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Torrent Suite™ Software 5.22

USER GUIDE

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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1000552 A (English)

Revision	Date	Description
A	17 April 2025	New document for *Torrent Suite™ Software 5.22 User Guide*

Contents

1 Introduction to Torrent Suite™ Software

Torrent Suite™ Software product information

Torrent Suite™ Software is used to plan and monitor runs, view instrument and run statuses, and review data from runs on the Ion Chef™ Instrument and Ion GeneStudio™ S5 Series sequencers, which include the Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, and Ion GeneStudio™ S5 Prime System.

IMPORTANT! Torrent Suite™ Software 5.18 is the last software release that can be used with Ion S5™ XL System and Ion S5™ System.

Torrent Suite™ Software 5.14 is the last software release that can be used with an Ion PGM™ System or Ion Proton™ System.

Torrent Suite™ Software and the sequencing instruments provide customizable and automated sample analysis and data storage solutions. You can plan, monitor, and track sequencing runs in the **Dashboard**, and reviewing the quality and accuracy of runs. In addition, you can perform variant calling and primary analysis including detection of SNPs, INDELs, CNVs and fusions.

Operating system compatibility

Torrent Suite™ Software 5.22 is compatible with only an Ion Torrent™ Server that uses the Ubuntu™ operating system, version 22.04.

Browser compatibility

Torrent Suite™ Software can be operated on all modern web browsers such as Microsoft™ Internet Explorer™, Microsoft™ Edge, Google™ Chrome™, Safari, and Firefox™.

Hardware compatibility

Torrent Suite™ Software 5.22 is supported for use with the following instruments.

- Ion GeneStudio™ S5 Series sequencers
- Ion Chef™ Instrument

IMPORTANT! Torrent Suite™ Software 5.18 is the last software release that can be used with Ion S5™ XL System and Ion S5™ System.

Torrent Suite™ Software 5.14 is the last software release that can be used with an Ion PGM™ System or Ion Proton™ System.

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 recommendations for Torrent Suite™ Software

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. Passwords must be reset at first sign in to the product. Change passwords according to the password policy of your organization.

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

The software checks a password when it is created to verify that the password meets requirements for password security. If requirements are not met, a message is shown with details about how to create a stronger password.

For more information, see these topics.

- "Register for a new user account" on page 13
- "Sign in to Torrent Suite™ Software" on page 13
- "Change your password for Torrent Suite™ Software" on page 14

2 Get started with Torrent Suite™ Software

Register for a new user account

Before you can sign in to Torrent Suite™ Software for the first time, you request a new user account. The request is sent to the administrator for approval. An account is not active until approval is granted.

1. On the sign in screen for Torrent Suite™ Software, click **Register**.
2. Enter the new user information, then click **Submit**.
   The administrator approves and creates the new account, and makes your user name available on the software sign in screen.
3. On the software sign in screen, select your user name from the list, enter your password, then click **Sign In**.

Sign in to Torrent Suite™ Software

After you register for a new user account, you can sign in to Torrent Suite™ Software.

You are allowed 3 failed attempts to sign in, after which you are temporarily locked out. After a few minutes, you can try again. If sign in continues to fail, you must change your password.

1. On the software sign in screen, select your user name from the list, then enter your password.
2. Click **Sign In**.

Change your password for Torrent Suite™ Software

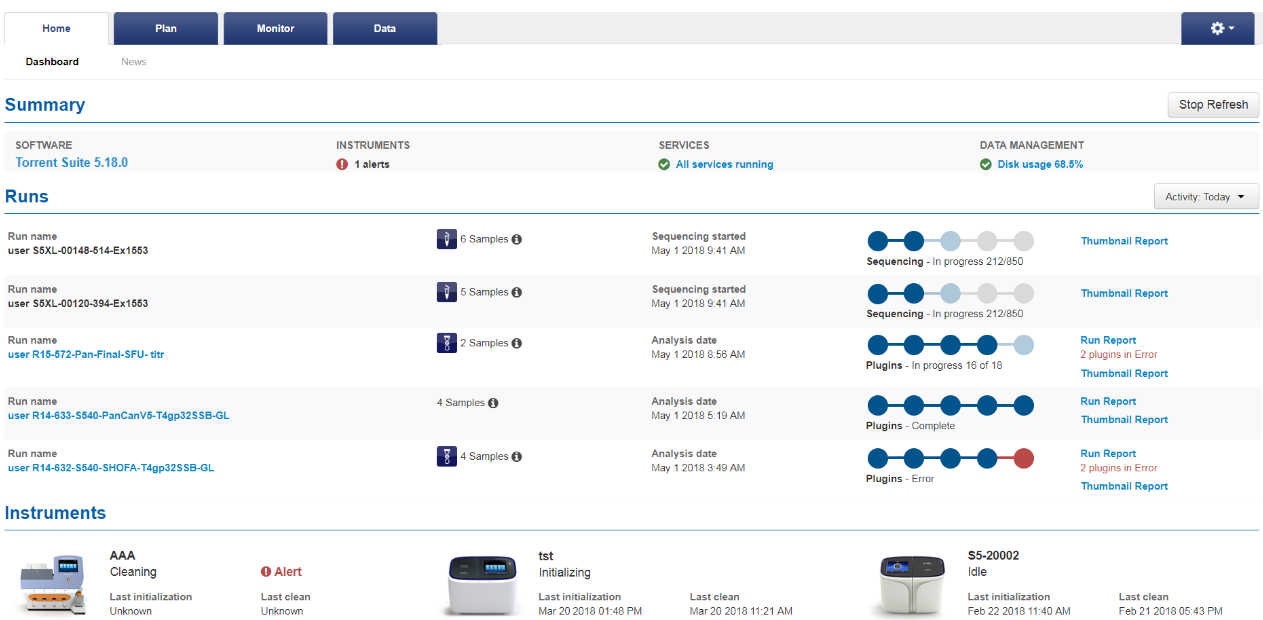
Users are required to change their password periodically based on company policies.

All users are required to change their password after a software upgrade from Torrent Suite™ Software 5.18 to 5.22.

1. Click **(Settings) ▸ Accounts**
2. In the **User Profile/Account Information** section, enter your old and new password, then confirm the new password.
3. Click **Save**
   The password is modified.
4. On the software sign in screen, select your user name from the list, enter your password, then click **Sign In**.

Dashboard at a glance

Use the Torrent Suite™ Software **Dashboard** on the **Home** tab to see active and recent sequencing runs. The **Dashboard** provides an overview of activity on all connected instruments. To view the **Dashboard**, click the **Home** tab, then click **Dashboard**.



The **Summary** section includes the following information.

- The **Software** column shows information and messages about the software. The software version link opens the **Releases** screen, where you can click **Update** to update your software when new versions are released, and scroll down to a list of links that contain support information. A message is shown with a link to change the password, if the password for the user account does not meet the recommended security policy.
- The **Instruments** column shows the number of instruments that are connected to the system or are offline, and those that have alerts.
- **Services** links—Report statuses, such as services that are running, nodes that are down, and Redundant Array of Independent Disks (RAID) storage statuses. Click a link to see details on the **Services** screen.
- **Data Management** links—Reports disk usage and data management activity. Click a link to see details on the **Data Management** screen.
- **Stop Refresh** stops the automatic refresh function, which occurs every 30 seconds by default. Stop the auto refresh function to review the screen without interruption from the refresh cycle.

The **Runs** section shows recent information from the **Data**, **Plan**, and **Monitor** tabs. However, it is only a subset of the recent information. The **Runs** section includes the following information.

- **Run Name link**—Takes you to the name of the run or the run report.

  The link is not functional until the run report is completed.
- **Samples** information icon—Shows names of and information about barcodes for samples.
- The third column shows the stage of the run, with the date that the run information was either last updated or completed.
- The fourth column shows the progress of instrument runs and plugin use. Blue circles indicate normal progress and red circles indicate instrument or plugin errors.
- The fifth column provides links to thumbnail reports and run reports. If an error appears in red under a run report, see "Troubleshoot Torrent Suite™ Software" on page 339 or contact Technical Support.
  - The **Run Report** links to completed run reports for the time interval selected. The link becomes active when the run report is complete.
  - The **Thumbnail Report** is an early run report that helps you determine the quality of a run that is in progress.
- The **Activity** list allows you to select a time interval for viewing instrument activity. Choices include **Last Hour**, **Today**, **Last 24 Hours**, and **Last 7 days**.

