see "Create a custom sample attribute" on page 44.

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 157.

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™, Mac OS™, 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 UtilityUser Guide (Pub. No. MAN0017648).

Import samples from Torrent Suite™ Dx Software

You can import samples into Ion Reporter™ Software that originate in the Ion PGM™ Dx System and are processed by Torrent Suite™ Dx Software. Only samples that are signed off in Torrent Suite™ Dx Software can be transferred to Ion Reporter™ Software. 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 157.

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 45.

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 46, "Define samples with a CSV file" on page 54, and "Demonstration samples" on page 159.

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.

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 additional 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.	1. Click the field in the column of the attribute heading. 2. Enter a value or select a value from a list.
Add an attribute and set its value.	1. Click **Add Attribute** and select an attribute from the list. 2. Click the field in the column of the attribute heading. 3. Enter a value or select a value from a list.

   You can create custom sample attributes that can be used for future analyses. For more information, see "Create a custom sample attribute" on page 44.

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 157.

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 51.

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.	1. Click the field in the column of the attribute heading. 2. Enter a value or select a value from a list.
Add an attribute and set its value.	1. Click **Add Attribute** and select an attribute from the list. 2. Click the field in the column of the attribute heading. 3. Enter a value or select a value from a list.

   You can create custom sample attributes that can be used for future analyses. For more information, see “Create a custom sample attribute“ on page 44.

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 157.

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:
   - The path to the sample BAM file.
   - 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 54.

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 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 on which sample attributes can be edited, see "Sample attributes" on page 38.

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 53.

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 filename 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.	1. Click the field in the column of the attribute heading. 2. Enter a value or select a value from a list.
Add an attribute and set its value.	1. Click **Add Attribute** and select an attribute from the list. 2. Click the field in the column of the attribute heading. 3. Enter a value or select a value from a list.
Add a new attribute.	For more information, see "Create a custom sample attribute" on page 44.

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 157.

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. In the **Define Samples** screen, click **Attributes** in the workflow bar. If the **Sample Type** attribute is not displayed, click **Add Attribute**.

3. In the **Sample Type** column, select **DNA NTC** or **RNA NTC**.

4. If you define the sample as **DNA NTC**, you must also add the **Percentage Cellularity** attribute, then set the percentage cellularity to 100%.

   The **Percentage 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.

5. When the sample information is complete, click **Next**.

6. In the **Samples ready to import!** screen, check 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 157.

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 presintalled demonstration samples that are provided with Ion Reporter™ Software. For more information, see "Demonstration samples" on page 159.

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.	1. Click the field in the column of the attribute heading. 2. Enter a value or select a value from a list.
Add an attribute and set its value.	1. Click **Add Attribute** and select an attribute from the list. 2. Click the field in the column of the attribute heading. 3. 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 custom sample attribute" on page 44.

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 157.

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 51.
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 157.

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.

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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 59.

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 custom 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 sample names returns a list of all samples with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and are therefore not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

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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 59.

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 custom 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 530 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**.
3. In the **Select Columns** dialog box, select the sample attributes that you want to add to the **Samples** table, then click **Apply**.
   Custom 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 custom 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, select the columns that you want to add to the **Samples** table, then click **Apply**.
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 60.

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 60.

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 38.

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 custom 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 custom 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** page.

To free up storage space, you can delete the sample after the transfer. For more information, see "Delete a sample" on page 65.

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 65.

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. Archived samples can be restored only to the Ion Reporter™ Server from which they were originally archived. This procedure cannot be used to transfer samples between multiple servers.

Available options include:

- 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**

- You must 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 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 `/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.

1. In the **Samples** tab, in the **Overview** screen, select the sample or samples to be archived.
2. In the **Details** pane, click **Actions ▸ Archive**.

   While the sample is being archived, 📥 **(Archive in progress)** appears in the analysis row.

   When the archive process is complete, 🖴 **(Archived)** appears in the analysis row.
3. Repeat for any other samples to archive.
4. Click **Yes** to confirm the archive.

The sample or samples are archived.

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

This information applies only to Ion Reporter™ Server.

If needed, you can restore the analyses that you have archived in Ion Reporter™ Software.

IMPORTANT! Ensure that you restore archived samples only on the Ion Reporter™ Server on which the samples were originally archived.

- The external storage device that contains archived samples must be mounted to your Ion Reporter™ Server.
- 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.

1. In the **Samples** tab, find the archived sample.
   An archived sample shows 🖴 **(Archived)** in the row of an analysis.
2. To restore the archived sample back onto the Ion Reporter™ Server, do one of the following:
   - Select the archived sample or samples, then click **Actions** ▸ **Restore**.
   - Click ↻ **(Restore)** to return the sample back to the Ion Reporter™ Server.

The sample or samples are restored.

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.

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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

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 *custom workflow*. You can also copy and edit, or edit a custom workflow.

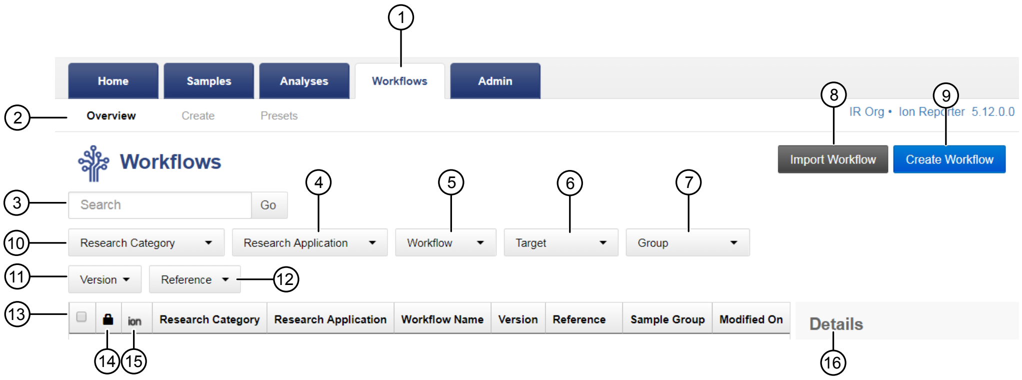
When an analysis workflow that contains samples is launched, Ion Reporter™ Software generates an analysis. Each analysis contains analysis results in various formats, including tables and visualizations in the software, and data files that can be downloaded.

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 one of the available workflows to create an analysis.

All analysis workflows that are available to your 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 "Create a custom analysis workflow from an existing analysis workflow" on page 75.

The **Presets** screen. For more information, see "Workflow presets" on page 129.

③ The **Search** box allows you to enter keywords or text strings to narrow the list of analysis workflows. For more information, see "Search for analysis workflows" on page 68.

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

⑤ The **Workflow** list allows you to filter by analysis workflows that are predefined, custom, or tagged for IonReporterUploader plugin.

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

⑦ The **Group** list allows you to filter by sample grouping type.

⑧ **Import Workflow** allows you to upload a file to create an analysis workflow.

⑨ **Create Workflow** allows you to create a new analysis workflow.

⑩ The **Research Category** filter allows you search by application category.

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

⑫ The **Reference** filter allows 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 additional 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 sample names returns a list of all samples with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and are therefore not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

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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 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 70.

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

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 custom analysis workflow" on page 90.

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 filename 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 filename of the hotspots file used in the analysis workflow.
Fusion Panel	The name and version of the fusion panel.
Annotations	The annotations that are used in the analysis workflow.
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.

Analysis workflow detail	Description
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 all of 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 your 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.
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™ 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™ CarrierSeq™ ECS Panel.

Research category	Description
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 and 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 less than 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 is 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 custom analysis workflow for an Ion AmpliSeq™ Exome tumor-normal pair analysis, we recommend that you do one of the following to ensure that the correct parameters that are applied:

1. Make a copy of the predefined analysis workflow or a custom analysis workflow for use with Ion AmpliSeq™ Exome tumor-normal pairs and edit any desired parameters. For more information, see "Create a custom analysis workflow from an existing analysis workflow" on page 75.
2. Use the predefined BED file to create a new custom analysis workflow. For more information, see "Create a custom analysis workflow without predefined settings" on page 76.

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 and aneuploidy detection

The CNV detection that is used in all predefined low-pass whole genome detection analysis workflows, including Ion ReproSeq™ analysis workflows for single-sample and two-sample Ion AmpliSeq™ panels, call copy number results down to the gene and subgene-level ploidy variants.

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

These low-pass whole genome detection anueploidy detection analysis workflows that are intended for Pre-Implantation Genetic Testing (PGT) based on low-pass whole genome preparation contain 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 custom 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.

Custom analysis workflows

There are three ways to create custom analysis workflows in Ion Reporter™ Software.

- Copy an existing analysis workflow, then edit its settings to create a custom analysis workflow. For more information, see "Create a custom analysis workflow from an existing analysis workflow" on page 75.
- Using the workflow bar that walks you through the process, create a unique custom analysis workflow that contains unique settings. For more information, see "Create a custom analysis workflow without predefined settings" on page 76.
- 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 custom analysis workflow, then save the analysis workflow before the custom workflow is used for analysis. For more information, see "Create a custom analysis workflow for use with Ion AmpliSeq™ HD panels" on page 77.

Create a custom analysis workflow from 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 custom analysis workflow.

We recommend that you start with a predefined analysis workflow or a custom analysis workflow to begin with an optimized set of parameters.

When you create a custom 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 custom 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 69.

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, confirm the research application and sample group, then click **Next**.
4. In the **Reference** step, confirm that the required files are selected, then click **Next**.
5. In the **Annotation** step, confirm that one annotation set is selected, then confirm or select a MyVariants database associated with the analysis workflow, then click **Next**.
6. In the **Filters** step, confirm or select a filter chain, then click **Next**.
7. In the **Copy Number** step, confirm or select a copy number baseline, then click **Next**.
8. In the **Fusion** step, for analysis workflows that apply to assays that include RNA Exon Tiling, confirm that the appropriate fusions baseline for the analysis workflow is selected, then click **Next**.
9. In the **Plugins** step, confirm or select plugins, then click **Next**.
10. In the **Final Report** step, confirm or select 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 analysis workflow was copied, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name to confirm that the custom analysis workflow is listed in the **Workflows** table.

Create a custom analysis workflow without predefined settings

You can create a custom analysis workflow in Ion Reporter™ Software that is not based on an existing analysis workflow that uses predefined settings. The settings that you start with are blank, and do not have a default selection.

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**.

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. For analysis workflows that apply to assays that include RNA Exon Tiling, confirm that the appropriate fusions baseline for the analysis workflow is selected in the **Fusion** step, then click **Next**.

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.

Create a custom analysis workflow for use with Ion AmpliSeq™ HD panels

To analyze Ion AmpliSeq™ HD sequencing data in Ion Reporter™ Software, you must create a custom analysis workflow for use with your Ion AmpliSeq™ HD panels. To create a custom analysis workflow, you must first copy one of the preinstalled Ion AmpliSeq™ HD analysis workflow templates, then add target regions files, and any available hotspots files or CNV baseline. For RNA samples, you must also add fusion panel files to your custom analysis workflow.

After you create the custom 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 on available Ion AmpliSeq™ HD analysis workflow templates, see "Analysis workflow templates for Ion AmpliSeq™ HD" on page 80.

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 with the **Research Application** and **Sample Group** preselected.

   Note: When you copy analysis workflow templates, you cannot change these settings.

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

Option	Description
Select a file that has been previously uploaded to Ion Reporter™ Software.	Select the **Target Regions** and, optionally, **Hotspot Regions** files from the dropdown lists.
Upload the entire package of the Ion AmpliSeq™ HD panel files directly from **AmpliSeq.com**. **Note:** When you use this option, a target regions file and any available hotspot regions or fusion files specific for your panel are uploaded.	Click ⤓ **AmpliSeq Import**.
Import a target regions file that you previously downloaded from **AmpliSeq.com** and saved to your local storage.	1. Under the **Target Regions** list, click **Upload**. 2. Click **Select file**, browse to, then select the target regions BED file, then click **Open**. 3. Select **Ion AmpliSeq™ HD**, then click **Upload**.
(Optional) Upload a hotspot regions file that was previously uploaded from **AmpliSeq.com** and saved to your local storage.	1. Click **Upload** under the **Hotspots Regions** list. 2. Click **Select file**, browse to, then select the hotspot regions BED file, then click **Open**. 3. Select **Ion AmpliSeq™ HD**, then click **Upload**.
If you use a panel that detects fusions, upload a FASTA file that was previously uploaded from **AmpliSeq.com** and saved to your local storage.	1. Click **Upload** under the **Fusion Panel** list. 2. Click **Select file**, browse to, then select the fusions ZIP file, then click **Open**. 3. Select **Ion AmpliSeq™ HD**, then click **Upload**.

4. Click **Next**.

5. In the **Annotation** step, confirm or select an annotation set, confirm or 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 is the default filter chain for Ion AmpliSeq™ HD analysis workflows. Use this filter chain for analysis results that report 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 FLT3ITD.
Called Hotspot Variants and Controls	Select this filter chain for analysis results that report all hotspot variants that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNP and INDEL.
Variant Matrix Summary	Select this filter chain for analysis results that replicate data that is shown for Ion AmpliSeq™ HD analysis results in the Variant Matrix Summary. Variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants.
Oncomine™ Variants (5.10 or later)	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. **IMPORTANT!** To add a copy number baseline, contact your Field Bioinformatics Specialist (FBS).

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

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

   In the **Plugins** step, the Oncomine™ Variant Annotator plugin is automatically selected. 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.

Analysis workflow templates for Ion AmpliSeq™ HD

Ion Reporter™ Software 5.10 or later contains predefined analysis workflow templates for use with Ion AmpliSeq™ HD panels. Identify the template that best matches your application, then use it to create a custom analysis workflow. For more information, see "Create a custom analysis workflow for use with Ion AmpliSeq™ HD panels" on page 77.

IMPORTANT! Ensure that the analysis workflow template name that you use matches the type of Ion AmpliSeq™ HD panel files that you include in your analysis. For example, if you use panel files for a liquid biopsy fusions single sample, copy the Ion AmpliSeq™ HD for Liquid Biopsy - w2.2 - Fusions - Single Sample analysis workflow template to create the custom analysis workflow.

Use the following guidelines to find Ion AmpliSeq™ HD analysis 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**.

Analysis workflow template	Description
Ion AmpliSeq™ HD for Tumor - w2.2 - 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.
Ion AmpliSeq™ HD for Tumor - w2.2 - 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.

Analysis workflow template	Description
Ion AmpliSeq™ HD for Tumor - w2.2 - 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.
Ion AmpliSeq™ HD for Tumor - w2.2 - 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, as well as gene fusions from matching targeted RNA libraries using Ion AmpliSeq™ HD technology. Compatible with DNA and RNA that is purified separately from tumor samples. Workflow 2.2 is released with Ion Reporter™ Software 5.12.
Ion AmpliSeq™ HD for Liquid Biopsy - w2.2 - DNA - Single Sample	Detects and annotates low frequency (to 0.1% 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.
Ion AmpliSeq™ HD for Liquid Biopsy - w2.2 - 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.
Ion AmpliSeq™ HD for Liquid Biopsy - w2.2 - DNA and Fusions (Single Library) - Single Sample	Detects and annotates low frequency (to 0.1% 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.
Ion AmpliSeq™ HD for Liquid Biopsy - w2.2 - DNA and Fusions (Separate Libraries) - Single Sample	Detects and annotates low frequency (to 0.1% 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. Workflow 2.2 is released with Ion Reporter™ Software 5.12.

Create a custom analysis workflow for use with Metagenomics research application

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

- Upload custom reference files, then map samples to any combination of custom 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 your samples for metagenomics analysis workflows.

Note: You can create an analysis workflow that does not contain any primer information, however, we recommend that you always add primer information to your metagenomics analysis workflow. When the primer information is missing, 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)* If you want to upload a custom reference database in FASTA format, in the **Reference** step, click **Upload**, then browse to, select, then upload the custom 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 of the 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 on the file format of the primers, see "Custom primer sequences for Metagenomics analysis workflows" on page 83.
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 on the file format of the primers, see "Custom primer sequences for Metagenomics analysis workflows" on page 83.

   a. Click **Select File**.

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

   c. Ensure that the correct filename 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 custom 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. Analysis workflows for use with the Oncomine™ Tumor Mutation Load Assay, such as

Oncomine™ Tumor Mutation Load - w3.0 - DNA - Single Sample analysis workflow, are enabled for tumor mutational burden by default.

Note: Tumor mutational burden calculation is available in Ion Reporter™ Software 5.10 or later.

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, 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 an analysis workflow template, you cannot change these settings.
3. Click **Next** multiple times to proceed to the **Parameters** step. Alternatively, click each step in the workflow bar to go to the parameter step.
4. 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)**.

      For parameter descriptions see “Annotation parameters“ on page 99.
5. Change other tumor mutational burden parameters, as needed.

   For more information, see “Annotation parameters“ on page 99.

   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 as desired. Contact your Field Bioinformatics Specialist (FSB) for assistance.
6. Click **Next**, then enter a **Workflow Name** and an optional **Description** for the analysis workflow.
7. Click **Save Workflow**.

   The custom analysis workflow is saved and is added to the **Workflows** table.

To ensure that the analysis workflow was saved, click the **Workflows** tab, then click **Overview**, and search for the analysis workflow name. To analyze samples with your new tumor mutational burden calculation enabled analysis workflow, see “Manually launch an analysis“ on page 160.

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 Oncomine™ Tumor Mutation Load - w3.0 - DNA - Single Sample analysis workflow

The Oncomine™ Tumor Mutation Load - w3.0 - 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. Such counts can affect whether the estimated SNP proportion is consistent with the deamination (mainly FFPE) number.

You can modify the Oncomine™ Tumor Mutation Load - w3.0 - 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.

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1. In the **Workflows** tab, click **Overview**.
2. In the **Overview** screen, you can use the **Oncology-ImmunOncology** 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.

Custom 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 339 and "Create an Expected Normal Ploidy Buffer filter chain" on page 320.

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 88.

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

Create a custom 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 adjustment 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 custom 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 88. 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 one of the following:
   - **Ion ReproSeq Low-Coverage Whole-Genome Baseline 1 Mbp**,
   - **Ion ReproSeq Low-Coverage Whole-Genome Baseline .5 Mbp**,
   - or a custom 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 custom 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 259.

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 anueploidy 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 anueploidy calls of ~20 Mbp. **Custom CNV Sensitivity** allows 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 **Anueploidy** 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**.

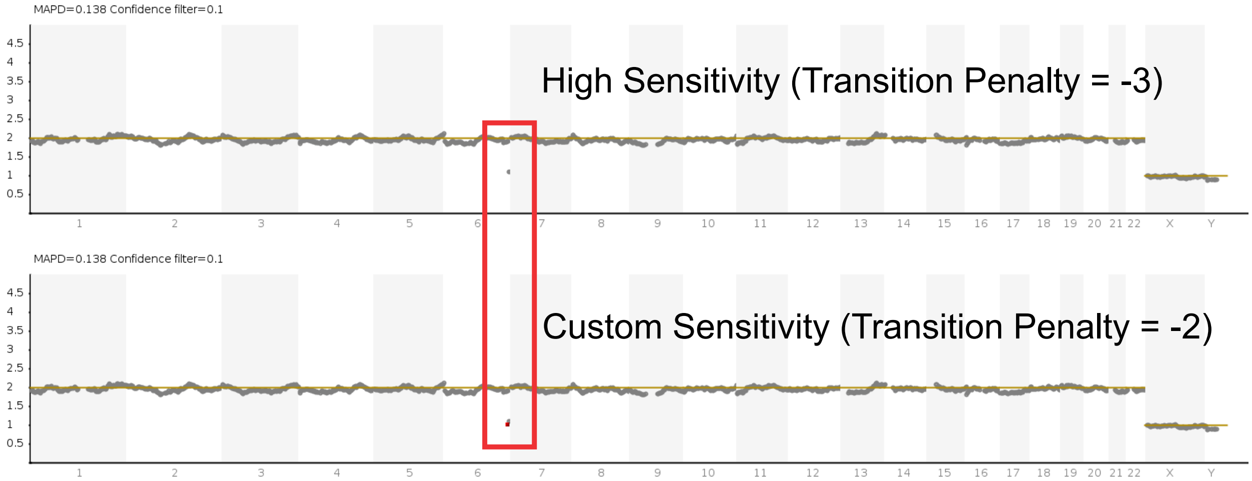
4. In the **Create Workflow** screen, advance to the **Parameters** step, then click the**Cnv Finding** link. In the **Analysis** section **Main** tab, change the **CNV Sensitivity** setting to **Custom**, then enter new values for the parameter.

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
   - -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 two customized workflows—one with the CNV Transition Penalty parameter set to -3, and a second with the parameter set to -2.



① In this visualization, a 7.9-Mb deletion that is called on chromosome 6 is visible with the slightly higher transition penalty.

Transfer a custom analysis workflow

Ion Reporter™ Software allows you export a custom 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 resuse the custom analysis workflow, instead of creating a new analysis workflow for each server or account.

You must 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 contain only one analysis workflow.

The target regions, hotspot regions, and CNV baseline files, are not included in the compressed directory 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 export directory. 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 custom 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 compressed directory of the files used for the workflow, then click **Export**.

   A compressed directory (ZIP) is saved to your 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 compressed directory (ZIP) file. The filename of the workflow directory is `workflow name.zip`.

   The workflow directory contains two 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 custom analysis workflow

You can edit a custom 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.

To edit an analysis workflow that is created in a version earlier than Ion Reporter™ Software 5.2, you must select a MyVariants database in the **Annotation** step to proceed.

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 **Fusion** step, for analysis workflows that apply to assays that include RNA Exon Tiling, confirm that the appropriate fusions 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 custom analysis workflow is listed in the **Workflows** table.

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.

Analysis workflow step	Description
Reference	Use this analysis workflow step to select a reference genome type and to upload the appropriate reference files that the analysis will use to focus the analysis in the custom analysis workflow. The predefined analysis workflows that use a human reference are designed to work with the hg19 human reference genome. Custom Ion AmpliSeq™ DNA panels can be designed to work with the GRCh38 human reference genome. For more information about uploading panel files, see "Import panel files from AmpliSeq.com" on page 96
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 custom analysis workflow for use with Metagenomics research application" on page 81 and "Custom primer sequences for Metagenomics analysis workflows" on page 83.
Annotation	Use this analysis workflow step to select annotation sets that are specific for the custom analysis workflow. Annotation sets contain 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 for selection, you can create a new annotation set from the **Workflow Presets** screen. For more information, see "Create annotation set workflow presets" on page 131.
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 identified in your analysis. Filters allow you to remove or include variants in your analysis results. For more information, see Chapter 8, "Filters and filter chains".

Analysis workflow step	Description
Copy Number	Use this analysis workflow step to select a copy number baseline to apply to the custom analysis workflow. The copy number baseline identifies the copy number variants (CNVs) in samples. This analysis workflow step applies only to DNA, aneuploidy, and some Oncomine™ analysis workflows. Analysis workflows that use copy number baseline controls can provide better copy number detection than 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 baseline workflow preset to an analysis workflow" on page 98
Fusion	Use this analysis workflow step to 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 must select an RNA baseline that corresponds to the panel content.
Plugins	Use this analysis workflow step to apply available plugins to a custom analysis workflow. Plugins provide additional functionality and content to an analysis workflow. Applying a plugin 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 blank, 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.

Analysis workflow step	Description
Final Report	Use this analysis workflow step to specify the final report template that will report 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 for selection in this step. If the final report template that you need is not available for selection, 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 133.
Parameters	Use this step to review or modify runtime parameters to refine and optimize the custom analysis workflow. Many fixed and community panels imported from AmpliSeq.com include optimized variant calling parameters. 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 99
Confirm	This analysis workflow step is the final step that you must 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 subsequent workflow steps.

The default options are recommended as best practice, but advanced users can adjust the recommended settings, if necessary.

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) rearrangements for research use.

Research application	Description
Metagenomics	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 that was released with Ion Reporter™ Software 5.6.
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 as best practice, but advanced users can adjust the recommended settings, if required.

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 8, "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 custom filter chain" on page 315.
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 panel files for Ion AmpliSeq™ and Ion AmpliSeq™ HD Made-to-Order, On-Demand, or Ready-to-Use research panels from AmpliSeq.com into Ion Reporter™ Software during analysis workflow creation. For more information, see "Ion AmpliSeq panel types" on page 18.

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

1. In the **Research Application** step, select an appropriate research application and sample group for your panel, then click **Next**.
2. In the **Reference** step, import a target regions file:

Option	Description
Custom Panel	1. Under **Target Regions**, 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 panel files. 3. Enter your user name and password and then click **List My AmpliSeq Panels**. 4. Select the panel files that you want to import from the dropdown list, then click **Import**.
Fixed Panel	1. Under **Target Regions**, click ⤓ **AmpliSeq Import**. 2. In the **Import for AmpliSeq** dialog box, select the **Fixed Panel** tab for On-Demand or Ready-to-Use panel files. 3. From the list, select the panel file that you want to import. 4. Click **Import**.

3. If your analysis includes fusions, import your fusion panel files.

Option	Description
Custom Panel	1. Under **Fusion Panel**, 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 fusion panel files. 3. Enter your user name and password, then click **List My AmpliSeq Panels**. 4. From the list, select the fusion panel file that you want to import, then click **Import**.
Fixed Panel	1. Click ⤓ **AmpliSeq Import** under the Fusion Panel field. 2. In the**Import for AmpliSeq** dialog box, select the ***Fixed Panel*** tab for On-Demand or Ready-to-Use fusion (RNA) panel files. 3. From the list, select the fusion panel file that you want to import. 4. Click **Import**.

4. Complete the remaining steps for analysis workflow creation.

   For more information, see "Create a custom analysis workflow without predefined settings" on page 76.

Your target regions and fusion panel files are added to the list of files selectable in the **Reference** workflow step.

Ion AmpliSeq panel types

AmpliSeq.com offers four design and ordering options for Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels:

- On -Demand Ion AmpliSeq™ research panels of optimized amplicons for germline analysis. Configurable to a specific human disease area, and ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ research panels of DNA and RNA designs for germline or somatic analysis of any genome, ordered in large reaction packs. Made-to-Order panels were formerly known as Custom Panels.
- Ready-to-Use Ion AmpliSeq™ research panels are predesigned DNA and RNA panels for germline and somatic analysis, ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ HD research panels are ultra high-sensitivity DNA and RNA designs for germline or somatic analysis of the human genome, including dual barcoded amplicons, and bidirectional sequencing.

Apply a 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! If you import a custom copy number baseline, the target regions file that was used to create the CNV baseline must be available in the software. The imported baseline then appears in the list of available copy number baselines.

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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 **Copy Number** workflow bar step, select an option from the **Baseline** dropdown list.

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

   Note: 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. Only baselines that were created with the same target regions file that is selected in the **Reference** step of the workflow bar are available.

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.

   Alternatively, you can click the **Workflow Presets** link, then configure a new copy number baseline preset. For more information, see "Create a copy number baseline workflow preset" on page 136.

3. Click **Next**.

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

   For more information, see "Steps in analysis workflow creation" on page 91.

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 for a analysis workflow to ensure that the filter chain is applied when the analysis workflow is launched. For more information, see .

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.

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 custom 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 modify the settings if needed.

Annotation parameters

You can modify the following annotation parameters to optimize your 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** **Suggested trial value: False**
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** **Suggested trial value: False**

Parameter	Description
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 could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto** **Suggested trial value: overlap**
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 could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto** **Suggested trial value: allele**
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 could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto** **Suggested trial value: locus**

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 could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto** **Suggested trial value: locus**
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 could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** • **overlap** • **locus** • **allele** • **auto** **Suggested trial value: overlap**
Gene Extension Size	Gene extension is the number of bases upstream and downstream of a transcript's start and end positions that should include the regulatory and control regions. **Allowed values:** 0 to unlimited **Suggested trial value:** 1,000

Parameter	Description
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 **Note:** Prior to Ion Reporter™ Software 5.10, we defined splicesite_5 and splicesite_3 as exon-centric. This was contrary to the common convention of splice site nomenclature that was intron-centric and would exchange the splicesite_5 and splicesite_3 designations. In Ion Reporter™ Software 5.10, splicesite_5 and splicesite_3 refers to the intron locations. In earlier releases of the software, you might have seen a 3' splice site in your results; you will now see a 5' splice site and vice versa.
Tumor Mutational Burden tab	
Tumor Mutational Burden Filter Chain	Filters out the potential germline variants and retain the somatic variants for tumor mutational burden calculation after the variant calling and variant annotation is completed 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 83. To enable the tumor mutational burden calculation for any other analysis workflow, you must select one of the 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) • Additional custom filter chains (if created on the server) **Suggested trial value:** TMB (Non-germline Mutations)
Minimum Base Coverage	The minimum depth of base coverage required for a variant to be counted for TMB calculation. **Allowed values:** 0 to unlimited **Suggested trial value:** 60
TMB Variant Minimum Allele Frequency	The minimum alternate allele frequency for a variant to be included for TMB calculation. **Allowed values:** 0 to 1 **Suggested trial value:** 0.05 You can use this parameter to reduce the affect of deamination in low-quality FFPEs and achieve 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 85.

Parameter	Description
TMB Variant Region Type	The type of region to include for TMB calculation. **Allowed values:** • Exonic regions only • Total target regions (exonic + intronic regions) **Suggested trial value:** Exonic regions only
TMB Variant Type	The variant types to be included for TMB calculation. **Allowed values:** • SNV • INDEL • MNV **Suggested trial value:** SNV and INDEL
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 **Suggested trial value:** Start with the default selections.
Deamination QC Threshold	The deamination value above which a sample is deemed failed for TMB reporting. **Allowed values:** 0 to unlimited **Suggested trial value:** 100
Calibration Minimum Cutoff	The lower limit of the range at which the germline calibration is applied. **Allowed values:** 0 to unlimited **Suggested trial value:** 25
Calibration Maximum Cutoff	The upper limit of the range at which the germline calibration is applied. **Allowed values:** 0 to unlimited **Suggested trial value:** 100
TMB Germline-Filter Calibraition Factor: Slope	The user-supplied value for the slope of the linear curve to which the number of somatic variants will be calibrated. **Allowed values:** 0 to unlimited **Suggested trial value:** 1.4637

Parameter	Description
TMB Germline-Filter Calibraition Factor: Intercept	The user-supplied value for the intercept of the linear curve to which the number of somatic variants will be calibrated. **Allowed values:** –2,000 to 2,000 **Suggested trial value:** 0
TMB-Low Threshold	The TMB (mut/mb) threshold below which a sample is defined as TMB-Low. **Allowed values:** 0 to unlimited **Suggested trial value:** 10
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. **Allowed values:** 0 to unlimited **Suggested trial value:** 20
TMB Standardization	Apply the standardization of the observed TMB value to fit a linear curve. **Allowed values: On** or **Off** **Suggested trial value: Off**
TMB Standarization Factor: Slope	The user-supplied value for the slope of the linear curve to which the observed final TMB value will be adjusted. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
TMB Standarization Factor: Intercept	The user-supplied value for the intercept of the linear curve to which the observed final TMB value will be adjusted. **Allowed values:** -2,000 to 2,000 **Suggested trial value:** 0

Bamstats parameters

You can adjust the following Bamstats parameters to optimize your 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	
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 **Suggested trial value:** 100,000
Maximum read length	The maximum read length. **Allowed values:** 700 to 20,000 **Suggested trial value:** 700
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 **Suggested trial value:** 255
Max mismatches	The maximum number of mismatches allowed in the alignments. Any alignment with more then the specified number of mismatches are ignored while generating reports related to the number of mismatches. **Allowed values:** 0 to 500 **Suggested trial value:** 500
Max base qv	The maximum base quality values. Any base with a base quality value of more then the specified value are ignored while generating reports. **Allowed values:** 0 to 100 **Suggested trial value:** 100
Maximum Medium Coverage (QC)	The maximum medium coverage value. Any base with a coverage value of more then the specified value are ignored while generating reports. The value of **Maximum Medium Coverage (QC)** should be greater than **Maximum Low Coverage (QC)**. **Allowed values:** 2 to unlimited **Suggested trial value:** 1,000

Parameter name	Description
Minimum Target Overlap Forward	The fraction of an alignment that must be overlapped by a target in order to be classified as on target. **Allowed values:** 0.000001 to 1 **Suggested trial value:** 0.000001
Maximum Low Coverage (QC)	The maximum low coverage value. Any base with a coverage value of more then the specified value is ignored while generating reports. **Value of Maximum Low Coverage (QC)** should be less than **Maximum Medium Coverage (QC)**. **Allowed values:** 1 to unlimited **Suggested trial value:** 500
Maximum Target Overlap Reverse	The fraction of an alignment that must be overlapped by a target in order to be classified as on target. **Allowed values:** 0.0001 to 1 **Suggested trial value:** 0.5

CNV Finding parameters

You can adjust Copy Number Variant (CNV) finding parameters to optimize your 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 (applies to all CNV finding algorithms)	
CNV Sensitivity	Sensitivity. Only when CUSTOM option is selected, the value of editable parameter Transition Penalty, available in Advanced tab in CNV parameters, will be utilized by the algorithm. **Allowed values:** Low, Medium, High, or Custom **Suggested trial value:** Medium
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 **Suggested trial value:** False

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 **Suggested trial value:** 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 **Suggested trial value:** 7
CNV Gender Min Mapping QV	Specifies min mapping qv of reads to consider in gender calling. **Allowed values:** 0 to 255 **Suggested trial value:** 30
CNV Gender Min Autosomes Count	Specifies min number of required filtered reads in autosomes. **Allowed values:** 0 to unlimited **Suggested trial value:** 25,000
CNV CHRM to Autosomes Ratio Min Mapping QV	Specifies min mapping qv of reads to consider in calculating chrM A Ratio. **Allowed values:** 0 to 255 **Suggested trial value:** 30
Sample Filtering (applies only to VCIB CNV finding algorithm)	
Read Count	User to enter a threshold number (integer). **Allowed values:** 10,000 to unlimited **Suggested trial value:** 100,000
min-mapping-qv	Minimum mapping quality value required for a read to be counted. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
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 **Suggested trial value:** 60

Parameter Name	Description
MAPD threshold Sample will fail if MAPD is above this threshold.	User to enter a threshold number (float). **Allowed values:** 0 to unlimited **Suggested trial value:** 0.5
Analysis (applies only to VCIB CNV finding algorithm)	
NPC	Number of Principal Components used for correction. **Allowed values:** 0 to 12 **Suggested trial value:** 4
CNV Shift Type	Methods of CNV shift: 'Median Amplicon' where median Copy Number of autosomal amplicons is set to 2; or 'Median Gene' where median Copy Number of the autosomal genes is set to 2; or 'VALUE_BASED_ON_MEDAMP' where the amount of subtraction in log2 ratio to the result of the CNV Shift method used; or 'MAXGENE' where the log2-normalized counts for each gene in the panel is adjusted by first setting the median of the highest counts gene to the expected normal value (log2N=0) and maintaining the relative copy number of the two BRCA genes. MAXGENE ensures that at least one gene is normal copy number internally, minimizing FP calls due to slight differences in copy number between the genes in germline samples; or 'FLATGENE' where the log2normalized counts for each gene is adjusted by setting median of each gene to the expected normal value (log2N=0) independently of the other gene. FLATGENE ensures that both genes have normal copynumber internally, making calling germline exon deletion variants possible in somatic whole gene deletion samples. **Allowed values:** MEDGENE, MEDAMP, VALUE_BASED_ON_MEDAMP, MAXGENE, or FLATGENE **Suggested trial value:** MEDGENE
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 **Suggested trial value:** 0
Analysis (applies only to aneuploidy analysis workflows) [1]	
Enable Mosaicism Detection	Enable Mosaicism Detection **Allowed values:** True or False **Suggested trial value:** False
Enable Smoothing	Enable Smoothing **Allowed values:** True or False **Suggested trial value:** False

Parameter Name	Description
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 **Suggested trial value:** 2,000,000
Hide called gender	Hide gender called by CNV gender calling. **Allowed values:** True or False **Suggested trial value:** False
Analysis	
Plot Y chromosome for Female or Unknown Gender?	Plot Y chromosome for Female or Unknown Gender. **Allowed values:** True or False **Suggested trial value:** 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 **Suggested trial value:** 0.30
Max Calls	Specifies the number of non-contiguous exon variant calls above which the sample will fail QC. **Allowed values:** 0 to 47 **Suggested trial value:** 4
Min Quality	Specifies the quality score below which a CNV variant is classified as a NOCALL. **Allowed values:** 0 to 100 **Suggested trial value:** 10
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 **Suggested trial value:** 0
Max Fold Difference for Loss	Maximum fold difference relative to reference for calling a loss. **Allowed values:** 0 to 1 **Suggested trial value:** 0.85

Parameter Name	Description
Min Fold Difference for Gain	Minimum fold difference relative to reference for calling a gain. **Allowed values:** 1 to unlimited **Suggested trial value:** 1.15
P-value for maximum calls	P-value for maximum calls. **Allowed values:** 0 to 1 **Suggested trial value:** 0.00001
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 **Suggested trial value:** -8
Analysis (applies only to VCIB CNV finding algorithm when custom panel is focal amplication)	
CNV Gain Threshold	Threshold value (greater than or equal to 0) for calling GAIN in autosomes **Allowed values:** 0 to unlimited **Suggested trial value:** 4
CNV Gain Threshold XY	Threshold value (greater than or equal to 0) for calling GAIN in X or Y for males. **Allowed values:** 0 to unlimited **Suggested trial value:** 3
Gain Confidence Level	Confidence level value (greater than 0 and less than 1) to be used to compare to gain-threshold or gain_threshold_xy. **Allowed values:** 0 to 1 **Suggested trial value:** 0.05
Analysis (applies only to Hidden Gender aneuploidy analysis workflow) [2]	
Male Normal Ploidy Lower Bound	Male Minimum Normal Ploidy for Hidden Gender aneuploidy analysis workflow. **Allowed values:** 0 to 100,000 **Suggested trial value:** 0.8

Parameter Name	Description
Male Normal Ploidy Upper Bound	Male Maximum Normal Ploidy for Hidden Gender aneuploidy analysis workflow **Allowed values:** 0 to 100,000 **Suggested trial value:** 1.2
Female Normal Ploidy Lower Bound	Female Minimum Normal Ploidy for Hidden Gender aneuploidy analysis workflow. **Allowed values:** 0 to 100,000 **Suggested trial value:** 1.8
Female Normal Ploidy Upper Bound	Female Maximum Normal Ploidy for Hidden Gender aneuploidy analysis workflow **Allowed values:** 0 to 100,000 **Suggested trial value:** 2.2

[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 paramaters apply only to the Ion ReproSeq™ No Gender PGS w1.1 analysis workflow.

Fusions parameters

You can adjust the following Fusion parameters to optimize your 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. **Suggested trial value:** Medium
Minimum Read Counts for Fusions	Threshold on the minimum number of valid reads aligned to specific fusion isoform sequence in order to call the isoform as present, provided that 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 **Suggested trial value:** 20

Parameter	Description
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 **Suggested trial value:** 0
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 **Suggested trial value:** 20,000
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) **Suggested trial value:** True
Minimum number of Valid pools for SampleQC	For multipool RNA pools, specify the minimum number of pools in a sample that have to pass QC in order 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 in order to qualify that sample. Similarly use value = 1 to proceed with the analysis even if one of the pools failed. **Allowed values:** ≥1 integers only **Suggested trial value:** 2
Minimum Total Valid mapped reads Per Pool	Minimum number of total valid mapped reads in each pool (in the case of multipool RNA panels) in order to qualify that primer pool as valid. **Allowed values:** ≥0 integers only **Suggested trial value:** 0
Advanced tab	
Minimum Read Counts for Non-Targeted Fusions	Threshold on minimum number of valid reads aligned to a nontargeted fusion sequence in order 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 **Suggested trial value:** 250
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 a expression control is >15, it is called present. **Allowed values:** ≥0 integers only **Suggested trial value:** 15

Parameter	Description
Minimum Total Control reads	Minimum number of housekeeping control reads required to compute Imbalance scores for 5p3p assays. **Allowed values:** ≥0 integers only **Suggested trial value:** 1,200
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 user's input using a Regular expression. **Suggested trial value:** ALK=0.001;RET=0.03;ROS1=0.2
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 user's input using a Regular expression. **Suggested trial value:** ALK=0.015;RET=0.55;ROS1=0.5
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 user's input using a Regular expression. **Suggested trial value:** ALK=5;RET=5;ROS1=5
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 user's input using a Regular expression. **Suggested trial value:** ALK=0.001;RET=0.3;ROS1=0.15
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 user's input using a Regular expression. **Suggested trial value:** ALK=0.01;RET=0.25;ROS1=0.5
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 **Suggested trial value:** 0.5

Parameter	Description
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 **Suggested trial value:**
Keep Intermediate files	Turn this flag on to keep the intermediate files generated when using the fusions analysis. **Allowed values:** True or False **Suggested trial value:** 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 **Suggested trial value:** True
Minimum Read Counts for Gene Expression targets	Threshold on minimum number of valid reads aligned to specific gene expression target in order to call it as present. **Allowed values:** ⩾0 integers only **Suggested trial value:** 10
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 **Suggested trial value:** 0
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 within gene metrics for RNAExonVariant targets. **Allowed values:** True or False **Suggested trial value:** True

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 **Suggested trial value:** 2
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 **Suggested trial value:** default 2, suggested value between 2 and 10
Minimum Family Coverage per Strand	Minimum required coverage of reads on each strand in a bidirectional molecular tag family. **Allowed values:** Integers only **Suggested trial value:** 1
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 **Suggested trial value:** 2
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 **Suggested trial value:** 1
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 **Suggested trial value:** 20
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 **Suggested trial value:** 2
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 Suggested trial value: 2.0 **Note:** Gene-specific parameters are enabled in the properties.txt file.

Parameter	Description
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.
Minimum average read counts for for all the Exon Tiling assays in a Driver Gene to proceed with Imbalance Score	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 Suggested trial value: 30.0. **Note:** Gene-specific parameters are enabled in the properties.txt file.

MSI parameters

Note: MSI parameters are available only for analysis workflows that will be released after Ion Reporter™ Software 5.12.

Parameter name	Description
Main tab	
MSI Marker Regions	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. It is blank by default until the release of analysis workflows that are designed for use with MSI detection.
MSI Baseline File	Select an MSI baseline file—this file contains baseline information that is used if the spike-in that is used does not meet the RMC performance threshold that is set for the sequencing run.
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]
MSS Threshold	The MSI score below which a sample will report MSS in analysis results. [2]

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

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 **Suggested trial value:** False
Tmap Realignment Enable Flag	Flag to indicate whether BAM Realignment should be invoked. **Allowed values:** True or False **Suggested trial value:** False
Advanced tab	
Mismatch Penalty	Specifies the mismatch penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 3
Soft Clipping Type	Specifies the type of soft-clipping to perform. **Allowed values:** 0 to 3 **Suggested trial value:** 2
Gap Open Penalty	Specifies the gap open penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 5
Match Score	Specifies the match score. **Allowed values:** 1 to 100 **Suggested trial value:** 1
Gap Extension Penalty	Specifies the gap extension penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 2
Context	Realign with context-dependent gap scores. **Allowed values:** True or False **Suggested trial value:** False

Variant Name	Description
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 **Suggested trial value:** 6
Flow Score Penalty	Specifies the flow score penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 2
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 **Suggested trial value:** 2147483647
Local Band Width	Specifies the Local band width. **Allowed values:** 1 to 100 **Suggested trial value:** 50
Do Repeat Clip	Trim repetitive sequence at the ends of alignment. **Allowed values:** True or False **Suggested trial value:** False
End Repair	Specifies to perform 3' end repair. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
Use BED file	Use bed file to capture INDELs near amplicon edges. **Allowed values:** True or False **Suggested trial value:** False
Output Filter	Specifies the output filter for the mapping. **Allowed values:** 0 to 3 **Suggested trial value:** 1
Maximum INDEL Size to Rescue	The maximum INDEL size to rescue with one large INDEL algorithm. **Allowed values:** 0 to unlimited **Suggested trial value:** 30

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 **Suggested trial value:** 6.5
Downsample to Coverage	Reduce coverage in over-sampled locations to this value. **Allowed values:** 1 to unlimited **Suggested trial value:** 4000
SNP Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited **Suggested trial value:** >=3
SNP Min Variant Score	Filter: Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited **Suggested trial value:** 10
SNP Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals) **Suggested trial value:** 0.0005 to 0.005 (TagSeq), 0.01 to 0.2 (other)
SNP Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited **Suggested trial value:** 2 (TagSeq), 5 to 20 (other)
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) **Suggested trial value:** 0.95
INDEL Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited **Suggested trial value:** >=3

Parameter Name	Description
INDEL Min Variant Score	Filter: Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** >=10
INDEL Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals) **Suggested trial value:** 0.0005 to 0.005 (TagSeq), 0.05 to 0.2 (other)
INDEL Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** 2 to 10,000 (TagSeq), 15 to 30 (other)
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) **Suggested trial value:** 0.95
Hotspot Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited (integers **Suggested trial value:** 3
Hotspot Min Variant Score	Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** ⩾10
Hotspot Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals) **Suggested trial value:** 0.0005 to 0.005 (TagSeq); 0.01 to 0.2 (other)
Hotspot Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** 2 to 10,000 (TagSeq); 5 to 20 (other)
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) **Suggested trial value:** 0.95

Parameter Name	Description
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 allelles. No indicates variants that are not PPA alleles. For more information, see "Locus View versus Allele View of variants" on page 193. **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) **Suggested trial value:** 1.0
Outlier Probability	Prior probability that a read comes from some other distribution. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.005 to 0.01
Heavy Tailed	How heavy the t-distribution tails are to allow for unusual spread in the data. **Allowed values:** 0 to unlimited (decimals) **Suggested trial value:** 3
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) **Suggested trial value:** 0.3 (30% of variant change size)
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) **Suggested trial value:** 0.2 (20% of variant change size)
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) **Suggested trial value:** 0.2 (20% of variant change size)

Parameter Name	Description
HP Max Length	Filter: homopolymer length involved in an in/del. Related VCF field: HRUN. **Allowed values:** 1 to unlimited (integers) **Suggested trial value:** 8
Do SNP Realignment	Realign reads in the vicinity of SNP candidates. Impact: True = do not realign, False = realign. **Allowed values:** True or False **Suggested trial value:** PGM: (germline) True, (somatic) False; Proton: (germline) False, (somatic) 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 **Suggested trial value:** False
SSE Probability Threshold	Filter out variants in motifs with error rates above this. **Allowed values:** 0 to 1 **Suggested trial value:** 0.2
MNP Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** >=3
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 **Suggested trial value:** 10
MNP Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1 **Suggested trial value:** 0.0005 to 0.005 (TagSeq); 0.01 to 0.2 (other)
MNP Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** 2 to 10,000 (TagSeq); 5 to 20 (other)
MNP Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1 **Suggested trial value:** 0.95

Parameter Name	Description
MNP Strand Bias Pval	Filter out mnps with pval below this [1.0] given strand bias > mnp-strand-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 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 **Suggested trial value:** 1
INDEL Strand Bias Pval	Filter out INDELs with pval below this [1.0] given strand bias > INDEL-strand-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 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 **Suggested trial value:** 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 **Suggested trial value:** 0.05
Position Bias	Filter out variants with position bias relative to soft clip ends in reads > position-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 0.75
Position Bias Pvalue	Filter out if position bias is above the Position Bias given pval less than Position Bias Pval. **Allowed values:** 0 to 1 **Suggested trial value:** 0.05
Use position bias	Enable the position bias filter. **Allowed values:** True or False **Suggested trial value:** False
INDEL As HPINDEL	Apply INDEL filters to non HP INDELs. **Allowed values:** True or False **Suggested trial value:** False

Parameter Name	Description
Do MNP Realignment	Realign reads in the vicinity of candidate mnp variants. **Allowed values:** True or False **Suggested trial value:** True
Realignment Threshold	Maximum allowed fraction of reads where realignment causes an alignment change. **Allowed values:** 0 to 1 **Suggested trial value:** 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 **Suggested trial value:** 1
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 **Suggested trial value:** 5 (TagSeq), 0 (other)
Min Cov Fraction	Do not count reads with fraction of covering any amplicons below this threshold. **Allowed values:** 0 to 1 **Suggested trial value:** 0.9 (TagSeq), 0 (other)
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 **Suggested trial value:** 0
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 **Suggested trial value:** 0
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 **Suggested trial value:** between 3 and 7
min-mapping-qv	Minimum mapping quality value required for a read to be counted. **Allowed values:** 0 to unlimited (integer) **Suggested trial value:** 20 (TagSeq); 0 (other)

Parameter Name	Description
Poisson	Use Poisson parameter estimation to estimate count of functional families. **Allowed values:** 0 to 1 **Suggested trial value:** 0
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 **Suggested trial value:** 2
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 **Suggested trial value:** 2
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 **Suggested trial value:** 2
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 **Suggested trial value:** 2
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 **Suggested trial value:** 2
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 **Suggested trial value:** 20
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 **Suggested trial value:** 0

Parameter Name	Description
Minimum Callable Probability	Minimum callable probability for LOD calculation. **Allowed values:** 0 to 1 **Suggested trial value:** 0.98
Suppress called allele LOD	Suppress the LOD reporting of a variant allele that is called. **Allowed values:** True or False **Suggested trial value:** 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 **Suggested trial value:** True
Allow SNPs	Enable SNPs in FreeBayes hypothesis generator. Allowed values: True = generate SNP hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** True
Allow MNPs	Enable MNPs in FreeBayes hypothesis generator. Allowed values: True = generate MNP hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** True
Allow Complex	Enable complex variants in FreeBayes hypothesis generator. Allowed values: True = generate MNP hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** 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) **Suggested trial value:** 4
Read SNP Limit	Read mismatch limit on number of mismatches: filter potential mis-mapped reads. **Allowed values:** 0 to unlimited (integer) **Suggested trial value:** 10
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) **Suggested trial value:** 1

Parameter Name	Description
Generate Min Alt Allele Freq	Generate variants with at least this frequency in the pileup. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.02 to 0.15
Generate Min INDEL Alt Allele Freq	Generate INDEL variants with at least this frequency in the pileup. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.02 to 0.15
Generate Min Coverage	Generate variants in locations with at least this depth of coverage. **Allowed values:** 0 to unlimited **Suggested trial value:** 6
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) **Suggested trial value:** 11 to 30
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) **Suggested trial value:** 3 to 30
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) **Suggested trial value:** between 4 and the value given to the kmer_len parameter
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) **Suggested trial value:** 2 to 30
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) **Suggested trial value:** 2 to 11
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) **Suggested trial value:** 0.1 to 0.4

Parameter Name	Description
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) **Suggested trial value:** 0.6 to 1.0
Output MNV	Enables reporting of complex variants. Impact: 1 = report complex variants, 0 = don't report. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0
Variant View	Represent variants with Locus view or Allele view. For more information, see "Locus View versus Allele View of variants" on page 193. **Allowed values:** Locus view or Allele view **Suggested trial value:** your preference
Analysis SSE File	Analysis strand specific error file. Select a mask from the list or upload a mask from **Upload**.

Import custom variantCaller parameters

If you would like to use custom 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.

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.

Workflow preset type	Description
Annotation Sets	A set of annotation sources to apply to variants for selection in the **Annotation** step of creating an analysis workflow. For more information, see "Create annotation set workflow presets" on page 131.
Annotation Source	Annotation sources to apply to variants for selection in the **Annotation** step of creating an analysis workflow. For more information, see "Create annotation set workflow presets" on page 131.
Filter Chains	A set of filters to apply to variants for selection in the **Filter** step of creating an analysis workflow For more information, see "Create a custom filter chain" on page 315
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 accessible in the **Copy Number** step when you create an analysis workflow. For more information, see "Create a copy number baseline workflow preset" on page 136
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 141.
Final Report Templates	Final report templates that are accessible for selection in the **Final Report** step of creating an analysis workflow. For more information, see "Create a final report template workflow preset" on page 133.
Fusion Panels	

Workflow preset type	Description
Target Region Files	Target regions files restrict analysis to only regions specified in the file. These BED files are accessible for selection in the **Reference** step of creating an analysis workflow. For more information, see "Upload a target regions BED file workflow preset" on page 143.
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 accessible for selection in the **Reference** step of creating an analysis workflow. For more information, see "Upload a hotspots BED file workflow preset" on page 144.

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.

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 a analysis workflow so that you can launch analyses that use the custom annotation set.

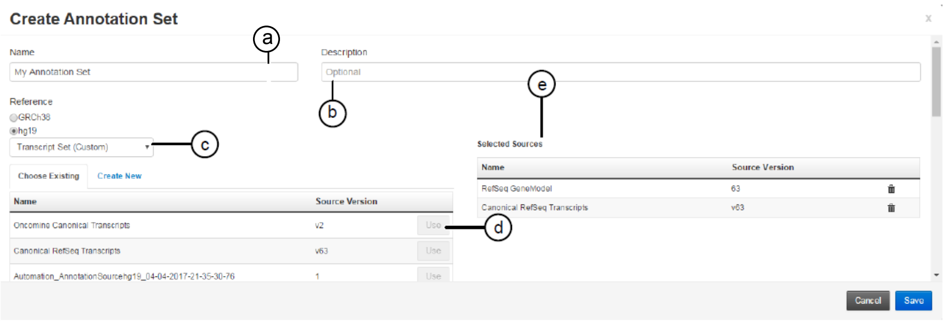
Ion Reporter™ Software includes two gene models: RefGene and Ensembl. To use an Ion Reporter™ Software canonical transcript set, you must use the compatible gene model when you select an annotation source. For example, use the RefGene gene model with the Refseq canonical transcript set.

Note: When you create an 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 400.

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

2. Click **Create Preset ▸ Annotation Set**.

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



Name: My Annotation Set

Description: Optional

Reference
○ GRCh38
● hg19

Transcript Set (Custom)

Name	Source Version
Oncomine Canonical Transcripts	v2
Canonical RefSeq Transcripts	v63
Automation_AnnotationSourcehg19_04-04-2017-21-35-30-76	1

Selected Sources

Name	Source Version
RefSeq GeneModel	63
Canonical RefSeq Transcripts	v63

   a. Enter a descriptive name.

   IMPORTANT! You can use the words AND, NOT or, OR in an annotation set name in Ion Reporter™ Software 5.12. Do not include these operators in an annotation set name if you use Ion Reporter Software 5.10 or earlier.

   ---

   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.

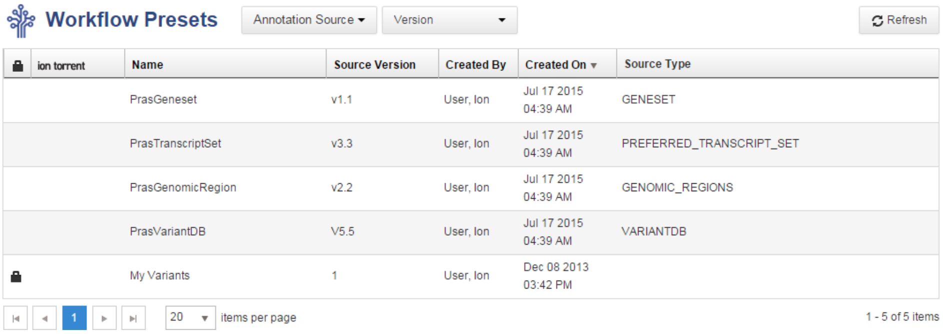
   f. Click **Save** to save the annotation set.
   The annotation set appears in the **Workflow Presets** table and is available in the **Annotation** step when you create an analysis workflow.

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 131.

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.



🔒	ion torrent	Name	Source Version	Created By	Created On	Source Type
		PrasGeneset	v1.1	User, Ion	Jul 17 2015 04:39 AM	GENESET
		PrasTranscriptSet	v3.3	User, Ion	Jul 17 2015 04:39 AM	PREFERRED_TRANSCRIPT_SET
		PrasGenomicRegion	v2.2	User, Ion	Jul 17 2015 04:39 AM	GENOMIC_REGIONS
		PrasVariantDB	V5.5	User, Ion	Jul 17 2015 04:39 AM	VARIANTDB
🔒		My Variants	1	User, Ion	Dec 08 2013 03:42 PM	

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 more flexible report template from your analysis results. For more information, see "Create a report template" on page 357.

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)* Enter an organization name.
   d. *(Optional)* Enter the organization address.
   e. *(Optional)* To add a logo to the report header, click **Upload** . In the **Upload Header Logo** dialog box, click **Select File** to browse to, then select your logo file. Acceptable formats are: PNG, GIF, and JPG.

f. 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) report contents" on page 368. You can also view and download these metrics in a separate report. For more information, see "View a Quality Control (QC) report" on page 367. The QC Metrics section is available in Ion Reporter™ Software 5.12 or later.
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 of the reported variants 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 additional 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 your 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 359.
6. Launch an analysis from your analysis workflow.

   For more information, see "Launch an analysis" on page 157.

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, per-amplicon attributes of GC proportion, and length of the amplicon insert. In practice, other variability exists that is not associated with known attributes, yet is systematic. Copy number baselines, when added to a workflow can correct the variability.

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.

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 in a "normal" sample, then makes further adjustments.

Known sources of variability include pool imbalance (when the assay has more than one pool of amplicons), total number of reads, and per 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 comprises the baseline.

You can create a Variability Correction Information Baseline (VCIB) baseline to detect copy number variation in samples. To try to get improved results with a baseline, you can create a custom baseline that uses your samples, 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 normal.

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. The samples do not need to be normal.

Note: If you use a single sample analysis workflow, you must use a copy number baseline to detect copy number variations. You must create a custom baseline if a baseline is not shipped with a panel.

To use a VCIB baseline that is included in Ion Reporter™ Software, you can select the baseline preset when you create an analysis workflow. For more information, see "Apply a baseline workflow preset to an analysis workflow" on page 98.

VCIB calculations

Copy number estimates are made using a proprietary algorithm.

The VCIB algorithm uses an informatics baseline, which is created using at least 48 diverse samples, to allow 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, see the user guide for the assay that you use.

Create a copy number baseline workflow preset

You can create a copy number 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 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 copy number baseline workflow preset.

If you import the custom copy number baseline, 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 **Copy Number** step of the **Create Copy Number Baseline** workflow bar, select the baseline type that corresponds to the type of libraries that you use.

Baseline Type	Description
AmpliSeq HD	Create copy number baselines for Ion AmpliSeq™ HD libraries. For more information, see "VCIB baseline workflow presets" on page 135.
AmpliSeq	Create copy number baselines for Ion AmpliSeq™ and Oncomine™ libraries (not Exome). For more information, see "VCIB baseline workflow presets" on page 135.
AmpliSeq-Exome	Create copy number baselines for Ion AmpliSeq™ Exome libraries.
TagSeq	Create copy number baselines for Ion TagSeq libraries. For more information, see "VCIB baseline workflow presets" on page 135.

Baseline Type	Description
TargetSeq-Exome	Create copy number baselines for Ion TargetSeq™ Exome or other targeted libraries.
Low-coverage Whole-Genome	Create copy number baselines for whole genome libraries with low coverage (for example, Aneuploidy).

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. Use the following options to import AmpliSeq libraries and panel files:

Option	Import DNA panel files
Custom Panel	1. Under **Target Regions**, 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 panel files. 3. Enter your user name and password and then click **List My AmpliSeq Panels**. 4. Select the panel files that you want to import from the dropdown list, then click **Import**.
Fixed Panel	1. Under **Target Regions**, click ⤓ **AmpliSeq Import**. 2. In the **Import for AmpliSeq** dialog box, select the **Fixed Panel** tab for On-Demand or Ready-to-Use panel files. 3. From the list, select the panel file that you want to import. 4. Click **Import**.

6. Click **Next**.

7. If you selected **AmpliSeq HD**, **AmpliSeq**, or **TagSeq** baseline types, you can use an existing baseline and augument the baseline with additional samples.
   a. In the **Altorithm** step, select **Select an existing CNV baseline** option to use an existing baseline that you can add more samples to.

8. In the **Samples** step, select the samples to be used in the baseline creation, based on the guidelines that are included in the software. If you do not see your samples in the table, see "Sample definition" on page 50 for information on how to upload or define a sample.

9. Click **Next**, then advance to the **Confirm** step.

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

11. Click **Create Baseline**.

The baseline creation job is started and the baseline with its status now appears on the screen. When the job completes, it is selectable in the **Copy Number** step for analysis workflow creation. For more information, see “Apply a baseline workflow preset to an analysis workflow“ on page 98.

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 Psuedo 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.

1. Create a normal male and chrX PAR CNV baseline.

   For more information, see "Create a copy number baseline workflow preset" on page 136.

2. Add the CNV baseline to an Oncomine™, Ion AmpliSeq™, or Ion AmpliSeq™ HD analysis workflow.

   For more information, see "Apply a baseline workflow preset to an analysis workflow" on page 98.

   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 157.

4. Visualize the analysis.

   For more information, see "IRGV tab" on page 281 or Chapter 7, "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 207.

Create an analysis workflow that contains a copy number baseline

Ion Reporter™ Software provides a workflow bar to guide you through the process to create an analysis workflow that contains a CNV baseline workflow preset. However, we recommend that you copy an existing predefined analysis workflow, then add the CNV baseline workflow preset to the new analysis workflow.

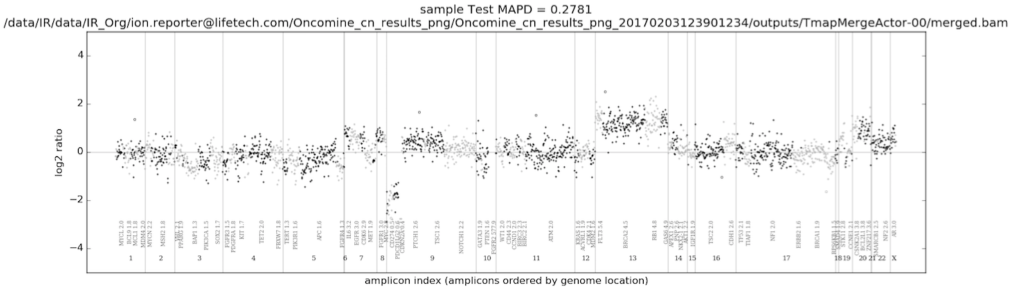
1. In the **Workflows** tab, click the **Overview** screen, then select an appropriate Oncomine™ analysis workflow.
2. Click **Actions** ▸ **Copy**, then click **Next** .
3. In the **Reference** step, ensure that **hg19** is the selected **Reference**, then select **Target Regions**, **Hotspot Regions**, and **Fusions** BED files, 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 a CNV baseline analysis workflow, 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 157. You can also optionally download the analysis results and view them visually in the `cn_results.png`.



These example analysis results shows a plot with log2 ratios across the genome and highlights panel CNV IDs, in this case gene symbols, to aid in verifying 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 numbers on the X axis are 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 above 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.

Augment an existing VCIB baseline workflow preset

You can augment an existing Variability Correction Information 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** 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. 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.

5. Click **Configure Parameters**.

6. 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.

7. Click **Done**, then click **Next**.

8. In the **Samples** step, select any additional samples to include in the CNV baseline workflow preset, then click **Next**.

9. 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 augumented baseline to an analysis workflow. For more information, see “Create an analysis workflow that contains a copy number baseline“ on page 139.

Sequence variant baselines

The filters that are used in Ion Reporter™ Software remove most sequencing errors. While 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 reduce 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 could lead to false positive variant calls.

Ion Reporter™ Software includes predefined sequence variant baselines. You can also create a custom 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 detect somatic variants. 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 540 chip and custom Ion AmpliSeq™ panel, then the germline samples used to create the SVB must have been sequenced with the 540 chip 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.

      All samples must be normal (non-tumor). It is recommended that at least 20 samples be used to create the custom sequence variant baseline. The use of multiple samples increases the robustness of the sequence variant baseline.

      If you do not see your samples in the table, see "Sample definition" on page 50 for information on 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 baseline workflow preset to an analysis workflow" on page 98.

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 the "available for use" message.
    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 a 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 custom 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 custom 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.

The analysis workflow is now locked and cannot be edited.

5

Analyses

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.

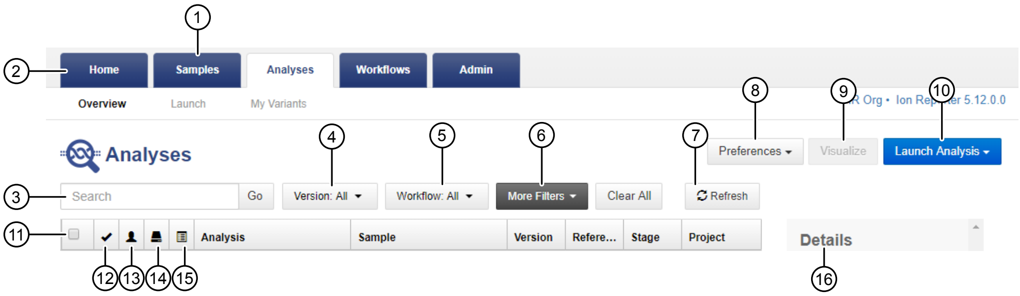
You can also generate, or *launch*, one or more analyses manually in Ion Reporter™ Software. Manual launches allow you to select an analysis workflow and one or more previously uploaded sample or samples for the analysis. For single-sample analysis workflows, you can select multiple samples for multiple 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 **Analyses** tab

② The **Overview** screen in the **Analyses** tab. Click the screen name to toggle between screens.
The **Launch** screen. For more information, see "Launch an analysis" on page 157.
The **MyVariants** screen. For more information, see "MyVariants" on page 163.

③ You can enter keywords or text strings in the **Search** box. For more information, see "Search for analyses" on page 156.

④ The **Version All** filter allows you to search for specific versions of analyses.

⑤ The **Workflow All** filter allows you to search for specific analysis workflows.

⑥ **More Filters** allows you to add additional filters to the **Analyses** tab. For more information, see "Filter analyses" on page 156.

⑦ ↻ **Refresh** refreshes the list of samples.

⑧ Apply **Preferences** to customize how the **Analyses** table is organized. For more information, see "Customize the Analyses table" on page 149.

⑨ **Visualize** allows you to view the data in Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV). For more information, see Chapter 7, "Visualize analysis results with Ion Reporter™ Software".

⑩ **Launch Analyses** allows you to manually launch one or more analyses (batch). For more information, see "Launch an analysis" on page 157.

⑪ 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 151.

⑫ ✓ **(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.

⑬ 👤 **(Analyze role)** Indicates that the analysis is shared by an Analyze role user.

⑭ 🗄 **(Archived)** Indicates that the analysis is archived.
📥 **(Archive in process)** Indicates that archive of the analysis is in process.

⑮ ☰ **(Notes)** Notes about the analysis.

⑯ **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 attribute columns to the **Analyses** table

You can add analysis attributes to the **Analyses** table that you view in Ion Reporter™ Software. If an analysis contains an attribute, columns in the **Analyses** table show the attribute values. An attribute can be associated with the analysis itself, with samples that are used in the analysis, or with the analysis workflow. Sample attributes can be inherited from Torrent Suite™ Software and include information such as chip type, sequencing run details, and custom attributes.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences ▸ Select Columns**.
3. In the **Select Columns** dialog box, select the analysis attributes that you want to add to the **Analyses** table, then click **Apply**.

The selected attributes are added to the **Analyses** table until you close the table. To save the table with the attributes that you added, create a preference. For ore information, see “Create an Analyses table preference“ on page 150.

Change the order of columns in the **Analyses** table

You can change the order of 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.
   The columns 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 allows you to view only the columns of data that are relevant to your research in an **Analyses** table.

The 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**.
3. In the **Select Columns** dialog box, select any available columns that you want to add, then click **Apply**.
   The selected columns are added on the right side of the table.
4. Save the custom table as a preference.

Option	Description
Preferences ▸ Save Table Preference As	This setting allows you to name the custom 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** table preference

You can apply table preferences to change the view of information in an **Analysis** 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 **Analysis** table changes to show your selected preferences.
4. *(Optional)* Create a new **Analysis** 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 lick **Save**.

   The new **Analysis** table preference is saved.

Restore default settings for the **Analysis** table

You can restore default table column headings in the **Analysis** 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 **Analysis** table preference

You can delete a custom **Analysis** table preference setting in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Click **Preferences ▸ Table Preferences**, then select the custom 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 custom 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.
📥	Archive in process (Ion Reporter™ Server only)—Indicates that the analysis is in the process of being transferred to the archive server.
🖴	Archived (Ion Reporter™ Server only)—Indicates that the analysis was transferred to the archive server.

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 biopsy was taken from an embryo.
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.

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 can not 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.

Column	Description
Percentage Cellularity	The percentage of tumor cells in the sample.
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 Archived	An indication of whether the sample was transferred to the archive server. • **Archived**—The sample has been archived. • **Available**—The sample is available to be archived.
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.

Column	Description
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**
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 attribute columns to the Analyses table" on page 149. 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 sample names returns a list of all samples with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and are therefore not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

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1. In the **Analysis** 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 custom 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 530 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

When samples are combined with an analysis workflow in Ion Reporter™ Software, and analysis results are generated, an analysis is *launched*. You can select which samples to analyze and select an analysis workflow, then launch an analysis to generate an analysis in Ion Reporter™ Software, or reanalyze a set of samples.

1. In the **Analyses** tab, click **Launch**.

    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. 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).	1. Select a Normal and Tumor sample pair. 2. Click **Add Samples**. 3. Enter a **Relationship Name** for the sample pair. 4. 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. For best results, select a control sample with no known CNVs in any region that is covered by the Ion AmpliSeq™ panel used. 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 actually has a CNV in that region. 1. Select a Sample and Control pair. 2. Click **Add Samples**. 3. Enter a **Relationship Name** for the sample pair. 4. Click **Add to Analysis**.
Select trio samples (for use with trio analysis workflows).	1. Select the three samples for the trio (**Mother**, **Father**, **Child**). 2. Click **Add Samples**. 3. Enter a name for the sample trio. 4. Click **Add to Analysis**.

4. Click **Next**.

5. *(Optional)* In the **Plugins** step, select a plugin, then click **Next**.

6. 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	1. In the **Dashboard** screen, click **Launch analysis**. 2. In the **Launch Analysis** screen, select a **Research Application**. 3. In the **Research Category** list, select a row with an analysis workflow name. 4. Click **Next**.
Analyses tab	1. Click **Launch Analysis**, then select **Manual** from the list. 2. In the **Launch Analysis** screen, select a **Research Application**. 3. Click in the analysis workflow row, then click **Next**.
Workflows tab	1. Select a **Research Application**. 2. 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 pluginplugin 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 161 .

   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
Version	`5.12`	Yes

Column name	Example	Required
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_5.12.0.1-IR1;ampliSeqRNA_5.12.0.1-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 157.

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 157. 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 165.

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)**	MyVariant 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 to variants of interest" on page 166. This column can be edited.
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.

Information type	Description
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 171 and "Import MyVariants" on page 171 .
- Use a MyVariants database to create various filter chains to narrow analysis results. For more information, see "MyVariants filter" on page 350.
- Apply the latest MyVariants information to older analysis results. For more information, see "Get the latest updates from the MyVariants database" on page 169.
- Create a MyVariants report in PDF format. For more information, see "Create a PDF report of MyVariants" on page 171.

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 ensure that a variant is always is flagged as **Important** and classified as **Benign**, and includes a note with other information.

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 166.

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. Notes are added automatically if no other note is available for the variant.
Classifications	A category that users can add to a variant. Classification options include: Unclassified, Unknown, Benign, Suspected Benign, Likely Benign, Pathogenic, Suspected Pathogenic, Likely Pathogenic, VUS, Uncertain Significance, Deleterious, and Suspected Deleterious. By default, classifications for each variant are blank until a value is selected.

Apply flags 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. We recommend 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** screen appears in **Summary** view.
2. (*Optional*) In the **Classification** column, you can 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**

3. (*Optional*) In the ▤ **(Note)** column, click + **(Add)** , click the **Notes** tab, then enter a note for the variant. When you are finished, click **Add Note**.

4. 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.

5. To check that the variants were added, 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. You can add a classification to variants, add notes, synchronize the MyVariants database, generate a report, and select variants and perform batch actions.

This topic shows how you can flag variants in a MyVariants database. You can change the **Important** or **Ignore** flags existing in a MyVariants database. However, keep in mind that the edits can affect the MyVariants flags that are set by other members of the organization if you are sharing 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.

1. In the **Analyses** tab, click **MyVariants**.
2. In the **MyVariants** table, select a specific MyVariants database from the list.
3. Review the MyVariants flags.

4. To change a MyVariants flag, click ⚑ ⌄ **(Flag list)**, then select an appropriate option.

Option	Description
None	Removes the existing Important or Ignore flag from the table and removes the variant from the MyVariants database for all analyses.
Important	Changes the existing flag to Important and stores the variant in the MyVariants database.
Ignore	Changes the existing flag to Ignore and stores the variant in the MyVariants database.

Get the latest updates from the MyVariants database

You can get the latest additions, deletions, or changes to the organization's MyVariants database 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 in Ion Reporter™ Software 5.10 and later 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.

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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 171.

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 170.

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 171.

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**.

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 350 .

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 report template" on page 357.
5. Enter required information into the sections. For more information, see "Available report sections" on page 363.
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" on page 175.

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, such as Oncomine™ Reporter Software. For more information, see "Increase storage space on Connect" on page 173.

You must 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 an aborted analysis

You can reanalyze the results for an analysis run that has been aborted.

1. In the **Analyses** tab, select an analysis of interest that has a status of **Aborted**.
2. Click **Actions** ▸ **Reanalyze**.

Archive analyses to an external storage device

This information applies only to Ion Reporter™ Server.

You can archive one or more analyses from an Ion Reporter™ Server to an external storage device to increase disk space. Available device options include:

- Torrent Storage™ NAS (Network Attached Server) device—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**

You can also archive samples. For more information, see "Archive samples to an external storage device" on page 63 .

- 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.

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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 the 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. In the **Confirm Archive** dialog box, click **Yes**.
   While the analysis is being archived, 📥 **(Archive in progress)** appears in the analysis row.
   When the archive process is complete, 🖴 **(Archived)** appears in the analysis row.

The analysis or analyses are archived. To use the analysis or analyses again, see "Restore archived analyses" on page 175.

Restore archived analyses

This information applies only to Ion Reporter™ Server.

If needed, you can restore the analyses that you have archived in Ion Reporter™ Software.

IMPORTANT! Ensure that you restore archived analyses and samples only on the Ion Reporter™ Server on which the analyses and samples were originally archived.

You can also restore samples that are archived. For more information, see "Restore archived samples from an external device to Ion Reporter™ Server" on page 64.

- The external storage device that contains archived analyses must be mounted to your Ion Reporter™ Server.
- If you have a new Ion Reporter™ Server or upgrade from a previous version, you must 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.

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1. In the **Analyses** tab, find the archived analysis.
   An archived analysis has 🖴 **(Archived)** in the row of an analysis.
2. Click 🖴 **(Archived)** to restore the analysis.
   A restored analysis has ↻ **(Restore)** in the row of an analysis.

The analysis is restored and can be reported on or launched. For more information, see "Send an analysis to the Report role" on page 177, or "Launch an analysis" on page 157.

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 can not 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 can not 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 174.

You can download a BAM file that is generated by Ion Reporter™ Software to review the file, or open it and inspect the data with a visualization tool outside of the software.

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)**.

The mapped 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 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 directory to access the folders and files.

   A compressed directory named `analysis name_All.zip` is downloaded.

   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 contain TXT, FASTA, PDF, CSV, or PNG files.
`QC`	• <bam_file_basename>.ionstats_alignment.json • analysis.bfmask.stats • explog_final.txt • raw_peak_signal • InitLog.txt
	The `basecaller_results` folder contains the following JSON files: • BaseCaller.json • datasets_basecaller.json • ionstats_tf.json • TFStats.json

Folder	Files
`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.

Variants file downloads

You can download a compressed directory of files from a successful analysis that includes variants results in a VCF file. You can use the files to analyze Ion Reporter™ Software data in downstream software, such as Oncomine™ Reporter, or examine VCF files directly. Each downloaded directory 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.

There are two types of variants directories available in Ion Reporter™ Software.

- **Filtered variants**—This directory contains a VCF file and other files, all of 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 8, "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.

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 in an analysis

- In the **Analyses** tab, click **Overview**, then select the analysis that contains the filtered variants.
- Use one of the following options to download a compressed directory of files that contains data for variants that are filtered:
  - Click **Actions ▸ Download Filtered Variants**.
  - Click the hyperlink for the name of the **Analysis** to open the **Analysis Results** page, then click **Download ▸ Filtered Variants**.
  - Click **Visualize**, then in the **Analysis Visualization** click **Download ▸ Filtered Variants**.

  A compressed directory that is named `analysis name_Filtered.zip` is downloaded.

- To download the compressed directory 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)**.

- Extract the contents of the directory to access the folders and files.

Folder	Description of contents
`CNV_VCIB`	– `cn_results.png`, a file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor. – `amplicon_data.txt`
`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 on 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 in PDF format. The same QC metrics are available in the Final Report of an analysis.
	`StatsActor` – – `amplicons_low_no_coverage_statistics.txt` – `analysis_low_no_coverage_statistics.txt` – `genes_low_no_coverage_statistics.txt`
	– `AnnotatorActor` – `variome-stats.csv`

Folder	Description of contents
`Variants`	Contains VCF and TSV files for the CNV variants. 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`. – `<analysis name><timestamp>.oncomine.tsv` – `<analysis name><timestamp>.vcf` – `<analysis name><timestamp>.full.tsv` – `SmallVariants.filtered.vcf` – `SmallVariants.vcf` – `Variant.filtered.genome.vcf`
`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 `fusions_RNAExonVariants_normCounts.png`
`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. – `Summary.tsv`—Provides a summary of results for MSI markers, as shown in the **Analysis Results** table, and additional information about the algorithm version that is used to calculate the Marker MSI Score and MSI Score. – `Details.tsv`—Provides marker-level information on microsatellite instability, such as individual marker MSI scores, and read coverage for individual MSI markers.

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 contain 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

`Plugin outputs`	Contains ZIP file of the plugin output directory.
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 that contains reads for the analyses in FASTA format, and other TXT files for the analysis.

You can view the extracted files individually, or upload a VCF file to a software application that requires VCF files, such as Oncomine™ Reporter Software.

Download all variants in an analysis

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Use one of the following options to download a compressed directory of files that contains data for all variants in the analysis:
   - Click **Actions ▸ Download All Variants**.
   - Click the hyperlink for the name of the **Analysis** to open the **Analysis Results** page, then click **Download ▸ All Variants**.
   - Click **Visualize**, then in **Analysis Visualization**, click **Download ▸ All Variants**.

     A compressed directory that is named `analysis name_All.zip` is downloaded.
3. To download the compressed directory 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 directory to access the folders and files.

Folder	Description of contents
`CNV_VCIB`	• `cn_results.png`, a file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor. • `amplicon_data.txt`
`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` • `diffCoverage.seg` • `RNAQCAndCalls.txt` • `TotalMappedFusionPanelReads.txt` • `qc_cnv_display.txt` • `<analysis name>_QC.pdf`
	`StatsActor` • – `amplicons_low_no_coverage_statistics.txt` – `analysis_low_no_coverage_statistics.txt` – `genes_low_no_coverage_statistics.txt`
	• `AnnotatorActor` – `variome-stats.csv`

Folder	Description of contents
`Variants`	Contains VCF and TSV files for the CNV variants. 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`. • `<analysis name><timestamp>.oncomine.tsv` • `<analysis name><timestamp>.vcf` • `<analysis name><timestamp>.full.tsv` • `SmallVariants.filtered.vcf` • `SmallVariants.vcf` • `Variant.filtered.genome.vcf`
`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 `fusions_RNAExonVariants_normCounts.png`
`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. • `Summary.tsv`—Provides a summary of results for MSI markers, as shown in the **Analysis Results** table, and additional information about the algorithm version that is used to calculate the Marker MSI Score and MSI Score. • `Details.tsv`—Provides marker-level information on microsatellite instability, such as individual marker MSI scores, and read coverage for individual MSI markers.

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 contain 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
`Plugin outputs`	Contains ZIP file of the plugin output directory.
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 that contains reads for the analyses in FASTA format, and other TXT files for the analysis.

You can view the extracted files individually, or upload a VCF file to a software that requires VCF files, such as Oncomine™ Reporter Software.

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 ouput 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.

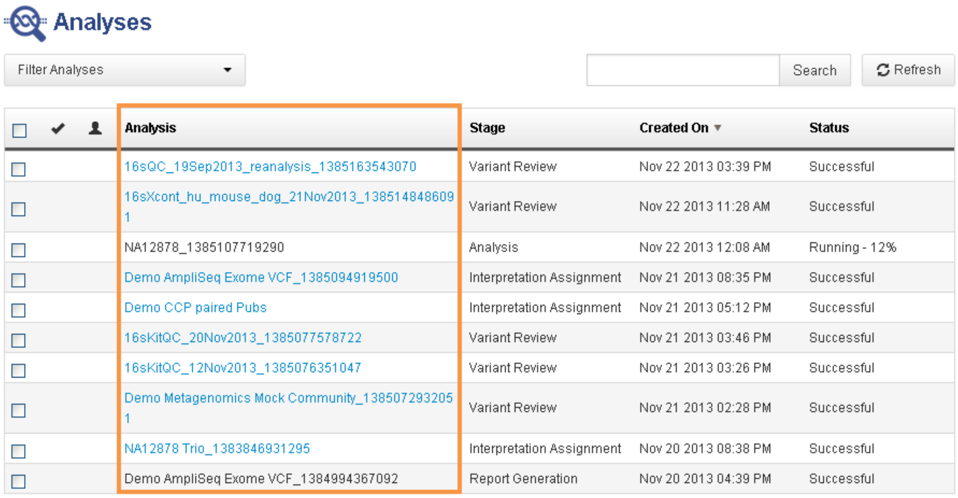
6

Analysis results

Open and review analyses results

To access the results of your DNA analysis:

1. In the **Analysis** tab, click **Overview**.
2. In the **Analyses** table, click the name of your analysis .



☐	✓	👤	Analysis	Stage	Created On ▾	Status
☐			16sQC_19Sep2013_reanalysis_1385163543070	Variant Review	Nov 22 2013 03:39 PM	Successful
☐			16sXcont_hu_mouse_dog_21Nov2013_1385148486091	Variant Review	Nov 22 2013 11:28 AM	Successful
☐			NA12878_1385107719290	Analysis	Nov 22 2013 12:08 AM	Running - 12%
☐			Demo AmpliSeq Exome VCF_1385094919500	Interpretation Assignment	Nov 21 2013 08:35 PM	Successful
☐			Demo CCP paired Pubs	Interpretation Assignment	Nov 21 2013 05:12 PM	Successful
☐			16sKitQC_20Nov2013_1385077578722	Variant Review	Nov 21 2013 03:46 PM	Successful
☐			16sKitQC_12Nov2013_1385076351047	Variant Review	Nov 21 2013 03:26 PM	Successful
☐			Demo Metagenomics Mock Community_1385072932051	Variant Review	Nov 21 2013 02:28 PM	Successful
☐			NA12878 Trio_1383846931295	Interpretation Assignment	Nov 20 2013 08:38 PM	Successful
☐			Demo AmpliSeq Exome VCF_1384994367092	Report Generation	Nov 20 2013 04:39 PM	Successful

This action opens the **Analysis Results** table in edit mode.
You cannot review analyses that are still running.
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.

Display different views of analysis results

You can see several different views of analysis results in Ion Reporter™ Software.

1. In the **Analysis** 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 % Frequency information.
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, UCSC Common SNPs, ExAC LAF, ExAc EAAF, ExAC OAF, ExAC EFAF, ExAC SAAF, ExAC ENFAF, ExAC AAF, and ExAC GAF 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, % Frequency, and Information.
QC	Provides a QC view of the analysis results, Includes columns for Classification, Locus, Genotype, Reference, Type, No Call Reason, Genes, p-value, Phred QUAL Score, Coverage, Allele Coverage, Allele Ratio, Ref+/Ref-/Var+/Var-, Homopolymer Length, and Subset of information.

Variants Table view

The contents of the Samples column vary by variant type:

- **SNVs and INDELs:** Genotype
- **CNVs:** Cytoband
- **Fusions:** Presence or Absence detection call

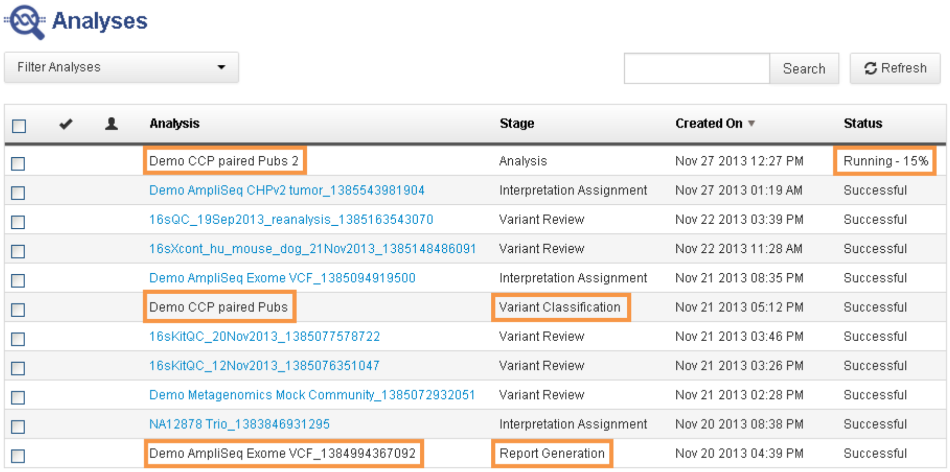
Note: In the Variants Table view, analysis columns are ordered from left to right starting with the first analysis that you selected. The annotation data from the variants in the first selected analysis is what populates the Location column of the table.

Restrictions on analysis selection

- Comparison and visualization are not supported for Metagenomics analyses. If one of these is among the analyses selected, then the Visualize option is not available in the Action menu.
- If a Failed or In-Progress analysis is among the analyses selected, then the Visualize option is not available in the Action menu.
- If only one set of variants is selected, the Visualization option is not available:
  - Trio and paired analyses each contain more than one set of variants. These analyses support visualization one a single trio or paired analysis.
  - A tumor-normal analysis has only one set of variants and does not support visualization. (A tumor-normal analysis reports only the set of variants for the tumor sample, not the set of variants for the normal sample.)

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.



☐	✓	👤	Analysis	Stage	Created On ▼	Status
☐			Demo CCP paired Pubs 2	Analysis	Nov 27 2013 12:27 PM	Running - 15%
☐			Demo AmpliSeq CHPv2 tumor_1385543981904	Interpretation Assignment	Nov 27 2013 01:19 AM	Successful
☐			16sQC_19Sep2013_reanalysis_1385163543070	Variant Review	Nov 22 2013 03:39 PM	Successful
☐			16sXcont_hu_mouse_dog_21Nov2013_1385148486091	Variant Review	Nov 22 2013 11:28 AM	Successful
☐			Demo AmpliSeq Exome VCF_1385094919500	Interpretation Assignment	Nov 21 2013 08:35 PM	Successful
☐			Demo CCP paired Pubs	Variant Classification	Nov 21 2013 05:12 PM	Successful
☐			16sKitQC_20Nov2013_1385077578722	Variant Review	Nov 21 2013 03:46 PM	Successful
☐			16sKitQC_12Nov2013_1385076351047	Variant Review	Nov 21 2013 03:26 PM	Successful
☐			Demo Metagenomics Mock Community_1385072932051	Variant Review	Nov 21 2013 02:28 PM	Successful
☐			NA12878 Trio_1383846931295	Interpretation Assignment	Nov 20 2013 08:38 PM	Successful
☐			Demo AmpliSeq Exome VCF_1384994367092	Report Generation	Nov 20 2013 04:39 PM	Successful

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**.

Apply a filter chain to analysis results

You can apply a filter chain to the analysis results to further refine your view to only variants of interest in Ion Reporter™ Software. Filter chains are preinstalled or customized 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 8, “Filters and filter chains“.

When you apply a filter chain to analysis results, the list of variants is filtered in real-time. You must save your filter chain to have the filter chain immediately alter the heat maps, other visualizations, and exported results of your variants of interest. If you do not save your 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 your 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 choose to publish it 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 choose to publish it at this point, because of filter chain settings.
5. *(Optional)* If you want to save these changes to your 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.

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 have the option to view variants per position, **Locus View**, or variants per allele, **Allele View**. For more information on how to set this view, see "Customize tuning parameters" on page 99 and "Variant Finding parameters" on page 119. 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 an individual 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:

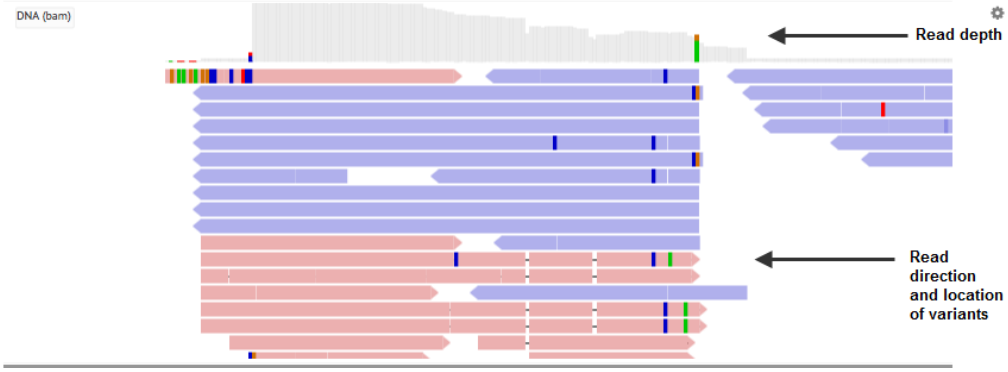
The subset column indicates that an allele, which is part of a larger variant that has been called, is present (the superset). The format is subset index (superset index). The larger variant can be found by using the value of the superset index counting into the alleles listed in the **Observed Allele** 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 counting in 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 foward 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 (/).

Coverage histogram

When you zoom in on an analysis, 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.



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.

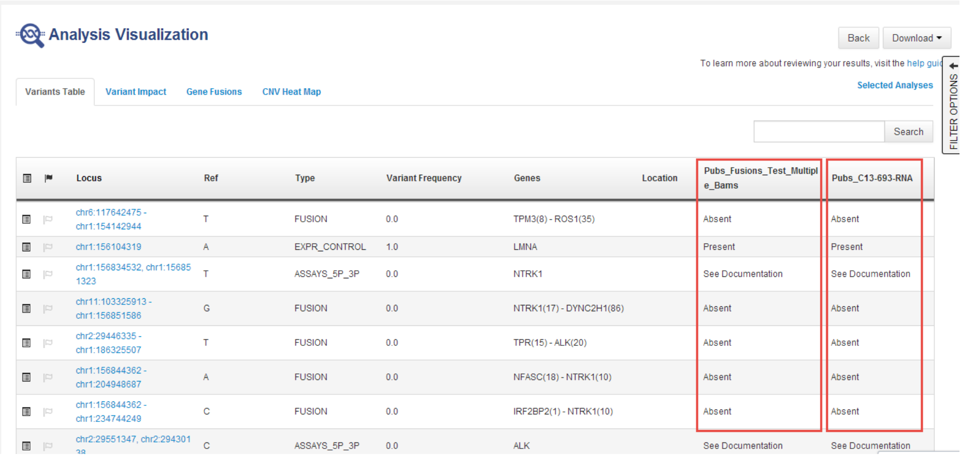
1. In the **Analyses** tab, click **Overview**.
2. Select multiple analyses, then click **Actions** ▸ **Visualize**.

The Visualize function allows you to review the results in a variety of ways. For more information, see “Compare results of single or multiple analyses“ on page 195.

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. Multianalysis visualization supports the following views, depending on analysis workflow type.

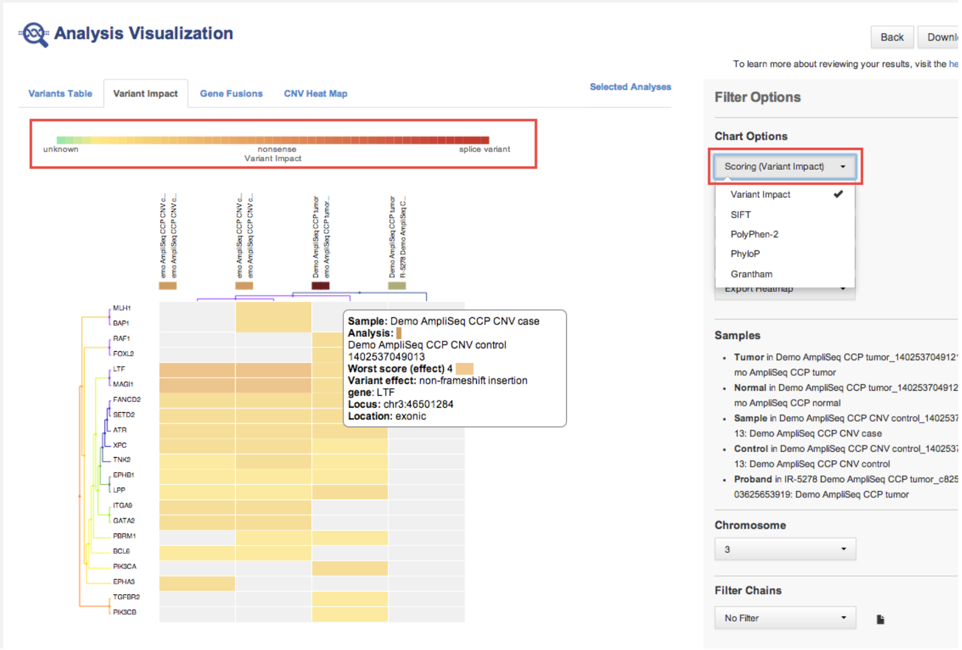
- In the **Variants Table** tab, the table view shows side-by-side columns to compare variant calls in different analyses:



Locus	Ref	Type	Variant Frequency	Genes	Location	Pubs_Fusions_Test_Multiple_Bams	Pubs_C13-693-RNA
chr6:117642475 - chr1:154142944	T	FUSION	0.0	TPM3(8) - ROS1(35)		Absent	Absent
chr1:156104319	A	EXPR_CONTROL	1.0	LMNA		Present	Present
chr1:156834532, chr1:156851323	T	ASSAYS_5P_3P	0.0	NTRK1		See Documentation	See Documentation
chr11:103325913 - chr1:156851586	G	FUSION	0.0	NTRK1(17) - DYNC2H1(86)		Absent	Absent
chr2:29446335 - chr1:186325507	T	FUSION	0.0	TPR(15) - ALK(20)		Absent	Absent
chr1:156844362 - chr1:204948687	A	FUSION	0.0	NFASC(18) - NTRK1(10)		Absent	Absent
chr1:156844362 - chr1:234744249	C	FUSION	0.0	IRF2BP2(1) - NTRK1(10)		Absent	Absent
chr2:29551347, chr2:29430138	C	ASSAYS_5P_3P	0.0	ALK		See Documentation	See Documentation

- In the **Variant Impact** tab, the variant impact heat map that displays is based on the predicted variant impact:

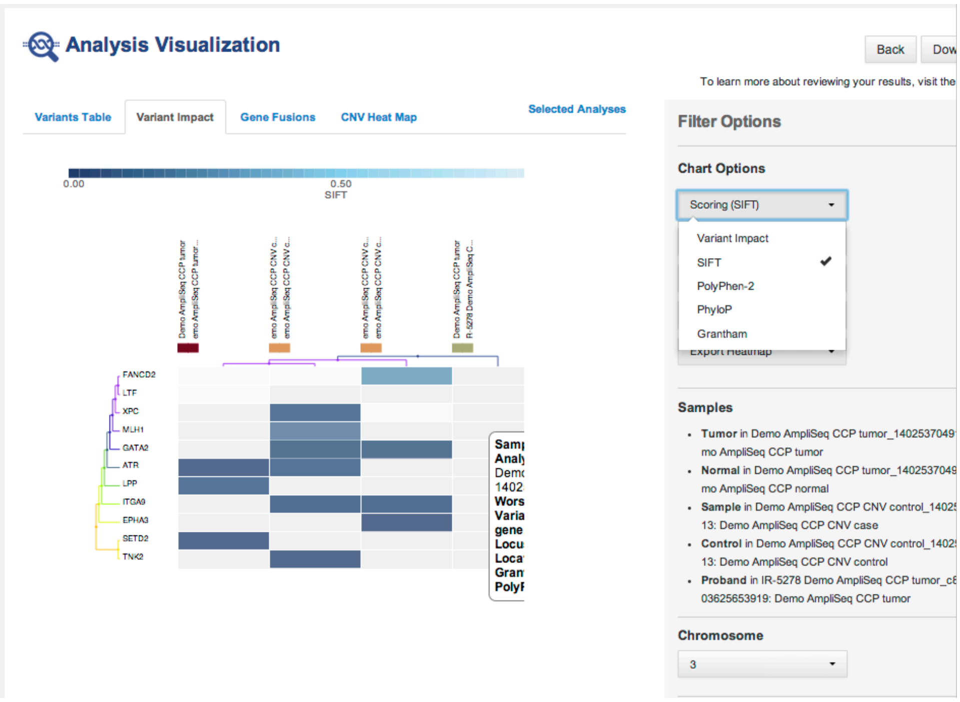
  The following is a variant impact heat map that is based on the predicted variant impact.



Sample: Demo AmpliSeq CCP CNV case
Analysis:
Demo AmpliSeq CCP CNV control 1402537049013
Worst score (effect) 4
Variant effect: non-frameshift insertion
gene: LTF
Locus: chr3:46501284
Location: exonic

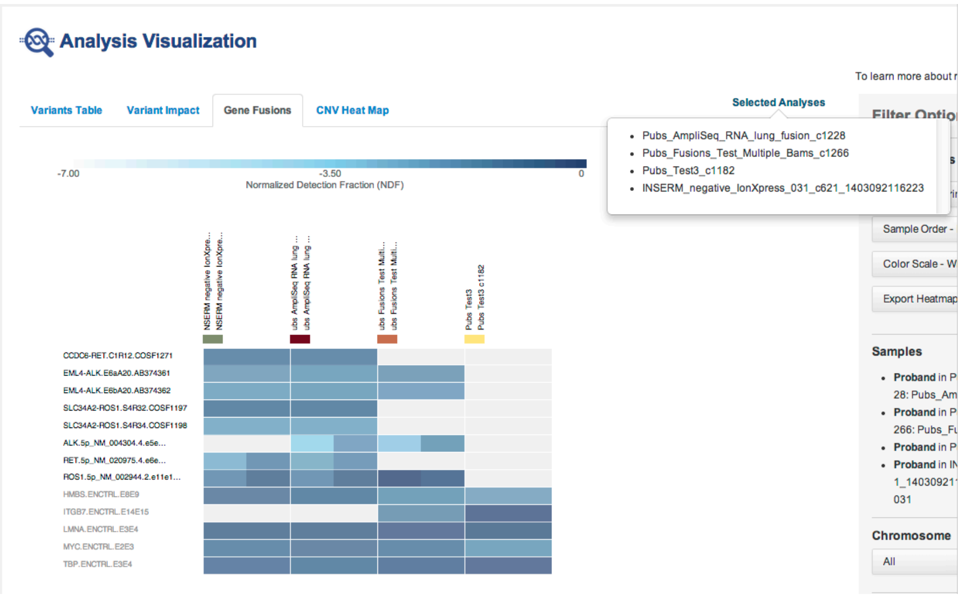
- **Tumor** in Demo AmpliSeq CCP tumor_140253704912
  mo AmpliSeq CCP tumor
- **Normal** in Demo AmpliSeq CCP tumor_140253704912
  mo AmpliSeq CCP normal
- **Sample** in Demo AmpliSeq CCP CNV control_1402537
  13: Demo AmpliSeq CCP CNV case
- **Control** in Demo AmpliSeq CCP CNV control_1402537
  13: Demo AmpliSeq CCP CNV control
- **Proband** in IR-5278 Demo AmpliSeq CCP tumor_c825
  03625653919: Demo AmpliSeq CCP tumor

The following is a variant impact heat map that is based on other scoring.



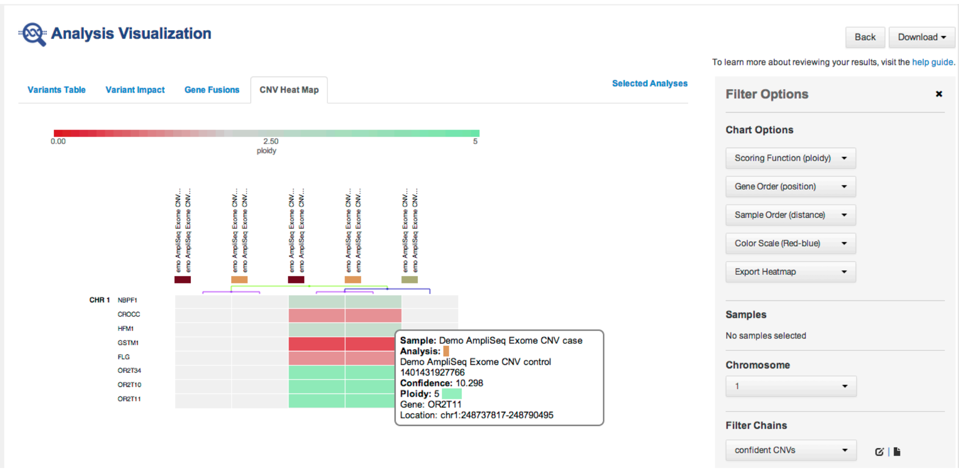
- **Tumor** in Demo AmpliSeq CCP tumor_1402537049 mo AmpliSeq CCP tumor
- **Normal** in Demo AmpliSeq CCP tumor_1402537049 mo AmpliSeq CCP normal
- **Sample** in Demo AmpliSeq CCP CNV control_1402 13: Demo AmpliSeq CCP CNV case
- **Control** in Demo AmpliSeq CCP CNV control_1402 13: Demo AmpliSeq CCP CNV control
- **Proband** in IR-5278 Demo AmpliSeq CCP tumor_c 03625653919: Demo AmpliSeq CCP tumor

The following is a Gene Fusions heat map



- Pubs_AmpliSeq_RNA_lung_fusion_c1228
- Pubs_Fusions_Test_Multiple_Bams_c1266
- Pubs_Test3_c1182
- INSERM_negative_IonXpress_031_c621_1403092116223

The following is CNV heat map.

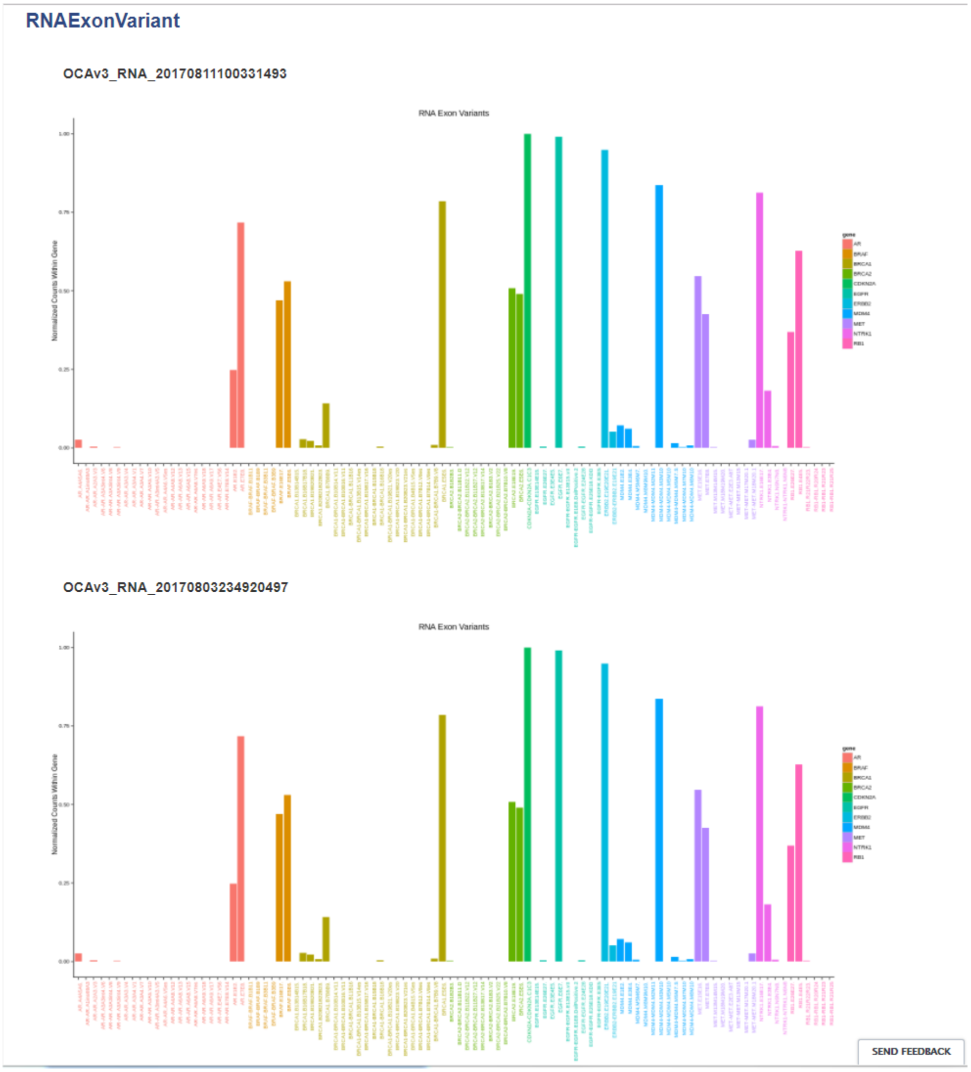


To learn more about reviewing your results, visit the help guide.

Sample: Demo AmpliSeq Exome CNV case
Analysis: 🟧
Demo AmpliSeq Exome CNV control
1401431927766
Confidence: 10.298
Ploidy: 5 🟩
Gene: OR2T11
Location: chr1:248737817-248790495

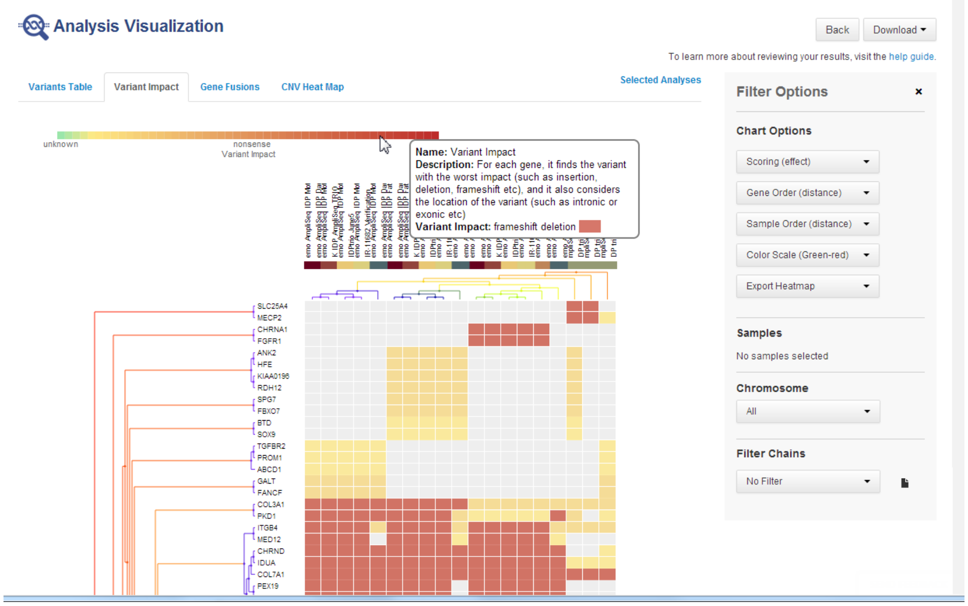
No samples selected

- RNA Exon Variants:



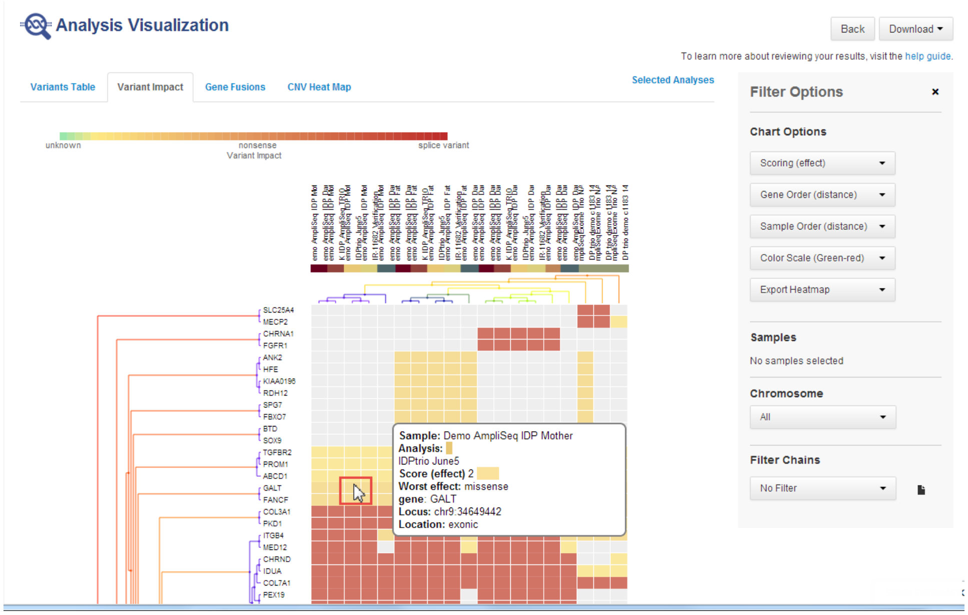
Note:

- In most cases, you will compare similar analyses. 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.
- Hover over the legend of a heat map:



Name: Variant Impact
Description: For each gene, it finds the variant with the worst impact (such as insertion, deletion, frameshift etc), and it also considers the location of the variant (such as intronic or exonic etc)
Variant Impact: frameshift deletion 🟥

Hover over the legend of a cell to see more information:



Sample: Demo AmpliSeq IDP Mother
Analysis: 🟨
IDPtrio June5
Score (effect) 2 🟨
Worst effect: missense
gene: GALT
Locus: chr9:34649442
Location: exonic

View original source of annotation information

You can view the original source of annotation information in Ion Reporter™ Software.

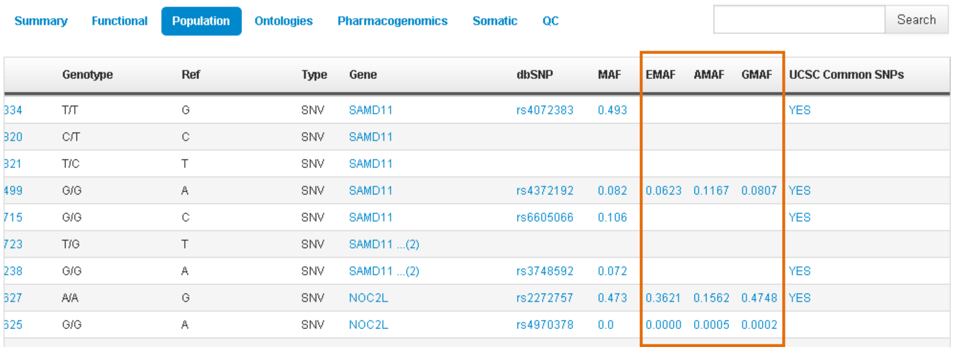
1. In the **Analyses** tab, click **Overview**.
2. Click the name of the analysis to open the **Analysis Results** table.
3. In the **Genes** column, click the link.

The link opens to a public database that contains the variant, for example, OMIM or COSMIC.

Links to external databases

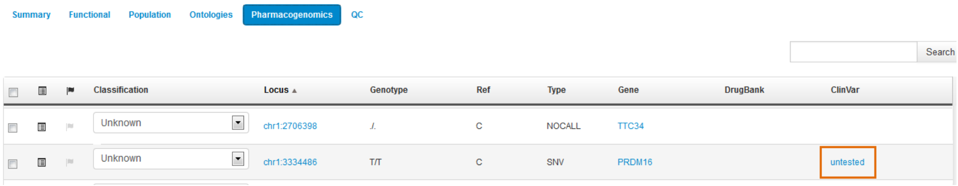
In the following cases, the links to external databases that are included in the **Analysis Results** screen can lead to database pages that are not specific, contain an error, or are blank.

- 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:



	Genotype	Ref	Type	Gene	dbSNP	MAF	EMAF	AMAF	GMAF	UCSC Common SNPs
334	T/T	G	SNV	SAMD11	rs4072383	0.493				YES
320	C/T	C	SNV	SAMD11						
321	T/C	T	SNV	SAMD11						
499	G/G	A	SNV	SAMD11	rs4372192	0.082	0.0623	0.1167	0.0807	YES
715	G/G	C	SNV	SAMD11	rs6605066	0.106				YES
723	T/G	T	SNV	SAMD11 ...(2)						
238	G/G	A	SNV	SAMD11 ...(2)	rs3748592	0.072				YES
327	A/A	G	SNV	NOC2L	rs2272757	0.473	0.3621	0.1562	0.4748	YES
325	G/G	A	SNV	NOC2L	rs4970378	0.0	0.0000	0.0005	0.0002	

- **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.**



☐			Classification	Locus ▲	Genotype	Ref	Type	Gene	DrugBank	ClinVar
☐			Unknown	chr1:2706398	./.	C	NOCALL	TTC34		
☐			Unknown	chr1:3334486	T/T	C	SNV	PRDM16		untested

- 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".

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 ▤ .

Customize the Analyses Results table

The **Analyses Results** table includes details that describe the samples, 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 **Analyses 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.

Default columns are included for all analyses. You can customize the **Analysis Results** table to more easily find analyses of interest, either by the date the analysis was run, or by sample, workflow, or analysis attributes. You can also add other available columns to customize the table.

Note: The **Subtype** column is not in use. It is reserved for future use.

Add attribute columns to the **Analysis Results** table

You can add analysis attributes to the **Analysis Results** table that you view in Ion Reporter™ Software. Columns in the **Analyses Results** table show the respective value for each attribute, if the analysis contains the attribute. An attribute can be associated with the analysis itself or with samples used in the analysis, or with the workflow that is used for the analysis. Sample attributes can be inherited from Torrent Suite™ Software and include information such as chip type and sequencing run details, and custom attributes.

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**.
4. In the **Select Columns** dialog box, select the analysis attributes that you want to add to the **Analysis Results** table, then click **Apply**.

The selected attributes 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 201.

Change the order of columns in the **Analysis Results** table

You can change the order 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.
   The column order remains until you close the screen.
4. (Optional) To 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 **Analysis Results** table preference is saved.

Create an **Analysis Results** table preference

You can create a custom table preference for the **Analysis Results** table in Ion Reporter™ Software. The table preference allows you to view only the columns of data that are relevant to your research.

The 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**.
4. In **Select Columns**, select any **Available Columns** that you want to add, then click **Apply**.
   The selected columns are added on the right side of the table.

5. To save the custom table as a preference:

Option	Description
Preferences Save Table Preference As	This setting allows you to name the custom 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 lick **Save**.

The new **Analysis Results** table preference is saved.

Delete an **Analysis Results** table preference

You can delete a custom 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 custom 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 custom 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.

Searches for the Analysis Results screen

Searches of analysis results can be performed in the **Analysis Results** screen with a controlled vocabulary query language. This allows advanced searches, including OR and AND searches. 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, rather than 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

Notation	Meaning
key:value*	key contains value
key:*	key exists (key has any value)
-key:value	key != value

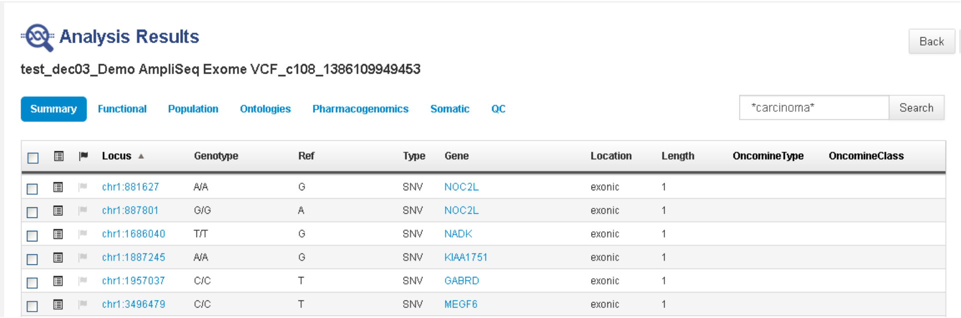
The following keys are supported:

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 your search string, matches at the beginning, middle, and end are all found.
- This example shows a search for *carcinoma*.

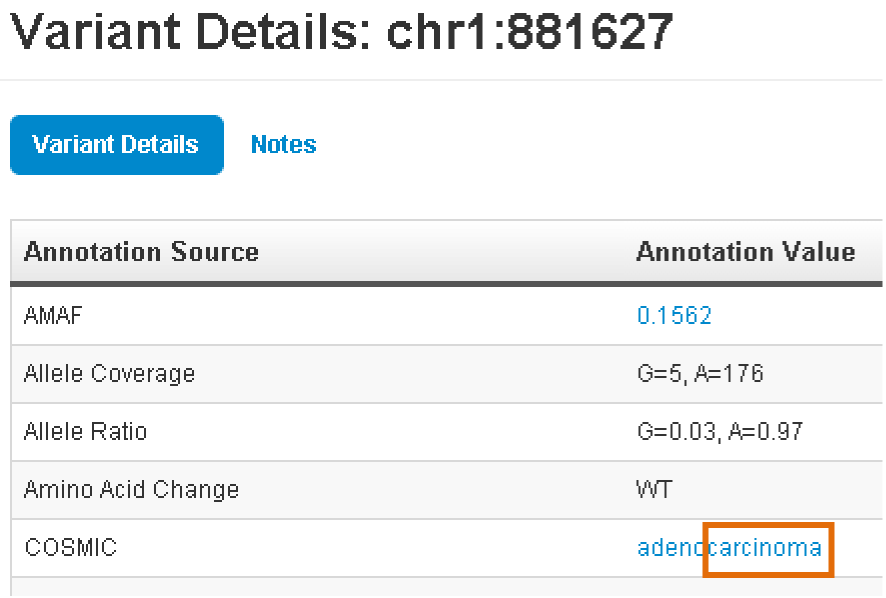


test_dec03_Demo AmpliSeq Exome VCF_c108_1386109949453

carcinoma

☐			Locus ▲	Genotype	Ref	Type	Gene	Location	Length	OncomineType	OncomineClass
☐			chr1:881627	A/A	G	SNV	NOC2L	exonic	1		
☐			chr1:887801	G/G	A	SNV	NOC2L	exonic	1		
☐			chr1:1686040	T/T	G	SNV	NADK	exonic	1		
☐			chr1:1887245	A/A	G	SNV	KIAA1751	exonic	1		
☐			chr1:1957037	C/C	T	SNV	GABRD	exonic	1		
☐			chr1:3496479	C/C	T	SNV	MEGF6	exonic	1		

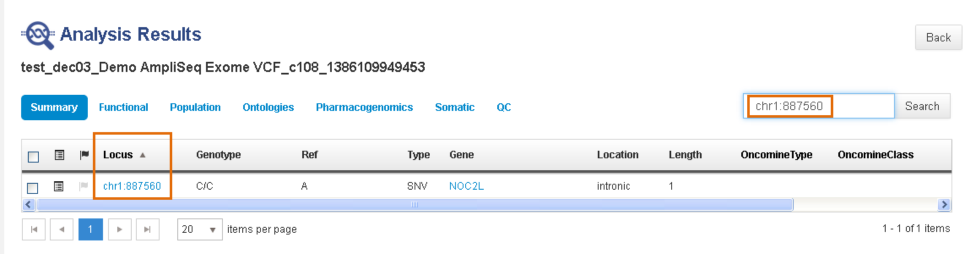
This search lists all variants that have an OMIM, COSMIC, ClinVar, DrugBank, and so on, annotation that contains "carcinoma" anywhere in the annotation. Click **(Details)** for one **(Flag)** of the matched variants to open its variant detail card. Scroll down to find the entries that contain "carcinoma".



Variant Details: chr1:881627

Annotation Source	Annotation Value
AMAF	0.1562
Allele Coverage	G=5, A=176
Allele Ratio	G=0.03, A=0.97
Amino Acid Change	WT
COSMIC	adenocarcinoma

- To search for a locus, enter the complete locus entry, with chromosome number and full position number.



test_dec03_Demo AmpliSeq Exome VCF_c108_1386109949453

chr1:887560

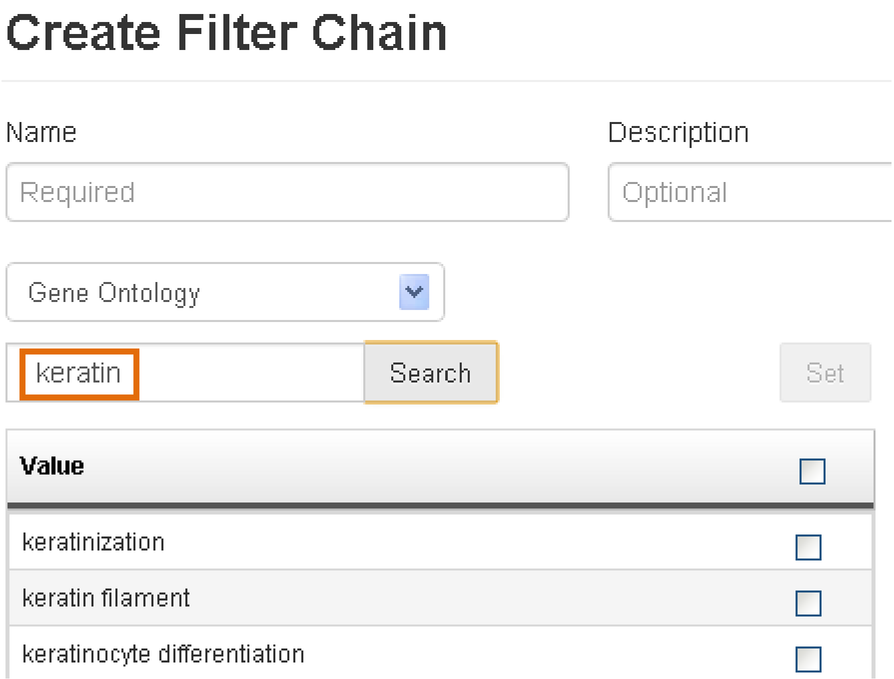
☐			Locus ▲	Genotype	Ref	Type	Gene	Location	Length	OncomineType	OncomineClass
☐			chr1:887560	C/C	A	SNV	NOC2L	intronic	1		

20 items per page

1 - 1 of 1 items

- 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.

- Search terms within a filter chain also do not allow an asterisk.



keratin

Value	☐
keratinization	☐
keratin filament	☐
keratinocyte differentiation	☐

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 less than 0.05 or are noval (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 your Oncomine™ assay user guide.

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.

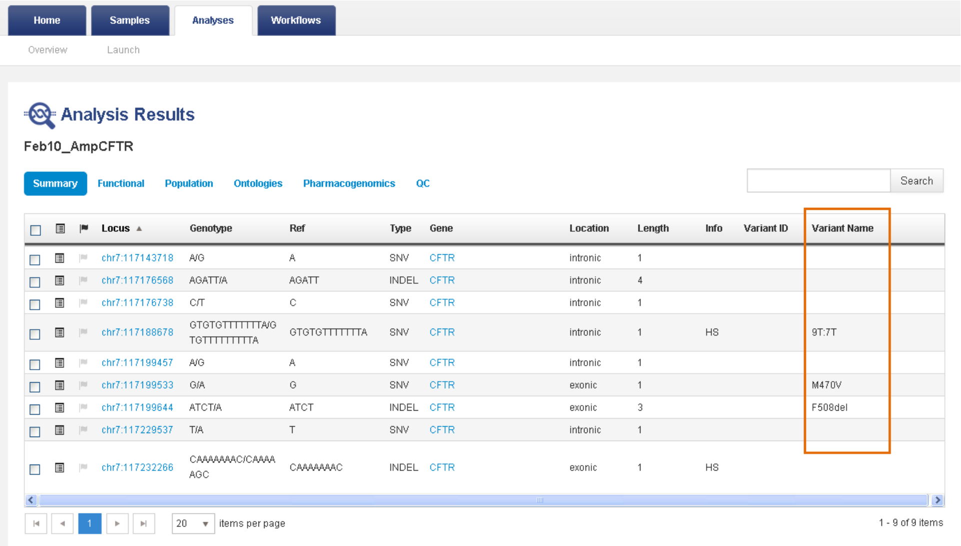
The CNV algorithm looks for heterogeneity and trends at the CNV_ID level, which may potentially, be indicative of data artifacts, and annotates the calls for CNV_IDs displaying these effects as SUSPECT as it is unclear whether the data for this CNV_ID indicates a true biological focal amplification.

- 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:

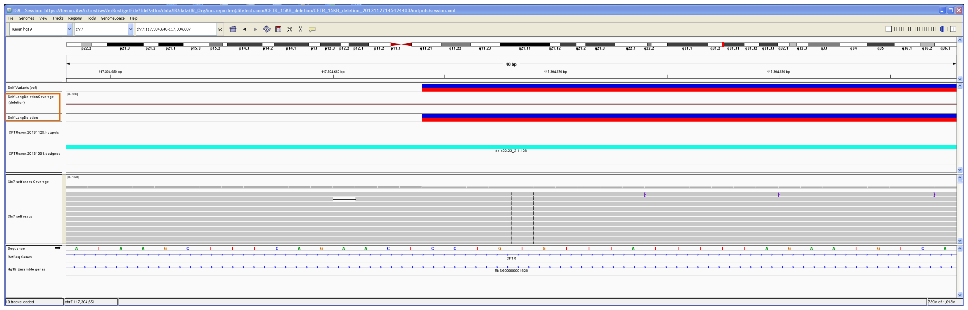
- The **Variant Name** column reports matches with the Named Variants annotation source.



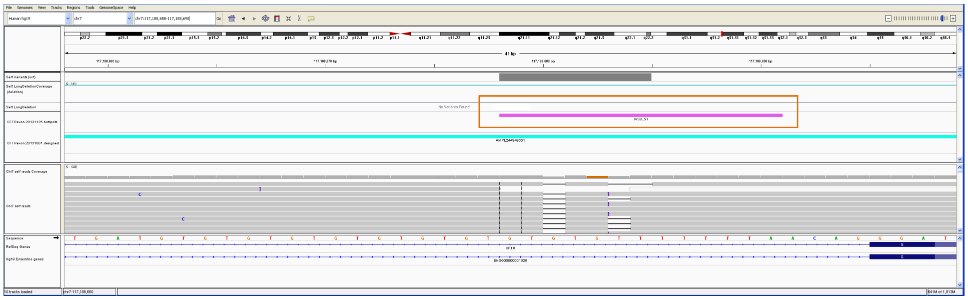
Feb10_AmpCFTR

Locus ▲	Genotype	Ref	Type	Gene	Location	Length	Info	Variant ID	Variant Name
chr7:117143718	A/G	A	SNV	CFTR	intronic	1			
chr7:117176568	AGATT/A	AGATT	INDEL	CFTR	intronic	4			
chr7:117176738	C/T	C	SNV	CFTR	intronic	1			
chr7:117188678	GTGTGTTTTTTTA/G TGTTTTTTTTTA	GTGTGTTTTTTTTA	SNV	CFTR	intronic	1	HS		9T:7T
chr7:117199457	A/G	A	SNV	CFTR	intronic	1			
chr7:117199533	G/A	G	SNV	CFTR	exonic	1			M470V
chr7:117199644	ATCT/A	ATCT	INDEL	CFTR	exonic	3			F508del
chr7:117229537	T/A	T	SNV	CFTR	intronic	1			
chr7:117232266	CAAAAAAAC/CAAAA AGC	CAAAAAAAC	INDEL	CFTR	exonic	1	HS		

- The following IGV visualization for CFTR analysis results include tracks for long deletions:

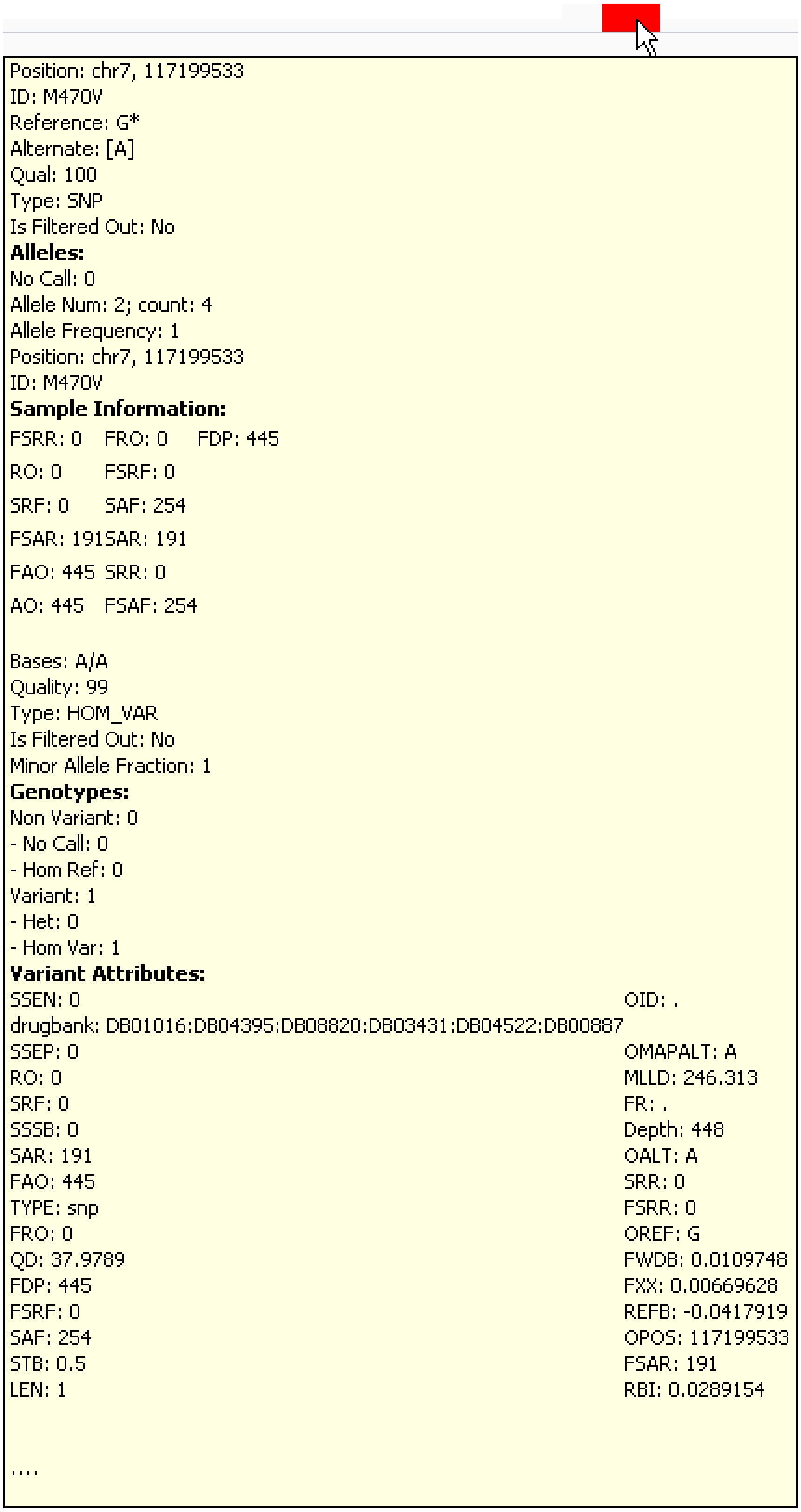


- The following IGV visualization for CFTR analysis results include a named variant:



- 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

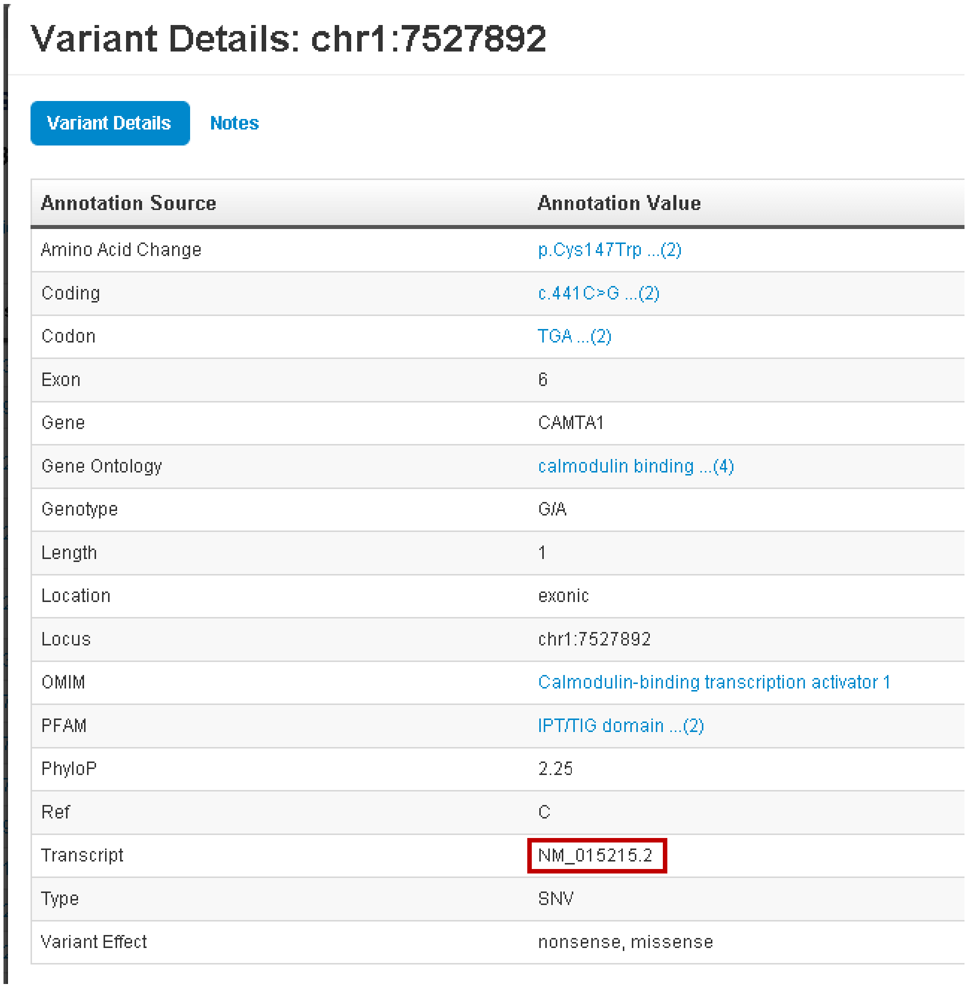
You can view the details about the annotations that Ion Reporter™ Software supports for a particular gene model transcript in the list of **Variant Details**, including the versions for the RefGene GeneModel and Ensembl GeneModel transcripts.

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: chr1:7527892

Annotation Source	Annotation Value
Amino Acid Change	p.Cys147Trp ...(2)
Coding	c.441C>G ...(2)
Codon	TGA ...(2)
Exon	6
Gene	CAMTA1
Gene Ontology	calmodulin binding ...(4)
Genotype	G/A
Length	1
Location	exonic
Locus	chr1:7527892
OMIM	Calmodulin-binding transcription activator 1
PFAM	IPT/TIG domain ...(2)
PhyloP	2.25
Ref	C
Transcript	NM_015215.2
Type	SNV
Variant Effect	nonsense, missense

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 your preferred transcript file.

Phred QUAL Score

Phred quality score column is added to the **Analysis Results** table.

Review analysis results for Ion AmpliSeq™ HD panels

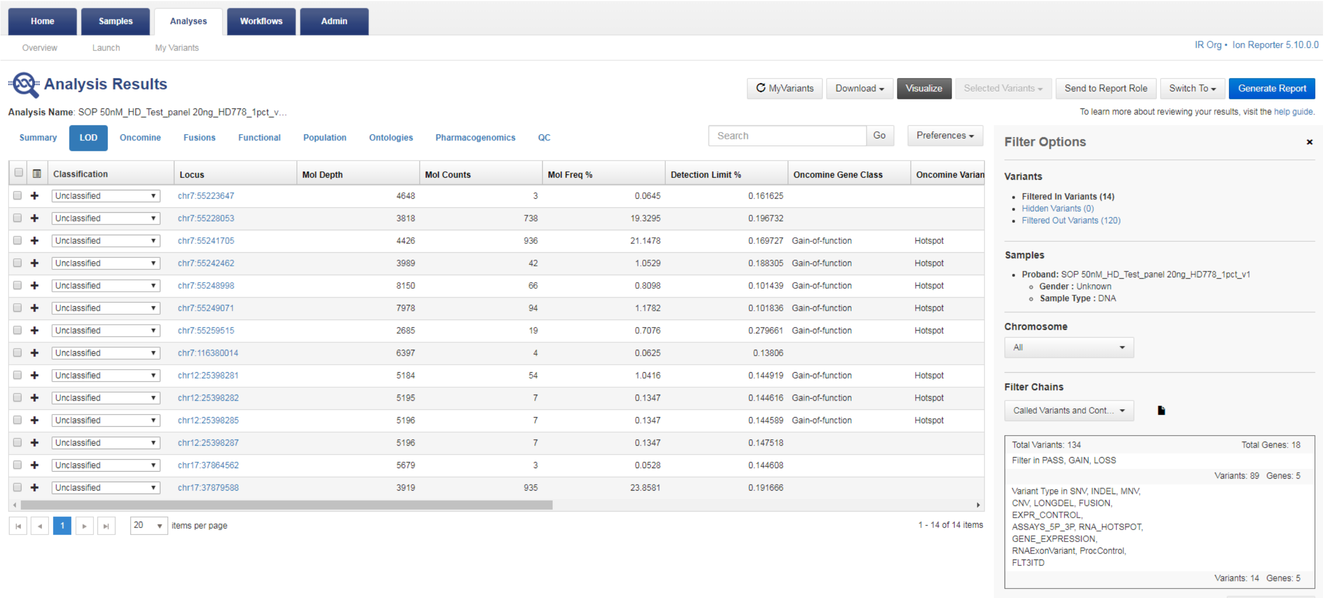
1. In the **Analyses** tab, click **Overview**.
   The **Analyses** table lists all of the available analyses results.
2. Click the column headings to sort the results. Alternatively, use the available filters or the **Search** function to limit the list of analyses.
3. In the **Analysis** column, click the link to open an analysis of interest.
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	Default filter chain for the Ion AmpliSeq™ HD analysis workflows. Use this filter chain for analysis results that report 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 FLT3ITD.
Called Hotpot Variants and Controls	Select this filter chain for analysis results that report all hotspot variants that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNP and INDEL.
Variant Matrix Summary	Select this filter chain for analysis results that replicate data that is shown for Ion AmpliSeq™ HD analysis results in the Variant Matrix Summary. Variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants.
Oncomine Variants (5.10 or later)	Apply the **Oncomine Variants (5.10 or later)** 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.