The **Instruments** section shows the instruments that are connected to the system and their operational status.

- **Connected**—Instruments that are connected but status is unknown.
- **Analyzing**—Instruments that are currently in use.
- **Cleaning**—Instruments that are currently undergoing a cleaning process.
- **Idle**—Instruments that are connected, but not currently in use.
- **Offline**—Instruments that are no longer connected. These instruments are shown when run reports for runs on these instruments exist in the software.

If an instrument has an operational error, place the pointer over the **Alert** to see details. Then, resolve the issue on the instrument.

News screen

Use the **News** screen on the **Home** tab to see Thermo Fisher Scientific announcements about new products, software releases, and other news. To view the **News** screen, click the **Home** tab, then click **News**.

Plan a run

The following steps describe how to use Planned Run templates and Planned Runs that fit into your sequencing workflows.

1. Decide on your sequencing application and sequencing product (such as an Ion AmpliSeq™ custom panel).

2. Select a preinstalled Planned Run template with defaults for your application and sequencing product, or create a user-defined Planned Run template to customize your Planned Run.

3. Create new Planned Runs from Planned Run templates, adding the names of the samples to be sequenced.

   Use Planned Run templates and Planned Runs to enter run information through Torrent Suite™ Software instead of directly on the sequencing instrument. The use of Planned Run templates and Planned Runs reduces the chance of errors and wasted runs, reduces setup time on the sequencing instrument, and increases instrument throughput.

4. Start the run on the sequencer.

   On the sequencer, information for a Planned Run is applied to the current **Run Info** screen automatically, or by selecting the Planned Run from a list of Planned Runs. You can also overwrite (change) Planned Run information directly on the sequencer.

3 Samples and sample sets

Torrent Suite™ Software uses samples and sample sets that sequencing instruments use to process the genetic material during instrument runs.

About samples and sample sets

Torrent Suite™ Software uses samples and sample sets that sequencing instruments use during instrument runs.

Samples in Torrent Suite™ Software are the information and attributes that characterize the data. Samples contain information, or attributes, that sequencing instruments use to process the genetic material during instrument runs. You can preselect the attributes that you want to associate with each sample before you start to plan your templating and sequencing runs.

After sample sequencing is complete, software programs, such as Ion Reporter™ Software, use the sample information for data analyses. Sample information can be transferred to Ion Reporter™ Software from the sequencer through Torrent Suite™ Software with the IonReporterUploader plugin or imported as individual BAM or VCF files. When an Ion Reporter™ Software analysis workflow is used to analyze a sample, the output in Ion Reporter™ Software is a set of analysis results.

You can set up samples in the software in two ways. You can manually enter sample information for each sample or import sample information from a CSV file that contains the sample information.

You can organize samples into sample sets. A sample set contains sample information that you enter one time, then you can reuse the sample set in other Planned Runs. This helps reduce data entry time and errors.

Grouping samples into sample sets is also helpful when you use barcodes that have attributes that are assigned to each individual barcode. If you create sample sets before you plan the run, you can enter barcodes and the barcode attributes only once in the sample sets. Then you can select one or more sample sets to reuse these barcodes when you create a Planned Run.

You can search for and find samples in the software, add sample sets to your Planned Runs, and view details about how the sample libraries were prepared if the run uses an Ion AmpliSeq™ library preparation kit. To change the sample files, you can edit information in the samples, update sample sets, and delete samples and sample sets.

Create a sample set manually

You can create a sample set by manually entering sample information into Torrent Suite™ Software without the use of an external CSV file. Entering sample information manually is useful for creating small samples sets.

To create a sample set manually, enter individual samples into the software, then create a new sample set and add samples to it. Alternatively, you can add new samples to an existing sample set.

For sample sets that include numerous samples, you can import samples from a CSV file. For more information, see "Create a sample set by importing samples from a CSV file" on page 19.

1. In the **Plan** tab, click **Samples**, then click **Add or Update Sample Set/Samples**.
2. Click **Enter New Sample**.
3. Complete the **Add Sample** dialog box, then click **Done**.
   For information about defining the samples, see "Sample attributes" on page 24.
   New samples and sample attributes appear in the **Enter Samples** list.
4. *(Optional)* Click **Enter New Sample** to enter more samples.
5. Add the new sample or samples to a sample set in one of the following ways.
   - Click **Add to existing Sample Set**, then select an existing sample set to contain the samples.
   - Click **Create Sample Set**. For information about defining the sample set, see "Sample set definitions" on page 28.
6. Click **Save Sample Set**.
   The set name appears in the **Sample Sets** table.

You can use the sample set to create a Planned Run. The information from the sample set and the samples in the sample set are prepopulated in the Planned Run workflow bar steps and Planned Run template. For more information, see "Plan by sample set" on page 58.

Create a sample set by importing samples from a CSV file

If you have many samples, you can import new samples into Torrent Suite™ Software, or update existing samples, using a CSV file that contains sample information. If you do not yet have a samples file, you can create one from a samples CSV template that is available in the software. During this process, you can also create a new sample set for the new samples.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.

   If you do not yet have a samples file, create a new samples file from an available samples CSV template. For more information, see "Create a samples file from a template" on page 19.

2. Upload the samples file, and optionally add a new sample set to receive the samples.
   a. In the **Import Samples** section, click **Choose file**, navigate to the sample import file, then upload the sample import file.
   b. Select a sample set CSV file, then click **Open**.
   c. Select a sample set to receive the samples.
   d. *(Optional)* To add a new sample set to receive the samples, click **Add Sample Set**, then complete the sample set information.

      For information about how to define a sample set, see "Sample set definitions" on page 28.

3. Click **Save & Finish**.

   The software loads, parses, and validates the file. If no errors are found, samples and sample sets are saved. If an error occurs, correct the file and resubmit the file.

Create a samples file from a template

If you do not already have a samples file on your computer to import samples from a CSV file, you can download a samples template and then use it to create a samples file.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.

2. In step 1 of the **Import Samples** section, click **Sample File Format** to download a sample CSV file.

   The sample file format CSV contains the version of the CSV file in the top row, and sample attributes in separate columns.

3. To create a new CSV file, copy and paste the contents of your existing sample CSV file into the new file format.

4. Open the CSV template and enter sample information into the cells.

   For information on how to define the samples, see "Sample attributes" on page 24.

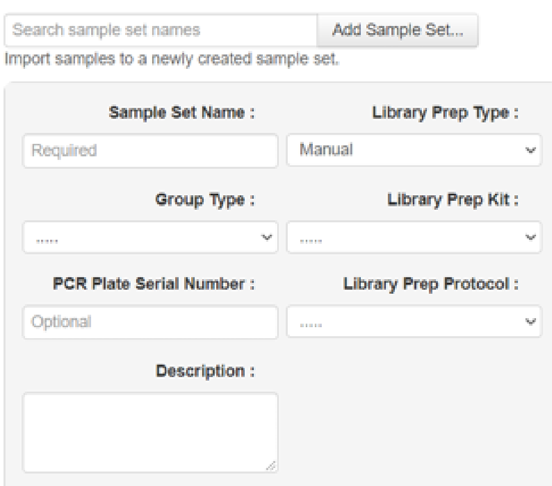
5. Save the file to your computer so that is available for use to create a sample set.

Create an Ion AmpliSeq™ on Ion Chef™ sample set

Samples that are in an Ion AmpliSeq™ on Ion Chef™ sample set can be tracked in Torrent Suite™ Software, from library preparation through sequencing and data analysis. First, create a sample set in the software, then use it to set up a library preparation run on the Ion Chef™ Instrument. Sample information is then transferred to the Planned Run when templating and sequencing the combined library.