Example of Analysis Results screen, LOD view, and Filter Options pane



Analysis Name: SOP 50nM_HD_Test_panel 20ng_HD778_1pct_v...

To learn more about reviewing your results, visit the help guide.

☐		Classification	Locus	Mol Depth	Mol Counts	Mol Freq %	Detection Limit %	Oncomine Gene Class	Oncomine Varian
☐	+	Unclassified	chr7:55223647	4648	3	0.0645	0.161625		
☐	+	Unclassified	chr7:55228053	3818	738	19.3295	0.196732		
☐	+	Unclassified	chr7:55241705	4426	936	21.1478	0.169727	Gain-of-function	Hotspot
☐	+	Unclassified	chr7:55242462	3989	42	1.0529	0.188305	Gain-of-function	Hotspot
☐	+	Unclassified	chr7:55248998	8150	66	0.8098	0.101439	Gain-of-function	Hotspot
☐	+	Unclassified	chr7:55249071	7978	94	1.1782	0.101836	Gain-of-function	Hotspot
☐	+	Unclassified	chr7:55259515	2685	19	0.7076	0.279661	Gain-of-function	Hotspot
☐	+	Unclassified	chr7:116380014	6397	4	0.0625	0.13806		
☐	+	Unclassified	chr12:25398281	5184	54	1.0416	0.144919	Gain-of-function	Hotspot
☐	+	Unclassified	chr12:25398282	5195	7	0.1347	0.144616	Gain-of-function	Hotspot
☐	+	Unclassified	chr12:25398285	5196	7	0.1347	0.144589	Gain-of-function	Hotspot
☐	+	Unclassified	chr12:25398287	5196	7	0.1347	0.147518		
☐	+	Unclassified	chr17:37864562	5679	3	0.0528	0.144608		
☐	+	Unclassified	chr17:37879588	3919	935	23.8581	0.191666		

1 | 20 items per page

1 - 14 of 14 items

Variants

- **Filtered In Variants (14)**
- Hidden Variants (0)
- Filtered Out Variants (120)

Samples

- **Proband:** SOP 50nM_HD_Test_panel 20ng_HD778_1pct_v1
  - **Gender :** Unknown
  - **Sample Type :** DNA

Chromosome

All

Filter Chains

Called Variants and Cont...

Total Variants: 134 Total Genes: 18
Filter in PASS, GAIN, LOSS
Variants: 89 Genes: 5
Variant Type in SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, FLT3ITD
Variants: 14 Genes: 5

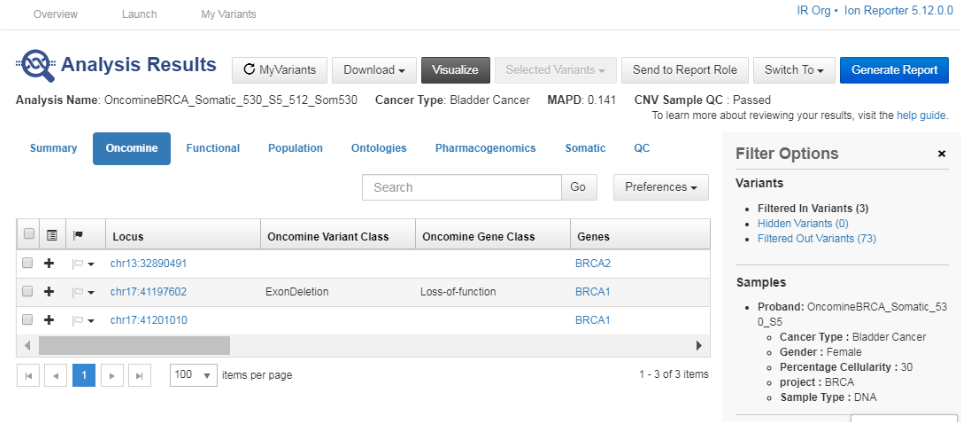
BRCA research analysis results

View BRCA analysis results

The Oncomine™ BRCA Research Assay that is used with Ion Reporter™ Software enables 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. Click the hyperlink for the analysis of interest to open a table of BRCA analysis results.



Analysis Name: OncomineBRCA_Somatic_530_S5_512_Som530 **Cancer Type**: Bladder Cancer **MAPD**: 0.141 **CNV Sample QC** : Passed

To learn more about reviewing your results, visit the help guide.

Locus	Oncomine Variant Class	Oncomine Gene Class	Genes
chr13:32890491			BRCA2
chr17:41197602	ExonDeletion	Loss-of-function	BRCA1
chr17:41201010			BRCA1

Filter Options

Variants

- Filtered In Variants (3)
- Hidden Variants (0)
- Filtered Out Variants (73)

Samples

- **Proband:** OncomineBRCA_Somatic_530_S5
  - **Cancer Type :** Bladder Cancer
  - **Gender :** Female
  - **Percentage Cellularity :** 30
  - **project :** BRCA
  - **Sample Type :** DNA

3. 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, and sort and select variants of interest. 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 by the sample attribute in Torrent Suite™ Software, if sample data is 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.

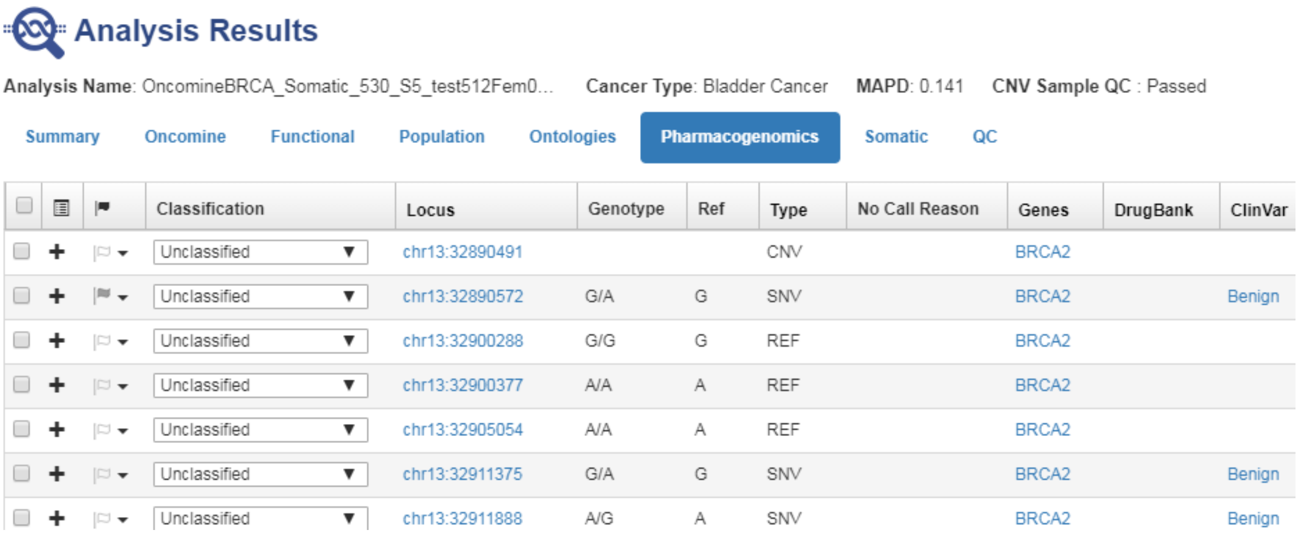
Item	Description
CNV Sample QC	A quality control score that is based on the MAPD threshold. **CNV Sample QC** is PASSED or FAILED. If failed, a reason is provided in a message.
Sample ID	**Note:** If the sample data is transferred from Torrent Suite™ Software and the SampleID plugin was run, a Sample ID is displayed at the top of the **Analysis Results** screen, and on the **Generate Report** page 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
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.



Analysis Results

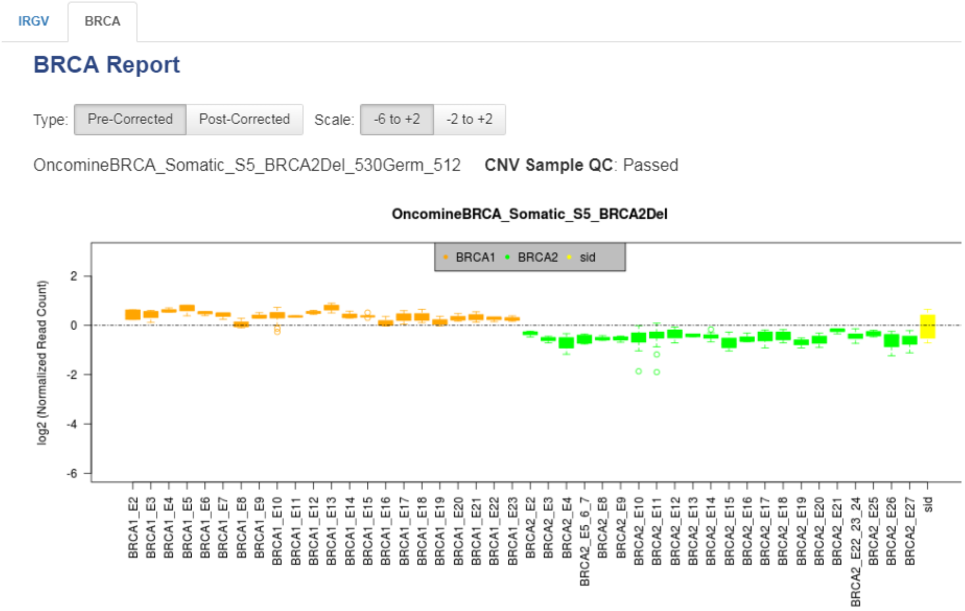
Analysis Name: OncomineBRCA_Somatic_530_S5_test512Fem0... **Cancer Type**: Bladder Cancer **MAPD**: 0.141 **CNV Sample QC** : Passed

☐			Classification	Locus	Genotype	Ref	Type	No Call Reason	Genes	DrugBank	ClinVar
☐	+		Unclassified	chr13:32890491			CNV		BRCA2		
☐	+		Unclassified	chr13:32890572	G/A	G	SNV		BRCA2		Benign
☐	+		Unclassified	chr13:32900288	G/G	G	REF		BRCA2		
☐	+		Unclassified	chr13:32900377	A/A	A	REF		BRCA2		
☐	+		Unclassified	chr13:32905054	A/A	A	REF		BRCA2		
☐	+		Unclassified	chr13:32911375	G/A	G	SNV		BRCA2		Benign
☐	+		Unclassified	chr13:32911888	A/G	A	SNV		BRCA2		Benign

BRCA Report

The Oncomine™ BRCA Research Assay used with Ion Reporter™ Software enables the detection and visualization of whole exon and multiple exon deletion in BRCA1 and BRCA2 genes in somatic and germline samples with high sensitivity.

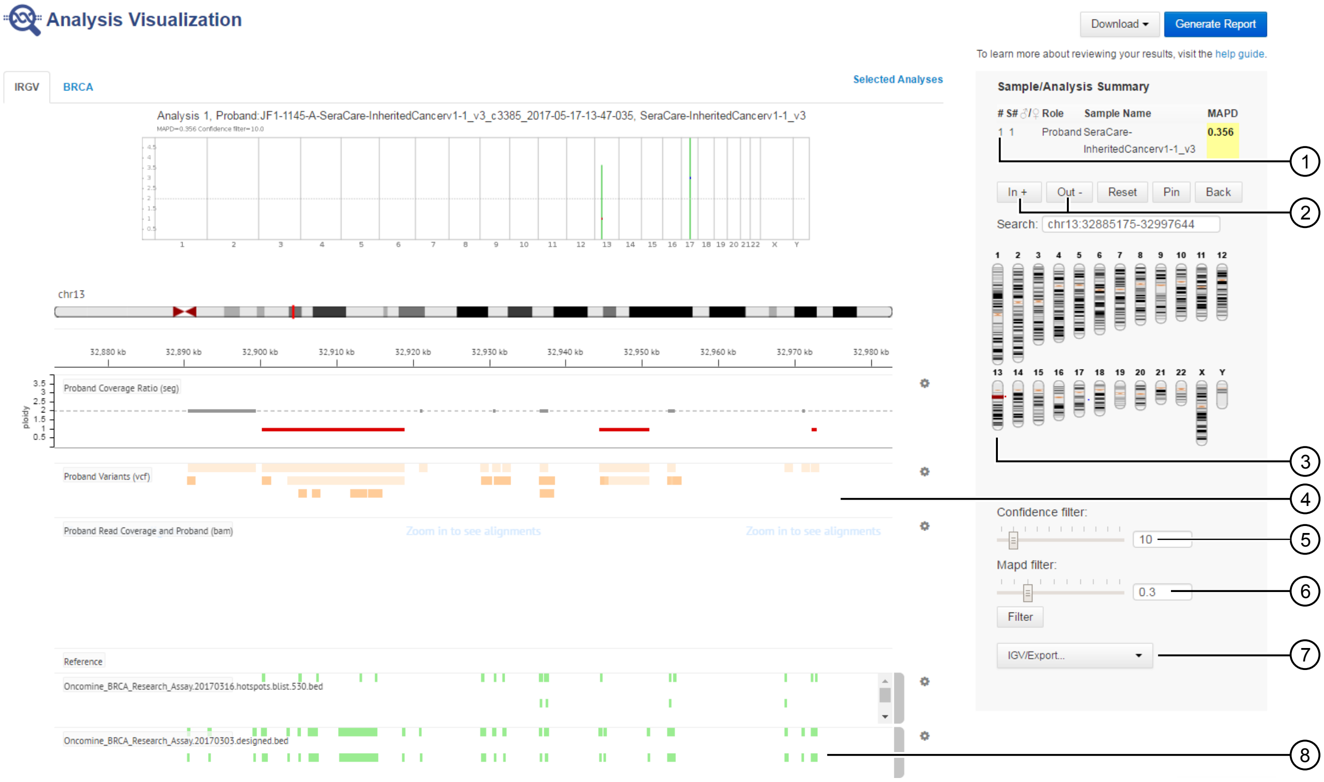
- When you open a visualization for BRCA analysis results, you can view a **BRCA Report** that shows read counts of BRCA1 and BRCA2 exons that are normalized to the Oncomine BRCA DNA Baseline.



BRCA Report

OncomineBRCA_Somatic_S5_BRCA2Del_530Germ_512 **CNV Sample QC**: Passed

- In the **IRGV** tab, you can view exon deletions or duplications on chromosomes 13 and 17.



To learn more about reviewing your results, visit the help guide.

Analysis 1, Proband:JF1-1145-A-SeraCare-InheritedCancerv1-1_v3_c3385_2017-05-17-13-47-035, SeraCare-InheritedCancerv1-1_v3

MAPD=0.356 Confidence filter=10.0

#	S#	♂/♀	Role	Sample Name	MAPD
1	1		Proband	SeraCare-InheritedCancerv1-1_v3	0.356

Search: chr13:32885175-32997644

Confidence filter: 10

Mapd filter: 0.3

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 Scroll down in this section to see more of the alignments or hotspots.

⑤ Slide the **Confidence filter** to change the value that is applied to the score that is reported for the CNV on the **Analysis Results** screen.

⑥ Slide the **MAPD filter** This filter applies to the entire sample. If the reported MAPD value is above the selected **MAPD filter** value, no CNVs are displayed in the BRCA Report.

⑦ 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.

⑧ Oncomine BRCA Research Assay designed BED file alignment. This **IRGV** track shows the alignment of the amplicons to the reference sequence.

Immune repertoire analysis results

Analyses that are performed in Ion Reporter™ Software with Oncomine™ Immune repertoire analysis workflows report the frequency and sequence features of clonotypes, and provide other data results, such as clone frequency and Jaccard Similarity indices. There are interactive plots and graphs 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 formats. Static plots in PDF format can be in slides for presentations.

The Immune Repertoire Results Report also includes sample and quality control results for each sample that is included in the analysis.

View Immune Repertoire analysis results

If you selected an Immune Repertoire analysis workflow in the Planned Run in Torrent Suite™ Software, and selected the option to automatically upload results to Ion Reporter™ Software, you can view the analysis results in Ion Reporter™ Software.

For instructions to manually launch an analysis, see "Manually launch an analysis" on page 160.

To perform a multi-sample analysis, see "Compare the immune repertoire between samples" on page 234.

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.

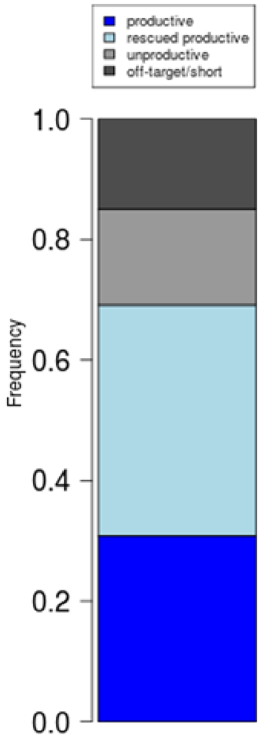
   You can further refine the list of analyses with other filters, or click column headings to sort the list.
3. Click the analyses name hyperlink.
4. In the **Immune Repertoire Results** summary screen, click the hyperlinked **Sample** name to open the **Immune Repertoire Results** for that sample.
5. Select the **Sample Results** , **Sample QC** , or **Plugin Results** tab, then select the graphical representation of the data from the **Views** list.

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.

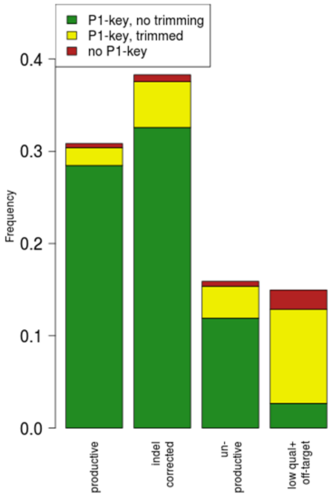
Read classification

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.	

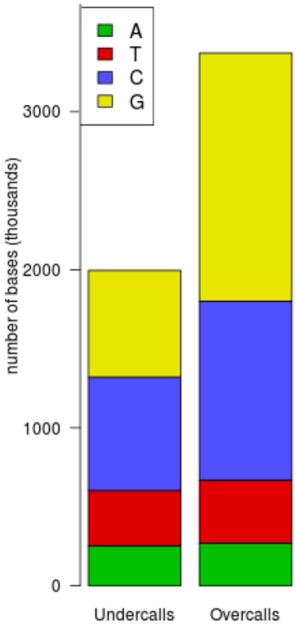
Proportion of full length, quality trimmed and reads lacking P1-key, by read classification

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

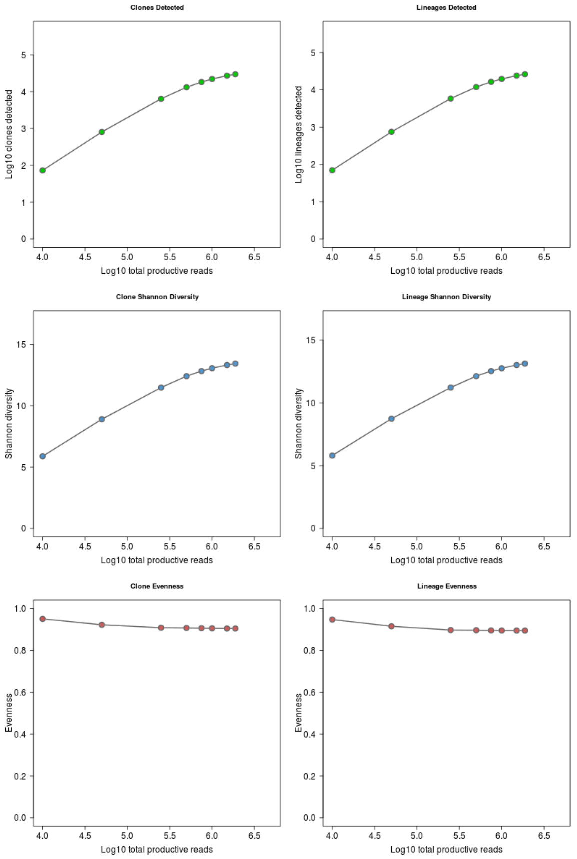
Stacked bar plot indicating the nucleotide composition of overcalled bases (base insertion sequencing errors) and undercalled bases (base deletion sequencing errors). Highly skewed nucleotide composition can indicate lower quality sequencing or low library diversity.

Color	Description	Example
Yellow	Guanidine (G)	
Blue	Cytosine (C)	
Ted	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.

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.

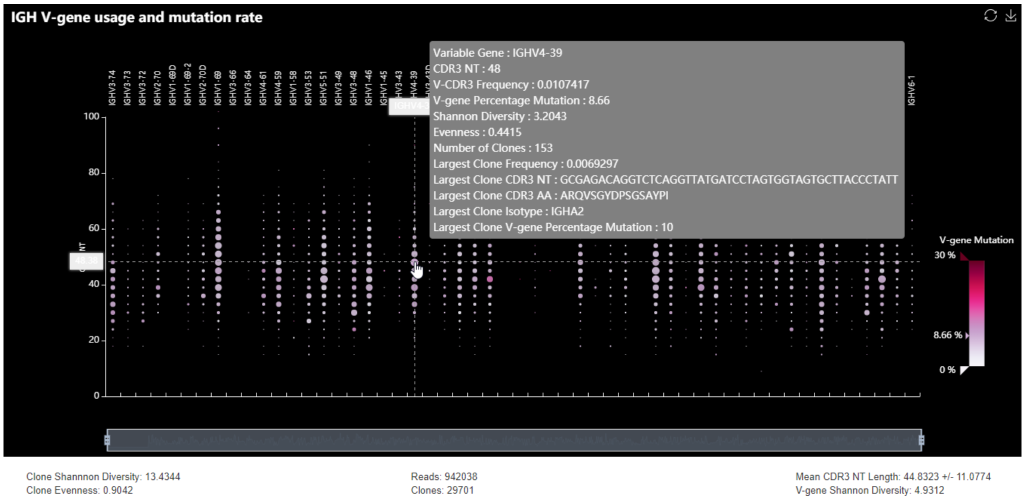
Spectratyping plots

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 forth metric specific to each graph (for example, Shannon Diversity, evenness, clone frequency). Key repertoire metrics are displayed along the lower margin of the plot.

In Ion Reporter™ Software, the spectratyping plots are interactive, allowing you to adjust the data and access clone details. Drag the ends of the horizontal bar below the X-axis to limit the region (v-genes) to view in the plot. Drag the ends of the color range up or down to limit the clones that are viewed. Hover your cursor over any dot to view the details of an individual clone. Click ⟳ to restore the default plot view, click ⤓ to download a static image of the plot (if you have adjusted the plot view, the adjusted plot is downloaded).

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 spetratyping plot.

IGH V-gene usage and mutation rate



Variable Gene : IGHV4-39
CDR3 NT : 48
V-CDR3 Frequency : 0.0107417
V-gene Percentage Mutation : 8.66
Shannon Diversity : 3.2043
Evenness : 0.4415
Number of Clones : 153
Largest Clone Frequency : 0.0069297
Largest Clone CDR3 NT : GCGAGACAGGTCTCAGGTTATGATCCTAGTGGTAGTGCTTACCCTATT
Largest Clone CDR3 AA : ARQVSGYDPSGSAYPI
Largest Clone Isotype : IGHA2
Largest Clone V-gene Percentage Mutation : 10

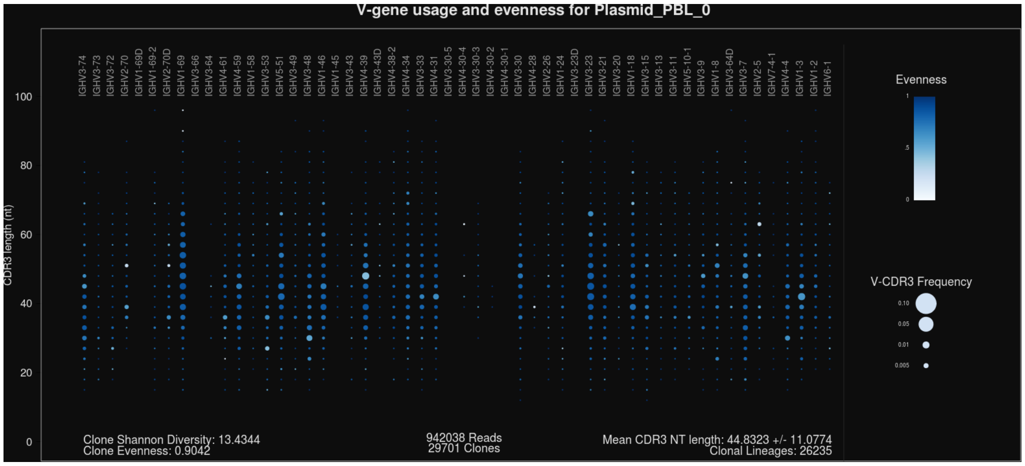
Clone Shannnon Diversity: 13.4344
Clone Evenness: 0.9042

Reads: 942038
Clones: 29701

Mean CDR3 NT Length: 44.8323 +/- 11.0774
V-gene Shannon Diversity: 4.9312

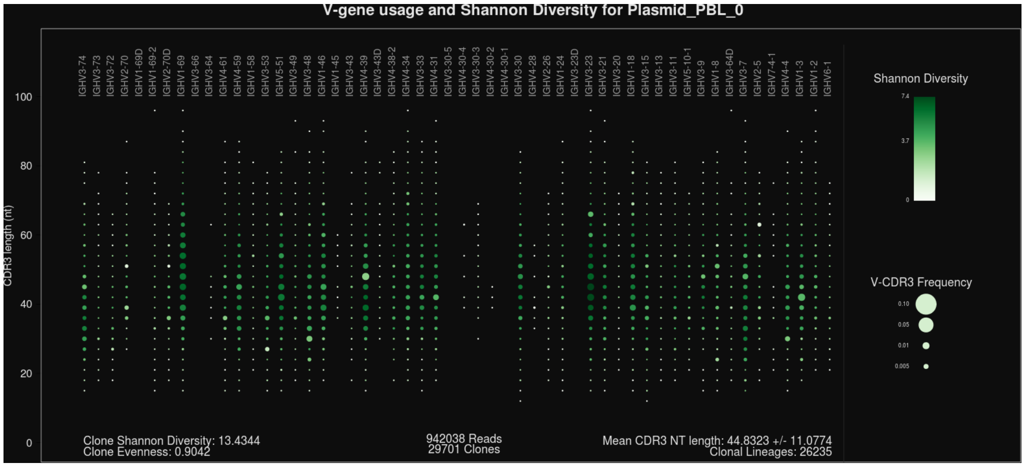
Spectratyping plot highlighting frequency of mutated bases over the variable gene of identified clones. Circle color indicates the average frequency of mutated bases for clones having a particular variable gene-CDR3 nucleotide length combination. B cells that have undergone isotype switching tend to 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 above the interactive spectratyping plot for Oncomine™ BCR IGH-LR Assay analyses.

IGH V-gene usage and evenness



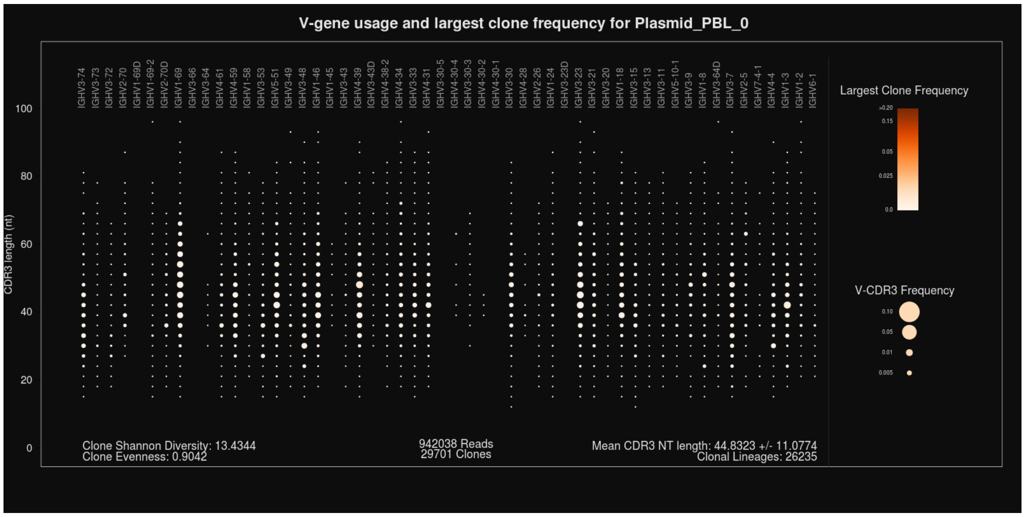
Spectratyping plot highlighting evenness of identified clone sizes (Normalized Shannon Entropy). Circle color indicates the evenness of clone sizes for clones having a particular variable gene-CDR3 nucleotide length combination. Values range from 0 to 1, with 1 indicating most even clone sizes. In this 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 above the interactive spectratyping plot for Oncomine™ BCR IGH-LR Assay analyses.

IGH V-gene usage and Shannon Diversity



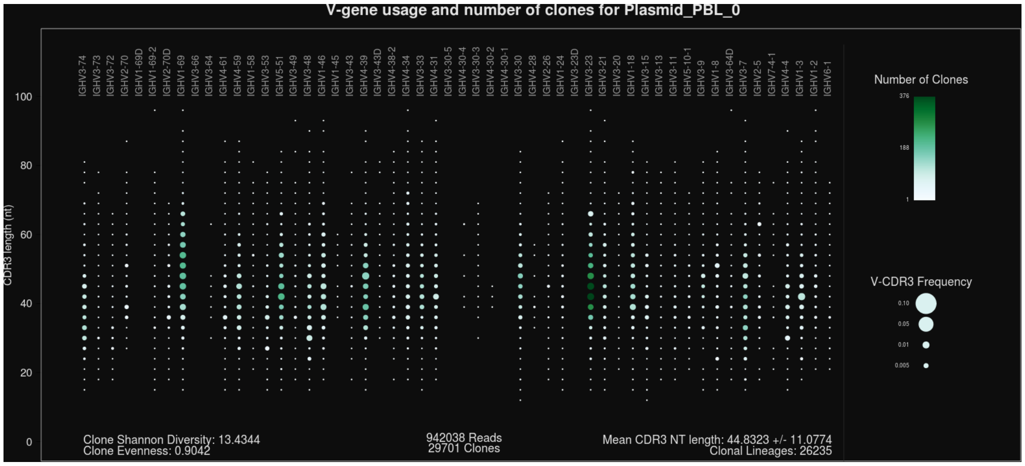
Spectratyping plot highlighting Shannon Diversity (entropy) of identified clones. Circle color indicates the Shannon Diversity of clones having a particular variable gene-CDR3 nucleotide length combination. Portions of the repertoire containing highly expanded clones typically have a corresponding low Shannon Diversity value. You can further partition the clones by isotype in Ion Reporter™ Software, with the buttons that above the interactive spectratyping plot for Oncomine™ BCR IGH-LR Assay analyses.

IGH V-gene usage and largest clone frequency



Spectratyping plot highlighting the frequency of the largest clone for each variable gene-CDR3 nucleotide length combination. 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 above the interactive spectratyping plot for Oncomine™ BCR IGH-LR Assay analyses.

IGH V-gene usage and number of clones



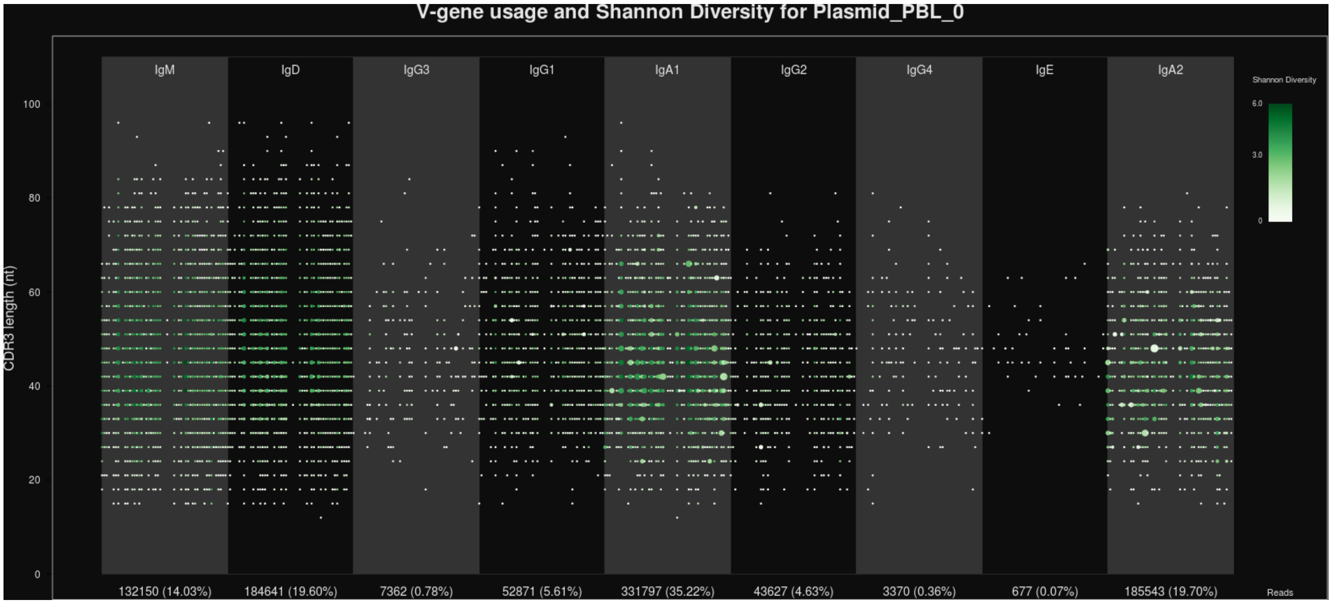
Spectratyping plot highlighting the number of clones that are identified for each variable gene-CDR3 nucleotide length combination. Circle color indicates the number of clones having a particular variable gene-CDR3 nucleotide length combination. Specific variable genes may more frequently participate 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 above the interactive spectratyping plot for Oncomine™ BCR IGH-LR Assay analyses.

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. Ordering of the isotypes reflects their position within the IGH locus.

Overview plots are generated for the Oncomine™ BCR IGH-LR Assay, and are available only in the compressed (zipped) results folder.

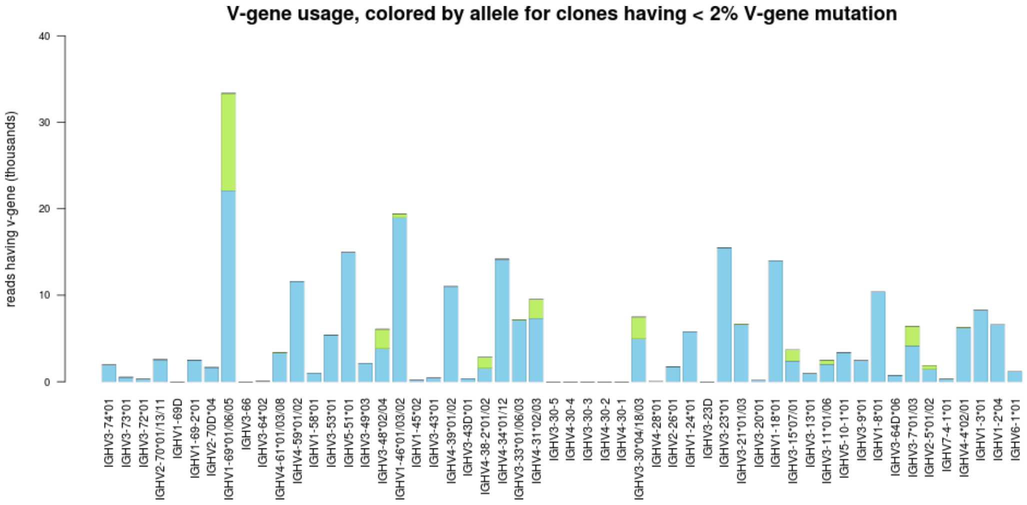
IGH V-gene usage and Shannon Diversity spectratyping overview plot



V-gene usage

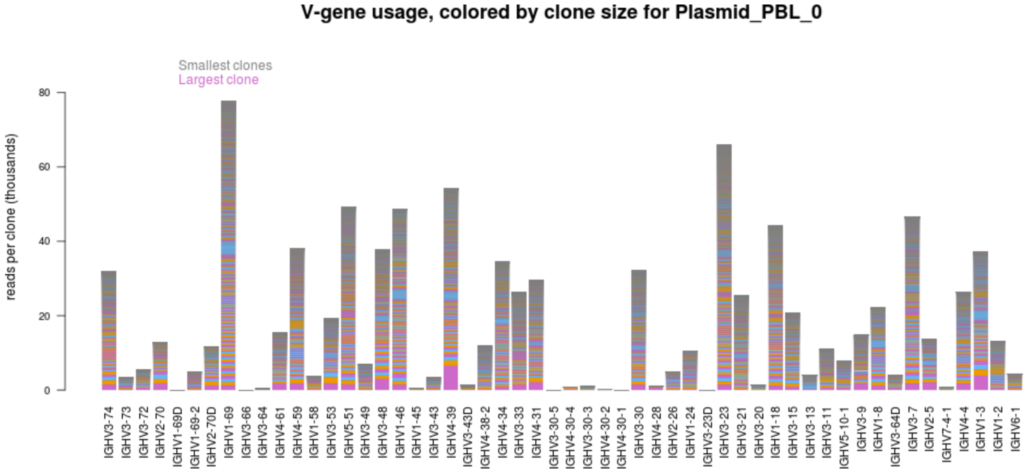
Immune Repertoire Analysis Results report the frequency of variable genes and the variable gene allele. Select **V-gene usage** from the **Views** list to see graphs that represent the results. Stacked barplots indicate the representation of variable genes among identified clones.

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

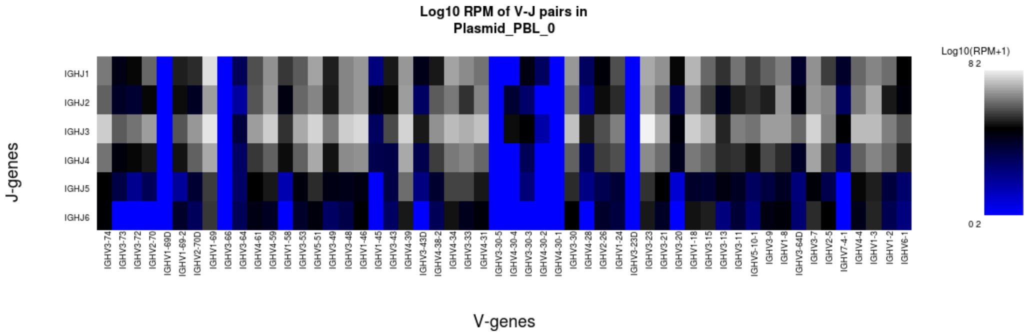


Color segments in each bar indicate the contribution of individual clones, arranged from smallest clones (top) to largest clones (bottom).

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

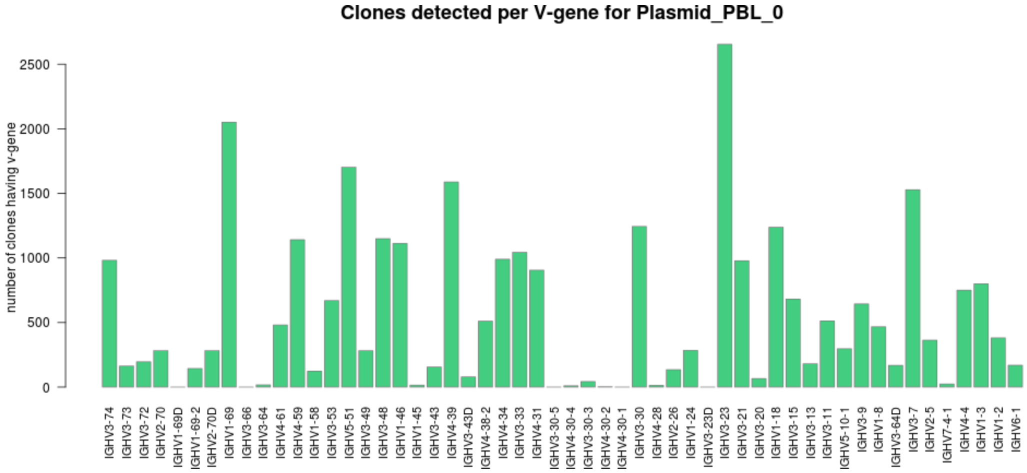


Heatmap illustrating the frequency of particular Variable gene-Joining gene combinations for identified clones. Frequencies are log transformed with pseudocount added. Over-represented Variable-Joining gene combinations (white) can indicate the presence of a highly expanded clone.

Clones detected per variable gene

The Oncomine™ BCR IGH Assay and Oncomine™ TCR Beta Assay 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.

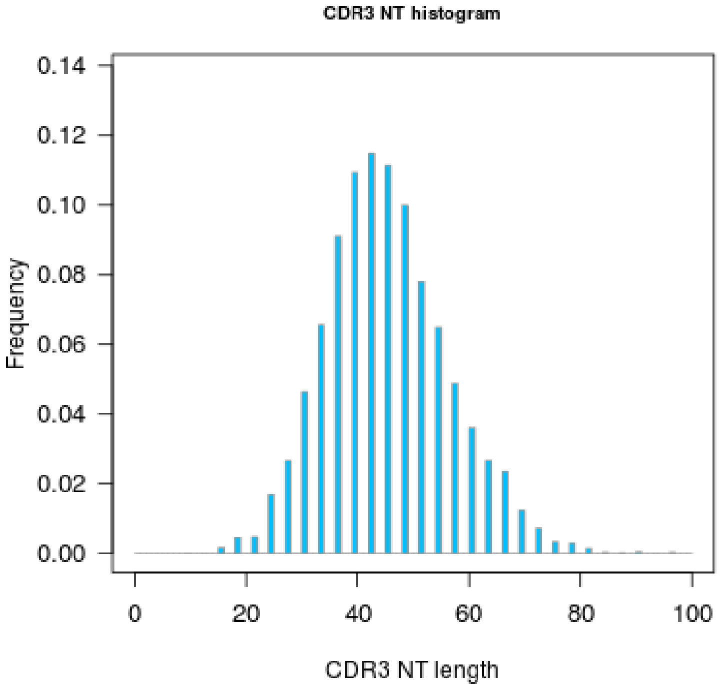


The bar plot indicates the number of identified clones having a particular variable gene. Ordering of variable genes reflects position within the IGH locus.

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)



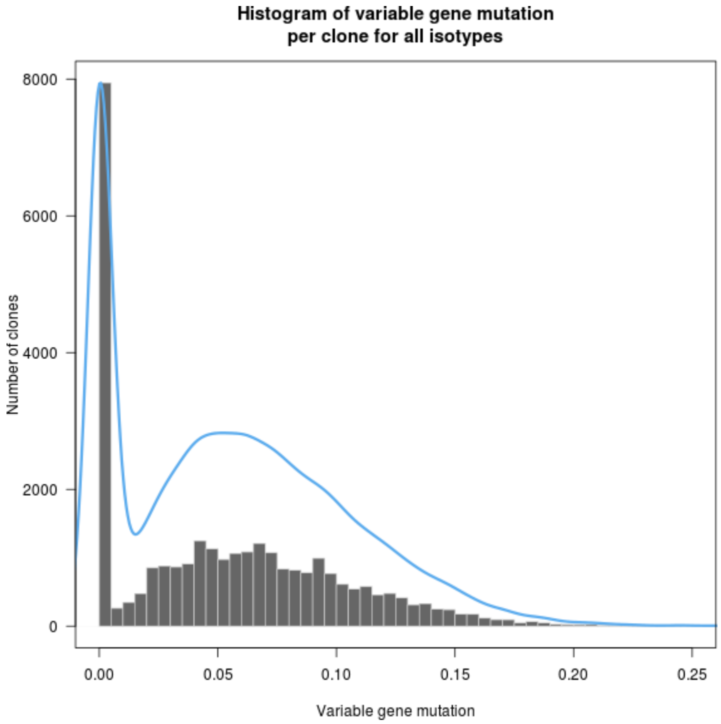
The histogram indicates the distribution of CDR3 lengths for clones that are identified in the sample.

Somatic hypermutation profile

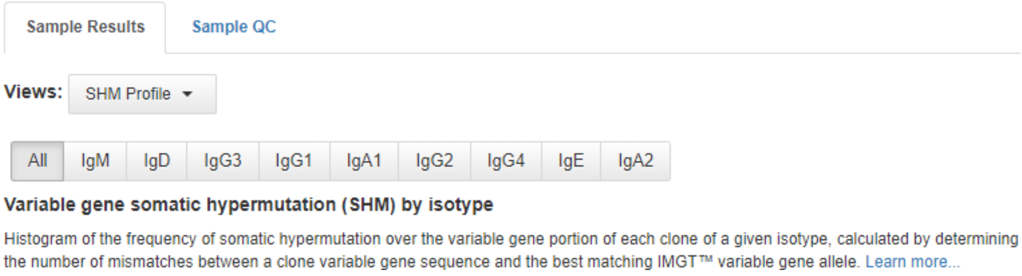
Immune Repertoire Analysis Results report the frequency of somatic hypermutation (SHM) over the variable gene portion of each clone of a given isotype. This view is available only for the Oncomine™ BCR IGH-LR Assay and Oncomine™ BCR IGH-SR Assay. Select **SHM profile** from the **Views** list to see a histogram that represents the data.

SHM is calculated by determining the number of mismatches between a clone variable gene sequence and the best 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.



Variable gene somatic hypermutation (SHM) by isotype

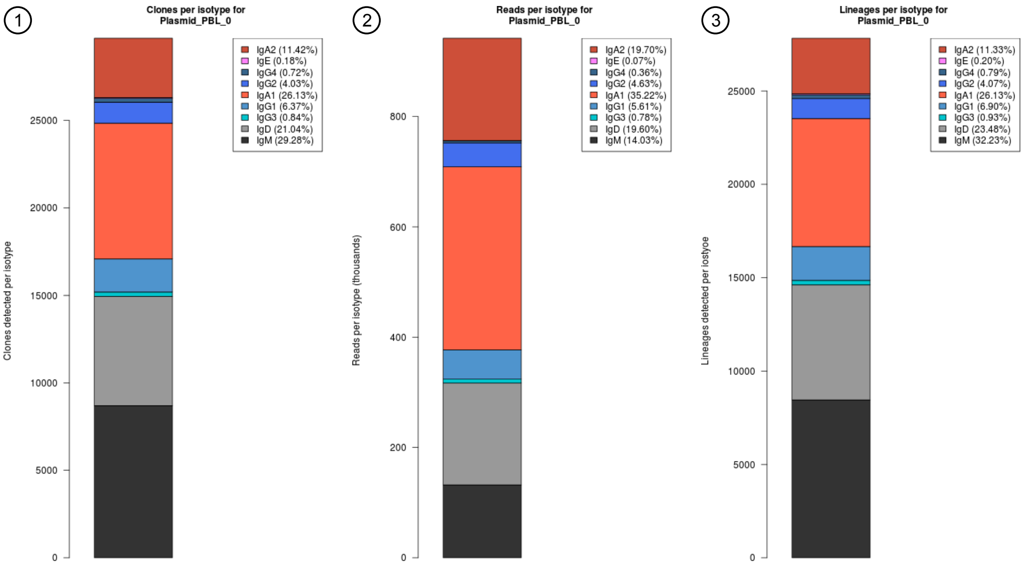
Histogram of the frequency of somatic hypermutation over the variable gene portion of each clone of a given isotype, calculated by determining the number of mismatches between a clone variable gene sequence and the best matching IMGT™ variable gene allele. Learn more...

Isotype usage

Immune Repertoire Analysis Results reports 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

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.

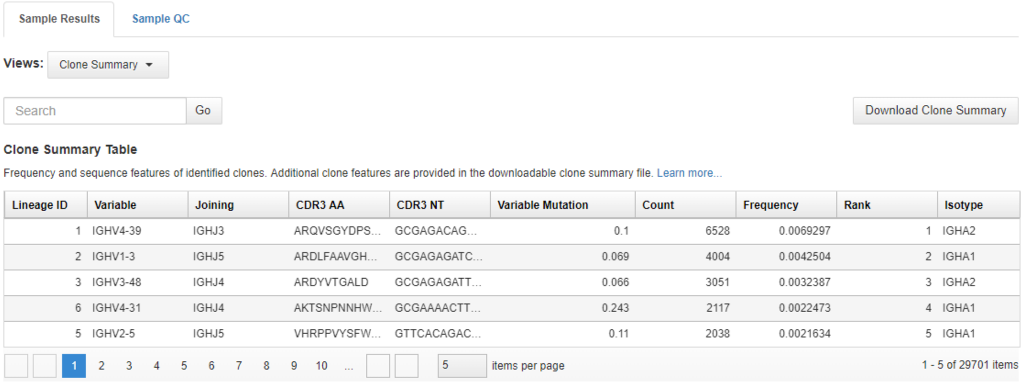
Each clone is assigned a Lineage ID. The ordering of the Lineage ID reflects the abundance ranking of the clonal lineage, which is calculated by determining the sum of the frequencies of all members of the clonal lineage. Lineage 1 corresponds to the most abundant lineage, followed by Lineage 2, until 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.



Clone Summary Table

Frequency and sequence features of identified clones. Additional clone features are provided in the downloadable clone summary file. Learn more...

Lineage ID	Variable	Joining	CDR3 AA	CDR3 NT	Variable Mutation	Count	Frequency	Rank	Isotype
1	IGHV4-39	IGHJ3	ARQVSGYDPS...	GCGAGACAG...	0.1	6528	0.0069297	1	IGHA2
2	IGHV1-3	IGHJ5	ARDLFAAVGH...	GCGAGAGATC...	0.069	4004	0.0042504	2	IGHA1
3	IGHV3-48	IGHJ4	ARDYVTGALD	GCGAGAGATT...	0.066	3051	0.0032387	3	IGHA2
6	IGHV4-31	IGHJ4	AKTSNPNNHW...	GCGAAAACTT...	0.243	2117	0.0022473	4	IGHA1
5	IGHV2-5	IGHJ5	VHRPPVYSFW...	GTTCACAGAC...	0.11	2038	0.0021634	5	IGHA1

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.

Variable	Joining	CDR3 AA	CDR3 NT	B212050_RNA_v1_c825_201...	B707172_RNA_v1_c753_201...
TRBV5-6	TRBJ2-2	ASSLGSGELF	GCCAGCAGCTTAGGTTCCGGGGAGCTGTTT	0.0874930	0.0000125
TRBV3-1	TRBJ1-3	ASSQKWQTGNTIY	GCCAGCAGCCAAAAGTGGCAGACCGGAAACACCATATAT	0.0163406	0

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.

Column name	Description
Variable	The best matching IMGT variable gene of the rearrangement.
Joining	The best 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 gene that differ from the best-matching IMGT variable gene. In B cells, such mismatching bases are largely derived from somatic hypermutation.
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 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) analyses 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.

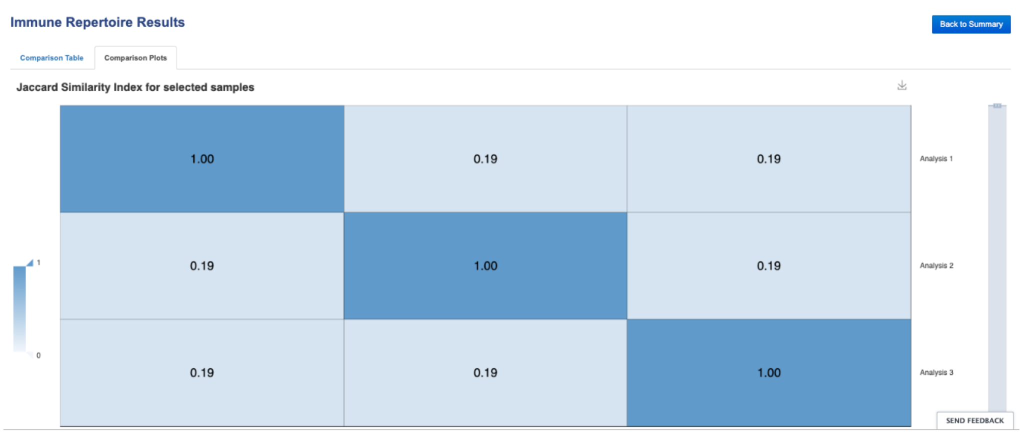
If you . . .	Then . . .
Select 2 samples, then click **Compare Samples**.	An interactive scatter plot for the selected samples is shown in the **Comparison Plots** tab.
Select 2 or more samples, then click **Compare Samples**.	A Jaccard Similarity Index for the selected samples is shown in the **Comparison 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 232.

  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 233.
- The interactive scatter plot indicates the frequency of clones across two samples. For more information, see "Example correlation and proportion of shared clones" on page 235.
- The Jaccard similarity index is determined for each pairwise comparison and is displayed in heat map form. For more information, see "Example Jaccard Similiarity Index for selected samples" on page 235.

Example Jaccard Similiarity Index for selected samples

This is an example of a **Comparison Plots** tab showing the Jaccard Similiarity Index for selected samples. The Jaccard similarity index is determined for each pairwise comparison and displayed in heatmap form. The Jaccard Similiarity Index ranges from 0 to 1 and is calculated as the total number of shared clones divided by the total number of unique clones across two samples.

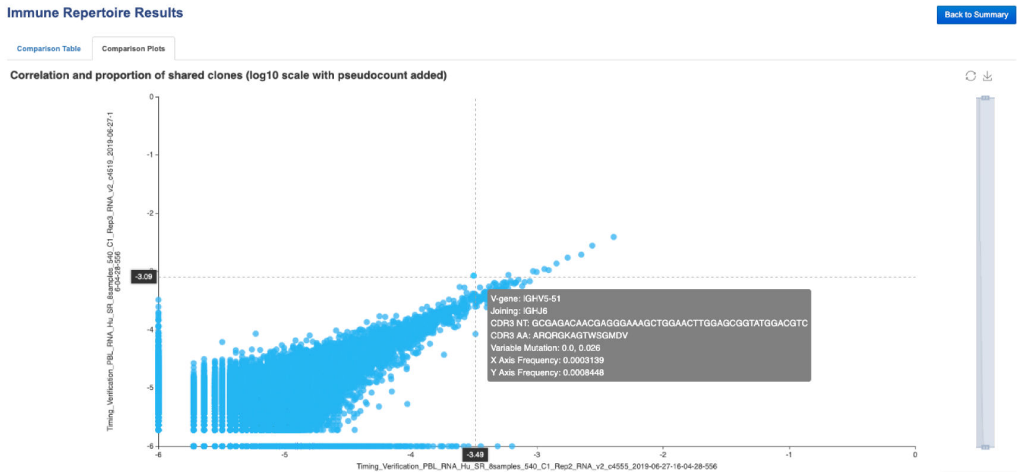


Jaccard Similarity Index for selected samples

1.00	0.19	0.19	Analysis 1
0.19	1.00	0.19	Analysis 2
0.19	0.19	1.00	Analysis 3

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. Hovering over a point reveals the CDR3NT and AA sequence of a clone, the variable mutation, and the frequency in either sample.



Correlation and proportion of shared clones (log10 scale with pseudocount added)

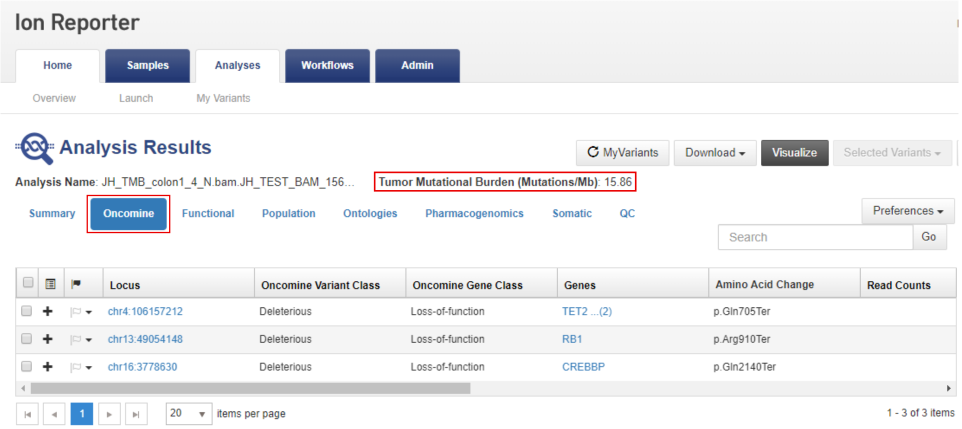
V-gene: IGHV5-51
Joining: IGHJ6
CDR3 NT: GCGAGACAACGAGGGAAAGCTGGAACTTGGAGCGGTATGGACGTC
CDR3 AA: ARQRGKAGTWSGMDV
Variable Mutation: 0.0, 0.026
X Axis Frequency: 0.0003139
Y Axis Frequency: 0.0008448

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.
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 displaying variants that are annotated with Oncomine™ annotated variants that are relevant to cancer with Oncomine™ Gene Class and Oncomine™ Variant Class information. The tumor mutational burden is displayed at the top of the results screen in mutations per Mb. QC Metrics are displayed in the **Analysis Visualization** screen. For more information, see “QC metrics for tumor mutational burden“ on page 267.
   See the Ion Reporter™ Software help for more options.



Analysis Name: JH_TMB_colon1_4_N.bam.JH_TEST_BAM_156...

Tumor Mutational Burden (Mutations/Mb): 15.86

☐			Locus	Oncomine Variant Class	Oncomine Gene Class	Genes	Amino Acid Change	Read Counts
☐	+		chr4:106157212	Deleterious	Loss-of-function	TET2 ...(2)	p.Gln705Ter	
☐	+		chr13:49054148	Deleterious	Loss-of-function	RB1	p.Arg910Ter	
☐	+		chr16:3778630	Deleterious	Loss-of-function	CREBBP	p.Gln2140Ter	

1 | 20 items per page | 1 - 3 of 3 items

5. In the **Analysis Results** table, sort or filter the data using the Oncomine™-specific annotations.
    a. The default **Filter Chain** is **Oncomine Variants (5.12)**, 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 332 .
        - 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, such as the results in Oncomine™ Reporter Software.

    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**, or **Current Results TSV**.

       For more information on the downloads, see "Variants file downloads" on page 180.

    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 263. 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 contain 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.

Folder	Contents of folder
Variants	- Contains intermediate and Oncomine™ annotated VCF files, which are used by Oncomine™ Reporter Software. For more information, see the *Oncomine™ Reporter 3.0 User Guide*. - TSV files that contain 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 the Oncomine™ Tumor Mutation Load - w3.0 - DNA - Single Sample is launched. 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.

1. Download, then extract a results file to your hard drive.

   For more information, see "Download analysis files" on page 179.