To create a sample set of this type, you can either import samples from a CSV file, or enter them manually. The following procedure is an example of how to import samples from a CSV file.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.
2. In the **Import Samples** screen, click **Sample File Format**.
   A CSV template file downloads to your computer in the Downloads folder.
3. Open the CSV template file, then enter the sample names, PCR plate positions, and barcodes used. Save the file to your computer with a new name.
   You can also enter sample names in the CSV file, then supply the plate position, barcode, and other information later by editing the sample set in the **Sample Sets** screen. For more information, see "Edit a sample set" on page 30.
4. In the **Import Samples** screen, click **Choose File**, select your new CSV file, then click **Open**.
5. Click **Add Sample Set**.
6. Complete the **Add Sample Set** dialog box.



   a. Enter a **Sample Set Name**.
   b. Select the appropriate **Group Type**.
   c. Set **Library Prep Type** to **AmpliSeq on Chef**.
   d. Set **Library Prep Kit** to **Ion AmpliSeq Kit on Chef DL8**.

e. In **PCR Plate Serial Number**, type or scan the PCR plate serial number.

f. *(Optional)* In **Description**, enter more information.

g. *(Optional)* Select the **Library Prep Protocol**.

h. Click **Save & Finish**

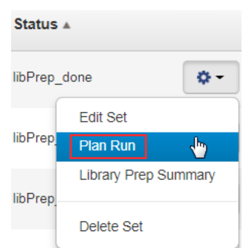
A new sample set is created.

7. In the **Plan** tab, click **Samples**.

The **Sample Sets** table lists the sample set run status in the **Status** column, indicating whether the sample set is ready for a library preparation run, a library preparation run with the sample set is currently running, or the combined library is ready for a template run.

Status	Indication
libPrep_pending	The new sample set is ready for a library preparation run.
libPrep_reserved	The sample set is currently running on an Ion Chef™ Instrument. You can monitor the run status at **Monitor ▸ Ion Chef**.
libPrep_done	The Ion Chef™ Instrument has finished the library preparation run, and the combined library is ready for a template run.

8. When the status of your sample set shows **libPrep_done**, in the row of the sample set, click **(Actions)**, then select **Plan Run**.



9. In the **Plan Run from Sample Set** dialog box, select an existing Planned Run template, or create a new template by selecting **Add new template**.
The sample information in the sample set automatically populates your new Planned Run.

For more information on how to create or edit a Planned Run template, see Chapter 4, “Plan and execute an instrument run”.

Sample information

When you add a sample to a sample set in Torrent Suite™ Software, you enter information to describe and identify each sample, with characteristics such as gender, sample type (RNA or DNA, for example), or relationship group. This sample information is referred to as sample attributes. You can add user-defined attributes to the sample attributes that are available in the software. Each attribute that you add appears in:

- Lists of samples and sample sets on the Ion Torrent™ Server.
- The **Add Sample** dialog box in the software.
- The CSV file that is used to import sample information.

A sample attribute can be made mandatory, in which case you are required to enter the attribute information for each new sample. After the attribute is added to the **Sample Attributes** list, you can edit or delete user-defined attributes. You can also choose whether the attribute is displayed or hidden from the sample listings and the **Add Sample** dialog box.

The attributes that you create are applied to individual samples and not to the sample sets.

View sample set attributes

You can view details about a sample set and review sample attributes for each sample that is in a sample set.

1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** table, find a sample set that you want to view.

   For more information about locating your sample set, see "Find a sample set" on page 29 and "Sort sample sets" on page 30.

   Each column in the **Sample Sets** table lists a specific attribute for that sample set.
3. Click the arrow in the row of the sample set to expand the table and view all samples in the sample set.
   Each column in the expanded table lists a specific attribute for each sample in the selected sample set.

Trio sample set

Some sample attributes are passed from Torrent Suite™ Software for use in Ion Reporter™ Software. Trio is an example of a sample set grouping that is used in an Ion Reporter™ Software analysis workflow. If you select the Trio sample set grouping, Torrent Suite™ Software selects a Trio Ion Reporter™ Software analysis workflow when you create a Planned Run.

In a Trio sample set, you can view attributes that include the following.

- **Gender**—The gender of the sample.
- **Type**—The Torrent Suite™ Software relationship type used for **Example Sample Set**.
- **Group**—The number is the sample set mechanism to mark the samples as related. Related means that in the eventual Ion Reporter™ Software analysis, these samples are analyzed in one analysis with a defined relationship between the samples, such as Tumor and Normal.

Add a sample attribute to a sample

1. In the **Plan** tab, click **Samples**, then click **Sample Attributes ▸ Add**.
2. Complete the **Add Attribute** dialog box.
   - **Attribute Name**
   - **Attribute Type**—If the **Attribute Type** is set to Integer, you can enter only numeric characters (whole numbers) for this attribute.
   - *(Optional)* **Attribute Description**
   - *(Optional)* **Is Mandatory**— Select this checkbox if you want the attribute to be required for every sample.
3. Click **Save**.

Manage sample attributes

If you add user-defined sample attributes to the Ion Torrent™ Server, you can manage the sample attributes

- Edit sample attributes.
- Choose whether the attributes are shown or hidden from the list of samples, sample sets, and the **Add Sample** dialog box.
- Delete sample attributes.

1. In the **Plan** tab, click **Samples**, then click **Sample Attributes ▸ Manage**.

2. In the **Sample Attributes** list, in the row of the attribute that you want to manage, click ⚙ **(Actions)**, then select an option.

Option	Description
Click **Edit**.	Edit the attribute. • In the **Edit Attribute** dialog box, edit one or all of the following information. a. **Attribute Name** b. **Attribute Type** c. **Attribute Description** • You can also change whether the attribute is required or optional by selecting or deselecting the **Is Mandatory** checkbox. The attribute that is mandatory is designated by the selected checkbox in the **Required** column of the **Sample Attributes** table. The absence of the selected checkbox designates the attribute as optional.
Click **Show/Hide**.	Hide a user-defined attribute from view without deleting it. • If you hide an attribute, that attribute no longer appears in the list of samples, sample sets, and the **Add Sample** dialog box. The attribute that is shown is designated by the selected checkbox in the **To Show** column of the **Sample Attributes** table. The absence of the checkbox designates the attribute as hidden. • If you hide a mandatory attribute, that attribute is no longer mandatory.
Click **Delete**.	Delete the sample attribute and permanently remove the sample attribute from the Ion Torrent™ Server.

Sample attributes

When you create a sample set, you can enter sample attributes into Torrent Suite™ Software manually using the **Add Sample** dialog box, or you can import samples with a CSV file. Most of the sample information is optional except for **Sample Name**. However, some information is required if you transfer data to Ion Reporter™ Software, and those attributes required by Ion Reporter™ Software are indicated in the following table.

Attribute	Description
Sample Name	The unique name of the sample. This attribute is used by Ion Reporter™ Software. Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_). A 255-character limit must be followed for Ion Reporter™ Software name validation. If you use Ion Reporter™ Software, and the actual sample name already exists in that software, a string such as _v1 or _v2, and so on, is added to the sample name.

(continued)

Attribute	Description
Sample ID	A unique identifier (SampleID) for each barcode in a sample. This identifier helps to track samples or possibly identify misassignment between samples and barcodes in a sequencing run. The SampleID is passed to Ion Reporter™ Software. 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. **Note:** This attribute is optional in Torrent Suite™ Software. However, this value can be required for planned runs that include automatic upload of data to Ion Reporter™ Software.
Case ID	A unique identifier for the case. Multiple sample IDs can be associated with a single case ID. **Note:** This attribute is optional in Torrent Suite™ Software. However, this value can be required for planned runs that include automatic upload of data to Ion Reporter™ Software.
PCR Plate Position	A unique identifier for the 96-well plate that is used for library preparation and templating. Select a plate position of **A** to **H**.
Barcode Kit	The name of the barcode kit used to make a library from the sample. The same barcode kit must be used for all samples in a sample set.
Barcode	The name of the specific barcode in the selected barcode kit. Assign a unique barcode to each sample in a sample set.
Control Type	The control type used when preparing the sample. If a value is selected, the sample is identified as a control sample. • **MSI On-Chip Control** • **No Template Control**
Basic Annotations	
Description	Typically, one or two sentences that describe the sample.
Nucleotide Type	The nucleic acid type or RNA variant type that is associated with the sample: • **Unspecified** • **DNA** • **RNA** • **TNA** • **Fusions**

(continued)

Attribute	Description
Sample Source	The source from which the sample is extracted. • **Blood** • **FFPE** • **Other**
Panel Pool Type	The type of pool that is used by the assay. • **Dual Pool** • **Single Pool**
Gender	The biological gender of the sample. This attribute is used by Ion Reporter™ Software. • **Female** • **Male** • **Unknown** **IMPORTANT!** If you use Ion Reporter™ Software, do not leave this blank. Select **Unknown** if the gender is not known. Several analysis workflows in Ion Reporter™ Software, for example, copy number variation detection and genetic disease research, are limited when the gender is not known. The analysis workflows can return unexpected results when the gender is incorrectly specified for a sample.
Type	The relationship type for this sample. Type is used with **Relationship Group**, described below. For example, a relationship group can include two samples, one with a type **Tumor** and another with a type **Normal**. The following sample relationships are supported by Ion Reporter™ Software: • **Control** • **Father** • **Mother** • **Normal** • **Sample** • **Self**—Use **Self** for both a single sample and for the proband sample in a trio. A single sample is not related to other samples and is analyzed by itself. • **Tumor**
Relationship Group	Use **Relationship Group** to designate a group of multiple related samples in the same sample set. For example, DNA and RNA samples from the same sample have the same **Relationship Group** number. Use a whole number to define a sample as part of a relationship group. It is used with **Type**. For example, a sample set can include 6 samples, consisting of 3 groups of 2 related samples each (of types **Tumor** and **Normal**). In this case, designate the two samples in each group as part of group 1, 2, or 3. This is identical to the Set ID in the IonReporterUploader plugin.

(continued)

Attribute	Description
Extra Annotations (used for specialized applications, such as preimplantation genetic screening (PGS) research or oncology research)	
Sample Collection Date	The date that the sample was drawn.
Sample Receipt Date	The date that the laboratory received the sample.
Cancer Type	The type of cancer that is present in the sample.
Population	The super population code assignment for a sample, as defined by the human 1000 genomes project (**http://www.internationalgenome.org/faq/which-populations-are-part-your-study/**). The population is relevant to the analysis of samples by the TCRB-LR assay workflow in Ion Reporter™ Software. The TCRB-LR analysis workflow produces a haplotype group assignment for samples having a population attribute of "European".
Mouse Strains	The name of a mouse strain. Choose from a select number of the most common strains. In Ion Reporter™ Software 5.12, the selected mouse strain does not affect Ion Reporter™ Software analysis workflows.
Cellularity %	The percentage of tumor cells in the sample. This is a whole number between 1 and 100.
Biopsy Days	The number of days after which a biopsy was collected. This is a whole number.
Cell Number	The cell count of the biopsied sample.
Couple ID	An identifier for use with the Reproductive research application.
Embryo ID	An identifier for use with the Reproductive research application.
User-defined Attributes	
<user defined>	If you create additional sample attributes, each attribute is listed here and in the CSV file. Attributes that are marked as mandatory must be entered for each sample. If you create an attribute of type integer, only numeric characters (whole numbers) can be entered for that attribute.

Sample set definitions

When you create a sample set, specify some or all of the following information about the sample set.

Item	Description
Sample Set Name	Enter a name for this sample set. Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_).
Group Type	Select the Group Type that describes this sample set. • **DNA Fusions** • **Other** • **Sample_Control** • **Self** • **Single Fusions** • **Trio** • **Tumor_Normal**
PCR Plate Serial Number	Enter the serial number for the PCR plate used for this sample set.
Description	Provide a unique description for this sample set. The description is limited to 1,024 characters. Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_).
Library Prep Type	Specify how your library is prepared for use with this sample set. Valid options are: • **Manual** • **AmpliSeq on Chef**
Library Prep Kit	Select the library preparation kit that is used to prepare your library for this sample set. • **Ion AmpliSeq Kit for Chef DL8** • **Precision ID Chef DL8** • **Ion Ampliseq HD DL8**
Library Prep Protocol	Select the library preparation protocol that is used to prepare your library for this sample set. • **——— (generic option)** • **Myeloid** • **2 Library Pools - OCA Plus**

View Ion AmpliSeq™ library preparation on an Ion Chef™ Instrument run

You can view a summary of details about how the libraries were prepared for a completed run on an Ion Chef™ Instrument that used an Ion AmpliSeq™ library preparation kit. Knowing this information can be useful for troubleshooting an Ion Chef™ Instrument run.

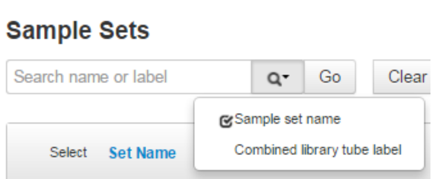
This information is not available if a library preparation kit is not selected when the sample is created, or if the run did not use an Ion AmpliSeq™ library preparation kit.

1. In the **Plan** tab, click **Samples**.
2. In the row that contains the sample set of interest, click **(Actions)** ▸ **Library Prep Summary**.
3. In the **Library Prep Summary** screen, view the information that is listed for the sample set. This information is provided by the Ion Chef™ Instrument; some items may be blank.
   - **Library Prep Type**
   - **PCR Plate Type**
   - **PCR Plate Serial Number**
   - **Combined Library Tube Label**
   - **Chef Last Updated**
   - **Chef Instrument Name**
   - **Tip Rack Barcode**
   - **Library Kit Type**
   - **Reagent Lot Number**
   - **Reagent Part Number**
   - **Reagent Expiration**
   - **Solution Lot Number**
   - **Solution Part Number**
   - **Solution Expiration**
   - **Chef Script Version**
   - **Chef Package Version**

   The **Chef Script Version** lists the version of the software script for the Ion Chef™ Instrument, and the **Chef Package Version** lists the software package that is used by the Ion Torrent™ Server. The release version for these scripts can differ if Torrent Suite™ Software was updated with an off-cycle release.

Find a sample set

1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** screen, click **(Search)** to filter the search, then select **Sample set name** or **Combined library tube label**.

3. In the search box, enter a search term for the name of a sample set or the unique barcode ID of a combined library tube label.

   The search term is not case sensitive. You do not need to know the exact name because a partial name returns results.

4. Click **Go** to display the results of the search.
5. Click **Clear All** to return to the complete list of sample sets.

Sort sample sets

1. In the **Plan** tab, click **Samples**.
2. In the **Samples Sets** table, click any column heading to sort the sample rows alphabetically or numerically.
3. Click the column heading again to reverse the order of the column contents.
4. Click **Clear** to undo the sort.

Edit a sample set

1. In the **Plan** tab, click **Samples**.
2. In the row of the sample set that you want to edit, click **(Actions)** ▸ **Edit Sample Set**.
3. (Optional) In the **Edit Sample Set** dialog box, make desired changes, then click **Done**.
4. (Optional) Edit an individual sample in a sample set.
   a. Click the small triangle to the left of the sample set name to expand the sample set row.
   b. Select a **Sample Name**, then then click **(Actions)** ▸ **Edit Sample in Set** in the row of the sample that you want to edit.
   c. Update the **Edit Sample** dialog box, then click **Done**.
   d. (Optional) To delete a sample from a set, click **(Actions)** ▸ **Remove Sample from Set** in the row of the sample that you want to delete, then click **Yes, Delete!**.

Delete a sample set

1. In the **Plan** tab, click **Samples**.
2. Click **(Actions)** ▸ **Delete Set** in the row of the sample set that you want to delete.
3. Click **Yes, Delete!**.

To delete a sample from a sample set, see "Edit a sample set"

4 Plan and execute an instrument run

Use Torrent Suite™ Software to plan and execute an instrument run with an existing run template or create your own template.

Planned Runs

Planned Runs are the digital instructions for the sequencing instrument. A Planned Run includes specifications for sample preparation, sequencing, data export, and post-sequencing data analysis. A Planned Run contains all settings that are used in a sequencing run. A Planned Run tracks samples, chips, and reagents throughout the sequencing workflow, from library preparation through subsequent data analysis.