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='V3.0'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
```

Tumor Mutational Burden calculation

Ion Reporter™ Software 5.12 uses TMB Algorithm v3.0:

$$\text{precalibration TMB} = \frac{(\text{SM x } 106^{6})}{\text{Total Exonic Bases with Sufficient Coverage}}$$

where

- TMB is Tumor Mutational Burden
- SM is Somatic Mutations

Note: In Ion Reporter™ Software 5.12, 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 will not be adjusted until the user provides values for the intercept and the slope.

Note: Standardization is independent of the germline calibration.

View metagenomics analysis results

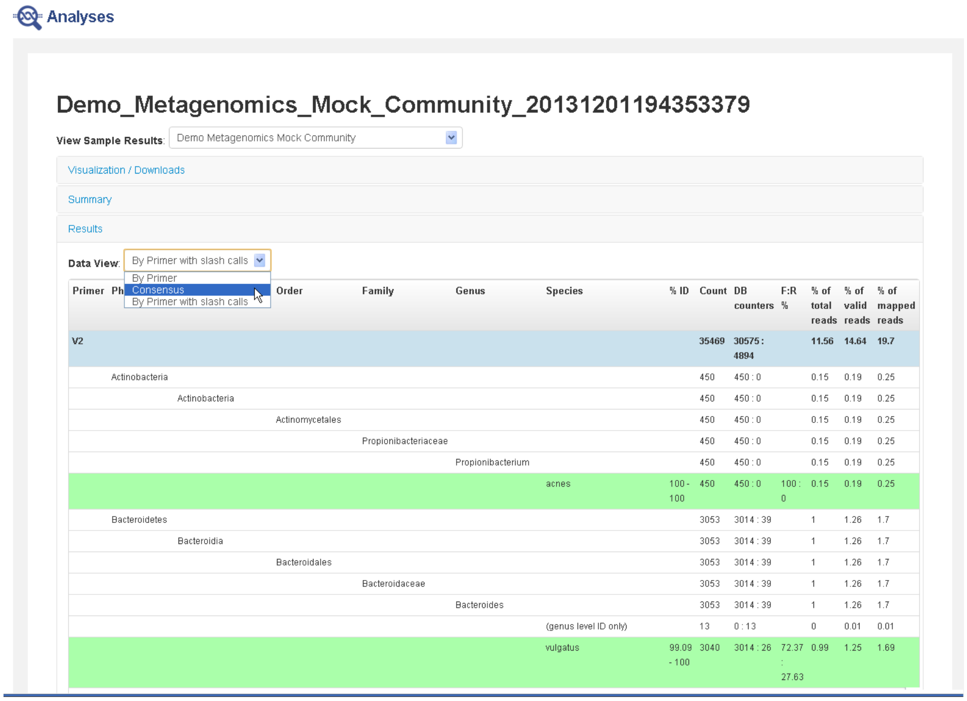
Sequences from different 16S databases may use different taxonomic hierarchies. For example, Greengenes may have *de novo* classified sequences labeled as Archaea> Crenarchaeota>Thaumarchaeota, where *Thaumarchaeota* is a class of *Crenarchaeota*. Records from other databases may treat both *Thaumarchaeota* 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 **Analysis** 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 download results files and to visualize results with Krona™. For more information, see "Reference output files for Metagenomics" on page 243 and "Visualize metagenomics analysis results with Krona charts" on page 247.
- **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.



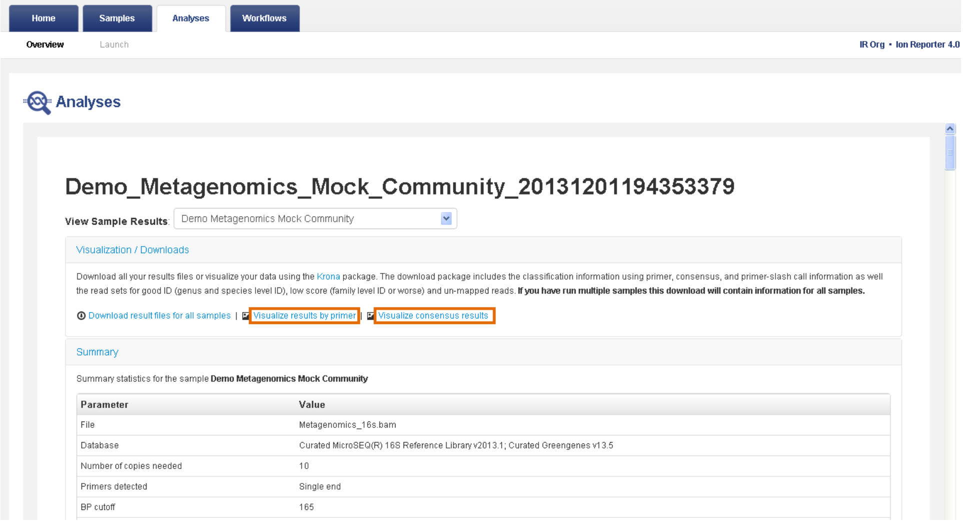
Demo_Metagenomics_Mock_Community_20131201194353379

View Sample Results: Demo Metagenomics Mock Community

Data View: By Primer with slash calls

Primer	Ph		Order	Family	Genus	Species	% ID	Count	DB counters	F:R %	% of total reads	% of valid reads	% of mapped reads
V2								35469	30575 : 4894		11.56	14.64	19.7
	Actinobacteria							450	450 : 0		0.15	0.19	0.25
		Actinobacteria						450	450 : 0		0.15	0.19	0.25
			Actinomycetales					450	450 : 0		0.15	0.19	0.25
				Propionibacteriaceae				450	450 : 0		0.15	0.19	0.25
					Propionibacterium			450	450 : 0		0.15	0.19	0.25
						acnes	100 - 100	450	450 : 0	100 : 0	0.15	0.19	0.25
	Bacteroidetes							3053	3014 : 39		1	1.26	1.7
		Bacteroidia						3053	3014 : 39		1	1.26	1.7
			Bacteroidales					3053	3014 : 39		1	1.26	1.7
				Bacteroidaceae				3053	3014 : 39		1	1.26	1.7
					Bacteroides			3053	3014 : 39		1	1.26	1.7
						(genus level ID only)		13	0 : 13		0	0.01	0.01
						vulgatus	99.09 - 100	3040	3014 : 26	72.37 : 27.63	0.99	1.25	1.69

5. In the **Visualization/Downloads** section, click one of the visualization links to view your results with Krona charts.



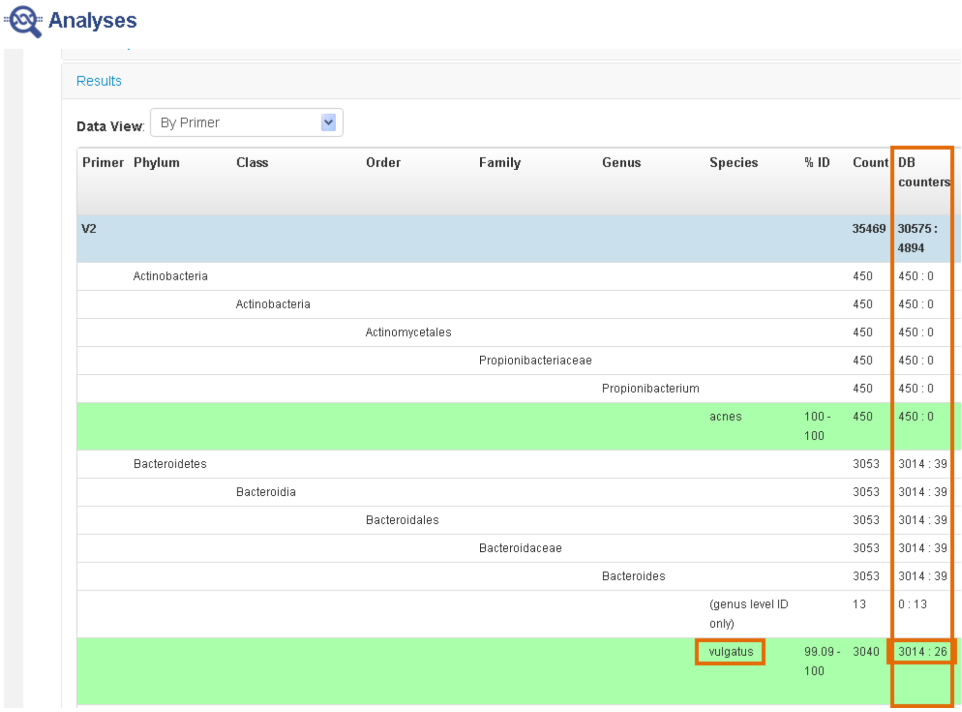
Download all your results files or visualize your data using the Krona package. The download package includes the classification information using primer, consensus, and primer-slash call information as well the read sets for good ID (genus and species level ID), low score (family level ID or worse) and un-mapped reads. **If you have run multiple samples this download will contain information for all samples.**

Summary statistics for the sample **Demo Metagenomics Mock Community**

Parameter	Value
File	Metagenomics_16s.bam
Database	Curated MicroSEQ(R) 16S Reference Library v2013.1; Curated Greengenes v13.5
Number of copies needed	10
Primers detected	Single end
BP cutoff	165

Reads included in Metagenomics analysis results

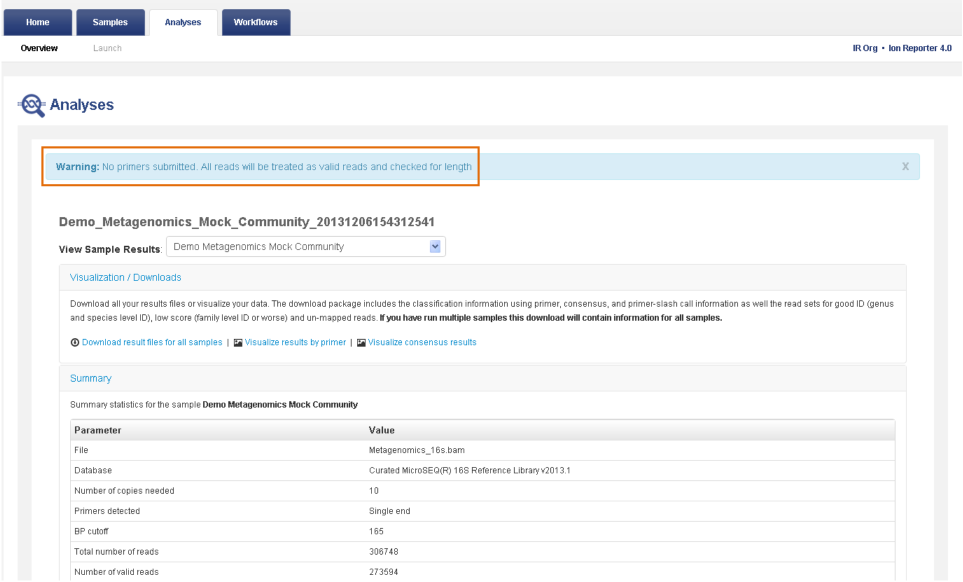
Note: When your metagenomics analysis workflow uses two databases, only reads that do not map to the first database are attempted with the second database. In the **Results** section of the screen, the **DB counters** column shows how many reads matched sequences in each database.



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.



Warning: No primers submitted. All reads will be treated as valid reads and checked for length

Demo_Metagenomics_Mock_Community_20131206154312541

View Sample Results: Demo Metagenomics Mock Community

Visualization / Downloads

Download all your results files or visualize your data. The download package includes the classification information using primer, consensus, and primer-slash call information as well the read sets for good ID (genus and species level ID), low score (family level ID or worse) and un-mapped reads. **If you have run multiple samples this download will contain information for all samples.**

Download result files for all samples | Visualize results by primer | Visualize consensus results

Summary

Summary statistics for the sample **Demo Metagenomics Mock Community**

Parameter	Value
File	Metagenomics_16s.bam
Database	Curated MicroSEQ(R) 16S Reference Library v2013.1
Number of copies needed	10
Primers detected	Single end
BP cutoff	165
Total number of reads	306748
Number of valid reads	273594

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

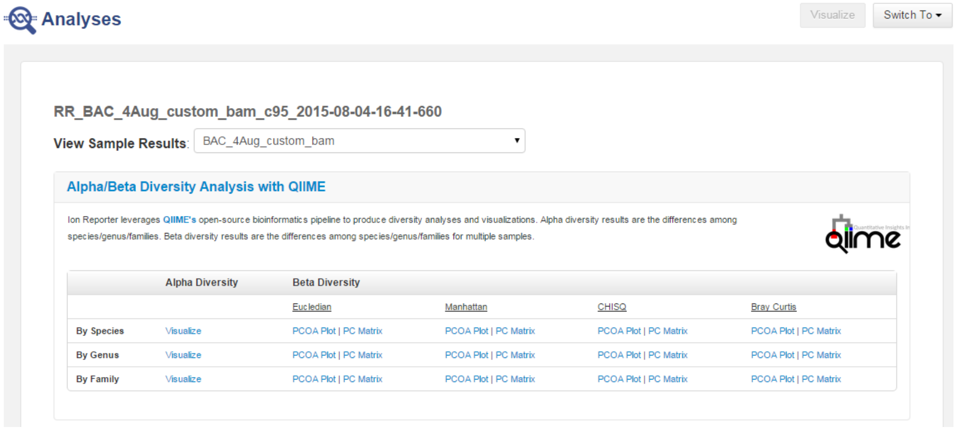
The Metagenomics 16S analysis workflow in Ion Reporter™ Software includes alpha diversity calculations and beta diversity calculations.

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.



RR_BAC_4Aug_custom_bam_c95_2015-08-04-16-41-660

View Sample Results: BAC_4Aug_custom_bam ▾

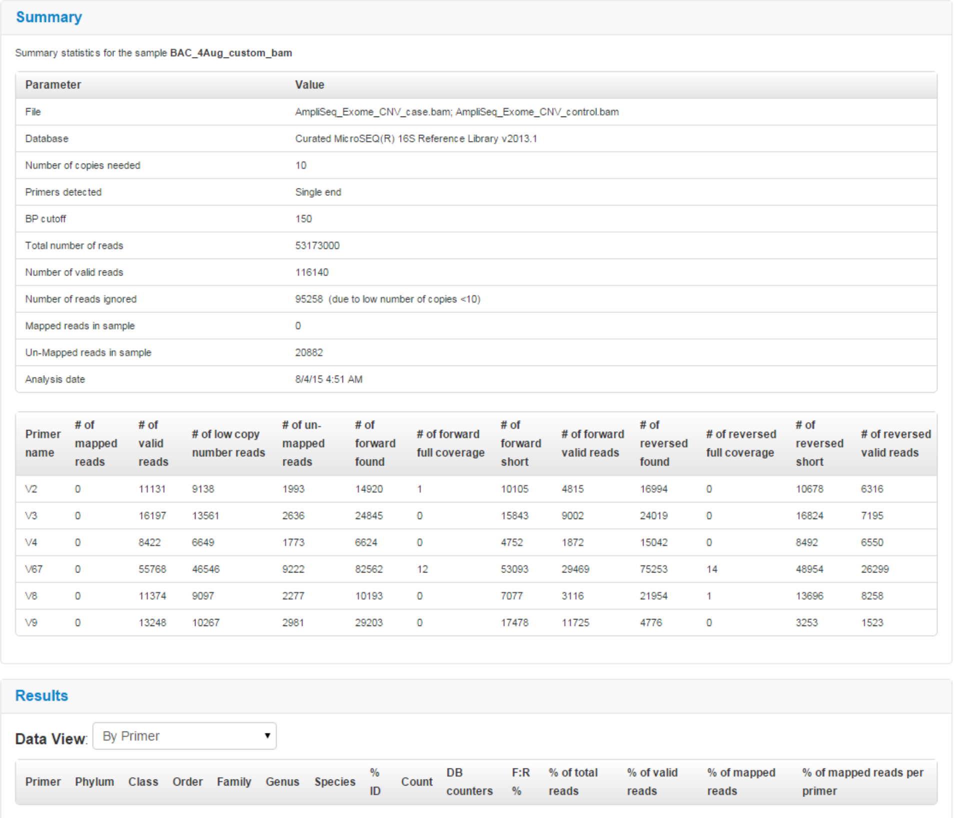
Alpha/Beta Diversity Analysis with QIIME

Ion Reporter leverages QIIME's open-source bioinformatics pipeline to produce diversity analyses and visualizations. Alpha diversity results are the differences among species/genus/families. Beta diversity results are the differences among species/genus/families for multiple samples.

	Alpha Diversity	Beta Diversity			
		Eucledian	Manhattan	CHISQ	Bray Curtis
By Species	Visualize	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix
By Genus	Visualize	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix
By Family	Visualize	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix	PCOA Plot | PC Matrix

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

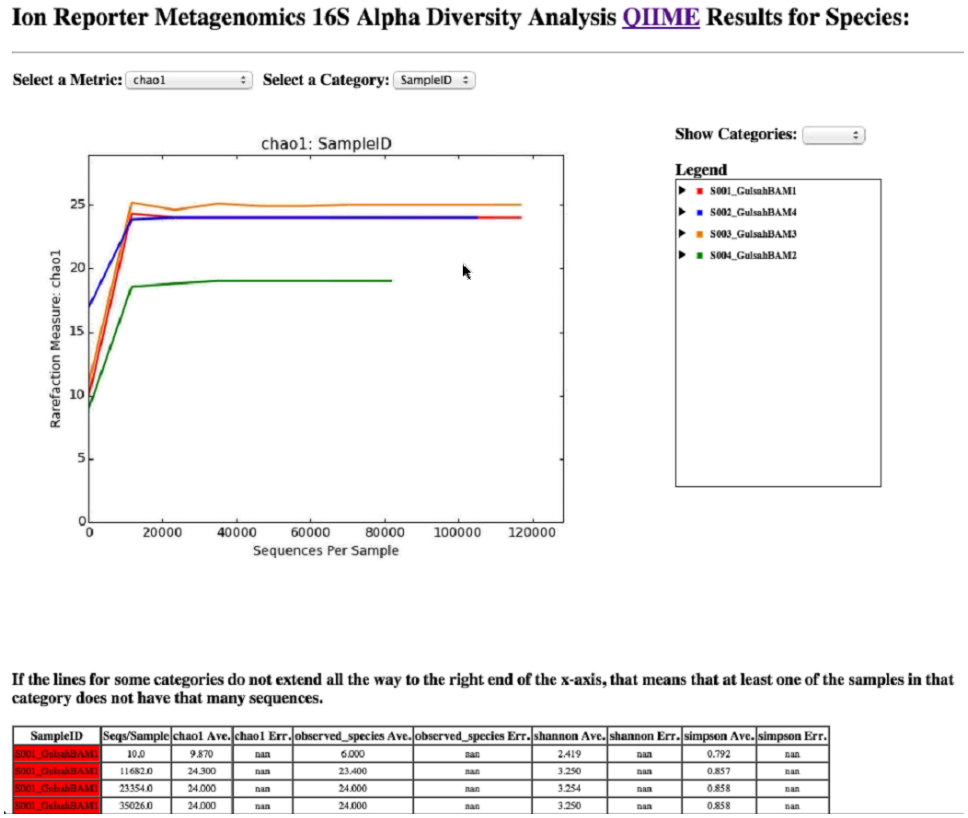
Primer name	# of mapped reads	# of valid reads	# of low copy number reads	# of un-mapped reads	# of forward found	# of forward full coverage	# of forward short	# of forward valid reads	# of reversed found	# of reversed full coverage	# of reversed short	# of reversed valid reads
V2	0	11131	9138	1993	14920	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.



Ion Reporter Metagenomics 16S Alpha Diversity Analysis QIIME Results for Species:

Select a Metric: chao1 **Select a Category:** SampleID

If the lines for some categories do not extend all the way to the right end of the x-axis, that means that at least one of the samples in that category does not have that many sequences.

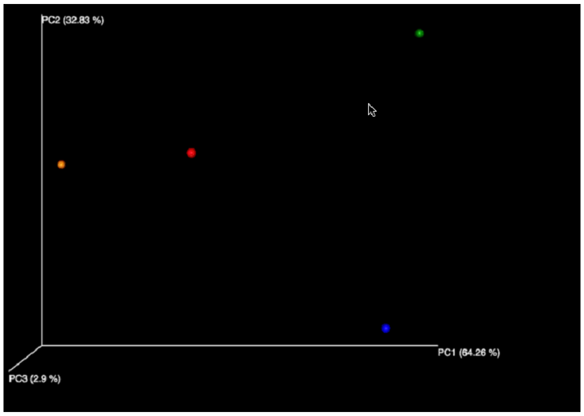
SampleID	Seqs/Sample	chao1 Ave.	chao1 Err.	observed_species Ave.	observed_species Err.	shannon Ave.	shannon Err.	simpson Ave.	simpson Err.
S001_GulsahBAM1	10.0	9.870	nan	6.000	nan	2.419	nan	0.792	nan
S001_GulsahBAM1	11682.0	24.300	nan	23.400	nan	3.250	nan	0.857	nan
S001_GulsahBAM1	23354.0	24.000	nan	24.000	nan	3.254	nan	0.858	nan
S001_GulsahBAM1	35026.0	24.000	nan	24.000	nan	3.250	nan	0.858	nan

5. In the **By Species** row, in the **Alpha Diversity** column, click **Visualize**, then in the resulting screen, select your metrics.

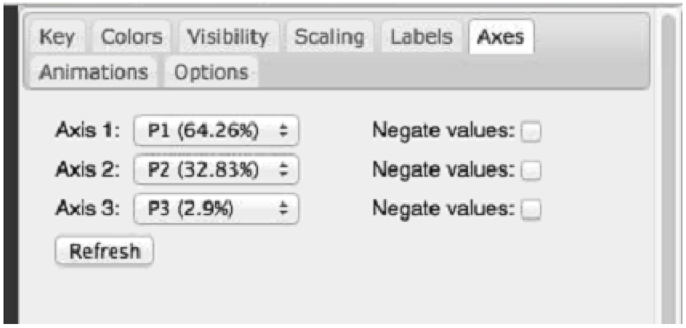
6. Repeat for **By Genus** and **By Family** links in the **Alpha Diversity** column to see the genus and families that are in the sample.

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.



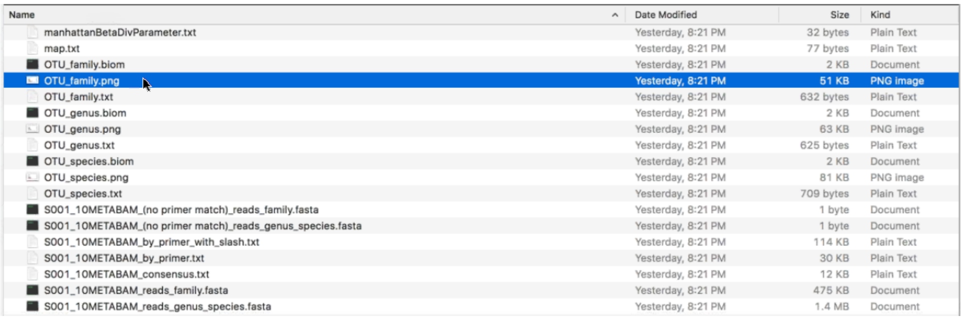
Axis 1: P1 (64.26%) Negate values: ☐
Axis 2: P2 (32.83%) Negate values: ☐
Axis 3: P3 (2.9%) Negate values: ☐

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.

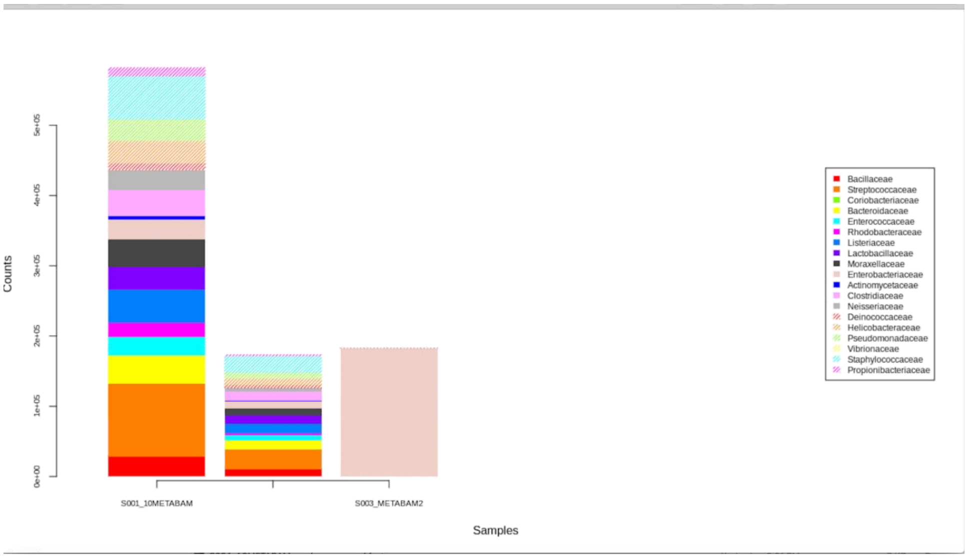
1. Download your results.

2. Click the PNG file of the results.



Name	Date Modified	Size	Kind
manhattanBetaDivParameter.txt	Yesterday, 8:21 PM	32 bytes	Plain Text
map.txt	Yesterday, 8:21 PM	77 bytes	Plain Text
OTU_family.biom	Yesterday, 8:21 PM	2 KB	Document
OTU_family.png	Yesterday, 8:21 PM	51 KB	PNG image
OTU_family.txt	Yesterday, 8:21 PM	632 bytes	Plain Text
OTU_genus.biom	Yesterday, 8:21 PM	2 KB	Document
OTU_genus.png	Yesterday, 8:21 PM	63 KB	PNG image
OTU_genus.txt	Yesterday, 8:21 PM	625 bytes	Plain Text
OTU_species.biom	Yesterday, 8:21 PM	2 KB	Document
OTU_species.png	Yesterday, 8:21 PM	81 KB	PNG image
OTU_species.txt	Yesterday, 8:21 PM	709 bytes	Plain Text
S001_10METABAM_(no primer match)_reads_family.fasta	Yesterday, 8:21 PM	1 byte	Document
S001_10METABAM_(no primer match)_reads_genus_species.fasta	Yesterday, 8:21 PM	1 byte	Document
S001_10METABAM_by_primer_with_slash.txt	Yesterday, 8:21 PM	114 KB	Plain Text
S001_10METABAM_by_primer.txt	Yesterday, 8:21 PM	30 KB	Plain Text
S001_10METABAM_consensus.txt	Yesterday, 8:21 PM	12 KB	Plain Text
S001_10METABAM_reads_family.fasta	Yesterday, 8:21 PM	475 KB	Document
S001_10METABAM_reads_genus_species.fasta	Yesterday, 8:21 PM	1.4 MB	Document

The data is displayed visually.



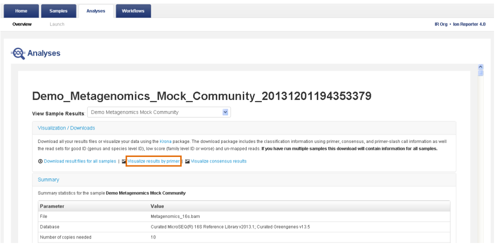
Visualize metagenomics analysis results with Krona charts

Use the Krona visualization feature to browse through your data using interactive zoomable pie charts. Krona documentation is available at **github**.

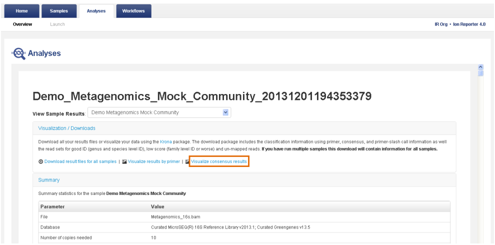
Note: The Snapshot button does not work on the Krona visualization page in the Metagenomics 16S analysis workflow. Krona documentation states that their charts are best viewed with the Firefox™ browser.

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 the link next to the **Krona piechart** label.
4. For the next steps, see the following table or Krona documentation on their **github site**.

This table describes ways to visualize metagenomics analysis results using Krona charts.

Task	Your action
Open your analysis data in a Krona chart	Click the **Visualize results by primer** link.  Demo_Metagenomics_Mock_Community_20131201194353379 View Sample Results: Demo Metagenomics Mock Community Visualization / Downloads Download all your results files or visualize your data using the Krona package. The download package includes the classification information using primer, consensus, and primer-slash call information as well the read sets for good ID (genus and species level ID), low score (family level ID or worse) and un-mapped reads. **If you have run multiple samples this download will contain information for all samples.** Download result files for all samples | Visualize results by primer | Visualize consensus results Summary Summary statistics for the sample **Demo Metagenomics Mock Community** Parameter: Value File: Metagenomics_16s.bam Database: Curated MicroSEQ(R) 16S Reference Library v2013.1; Curated Greengenes v13.5 Number of copies needed: 10

<table>
<tr><th>Task</th><th>Your action</th></tr>
<tr><td>Open Krona on your consensus data</td><td>Click the Visualize consensus results link.



Demo_Metagenomics_Mock_Community_20131201194353379

View Sample Results: Demo Metagenomics Mock Community

Visualization / Downloads

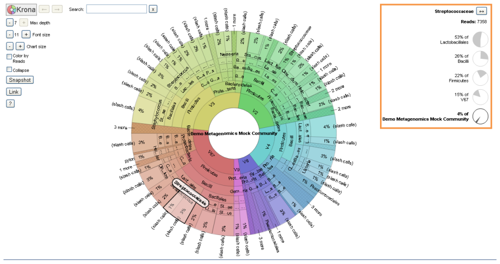
Download all your results files or visualize your data using the Krona package. The download package includes the classification information using primer, consensus, and primer-slash call information as well the read sets for good ID (genus and species level ID), low score (family level ID or worse) and un-mapped reads. If you have run multiple samples this download will contain information for all samples.

Download result files for all samples | Visualize results by primer | Visualize consensus results

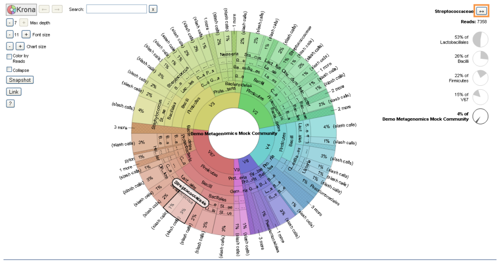
Summary

Summary statistics for the sample Demo Metagenomics Mock Community

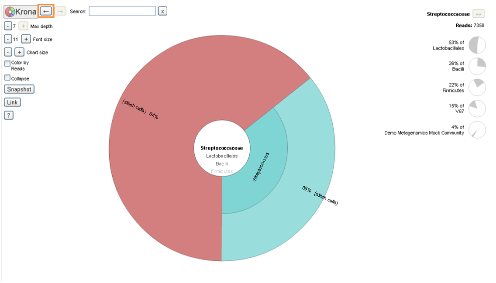
Parameter	Value
File	Metagenomics_16s.bam
Database	Curated MicroSEQ(R) 16S Reference Library v2013.1; Curated Greengenes v13.5
Number of copies needed	10
</td></tr>
<tr><td>Display percentages for one area of the chart</td><td>Click that area of the chart. The percentages for that area are displayed in the top right of the page.



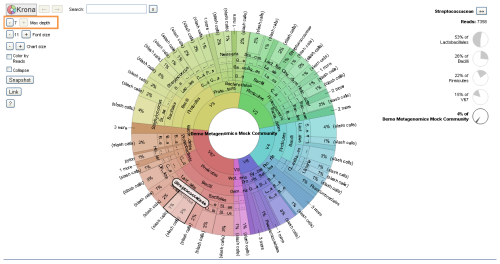
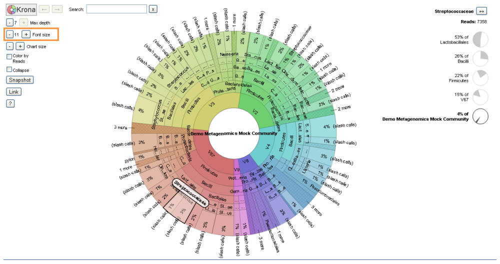
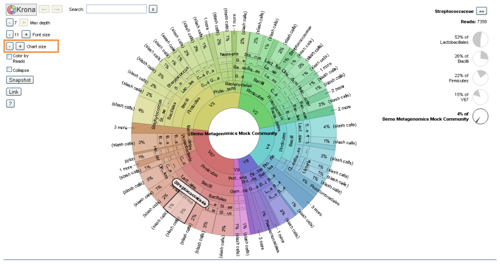
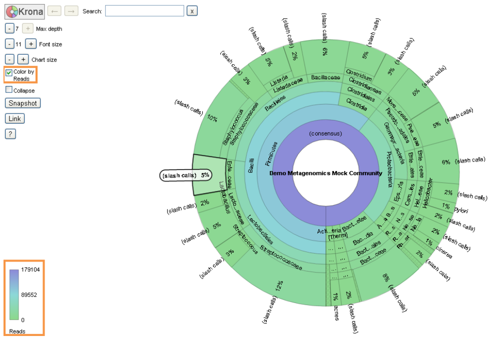
</td></tr>
<tr><td>Make that area the new focus of the chart</td><td>Click the expand box in the top right of the page.

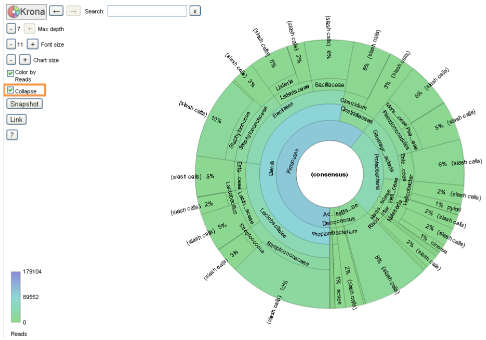
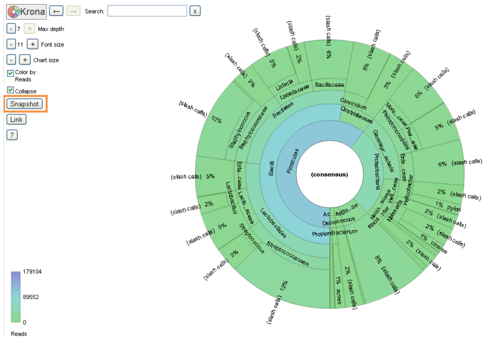
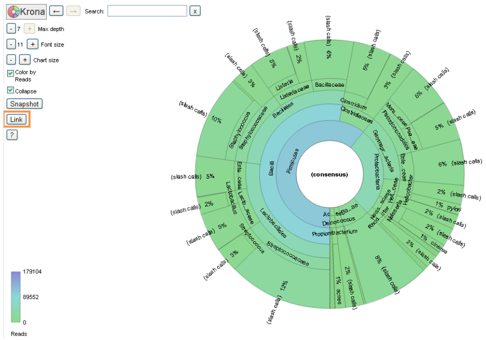


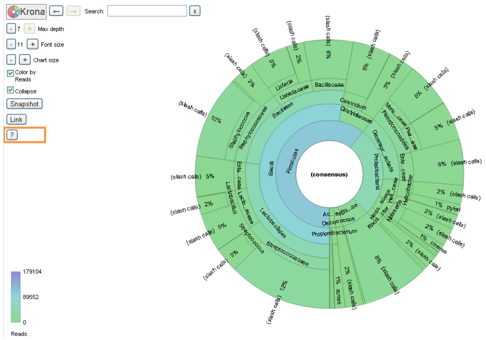
</td></tr>
<tr><td>Return to the previous chart focus</td><td>Click the back arrow ⟵ near the Krona logo in the top left of the page. (Only one go-back is supported at a time.)



</td></tr>
</table>

Task	Your action
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. 
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.) 

Task	Your action
Simplify wedges	Select the **Collapse** checkbox. This selection combines redundant wedges (that contain 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 Krona documentation on their **github site** for browser support. (For example, on Chrome™ an SVG file is not created directly. You have to save the page as a PDF file.)
Create a sharable link to the chart	Click **Link**. 

Task	Your action
Open Krona documentation	Click the question mark button. This link opens the Krona sourceforge documentation in a new tab. 

7

Visualize analysis results with Ion Reporter™ Software

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 253 and "Set IRGV preferences" on page 253.

Set IRGV or IGV as the default viewer

You can set a preference for your 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.

To view . . .	Do this . . .
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** in the upper right corner of the screen. 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 Visualization** screen, click **Variants Table**, then click on 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 JNLP file is downloaded when you click the locus in the **Variants Table**. Double-click the file to launch IGV as a standalone application on your computer.

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** 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 will be 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.

Option	Description
Analysis limit for BAM tracks	Maximum number of analysis that will include BAM tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Analysis limit for BED tracks	Maximum number of analysis that will include BED tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Limit for coverage data	By default, the maximum limit of 25000 is used. Reduce 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 choose to display fixed ploidy lines at copy number=1 and copy number=3 across the Whole Genome View. • **true**—Select this default setting to display fixed ploidy lines at CN=1 and CN=3 across the Whole Genome View. • **false**—Select this setting to display ploidy lines that are not fixed.
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 is 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 your 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 your 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 an Ion Reporter™ Genomic Viewer (IRGV) 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.

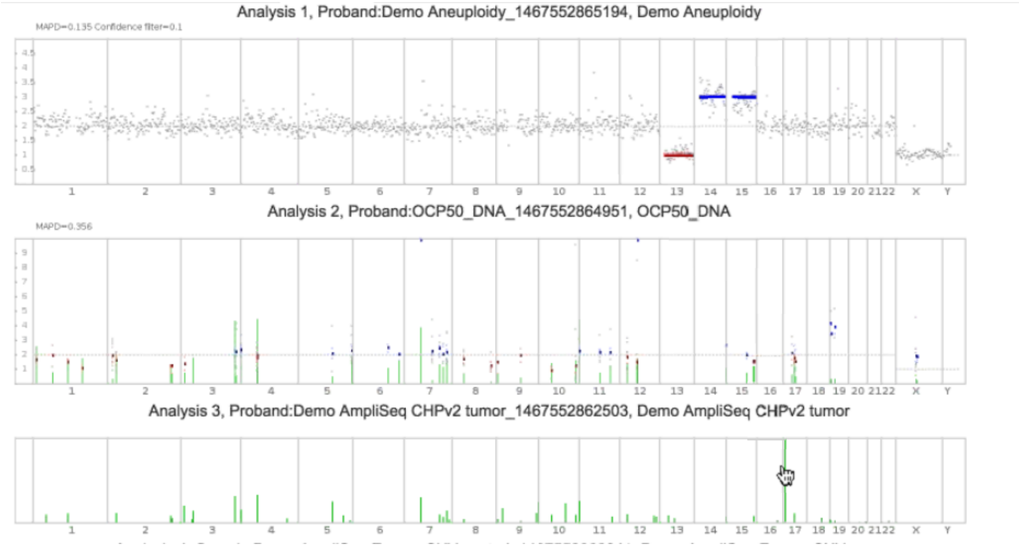
   If you use IRGV, drag, then drop, the newly opened IRGV tab to view the tab as a separate window.

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.

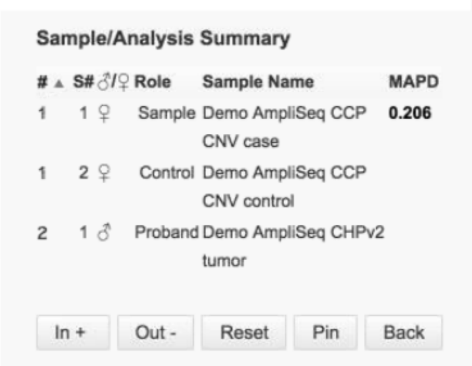


The first analysis on the whole genome view graph is an aneuploidy analysis. The graph displays CNV calls. It also displays **MAPD value**, productive read count of the sample, and the confidence filter used in the default filter chain. Blue and red bars in the whole genome view represent CNV calls. 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 graph is an Oncomine analysis where somatic CNV calls have been made. Instead of using a confidence filter, this 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 on any green density plot and the software zooms and displays the area in detail.

The various types of analyses also produce differing summary tables.

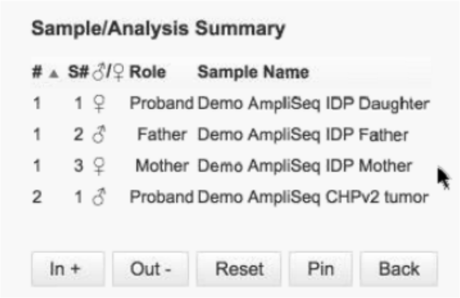
This summary table is from a CNV analysis:



Sample/Analysis Summary

# ▲	S# ♂/♀	Role	Sample Name	MAPD
1	1 ♀	Sample	Demo AmpliSeq CCP CNV case	**0.206**
1	2 ♀	Control	Demo AmpliSeq CCP CNV control	
2	1 ♂	Proband	Demo AmpliSeq CHPv2 tumor	

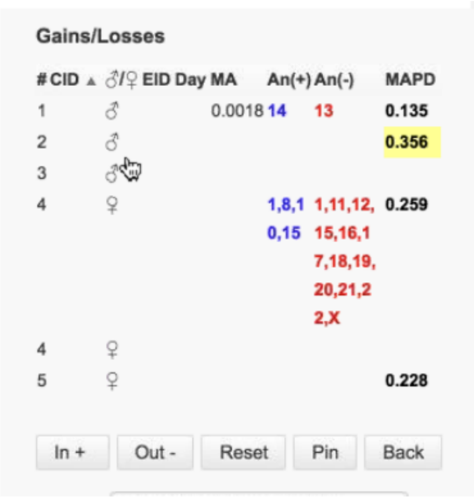
This summary table is from a Trio analysis:



Sample/Analysis Summary

# ▲	S# ♂/♀	Role	Sample Name
1	1 ♀	Proband	Demo AmpliSeq IDP Daughter
1	2 ♂	Father	Demo AmpliSeq IDP Father
1	3 ♀	Mother	Demo AmpliSeq IDP Mother
2	1 ♂	Proband	Demo AmpliSeq CHPv2 tumor

This summary table is from an aneuploidy analysis:



Gains/Losses

# CID ▲	♂/♀	EID	Day	MA	An(+)	An(-)	MAPD
1	♂			0.0018	14	13	0.135
2	♂						0.356
3	♂						
4	♀				1,8,10,15	1,11,12,15,16,17,18,19,20,21,22,X	0.259
4	♀						
5	♀						0.228

This summary table is from the RNA View option in a DNA analysis:

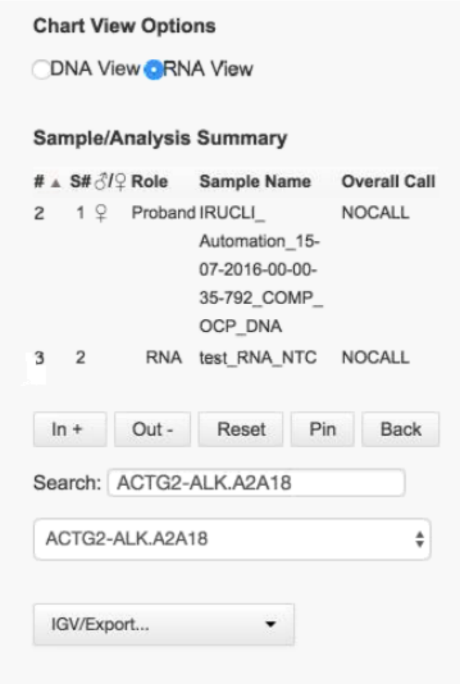


Chart View Options

○ DNA View ● RNA View

Sample/Analysis Summary

# ▲	S#	♂/♀	Role	Sample Name	Overall Call
2	1	♀	Proband	IRUCLI_Automation_15-07-2016-00-00-35-792_COMP_OCP_DNA	NOCALL
3	2		RNA	test_RNA_NTC	NOCALL

Search: ACTG2-ALK.A2A18

ACTG2-ALK.A2A18

Visualize aneuploidy analysis results

You can visualize aneuploidy results and generate reports with the Ion Reporter™ Genomic Viewer (IRGV). With this viewer, you can visualize multiple analyzes at once.

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 253.

1. Under the **Analyses** tab, click **Overview**.
2. Select one or more analyses then click **Actions ▸ Visualize**.

   The **Analysis Visualization** screen opens to the **IRGV** tab. A copy number histogram for each analysis selected appears, with ploidy maps for selected chromosomes or chromosome regions, and karyograms that show copy number gains and losses.

   Above the whole genome view for each analysis is a metric known as Productive Reads. It is 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 Remove Duplicates CNV finding parameter is set to True, then the Productive reads metric excludes duplicates. Productive reads are the specific reads that are used to establish the relative coverage of tiles across the sample.

Analysis 1, proband:Auto_BAC_Reproseq_26-05-2018-01-53-52-195, Demo Aneuploidy

MAPD=0.135 Productive Read Count=299009 Confidence filter=0.1 Filter Chain=CNVs of Confidence >= 0.1 - Germline - CNVs only

Gains / Loss

#	CID	♂/♀	EID	Day	Cell #	MA	An(+)	An(-)	MAPD
1		SxChrNrml				0.0018	14,15	13	0.135

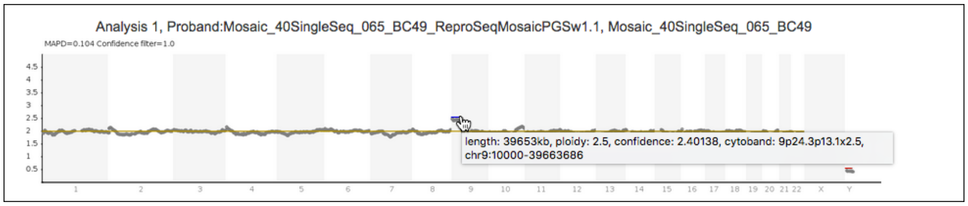
Search: chr13:19020000-114325993

MAPD filter: 0.3

Filter Chains: CNVs of Confidence >= ...

Note: If you select multiple analyses generated from analysis workflows with different filter chains with different confidence settings, the software will use the filter chain with the lowest confidence setting and apply that filter chain across the group. You can adjust the Filter Chain and Mapd filters with the controls in the lower right corner of the screen.

Example of an IRGV plot showing non-integer ploidy of the short arm of chromosome 9, indicating that the sample was mosaic.



Analysis 1, Proband:Mosaic_40SingleSeq_065_BC49_ReproSeqMosaicPGSw1.1, Mosaic_40SingleSeq_065_BC49

MAPD=0.104 Confidence filter=1.0

length: 39653kb, ploidy: 2.5, confidence: 2.40138, cytoband: 9p24.3p13.1x2.5, chr9:10000-39663686

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.

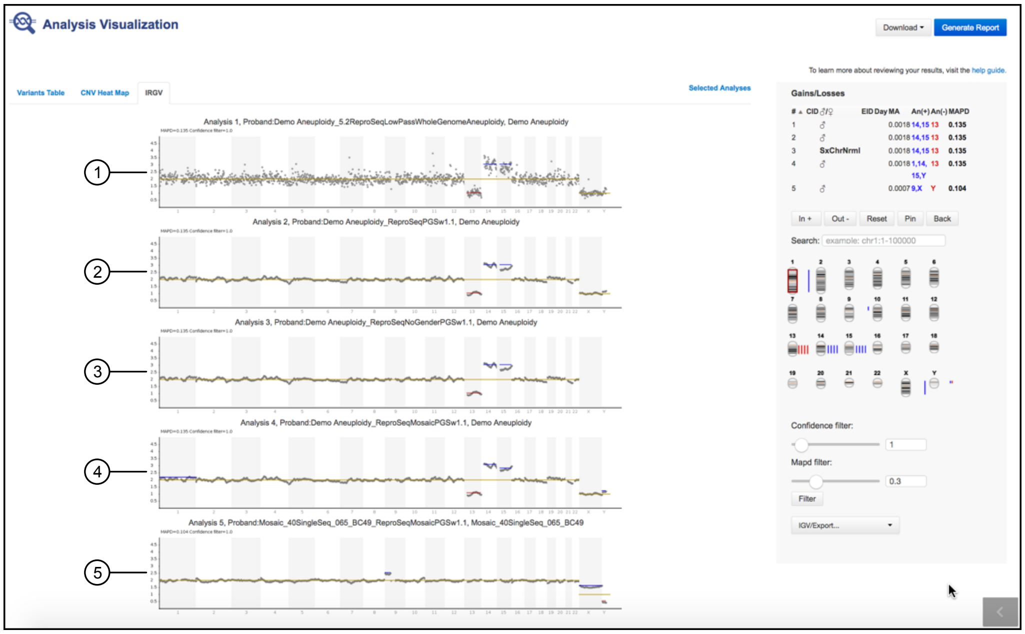
1. Under the **Analyses** tab, click ***Overview***.
2. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.
3. In the **IRGV** tab, scroll to the bottom of the visualization, then click **IRGV/Export & Preferences ▸ Show IRGV preferences** to expand the **Preferences** 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

This image shows five aneuploidy analyses run in various Ion Reporter™ Software aneuploidy analysis workflows that show how smoothing, no gender, and mosaicism appear in Ion Reporter™ Genomic Viewer (IRGV) visualizations. Visualization of aneuploidy detection is enhanced by tile-by-tile data points that are displayed 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 has no impact on the calls that are made by the aneuploidy pipeline in the software. Smoothing can be turned on or off in any analysis workflow.



Gains/Losses

# ▲	CID	♂/♀	EID	Day	MA	An(+)	An(-)	MAPD
1		♂			0.0018	14,15	13	0.135
2		♂			0.0018	14,15	13	0.135
3		SxChrNrml			0.0018	14,15	13	0.135
4		♂			0.0018	1,14, 15,Y	13	0.135
5		♂			0.0007	9,X	Y	0.104

Confidence filter: 1

Mapd filter: 0.3

① An analysis performed with the Ion Reporter™ Software 5.2 version of 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 an Ion Reporter™ Software 5.2 analysis workflow version that does not include smoothing of the discrete tile data.

② An analysis of the Demo Aneuploidy sample run through the default ReproSeq analysis workflow in Ion Reporter™ Software.

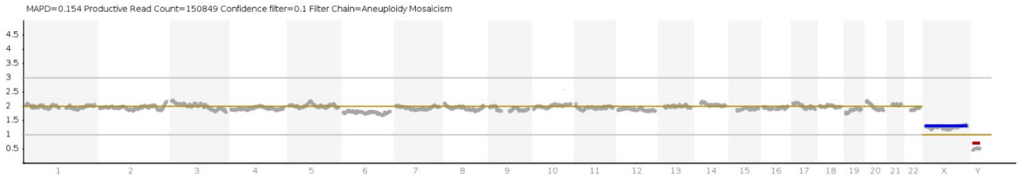
③ An analysis of the Demo Aneuploidy sample run through the ReproSeq No Gender analysis workflow. Use of the no gender analysis workflow generates analysis results that do not record or display the called gender of the sample in display or in data files. Instead, a called gender value of SxChrNrml is 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 of the Demo Aneuploidy sample run through the ReproSeq Mosaic analysis workflow. Note that 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 close to 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, while the other sample was normal ploidy=2 for chr 9. The mixing of two samples of different gender 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). Mosiac 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

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.

Note: For best results, use the **Expanded** option to click and view hover help with details about variants and base calls that are visualized in each read coverage track

In an open **Analysis Visualization**, in the **IRGV** tab, click ⚙ **Actions** next to the read coverage track, then select an option to adjust the view of the track.

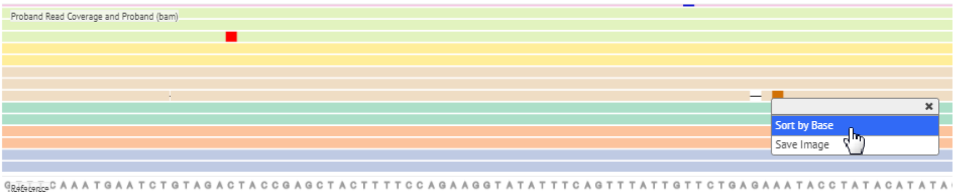
Option	Description
Expanded	Select this option to view the read coverage track with the maximum visible height for each BAM track.
Squish	Select this option to view reads in the coverage track in a single line that has minimal height for each BAM track.
Collapse	Select this option to view no BAM tracks 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. Mouse over the blue line to display context-sensitive information. For some CNV segment analyses, two start, two end, and two ploidy values are displayed, as shown in this example.

#	S#	♂/♀	Role	Sample Name	MAPD
1	1	♂	Proband	OCP50_DNA	0.356

Start 58142052
End 58146027
Ploidy 19.08
Confidence Interval 14.9456: 24.2231
Expected value 2
Start 58142051
End 58142160
Ploidy 12.4504

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 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. For more information, see *Ion Reporter™ Software Help*.

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**.

   The **Analysis Visualization** screen opens to the **TMB** tab. The **Tumor Mutational Burden (Mutations/Mb)** result is shown at the top of the screen.

   Multiple sample results are listed sequentially. You must scroll to view all the analyses.

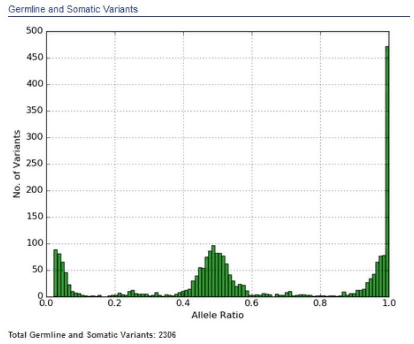
2. Scroll down to view graphical representations of the analysis results and QC metrics, and download additional files.
   - Click **Download Report** to download the **Tumor Mutational Burden Analysis Report** in PDF format, which includes the results with graphs and metrics.
   - Click **Download Variant Details TSV** in the lower right corner of the screen 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™.

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*.



Germline and Somatic Variants

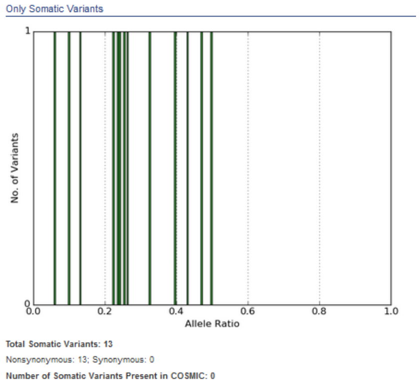
Total Germline and Somatic Variants: 2306

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)**.
- The number of variants 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 the *Ion Reporter™ Software Help*.



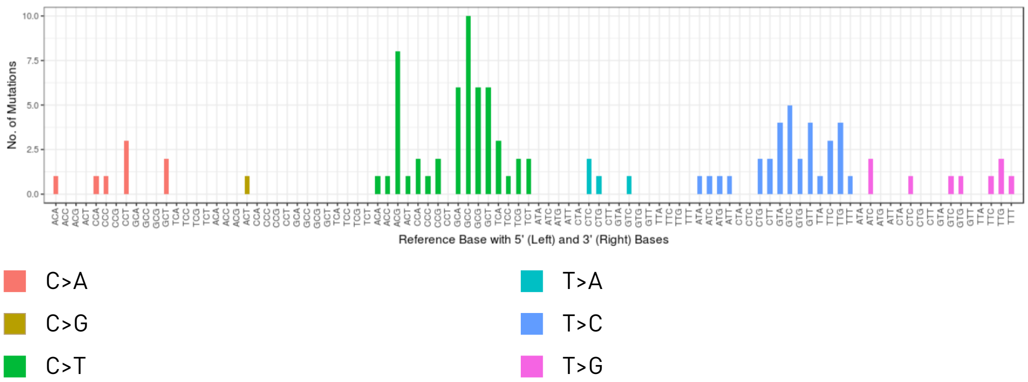
Only Somatic Variants

Total Somatic Variants: 13

Nonsynonymous: 13; Synonymous: 0

Number of Somatic Variants Present in COSMIC: 0

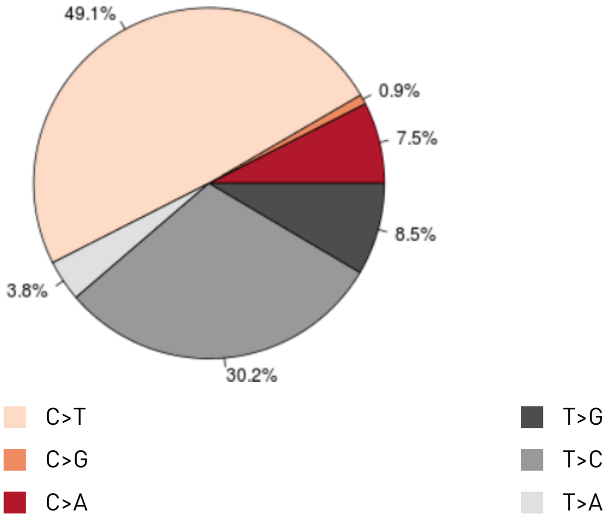
Substitution Type and Context of Somatic Mutations



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.

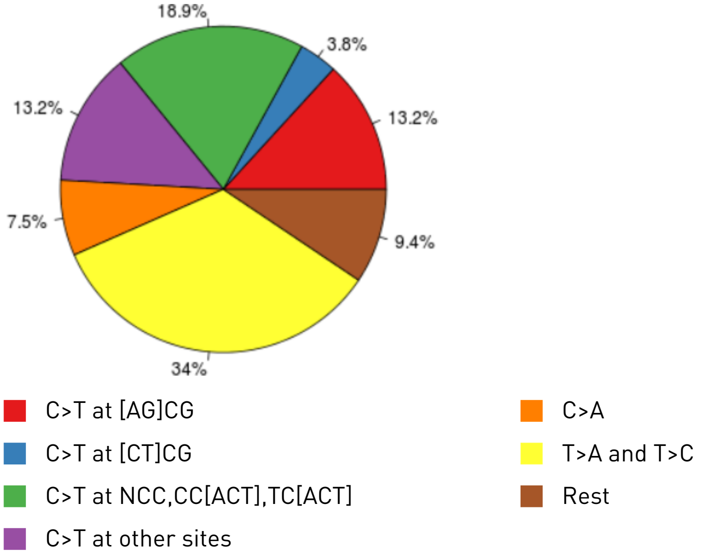
Substitution Type of Somatic Mutations

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.

- High C>T at C̲pC, CpC̲, TpC̲, 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 C̲pG 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: Underlined bases represent the reference base being substituted (for example, C̲pG in the first bullet is same as to [ACGT]CG).

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
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 exonic regions covered by the panel to be used instead of all genomic regions. See the *Ion Reporter™ Software Help* 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*.
Deamination score	Deamination is reported as the estimated SNP proportion consistent with deamination (low allele frequency C:G>T:A SNVs). 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*. 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*.

Variants Table view

The contents of the Samples column vary by variant type:

- **SNVs and INDELs:** Genotype
- **CNVs:** Cytoband
- **Fusions:** Presence or Absence detection call

Note: In the Variants Table view, analysis columns are ordered from left to right starting with the first analysis that you selected. The annotation data from the variants in the first selected analysis is what populates the Location column of the table.

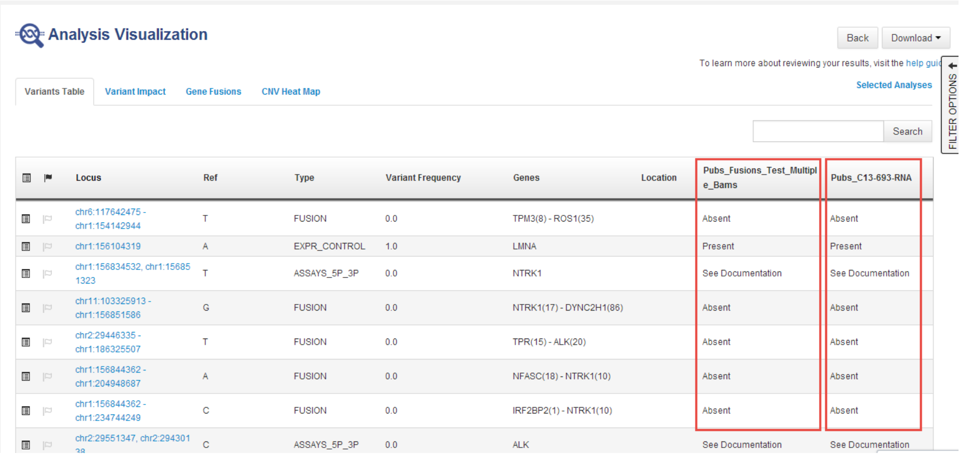
Restrictions on analysis selection

- Comparison and visualization are not supported for Metagenomics analyses. If one of these is among the analyses selected, then the Visualize option is not available in the Action menu.
- If a Failed or In-Progress analysis is among the analyses selected, then the Visualize option is not available in the Action menu.
- If only one set of variants is selected, the Visualization option is not available:
  - Trio and paired analyses each contain more than one set of variants. These analyses support visualization one a single trio or paired analysis.
  - A tumor-normal analysis has only one set of variants and does not support visualization. (A tumor-normal analysis reports only the set of variants for the tumor sample, not the set of variants for the normal sample.)

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. Multianalysis visualization 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:

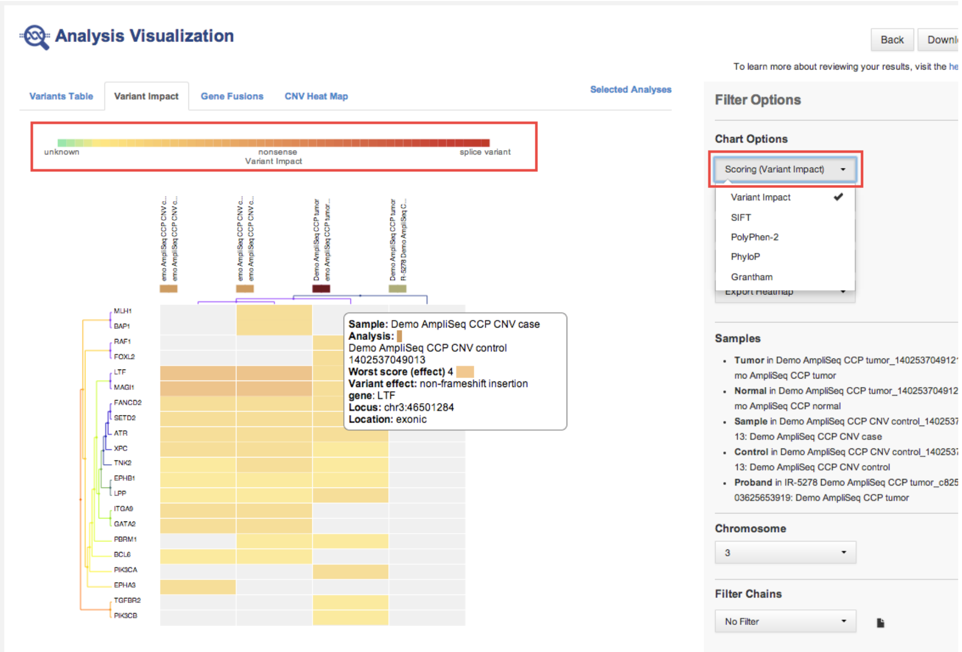


To learn more about reviewing your results, visit the help gui

		Locus	Ref	Type	Variant Frequency	Genes	Location	Pubs_Fusions_Test_Multiple_Bams	Pubs_C13-693-RNA
		chr6:117642475 - chr1:154142944	T	FUSION	0.0	TPM3(8) - ROS1(35)		Absent	Absent
		chr1:156104319	A	EXPR_CONTROL	1.0	LMNA		Present	Present
		chr1:156834532, chr1:156851323	T	ASSAYS_5P_3P	0.0	NTRK1		See Documentation	See Documentation
		chr11:103325913 - chr1:156851586	G	FUSION	0.0	NTRK1(17) - DYNC2H1(86)		Absent	Absent
		chr2:29446335 - chr1:186325507	T	FUSION	0.0	TPR(15) - ALK(20)		Absent	Absent
		chr1:156844362 - chr1:204948687	A	FUSION	0.0	NFASC(18) - NTRK1(10)		Absent	Absent
		chr1:156844362 - chr1:234744249	C	FUSION	0.0	IRF2BP2(1) - NTRK1(10)		Absent	Absent
		chr2:29551347, chr2:29430138	C	ASSAYS_5P_3P	0.0	ALK		See Documentation	See Documentation

- In the **Variant Impact** tab, the variant impact heat map that displays is based on the predicted variant impact:

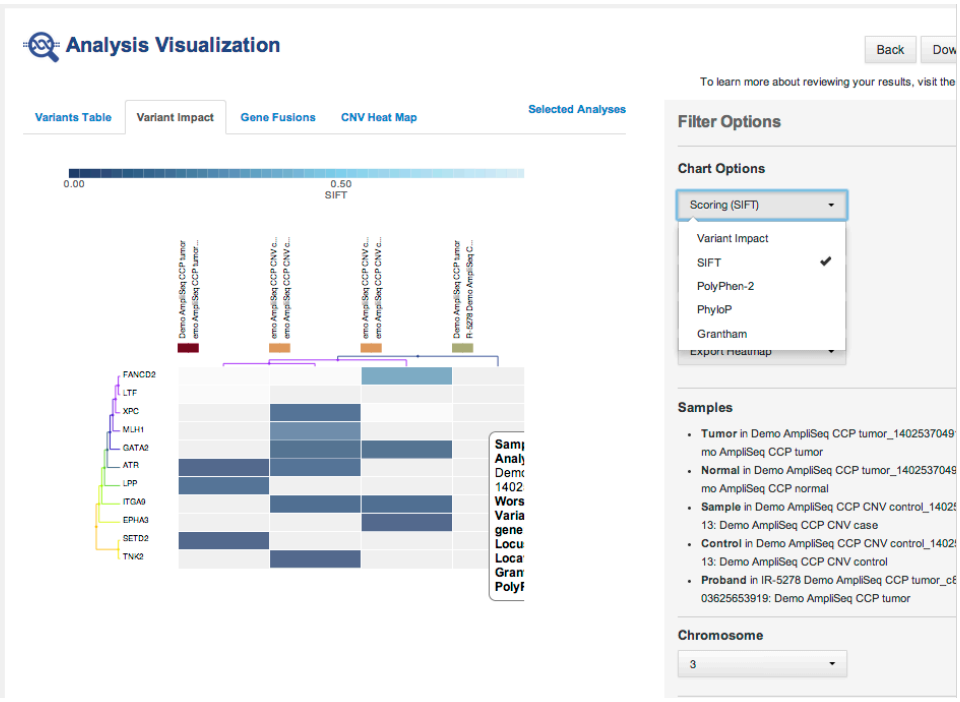
  The following is a variant impact heat map that is based on the predicted variant impact.



Sample: Demo AmpliSeq CCP CNV case
Analysis:
Demo AmpliSeq CCP CNV control
1402537049013
Worst score (effect) 4
Variant effect: non-frameshift insertion
gene: LTF
Locus: chr3:46501284
Location: exonic

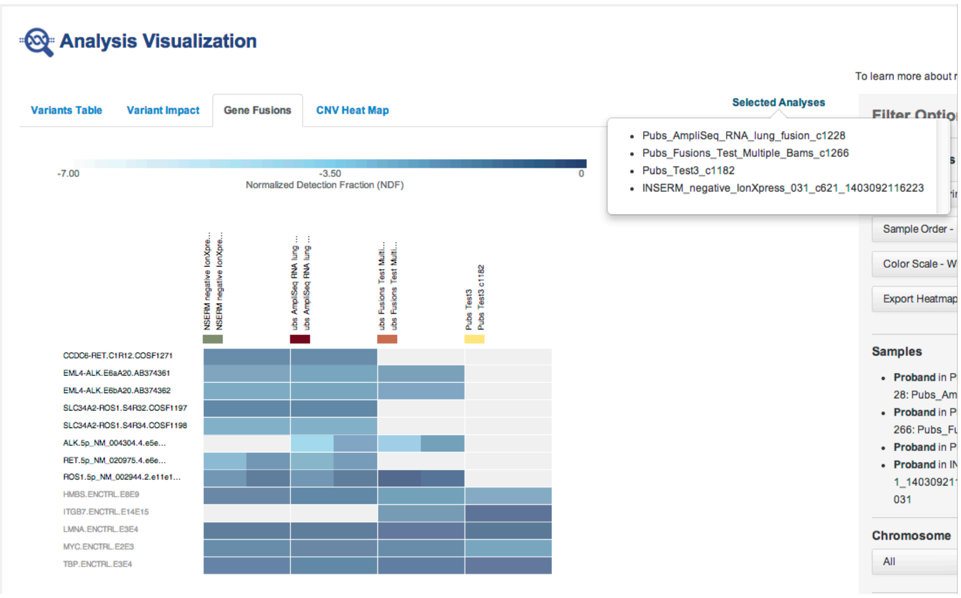
- **Tumor** in Demo AmpliSeq CCP tumor_140253704912
mo AmpliSeq CCP tumor
- **Normal** in Demo AmpliSeq CCP tumor_140253704912
mo AmpliSeq CCP normal
- **Sample** in Demo AmpliSeq CCP CNV control_1402537
13: Demo AmpliSeq CCP CNV case
- **Control** in Demo AmpliSeq CCP CNV control_1402537
13: Demo AmpliSeq CCP CNV control
- **Proband** in IR-5278 Demo AmpliSeq CCP tumor_c825
03625653919: Demo AmpliSeq CCP tumor

The following is a variant impact heat map that is based on other scoring.



- **Tumor** in Demo AmpliSeq CCP tumor_1402537049
mo AmpliSeq CCP tumor
- **Normal** in Demo AmpliSeq CCP tumor_1402537049
mo AmpliSeq CCP normal
- **Sample** in Demo AmpliSeq CCP CNV control_1402
13: Demo AmpliSeq CCP CNV case
- **Control** in Demo AmpliSeq CCP CNV control_1402
13: Demo AmpliSeq CCP CNV control
- **Proband** in IR-5278 Demo AmpliSeq CCP tumor_c8
03625653919: Demo AmpliSeq CCP tumor

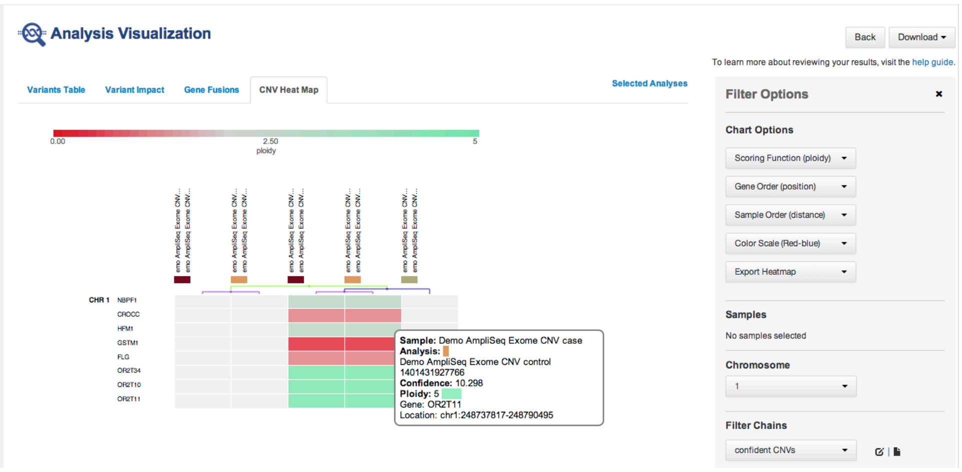
The following is a Gene Fusions heat map



- Pubs_AmpliSeq_RNA_lung_fusion_c1228
- Pubs_Fusions_Test_Multiple_Bams_c1266
- Pubs_Test3_c1182
- INSERM_negative_IonXpress_031_c621_1403092116223

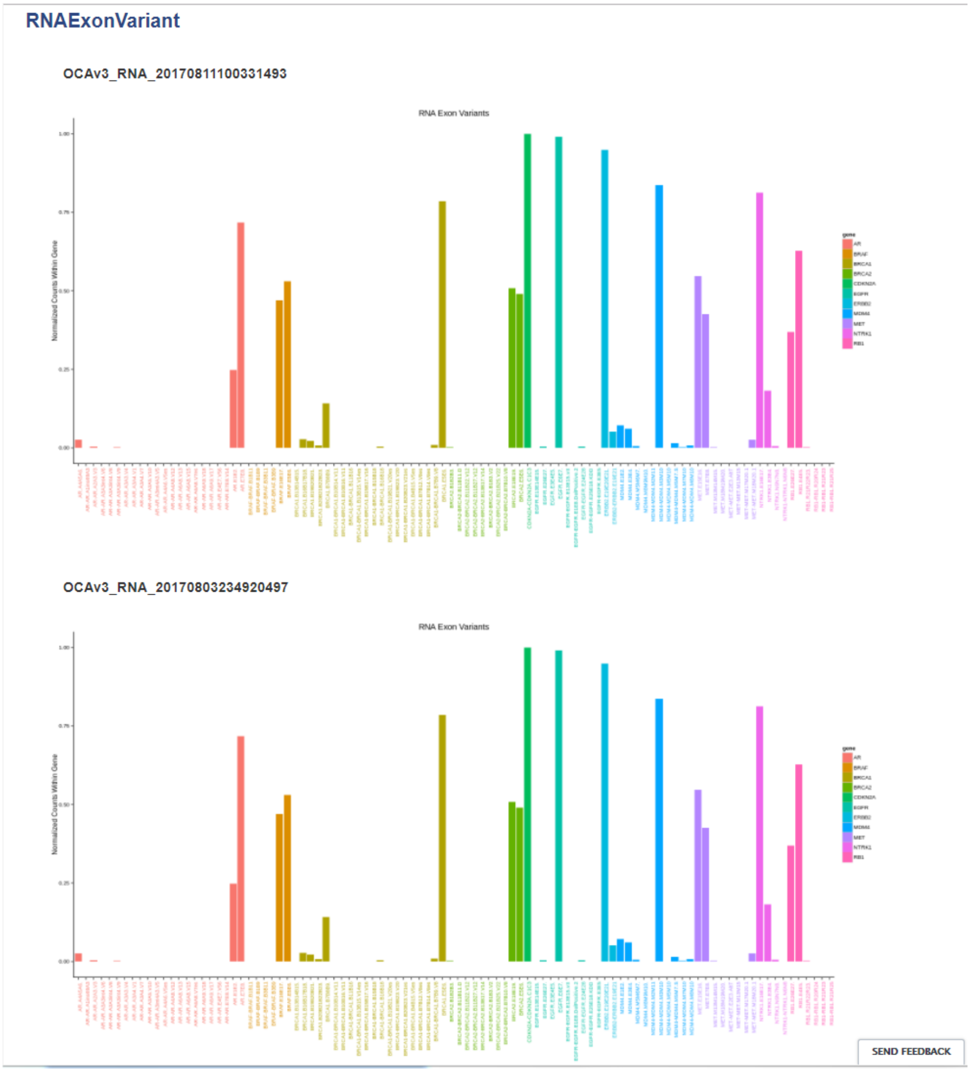
- **Proband** in P
28: Pubs_Am
- **Proband** in P
266: Pubs_Fu
- **Proband** in P
- **Proband** in IN
1_140309211
031

The following is CNV heat map.



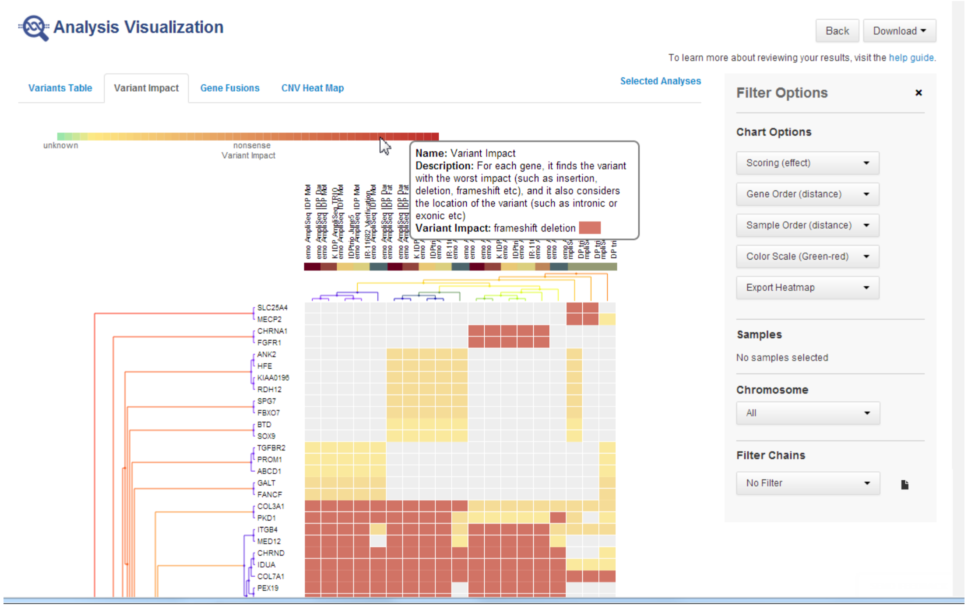
Sample: Demo AmpliSeq Exome CNV case
Analysis:
Demo AmpliSeq Exome CNV control 1401431927766
Confidence: 10.298
Ploidy: 5
Gene: OR2T11
Location: chr1:248737817-248790495

- RNA Exon Variants:



Note:

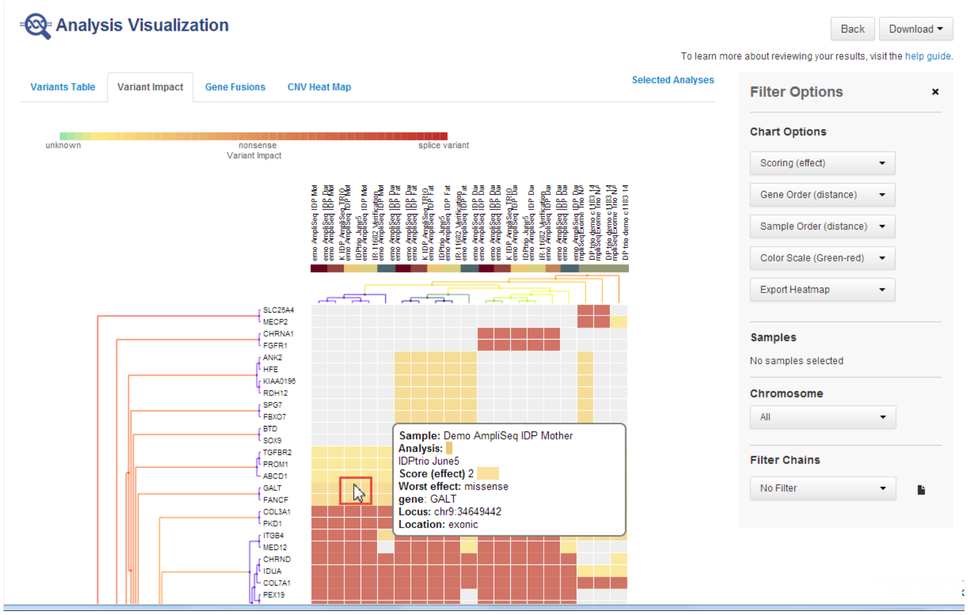
- In most cases, you will compare similar analyses. However, Ion Reporter™ Software does not restrict comparisons to only similar analyses.
- You can compare one trio or one paired analysis without selecting multiple analyses. Each trio or paired analysis contains more than one set of variants.
- Hover over the legend of a heat map:



Name: Variant Impact
Description: For each gene, it finds the variant with the worst impact (such as insertion, deletion, frameshift etc), and it also considers the location of the variant (such as intronic or exonic etc)
Variant Impact: frameshift deletion 🟥

Scoring (effect)
Gene Order (distance)
Sample Order (distance)
Color Scale (Green-red)
Export Heatmap
No samples selected
All
No Filter

Hover over the legend of a cell to see more information:



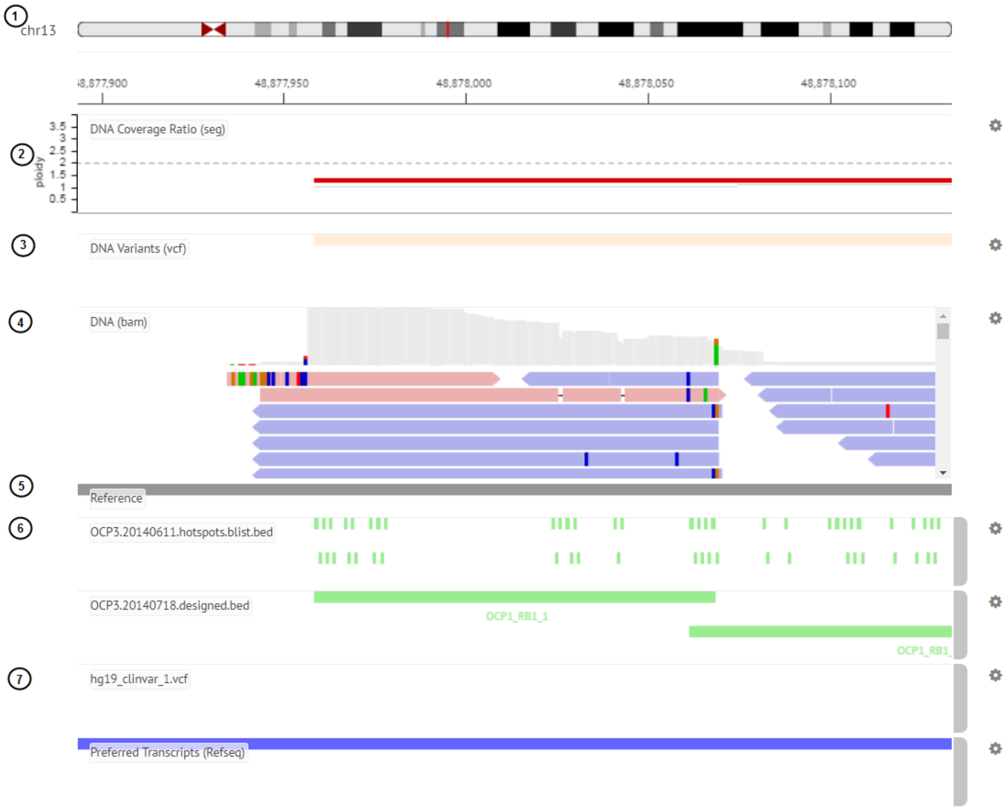
Sample: Demo AmpliSeq IDP Mother
Analysis:
IDPtrio June5
Score (effect) 2
Worst effect: missense
gene: GALT
Locus: chr9:34649442
Location: exonic

Scoring (effect)
Gene Order (distance)
Sample Order (distance)
Color Scale (Green-red)
Export Heatmap
No samples selected
All
No Filter

Track order for non-aneuploidy analyses

Note: In the GRCh38 genome reference, there exist alternate loci which are not part of the 1-22, X,Y chromosome contigs, and so are not annotated by the software (by design), for example chr22_K1270879v1_alt. Ion Reporter™ Software does, however, annotate the GSTT1 gene which is also located on an alternate loci.

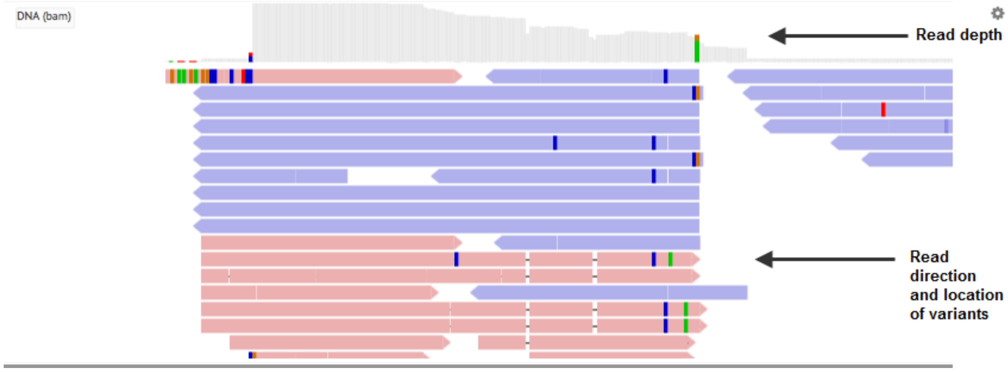
After the Whole Genome View of each non-aneuploidy analysis, the various data tracks are loaded in this order by default:



① Selected chromosome ideogram
② Copy Number segment (.seg) file
③ .vcf file
④ .bam track for each sample
⑤ genome reference track
⑥ design .bed files and hotspot files
⑦ annotation tracks (COSMIC, ClinVar, and preferred transcript annotation tracks)

Coverage histogram

When you zoom in on an analysis, 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.



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.

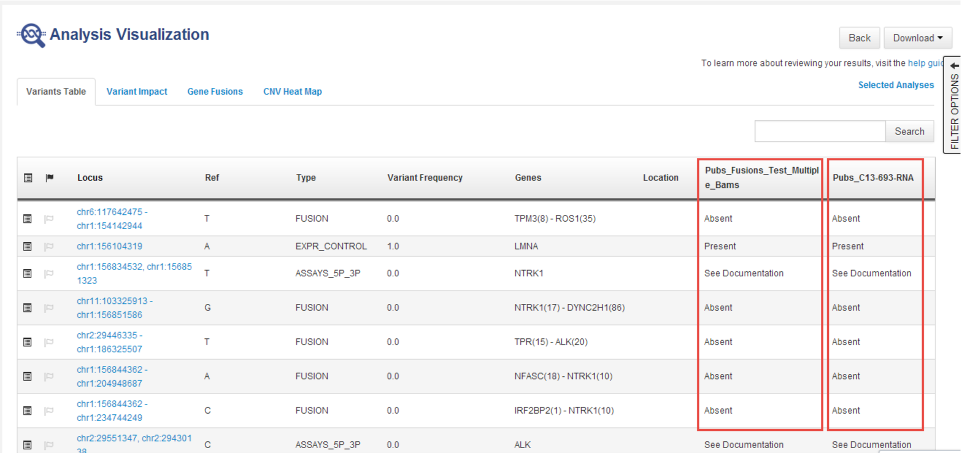
1. In the **Analyses** tab, click **Overview**.
2. Select multiple analyses, then click **Actions** ▸ **Visualize**.

The Visualize function allows you to review the results in a variety of ways. For more information, see "Compare results of single or multiple analyses" on page 195.