The details that you specify for items depend on the assay and the sequencing instrument that you use. For example, the Planned Run template specifies a default chip type, but you can change the chip type based on throughput needs. Changing the chip type makes changes in the sequencing kit and template kits.

For more information, see the user guides for the specific assays and sequencing instrument that you plan to use.

Planned Run templates

A Planned Run template is a reusable experimental design that can be saved and used to create many Planned Runs. Planned Run templates play an important role in enabling rapid throughput across your sequencing instrument. Planned Run templates also help reduce the chances of errors, because information is stored and then applied to Planned Runs, instead of entered manually for each run.

When planning a run, you can use a system Planned Run template that is preloaded in Torrent Suite™ Software and is suitable for use with specific types of research applications. If a preinstalled Planned Run template does not meet your needs, you can create your own unique Planned Run template.

Planned Run templates for specific research applications

Torrent Suite™ Software is preloaded with many Planned Run templates that contain predefined settings for common sequencing applications. These preinstalled Planned Run templates are categorized into research applications that describe the sequencing techniques, or specialized products, for which each system Planned Run template is used.

If a preinstalled Planned Run template does not meet your needs, you can download a template from **AmpliSeq.com**, or advanced users can create unique Planned Run templates. For assistance, contact your local Field Service Engineer.

Planned Run templates are available for use on the **Plan** tab under **Templates**. On this screen, the following categories of research applications filter the list of Planned Run templates that are available for use. Templates may be available in multiple research application categories.

Research application	Description
AmpliSeqDNA	Ion AmpliSeq™ research applications (DNA and exome), including the Ion AmpliSeq™ On-Demand Panels, and Ion AmpliSeq™ Made-to-Order and Community Panels.
AmpliSeqRNA	Ion AmpliSeq™ research applications (RNA), including the Oncomine™ Immune Response Research Assay.
AmpliSeq HD	Ion AmpliSeq™ HD libraries (DNA and RNA).
DNA and Fusions	Ion AmpliSeq™ research applications such as Oncomine® Focus Fusions, Oncomine® Focus DNA & Fusions, Colon and Lung Research Panel v2.
Generic Sequencing	Research applications that do not fit in the other categories. Use this research application to provide all choices for the experiment. The choices are not restricted based on a common application workflow.
Human Identification	Applied Biosystems™ Human Identification panels.
Immune Repertoire	Ion AmpliSeq™ Immune Repertoire Research Assay.
Inherited Disease	Ion AmpliSeq™ Inherited Disease Panel and Oncomine™ BRCA research panels.
Mutation Load	Oncomine™ Tumor Mutation Load Assay.
Oncology – HemeOnc	All blood-related oncology research applications, for example, Oncomine™ Myeloid Research Assay.
Oncology – ImmunoOncology	All ImmunoOncology research applications, for example, Oncomine™ Immune Response Research Assay.
Oncology – Liquid Biopsy	Liquid biopsy oncology research assays.
Oncology – Solid Tumor	Solid tumor oncology research assays.
Pharmacogenomics	Ion AmpliSeq™ Pharmacogenomics Research Panel that is imported from **AmpliSeq.com**.
Reproductive	Ion ReproSeq™ PGS kits for Aneuploidy Detection.

(continued)

Research application	Description
RNA Seq	RNA sequencing research assays.
TargetSeq	TargetSeq™ research applications, with parameters optimized for hybridization-based target enrichment.
Whole Genome	Whole genome sequencing research applications, such as Ion ReproSeq™ Aneuploidy, that do not assume enrichment and do not require a target regions file.
16S rRNA Profiling	For use with: • Ion AmpliSeq™ Microbiome Health Research 540 Kit • Ion AmpliSeq™ Microbiome Health Research 550 Kit • Ion 16S™ Metagenomics Kit. For more information, see the *Ion AmpliSeq™ Microbiome Health Research Kits User Guide*.
16S Target Sequencing	For use with: • Ion AmpliSeq™ Microbiome Health Research 540 Kit • Ion AmpliSeq™ Microbiome Health Research 550 Kit • Ion 16S™ Metagenomics Kit. For more information, see the *Ion AmpliSeq™ Microbiome Health Research Kits User Guide*.
Carrier Seq	Expanded carrier screening (ECS) applications, including Ion Torrent™ Ion CarrierSeq™ ECS Kits and Ion AmpliSeq™ Ion CarrierSeq™ ECS Panel.

Create a user-defined Planned Run template

Create a user-defined Planned Run template that you can reuse when the same conditions can be used for multiple runs. To create the template, copy an existing preinstalled Planned Run template, then edit the settings to meet the requirements for your Planned Run.

IMPORTANT! Before you create the template, ensure that the most current reference sequences and the target regions and hotspots BED files are on the Ion Torrent™ Server. For more information about installing these files, see Chapter 10, "References management". Contact your local Field Service Engineer to obtain the most current BED files.

1. In the **Plan** tab, click **Templates**, then select the desired type of research application from the left navigation menu.

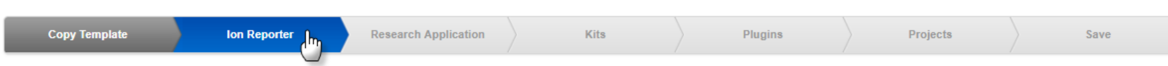
2. In the list of templates, find a preinstalled Planned Run template to use to create a user-defined template, then click ⚙ **(Actions)** ▸ **Copy**.
   The **Copy Template** workflow bar opens to the **Save** step.

3. In the **Save** step, enter or select the required information. The information is based on the selected template.

   The settings are updated in the **Summary** pane.

Item	Description
Template Name	Enter a name for the template.
Set as Favorite	Select the **Set as Favorite** checkbox to add the template to the **Favorites** list.
Analysis Parameters	Select **Default (Recommended)** to accept default analysis parameter settings. Advanced users can select **Custom** to edit analysis parameters. For more information, see “Copy an analysis parameter set” on page 57.
Reference Library	Select the reference library file that is appropriate for the sample. Based on the application, you might have to select separate DNA, RNA, and fusions reference library files.
Target Regions	Select the target regions BED file appropriate for the sample. Based on the application, you might have to select separate **DNA Target Regions** and **Fusions Target Regions** files. Check with your local Field Service Engineer for updates to ensure that the most current files are being used. For BED file installation instructions, see “Upload a target regions file” on page 248.
Hotspot Regions	Select the hotspots (BED or VCF) file that is appropriate for the sample. For BED file installation instructions, see “Upload a hotspots file” on page 250.
Add a note	Add an optional note about the Planned Run
Add LIMS Meta Data	If using LIMS metadata, enter the text that is associated with the Planned Run.
Monitoring Thresholds	Adjust **Bead Loading (%)**, **Key Signal (1-100)**, and **Usable Sequence (%)** minimum thresholds for your Planned Run.

4. In the **Copy Template** workflow bar, click the **Ion Reporter** step, then set up the transfer of the completed run results to a specified Ion Reporter™ Server.

   For more information, see “Ion Reporter step in the workflow bar” on page 40.

5. Click **Next**.

6. In the **Research Application** step, ensure that the correct **Research Application** and **Target Technique** are selected, then click **Next**.

   For more information, see “Research Application step in the workflow bar” on page 42.

7. In the **Kits** step, enter or select the required information, then click **Next**.

   For more information, see “Kits step in the workflow bar” on page 44.

8. In the **Plugins** step, select from the available plugins, configure the selected plugins as required, then click **Next**.

   For more information, see “Plugin configuration” on page 121.

   For more information about the **Plugins** step, see “Plugins step in the workflow bar” on page 47.

9. In the **Projects** step, select the project or projects to receive data from the runs that use this template, then click **Next**.

   For more information, see “Projects step in the workflow bar” on page 48.

10. In the **Save** step, click **Copy Template** to save the new Planned Run template.

The user-defined template is available in the **Templates** screen in the **Research Application** group from which you copied the preinstalled Planned Run template. If you selected the **Set as Favorite** option, the template also appears in the **Favorites** list. The Planned Run can be used in an instrument run.