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. Multianalysis visualization supports the following views, depending on analysis workflow type.

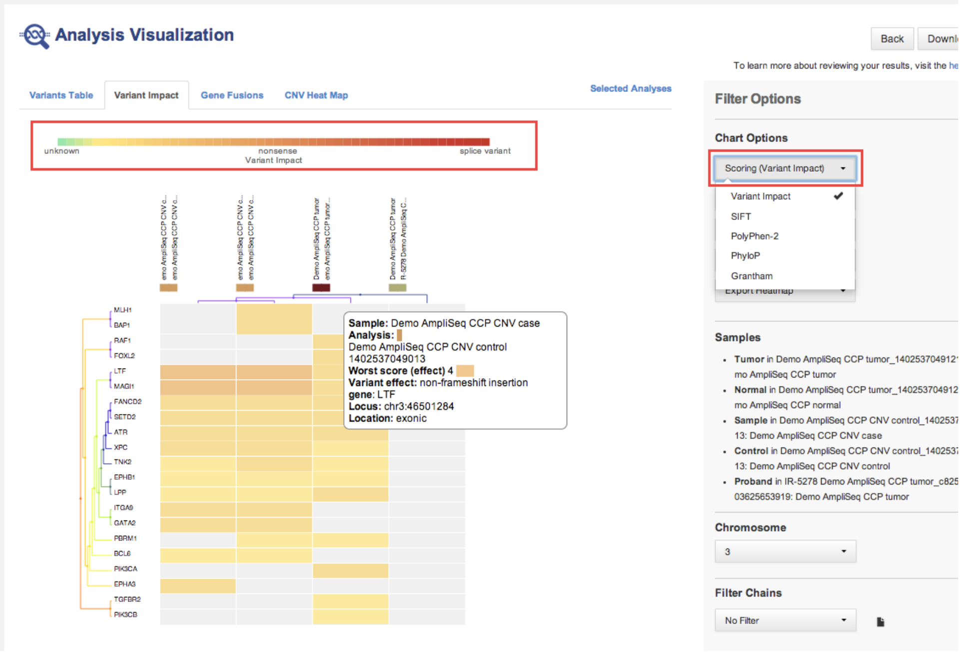
- In the **Variants Table** tab, the table view shows side-by-side columns to compare variant calls in different analyses:



Locus	Ref	Type	Variant Frequency	Genes	Location	Pubs_Fusions_Test_Multiple_Bams	Pubs_C13-693-RNA
chr6:117642475 - chr1:154142944	T	FUSION	0.0	TPM3(8) - ROS1(35)		Absent	Absent
chr1:156104319	A	EXPR_CONTROL	1.0	LMNA		Present	Present
chr1:156834532, chr1:156851323	T	ASSAYS_5P_3P	0.0	NTRK1		See Documentation	See Documentation
chr11:103325913 - chr1:156851586	G	FUSION	0.0	NTRK1(17) - DYNC2H1(86)		Absent	Absent
chr2:29446335 - chr1:186325507	T	FUSION	0.0	TPR(15) - ALK(20)		Absent	Absent
chr1:156844362 - chr1:204948687	A	FUSION	0.0	NFASC(18) - NTRK1(10)		Absent	Absent
chr1:156844362 - chr1:234744249	C	FUSION	0.0	IRF2BP2(1) - NTRK1(10)		Absent	Absent
chr2:29551347, chr2:29430138	C	ASSAYS_5P_3P	0.0	ALK		See Documentation	See Documentation

- 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.

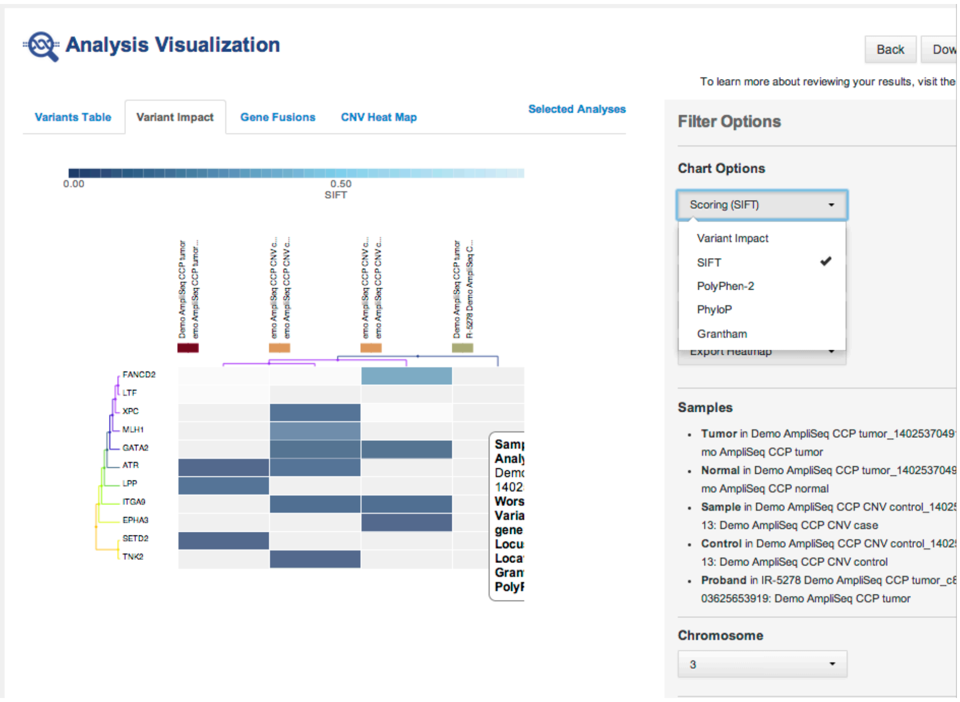


Sample: Demo AmpliSeq CCP CNV case
Analysis:
Demo AmpliSeq CCP CNV control 1402537049013
Worst score (effect) 4
Variant effect: non-frameshift insertion
gene: LTF
Locus: chr3:46501284
Location: exonic

Samples

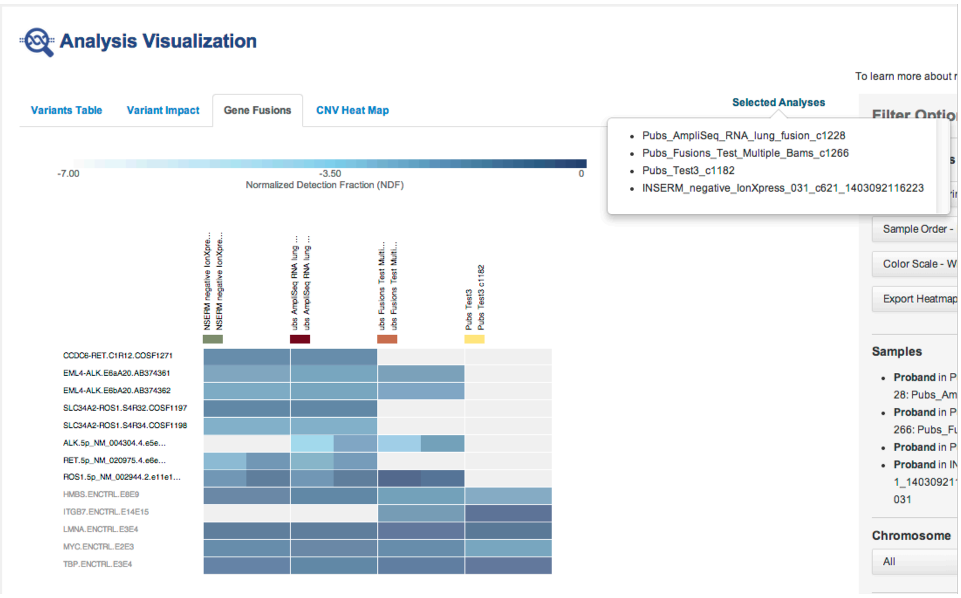
- **Tumor** in Demo AmpliSeq CCP tumor_140253704912 mo AmpliSeq CCP tumor
- **Normal** in Demo AmpliSeq CCP tumor_140253704912 mo AmpliSeq CCP normal
- **Sample** in Demo AmpliSeq CCP CNV control_1402537 13: Demo AmpliSeq CCP CNV case
- **Control** in Demo AmpliSeq CCP CNV control_1402537 13: Demo AmpliSeq CCP CNV control
- **Proband** in IR-5278 Demo AmpliSeq CCP tumor_c825 03625653919: Demo AmpliSeq CCP tumor

The following is a variant impact heat map that is based on other scoring.



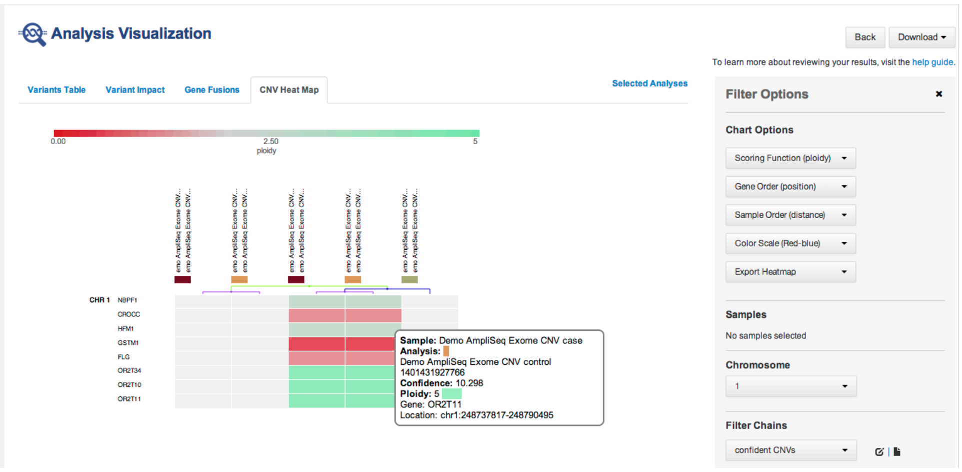
- **Tumor** in Demo AmpliSeq CCP tumor_1402537049 mo AmpliSeq CCP tumor
- **Normal** in Demo AmpliSeq CCP tumor_1402537049 mo AmpliSeq CCP normal
- **Sample** in Demo AmpliSeq CCP CNV control_1402 13: Demo AmpliSeq CCP CNV case
- **Control** in Demo AmpliSeq CCP CNV control_1402 13: Demo AmpliSeq CCP CNV control
- **Proband** in IR-5278 Demo AmpliSeq CCP tumor_c 03625653919: Demo AmpliSeq CCP tumor

The following is a Gene Fusions heat map



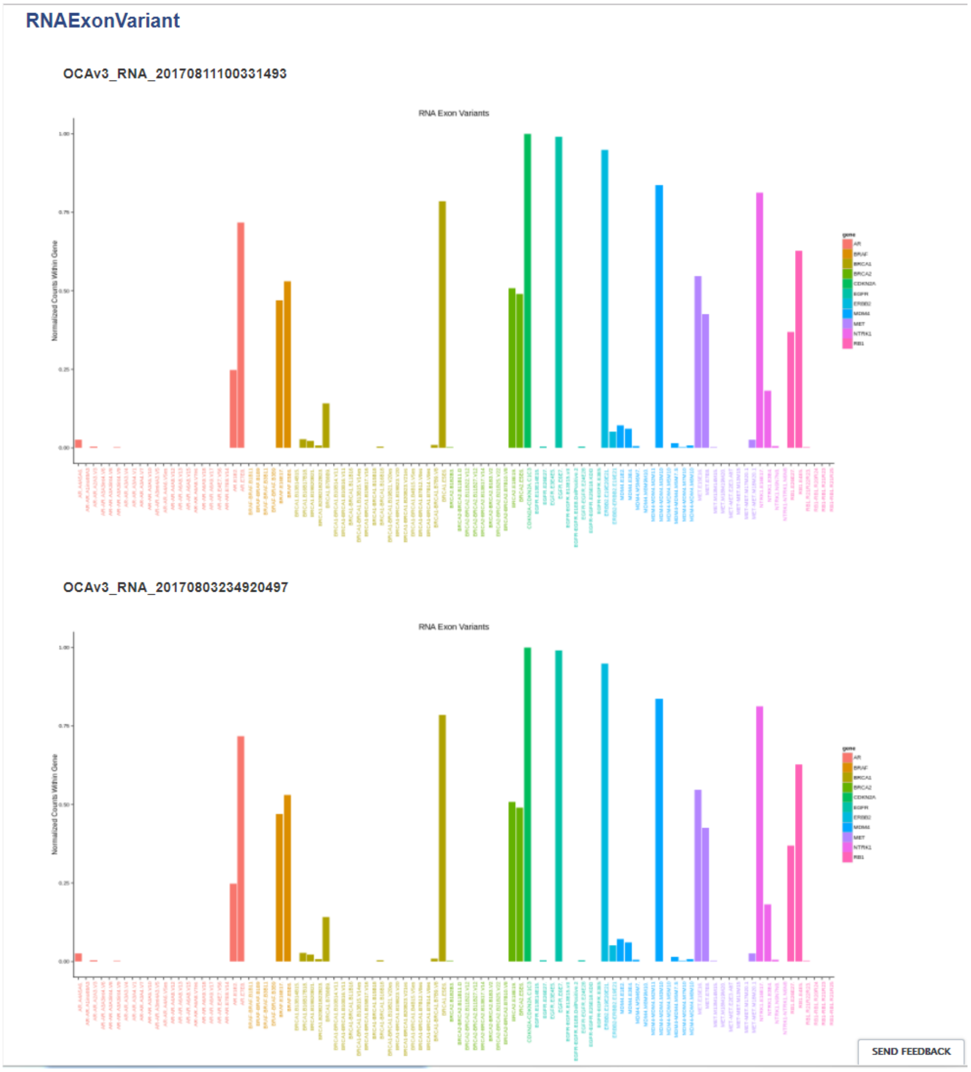
- Pubs_AmpliSeq_RNA_lung_fusion_c1228
- Pubs_Fusions_Test_Multiple_Bams_c1266
- Pubs_Test3_c1182
- INSERM_negative_IonXpress_031_c621_1403092116223

The following is CNV heat map.



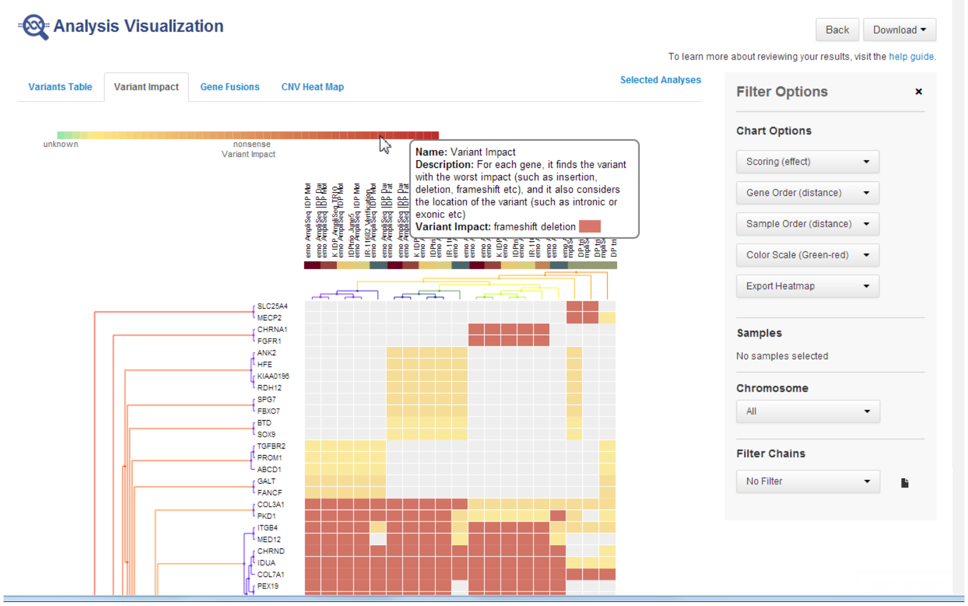
Sample: Demo AmpliSeq Exome CNV case
Analysis: 🟧
Demo AmpliSeq Exome CNV control
1401431927766
Confidence: 10.298
Ploidy: 5 🟩
Gene: OR2T11
Location: chr1:248737817-248790495

- RNA Exon Variants:



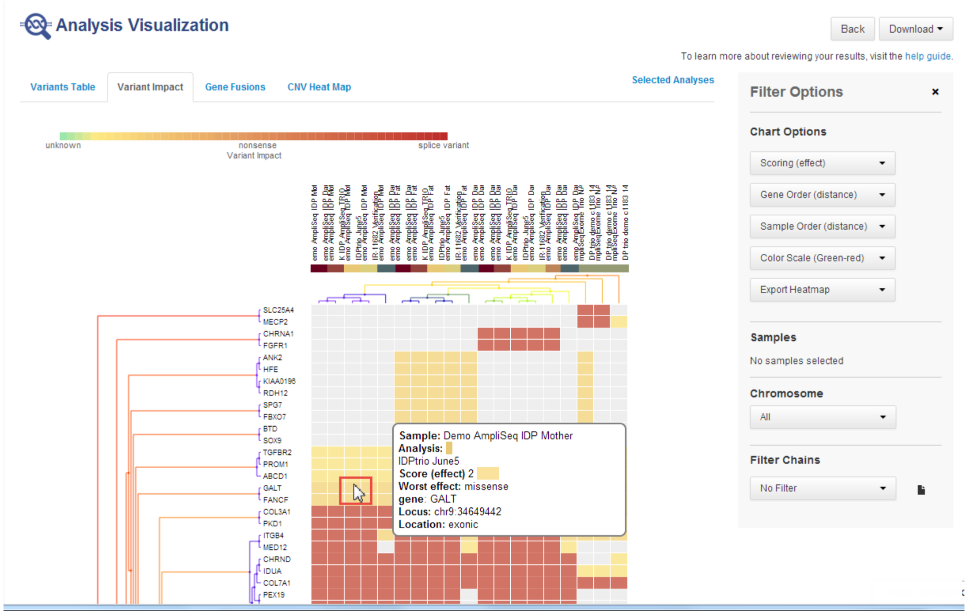
Note:

- In most cases, you will compare similar analyses. 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.
- Hover over the legend of a heat map:



Name: Variant Impact
Description: For each gene, it finds the variant with the worst impact (such as insertion, deletion, frameshift etc), and it also considers the location of the variant (such as intronic or exonic etc)
Variant Impact: frameshift deletion 🟥

Hover over the legend of a cell to see more information:



Sample: Demo AmpliSeq IDP Mother
Analysis: 🟨
IDPtrio June5
Score (effect) 2 🟨
Worst effect: missense
gene: GALT
Locus: chr9:34649442
Location: exonic

Compare multiple analyses and download TSV file

Follow these steps to compare multiple analyses:

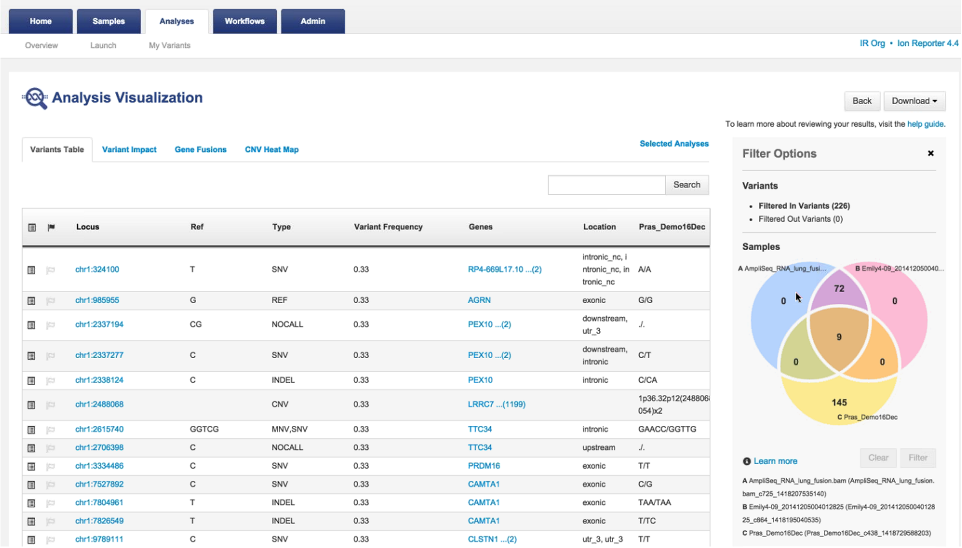
1. In the Analysis > Overview tab, select multiple analyses.
2. *(Optional)* Use the Filter Applications menu to view only analyses of interest.
3. In the **Actions** menu on the right, select **Visualize**.

☐						
☑	Exp10_HCC1143_HCC1143BL_50_50--G99OA_IonXpress_003_c2325_1406993948633	4.2	Variant Review	Exp10_All_38_Samples	Aug 02 2014 08:41 AM	Successful
☑	Exp10_HCC2218_100--TDRDL_IonXpress_006_c2312_1406993948633	4.2	Variant Review	Exp10_All_38_Samples	Aug 02 2014 08:41 AM	Successful
☐	Exp10_HCC2218BL_100--KNRVK_IonXpress_007	4.2	Variant Review	Exp10_All_38_Samples	Aug 02 2014 08:41 AM	Successful

Not all actions are available
analysis types or incomplete
Exome scale analyses or le

Number of selected analys

4. All results are shown initially.



To learn more about reviewing your results, visit the help guide.

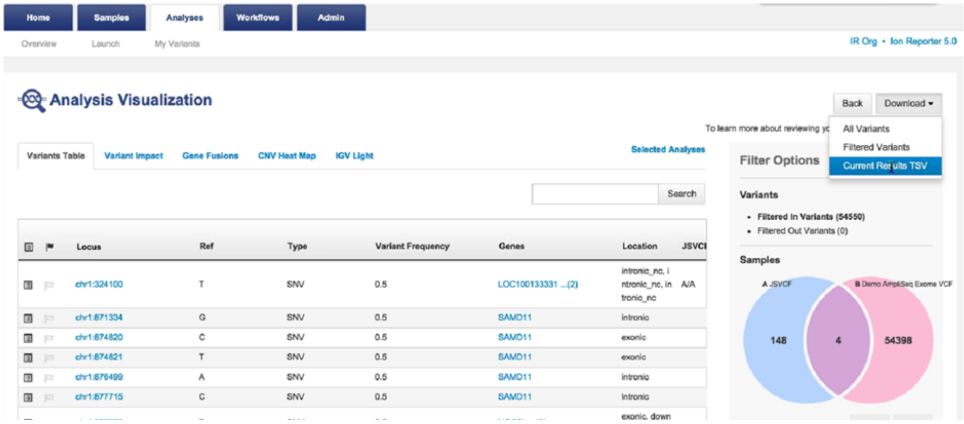
Locus	Ref	Type	Variant Frequency	Genes	Location	Pras_Demo16Dec
chr1:324100	T	SNV	0.33	RP4-669L17.10 ...(2)	intronic_nc, intronic_nc, intronic_nc	A/A
chr1:985955	G	REF	0.33	AGRN	exonic	G/G
chr1:2337194	CG	NOCALL	0.33	PEX10 ...(2)	downstream, utr_3	./.
chr1:2337277	C	SNV	0.33	PEX10 ...(2)	downstream, intronic	C/T
chr1:2338124	C	INDEL	0.33	PEX10	intronic	C/CA
chr1:2488068		CNV	0.33	LRRC7 ...(1199)		1p36.32p12(248806054)x2
chr1:2615740	GGTCG	MNV,SNV	0.33	TTC34	intronic	GAACC/GGTTG
chr1:2706398	C	NOCALL	0.33	TTC34	upstream	./.
chr1:3334486	C	SNV	0.33	PRDM16	exonic	T/T
chr1:7527892	C	SNV	0.33	CAMTA1	exonic	C/G
chr1:7804961	T	INDEL	0.33	CAMTA1	exonic	TAA/TAA
chr1:7826549	T	INDEL	0.33	CAMTA1	exonic	T/TC
chr1:9789111	C	SNV	0.33	CLSTN1 ...(2)	utr_3, utr_3	T/T

Variants
- Filtered In Variants (226)
- Filtered Out Variants (0)

A AmpliSeq_RNA_lung_fusion.bam (AmpliSeq_RNA_lung_fusion.bam_c725_1418207535140)
B Emily4-09_20141205004012825 (Emily4-09_20141205004012825_c864_1418195040535)
C Pras_Demo16Dec (Pras_Demo16Dec_c438_1418729588203)

5. Click the numbers in the Venn components to toggle for inclusion.
6. Then use the Filter button to update the results to include only toggled components.
7. Use the Clear button to deselect all Venn components.
8. When no components are selected, no Venn filtering is performed.
9. Hover over a sample name to see the analysis to which it belongs.

10. To download the results, go to **Download ▸ Current Results TSV**.



Locus	Ref	Type	Variant Frequency	Genes	Location	JSVC
chr1:324100	T	SNV	0.5	LOC100133331 ...(2)	intronic_nc, intronic_nc, intronic_nc	A/A
chr1:871334	G	SNV	0.5	SAMD11	intronic	
chr1:874820	C	SNV	0.5	SAMD11	exonic	
chr1:874821	T	SNV	0.5	SAMD11	exonic	
chr1:876499	A	SNV	0.5	SAMD11	intronic	
chr1:877715	C	SNV	0.5	SAMD11	intronic	

The software outputs a zipped TSV file of all the analyses for comparison.

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. Thereafter hierarchical clustering is conducted for clustering genes and samples within the heat map.

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 .

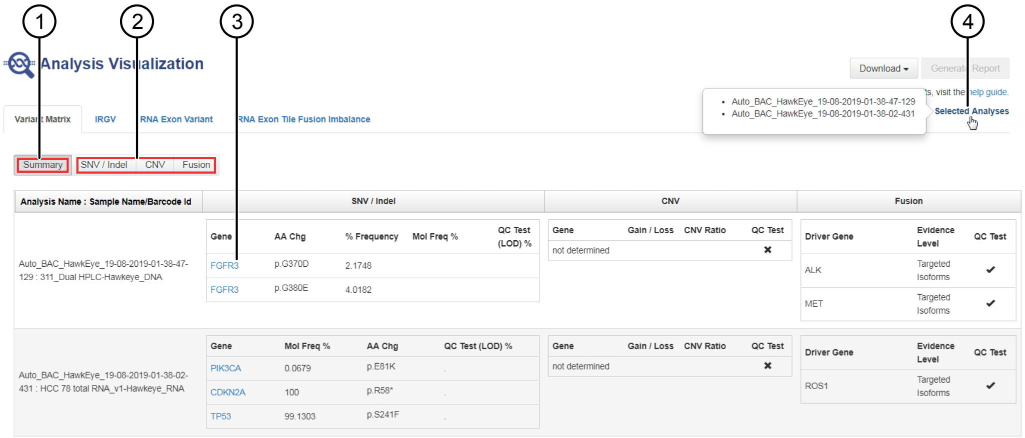
Use the menus in the **Filter Options** pane to limit the data that is displayed in the Variants Table.

- For more information on the **Samples** Venn diagram, see "Samples Venn diagram in Filter Options" on page 295.
- For more information on using the **Chromosome** filter, see "Chromosome filter in the Filter Options" on page 295.
- For more information on using the **Filter Chains** filter, see "Filter Chains filter in the Filter Options" on page 295.

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 1 and Figure 2). For more information, see "Visualize variants in an analysis run with an Ion AmpliSeq™ HD analysis workflow" on page 296.

Note: To review variant information for analyses with Ion AmpliSeq™ workflows, see "Variants Table tab" on page 280.



- Auto_BAC_HawkEye_19-08-2019-01-38-47-129
- Auto_BAC_HawkEye_19-08-2019-01-38-02-431

Analysis Name : Sample Name/Barcode Id: Auto_BAC_HawkEye_19-08-2019-01-38-47-129 : 311_Dual HPLC-Hawkeye_DNA

SNV / Indel

Gene	AA Chg	% Frequency	Mol Freq %	QC Test (LOD) %
FGFR3	p.G370D	2.1748		
FGFR3	p.G380E	4.0182		

CNV

Gene	Gain / Loss	CNV Ratio	QC Test
not determined			✖

Fusion

Driver Gene	Evidence Level	QC Test
ALK	Targeted Isoforms	✓
MET	Targeted Isoforms	✓

Analysis Name : Sample Name/Barcode Id: Auto_BAC_HawkEye_19-08-2019-01-38-02-431 : HCC 78 total RNA_v1-Hawkeye_RNA

SNV / Indel

Gene	Mol Freq %	AA Chg	QC Test (LOD) %
PIK3CA	0.0679	p.E81K	.
CDKN2A	100	p.R58*	.
TP53	99.1303	p.S241F	.

CNV

Gene	Gain / Loss	CNV Ratio	QC Test
not determined			✖

Fusion

Driver Gene	Evidence Level	QC Test
ROS1	Targeted Isoforms	✓

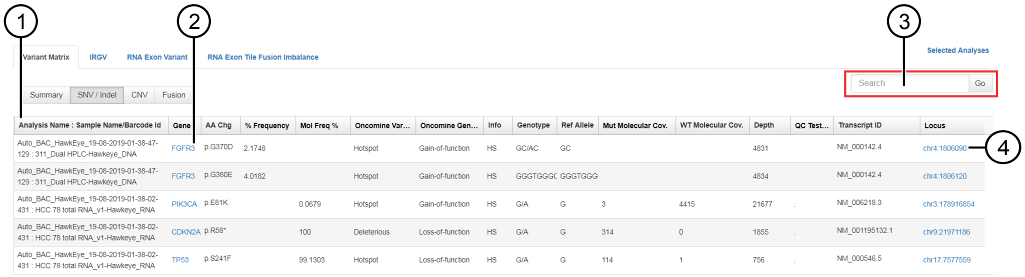
Figure 1 Representative **Summary** screen in the **Variant Matrix** tab

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 2).

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.



Analysis Name : Sample Name/Barcode Id	Gene	AA Chg	% Frequency	Mol Freq %	Oncomine Var...	Oncomine Gen...	Info	Genotype	Ref Allele	Mut Molecular Cov.	WT Molecular Cov.	Depth	QC Test...	Transcript ID	Locus
Auto_BAC_HawkEye_19-08-2019-01-38-47-129 : 311_Dual HPLC-Hawkeye_DNA	FGFR3	p.G370D	2.1748		Hotspot	Gain-of-function	HS	GC/AC	GC			4831		NM_000142.4	chr4:1806090
Auto_BAC_HawkEye_19-08-2019-01-38-47-129 : 311_Dual HPLC-Hawkeye_DNA	FGFR3	p.G380E	4.0182		Hotspot	Gain-of-function	HS	GGGTGGGC	GGGTGGG			4834		NM_000142.4	chr4:1806120
Auto_BAC_HawkEye_19-08-2019-01-38-02-431 : HCC 78 total RNA_v1-Hawkeye_RNA	PIK3CA	p.E81K		0.0679	Hotspot	Gain-of-function	HS	G/A	G	3	4415	21677	.	NM_006218.3	chr3:178916854
Auto_BAC_HawkEye_19-08-2019-01-38-02-431 : HCC 78 total RNA_v1-Hawkeye_RNA	CDKN2A	p.R58*		100	Deleterious	Loss-of-function	HS	G/A	G	314	0	1855	.	NM_001195132.1	chr9:21971186
Auto_BAC_HawkEye_19-08-2019-01-38-02-431 : HCC 78 total RNA_v1-Hawkeye_RNA	TP53	p.S241F		99.1303	Hotspot	Loss-of-function	HS	G/A	G	114	1	756	.	NM_000546.5	chr17:7577559

Figure 2 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.

IRGV tab

You can view visualized analysis results in the Ion Reporter™ Genomic Viewer (IRGV) tab. Click the **IRGV** view the visualization of the results.

#	S#	Role	Sample Name	MAPD
1	1	Self	IR421_bam_definesample	
2	1		DNAOCPv2_DNA_dayu	0.261

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 8, "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.

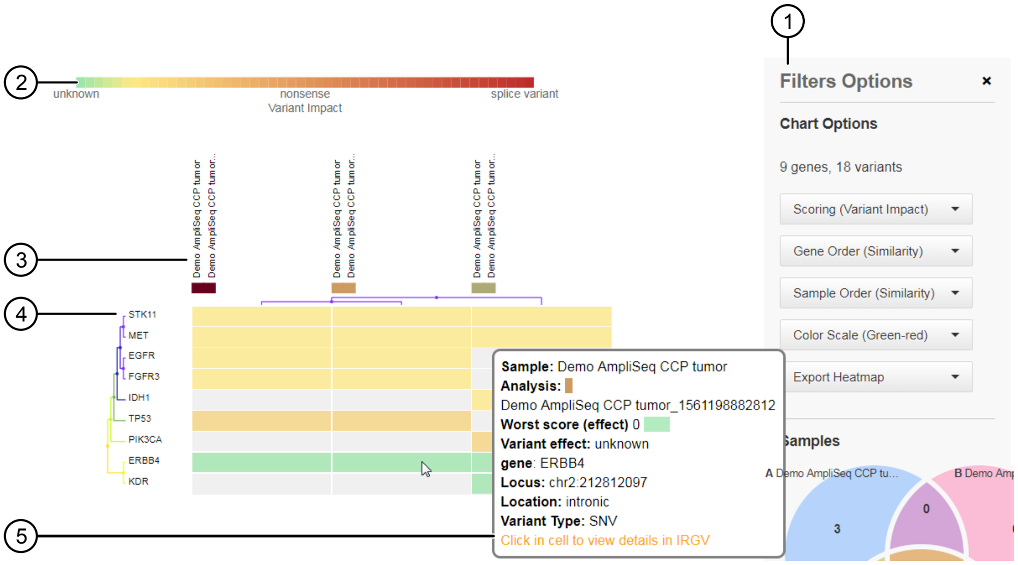
⑪ IGV/Export—Use multiple export options, including an export to IGV, and access to preferences. For more infromation, see "Visualize variants with IGV" on page 306 and "Set IRGV preferences" on page 253.

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 295.
- For more information on using the **Chromosome** filter, see "Chromosome filter in the Filter Options" on page 295.
- For more information on using the **Filter Chains** filter, see "Filter Chains filter in the Filter Options" on page 295.



Sample: Demo AmpliSeq CCP tumor
Analysis:
Demo AmpliSeq CCP tumor_1561198882812
Worst score (effect) 0
Variant effect: unknown
gene: ERBB4
Locus: chr2:212812097
Location: intronic
Variant Type: SNV
Click in cell to view details in IRGV

Figure 3 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 • Nnonsense: 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 alignmen 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.
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.

Chart Option	Description
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.

Variant Impact tab

The legend at the top of the heat map is color-coded for the following variant impacts using the associated score values:

- 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

Set the Scoring menu to a different option, such as SIFT or PolyPhen2 , to base the legend and the heat map on those scores.

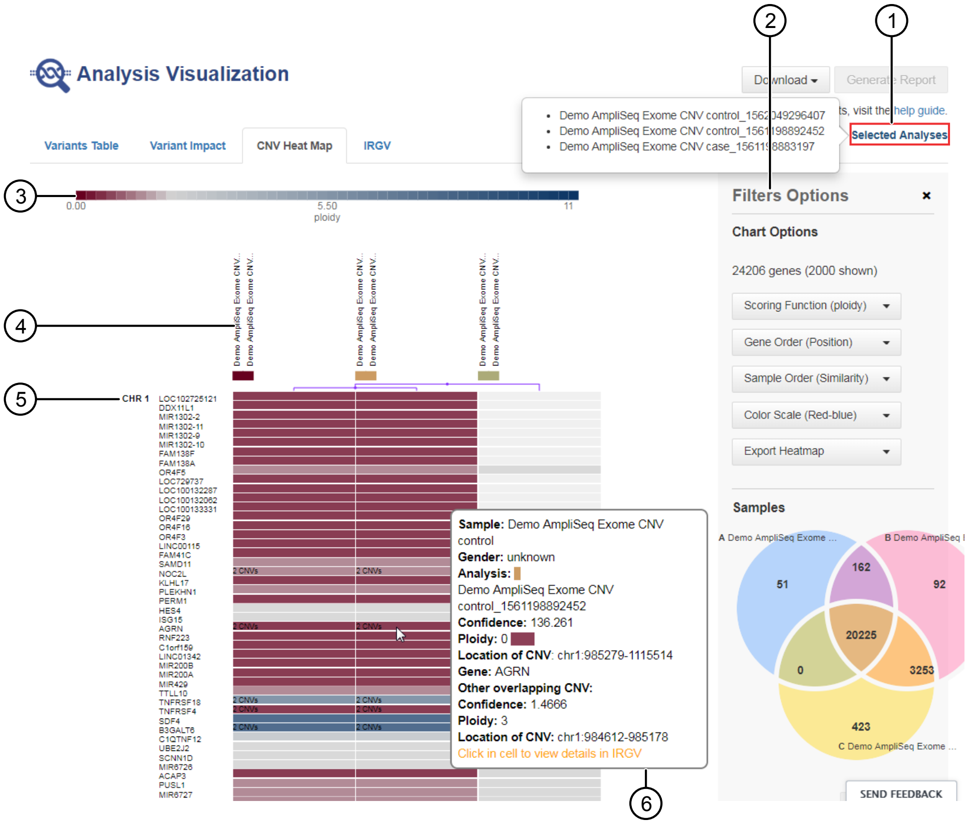
Clustering in Variant Impact heatmap For variant impact heat maps, based on the scoring criteria selected from chart options, most deleterious score is picked for every gene-sample pair to generate the heat map. Thereafter hierarchical clustering is conducted for clustering genes and samples within the heat map.

CNV Heat Map tab

The CNV Heat Map tab provides a visual representation of copy number variation (CNV) data in the form of a heatmap. 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 on the **Samples** Venn diagram, see "Samples Venn diagram in Filter Options" on page 295.
- For more information on using the **Chromosome** filter, see "Chromosome filter in the Filter Options" on page 295.
- For more information on using the **Filter Chains** filter, see "Filter Chains filter in the Filter Options" on page 295.



- Demo AmpliSeq Exome CNV control_1562049296407
- Demo AmpliSeq Exome CNV control_1561198892452
- Demo AmpliSeq Exome CNV case_1561198883197

Sample: Demo AmpliSeq Exome CNV control
Gender: unknown
Analysis: 🟧
Demo AmpliSeq Exome CNV control_1561198892452
Confidence: 136.261
Ploidy: 0 🟥
Location of CNV: chr1:985279-1115514
Gene: AGRN
Other overlapping CNV:
Confidence: 1.4666
Ploidy: 3
Location of CNV: chr1:984612-985178
Click in cell to view details in IRGV

① Click **Selected Analyses** to view the list of the analyses that are visualized.
② Heat map **Filter Options**.
③ Heat map legend: ploidy. Hover over the legend the view the relationship between the ploidy value 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 Genes: hover over a gene ID to view the details for that gene or click on 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: 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. If >1 CNVs are detected for the same gene, the number of CNVs is listed in the cell.

Use the following **Chart Options** to display 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 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.

Filter impact on the variants displayed in heat maps

If you save your filter chain on the Analysis Results page, then the heat maps and other multiple analysis visualizations show only filtered in variants.

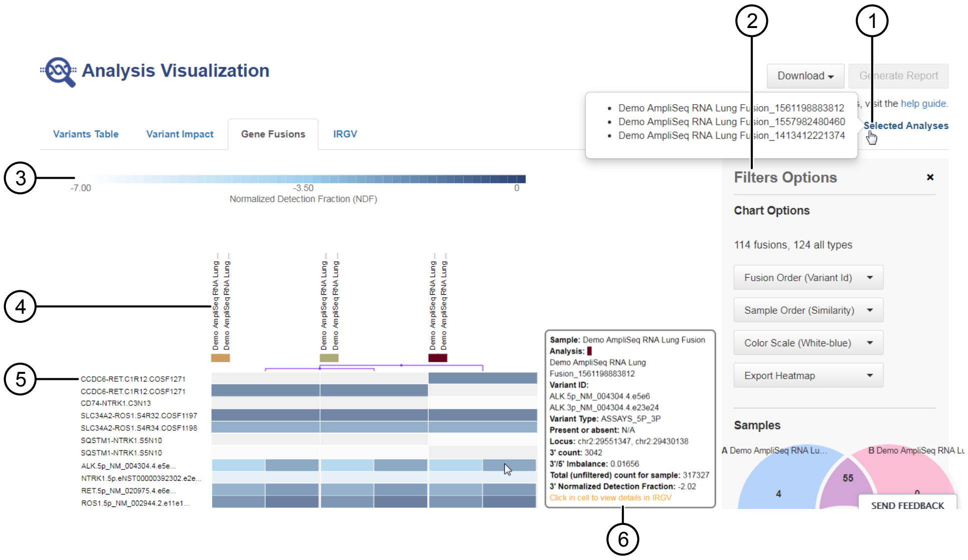
If you do not save your filter on the Analysis Results page, then the heat maps and other multiple analysis visualizations show only the entire result sets (nothing is filtered out).

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 295.
- For more information on using the **Chromosome** filter, see “Chromosome filter in the Filter Options“ on page 295.
- For more information on using the **Filter Chains** filter, see “Filter Chains filter in the Filter Options“ on page 295.



Sample: Demo AmpliSeq RNA Lung Fusion
Analysis: ■
Demo AmpliSeq RNA Lung Fusion_1561198883812
Variant ID:
ALK.5p_NM_004304.4.e5e6
ALK.3p_NM_004304.4.e23e24
Variant Type: ASSAYS_5P_3P
Present or absent: N/A
Locus: chr2:29551347, chr2:29430138
3' count: 3042
3'/5' Imbalance: 0.01656
Total (unfiltered) count for sample: 317327
3' Normalized Detection Fraction: -2.02
Click in cell to view details in IRGV

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.

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.

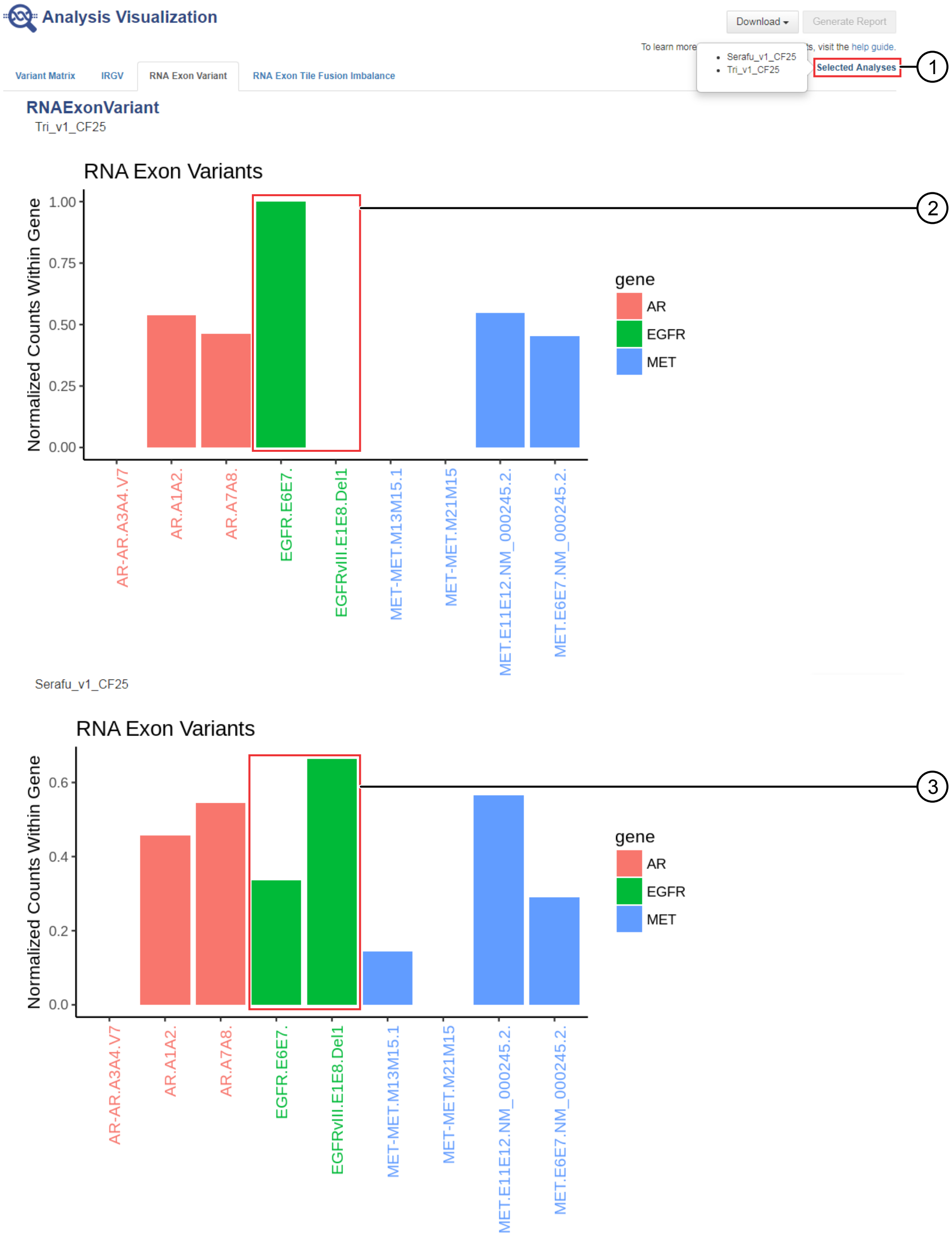


Figure 4 Representative RNA Exon Variant bar graph

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.

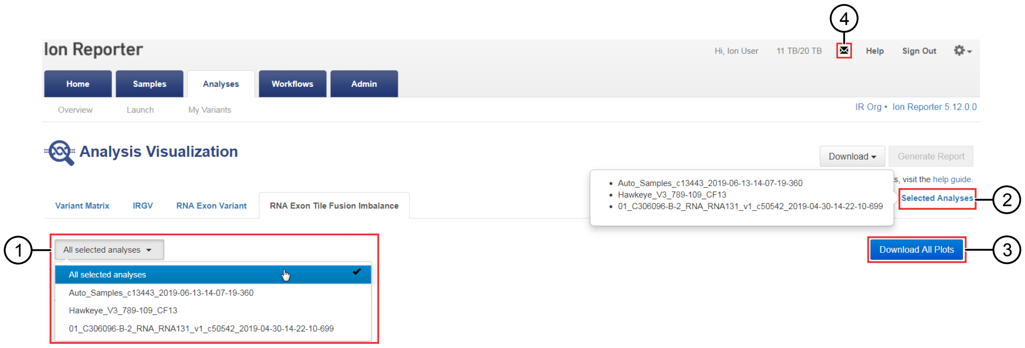
① Click **Selected Analyses** to view a list of the analyses that you selected for visualization.

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)**.

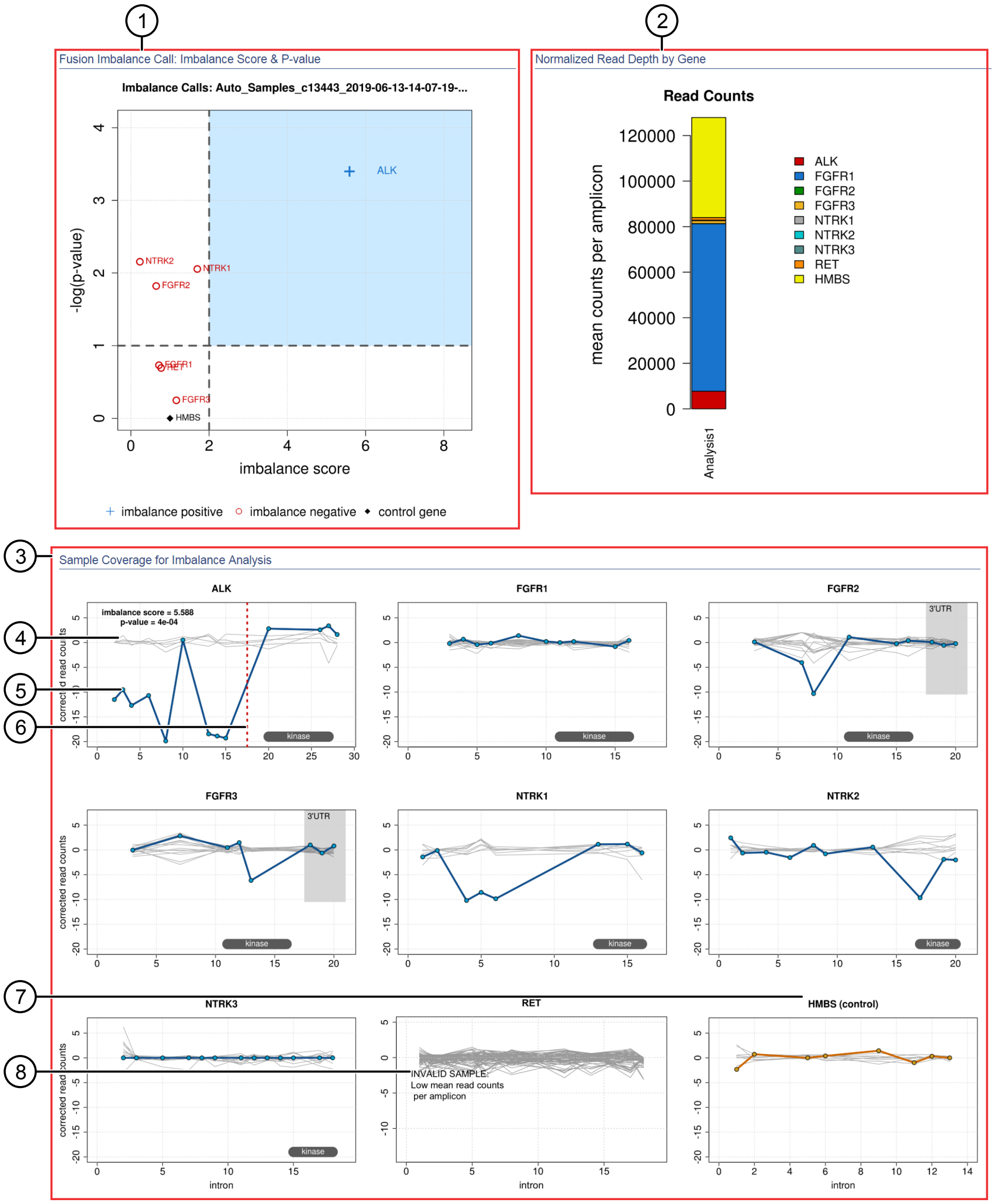


Figure 5 Single-sample analysis visualization

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.

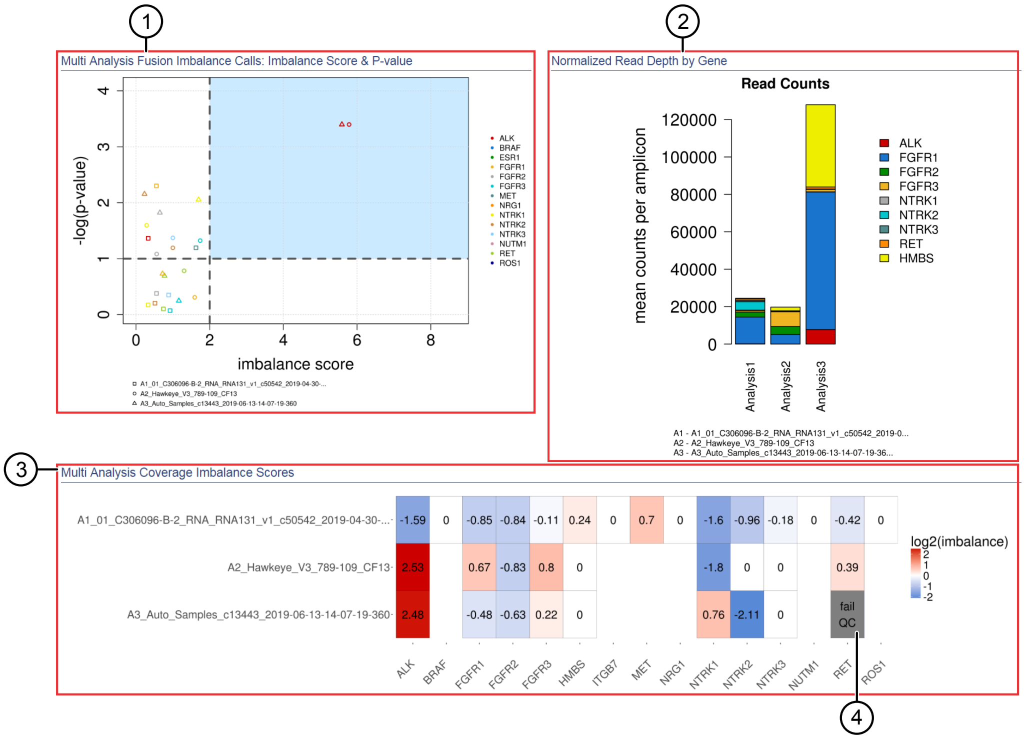


Figure 6 Multi-sample analyses visualization

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 5, 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 5, callout 8).

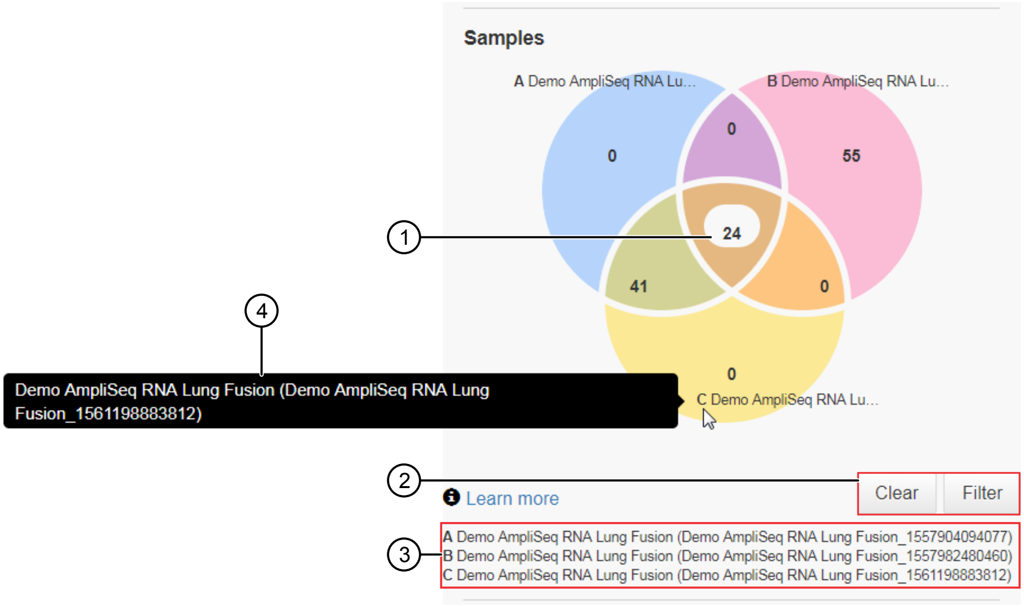
Filter Options

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 2 or 3 analyses side by side.



Demo AmpliSeq RNA Lung Fusion (Demo AmpliSeq RNA Lung Fusion_1561198883812)

A Demo AmpliSeq RNA Lung Fusion (Demo AmpliSeq RNA Lung Fusion_1557904094077)
B Demo AmpliSeq RNA Lung Fusion (Demo AmpliSeq RNA Lung Fusion_1557982480460)
C Demo AmpliSeq RNA Lung Fusion (Demo AmpliSeq RNA Lung Fusion_1561198883812)

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 .

Visualize variants in an analysis run with an Ion AmpliSeq™ HD analysis workflow

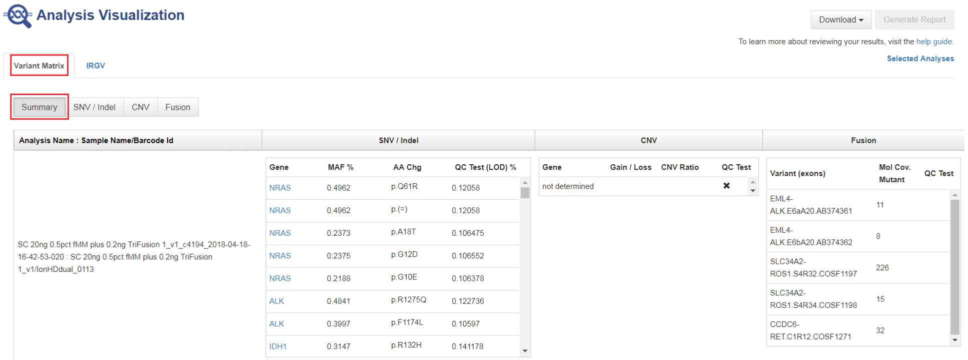
You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants.

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 two or more analyses if you want 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.



Analysis Name : Sample Name/Barcode Id

SC 20ng 0.5pct fMM plus 0.2ng TriFusion 1_v1_c4194_2018-04-18-16-42-53-020 : SC 20ng 0.5pct fMM plus 0.2ng TriFusion 1_v1/IonHDdual_0113

SNV / Indel

Gene	MAF %	AA Chg	QC Test (LOD) %
NRAS	0.4962	p.Q61R	0.12058
NRAS	0.4962	p.(=)	0.12058
NRAS	0.2373	p.A18T	0.106475
NRAS	0.2375	p.G12D	0.106552
NRAS	0.2188	p.G10E	0.106378
ALK	0.4841	p.R1275Q	0.122736
ALK	0.3997	p.F1174L	0.10597
IDH1	0.3147	p.R132H	0.141178

CNV

Gene	Gain / Loss	CNV Ratio	QC Test
not determined			✖

Fusion

Variant (exons)	Mol Cov. Mutant	QC Test
EML4-ALK.E6aA20.AB374361	11	
EML4-ALK.E6bA20.AB374362	8	
SLC34A2-ROS1.S4R32.COSF1197	226	
SLC34A2-ROS1.S4R34.COSF1198	15	
CCDC6-RET.C1R12.COSF1271	32	

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.
   - Click **SNV/INDEL**, **CNV**, or **Fusion** to view detailed analysis metrics. For a description of each metric, see "Detailed analysis metrics" on page 308.

- 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 7 Example SNV/Indel visualization in IRGV

① Variant density overview, illustrated 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

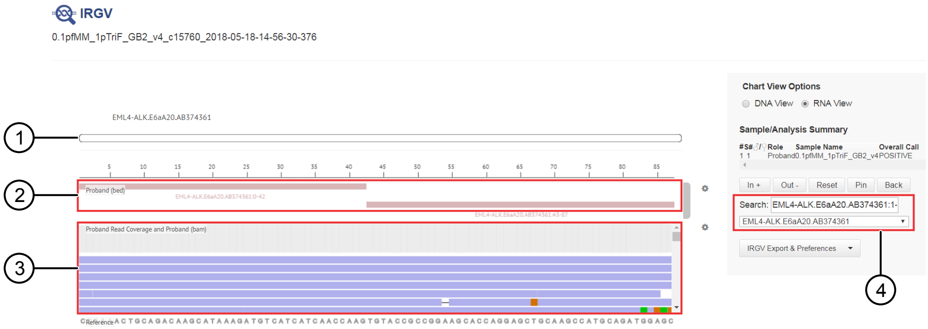


Figure 8 Example fusion visualization in IRGV

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 cursor 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 261.
Review detailed data about a BAM read	Single-click on 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, and total number of molecules, the distribution of single nucleotides at that position, and the 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 additional processing by the variant detection pipeline.

Visualize MSI analysis results

1. There are two ways to visualize MSI analysis results in Ion Reporter™ Software:

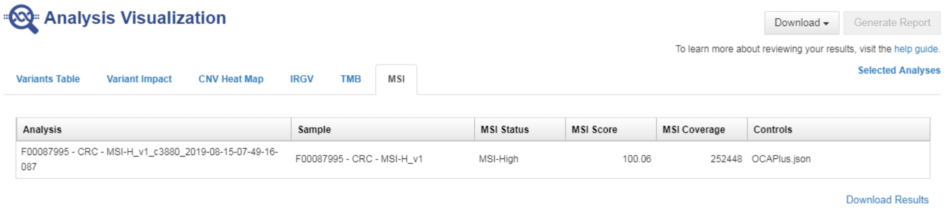
Option	Description
Visualize MSI results from one or more analyses simultaneously from the **Analyses** table.	In the **Analyses** table, select a row for an analysis or select the checkbox next to one or more analyses that you want to visualize simultaneously, then click **Visualize**. Alternatively, 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 **Tumor Mutational Burden** tab.

2. Click the **MSI** tab.

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-Low • MSS (MSI stable) • No Call (MSI status is intermediate, i.e., between the thresholds) • QC Fail MSI parameter settings include thresholds that determine whether the status is MSI-High, MSI-Low, or MSS. A status of No Call or QC Fail is based on the MSI marker coverage.
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 Coverage	A combined sample-level coverage that is calculated with the individual MSI marker-level coverage.

A table with **MSI Status**, **MSI Score**, and **MSI Coverage** is shown.



Analysis	Sample	MSI Status	MSI Score	MSI Coverage	Controls
F00087995 - CRC - MSI-H_v1_c3880_2019-08-15-07-49-16-087	F00087995 - CRC - MSI-H_v1	MSI-High	100.06	252448	OCAPlus.json

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**.

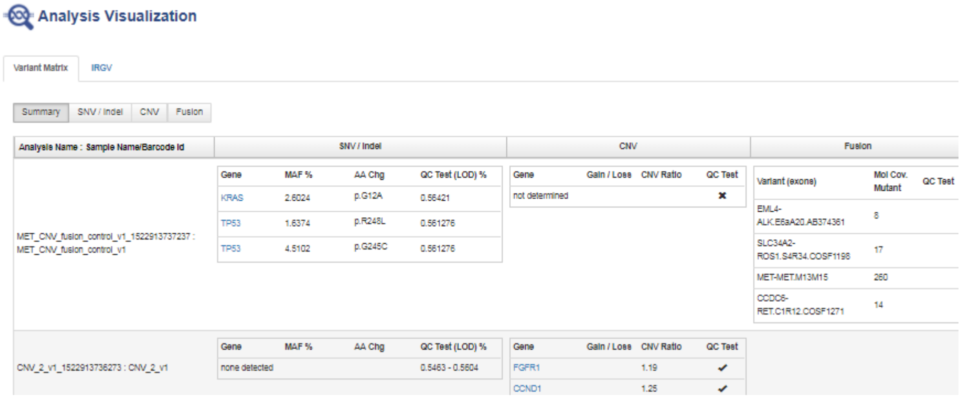
2. Click the column headings to sort the results, or use the available filters to limit the list of analyses.

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. Cclick **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.



Analysis Name : Sample Name/Barcode Id: MET_CNV_fusion_control_v1_1522913737237 : MET_CNV_fusion_control_v1

SNV / Indel

Gene	MAF %	AA Chg	QC Test (LOD) %
KRAS	2.6024	p.G12A	0.56421
TP53	1.6374	p.R248L	0.561276
TP53	4.5102	p.G245C	0.561276

CNV

Gene	Gain / Loss	CNV Ratio	QC Test
not determined			✖

Fusion

Variant (exons)	Mol Cov. Mutant	QC Test
EML4-ALK.E6aA20.AB374361	8	
SLC34A2-ROS1.S4R34.COSF1196	17	
MET-MET.M13M15	260	
CCDC6-RET.C1R12.COSF1271	14	

Analysis Name : Sample Name/Barcode Id: CNV_2_v1_1522913736273 : CNV_2_v1

SNV / Indel

Gene	MAF %	AA Chg	QC Test (LOD) %
none detected			0.5463 - 0.5604

CNV

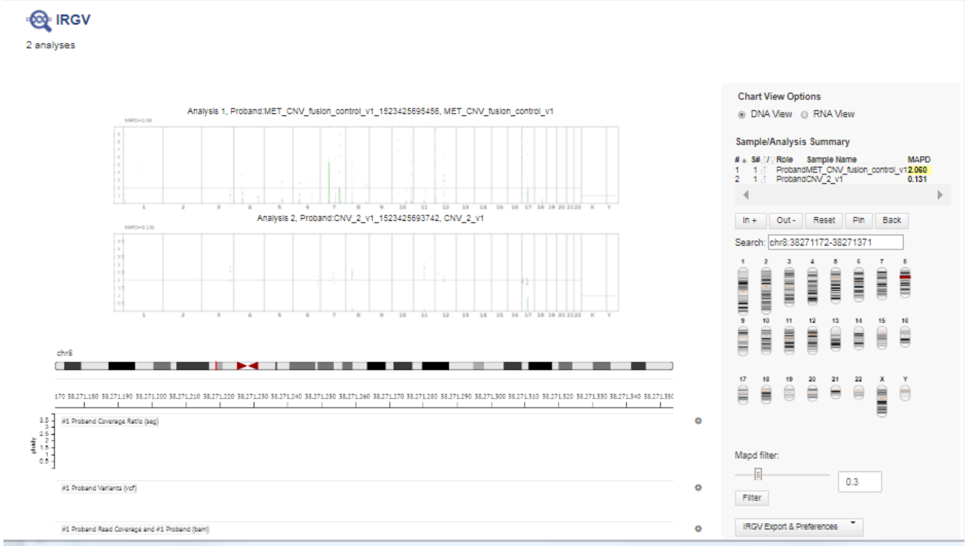
Gene	Gain / Loss	CNV Ratio	QC Test
FGFR1		1.19	✓
CCND1		1.25	✓

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).



#	S#	Role	Sample Name	MAPD
1	1	Proband	MET_CNV_fusion_control_v1	2.060
2	1	Proband	CNV_2_v1	0.131

Search: chr8:38271172-38271371

Mapd filter: 0.3

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.

View analysis results for a single sample

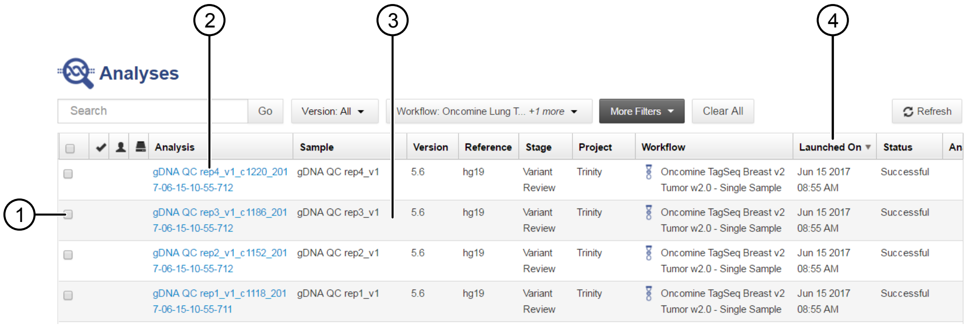
Use this procedure to view and manage the extended analysis results of a single sample.

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 299.

1. Click the **Analyses** tab, then click **Overview**.

2. In the **Analyses** screen you can:

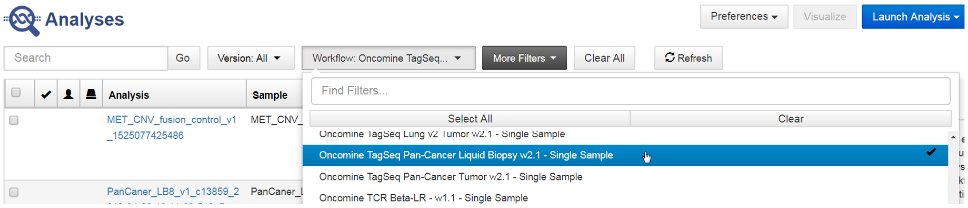
To	Action
Select an analysis	Enable the checkbox.
Open an **Analysis Results** screen.	Click the hyperlink (in the **Analysis** column).
View details	Click anywhere in the analysis' row, except on the hyperlink.
Sort	Click column headings to sort the analyses based on the column contents.



☐	✔	👤		Analysis	Sample	Version	Reference	Stage	Project	Workflow	Launched On ▾	Status	An
☐				gDNA QC rep4_v1_c1220_2017-06-15-10-55-712	gDNA QC rep4_v1	5.6	hg19	Variant Review	Trinity	Oncomine TagSeq Breast v2 Tumor w2.0 - Single Sample	Jun 15 2017 08:55 AM	Successful	
☐				gDNA QC rep3_v1_c1186_2017-06-15-10-55-712	gDNA QC rep3_v1	5.6	hg19	Variant Review	Trinity	Oncomine TagSeq Breast v2 Tumor w2.0 - Single Sample	Jun 15 2017 08:55 AM	Successful	
☐				gDNA QC rep2_v1_c1152_2017-06-15-10-55-712	gDNA QC rep2_v1	5.6	hg19	Variant Review	Trinity	Oncomine TagSeq Breast v2 Tumor w2.0 - Single Sample	Jun 15 2017 08:55 AM	Successful	
☐				gDNA QC rep1_v1_c1118_2017-06-15-10-55-711	gDNA QC rep1_v1	5.6	hg19	Variant Review	Trinity	Oncomine TagSeq Breast v2 Tumor w2.0 - Single Sample	Jun 15 2017 08:55 AM	Successful	

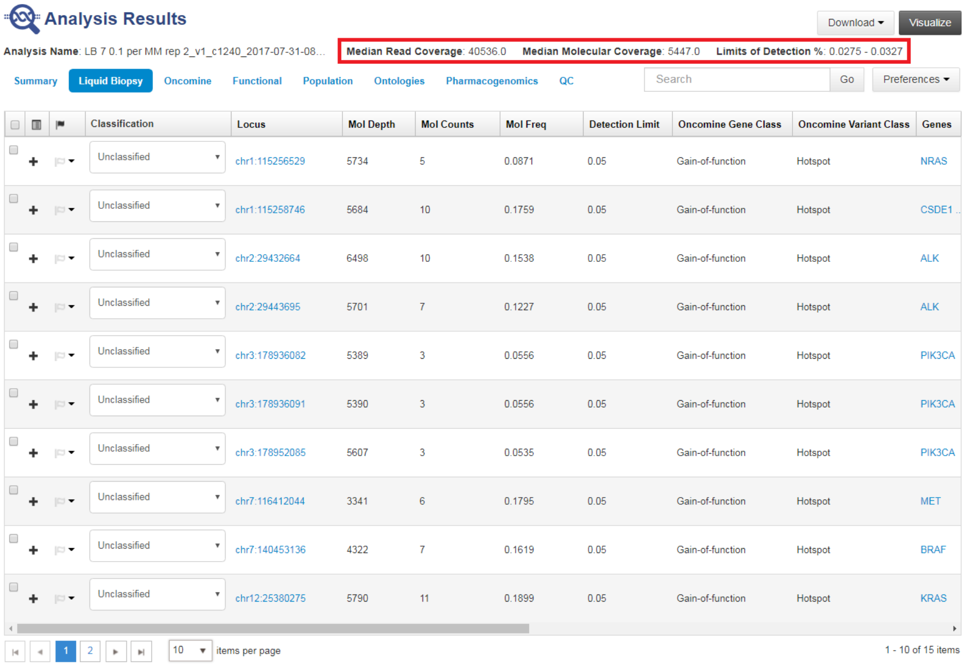
① Select analysis
② Open **Analysis Results** screen
③ View details
④ Sort a column

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.



Analysis Name: LB 7 0.1 per MM rep 2_v1_c1240_2017-07-31-08...

Median Read Coverage: 40536.0 **Median Molecular Coverage**: 5447.0 **Limits of Detection %**: 0.0275 - 0.0327

Classification	Locus	Mol Depth	Mol Counts	Mol Freq	Detection Limit	Oncomine Gene Class	Oncomine Variant Class	Genes
Unclassified	chr1:115256529	5734	5	0.0871	0.05	Gain-of-function	Hotspot	NRAS
Unclassified	chr1:115258746	5684	10	0.1759	0.05	Gain-of-function	Hotspot	CSDE1 ..
Unclassified	chr2:29432664	6498	10	0.1538	0.05	Gain-of-function	Hotspot	ALK
Unclassified	chr2:29443695	5701	7	0.1227	0.05	Gain-of-function	Hotspot	ALK
Unclassified	chr3:178936082	5389	3	0.0556	0.05	Gain-of-function	Hotspot	PIK3CA
Unclassified	chr3:178936091	5390	3	0.0556	0.05	Gain-of-function	Hotspot	PIK3CA
Unclassified	chr3:178952085	5607	3	0.0535	0.05	Gain-of-function	Hotspot	PIK3CA
Unclassified	chr7:116412044	3341	6	0.1795	0.05	Gain-of-function	Hotspot	MET
Unclassified	chr7:140453136	4322	7	0.1619	0.05	Gain-of-function	Hotspot	BRAF
Unclassified	chr12:25380275	5790	11	0.1899	0.05	Gain-of-function	Hotspot	KRAS

1 - 10 of 15 items

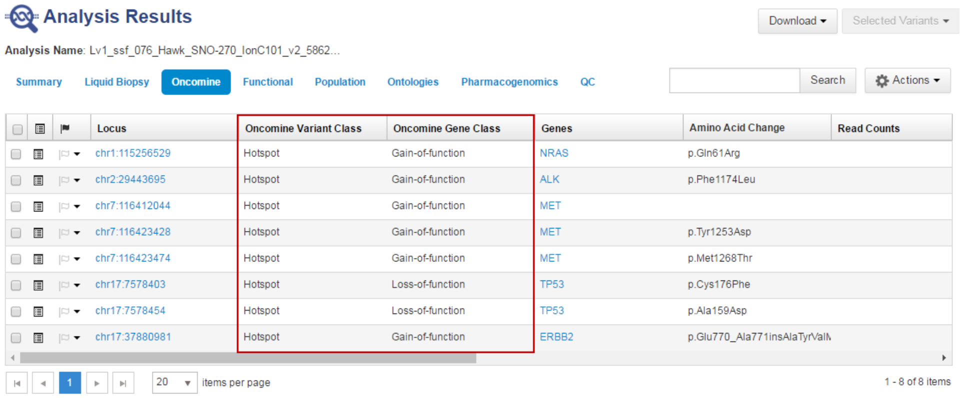
Column	Description
Median Read Coverage	Reports median coverage across targets. Median Molecular Coverage reports median number of individual interrogated DNA molecules across targets.
Median Molecular Coverage	Directly influences the limit of detection in a sample run. We always require two independent 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.

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**.

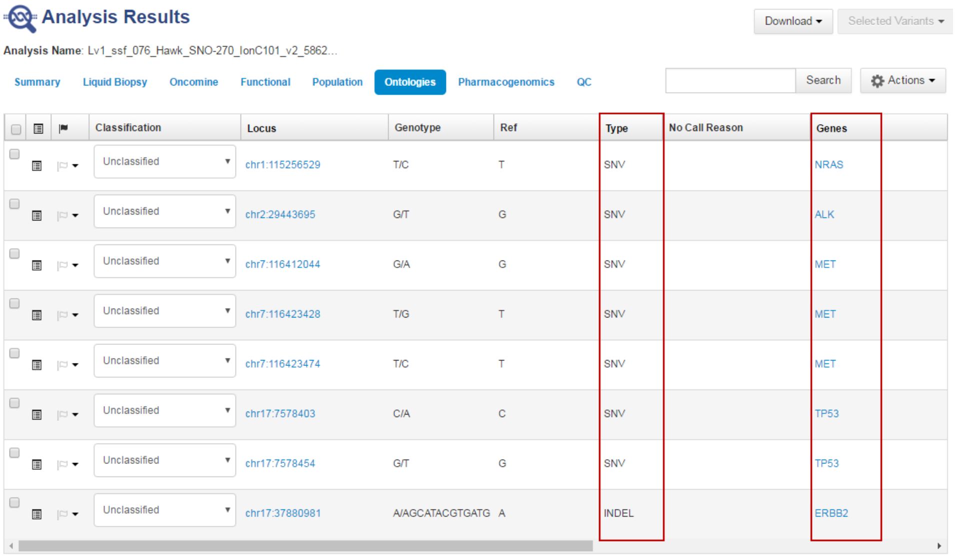


Analysis Name: Lv1_ssf_076_Hawk_SNO-270_IonC101_v2_5862...

Locus	Oncomine Variant Class	Oncomine Gene Class	Genes	Amino Acid Change	Read Counts
chr1:115256529	Hotspot	Gain-of-function	NRAS	p.Gln61Arg	
chr2:29443695	Hotspot	Gain-of-function	ALK	p.Phe1174Leu	
chr7:116412044	Hotspot	Gain-of-function	MET		
chr7:116423428	Hotspot	Gain-of-function	MET	p.Tyr1253Asp	
chr7:116423474	Hotspot	Gain-of-function	MET	p.Met1268Thr	
chr17:7578403	Hotspot	Loss-of-function	TP53	p.Cys176Phe	
chr17:7578454	Hotspot	Loss-of-function	TP53	p.Ala159Asp	
chr17:37880981	Hotspot	Gain-of-function	ERBB2	p.Glu770_Ala771insAlaTyrValM	

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.



Analysis Name: Lv1_ssf_076_Hawk_SNO-270_IonC101_v2_5862...

☐	Classification	Locus	Genotype	Ref	Type	No Call Reason	Genes
☐	Unclassified	chr1:115256529	T/C	T	SNV		NRAS
☐	Unclassified	chr2:29443695	G/T	G	SNV		ALK
☐	Unclassified	chr7:116412044	G/A	G	SNV		MET
☐	Unclassified	chr7:116423428	T/G	T	SNV		MET
☐	Unclassified	chr7:116423474	T/C	T	SNV		MET
☐	Unclassified	chr17:7578403	C/A	C	SNV		TP53
☐	Unclassified	chr17:7578454	G/T	G	SNV		TP53
☐	Unclassified	chr17:37880981	A/AGCATACGTGATG	A	INDEL		ERBB2

Use filter chains to change analysis results

You can review the results of an Oncomine™ analysis run through a TagSeq analysis workflow, then apply different filter chains to alter 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 is the default filter chain for the TagSeq analysis workflows. Use this filter chain for analysis results that report all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL_ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
Called Hotspot Variants and Controls	Select this filter chain for analysis results that report all hotspot variants that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNP and INDEL.
Variant Matrix Summary	Select this filter chain for analysis results that generate a visualization that contains the same set of variants that are included in the Analysis Results table. This filter chain allows results to be exported. Import the exported results file into Oncomine™ Reporter (OKR) to include the variants shown in the Variant Matrix Summary view in OKR reports. Filter variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants.

3. Review the results.

Visualize variants with IGV

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.

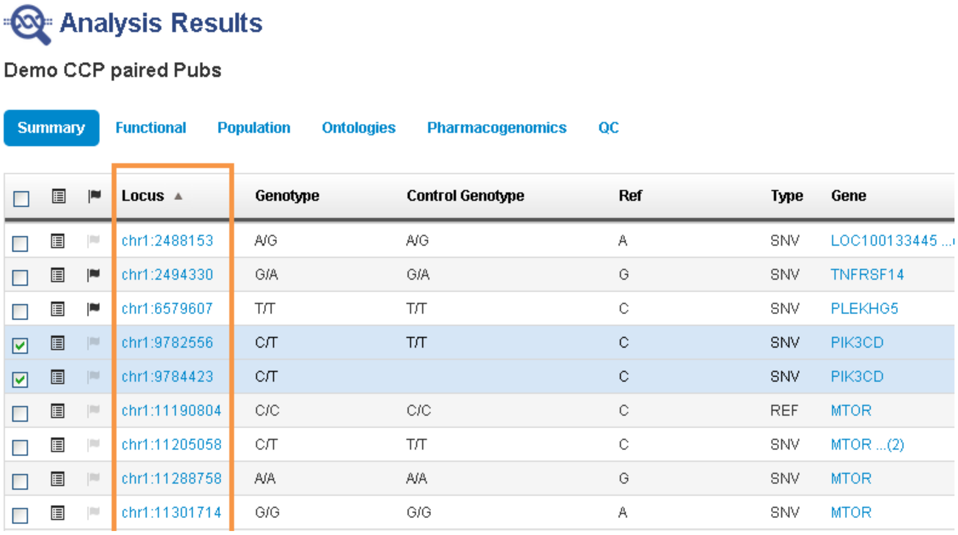
Note: In an IGV visualization, hotspots files that are in shown in a track in can include IGV annotations for hotspots that were not used to call bases in the data.

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 253.

The following steps are for a Chrome™ browser on the Windows™ operating system. The instructions vary slightly based on the operating system and browser that you use.

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.



☐	Locus ▲	Genotype	Control Genotype	Ref	Type	Gene
☐	chr1:2488153	A/G	A/G	A	SNV	LOC100133445 ...
☐	chr1:2494330	G/A	G/A	G	SNV	TNFRSF14
☐	chr1:6579607	T/T	T/T	C	SNV	PLEKHG5
☑	chr1:9782556	C/T	T/T	C	SNV	PIK3CD
☑	chr1:9784423	C/T		C	SNV	PIK3CD
☐	chr1:11190804	C/C	C/C	C	REF	MTOR
☐	chr1:11205058	C/T	T/T	C	SNV	MTOR ...(2)
☐	chr1:11288758	A/A	A/A	G	SNV	MTOR
☐	chr1:11301714	G/G	G/G	A	SNV	MTOR

3. In the notice that appears in the browser download bar, click**Keep**.

4. Do one of the following steps to open an IGV visualization for a variant:
   - Click the **igv.jnlp** link on the viewer download bar.
   - Click the **igv.jnlp** filename in the viewer.

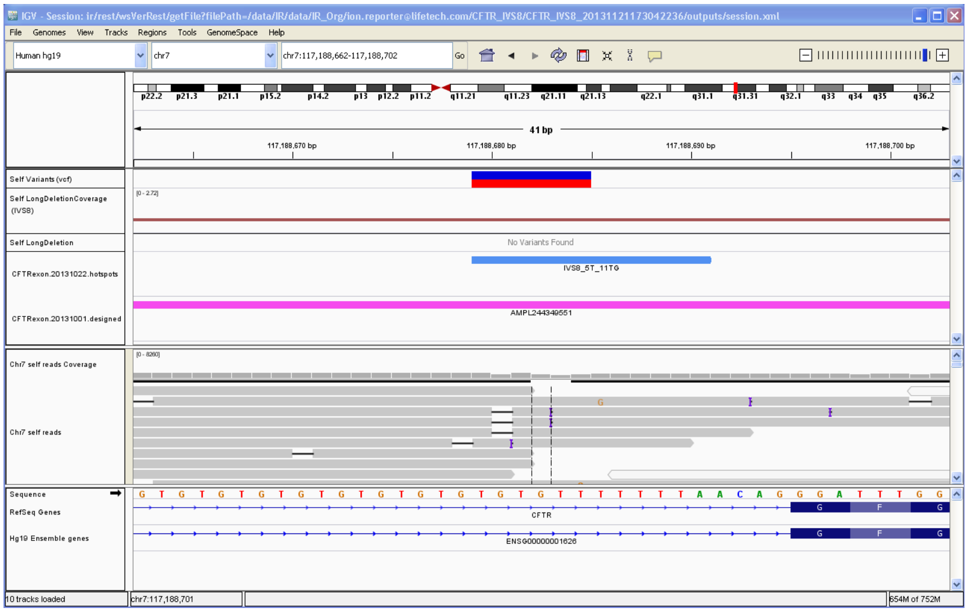


Figure 9 Example of an IGV visualization of a variant

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***.

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.

Metric	Description
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.
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	Cancer driver gene descriptions as reported when the Oncomine™ Variant Annotator plugin is run in Ion Reporter™ Software.
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.

Metric	Description
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).

Quality control (QC) thresholds

QC Test	Detection threshold
SNV/Indel	
A limit of detection (LOD) is calculated and displayed for each variant call. LOD is determined by the level of molecular amplicon coverage. If no variant call is detected, the LOD range is displayed across entire amplicon.	Molecular coverage must be at least 2 with a minimum detection cutoff frequency of 0.035% and 0.05% for lung and breast panels, respectively.
CNV	
The MAPD metric is a measure of read coverage noise detected across all amplicons in a panel. Higher MAPD typically translates to lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. MAPD score is viewable in downloadable VCF file or review of the **Analysis Results** of a single sample extended analysis.	To make a CNV call the following criteria must be met: • MAPD <0.4 • P-value $<10^{-5}$ • CNV Ratio for a copy number gain must be >1.15 • CNV Ratio for a copy number loss must be <0.85 **Note:** The CNV Ratio call thresholds were derived empirically using plasma samples from healthy donors with normal CNV status.
Fusions/Exon Skipping[1]	
• Fusions—Panel includes 2 process control target genes, TBP and HMBS. At least 1 control must have a molecular count of >2 to pass QC. • MET Exon Skipping—Panel includes 2 MET Wild Type control amplicons (gene name has WT at the end). At least 1 of these controls must have a molecular count >2 to pass QC.	Fusion and Exon Skipping amplicons must have >2 molecular counts to be reported.

[1] These variant types are included in the Oncomine™ Lung cfNA Assay, derived from RNA reverse-transcribed into cDNA during library preparation.

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. For QC and CNV calling rules, see page 310 .
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. For QC and Fusion calling rules, see page 310.
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.
Analysis detail	• MET exon 14 targets are reported as **RNAExonVariant** in the **Type** column. • For QC and MET exon 14 skipping calling rules, see page 310.

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. For QC and CNV calling rules, see page 310.
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.

8 Filters and filter chains

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 a 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 99.

You can also create custom 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 340 and “Data type filters“ on page 343. After you create a custom filter chain, you can add it to a 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 custom 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. Custom filter chains are created by combining filters to make a filter chain that meets your 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 that are applied to the filter chain.

- When you refine analysis results, you can view 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 have been applied when the analysis workflow is launched, or applied to the 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 custom filter chain

You can create a custom filter chain to refine your 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 custom 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 custom filter chain, use one of the following procedures to get started:

Option	Description
Workflow Presets	1. In the **Workflows** tab, click **Presets**. 2. Click **Create Preset ▸ Filter Chain**.
Analysis Results	1. In the **Analysis** tab, click **Overview**. 2. Click the link of an analysis of interest. The **Analysis Results** screen appears. 3. 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 340 and "Data type filters" on page 343.

  b. If additional settings are available, set the appropriate conditions for the selected filter.
     For more information, see "Annotation source filters" on page 340.
  c. Click **Add** to add the filter to the **Selected Filters** list.
  d. Repeat to add additional filters.
     The filter count is incremented (for example, --2) as you add additional 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 96 and "Change the default filter chain for an analysis workflow" on page 99.

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 192 and "Use filter chains to change analysis results" on page 305.

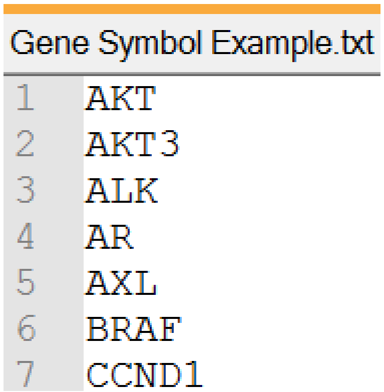
Create a gene symbol filter

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 321.

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:



```
AKT
AKT3
ALK
AR
AXL
BRAF
CCND1
```

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.

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 96.

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 192 and “Use filter chains to change analysis results“ on page 305.

Edit a filter chain

You can edit a custom 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 custom filter chain, use one of the following procedures to get started.

Option	Description
Workflow Presets	1. In the **Workflows** tab, click **Presets**. 2. Select **Filter Chains** from the list. 3. Select the row of the filter chain that you want to modify, then click **Edit**.
Analysis Results	1. In the **Analyses** tab, click **Overview**. 2. Click the link of a analysis of interest. The **Analysis Results** screen appears. 3. In the right pane, in the **Filter Chains** section, select the custom filter chain that you want to edit, then click ✎ **(Edit)**.
Visualize	1. In the **Analyses** tab, click **Overview**. 2. Click the link of an analysis of interest. The **Analysis Results** screen appears. 3. Click **Visualize**, then select the **IRGV** tab. 4. In the right pane, in the **Filter Chains** section, select the custom filter chain that you want to edit, then click ✎ **(Edit)**.

2. Update the **Edit Filter Chain** 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 **FilterChain Query**, change the order in which filters are applied by typing in the text box.
Modify filter conditions	In the **Choose Filter** list, select a filter that is included in the filter chain. Modify 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 96 and "Change the default filter chain for an analysis workflow" on page 99.

It can also be applied to analysis results or a visualization. For more information, see "Apply a filter chain to analysis results" on page 192 and "Use filter chains to change analysis results" on page 305.

Lock a custom filter chain in workflow presets

After you create a custom 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 custom filter chain in workflow presets

You can delete a custom filter chain from the available filter chains list in Ion Reporter™ Software. You cannot delete a predefined filter chain.

Custom 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.

Custom filter chain examples

You can create different types of custom 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 347.

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 96, “Create a custom analysis workflow for mosaicism, No Gender, or different tile sizes“ on page 87, and “Launch an analysis“ on page 157.

Create a gene symbol filter chain

You can create a custom 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 316.

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.

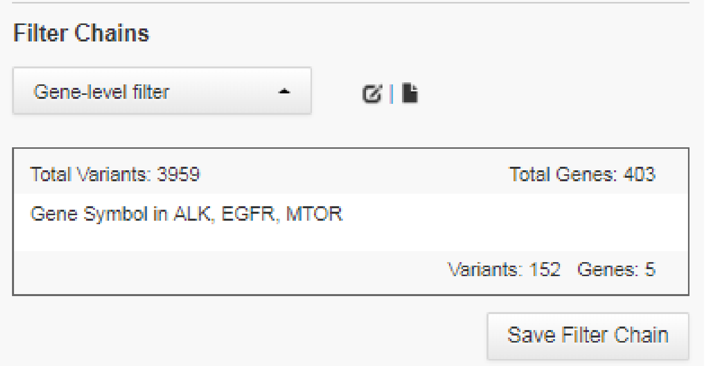
4. Select a **Reference**.

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 all of 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 additional genes.
9. *(Optional)* Use **Update** to configure and replace the last filter in the list without changing the filter count.
10. Click **Save**.

Your 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 96, “Apply a filter chain to analysis results“ on page 192, and “Use filter chains to change analysis results“ on page 305.

Example of a gene symbol filter chain that is applied to analysis results.



Total Variants: 3959 Total Genes: 403

Gene Symbol in ALK, EGFR, MTOR

Variants: 152 Genes: 5

Create a filter chain query

You can create custom 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.

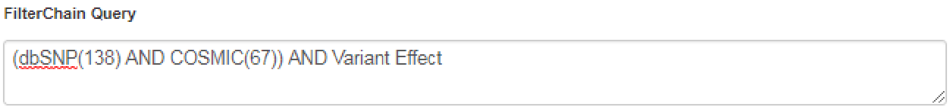
d. Add a third filter, set its value to **In**, then click **Add**.
   The third filter name appears on the right side of the **Create Filter Chain** dialog box.

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.

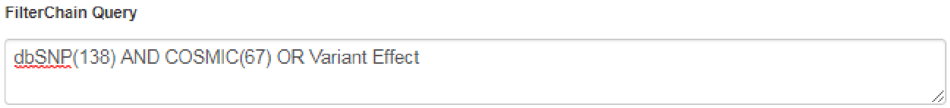
   In this example, the dbSNP and COSMIC filters are applied first, and then the Variant Effect filter is applied.



FilterChain Query

(dbSNP(138) AND COSMIC(67)) AND Variant Effect

   - 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.



FilterChain Query

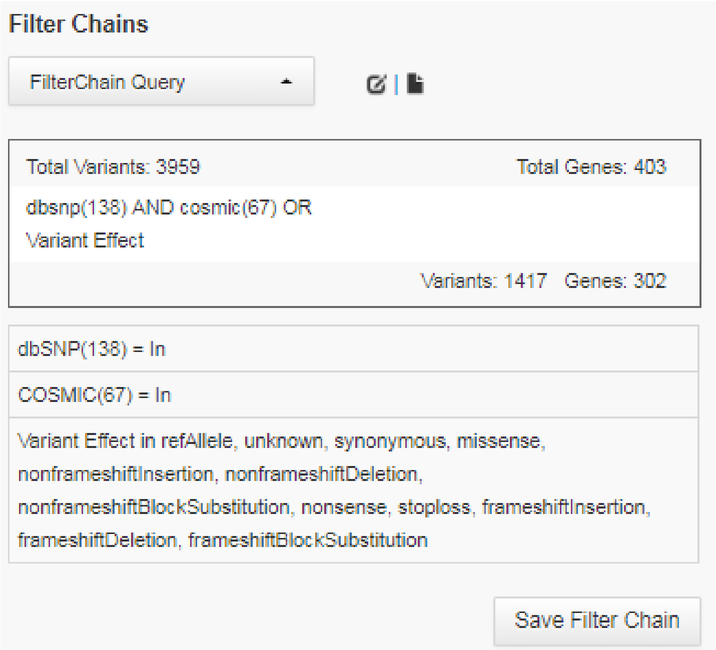
dbSNP(138) AND COSMIC(67) OR Variant Effect

   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 96, “Apply a filter chain to analysis results“ on page 192, and “Use filter chains to change analysis results“ on page 305.

Example of a filter chain query applied to analysis results.



Total Variants: 3959 Total Genes: 403

dbsnp(138) AND cosmic(67) OR
Variant Effect

Variants: 1417 Genes: 302

dbSNP(138) = In

COSMIC(67) = In

Variant Effect in refAllele, unknown, synonymous, missense, nonframeshiftInsertion, nonframeshiftDeletion, nonframeshiftBlockSubstitution, nonsense, stoploss, frameshiftInsertion, frameshiftDeletion, frameshiftBlockSubstitution

Create a MyVariants and Variant Classification filter chain

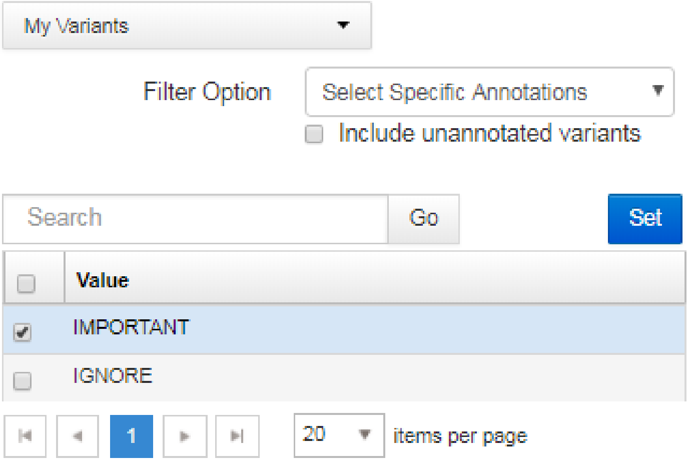
You can create a custom filter chain to narrow the analysis results to include only the variants that are flagged and classified by members of your Ion Reporter™ Software organization. This example shows how to set up a filter chain for MyVariants that are flagged as **Important** and classified as **Deleterious** 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**.



My Variants

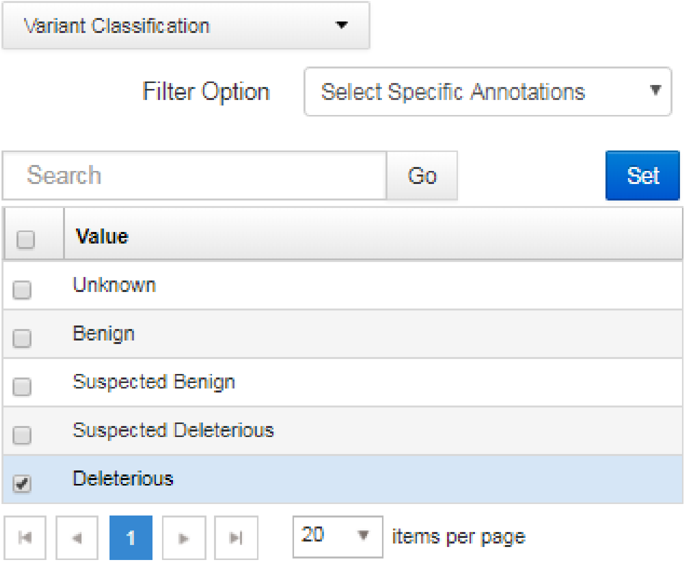
Filter Option: Select Specific Annotations

☐ Include unannotated variants

☐	Value
☑	IMPORTANT
☐	IGNORE

    b. Click **Set**.

5. In the **Choose Filter** list, select **Variant Classification**.
    a. Select **Deleterious**.



Variant Classification

Filter Option: Select Specific Annotations

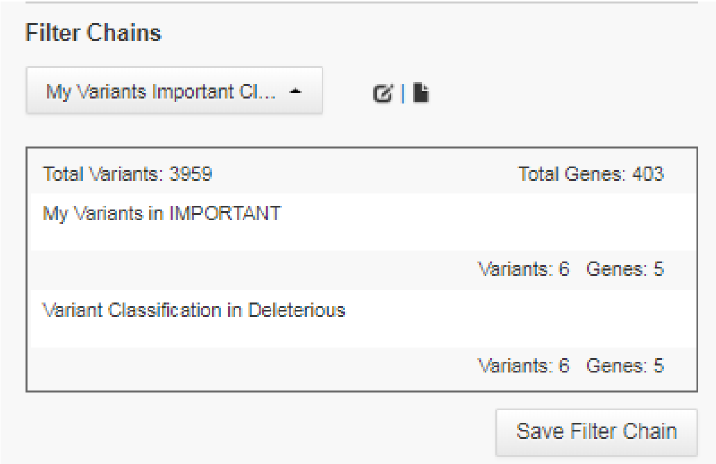
☐	Value
☐	Unknown
☐	Benign
☐	Suspected Benign
☐	Suspected Deleterious
☑	Deleterious

    b. Click **Set**.

6. Click **Save**.

The custom 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 96, "Apply a filter chain to analysis results" on page 192, and "Use filter chains to change analysis results" on page 305.

Example of a MyVariants and Variant Classification filter chain applied to analysis results.



Filter Chains

My Variants Important Cl...

Total Variants: 3959 Total Genes: 403

My Variants in IMPORTANT

Variants: 6 Genes: 5

Variant Classification in Deleterious

Variants: 6 Genes: 5

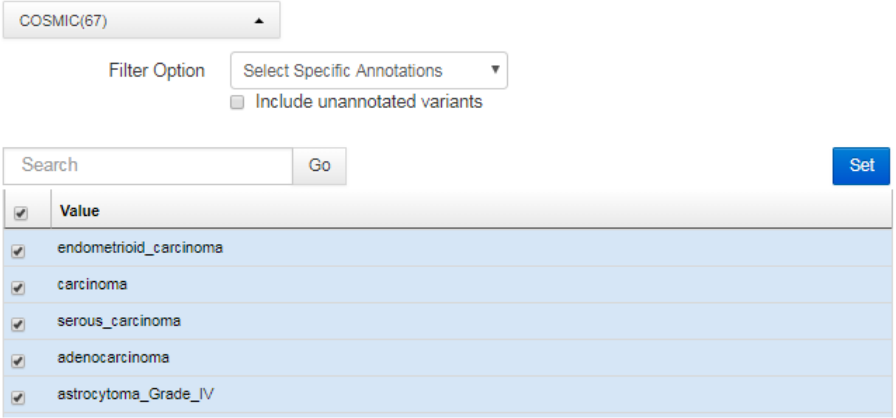
Create a COSMIC, ClinVar, MAF, and Variant Effect filter chain

You can create a custom 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.



COSMIC(67)

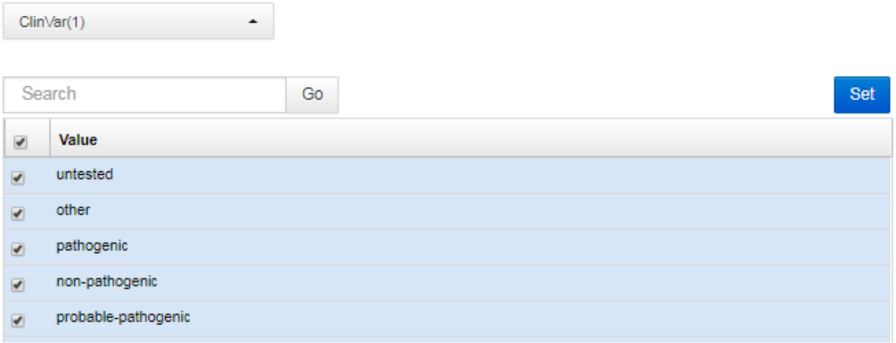
Filter Option: Select Specific Annotations

☐ Include unannotated variants

☑	Value
☑	endometrioid_carcinoma
☑	carcinoma
☑	serous_carcinoma
☑	adenocarcinoma
☑	astrocytoma_Grade_IV

   b. Click **Set**.

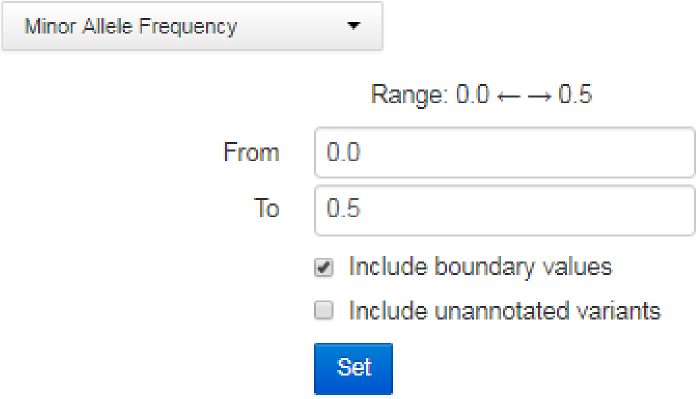
5. In the **Choose Filter** list, select **ClinVar**.
   a. Select the top checkbox to select all values.



☑	Value
☑	untested
☑	other
☑	pathogenic
☑	non-pathogenic
☑	probable-pathogenic

   b. Click **Set**.

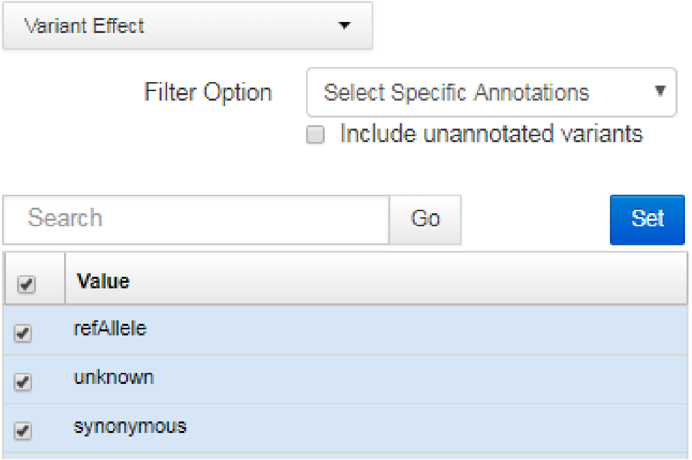
6. In the **Choose Filter** list, select **Minor Allele Frequency**.
   a. Enter a range value, then select **Include boundary values**.



Range: 0.0 ← → 0.5
From 0.0
To 0.5
☑ Include boundary values
☐ Include unannotated variants

   b. Click **Set**.

7. In the **Choose Filter** list, select **Variant Effect**.
   a. Select the top checkbox to select all values.



Filter Option Select Specific Annotations
☐ Include unannotated variants

☑	Value
☑	refAllele
☑	unknown
☑	synonymous

   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 96, “Apply a filter chain to analysis results“ on page 192, and “Use filter chains to change analysis results“ on page 305.

Example of a COSMIC, ClinVar, MAF, and Variant Effect filter chains applied to analysis results.

Total Variants: 3959 Total Genes: 403

COSMIC(67) in endometrioid_carcinoma, carcinoma, serous_carcinoma, adenocarcinoma, astrocytoma_Grade_IV, squamous_cell_carcinoma, malignant_melanoma, clear_cell_renal_cell_carcinoma, acute_myeloid_leukaemia, small_cell_carcinoma, chronic_lymphocytic_leukaemia-small_lymphocytic_lymphoma, primitive_neuroectodermal_tumour-medulloblastoma, hepatocellular_carcinoma, NS, acute_lymphoblastic_leukaemia, non_small_cell_carcinoma, ductal_carcinoma, carcinoid-endocrine_tumour, adenoid_cystic_carcinoma, superficial_spreading

Variants: 2482 Genes: 219

ClinVar(1) in untested, other, pathogenic, non-pathogenic, probable-pathogenic, unknown, probable-non-pathogenic, drug-response, histocompatibility

Variants: 8 Genes: 6

0.0 <= Minor Allele Frequency <= 0.5

Variants: 3 Genes: 3

Variant Effect in refAllele, unknown, synonymous, missense, nonframeshiftInsertion, nonframeshiftDeletion, nonframeshiftBlockSubstitution, nonsense, stoploss, frameshiftInsertion, frameshiftDeletion, frameshiftBlockSubstitution

Variants: 3 Genes: 3

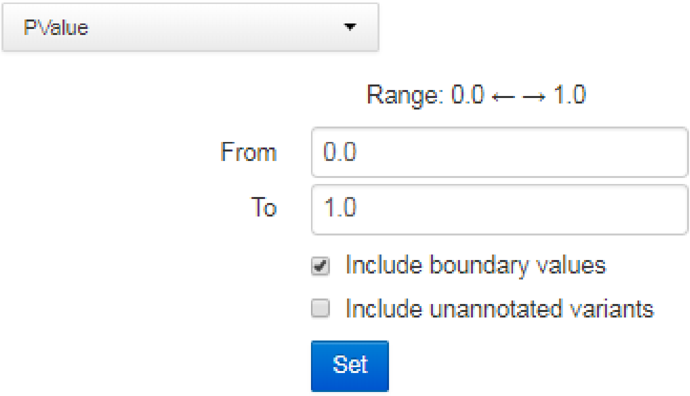
Create a PValue, dbSNP, and Variant Type filter chain

You can create a custom 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**.



PValue

Range: 0.0 ←→ 1.0

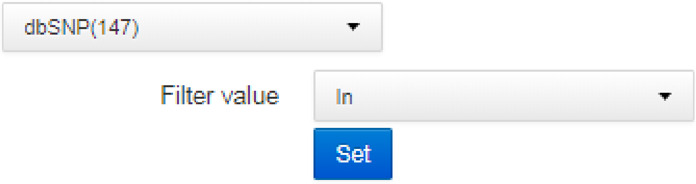
From 0.0

To 1.0

☑ Include boundary values

☐ Include unannotated variants

   b. Click **Set**.
5. In the **Choose Filter** list, select **dbSNP**.
   a. Set **Filter value** to **In**.

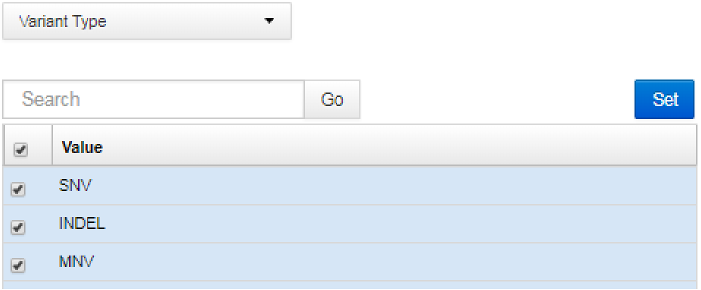


dbSNP(147)

Filter value In

   b. Click **Set**.

6. In the **Choose Filter** list, select **Variant Type**.
    a. Select the checkbox to select all values.



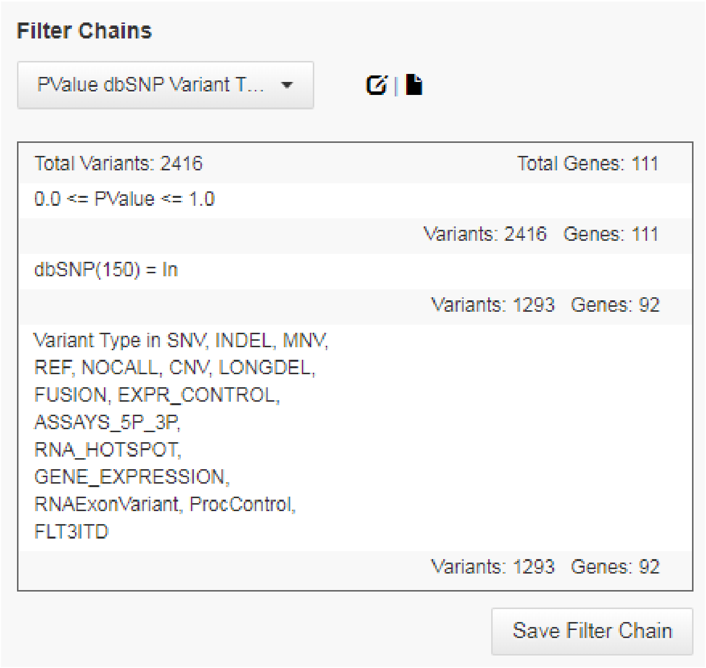
☑	Value
☑	SNV
☑	INDEL
☑	MNV

    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 96, "Apply a filter chain to analysis results" on page 192, and "Use filter chains to change analysis results" on page 305.

Example of a PValue, dbSNP, and Variant Type filter applied to analysis results.



Total Variants: 2416 Total Genes: 111
0.0 <= PValue <= 1.0
Variants: 2416 Genes: 111
dbSNP(150) = In
Variants: 1293 Genes: 92
Variant Type in SNV, INDEL, MNV, REF, NOCALL, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, FLT3ITD
Variants: 1293 Genes: 92

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 340 and "Data type filters" on page 343.

You cannot edit predefined filter chains, but you can create custom filter chains that are built from predefined filter chains, or from one or more filters. For more information, see "Create a custom filter chain" on page 315.

Predefined filter chain name	Description
AmpliSeq Exome Tumor Normal v1	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	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	This filter chain reports all hotspot variants that pass the filter and are not called as reference or NOCALL. Variant types include SNP and INDEL. This filter chain displays the same set of variants in the **Analysis Results** view as in the **SNP/INDEL** summary table in visualization view.
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, and FLT3ITD.
CNVs of Confidence ≥0.1–Germline–CNVs only	For germline analyses, this filter chain narrows your analysis results to copy number variants with a confidence value of ≥0.1.

Predefined filter chain name	Description
Confident CNVs—CNVs Only	This filter chain returns the variants whose minimum ploidy gain (5% CI) is over expected, which is 1.0, OR minimum ploidy loss (95% CI) is under expected, which is also 1.0.
Confident Germline CNVs–CNVs Only	For germline analyses, this filter chain narrows your analysis results to copy number variants with a confidence value of ≥10. Not a default filter.
Confident Somatic CNVs–CNVs Only	This filter chain includes 5% confidence interval range and 95% confidence interval range. **Filter chain logic** 10.0 <= CNV Confidence Range <= 1.0E7
Default CarrierSeq View	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™ CarrierSeq™ ECS Kits User Guide*, Pub. No. MAN0018483. **Filter chain logic** Variant Type in [MNV, SNV, INDEL, NOCALL] OR (Variant Type in CNV AND Filter in [GAIN, LOSS, NOCALL])
Default DNA and Fusions View	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	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

Predefined filter chain name	Description
Default Variant View	This is the default filter chain for the following analysis workflows: • Ion AmpliSeq™ Exome single-sample (both Germline and Somatic) • TargetSeq Exome v2 single-sample • Ion AmpliSeq™ CHPv1 tumor-normal pair • Ion AmpliSeq™ CHPv2 tumor-normal pair • Ion AmpliSeq™ CCP tumor-normal pair • Ion AmpliSeq™ Exome paired sample • Ion AmpliSeq™ CCP paired sample • non-ReproSeq Low-pass whole-genome aneuploidy This filter chain narrows your analysis results to confident variant types, which are not CNVs, but could include SNV, INDEL, MNV, REF, NOCALL, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
Genetic Disease Variants	This filter chain narrows your 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.
Mutation Load (Somatic Mutations)	This filter chain is for use only with Ion Reporter™ Software 5.10 analysis workflows.

Predefined filter chain name	Description
Mutation Load (Somatic SNVs)	This filter chain returns results for somatic SNVs based upon dbSNP, 5000Exomes, ExAC, and UCSC Common SNPs annotation source databases. The minor allele frequencies range lies between 0.0 and 1.0E-6. This filter chain also filters out variants of homopolymer lengths greater than 7, coverage lower than 60, and allele frequency less than 0.05. The **Tumor Mutational Burden Filter Chain** parameter for this filter chain must be enabled for Ion Reporter™ Software to generate mutation load analysis results. By default, the tumor mutational burden calculation is disabled. You must also copy and edit either a DNA–Single Sample analysis workflow or a DNA and Fusions–Single Sample analysis workflow to enable mutation load calculations on DNA samples. **Filter chain logic** (Filtered Coverage >= 60) AND (Variant Type in SNV) AND (0.0 <= Minor Allele Frequency <= 1.0E-6) AND (0.0 <= 5000Exomes Global MAF <= 1.0E-6) AND (0.0 <= ExAC GAF <= 1.0E-6) AND (UCSC Common SNPs = Not In) AND (0 <= Homopolymer Length <= 7) AND (0.05 <= Allele Frequency <= 1.0) This filter chain is for use only with Ion Reporter™ Software 5.10 analysis workflows.
Oncomine™ BRCA	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.

Predefined filter chain name	Description
Oncomine™ Extended	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: • Nontargeted fusions. • Likely somatic mutations based upon dbSNP, 5000Exomes, ExAC, 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. **Filter chain logic**. Note that *** means that the Include Unannotated Variants option was checked: Oncomine = IN OR (Variant Type in FUSION AND Filter in PASS) OR (Variant Type in SNV, INDEL, MNV, LONGDEL AND 0.0 <= Minor Allele Frequency*** <= 1.0E-6 AND 0.0 <= 5000Exomes Global MAF(20161108)*** <= 1.0E-6 AND 0.0 <= ExAC GAF(1)*** <= 1.0E-6 AND UCSC Common SNPs = "Not In" AND Variant Effect in missense, frameshiftDeletion, frameshiftInsertion, nonframeshiftDeletion, nonframeshiftInsertion, nonsense, nonframeshiftBlockSubstitution, frameshiftBlockSubstitution, stoploss AND Location in exonic, splicesite_3, splicesite_5) OR ClinVar(20180729) in "Pathogenic", "Likely pathogenic", "Pathogenic/Likely pathogenic", "Pathogenic, other", "Uncertain significance,Likely pathogenic", "Pathogenic,Conflicting interpretations of pathogenicity", "Pathogenic,Uncertain significance", "Pathogenic,Likely pathogenic",

Predefined filter chain name	Description
	"Conflicting interpretations of pathogenicity,Likely pathogenic", "Pathogenic,other", "Pathogenic,Benign", "Pathogenic,Pathogenic/Likely pathogenic", "Likely pathogenic, drug response", "Pathogenic,Benign/Likely benign", "Pathogenic/Likely pathogenic, drug response", "Pathogenic,not provided", "Pathogenic, risk factor", "Pathogenic, Affects", "Likely pathogenic, risk factor", "Pathogenic/Likely pathogenic, risk factor", "Pathogenic, other, risk factor", "Likely pathogenic, association", "Pathogenic, protective", "Pathogenic,Conflicting interpretations of pathogenicity, other,Benign", "Pathogenic,Conflicting interpretations of pathogenicity,Likely pathogenic", "Pathogenic, protective, risk factor", "Pathogenic/Likely pathogenic, other", "Likely pathogenic, other", "Pathogenic,Conflicting interpretations of pathogenicity,Uncertsignificance", "Pathogenic,Likely benign", "Pathogenic,Conflicting interpretations of pathogenicity, risk factor", "Pathogenic,other,association", "Pathogenic,drug response", "Pathogenic,Conflicting interpretations of pathogenicity, other", "Pathogenic/Likely pathogenic,Likely pathogenic", "Pathogenic,Pathogenic, other", "Pathogenic/Likely pathogenic, risk factor,Pathogenic", "Pathogenic, drug response", "Benign,Likely pathogenic", "Benign/Likely benign,Likely pathogenic",

Predefined filter chain name	Description
	"Pathogenic,Conflicting interpretations of pathogenicity, other,other", "Pathogenic,Conflicting interpretations of pathogenicity, rfactor,Likely pathogenic", "Pathogenic, association, protective", "Pathogenic,Benign,Pathogenic/Likely pathogenic", "Benign,Pathogenic/Likely pathogenic"
Oncomine™ Variants	This filter chain returns results for INDELs and SNV variant types, and minor allele frequencies between 0.0 and 1.0E-6 based on 5000Exomes and ExAC annotation source databases that have homopolymer lengths less than or equal to 7 and allele frequencies between 0.05 and 1.0.
Oncomine™ Variants, 5% CI CNV ploidy ≥gain of 2 over normal	This filter chain restricts copy number variants to gains of greater than 2 based on the 5% confidence interval level. It also returns Oncomine™-annotated variants.

Predefined filter chain name	Description
TMB (Non-germline Mutations)	This filter chain returns results for somatic variants based upon dbSNP, 5000Exomes, ExAC, 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 greater than 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 though the use of this filter chain cannot be changed after an analysis is complete. The filter chain is applied prior to the parameter application. The filtered variant file is passed to the TML script that further applies the user parameters. **Filter chain logic** (0 <= Homopolymer Length <= 4) AND (0.0 <= Minor Allele Frequency <= 1.0E-6) AND (0.0 <= 5000 Exomes Global MAF <= 1.0E-6) AND (0.0 <= ExAC GAF <= 1.0E-6) AND (UCSC Common SNPs = Not In)
Variant Matrix Summary	For visualizations that include the Variant Matrix, such as TagSeq and Ion AmpliSeq™ HD analyses, this filter chain returns results in a visualization that contains the same set of variants that are included in the **Variant Matrix Summary** tab. This filter chain allows results to be exported. Import the exported results file into Oncomine™ Reporter (OKR) to include the variants shown in the **Variant Matrix Summary** view in OKR reports. Variant types returned are SNV/INDEL, CNV, fusions, and RNAExonVariants.

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:

- 5000Exomes AfricanAmerican MAF
- 5000Exomes EuropeanAmerican MAF
- 5000Exomes Global MAF

Filter conditions include the ability to set minor allele frequency ranges from 0.0 to 0.5, to include or exclude boundary values in the range, and to include or exclude unannotated variants 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 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.

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.

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).

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.

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

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
- ExAC EAAF
- ExAC EFAF
- ExAC ENFAF
- ExAC GAF
- ExAC LAF
- ExAC OAF
- ExAC SAAF

Filter conditions include the ability to set a range from 0.0 to 1.0, to include or exclude boundary values from the ranges, and to include or exclude unannotated variants 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 Score 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.

Filter conditions include the ability to set a range from 0.0 to 1.0 for the score, to include or exclude boundary values in the ranges, and to include or exclude unannotated variants 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).

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.

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, to include or exclude range boundary values, and to include or exclude unannotated variants 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.

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.

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.

Filter conditions include the ability to set a score range from −14.0 to 3.0, to include range boundary values, and to include or exclude unannotated variants in filtered 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 are based on variant data types in the software, such as allele frequency and allele ratio. Many data types that are included in the results tables are available as filters.

Allele frequency filter

This 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, to include or exclude boundary values in the range, and to include or exclude unannotated variants 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 to include or exclude boundary values in the range, and to include or exclude unannotated variants 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, to include or exclude boundary values in the range, and to include or exclude unannotated variants 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, to include or exclude boundary values in the range, and to include or exclude unannotated variants 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, to include or exclude boundary values in the range, and to include or exclude unannotated variants in filtered analysis results.

The filter returns only copy number variants with high confidence levels.

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:

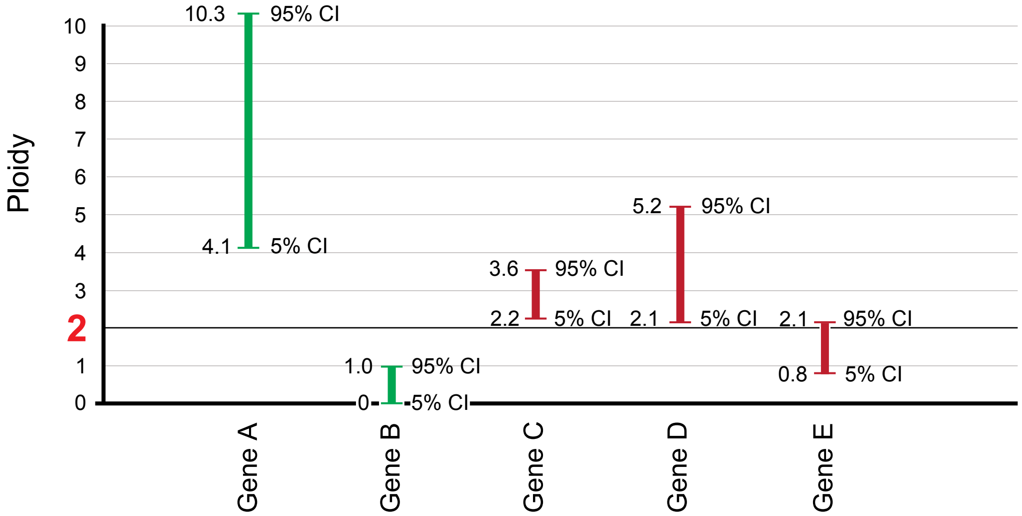
Gene A—A gene with suspected gain of 5% CI = 4.1 and 95% CI = 10.3 filtered in.

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).



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, and to include or exclude boundary values for either of the lower and upper confidence ranges in filtered analysis results.

The filter returns only somatic copy number variants within the lower and upper limits of the set confidence ranges.

Confident Somatic Variants filter

This filter can be added to a filter chain in the software to return variants that algorithms identify as confident somatic variants.

Filter conditions include the ability to include the confident somatic variants (In), or to exclude the confident somatic variants in analysis results (Not In) in filtered analysis results.

The filter detects confident somatic variants to include or exclude 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 Fusion View filter

This filter can be added to filter chains in the sofstware 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.

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 your coverage threshold.

Filter conditions include the ability to set a coverage threshold and 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.

The filter reports all results with a coverage threshold greater than 100 (or other threshold that is set).

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, to include or exclude range boundary values, and to include or exclude unannotated variants in filtered analysis results.

This filter returns INDEL variants in samples that meet the specified homopolymer length.

Hotspot filter

This filter can be added to filter chains in the software to detect variants that overlap specific hotspots.

Filter conditions include the ability to include (In) or exclude (Not In) hotspots as defined by a hotspots file in filtered analysis results.

The filter returns variants that overlap hotspots included 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.

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.

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 blank, 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.

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, to include the range boundary values, and to include or exclude the unannotated variants 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:

- Unknown
- Benign
- Suspected Benign
- Suspected Deleterious
- Deleterious

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.

9

Create and view reports

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.

You must have the Report role to view and create reports. Otherwise, you can send completed analyses to the person on your team who has the Report role. For more information, see "Send an analysis to the Report role" on page 177.

Final reports

Final reports include information on an analysis and samples, including variant details, background information, quality control metrics, disclaimers, comments, and electronic signatures. 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 flexible report directly from a completed analysis. The report is completely customizable. For more information, see "Create a flexible report" on page 355.
- Predesign a report template and attach it to an analysis workflow. The resulting report is not as customizable as a flexible report. For more information, see"Create a report template" on page 357.
- Predesign a report template as a Ion Reporter™ Software workflow preset. The resulting report is not as customizable as a flexible report. For more information, see "Create a final report template workflow preset" on page 133.

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 **View**.
5. In the report preview, review the report.

   You can:

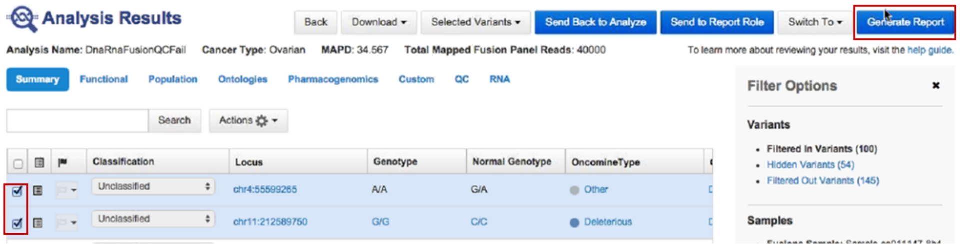
   - Click **Download** to download a PDF copy of the report.
   - Click **Switch To** to view a QC report or audit log. For more information, see “View a Quality Control (QC) report“ on page 367 and “View and download audit logs“ on page 375

Create a flexible report

You can create flexible reports directly from analysis results. You can create a report with an existing report template or you can design a flexible report. A flexible report allows you to customize the report, select variants, reorder sections, reorder section content, add notes, and remove sections. You can save the flexible report as a template.

Only one final report for each analysis can be published for later use. After a report has been published, you cannot edit MyVariants, classifications, or notes that are associated with the analysis results. A report that is published to PDF format cannot be edited. To create a different report for the analysis, you must reanalyze the sample using the same analysis workflow, then create and publish the report.

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.



Analysis Name: DnaRnaFusionQCFail **Cancer Type:** Ovarian **MAPD:** 34.567 **Total Mapped Fusion Panel Reads:** 40000

To learn more about reviewing your results, visit the help guide.

☐	Classification	Locus	Genotype	Normal Genotype	OncomineType
☑	Unclassified	chr4:55599265	A/A	G/A	Other
☑	Unclassified	chr11:212589750	G/G	C/C	Deleterious

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 report template. For more information, see "Create a report template" on page 357.

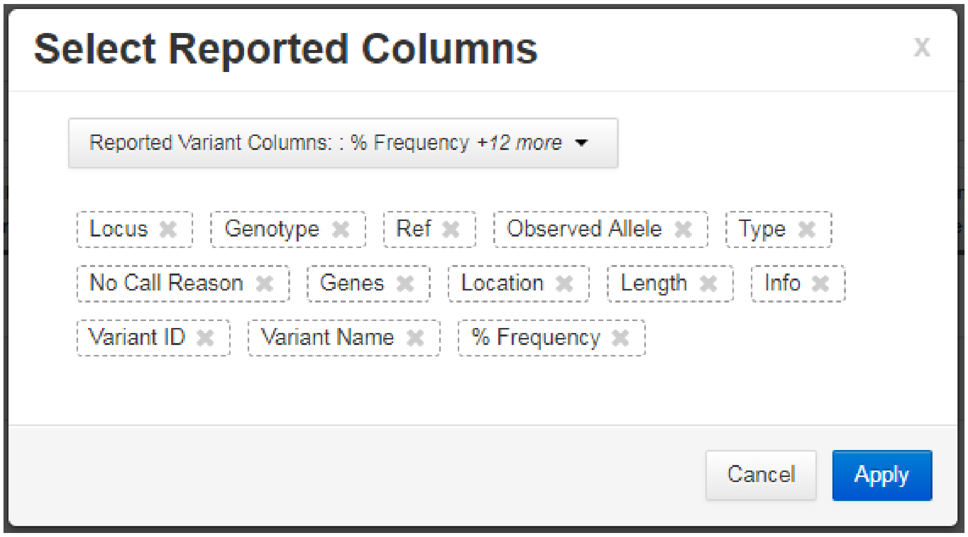
   If a report template is not associated with the analysis workflow, go to the next step.

5. Enter information into the sections.

   For more information, see "Available report sections" on page 363.

6. *(Optional)* Scroll to the **Reported Variants** section, then click **Select Columns**.

   In the **Select Reported Columns** dialog box, you can:
   - 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**.



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. When you are satisfied with your report design, click **Next**.

   A preview of the PDF report opens. If you need to change the report sections before publishing, click **Configuration** to go back to the previous screen.

9. 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 your 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 MyVariants, classifications, or notes that are in the analysis.

10. Click **Publish** to create the final report.

11. Click **Download** to download the published report.

Create a report template

You can create a new report template and save it for future analysis reports. This flexible approach allows you to reorder sections and remove unnecessary sections.

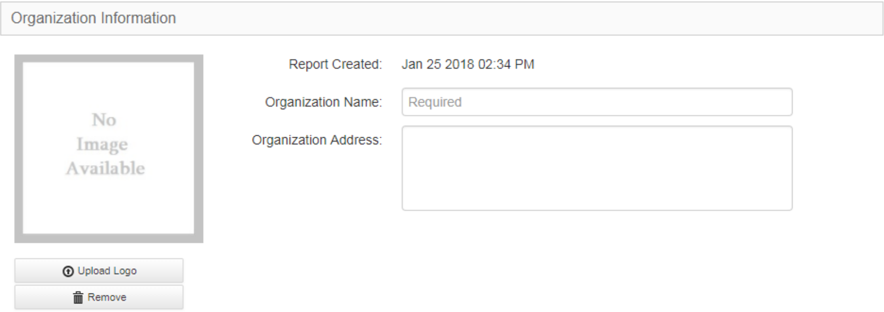
You must have the Report role to generate reports or report templates.

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 your report.
   a. In the **Organization Information** section,
      - Enter your organization name.
      - *(Optional)*If you want to use a logo on your report, click **Upload Logo**, then in the **Upload Header Logo** dialog box, click **Select File** to browse to your logo file. Acceptable formats are PNG, GIF, and JPG.
      - *(Optional)* Enter your organization address.

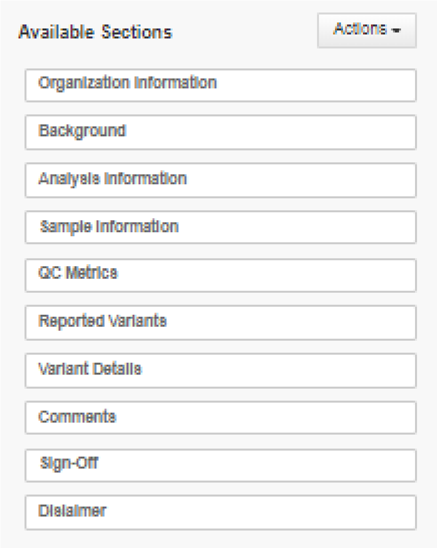


Report Created: Jan 25 2018 02:34 PM
Organization Name: Required
Organization Address:

   b. *(Optional)* Enter other standard information for the **Background**, **Comments**, **Sign-Off**, and **Disclaimer** sections.

5. In each section, use the up, down, and X icons to move or delete sections from the template.

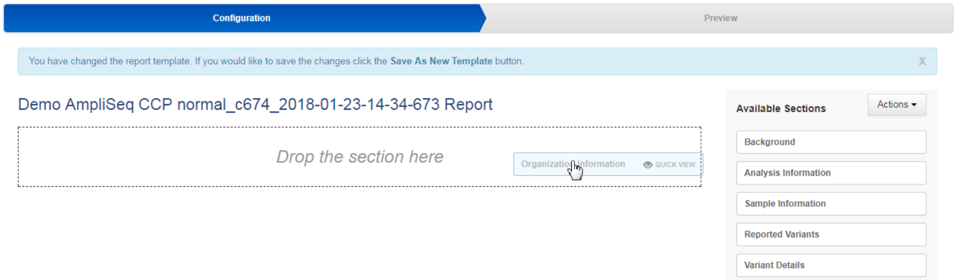
   Or, click **Actions**, then select one of the following:



Option	Description
Add all	Add all of the available report template sections to your customized template or report.
Remove all	Remove all of the report template sections from your customized template or report.
Reset	Return to the template to the default section configuration.

   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.

   The sections that you can add to the report template are listed under **Available Sections**. For more information, see “Available report sections“ on page 363.



You have changed the report template. If you would like to save the changes click the **Save As New Template** button.

6. Click **Save As New Template**.

7. Enter a template name, then click **Save**.

8. To use the template in future analyses, you must add it to your analysis workflows.

   For more information, see “Add a report template to an analysis workflow“ on page 359.

Search for a report template

You can review your existing custom and factory 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
Ion factory templates	Factory 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 for 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 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 report template" on page 357 or "Create a final report template workflow preset" on page 133.

2. In the **Workflows** tab, click **Create Workflow**.
3. Make the appropriate selections in the **Research Application**, **Reference**, **Annotation**, **Filters**, **Copy Number**, and **Plugins** steps.

   For more information, see "Create a custom analysis workflow without predefined settings" on page 76.

4. In the **Final Report** step, under **Final Report Template**, select the report template that you want to associate with the analysis workflow.
5. Make appropriate selections in the **Parameters** step, then confirm.

   Your analysis workflow with the corresponding report template is now available for use when you review analysis results.

Edit a report template

You can edit custom report templates that exist in your 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, select sections to include or exclude, then drag and drop Reported Variants details.
5. 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 359.

Lock a report template

You can lock custom 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**.
   The final report template now displays a lock icon in its row 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 359.

Delete a report template

You can delete unwanted custom 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 analysis report and approve with an electronic signature

If an analysis report uses a template that requires an electronic signature, a user with the Report role generates and electronically signs the report. After a final analysis 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.
3. In the **Analysis** column, click the link, or click the **Edit** in the **Details** panel.
4. Select some variants to include in the report, then click **Generate Report**.
   A report preview is created.
5. In the **Generate Report** dialog box, name the organization, then scroll down to review the various sections of the report.
6. Scroll to the bottom of the report, then click **Next**.
7. In the **Lock and Publish** dialog box, enter your user email address and password, enter any comments, then click **Publish**.
   The report is downloaded to your computer in PDF file format.
8. Open the downloaded PDF file.
   The electronic signature and comment appears 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** tab of the **Analysis Visualization** screen. When you generate a visualization report, the analysis is not locked.

Note: You cannot create visualization reports when Ion Reporter™ Genomic Viewer (IRGV) is launched from the **Locus** link in analysis results.

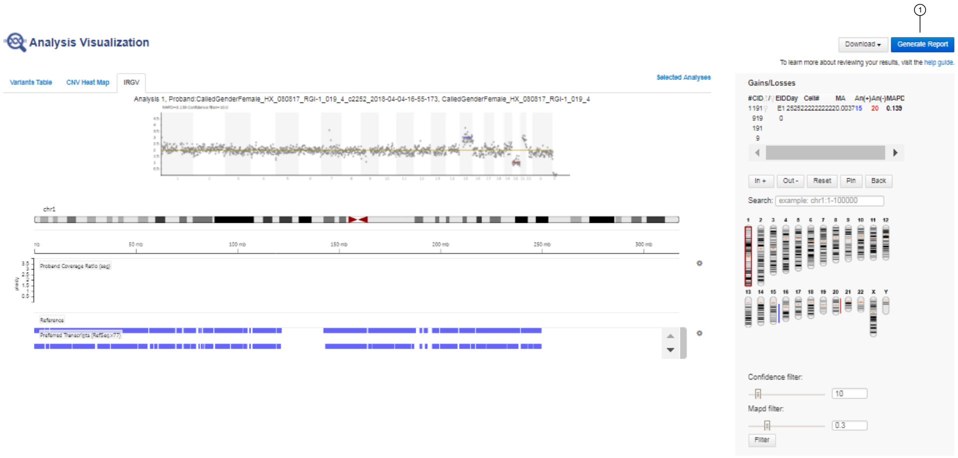
Items that are available from each visualization depend on the type of analysis that is run. The following sections are examples of sections can be added from a visualization:

- 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

For more information on other sections that can be added to the report, see "Available report sections" on page 363.

Create a visualization report

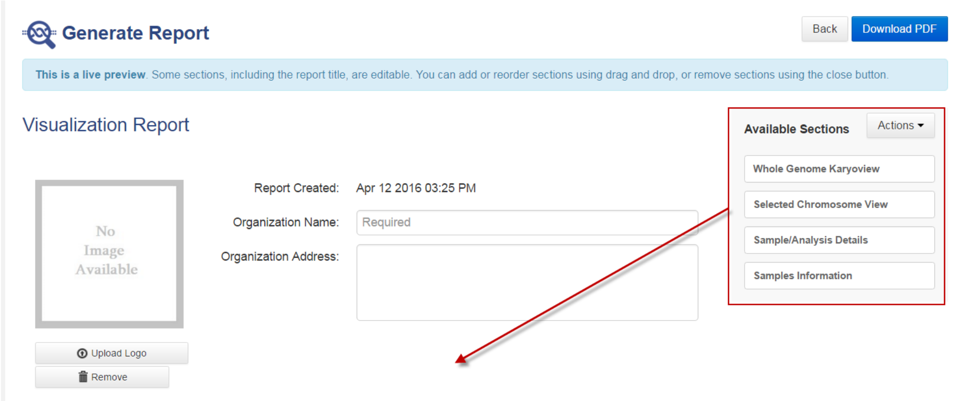
1. On the **Analyses** tab, click **Overview**, then select one or more analyses that you want to visualize in Ion Reporter™ Genomic Viewer.
2. Click **Actions ▸ Visualize**.
   The visualization of the data are viewable in the **IRGV** tab of the **Analysis Visualization** screen. For example, a visualization 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, choose a predesigned report template or create a new report template.
   A preview of the report is shown. You can customize the report.
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.



This is a live preview. Some sections, including the report title, are editable. You can add or reorder sections using drag and drop, or remove sections using the close button.

Report Created: Apr 12 2016 03:25 PM
Organization Name: Required
Organization Address:

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. When you are satisfied with the design of the visualization report, click **Next**.
   A preview of the PDF report opens. If you need to change the report sections before publishing, click **Configuration** to go back to the previous screen.

9. 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 your browser settings.

Note: When you generate a visualization report, the analysis is not locked.

Available report sections

The following sections are available to add to report templates, final reports, or visualization reports.

Note: Additional sections are available if you create a visualization report.

Category	Description
Organizational Information	• **Required**: Organizational name. • *(Optional)* Upload a logo and/or enter the organization address.
Background	*(Optional)* Enter background information.
Analysis Information	Includes software version number, report generation date, name of person who launched the report, analysis workflow, name of person who analyzed the data, list of annotations, and reference information.
Sample Information	Includes all sample attributes that are included in the Samples table when the data is viewed in Ion Reporter™ Software.
QC Metrics	*(Optional)* Generates a table coverage metrics for hotspots, amplicons and genes. For a complete list of the metrics that are included, see "Quality Control (QC) report contents" on page 368. You can also view and download these metrics in a separate report. See "View a Quality Control (QC) report" on page 367 for more information. **Note:** The QC Metrics section is available in Ion Reporter™ Software 5.12 or later.

Category	Description
Reported Variants	Generates a table of reported variants and includes columns of variant details.
Variant Details	Includes name and description of variants as well as which samples they came from. In addition, it lists Annotations and Notes for each variant.
Comments	*(Optional)* 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 additional required signatures, until all designated signers are added.
Disclaimer	*(Optional)* Enter a custom legal disclaimer.

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**.

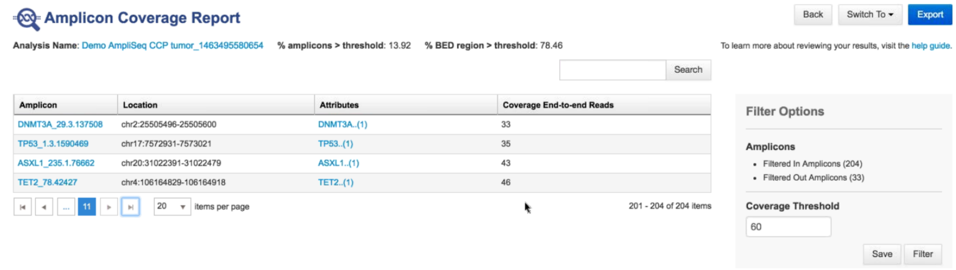
   Alternatively, you can click the analysis of interest to open the **Analysis Results** table, then click **Switch To** ▸ **Coverage Report**.

   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 mplicon 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.



Analysis Name: Demo AmpliSeq CCP tumor_1463495580654 **% amplicons > threshold:** 13.92 **% BED region > threshold:** 78.46

To learn more about reviewing your results, visit the help guide.

Amplicon	Location	Attributes	Coverage End-to-end Reads
DNMT3A_29.3.137508	chr2:25505496-25505600	DNMT3A..(1)	33
TP53_1.3.1590469	chr17:7572931-7573021	TP53..(1)	35
ASXL1_235.1.76662	chr20:31022391-31022479	ASXL1..(1)	43
TET2_78.42427	chr4:106164829-106164918	TET2..(1)	46

20 items per page

201 - 204 of 204 items

Amplicons
- Filtered In Amplicons (204)
- Filtered Out Amplicons (33)

Coverage Threshold: 60

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 an 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.
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.

Open an amplicon coverage report in (IRGV)

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 , depending on your viewer settings. For more information, see "Set IRGV or IGV as the default viewer" on page 253.

Figure 10 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:

3. To download a PDF of the QC report:
    1. Click **Export ▸ PDF**.
    2. Download the report from the **Notifications** page. The filename 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 directory that you use for downloads, depending on your browser settings.

4. To download a compressed directory 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:
    1. Click **Export ▸ QC Package**.
    2. Download the compressed directory from the **Notifications** page. The downloaded filename is `qc-report-id.zip`, where `id` is a system-generated analysis identifier.

       You can also view the QC report in your browser, or find the PDF in the directory that you use for downloads, depending on your browser settings.

Quality Control (QC) report contents

Quality Control (QC) reports include metrics that are based on which data are included in an analysis. For example, metrics about fusions are available only for analyses with fusions data.

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.

QC Metrics	Description
Number of Homozygous INDELs	The number of homozygous INDEL calls.
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 thousand 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 thousand 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 all of 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 fields are 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 metrics are included in a Quality Control report 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.

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.

Coverage analysis metric	Description
Mapped Reads	The total number of reads that were mapped to the reference in Torrent Suite™ Software. Reads that are non-uniquely mapped can have equally well-aligned reads that are mapped to multiple locations, and are typically mapped randomly to one.
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. An example of a read that is not counted is as follows: If a read overlaps a targeted region on a region that includes a flanking sequence that is aligned, due to poor matching of 5' bases of the read, the read is not counted.
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 if their (5') mapping location being sufficiently close to the end of the amplicon region, taking the read direction (mapping strand) into account.
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 if the molecularCoverageAnalysis 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.

Note: To download a report that contains the metrics, click the **Download** link in the **Molecular coverage analysis report** column.

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.
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.

QC package coverage columns

Quality Control (QC) statistics files report coverage distribution. 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 .

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

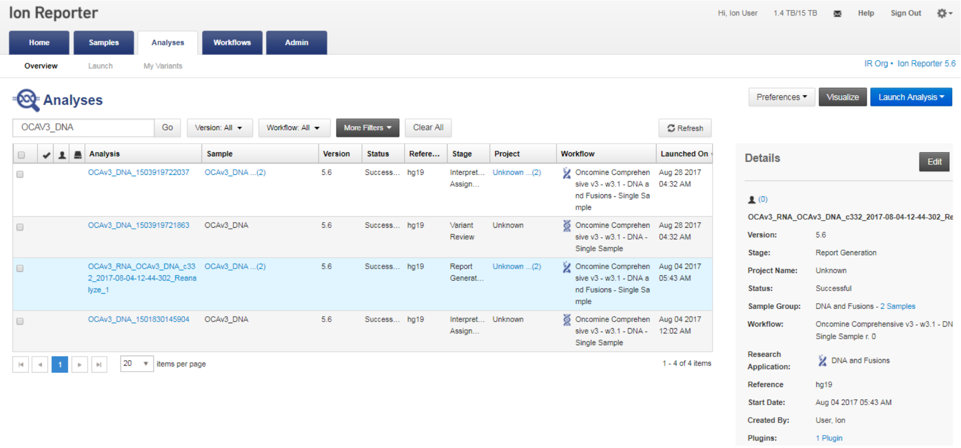
Download Quality Control (QC) files

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) report contents" on page 368.
- `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.



☐	✓			Analysis	Sample	Version	Status	Refere...	Stage	Project	Workflow	Launched On
☐				OCAv3_DNA_1503919722037	OCAv3_DNA ...(2)	5.6	Success...	hg19	Interpret... Assign...	Unknown ...(2)	Oncomine Comprehensive v3 - w3.1 - DNA and Fusions - Single Sample	Aug 28 2017 04:32 AM
☐				OCAv3_DNA_1503919721863	OCAv3_DNA	5.6	Success...	hg19	Variant Review	Unknown	Oncomine Comprehensive v3 - w3.1 - DNA - Single Sample	Aug 28 2017 04:32 AM
☐				OCAv3_RNA_OCAv3_DNA_c332_2017-08-04-12-44-302_Reanalyze_1	OCAv3_DNA ...(2)	5.6	Success...	hg19	Report Generat...	Unknown ...(2)	Oncomine Comprehensive v3 - w3.1 - DNA and Fusions - Single Sample	Aug 04 2017 05:43 AM
☐				OCAv3_DNA_1501830145904	OCAv3_DNA	5.6	Success...	hg19	Interpret... Assign...	Unknown	Oncomine Comprehensive v3 - w3.1 - DNA - Single Sample	Aug 04 2017 12:02 AM

(0)
OCAv3_RNA_OCAv3_DNA_c332_2017-08-04-12-44-302_Re

Version:	5.6
Stage:	Report Generation
Project Name:	Unknown
Status:	Successful
Sample Group:	DNA and Fusions - 2 Samples
Workflow:	Oncomine Comprehensive v3 - w3.1 - DN Single Sample r. 0
Research Application:	DNA and Fusions
Reference	hg19
Start Date:	Aug 04 2017 05:43 AM
Created By:	User, Ion
Plugins:	1 Plugin

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 ✉ to open the notifications list, then find and 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.

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.

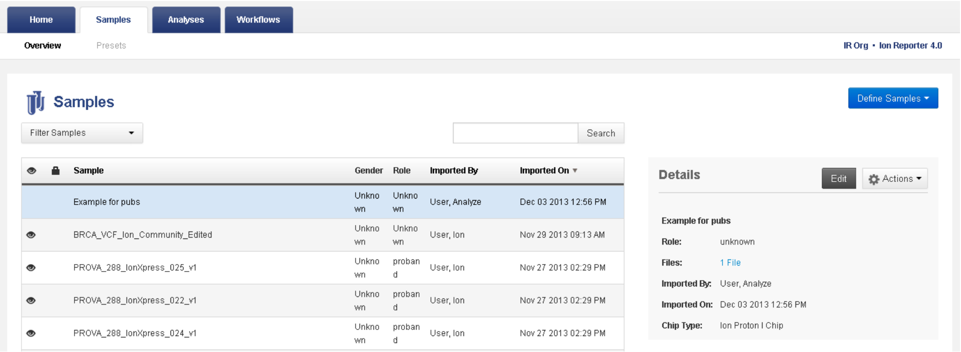
This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

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.

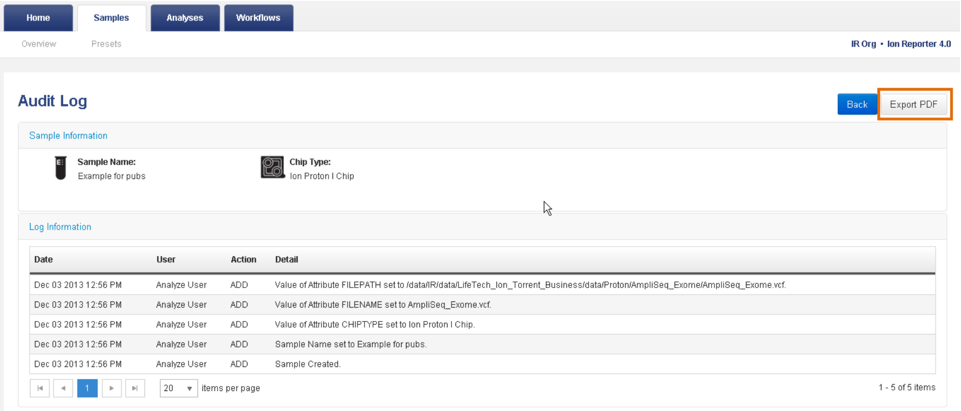
1. In the **Samples** tab, click **Overview**.
2. Select the row of the sample that you want to report on.



Sample	Gender	Role	Imported By	Imported On
Example for pubs	Unknown	Unknown	User, Analyze	Dec 03 2013 12:56 PM
BRCA_VCF_Ion_Community_Edited	Unknown	Unknown	User, Ion	Nov 29 2013 09:13 AM
PROVA_288_IonXpress_025_v1	Unknown	proband	User, Ion	Nov 27 2013 02:29 PM
PROVA_288_IonXpress_022_v1	Unknown	proband	User, Ion	Nov 27 2013 02:29 PM
PROVA_288_IonXpress_024_v1	Unknown	proband	User, Ion	Nov 27 2013 02:29 PM

Example for pubs
Role: unknown
Files: 1 File
Imported By: User, Analyze
Imported On: Dec 03 2013 12:56 PM
Chip Type: Ion Proton I Chip

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.



Sample Name: Example for pubs
Chip Type: Ion Proton I Chip

Date	User	Action	Detail
Dec 03 2013 12:56 PM	Analyze User	ADD	Value of Attribute FILEPATH set to /data/IR/data/LifeTech_Ion_Torrent_Business/data/Proton/AmpliSeq_Exome/AmpliSeq_Exome.vcf.
Dec 03 2013 12:56 PM	Analyze User	ADD	Value of Attribute FILENAME set to AmpliSeq_Exome.vcf.
Dec 03 2013 12:56 PM	Analyze User	ADD	Value of Attribute CHIPTYPE set to Ion Proton I Chip.
Dec 03 2013 12:56 PM	Analyze User	ADD	Sample Name set to Example for pubs.
Dec 03 2013 12:56 PM	Analyze User	ADD	Sample Created.

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.

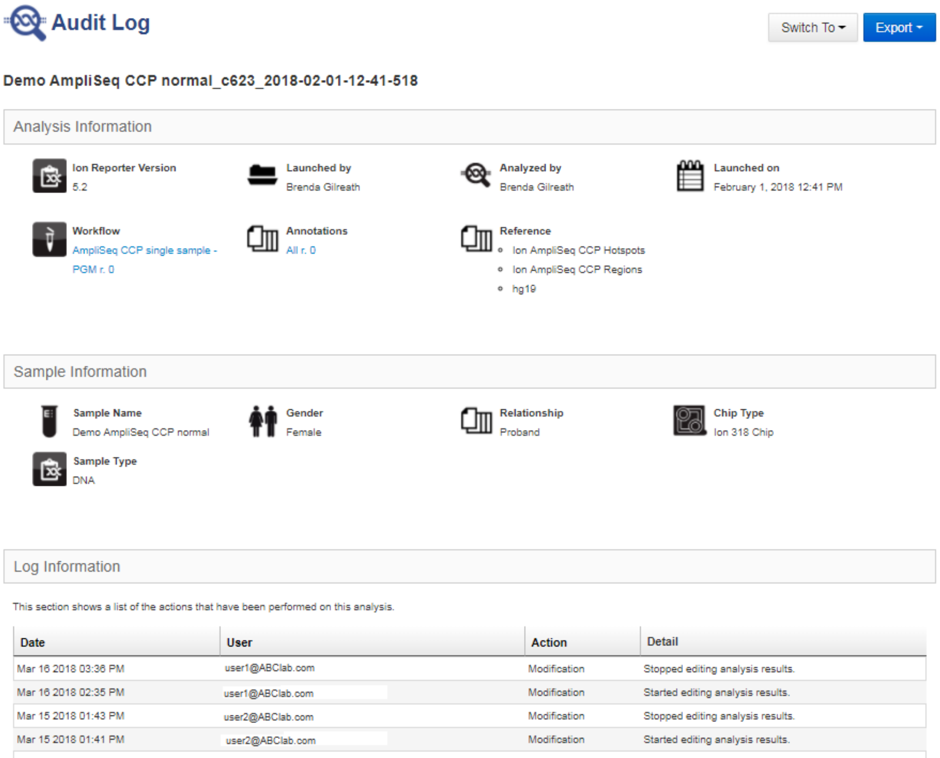
This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

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:



Demo AmpliSeq CCP normal_c623_2018-02-01-12-41-518

Analysis Information

Ion Reporter Version
5.2

Launched by
Brenda Gilreath

Analyzed by
Brenda Gilreath

Launched on
February 1, 2018 12:41 PM

Workflow
AmpliSeq CCP single sample - PGM r. 0

Annotations
All r. 0

Reference
- Ion AmpliSeq CCP Hotspots
- Ion AmpliSeq CCP Regions
- hg19

Sample Information

Sample Name
Demo AmpliSeq CCP normal

Gender
Female

Relationship
Proband

Chip Type
Ion 318 Chip

Sample Type
DNA

Log Information

This section shows a list of the actions that have been performed on this analysis.

Date	User	Action	Detail
Mar 16 2018 03:36 PM	user1@ABClab.com	Modification	Stopped editing analysis results.
Mar 16 2018 02:35 PM	user1@ABClab.com	Modification	Started editing analysis results.
Mar 15 2018 01:43 PM	user2@ABClab.com	Modification	Stopped editing analysis results.
Mar 15 2018 01:41 PM	user2@ABClab.com	Modification	Started editing analysis results.

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

Annotation sources

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 your 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 are available in the analysis workflow, and in the TSV file. Every analysis workflow also has an associated annotation set that lists all of the annotation sources used, along with their versions.

To add custom annotation sources to an annotation set, see "Import a custom annotation source" on page 385.

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 385for 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

Annotations	Description	Source[1]
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 then have be classified as a UCSC Common SNP.	dbSNP
DGV	Database of Genomic Variants: A curated database of human genomic structural variation	DGV
DrugBank	List of drugs known to target the gene(s) 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 the latest version available in Ion Reporter™ Software 5.12 for hg19. If you use the DrugBank version 20150107 in an hg19 annotation set, you will not be able to 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

Annotations	Description	Source[1]
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 within 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
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
Grantham score	A measure of evolutionary distance. See "Grantham score" on page 405.	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 within 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

Annotations	Description	Source[1]
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**
PhylopP	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
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 your 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 then have 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 particular 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.

For both GRCh38 and hg19 references, the default canonical transcript for the BRCA1 gene has changed in Ion Reporter™ Software 5.12 for both REFSEQ and ENSEMBL canonical transcripts.

- In Ion Reporter™ Software 5.10 and earlier, BRCA1 default REFSEQ canonical transcript is: NM_007300.3. In Ion Reporter™ Software 5.12 and later, the default BRCA1 REFSEQ canonical transcript is: NM_007294.3.
- In Ion Reporter™ Software 5.10 and earlier, BRCA1 default ENSEMBL canonical transcript is: ENST00000471181.7. In Ion Reporter™ Software 5.12 and later, the default BRCA1 ENSEMBL canonical transcript is: ENST00000357654.8.

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_000141.4
FGFR3	NM_001163213.1	NM_000142.4
ABL1	NM_007313.2	NM_005157.4
ARAF	NM_001256196.1	NM_001654.4
CHEK2	NM_001005735.1	NM_007194.3
GNAS	NM_080425.2	NM_000516.4
TP53	NM_001276760.1	NM_000546.5

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

Contig Name	Length (bps)
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
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 5.12 are both dbsnp versions 151. However, since the UCSC genome browser did not have the UCSC common genes available for dbSNP version 151 when Ion Reporter™ Software 5.12 was being released, UCSC common genes version 150 are used to set the UCSC common genes in Ion Reporter™ Software 5.12.

Import a custom annotation source

You can use your own annotation source files in Ion Reporter™ Software. If you have annotation data that you want to use, you must first create a new annotation set for the custom annotation source. Then, you can import the files into the new annotation set.

There are four 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 drop-down 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! You can use the words AND, NOT or, OR in an annotation source name in Ion Reporter™ Software 5.12. Do not include these operators in an annotation source name if you use Ion Reporter Software 5.10 or earlier.

   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 browse to and select your 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 TSF variant files, information from the **Annotation values** column is added to matching variants in your analysis results.
- In the Analysis Results screen, you can create a filter based on the first key-value pair in the . (Only the first key-value pair can be used as a filter.)

Here is an example file:

```
#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 385

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 your analysis results:

- Information from the second column of the gene set file is added to matching variants in your analysis results. The category name that is assigned to the gene is added to the Analysis Results screen and to downloaded TSF variant files.
- In the Analysis Results screen, you can create a filter that is based on the gene category name. You can filter your 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 385.

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. In Ion Reporter™ Software 5.4 and later, the variant is annotated if the transcript names match, even if the version numbers of the custom transcripts do not match.

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
```

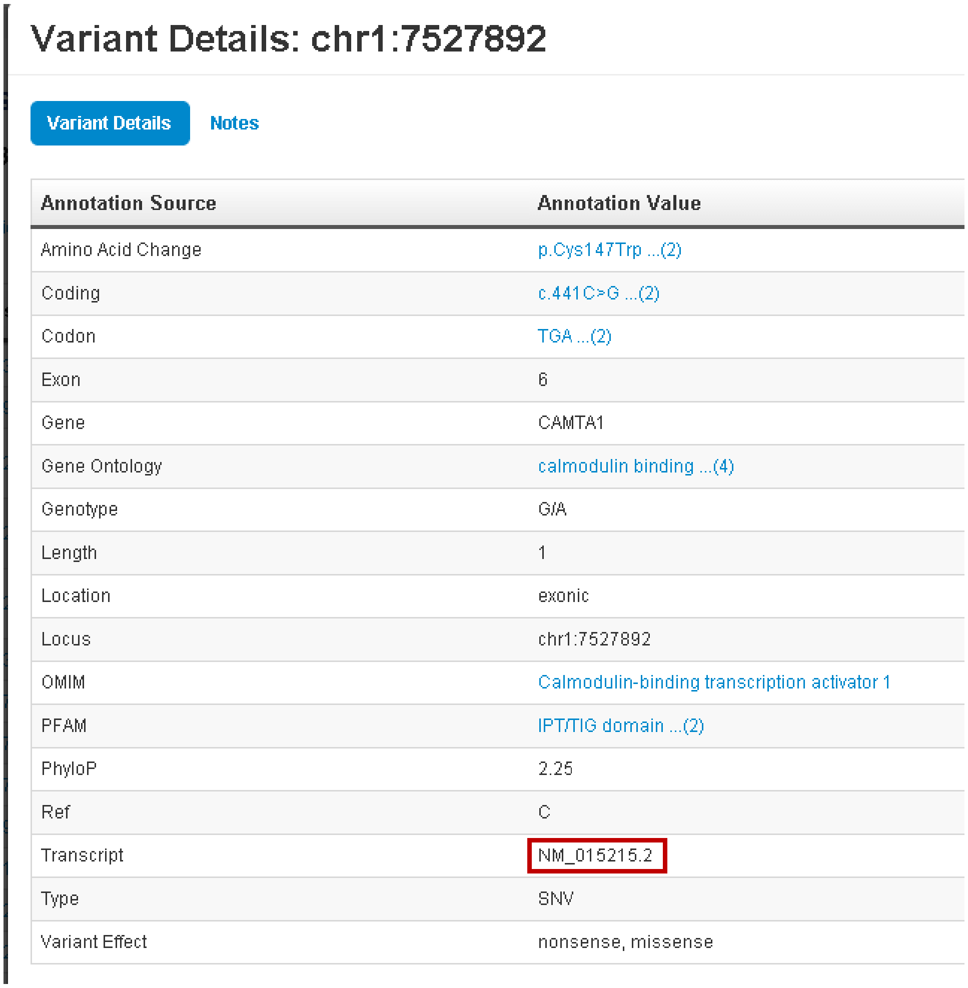
RefGene GeneModel and Ensembl GeneModel transcript versions

You can view the details about the annotations that Ion Reporter™ Software supports for a particular 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: chr1:7527892

Annotation Source	Annotation Value
Amino Acid Change	p.Cys147Trp ...(2)
Coding	c.441C>G ...(2)
Codon	TGA ...(2)
Exon	6
Gene	CAMTA1
Gene Ontology	calmodulin binding ...(4)
Genotype	G/A
Length	1
Location	exonic
Locus	chr1:7527892
OMIM	Calmodulin-binding transcription activator 1
PFAM	IPT/TIG domain ...(2)
PhyloP	2.25
Ref	C
Transcript	NM_015215.2
Type	SNV
Variant Effect	nonsense, missense

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 your 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 should be combined to ensure unique names.

```
Name: variantdbA_v1     Version: v1
Name: variantdbA_v2     Version: v2
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 your input VariantDB file is used in the following ways in your analysis results:

- In a downloaded variants TSV file, the content in your ID, REF, ALT, and INFO fields are added to the variant.
- In the Analysis Results screen, the content in your 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 your ID field. If the content of the ID field does not contain a value (contains only a period), then the first key-value pair of your INFO field is used.

Further information on 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 may 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 could 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 mandatory according to the official VCF specification. However, in order to perform a "genotype" hit level match in Ion Reporter™ Software, you must 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 will try to find the most specific hit level match possible. However, if no GT value is supplied, the most specific hit level possible will be 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 missing.
  - Consider the example below:

    ```
    ##fileformat=VCFv4.1

    ##FORMAT=<ID=GT,Number=1,Type=String,Description="Genoty
    pe">
                                    #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 in order to make a VariantDB in Ion Reporter™ Software, you will be able to filter on the AMPID field, since the AMPID key is the first INFO key present in the INFO field of the VCF file and the ID fields are missing (represented by dots).
  - **Option 2:**

    If the INFO field is not populated, filtering will be automatically enabled on the ID column.

    Consider the example below, in which the INFO field is missing and represented with a dot:

    ```
    ##fileformat=VCFv4.1
    ```

```
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genoty
pe">
                                        #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 in order to make a VariantDB in Ion Reporter™ Software, you will be able to filter on the ID field, since the INFO field of the VCF file is not populated.

See also the sections on "MyVariants" for marking and tracking of variant annotation beyond the VariantDB annotation presets in analysis workflows.

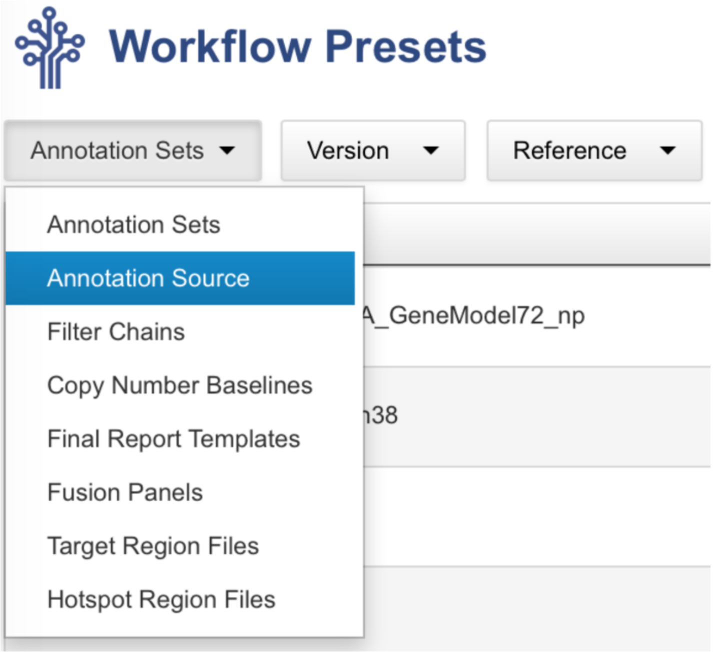
Delete custom annotation sources

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 a 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**.

Oncomine™ Variant Annotator plugin

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 blank, 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.

Preferred transcripts in default analysis workflows

The default 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:

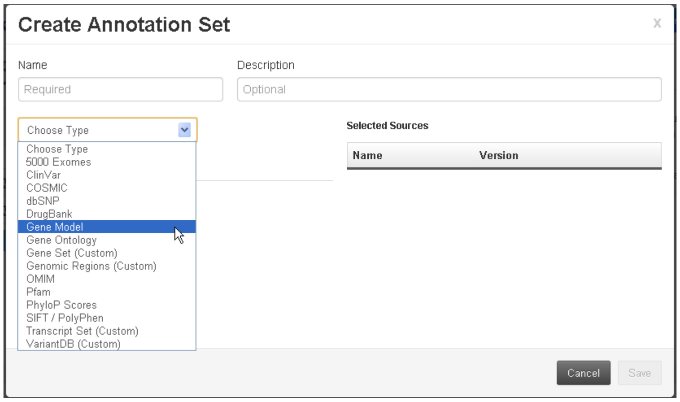
- RefGene
- Functional
- Canonical
- Transcript Scores

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 395.

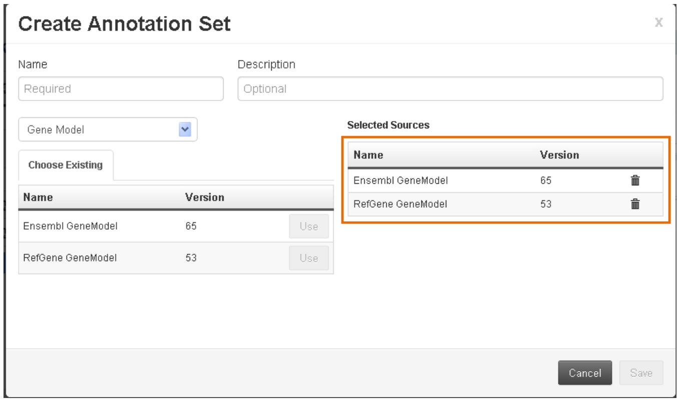
Create annotation set without preferred transcript sets

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.



Name	Version
Ensembl GeneModel	65
RefGene GeneModel	53

Selected Sources

Name	Version
Ensembl GeneModel	65
RefGene GeneModel	53

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 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 made by the VariantCaller. For example, a VariantCaller quality score of 20 is associated with a p-value of 0.01. A 30 is associated with a p-value is 0.001.

Pfam annotations

Ion Reporter™ Software has three Pfam versions: version 26, version 30 and version 31. Refer to the following website for more information on PFAM:

http://pfam.xfam.org/

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!

Annotation versions and calculations

This section provides details about the annotation scores used in Ion Reporter™ Software and how these scores are calculated.

SIFT versions

- **SIFT version**—5.1.1
- **Protein database**—UniprotTrEMBL, downloaded on July 11, 2011. Updated on March 14, 2014.
- **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 on August 12, 2011 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 flatfiles 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 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 131.

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 proband's parents at this variant position.

The category HasDeNovoNonRefAllele is assigned to a proband variant if the variant meets all of the following criteria:

- The variant has an allele that:
  - Is not 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_nonref_allele_min_father_coverage.
- The coverage of the mother's genotype at this variant position is at least denovo_nonref_allele_min_mother_coverage.

Note: The coverage parameters help avoid false-positive calls that might arise simply 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 we cannot exactly determine that the allele for the proband is *de novo*. To establish evidence of absence, we require that the parents be covered well enough that variants are called if actually present. Therefore we require 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 400.

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 father's genotype at this variant position and its other allele is present in the mother's genotype at this variant position.

A proband variant is assigned the category InconsistentWithParents if all of the following are true:

- The variant has coverage at least consistencyMinCoverage.
- The father's genotype has coverage at least consistencyMinCoverage at this variant position.
- The mother's genotype has coverage at least consistencyMinCoverage at this variant position.
- The variant is not consistent with it 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 409 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

11

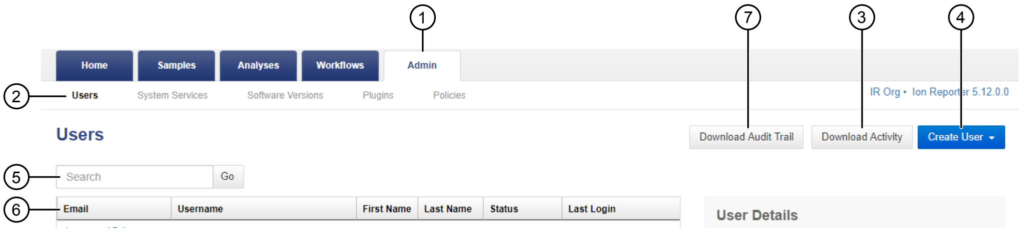
Administer Ion Reporter™ Software

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 412.
The **System Services** screen for managing system functions. For more information, see "Manage system services" on page 417.
The **Software Versions** screen for viewing software package version information. For more information, see "View software version information" on page 418.
The **Plugins** screen for managing plugins. For more information, see "Manage plugins" on page 418.
The **Policies** screen for managing e-signatures and password rules. For more information, see "Manage policies" on page 419.

③ **Download Activity** allows you to download logs of recent activity. For more information, see "Download an audit log of system activities" on page 423.

④ **Create User** allows 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** allows 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 415.
  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**.

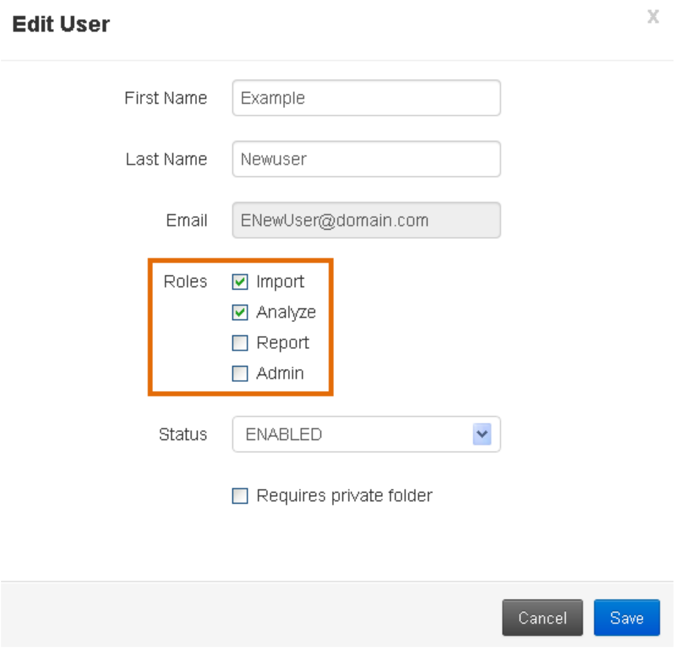
     The information in the spreadsheet is uploaded to the software. The new users appear on the **Users** screen.

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.



First Name: Example
Last Name: Newuser
Email: ENewUser@domain.com
Roles: ☑ Import ☑ Analyze ☐ Report ☐ Admin
Status: ENABLED
☐ Requires private folder

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 415.
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.

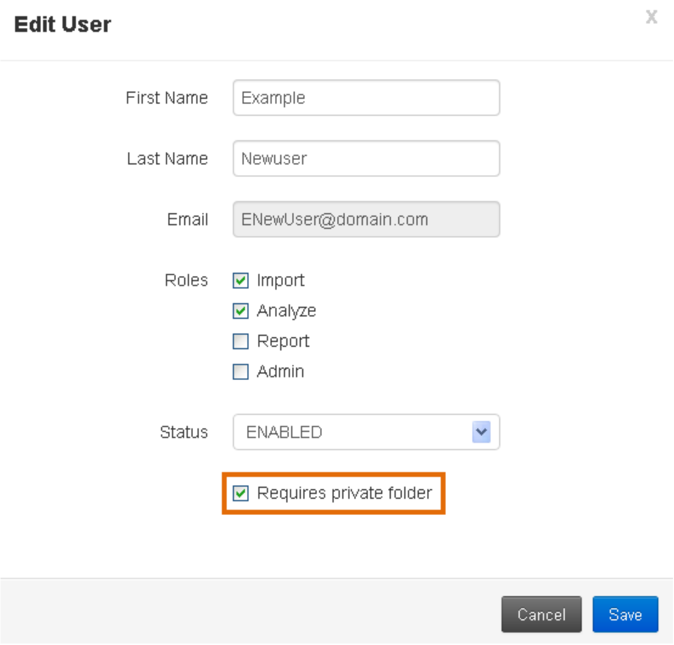
After a private folder is enabled, the data, analysis, and analysis workflows created by that user and generated in that folder are visible only to the user who owns the private folder, and to the administrator who set up the private folder.

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 anytime 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**.



Edit User

First Name: Example
Last Name: Newuser
Email: ENewUser@domain.com
Roles: ☑ Import ☑ Analyze ☐ Report ☐ Admin
Status: ENABLED
☑ Requires private folder

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. There are two mechanisms to set or reset the password for a user account.

After a password is reset, the administrator must notify the user about the new or reset password.

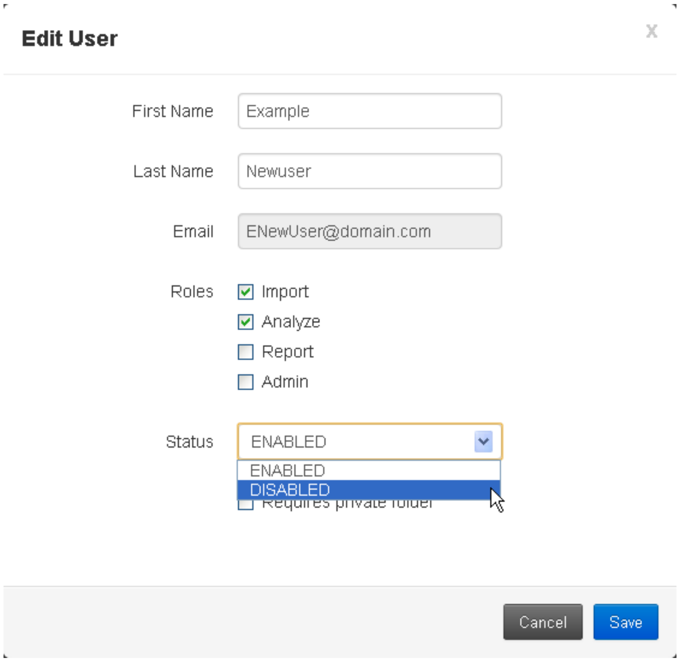
1. On the Ion Reporter™ Software server, sign in to Ion Reporter™ Software, then click the **Admin** tab.
2. In the **Users** screen, select the user account (without clicking the hyperlink for the account), then in **User Details** select **Actions ▸ Reset Password**.
3. In the **Reset User Password** 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**.



Edit User

First Name: Example

Last Name: Newuser

Email: ENewUser@domain.com

Roles:
☑ Import
☑ Analyze
☐ Report
☐ Admin

Status: ENABLED

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.

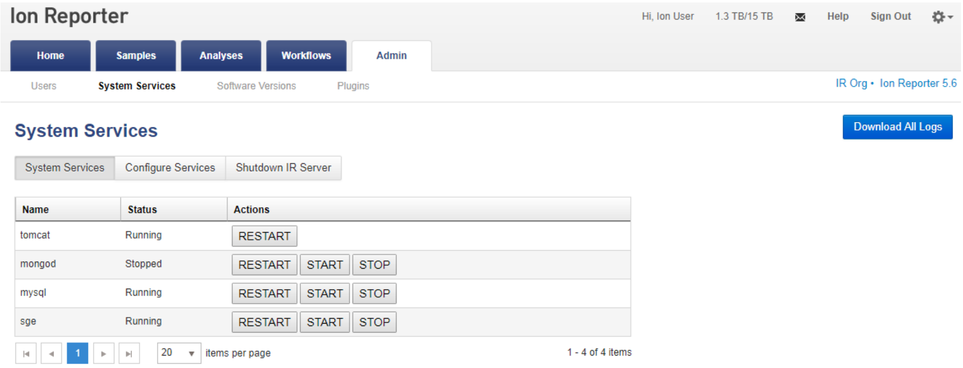
The user account is deleted.

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**.



Name	Status	Actions
tomcat	Running	RESTART
mongod	Stopped	RESTART START STOP
mysql	Running	RESTART START STOP
sge	Running	RESTART START STOP

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 an service. • **Stop**: stop an 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 plugins

An administrator can download plugins from Connect, then install the plugins into the Ion Reporter™ Software.

For information on how to create custom plugins for Ion Reporter™ Software, see Appendix C, "Programming guidelines for Ion Reporter™ Software plugins".

Download plugins from Connect

An administrator can download plugins from Connect. The ampliSeqRNA and RNASeq plugins can be downloaded from Connect, then installed into the Ion Reporter™ Software.

1. In a web browser, go to **https://apps.thermofisher.com/** , then sign in.

2. Click the apps icon (▦).

3. In the apps dashboard, click **Plugins**.

4. *(Optional)* Click a category at the top of page.
   The list of plugins is narrowed to only plugins that are included in the selected category.
5. Click ⬇ to download the plugin. Select the checkbox to indicate that you agree to the End User License Agreement (EULA), then click **Download Plugin**.
   A compressed directory that contains the plugin is downloaded to your local machine.

For details on how to install the plugins, see "Install and uninstall plugins" on page 419.

Install and uninstall plugins

Only administrators can install and uninstall plugins in their Ion Reporter™ Software organization.

1. In the **Admin** tab, click **Plugins**.
2. Click **Install Plugin**.
3. In the **Upload Plugin file** dialog box, click **Select file**.
4. Navigate to a plugin ZIP file that you have downloaded from Connect, select the ZIP file, then click **Open**.
   The file to be uploaded appears in the file text box.
5. Click **Upload**.
   A confirmation appears in the **Upload Plugin file** dialog box.
6. Click **Close**.
   - The plugin is added to the list of plugins.
   - Installed plugins are automatically enabled.
7. *(Optional)* Manage an installed plugin.
   a. Select a plugin by selecting the checkbox in the left-most column.
   b. To disable the selected plugin, click **Actions**, then select **Disable**.
   c. To uninstall the selected plugin, click **Actions**, then select **Uninstall**.

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

Password policy	Description	Default value
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
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 identify 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 375.

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.
    1. In **Screen Name**, select **Admin Policies** from the list.
    2. In **Date Range**, select a date range, or specify from and to dates using the calendar.
    3. (Optional) In the audit report for the selected time period, click **Filter** to refine the results.
    4. 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.

Manage the API token

The API token is used to upload data to the server version of Ion Reporter™ Server and access data from the API.

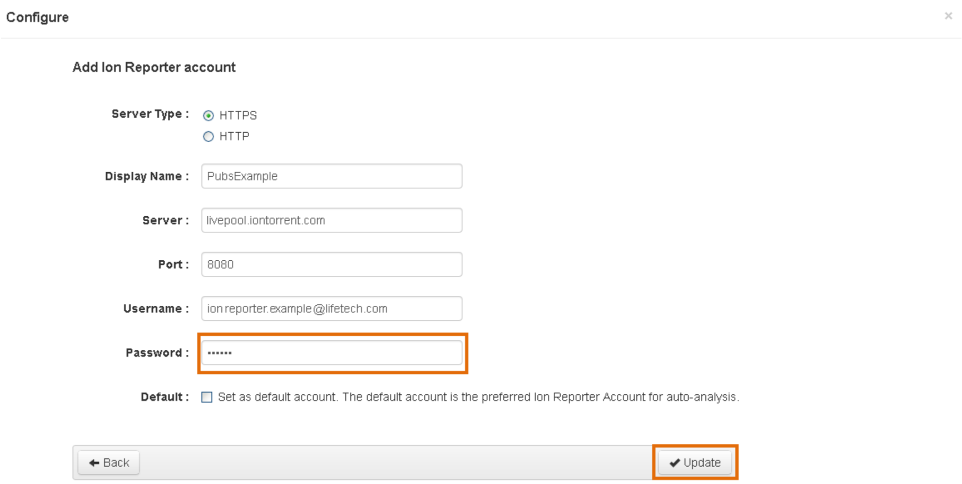
These steps can be performed by either an administrative user or a regular user.

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 token. The functions and the steps to recover functionality are as follows:

- IonReporterUploader plugins that are configured with your Ion Reporter™ Software account. To recover, in IonReporterUploader plugin configuration in Torrent Suite™ Software, retype your password, then click **Update** .



Add Ion Reporter account

Server Type : ● HTTPS ○ HTTP
Display Name : PubsExample
Server : livepool.iontorrent.com
Port : 8080
Username : ion reporter.example@lifetech.com
Password : ••••••
Default : ☐ Set as default account. The default account is the preferred Ion Reporter Account for auto-analysis.

- Scripts or code that access the Ion Reporter™ Software API with your previous token. To recover, use your new token with those scripts.

Follow these steps to access your API token:

1. Torrent Suite™ Software, click the **Admin** tab.
2. Click ⚙**Actions** ▸ **Manage API Token**.
3. In the **Manage API Token** dialog box, if the **API Token** field is blank, click **Generate** to create your token.

   If a token already exists, click **Generate** to generate a new one.

A new token is created. The functions noted above will no longer work with the new token until you perform the described actions.

12

Troubleshooting

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.

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.
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`.

Observation	Possible cause	Recommended action
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`.
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`.

Observation	Possible cause	Recommended action
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`.

A

Fusion analyses

This appendix applies to analyses that are based on the following Ion AmpliSeq™ and Oncomine™ fusion panels:

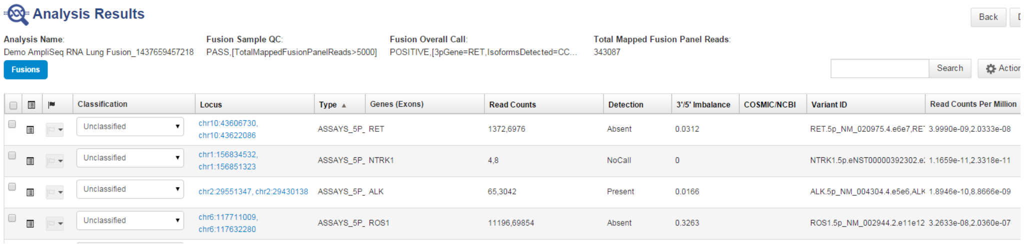
- Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2 and Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel.
- Custom Ion AmpliSeq™ and Ion AmpliSeq™ HD fusion panels designed by Ion Torrent White Glove team and Ion AmpliSeq™ Designer.
- Oncomine™ fusion panels for solid tumor, liquid biopsy, heme oncology, and immuno-oncology research applications.

For information on importing fusion panels, see "Import panel files from AmpliSeq.com" on page 96.

Gene fusion analysis results

Fusion Sample QC, Fusion Overall Call and Total Mapped Fusion Panel Reads, are included in Ion Reporter™ Software.

Analysis results are available in a table.



Analysis Name: Demo AmpliSeq RNA Lung Fusion_1437659457218

Fusion Sample QC: PASS,[TotalMappedFusionPanelReads>5000]

Fusion Overall Call: POSITIVE,[3pGene=RET,IsoformsDetected=CC...

Total Mapped Fusion Panel Reads: 343087

☐			Classification	Locus	Type ▲	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID	Read Counts Per Million
☐			Unclassified	chr10:43606730, chr10:43622086	ASSAYS_5P_	RET	1372,6976	Absent	0.0312		RET.5p_NM_020975.4.e6e7,RE	3.9990e-09,2.0333e-08
☐			Unclassified	chr1:156834532, chr1:156851323	ASSAYS_5P_	NTRK1	4,8	NoCall	0		NTRK1.5p.eNST00000392302.e	1.1659e-11,2.3318e-11
☐			Unclassified	chr2:29551347, chr2:29430138	ASSAYS_5P_	ALK	65,3042	Present	0.0166		ALK.5p_NM_004304.4.e5e6,ALK	1.8946e-10,8.8666e-09
☐			Unclassified	chr6:117711009, chr6:117632280	ASSAYS_5P_	ROS1	11196,69854	Absent	0.3263		ROS1.5p_NM_002944.2.e11e12	3.2633e-08,2.0360e-07

Note: In the table view, you can close the Summary Panel on the right, to provide more room for the table columns.

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:

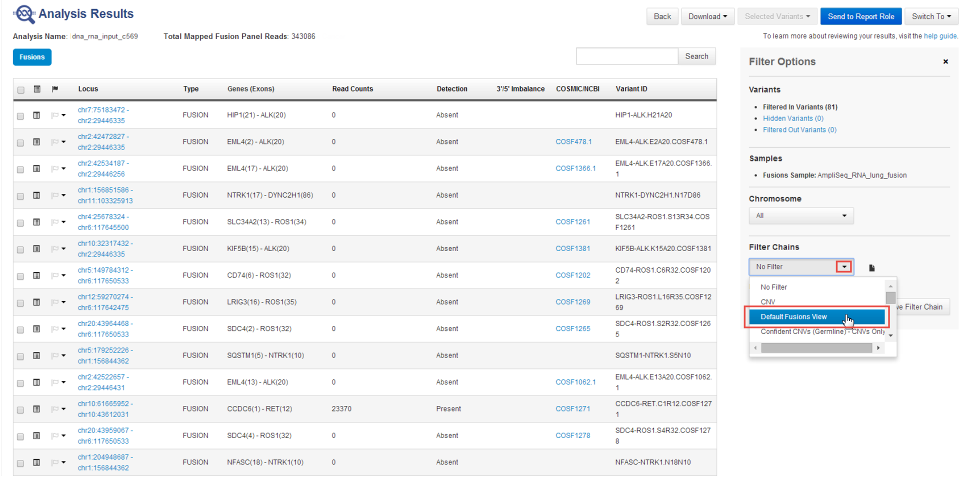
Fusion Isoforms	RET/ROS1/ALK Imbalance Score	Final Call
Positive	Positive	Positive
Positive	No Call/Negative	Positive
Negative	Positive	Positive
Negative	Positive	Positive
Negative	No Call	No Call
Negative	Negative	Negative

Filters

This view shows targeted fusions that are not found in your sample (and are reported as Absent in the Detection column), as well as other variant types.

For a view that includes only Present fusion calls:

1. Click the **Filter** menu.



Analysis Name: dna_rna_input_c569 Total Mapped Fusion Panel Reads: 343086

To learn more about reviewing your results, visit the help guide.

Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
chr7:75183472 - chr2:29446335	FUSION	HIP1(21) - ALK(20)	0	Absent			HIP1-ALK.H21A20
chr2:42472827 - chr2:29446335	FUSION	EML4(2) - ALK(20)	0	Absent		COSF478.1	EML4-ALK.E2A20.COSF478.1
chr2:42534187 - chr2:29446256	FUSION	EML4(17) - ALK(20)	0	Absent		COSF1366.1	EML4-ALK.E17A20.COSF1366.1
chr1:156851586 - chr11:103325913	FUSION	NTRK1(17) - DYNC2H1(86)	0	Absent			NTRK1-DYNC2H1.N17D86
chr4:25678324 - chr6:117645500	FUSION	SLC34A2(13) - ROS1(34)	0	Absent		COSF1261	SLC34A2-ROS1.S13R34.COSF1261
chr10:32317432 - chr2:29446335	FUSION	KIF5B(15) - ALK(20)	0	Absent		COSF1381	KIF5B-ALK.K15A20.COSF1381
chr5:149784312 - chr6:117650533	FUSION	CD74(6) - ROS1(32)	0	Absent		COSF1202	CD74-ROS1.C6R32.COSF1202
chr12:59270274 - chr6:117642475	FUSION	LRIG3(16) - ROS1(35)	0	Absent		COSF1269	LRIG3-ROS1.L16R35.COSF1269
chr20:43964468 - chr6:117650533	FUSION	SDC4(2) - ROS1(32)	0	Absent		COSF1265	SDC4-ROS1.S2R32.COSF1265
chr5:179252226 - chr1:156844362	FUSION	SQSTM1(5) - NTRK1(10)	0	Absent			SQSTM1-NTRK1.S5N10
chr2:42522657 - chr2:29446431	FUSION	EML4(13) - ALK(20)	0	Absent		COSF1062.1	EML4-ALK.E13A20.COSF1062.1
chr10:61665952 - chr10:43612031	FUSION	CCDC6(1) - RET(12)	23370	Present		COSF1271	CCDC6-RET.C1R12.COSF1271
chr20:43959067 - chr6:117650533	FUSION	SDC4(4) - ROS1(32)	0	Absent		COSF1278	SDC4-ROS1.S4R32.COSF1278
chr1:204948687 - chr1:156844362	FUSION	NFASC(18) - NTRK1(10)	0	Absent			NFASC-NTRK1.N18N10

Variants
- Filtered In Variants (81)
- Hidden Variants (0)
- Filtered Out Variants (0)

Samples
- Fusions Sample: AmpliSeq_RNA_lung_fusion

Chromosome: All

Filter Chains: No Filter
- No Filter
- CNV
- Default Fusions View
- Confident CNVs (Germline) - CNVs Only

2. Select the **Default Fusions View** filter.

Note: The following 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
- RNA_HOTSPOT
- GENE_EXPRESSION

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**: Greater than 20 supporting reads are required to report Present.
- **Expression controls**: Greater than 15 supporting reads are required to report Present.

For example, if the threshold of the Detection column is set to greater than 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 fewer than 21 reads support the fusion.
- **Present**: Greater than 20 reads provide evidence for the fusion.
- **Present-Non-Targeted**: Greater than 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 contain a specific primer pair, but it was picked up by a combination of two of the primers used for two 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 there is very strong evidence for non-targeted fusions, they may be worth following up with more detailed investigation. A literature search or a search of COSMIC or other databases may be helpful in determining whether such fusions have ever previously been observed, and may provide guidance in interpretation.
- **No Call**: Not enough evidence to determine if a fusion is present or not.

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).

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 web sites.

- The variant ID column reports our shorthand 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 demo 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

Interpret a fusion call

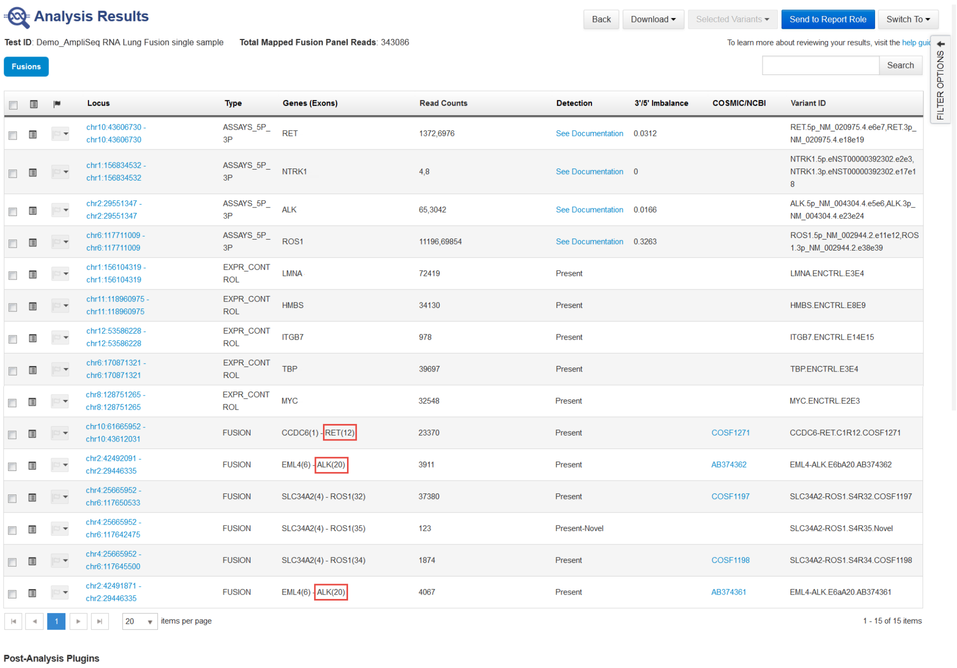
The analysis results provide several ways to confirm a fusion call, including Imbalance values, visualization, and Normalized Detection Fractions.

Imbalance values

Imbalance values are good predictors of fusion calls that involve ALK, RET, or ROS1 driver genes. We have not found Imbalance values to be predictors of fusion calls that involve a NTRK1 driver gene.

Follow these steps to find the Imbalance value for a specific fusion call:

1. Find the fusion's driver gene (the second gene listed in the Genes (Exons) column.



Analysis Results

Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

To learn more about reviewing your results, visit the help guid

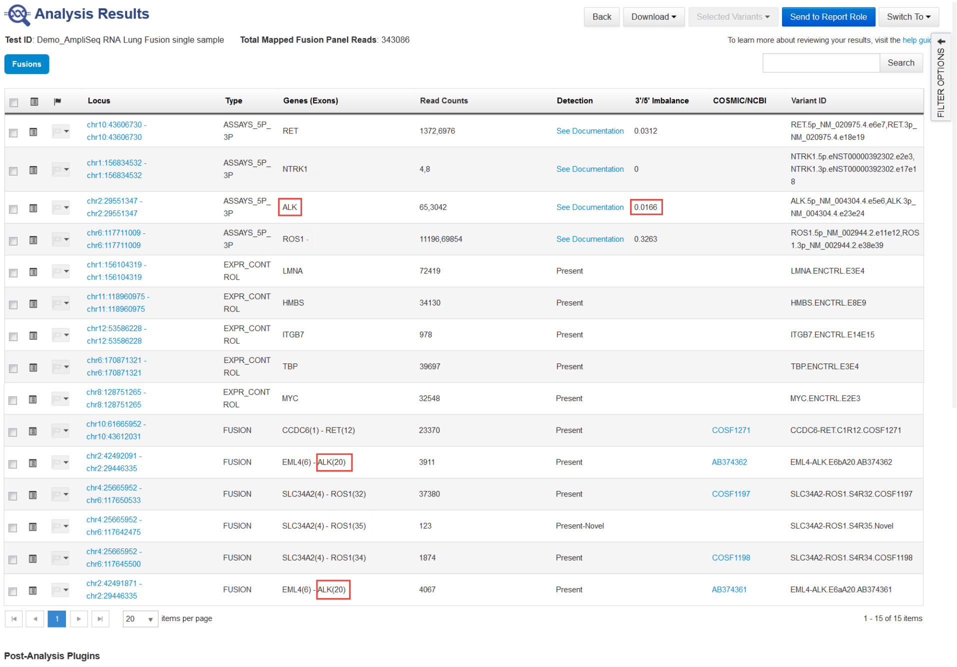
Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312		RET.5p_NM_020975.4.e6e7,RET.3p_NM_020975.4.e18e19
chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0		NTRK1.5p.eNST00000392302.e2e3, NTRK1.3p.eNST00000392302.e17e18
chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166		ALK.5p_NM_004304.4.e5e6,ALK.3p_NM_004304.4.e23e24
chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1	11196,69854	See Documentation	0.3263		ROS1.5p_NM_002944.2.e11e12,ROS1.3p_NM_002944.2.e38e39
chr1:156104319 - chr1:156104319	EXPR_CONTROL	LMNA	72419	Present			LMNA.ENCTRL.E3E4
chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present			HMBS.ENCTRL.E8E9
chr12:53586228 - chr12:53586228	EXPR_CONTROL	ITGB7	978	Present			ITGB7.ENCTRL.E14E15
chr6:170871321 - chr6:170871321	EXPR_CONTROL	TBP	39697	Present			TBP.ENCTRL.E3E4
chr8:128751265 - chr8:128751265	EXPR_CONTROL	MYC	32548	Present			MYC.ENCTRL.E2E3
chr10:61665952 - chr10:43612031	FUSION	CCDC6(1) - RET(12)	23370	Present		COSF1271	CCDC6-RET.C1R12.COSF1271
chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present		AB374362	EML4-ALK.E6bA20.AB374362
chr4:25665952 - chr6:117650533	FUSION	SLC34A2(4) - ROS1(32)	37380	Present		COSF1197	SLC34A2-ROS1.S4R32.COSF1197
chr4:25665952 - chr6:117642475	FUSION	SLC34A2(4) - ROS1(35)	123	Present-Novel			SLC34A2-ROS1.S4R35.Novel
chr4:25665952 - chr6:117645500	FUSION	SLC34A2(4) - ROS1(34)	1874	Present		COSF1198	SLC34A2-ROS1.S4R34.COSF1198
chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present		AB374361	EML4-ALK.E6aA20.AB374361

1 - 15 of 15 items

Post-Analysis Plugins

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.



Analysis Results

Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

To learn more about reviewing your results, visit the help guid

☐	Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
☐	chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312		RET.5p_NM_020975.4.e6e7,RET.3p_NM_020975.4.e18e19
☐	chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0		NTRK1.5p.eNST00000392302.e2e3,NTRK1.3p.eNST00000392302.e17e18
☐	chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166		ALK.5p_NM_004304.4.e5e6,ALK.3p_NM_004304.4.e23e24
☐	chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	11196,69854	See Documentation	0.3263		ROS1.5p_NM_002944.2.e11e12,ROS1.3p_NM_002944.2.e38e39
☐	chr1:156104319 - chr1:156104319	EXPR_CONTROL	LMNA	72419	Present			LMNA.ENCTRL.E3E4
☐	chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present			HMBS.ENCTRL.E8E9
☐	chr12:53586228 - chr12:53586228	EXPR_CONTROL	ITGB7	978	Present			ITGB7.ENCTRL.E14E15
☐	chr6:170871321 - chr6:170871321	EXPR_CONTROL	TBP	39697	Present			TBP.ENCTRL.E3E4
☐	chr8:128751265 - chr8:128751265	EXPR_CONTROL	MYC	32548	Present			MYC.ENCTRL.E2E3
☐	chr10:61665952 - chr10:43612031	FUSION	CCDC6(1) - RET(12)	23370	Present		COSF1271	CCDC6-RET.C1R12.COSF1271
☐	chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present		AB374362	EML4-ALK.E6bA20.AB374362
☐	chr4:25665952 - chr6:117650533	FUSION	SLC34A2(4) - ROS1(32)	37380	Present		COSF1197	SLC34A2-ROS1.S4R32.COSF1197
☐	chr4:25665952 - chr6:117642475	FUSION	SLC34A2(4) - ROS1(35)	123	Present-Novel			SLC34A2-ROS1.S4R35.Novel
☐	chr4:25665952 - chr6:117645500	FUSION	SLC34A2(4) - ROS1(34)	1874	Present		COSF1198	SLC34A2-ROS1.S4R34.COSF1198
☐	chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present		AB374361	EML4-ALK.E6aA20.AB374361

1 20 items per page 1 - 15 of 15 items

Post-Analysis Plugins

Imbalance score for assay calls

The Imbalance score is a metric that provides a measurement of the strength of fusion calls that involve ALK, RET, or ROS1 driver genes.

Several metrics and found Imbalance values were investigated and are considered a strong predictor of true fusions calls. Other predictors for fusions that involve NTRK1 driver genes are continually investigated.

Imbalance values are reported in the 3'/5' Imbalance column. The Imbalance score for an assay applies to all fusion calls with the driver gene that is reported in the Genes (Exons) column.

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.

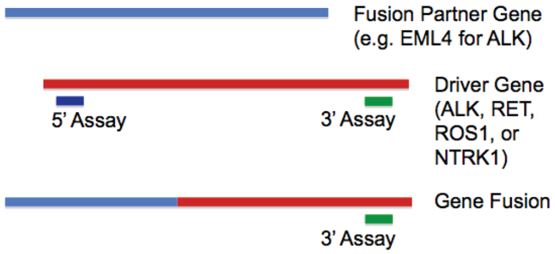
Note: Imbalance values provide information that is supplementary to the detection calls of "Present" or "Absent". If a fusion call is "Absent", then we recommended that you check the imbalance value to see if there is supplemental evidence of the presence of a fusion.

Interpret 3'/5' Imbalance values

The 3'/5' Imbalance values reporter in Ion Reporter™ Software generates analysis results for Ion AmpliSeq™ gene fusions, such as the Ion AmpliSeq™ RNA Lung Fusion analysis workflow. The imbalance values reporter measures 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 (see the following figure). The 3'/5' Imbalance assay is therefore included for two alternate purposes:

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 panel.

1. To confirm presence of a fusion from the ALK, RET, or ROS1 driver genes included in the panel, or



2. 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 441
- “Sensitivity“ on page 441
- “High 5' expression“ on page 441

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.

We currently do not have sufficient data to determine if the 3'/5' Imbalance value is a predictor for the NTRK1 gene.

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 Panel, 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	0.03	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 pages 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:

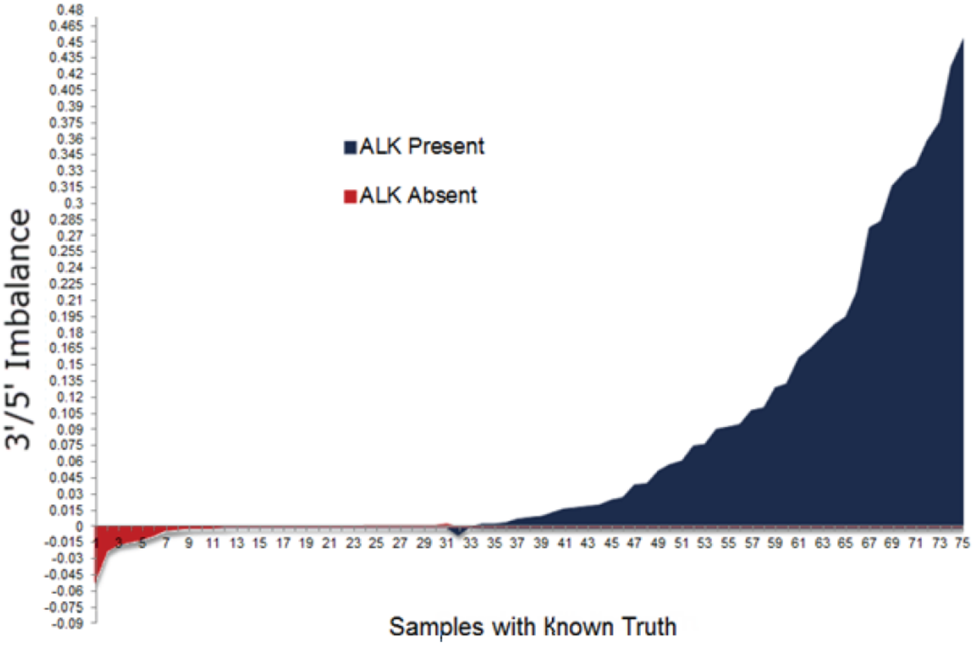
- In normal samples (without a fusion), imbalance values are very low (either close to zero or negative).
- In samples that contain a fusion, imbalance values are higher, with gene-specific thresholds shown in the above table.

In some cases, lower imbalance values are also good predictors of the presence of fusions, as shown in the plots linked below.

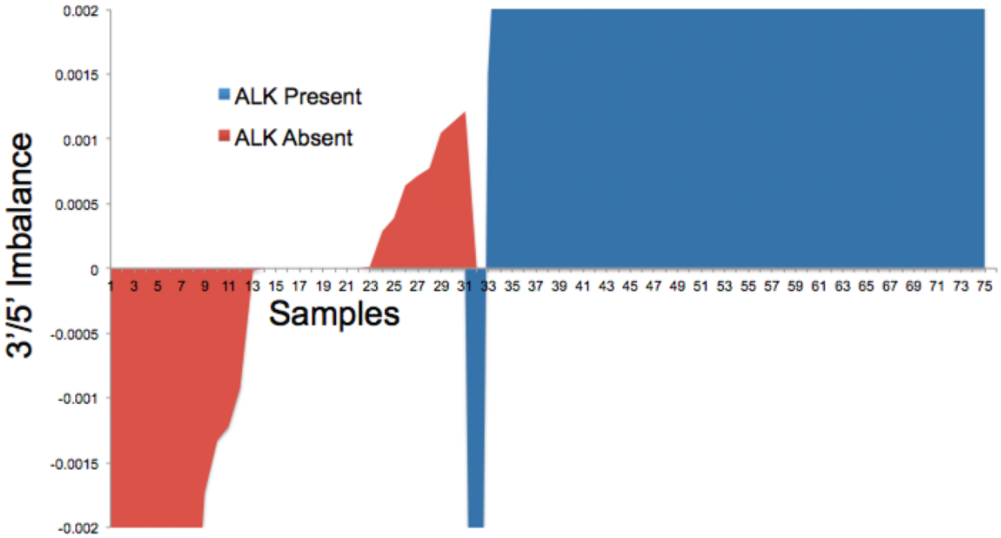
Note: These guidelines apply to samples that meet the criteria described in the sections: Minimum number of reads, Sensitivity, and High 5' expression. The values in the plots below have been based on results obtained from 75 FFPE lung cancer research samples previously tested with other technologies like FISH, IHC, and RT-PCR.

Example plot for ALK driver gene

The image linked here 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.



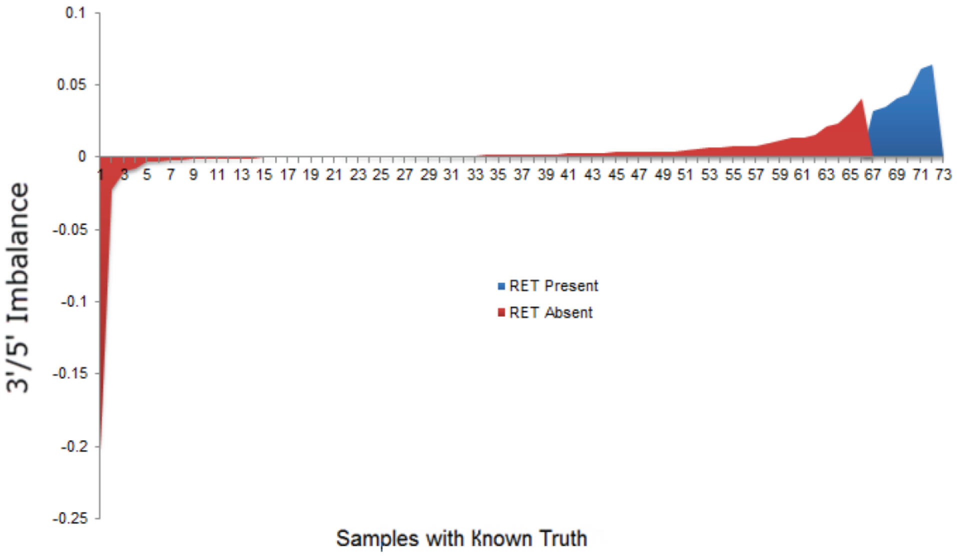
Zoomed in view:



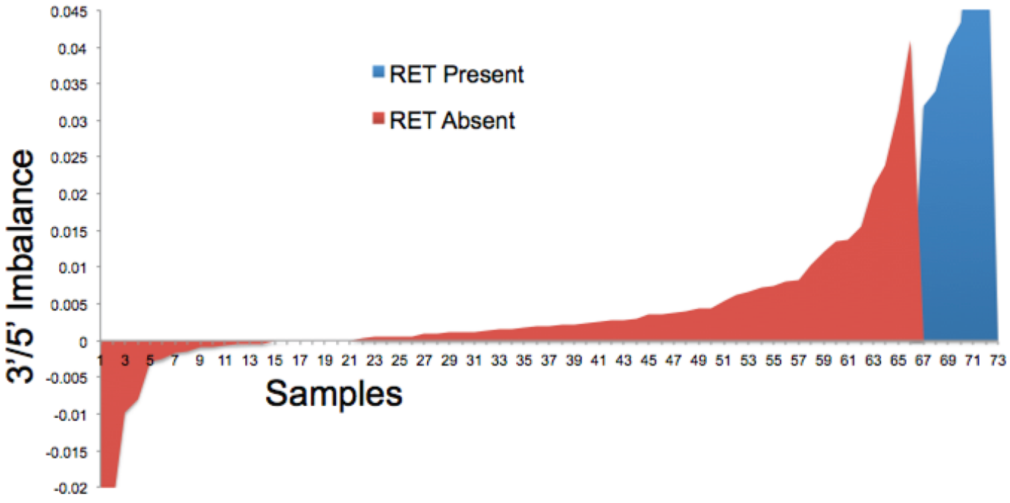
- A 3'/5' Imbalance value of 0.025 or greater indicates the likely presence of an ALK fusion.
- Below 0.001, there is no evidence that an ALK fusion is present.
- The range of 3'/5' Imbalance values between 0.001 and 0.025 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a fusion is present.

Example plot for RET driver gene

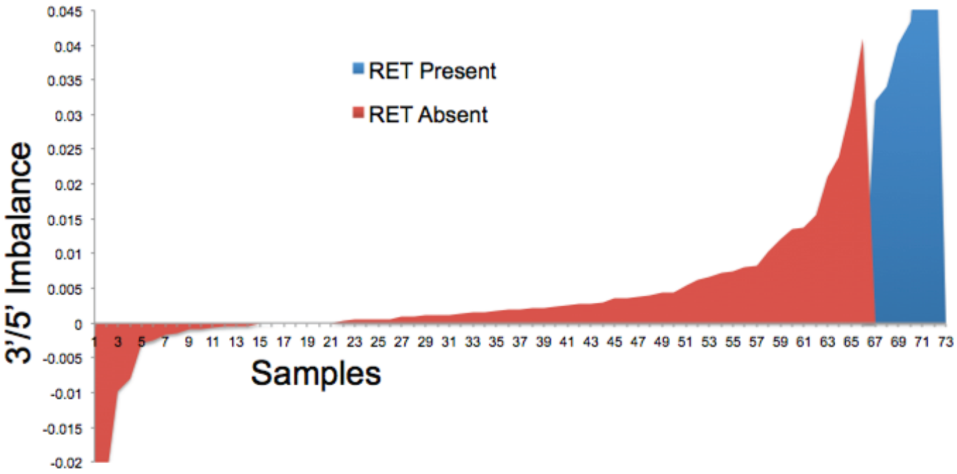
The image linked here shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that contain a fusion (in blue) all contain higher imbalance values.



Zoomed in view:



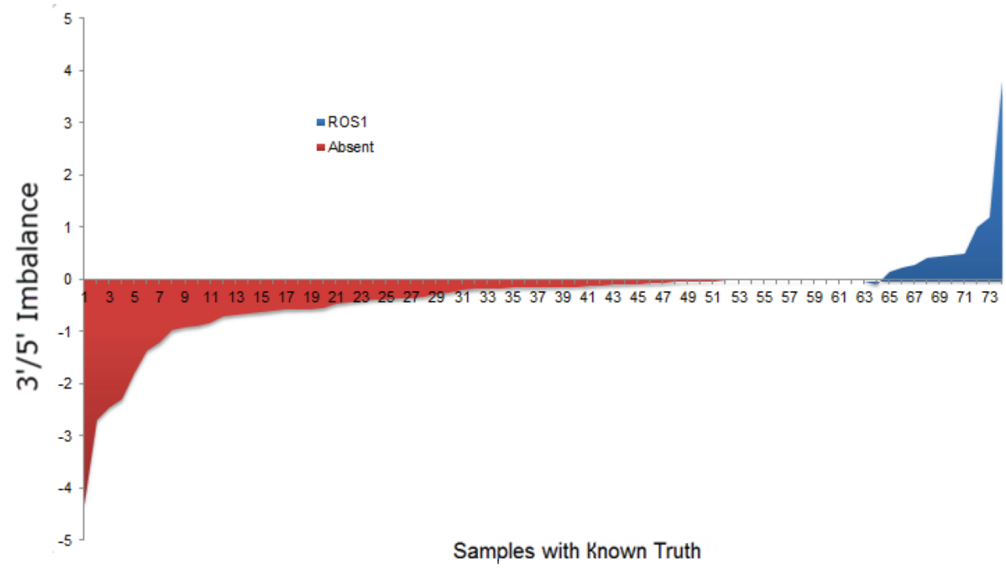
- A 3'/5' Imbalance value of 0.045 or greater indicates the likely presence of a RET fusion.



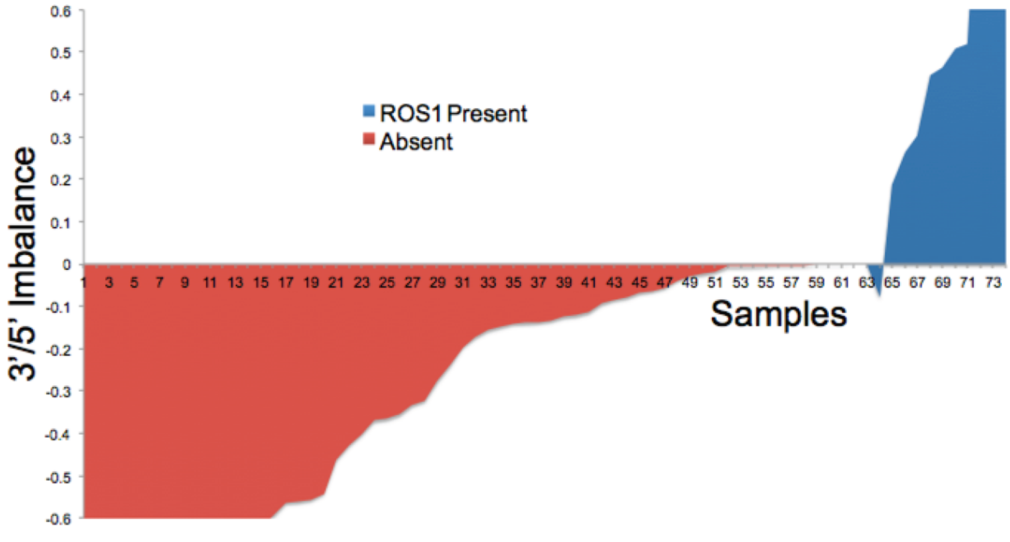
- With a 3'/5' Imbalance value of 0.03, there is no evidence that a RET fusion is present.
- The range of 3'/5' Imbalance values between 0.045 and 0.03 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a RET fusion is present.

Example plot for ROS1 driver gene

The image linked here 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.



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.

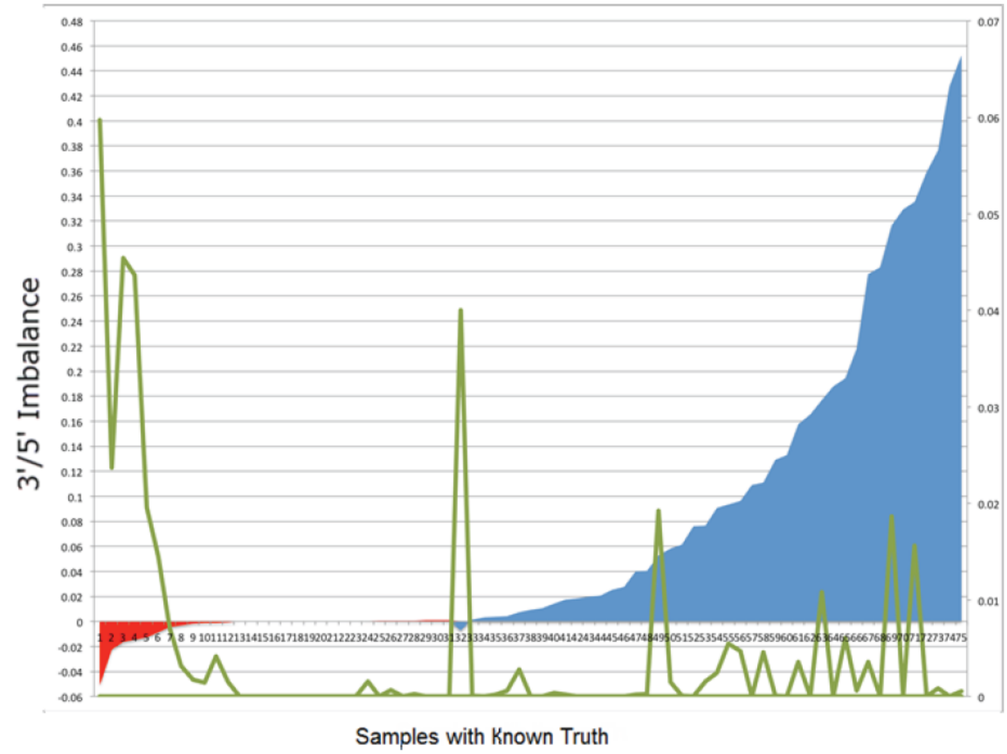
Check for High 5' expression and also check for the minimum number of reads before assuming that a fusion call of 'Present' is confirmed.

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.

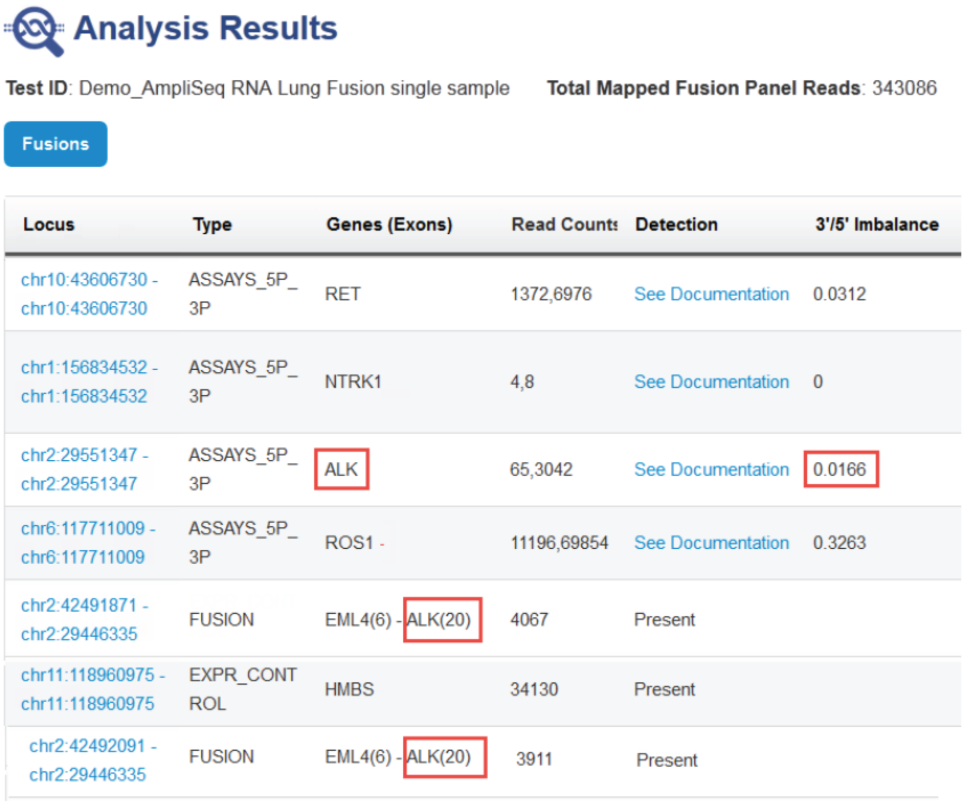
The plot linked here shows a false negative in sample 32. The green line tracks the 5' expression as 5' read counts divided by EXPR_CONTROL read counts. Note the spike of high 5' expression at the false negative for sample 21.

We have not seen a high 5' expression to be associated with a false positive.


3'/5' Imbalance
0.48
0.46
0.44
0.42
0.4
0.38
0.36
0.34
0.32
0.3
0.28
0.26
0.24
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
-0.04
-0.06
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Samples with Known Truth

How do I find the 3'/5' Imbalance value for a specific fusion?

Imbalance values are reported for ASSAY_5P_3P calls. The ASSAY_5P_3P's Imbalance value applies to all fusions with the driver gene that appears in the ASSAY_5P_3P's Genes (Exons) column:



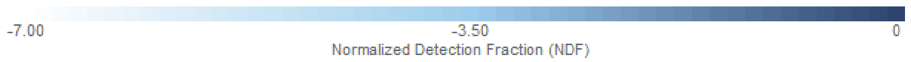
Analysis Results

Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance
chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312
chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0
chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166
chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	11196,69854	See Documentation	0.3263
chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present	
chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present	
chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present	

Normalized Detection Fractions (NDF)

Normalized Detection Fractions provide the color-coding of calls in heatmaps.

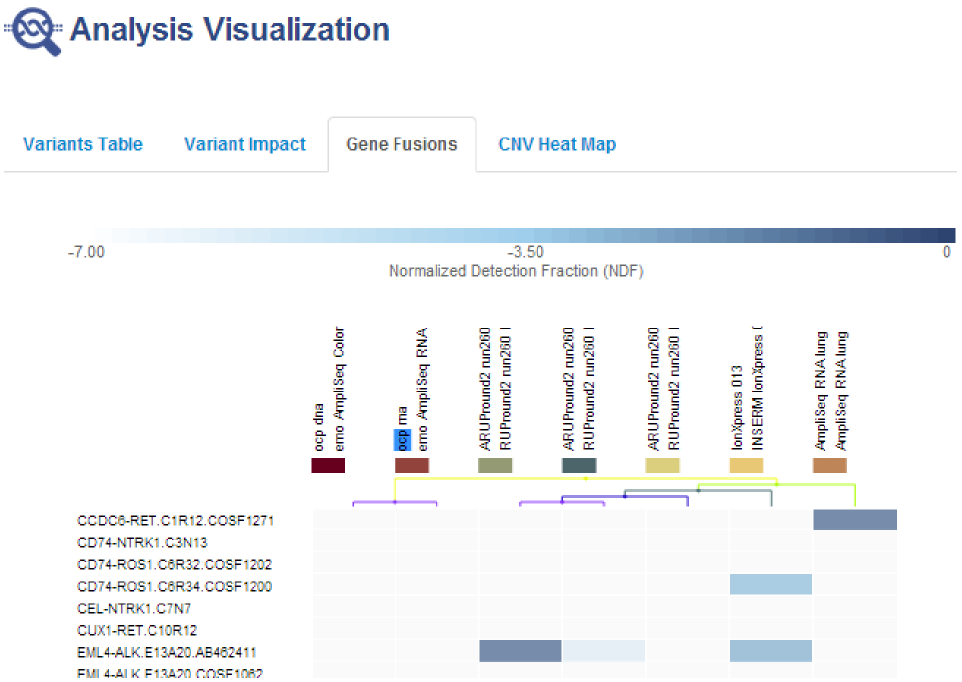


Normalized Detection Fractions are interpreted as follows:

- **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.

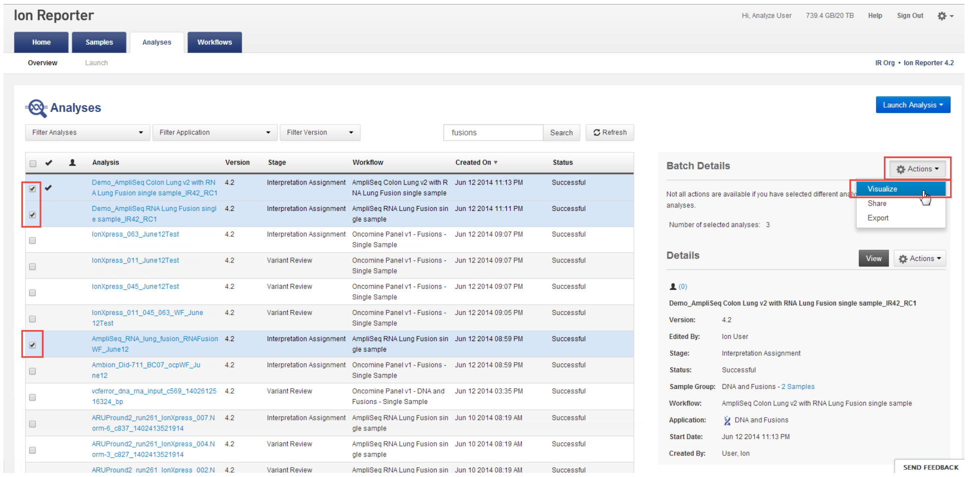
Visualize and compare fusion results of multiple analyses with heatmaps

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 your results of multiple fusion analyses:

1. In the Analysis Overview page, enable the checkboxes of the analyses with the results you want to visualize.



☐	✓		Analysis	Version	Stage	Workflow	Created On ▾	Status
☑	✓		Demo_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_IR42_RC1	4.2	Interpretation Assignment	AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample	Jun 12 2014 11:13 PM	Successful
☑			Demo_AmpliSeq RNA Lung Fusion single sample_IR42_RC1	4.2	Interpretation Assignment	AmpliSeq RNA Lung Fusion single sample	Jun 12 2014 11:11 PM	Successful
☐			IonXpress_063_June12Test	4.2	Interpretation Assignment	Oncomine Panel v1 - Fusions - Single Sample	Jun 12 2014 09:07 PM	Successful
☐			IonXpress_011_June12Test	4.2	Variant Review	Oncomine Panel v1 - Fusions - Single Sample	Jun 12 2014 09:07 PM	Successful
☐			IonXpress_045_June12Test	4.2	Variant Review	Oncomine Panel v1 - Fusions - Single Sample	Jun 12 2014 09:07 PM	Successful
☐			IonXpress_011_045_063_WF_June12Test	4.2	Variant Review	Oncomine Panel v1 - Fusions - Single Sample	Jun 12 2014 09:05 PM	Successful
☑			AmpliSeq_RNA_lung_fusion_RNAFusionWF_June12	4.2	Interpretation Assignment	AmpliSeq RNA Lung Fusion single sample	Jun 12 2014 08:59 PM	Successful
☐			Ambion_Did-711_BC07_ocpWF_June12	4.2	Interpretation Assignment	Oncomine Panel v1 - Fusions - Single Sample	Jun 12 2014 08:59 PM	Successful
☐			vcferror_dna_rna_input_c569_1402612516324_bp	4.2	Variant Review	Oncomine Panel v1 - DNA and Fusions - Single Sample	Jun 12 2014 03:35 PM	Successful
☐			ARUPround2_run261_IonXpress_007.Norm-6_c837_1402413521914	4.2	Interpretation Assignment	AmpliSeq RNA Lung Fusion single sample	Jun 10 2014 08:19 AM	Successful
☐			ARUPround2_run261_IonXpress_004.Norm-3_c827_1402413521914	4.2	Variant Review	AmpliSeq RNA Lung Fusion single sample	Jun 10 2014 08:19 AM	Successful
			ARUPround2_run261_IonXpress_002.N	4.2	Variant Review	AmpliSeq RNA Lung Fusion sin	Jun 10 2014 08:19 AM	Successful

Batch Details

Not all actions are available if you have selected different analyses.

Number of selected analyses: 3

Details

Demo_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_IR42_RC1

Version:	4.2
Edited By:	Ion User
Stage:	Interpretation Assignment
Status:	Successful
Sample Group:	DNA and Fusions - 2 Samples
Workflow:	AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample
Application:	DNA and Fusions
Start Date:	Jun 12 2014 11:13 PM
Created By:	User, Ion

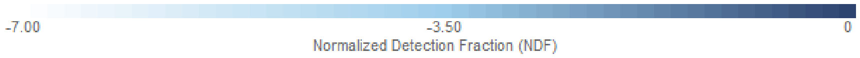
2. In the Action menu on the right, click the **Visualize** option.

   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:

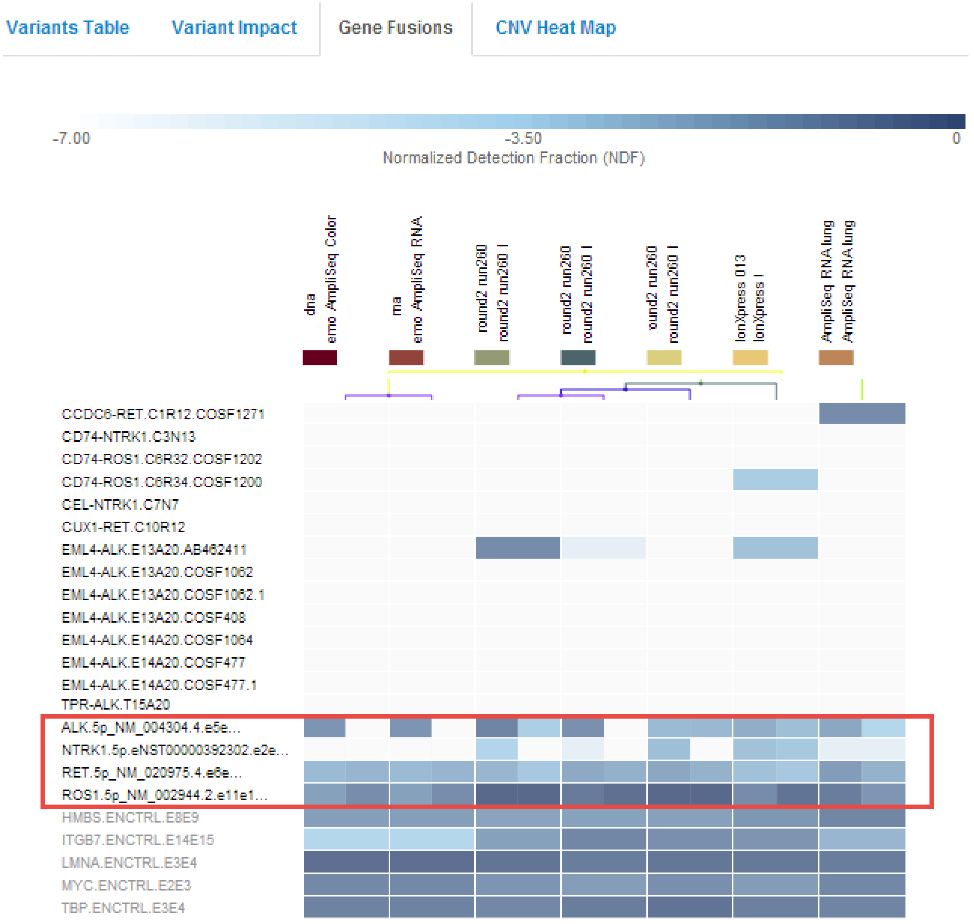
- Interpret each cell according to the color scale above the chart. Cells with the rightmost color are the strongest fusion calls.



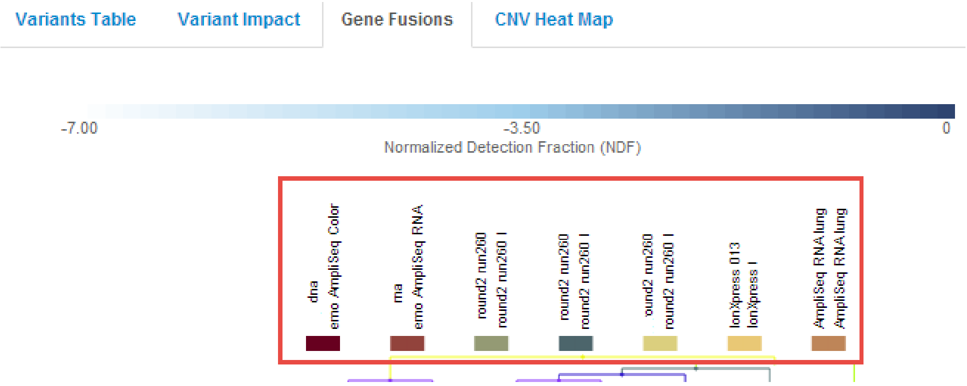
- Normalized Detection Fraction (NDF) values are calculated as follows (for a fusion F in sample S):