Create a Planned Run from a Planned Run template

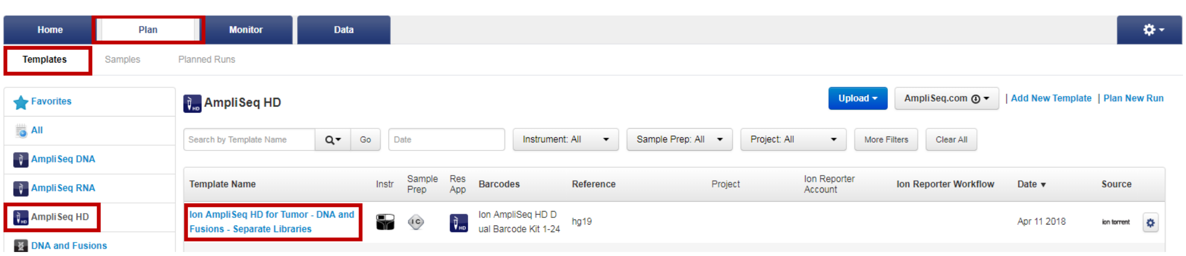
You can create a Planned Run from a preinstalled or user-defined Planned Run template that contains run settings. The preinstalled Planned Run templates are included in the software for standard research applications and kits, such as Ion AmpliSeq™ or Oncomine™ workflows. You can create a template as described in “Create a user-defined Planned Run template” on page 33

When you create a Planned Run from a preinstalled Planned Run template, some settings are predefined by the template and some can be selected or completed. The predefined settings can vary between templates. You can change any of the settings to create your Planned Run, even those settings that are predefined in the Planned Run template.

Most Planned Run templates have a corresponding Ion AmpliSeq™ or Oncomine™ panel. When you create a Planned Run from a Planned Run template, the Create Plan workflow bar opens to the **Plan** step, which is the final step in the Planned Run creation process. You can accept all Planned Run template settings if you enter only a run plan name and sample names then save a Planned Run from the template. Or, you can further customize the Planned Run if you open other steps and change information in the Planned Run workflow bar.

1. In the **Plan** tab, click **Templates**, then select the desired research application from the left navigation menu.

2. In the **Template Name** column of the templates table, click the template name. Alternatively, you can click **Favorites**, then click the template name, if the template is in the favorites list.

   The **Create Plan** workflow bar opens to the **Plan** step.

3. The **Run Plan Name** is prepopulated with the template name. Enter new text to create a unique Planned Run name.

4. Complete additional settings in the **Plan** step if needed.

   For more information about the individual settings, see “Plan step in the workflow bar” on page 49.

   As you make selections, the settings are updated in the **Summary** pane. You can also use the information in this pane to review the settings that are predefined by the template.

5. Click other steps in the workflow bar to enter or change settings if needed.

   For more information, see “Steps in the workflow bar” on page 39.

6. When you have completed your selections, review the settings in the **Summary** pane, then click **Plan Run** at the bottom of the **Plan** step.

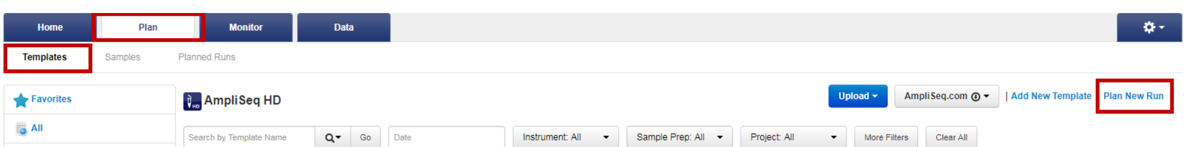
   The Planned Run is added to the **Planned Runs** table and can be used in an instrument run.

Create a Planned Run without a Planned Run template

If you do not create a Planned Run from a preinstalled or user-defined Planned Run template, you can create a Planned Run that includes the settings that you select to meet your needs.

Create a Planned Run from preinstalled or user-defined Planned Run templates. Planned Run templates contain predefined settings that you can use to prepare multiple runs of the same type. For example, use a predefined template if you perform the same type of sequencing analysis that uses the same instruments and reagents on multiple samples. For more information, see “Create a user-defined Planned Run template” on page 33 and “Create a Planned Run from a Planned Run template” on page 35.

1. In the **Plan** tab, in the **Templates** screen, click **Plan New Run**.

   The **Create Plan** workflow bar opens to the **Ion Reporter** step.

2. In the **Ion Reporter** step, set up the transfer of the completed run results to a specified Ion Reporter™ Server. As you make your selections, your settings are updated in the **Summary** pane.

   For more information, see “Ion Reporter step in the workflow bar” on page 40.

3. Click **Next**.

4. In the **Research Application** step, ensure that the correct **Research Application** and **Target Technique** are selected, then click **Next**.

   For more information, see “Research Application step in the workflow bar” on page 42.

5. In the **Kits** step, enter or select the required information, then click **Next**.

   For more information, see “Kits step in the workflow bar” on page 44.

6. In the **Plugins** step, select from the available plugins, configure the selected plugins as required, then click **Next**.

   For more information, see "Plugin configuration" on page 121 or "Plugins step in the workflow bar" on page 47.

7. In the **Projects** step, select the project or projects that receive data from the runs that use this template, then click **Next**.

   For more information, see "Projects step in the workflow bar" on page 48.

8. In the **Plan** step, enter a name for the plan in **Run Plan Name**, specify the reference and BED files, then enter or upload your sample information.

   For more information about the individual settings, see "Plan step in the workflow bar" on page 49.

9. Review the settings in the **Summary** pane, then click **Plan Run** at the bottom of the **Plan** step.

   The Planned Run is added to the list on the **Planned Runs** screen.

Search for a Planned Run template

You can search, sort, or filter the **Templates** list to find a Planned Run template of interest.

1. In the **Plan** tab, click **Templates**, then complete the selections for the following options.

Option	Selection
Search the list	a. Click **All** in the research applications menu on the left to search all templates. Alternatively, select a research application from the menu to search for templates in a specific research application. b. In **Search by Template Name**, enter a search term, then click **Go**. The templates that include the search term in the template name are listed.
Sort the list	Click any bolded column heading in the list of templates to sort the order in which the templates appear. Click the column heading a second time to reverse the sort order.
Limit the list to recent runs	In **Date**, select a preset range, or click **Date Range**, then select the start and end dates.
Filter the list	Select from one or more filters to limit the list of templates. Click **More Filters** to see all available filters. In a filter, enter text into **Find** to limit the filter choices. To remove a filter, remove the filter choice or click **Clear** in the filter list. In some cases, you can select more than one choice in a single filter category.

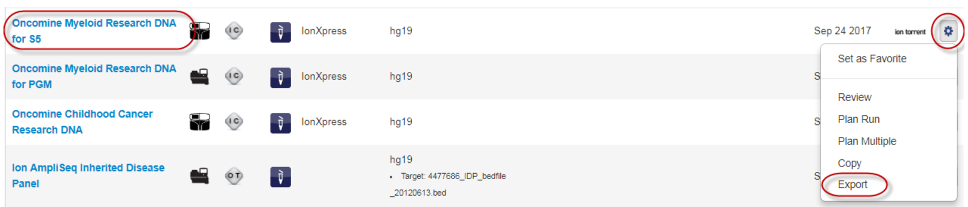
2. Click **Clear All** to remove filters and restore all results.

Export a Planned Run template

You can export the settings from a Planned Run template to a CSV file. You can then transfer the file to a different Ion Torrent™ Server and import the template into Torrent Suite™ Software on that server. For more information, see “Import a Planned Run template” on page 38.

You can also open the file in a spreadsheet application such as Microsoft™ Excel™ and edit the settings before import. Preserve the column headings and layout to ensure that the template file is correct.

1. On the **Templates** screen, on the **Plan** tab, locate the template that you want to export.
2. Click **(Actions)** in the row of the template, then select **Export**.



Based on your browser settings, the CSV file may be created and downloaded automatically, or you may be prompted to save the file.

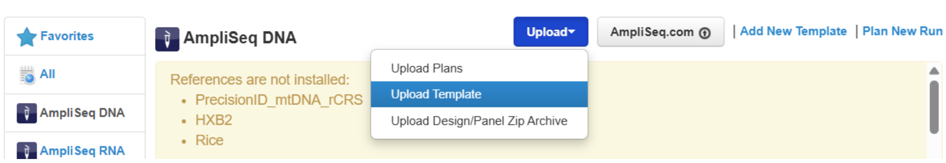
Exported Planned Run templates have "exported" appended to the front of the original template name, unless you edit the name in the CSV file.

Import a Planned Run template

You can import Planned Run template settings that have been exported to a CSV file. Use this procedure for transferring settings between different Ion Torrent™ Servers. You can change the settings in an exported CSV file and then import the Planned Run template. You must preserve the column headings and layout to ensure that the template file is correct.

To export Planned Run template settings to a CSV file, see “Export a Planned Run template” on page 38.

1. In the **Plan** tab, in the **Templates** screen, select the research application group that you want to import the template into.
2. Click **Upload ▸ Upload Template**.



3. In the **Import Plan Template** dialog box, click **Choose File**, select the CSV file to import, then click **Load**.
   The template is listed in the application group. Exported Planned Run templates have "exported" appended to the front of the original template name, unless the name has been edited in the CSV file.

4. *(Optional)* Edit the template name, by clicking **(Actions)** ▸ **Edit**.

Save a Planned Run template to Favorites

You can save Planned Run templates to **Favorites** to make it easier to find your frequently used templates within Torrent Suite™ Software.

1. In the **Plan** tab, click **Templates**, then search for a template that you want to add to **Favorites**.
   For more information, see "Search for a Planned Run template" on page 37.

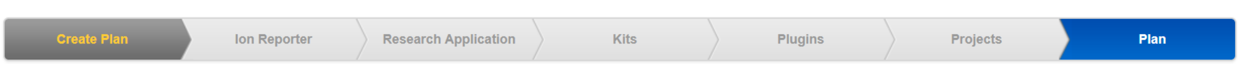
2. In the row of the template, click **(Actions)** ▸ **Set as Favorite** to add your Planned Run template to the **Favorites** list.

3. To see the list of saved favorites, in the **Plan** tab, click **Templates**, then click **Favorites** at the top of the research applications list.

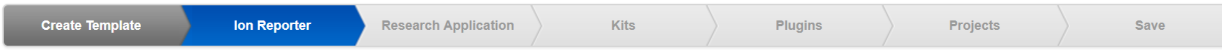
Steps in the workflow bar

Torrent Suite™ Software guides you through steps to provide the information that is required to create Planned Runs and Planned Run templates, and then to perform a Planned Run. You can work through the steps in workflow order, or you can use only the steps that you need.

Use the steps in the **Create Plan** workflow bar to create a Planned Run that is based on a predefined Planned Run template that is prepopulated with information specific for your instrument run. For more information, see "Create a Planned Run from a Planned Run template" on page 35.



Use the steps in the **Create Template** workflow bar to do the following.



- Create a user-defined Planned Run template that fits your sequencing needs, then save the template to reuse to create Planned Runs in the future. For more information, see “Create a user-defined Planned Run template” on page 33.
- Create a unique Planned Run that is not based on a Planned Run template. For more information, see “Create a Planned Run without a Planned Run template” on page 36.

After you create a Planned Run in Torrent Suite™ Software, the Planned Run is sent to the Ion Torrent™ sequencer to be executed.

You can also download a CSV file, customize the file to create multiple Planned Runs, then upload the file into the software. For more information, see “Create multiple Planned Runs” on page 68.

Ion Reporter step in the workflow bar

If you are an Ion Reporter™ Software user, when you create a Planned Run or a Planned Run template, use the **Ion Reporter** step to add settings to transfer automatically run results to Ion Reporter™ Software for further analysis and visualization.

You can directly transfer results in one of the following ways.

- On the **Ion Reporter** step in the workflow bar, if you select an Ion Reporter™ Software account and an analysis workflow, then also select the option to upload files automatically after run completion. An Ion Reporter™ Software analysis launches immediately after the run. Successful analyses are then available in Ion Reporter™ Software when you sign in to the selected account.
- You can also transfer the output files (BAM files and VCF files) from the run to Ion Reporter™ Software without selecting an Ion Reporter™ Software analysis workflow if you use the **Upload Only** option. In this case, you can launch the analysis manually in Ion Reporter™ Software. This approach is commonly used to annotate the VCF files with the Annotation-only analysis workflow in the software.

  For details, see the Ion Reporter™ Software help system, or see the Ion Reporter™ Software user guide that is available on the NGS Software Support page (**https://www.thermofisher.com/us/en/home/technical-resources/technical-reference-library/next-generation-sequencing-support-center/ngs-software-support.html**).

In the **Ion Reporter** step in the workflow bar, you select the Ion Reporter™ Software account to receive data from the completed run. If you are creating a Planned Run template, select the account to receive data from every run that is created from this Planned Run template.

Selections in the **Ion Reporter** step apply only to Ion Reporter™ Software users. The Ion Reporter™ Software is not included with Torrent Suite™ Software and is available under separate license. For details, see Chapter 9, “Integration with Ion Reporter™ Software”.

Use the plan by sample set feature when you configure Ion Reporter™ Software in the Planned Run or template. For details, see “Plan by sample set” on page 58.

1. In the **Ion Reporter** step in the workflow bar, select the Ion Reporter™ Software account that you want to transfer output files to for analysis.

   The selected account is the one to use to view and further analyze the files in Ion Reporter™ Software.

If the Ion Reporter™ Software account is not configured, click **Configure** to add another account. For more information, see “Set up an account for IonReporterUploader plugin” on page 221.

2. Select a **Sample Grouping** that corresponds to the sample relationship in Ion Reporter™ Software.

   When you select a **Sample Grouping**, the workflow bar in Ion Reporter™ Software shows only the analysis workflows that are appropriate for the sample.

3. Select an **Existing Workflow** option.

Option	Description
Upload Only	Use this option to transfer only the output files from the sequencing run to Ion Reporter™ Software. If you use this option, you can access the samples in Ion Reporter™ Software. VCF files are also available if you run the variantCaller plugin.
Ion Reporter workflow for your sample type	With this option, the analysis workflow is automatically launched in Ion Reporter™ Software with the sample data from the run. Successful analyses are available in Ion Reporter™ Software with the account and organization that you selected.

4. *(Optional)* To create a new analysis workflow, click **Create New Workflow** to open Ion Reporter™ Software in a new browser window. In Ion Reporter™ Software, create a new analysis workflow, then save it. When you return to Torrent Suite™ Software, refresh the browser. You can then select the newly created analysis workflow in the **Existing Workflow** list.

5. Under **Ion Reporter Upload Options**, select an option.

Option	Description
Review results after run completion, then upload to Ion Reporter	Use this option to review the completed run results and then manually upload the data to Ion Reporter™ Software. IMPORTANT! Review the results in **Completed Runs & Reports**, then click **Upload to IR ▸ Upload as Planned** to upload the data to Ion Reporter™ Software.
Automatically upload to Ion Reporter after run completion	With this option, run results are automatically uploaded to Ion Reporter™ Software when a run is complete. If you select an analysis workflow, an analysis is launched immediately after the run. Successful analyses are then available to you when you sign in to Ion Reporter™ Software with the account used in the setup.

6. Click **Next** in the workflow bar to continue with the Planned Run or with Planned Run template creation.

Research Application step in the workflow bar

Use the **Research Application** step in the workflow bar to select research applications and target techniques that apply to your template or plan. Available options in subsequent steps are restricted to those options that are compatible with the selected research application and target technique.