```
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**.

Samples

- **Proband** in Pround2_run260_IonXpress_002_c777_1402413521913: round2_run260_IonXpress_0
- **Proband** in AmpliSeq_RNA_lung_fusion_RNAFusionWF_June12: AmpliSeq_RNA_lung_fusion
- **Proband** in Demo_AmpliSeq RNA Lung Fusion single sample_IR42_RC1: rna
- **DNA Sample** in Demo_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_IR42_RC1: dna
- **Fusions Sample** in Demo_AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample_IR42_RC1: rna
- **Proband** in round2 run260 IonXpress 009.c807 140241352 round2_run260_IonXpress_009
- **Proband** in round2_run260_IonXpress_008.Norm-7_c787_1402413521913: round2_run260_IonXpress_0ess_008
- **Proband** in IonXpress_013_Rajesh: IonXpress_013

- Click **Selected Analyses** to see the list of analyses in the heatmap.

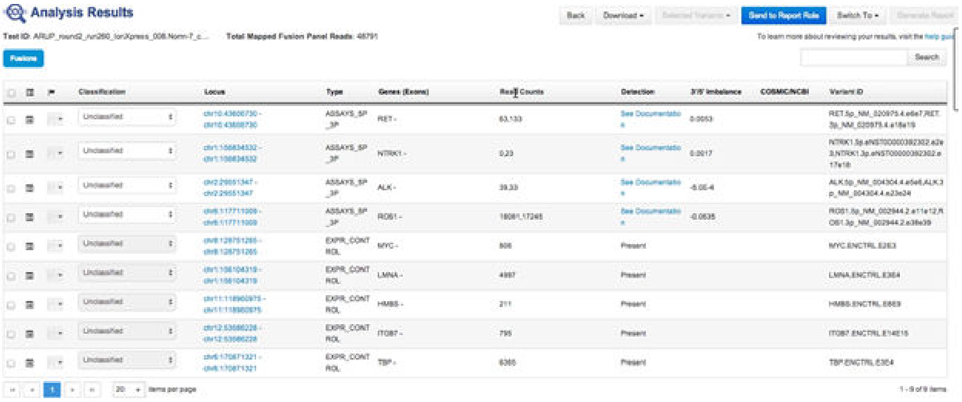
- You can change the order of the color scheme used in the chart with the **Color Scale** menu.

  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.



Test ID: ARUP_round2_run260_IonXpress_008.Norm-7_c... Total Mapped Fusion Panel Reads: 48791

To learn more about reviewing your results, visit the help guid

Classification	Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
Unclassified	chr10:43608730 - chr10:43608730	ASSAYS_5P_3P	RET -	63,133	See Documentation	0.0053		RET.5p_NM_020975.4.e6e7.RET.3p_NM_020975.4.e18e19
Unclassified	chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1 -	0.23	See Documentation	0.0017		NTRK1.5p.eNST00000392302.e2e3.NTRK1.3p.eNST00000392302.e17e18
Unclassified	chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK -	39.33	See Documentation	-6.0E-4		ALK.5p_NM_004304.4.e5e6.ALK.3p_NM_004304.4.e23e24
Unclassified	chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	18081,17245	See Documentation	-0.0635		ROS1.5p_NM_002944.2.e11e12.ROS1.3p_NM_002944.2.e38e39
Unclassified	chr8:128751265 - chr8:128751265	EXPR_CONTROL	MYC -	606	Present			MYC.ENCTRL.E2E3
Unclassified	chr1:156104319 - chr1:156104319	EXPR_CONTROL	LMNA -	4997	Present			LMNA.ENCTRL.E3E4
Unclassified	chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS -	211	Present			HMBS.ENCTRL.E8E9
Unclassified	chr12:53586228 - chr12:53586228	EXPR_CONTROL	ITGB7 -	795	Present			ITGB7.ENCTRL.E14E15
Unclassified	chr6:170871321 - chr6:170871321	EXPR_CONTROL	TBP -	6365	Present			TBP.ENCTRL.E3E4

1 - 9 of 9 items

Note: When you sort on the Gene column, you may believe you are getting inconsistent results. However, the sort is occurring on both of the genes involved in the fusion. The lexicographically smaller gene is used for an ascending sort, while the lexicographically larger gene is used for a descending sort.

Analysis configuration file for gene fusion analysis

An analysis configuration file is available to use to set individual assay-specific thresholds for these six properties, as applicable for the type of that individual assay:

- Minimum read count
- Minimum normalized read count
- Minimum wild type ratio
- Make calls
- Do not report
- Maximum read count negative
- Minimum Imbalance Score, for targets of type RNAExon Tile
- Minimum Imbalance p-value, for targets of type RNAExon Tile
- Minumum average read count for Exon Tiling QC, for targets of type RNAExon Tile

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 modify the file to set custom thresholds.

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 custom 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.
6. Save the file to a local directory, then open the file and make edits to any of the following properties:
   - Minimum read count
   - Minimum normalized read count
   - Minimum wild type ratio
   - Make calls
   - Do not report
   - Max read count negative
   - Minimum Imbalance Score, for targets of type RNAExon Tile
   - Minimum Imbalance p-value, for targets of type RNAExon Tile
   - Minumum average read count for Exon Tiling QC, for targets of type RNAExon Tile

   For more information, see "Editable parameters in the analysis configuration file" on page 450
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 drop-down 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.

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.

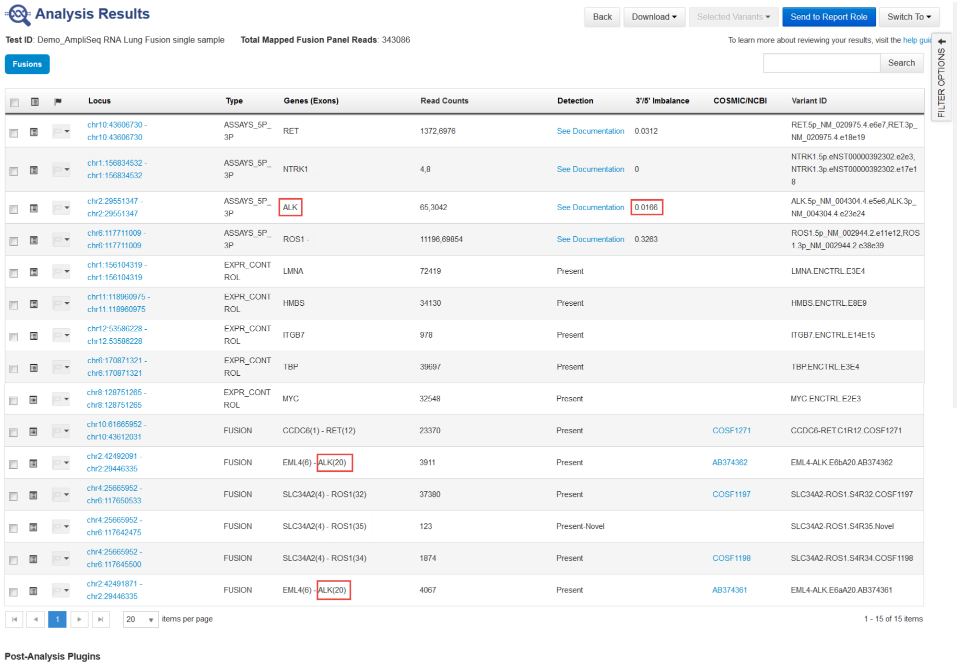
Data types for gene fusions analyses

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.

  Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

  To learn more about reviewing your results, visit the help guic

Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312		RET.5p_NM_020975.4.e6e7,RET.3p_NM_020975.4.e18e19
chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0		NTRK1.5p.eNST00000392302.e2e3, NTRK1.3p.eNST00000392302.e17e18
chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166		ALK.5p_NM_004304.4.e5e6,ALK.3p_NM_004304.4.e23e24
chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	11196,69854	See Documentation	0.3263		ROS1.5p_NM_002944.2.e11e12,ROS1.3p_NM_002944.2.e38e39
chr1:156104319 - chr1:156104319	EXPR_CONTROL	LMNA	72419	Present			LMNA.ENCTRL.E3E4
chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present			HMBS.ENCTRL.E8E9
chr12:53586228 - chr12:53586228	EXPR_CONTROL	ITGB7	978	Present			ITGB7.ENCTRL.E14E15
chr6:170871321 - chr6:170871321	EXPR_CONTROL	TBP	39697	Present			TBP.ENCTRL.E3E4
chr8:128751265 - chr8:128751265	EXPR_CONTROL	MYC	32548	Present			MYC.ENCTRL.E2E3
chr10:61665952 - chr10:43612031	FUSION	CCDC6(1) - RET(12)	23370	Present		COSF1271	CCDC6-RET.C1R12.COSF1271
chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present		AB374362	EML4-ALK.E6bA20.AB374362
chr4:25665952 - chr6:117650533	FUSION	SLC34A2(4) - ROS1(32)	37380	Present		COSF1197	SLC34A2-ROS1.S4R32.COSF1197
chr4:25665952 - chr6:117642475	FUSION	SLC34A2(4) - ROS1(35)	123	Present-Novel			SLC34A2-ROS1.S4R35.Novel
chr4:25665952 - chr6:117645500	FUSION	SLC34A2(4) - ROS1(34)	1874	Present		COSF1198	SLC34A2-ROS1.S4R34.COSF1198
chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present		AB374361	EML4-ALK.E6aA20.AB374361

  1 20 items per page 1 - 15 of 15 items

  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 455 and "Fusion calls in analysis results" on page 431.

- 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 455.

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

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.

Process Control Total Reads

Fusion detection methods

For some panels, Ion Reporter™ Software uses the targeted panel design with software algorithms to detect known and novel gene fusion isoforms. The assay uses the following methods to detect fusions.

Targeted 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 method

In the non-targeted fusion detection method, the panel primers are used to detect fusions between novel combinations of known diver 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.

Exon tiling method

The exon tiling method is a partner agnostic fusion detection method that enables the discovery of novel fusion isoforms and breakpoints. In this method, the primers are designed to target each exon-exon junction of every driver gene. Each driver gene in the test sample 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 (normal sample). 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.

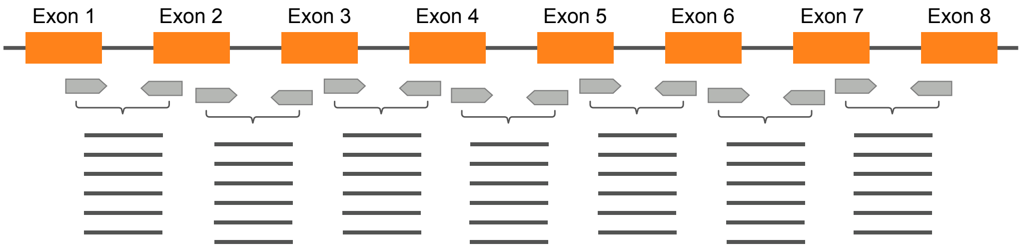


Figure 11 Representative primer design for an exon tiling assay

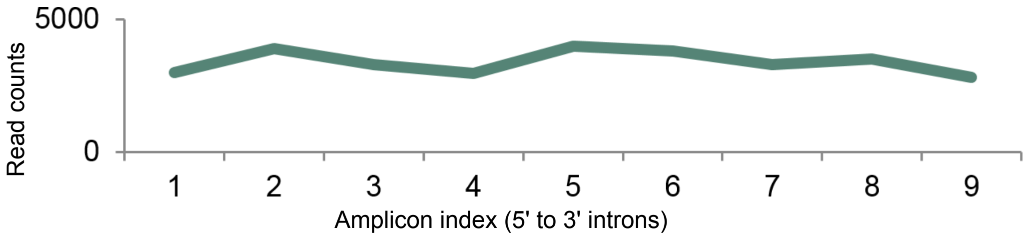


Figure 12 Example coverage profile for a sample with no fusion present

In this example, no fusion is present in the sample. The wild type transcript has uniform coverage of 3′ and 5′ introns.

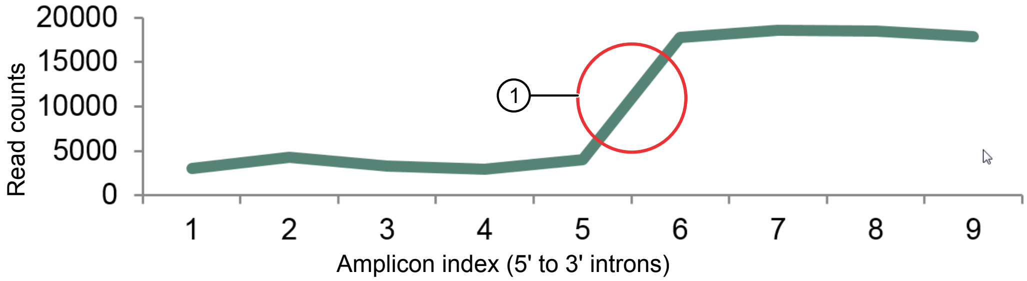


Figure 13 Example coverage profile for a sample with fusion present

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 for like 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.

Partner genes in the Ion AmpliSeq™ RNA Fusion panels

This panel contains the following four acceptor (driver) genes (in addition to many donor genes):

- ALK
- NTRK1
- RET
- ROS1
  In analyses that are based on this panel, fusion calls report other genes targeted in this panel that fused to these partner genes.

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	EGFRvlll	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		

B

Ion Reporter™ Software web services API

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 a JSON response object with status and message. The response object can be converted to a JSON object.
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://<your server name>/api/v1/
isAnalysisRunning -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d "userId=100"
```

Check if a sample exists

isSampleExists	
URL	`/api/v1/isSampleExists`
Description	IonReporterUploader plugin checks whether there is an existing sample that uses the sample that is given in the call.
Method	`POST`
Request query parameters	`sampleName` String. *Mandatory*. Valid sample name.
Example request	`https://ionreporter.thermofisher.com/api/v1/isSampleExists`
Request headers	`Content-Type`:application/x-www-form-urlencoded `Authorization:API Token`
Returns	`true` if sample with given name already exists in the organization given the API token for the user in headers. `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://<your server name>/api/v1/
isSampleExists -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d
"sampleName=Poo_C03-740_DNA_009_think3_IRUCLI"
```

Download a BAM file

downloadBAM	
URL	`/api/v1/downloadBAM`
Description	Download a BAM file for a given sample or analysis. This procedure applies to Ion Reporter™ Server only.
Method	`GET`
Request query parameters	• `filePath` String. *Mandatory*. Filepath of bam file got using / getAssociatedBamfiles AP. • `type` String. *Optional*. Valid values are "pdf" or "" (by default creates a ZIP file and downloads it.)

Example request	`https://ir-dev.ionreporter.thermofisher.com:80/api/v1/downloadBAM?`
	`filePath=/shared/data/ShazIndia-Labs/data/IRU_Uploads/`
	`20180302_01_06_30/v1/CFTR_15KB_deletion/`
	`1_AmpCFTR_contig7_15KBDeletions.bam"`

downloadBAM	
Request headers	`Content-Type`:application/x-www-form-urlencoded `Authorization:API Token`
Returns	Downloads the given BAM file. User can modify the BAM file name in curl command instead of "download.bam".

Returns

```
(INBL2-0QFG8WP:bamDownLaod_test dasd$ curl -0 download.bam _L -k _X
GET "https://juicebox.itw:443/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:
ZWJjODU2ODIwYWY40Tk2Yjg2MzI5NTU1MzIxZTA0NGVlYTZlnjIyYTJllGJlNDMwMGI3oWM
4ZGNhZTNlNzg1Mw" -H "Connection: close"
                     % Total % Received %Xferd Average Speed Time Time
Current 100 43.2M 100 43.2M 0 0 186k 0 0:03:58 0:03:58 --:--:-- 242k
[INBL2-0QFG8WP:bamDownLoad_test dasd$ ls -lrt total 88608 -rw-r-r 1
dasd 1153344739 45363244 Mar 29 21:09 download.bam
INBL2-0QFG8WP:bamDownLoad_test dasd$
```

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://<your 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:
ZWJjODU2ODIwYWY4OTk2Yjg2MzI5NTU1MzIxZTA0NGVlYTZlNjIyYTJlMGJlNDM
wMGI3OWM4ZGNhZTNlNzg1Mw" -H "Connection: close"
```

Download a file

download	
URL	`/api/v1/download`
Description	Gets the file, creates a compressed archive file in ZIP format and downloads it.
Method	`GET`

download	
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://ionreporter.thermofisher.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://<your 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:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Download QC and final reports

To retrieve one Quality Control (QC) report in JSON format, enter:

```
curl --request GET -k -H
"Authorization:ZDMxNDUwZGYyNjM4NGRlZjY0NjAyNTc1YTE1ZjU2MzFkNjVm
YzQxYjJhOTQwNTYyYWM3OTJmZDY2YTU5NGQwNA"  -O "https://<your
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 will resemble this:

```
[
    {
        "reports": {
            "qc": {
                "link": "https://<your 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://<your 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://<your 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 --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFm
Zjk3NjExNDA0ODVmYjYwMjA4YzQ5MGEwNDEzZg" https://
swanseaone.itw/api/v1/qcreport?format=json
```

To retrieve one final report in JSON format, enter:

```
curl --request GET -k -H
"Authorization:YTJjNGVmYWYyNDcxNTgyMmU3NzMxZmY0OWVjY2ZhYTA0ZDM2
YTk1OTQ4ZjUxZDlmMzFkZjFjM2UwNzZhNjliMg" "https://
teemo.itw/api/v1/finalreport?
format=json&name=testDNAFusion_c255_1421470231458"
```

To retrieve all of the final reports in JSON format, enter:

```
curl --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFm
Zjk3NjExNDA0ODVmYjYwMjA4YzQ5MGEwNDEzZg" https://
swanseaone.itw/api/v1/finalreport?format=json
```

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`

analysis	
Request query parameters	• `format` String, *Optional*. Response type, current version supports only json. • `type`. String. *Optional*. Valid values are "sample" or "analysis". • `name` String. *Conditional*. If **type** parameter is "sample" then **name** is Mandatory. • `id` String. *Optional*. Id of an analysis • `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 "".

Example request	`https://ionreporter.thermofisher.com/api/v1/analysis?`
	`format=json&name=xyzsampletest`
	`&exclude=reports,filteredvariants,unfilteredvariants&start_date=2016-01`
	`-01`
	`&end_date=2016-02-01`

analysis	
Request headers	`Content-Type`:application/x-www-form-urlencoded `Authorization:API Token`
Returns	

```
[

  {
    "reports": {
      "qc": {
        "link": "http://dev.ionreporter.thermofisher.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://
dev.ionreporter.thermofisher.com/api/v1/download?
                            filePath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/
                            Demo_AmpliSeq_Exome_VCF/
                            Demo_AmpliSeq_Exome_VCF_20160411134952720/
                            Demo_AmpliSeq_Exome_VCF_1460407585676.zip",
      "unfiltered_variants": "http://
dev.ionreporter.thermofisher.com/api/v1/download?
                            filePath=/data/IR/data/IR_Org/
ion.reporter@lifetech.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": "5.2",
    "started_by": "Ion User",
    "start_date": "April 11, 2016",
    "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 "http://<your server name>/api/v1/analysis?
format=json&name=xyzsampletest
&exclude=reports,filteredvariants,unfilteredvariants&start_date
=2016-01-01
```

```
&end_date=2016-02-01" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4GM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Download all analyses with one call

To retrieve all analyses in JSON format:

```
curl --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFm
Zjk3NjExNDA0ODVmYjYwMjA4YzQ5MGEwNDEzZg" https://
swanseaone.itw/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:ZTRhNWYyNjkzZjhlYjMxMTdmM2FkZGM1ZmQxYmRkNzU2ZTIx
ODk5OGRjZWM5ZDY2MjMzMTBiOGIwZWE5NmNlNg" "https://
baseline.itw/api/v1/analysis?
format=json&name=IR50_Rc10_Ane_BC18_DeganCases_1014_np"
```

The output is in JSON format:

```
[
    {
        "reports": {
            "qc": {
                "link": "https://baseline.itw/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://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___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://baseline.itw/api/v1/download?filepath=/data/IR/data/IR_Org/download/pdf/98ccb759-010f-4c92-acba-2da2d8aa9f4d/ff8081815067ca42015068f99193000d_QC.pdf"
- 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"
- filtered_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___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 --request GET -k -H
"Authorization:ZTRhNWYyNjkzZjhlYjMxMTdmM2FkZGM1ZmQxYmRkNzU2ZTIx
ODk5OGRjZWM5ZDY2MjMzMTBiOGIwZWE5NmNlNg" -O "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___4f776c35-4a9f-4a0f-
a787-6ee28ebb8c7c.zip"
```

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`

getAssociatedBamfiles	
Request query parameters	• `name` String, *Mandatory*. Single/multiple names of either sample or analysis. • `type` String. *Conditional*. If the type parameter is not provided, name is considered as analysis name, For sample name(s), type is mandatory (For example, `type=sample`).

Sample request	`https://dev.ionreporter.thermofisher.com/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`

Analysis request	`http://dev.ionreporter.thermofisher.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`

getAssociatedBamfiles	
Returns	Sample Response

```
[

    {
       "sample": "1194954B_Bladder_ManLib",
        "bam_links": [
            "http://dev.ionreporter.thermofisher.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://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/Zyklus/data/IRU_Uploads/
2017-12-29_1_51_48/v1/e5272-wfa-l165_v3/rawlib.bam",
            "http://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/Zyklus/data/IRU_Uploads/
20171229_14_54_21/v1/sample_test16/1_IonDx-9_rawlib12.bam"
        ]
    },
    {
        "sample": "Demo AmpliSeq Exome VCF",
        "bam_links": [
            "Sample or Bam file could not be found for the specified
name."
        ]
    }
]
```

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://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/demodata/PGM/
AmpliSeq_IDP/AmpliSeq_IDP_daughter.bam"
                ],
                "processedBam": [
                    "http://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
uattestsskadmuser@gmail.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_M
other/Demo_AmpliSeq_IDP_Daughter_20180301150555171/outputs/
VariantCallerActorProband-00/merged.bam.ptrim.bam"
                ]
            },
            {
                "sampleName": "Demo AmpliSeq IDP Father",
                "sampleRole": "father",
                "inputBam": [
                    "http://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/demodata/PGM/
AmpliSeq_IDP/AmpliSeq_IDP_father.bam"
                ],
                "processedBam": [
                    "http://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
uattestsskadmuser@gmail.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_M
```

getAssociatedBamfiles

Returns

```
other/Demo_AmpliSeq_IDP_Daughter_20180301150555171/outputs/
VariantCallerActorFather-00/merged.bam.ptrim.bam"
                ]
            },
            {
                "sampleName": "Demo AmpliSeq IDP Mother",
                "sampleRole": "mother",
                "inputBam": [
                    "http://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/demodata/PGM/
AmpliSeq_IDP/AmpliSeq_IDP_mother.bam"
                ],
                "processedBam": [
                    "http://dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
uattestsskadmuser@gmail.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_M
other/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://<your 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:ODA5YTVlZDcyNjkyNDg5Mjg2YmZiNjI3ODExZWFmM2I3Nzhk
Nzg0MDU5M2U
0YmIxOGU2YjQ2OTRmYmIxZjAxNw"
```

```
curl -X GET -k "http://<your 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:ODA5YTVlZDcyNjkyNDg5Mjg2YmZiNjI3ODExZWFmM2I3Nzhk
Nzg0MDU5M2U0YmIxOGU2YjQ2OTRmYmIxZjAxNw
```

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://ionreporter.thermofisher.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://<your server name>/api/v1/
getAvailableCancerType -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

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://ionreporter.thermofisher.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://<your server name>/api/v1/
getAvailableStorageSpace -H "Content-Type:application/x-www-
form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get current results TSV file

getcurrentResultTsv	
URL	`api/v1/currentResultTsv?parameters`
Description	Returns the Current Results TSV file path in the same column order as shown in the analysis results in Ion Reporter™ Software.
Method	`GET`

getcurrentResultTsv	
Request parameters	- `format`. String. *Optional*. Response type, current version supports only json - `type` String, *Optional*. Valid values are "sample" or "analysis". - `name` String, *Conditional*. If `type` parameter is "sample" then **name** is Mandatory. - `format=json` - `name=NAME OF THE ANALYSIS` 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://dev.ionreporter.thermofisher.com/api/v1/currentResultTsv?format=json&name=xyzsampletest`
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://localhost:443/api/v1/
download?filePath=/data/IR/data/IR_Org/ion.reporter@lifetech.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.t
sv"
        },
        "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": "5.10",
        "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://your server name/api/v1/
currentResultTsv?format=json&name=BRCA_Plus_RR_jul29" -H
"Content-Type:application/x-www-form-urlencoded" -H "A
```

Get data upload path with authentication

getDataUploadPathWithAuth	
URL	`/api/v1/getDataUploadPathWithAuth`
Description	Used by the IonReporterUploader plugin to return the current data upload path 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://ionreporter.thermofisher.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=NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5N
zVjODU3YjdlMWM4Mjk3OGYzODE4Yw" --header
"Content-Type: application/x-www-form-urlencoded" -k https://
<your 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`

finalreport	
Request query parameters	• `format`. String. *Optional*. Response type, current version supports only json • `type` String, *Optional*. Valid values are "sample" or "analysis". • `name` String, *Conditional*. If `type` parameter is "sample" then **name** is Mandatory. • `id` String, *Optional*. Id of an Analysis by default. If `type` is sample, this corresponds to the sample id. • `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://ionreporter.thermofisher.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`

finalreport	
Authorization	API Token
Returns	

```
[
  {
    "summary": "",
    "final_report_template": "Default Final Report Template",
    "report_pdf": "http://ionreporter.thermofisher.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://ionreporter.thermofisher.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://<your server name>/api/v1/
finalreport?
format=json&name=BRCA_Plus_RR_jul29&type=analysis&start_date=20
16-07-25&end_date=2016-07-31" -H "Content-Type:application/x-
www-form-urlencoded" -H
```

```
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

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	• `format`. String. *Optional*. Response type, current version supports only json. • `type` String, *Optional*. Valid values are "sample" or "analysis". • `name` String, *Conditional*. If type parameter is "sample" then name is Mandatory. • `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://ionreporter.thermofisher.com/api/v1/qcreport?`
	`fomat=json&type=analysis&`
	`name=Demo AmpliSeq Exome`
	`VCF_1460407585676&start_date=2016-01-30&end_date=2016-04-12`

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": "5.2",
    "samples": {
      "PROBAND": "Demo AmpliSeq Exome VCF"
    },
    "qc_report_pdf": "http://ionreporter.thermofisher.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://ionreporter.thermofisher.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_2016-04-12 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://<your server name>/api/v1/qcreport?
format=json&name=BRCA_Plus_RR_jul29&type=analysis&start_date=20
16-07-25&end_date=2016-07-31" -H "Content-Type:application/x-
www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

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://ionreporter.thermofisher.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://<your 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://ionreporter.thermofisher.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://<your server name>/api/v1/
getUserDetailsWithAuth -H "Content-Type:application/x-www-form-
urlencoded" -d
"auth=NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5N
zVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

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`

getvcf	
Request query parameters	• `format` String. *Optional*. Response type, current version supports only json. • `type` String. *Optional*. Valid values are "sample" or "analysis". • `name` String. *Conditional*. If type parameter is "sample" then name is *Mandatory*. • `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`

Request headers	`Content-Type:`application/x-www-form-urlencoded `Authorization:API Token`
Returns	

```
[
  {
    "data_links": "http://ionreporter.thermofisher.com/api/v1/download?
      filePath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/
JohnSmithSample
      /JohnSmithSample_20160429014705727/
JohnSmithSample_c150_2016-04-29-14-16-534.zip",
    "name": "JohnSmithSample_c150_2016-04-29-14-16-534",
    "id": "ff808181545d90790154613336be0008"
  }
]
```

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://<your server name>/api/v1/getvcf?
format=json&name=BRCA_Plus_RR_jul29&start_date=2016-07-25&end_d
ate=2016-07-31" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

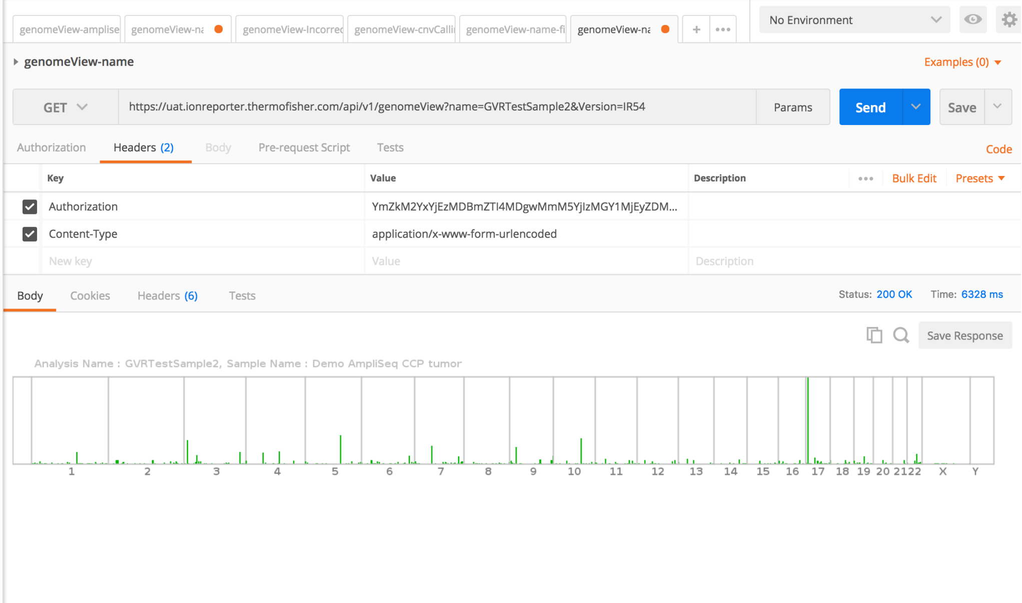
Get whole genome view PNG

genomeView	
URL	`/api/v1/genomeView`
Description	Gets the whole genome view PNG image file of the analysis.
Method	`GET`

genomeView	
Request query parameters	• `id` String. *Optional*. Id of an analysis. If the `name` parameter is empty, then `id` is *Mandatory*. • `name` String. *Optional*. Name of an analysis. If the `id` parameter is empty, then `name` is *Mandatory*. – If both `id` and `name` are given as input, then the genome-view PNG will be displayed for `id`, and `name` won't 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] • `version` String. *Conditional*. 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://ionreporter.thermofisher.com/api/v1/genomeView?id=ff8081815ba8eac4015ba8ead9ce005d`
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.



genomeView-name

GET https://uat.ionreporter.thermofisher.com/api/v1/genomeView?name=GVRTestSample2&Version=IR54

	Key	Value	Description
☑	Authorization	YmZkM2YxYjEzMDBmZTI4MDgwMmM5YjIzMGY1MjEyZDM...	
☑	Content-Type	application/x-www-form-urlencoded	
	New key	Value	Description

Status: 200 OK Time: 6328 ms

Analysis Name : GVRTestSample2, Sample Name : Demo AmpliSeq CCP tumor

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://
uat.ionreporter.thermofisher.com/api/v1/genomeView?
name=GVRTestSample2&Version=IR54"-H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:YmZkM2YxYjEzMDBmZTI4MDgwMmM5YjIzMGY1MjEyZDMyMGI3
ZTU5MmQyZGY4ZmIxZWI5M2ViYzIxZWNhOWU3NQ"
```

Get workflow names

getWorkflowNamesWithApplicationTypeWithAuth	
URL	`/api/v1/getWorkflowNamesWithApplicationTypeWithAuth`
Description	Used by IonReporterUploader plugin to get a list of workflows that are tagged with IRU for the given version. Returns the list of 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`: IR52
Returns	

```
[
  {
    "ApplicationType": "Amplicon Sequencing",
    "Workflow": "RK_GSTT1_GRCh38_APR06",
    "irVersion": "52",
    "tag_isFactoryProvidedWorkflow": "false",
    "irReference": "GRCh38"
  }
]
```

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://<your server name>/api/v1/
getWorkflowNamesWithApplicationTypeWithAuth -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -H "Version:52"
```

Start an analysis

startAnalysis	
URL	`/api/v1/startAnalysis`
Description	Used by the IonReporterUploader plugin to start an analysis for the given `setId`. 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 IRU. • `containerName` String. *Mandatory*. User-specified unique name for the analysis that will show up on the Ion Reporter™ Software user interface.
Example request	`https://ionreporter.thermofisher.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://<your server name>/api/v1/
startAnalysis -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -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 analyses. Returns a JSON file with status and message.
Method	`POST`
Request query parameters	`userId` String. *Mandatory*. UserId
Example request	`https://ionreporter.thermofisher.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://<your server name>/api/v1/
unsharedAnalyses" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
```

```
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d
"userId=ff8081815547ceb401554d62c0ec0493"
```

View=summary

To get a list of all analyses that a user has access to without the links to the actual analyses, enter:

```
view=summary
```

Example:

```
webservices_42/rest/api/analysis?format=json&view=summary
```

This approach saves time over the original calling API.

C

Programming guidelines for Ion Reporter™ Software plugins

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.

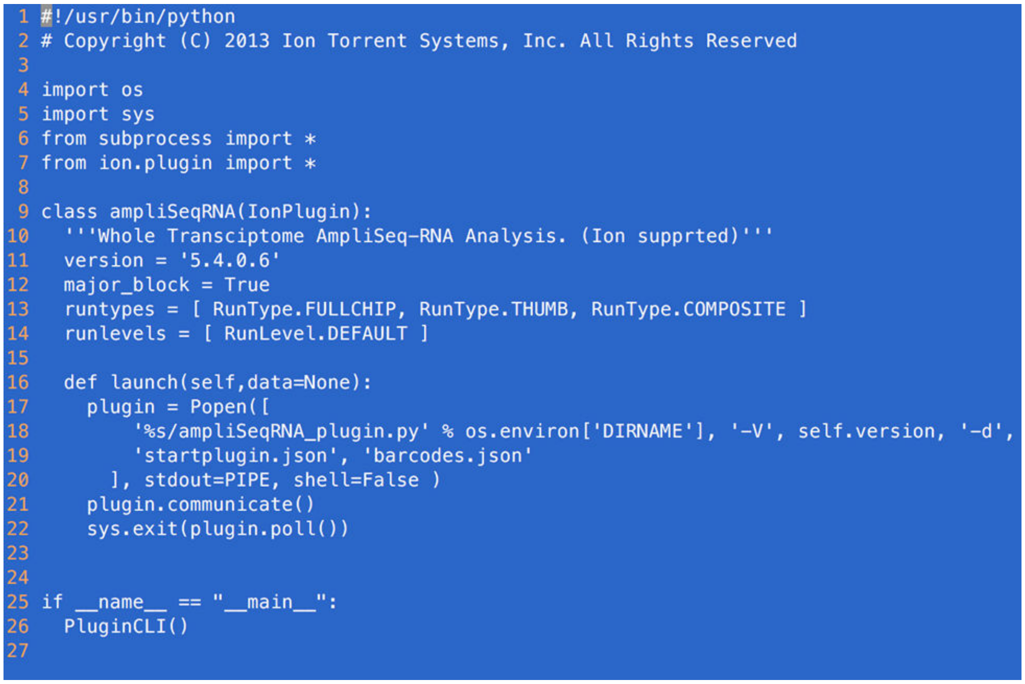
The Python class that inherits from the IonPlugin base class should call the main as shown in the sample code in lines 9 and 10.

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

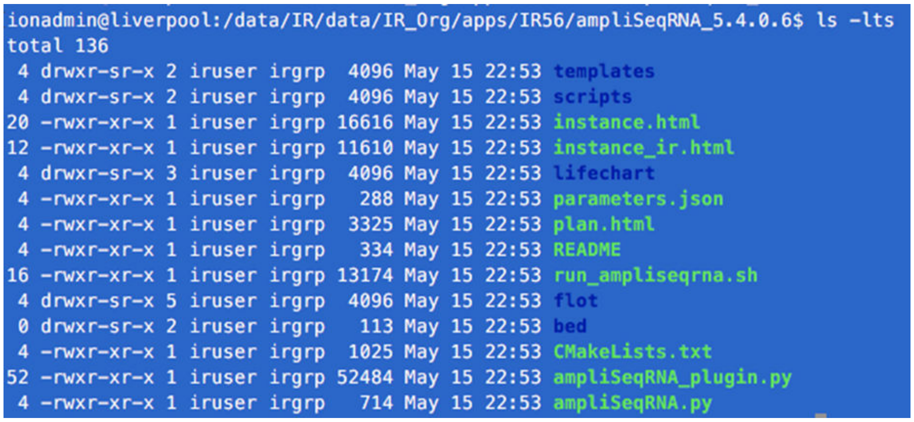
-
- IR plugin framework has some validations against how the zip file should be named.
- After compressing the plugin contents into the zip, the zip name should be given as <plugin_name>_<version>.zip.
- For example: From the above code snippet, the plugin zip file name should look like **MyIonPlugin_5.4.0.1.zip.**

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. Please refer the instance_ir.html in ampliSeqRNA plugin for more info.
   - **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

The information in this appendix applies to customers who use the Oncomine™ analysis workflows in Ion Reporter™ Software. The information 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"

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number exon deletion	Loss-of-Function	ExonDeletion	• SVTYPE = "CNV" • FILTER = "LOSS" • SUBTYPE = "BigDel"
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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

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
RNA exon variant	Gain-of-Function	RNAExonVariant	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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

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
Promoter hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined promoter hotspot list
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

Oncomine™ Colon cfDNA Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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	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™ Comprehensive 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 function
Gene fusion	Gain-of-Function	Fusion	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
RNA exon variant	Gain-of-Function	RNAExonVariant	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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	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
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

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 function

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
RNA exon variant	Gain-of-Function	RNAExonVariant	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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	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
Promoter hotspot mutations	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined promoter hotspot list
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

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

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
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
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	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list
MNV hotspot mutation	Gain-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

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
RNA exon variant	Gain-of-Function	RNAExonVariant	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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	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
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

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
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

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Synonymous hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Functional impact is synonymous • Transcript and coding syntax occur in pre-defined synonymous hotspot list
Intronic hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list

Oncomine™ Myeloid Research Assay

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
Loss-of-function mutation	Loss-of-Function	Deleterious	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Variant occurs in a designated 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
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
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
FLT3 internal tandem duplication	Gain-of-Function	Hotspot	• Positive mutation call • Functional impact is a non-frameshift insertion in exon 14/15 of FLT3 or SVTYPE = "FLT3ITD"

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
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Variant's 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, location, and coding syntax 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

Custom Ion AmpliSeq™ HD 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

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
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
Loss-of-function mutation	Loss-of-Function Unclassified	Deleterious	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a designated 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
Truncating hotspot mutation	Gain-of-Function Unclassified	Hotspot	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating 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
Promoter hotspot mutation	Gain-of-Function Loss-of-Function Unclassified	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined promoter hotspot list
Intronic hotspot mutation	Gain-of-Function Loss-of-Function Unclassified	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
MNV hotspot mutation	Gain-of-Function Loss-of-Function Unclassified	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

Oncomine™ Tumor Mutation Load Assay

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Loss-of-function mutation	Loss-of-Function	Deleterious	• 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	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
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

Documentation and support

Related documentation

Document	Publication number	Description
Torrent Suite™ Software 5.12 Help	MAN0017973	Describes the Torrent Suite™ Software and provides procedures for common tasks.
Torrent Suite™ Software 5.12 User Guide	MAN0017972	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.
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	MAN0014890	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™ cfDNA Assay.

Document	Publication number	Description
Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide	MAN0016158	Describes how to use Ion Reporter™ Software for data analysis of the Ion ReproSeq™ assay.
Oncomine™ Reporter 3.0 User Guide	MAN0017300	Describes how to generate reports in Oncomine™ Reporter Software.
Oncomine™ Reporter User Guide	MAN0016808	Describes how to generate reports in Oncomine™ Reporter Software.

Library preparation documentation

- *Ion AmpliSeq™ Library Kit 2.0 User Guide*, (Pub. No. MAN0006735)
- *Ion AmpliSeq™ HD Library Kit User Guide*, (Pub. No. MAN0017392)

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 **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=2**.
- *Reproductive genomics and inherited disease research*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=3**.
- *Rapid sequencing of microorganisms*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=4**.

Customer and technical support

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  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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. A SAM file (.sam) 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 a 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 target 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.
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 regions in the gene that typically contain variants and enables Ion Reporter™ Software to identify if a specific variant is present or absent. See also *target regions file*.
hotspots file	A BED or a VCF file that defines regions in the gene that typically contain variants. Specifying a hotspots file to use in a run enables Torrent Variant Caller to identify if a specific variant is present or absent. A hotspot file instructs the Torrent Variant Caller to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspot file affects only the variantCaller plugin, not other parts of the analysis pipeline. If you don't specify a hotspots file, the software tells only the difference between your sequence and the reference genome.
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	Acronym for median absolute pairwise difference. Assuming that adjacent amplicons in the genome most likely have the same underlying copy number in a sample, the difference between the log2(read count ratio) values against the reference baseline for all adjacent amplicons contains information for the noise level of the data. The median of the absolute values of all such difference in log2(read count ratio) is the measure for how informative the results fare for copy number estimates.
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 acrynym 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.
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	A BED file that specifies all of the regions that a panel represents such as the amplified regions that are used with target sequencing. The complete software analysis pipeline, including plugins, is restricted to only these specified regions instead of analyzing the entire reference library.
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.
TMB	Tumor mutation burden. The number of nonsynonymous somatic SNVs ("missense" and "nonsense" SNVs) and nonsynonymous somatic INDELs ("frameshift" and "non-frameshift" insertions and deletions) per megabase of the tumor genome.
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 only the differences between the BAM file and the reference file.
VCIB	Variability Correction Information 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.
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.

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