1. Select the research application used in your plan.

Research application	Description
DNA	Detects and annotates low frequency (to 0.5% limit of detection) somatic variants (SNPs, INDELs, and CNVs) from targeted DNA libraries that use Ion AmpliSeq™ HD technology. This method is compatible with DNA that is purified from tumor or liquid biopsy research samples.
DNA and Fusions (Separate Libraries)	Detects and annotates low frequency (to 0.5% limit of detection) somatic (SNPs, INDELs, and CNVs) variants from targeted DNA libraries, and gene fusions from matching targeted RNA libraries that use Ion AmpliSeq™ HD technology. This method is compatible with DNA and RNA that is purified separately from tumor or liquid biopsy research samples.
DNA and Fusions (Single Library)	Detects and annotates low frequency (to 0.5% limit of detection) somatic variants (SNPs, INDELs, CNVs, and fusions) from targeted nucleic acid libraries that use Ion AmpliSeq™ HD technology. This method is compatible with DNA and RNA that is purified together from tumor or liquid biopsy research samples.
Fusions	Detects and annotates gene fusions from targeted RNA libraries that use Ion AmpliSeq™ HD technology. This method is compatible with RNA that is purified from tumor or liquid biopsy research samples
Human Identification	For templates to run Applied Biosystems™ Precision ID set of panels.
Immune Repertoire	For use with the Ion AmpliSeq™ Immune Repertoire panel.
Metagenomics	Reserved for future use with Ion Reporter™ Software.
Mutation Load	For use with the Oncomine™ Tumor Mutation Load Assay panel.
Oncology - Liquid Biology	For use with liquid biopsy oncology research panels.
Pharmacogenomics	For use with the Ion AmpliSeq™ Pharmacogenomics Research Panel that is imported from **AmpliSeq.com**.
RNA	For use with RNA (fusions) libraries.
Typing	For use to perform molecular fingerprinting to detect viral or bacterial strains for research purposes.

2. Select the **Target Technique** that pertains to the selected research application.

Research Application selected	Available **Target Technique**
DNA	**AmpliSeq DNA** **AmpliSeq Exome** **AmpliSeq HD - DNA** **Other** **TargetSeq** **Whole Genome**
DNA and Fusions (Separate Libraries)	**AmpliSeq DNA and Fusions** **AmpliSeq HD - DNA and Fusions (Separate Libraries)** **AmpliSeq RNA**
DNA and Fusions (Single Library)	**AmpliSeq HD - DNA and Fusions (Single Library)**
Fusions	**AmpliSeq HD - Fusions**
Human Identification	**AmpliSeq DNA**
Immune Repertoire	**AmpliSeq RNA** **Mixed Samples (DNA/RNA)**
Metagenomics	**16S Targeted Sequencing** **Other**
Mutation Load	**AmpliSeq DNA**
Oncology - Liquid Biology	**Tag Sequencing**
Pharmacogenomics	**AmpliSeq DNA**
RNA	**AmpliSeq RNA** **RNA Sequencing**
Typing	**Other** **Whole Genome**

3. Click **Next** in the workflow bar to continue with the Planned Run or with Planned Run template creation.

Kits step in the workflow bar

Use the **Kits** step in the workflow bar to set up information that is needed for sample preparation and sequencing, including the chip and the kits that are used. Options that are available in the **Kits** step depend on the instrument used. Selections in this step affect how the data are analyzed after sequencing.

Note: Some Planned Run templates have parameter settings in the **Kits** step that cannot be changed. To change locked parameter settings, create a new template. For more information, see "Create a Planned Run without a Planned Run template" on page 36.

1. In the **Kits** step in the workflow bar, enter or select the following information.

Item	Selection
Instrument	Select the sequencing instrument system to be used, for example, Ion GeneStudio™ S5 Plus System or Ion GeneStudio™ S5 Prime System.
Sample Preparation Kit	Select the sample preparation kit, if one is to be used.
Library Kit Type	Select the kit to be used to prepare the library, for example, Ion AmpliSeq™ Library Kit Plus.
Template Kit	Select the instrument system to be used: **OneTouch**, **IonChef**, or **IA**. Select the templating kit to be used.
Sequencing Kit	Select the sequencing kit to be used, for example, **Ion S5™ Sequencing Kit**.
Chip Type	Select the sequencing chip type to be used, for example, **Ion 540™ Chip**.
Control Sequence	Select the control sequence to be added to the library preparation. Leave blank if not used.
Barcode Set	Select the barcode set to be used, for example, Ion Xpress™.
Flows	Enter the number of nucleotide reagent flows required to complete the sequencing run (for example, 400).
Mark as Duplicates Reads	Select this option to mark duplicate reads in the BAM file after a run is complete. Do not use with Ion AmpliSeq™ data. For more information, see "About the Mark as Duplicate Reads option" on page 47. To remove marked duplicates from the BAM file, select the FilterDuplicates plugin in the **Plugins** step in the workflow bar. For more information, see "Plugins step in the workflow bar" on page 47.
Enable Realignment	Select this option to perform realignment, an optional step that is executed immediately after TMAP. This step adjusts the alignment, primarily in the CIGAR string. For more information, see "TMAP modules" on page 292.

2. *(Optional)* In **Advanced Settings**, click **Customize** to customize the **Advanced Settings** parameters.

   For more information, see "Advanced Settings—Kits step in the workflow bar" on page 46.

   IMPORTANT! We recommend that you use the default settings. Consult your local Field Service Engineer before you modify advanced settings.

Item	Selection
Templating Protocol	Script that the Ion Chef™ Instrument follows to perform the templating reaction. We recommend that you do NOT change this setting.
Forward Library Key	Select your forward library key, if you have one.
Test Fragment Key	Enter your test fragment key.
Base Calibration Mode	Select one of the following base calibration options. • **Default Calibration**—Allows a random subset of wells to be used for base calibration. This option uses TMAP to align the training subset of wells and is recommended if a good reference for the template is available. • **Enable Calibration Standard**—Allows wells belonging to the Calibration Standard to be selected as the training subset. • **Blind Calibration**—Uses the same random subset of wells as Default Calibration but does not require an alignment step to generate the calibration model. This option is recommended if the template does not align well to a reference genome or if no reference is specified. • **No Calibration** See your template kit user guide for more details.
Forward 3' Adapter	Select your forward 3' adapter.
Flow Order	Select the flow order. For more information, see "flow order" on page 362.

3. Click **Next** in the workflow bar to continue with the Planned Run or with Planned Run template creation.

Advanced Settings—Kits step in the workflow bar

You can customize the kit advanced settings in the **Kits** step in the workflow bar.

IMPORTANT! We recommend that you use the default settings. Consult your local Field Service Engineer before you modify advanced settings.

1. In the **Kits** step in the workflow bar, expand the **Advanced Settings** box, then select **Customize**.
2. Edit one of the following settings.

Item	Selection
Templating Protocol	Script that the Ion Chef™ Instrument follows to perform the templating reaction. We recommend that you do NOT change this setting.
Forward Library Key	Select your forward library key, if you have one.
Test Fragment Key	Enter your test fragment key.
Base Calibration Mode	Select one of the following base calibration options. • **Default Calibration**—Allows a random subset of wells to be used for base calibration. This option uses TMAP to align the training subset of wells and is recommended if a good reference for the template is available. • **Enable Calibration Standard**—Allows wells belonging to the Calibration Standard to be selected as the training subset. • **Blind Calibration**—Uses the same random subset of wells as Default Calibration but does not require an alignment step to generate the calibration model. This option is recommended if the template does not align well to a reference genome or if no reference is specified. • **No Calibration** See your template kit user guide for more details.
Forward 3' Adapter	Select your forward 3' adapter.
Flow Order	Select the flow order. For more information, see “flow order” on page 362.

About the Mark as Duplicate Reads option

Use the **Mark as Duplicate Reads** option to mark duplicate reads in the BAM file after a run is completed.

For some applications, duplicate reads coming from PCR cause problems in downstream analysis. The presence of duplicate reads can create the appearance of multiple independent reads supporting a specific interpretation, when some of the reads are, in fact, duplicates of each other with no additional evidence for the interpretation.

Torrent Suite™ Software uses an Ion-optimized approach, which considers the read start and end positions of both the 5′ alignment start site and the flow in which the 3′ adapter is detected. Duplicate reads are flagged in the BAM file in a dedicated field. This method is recommended over other approaches, which consider only the 5′ alignment start site.

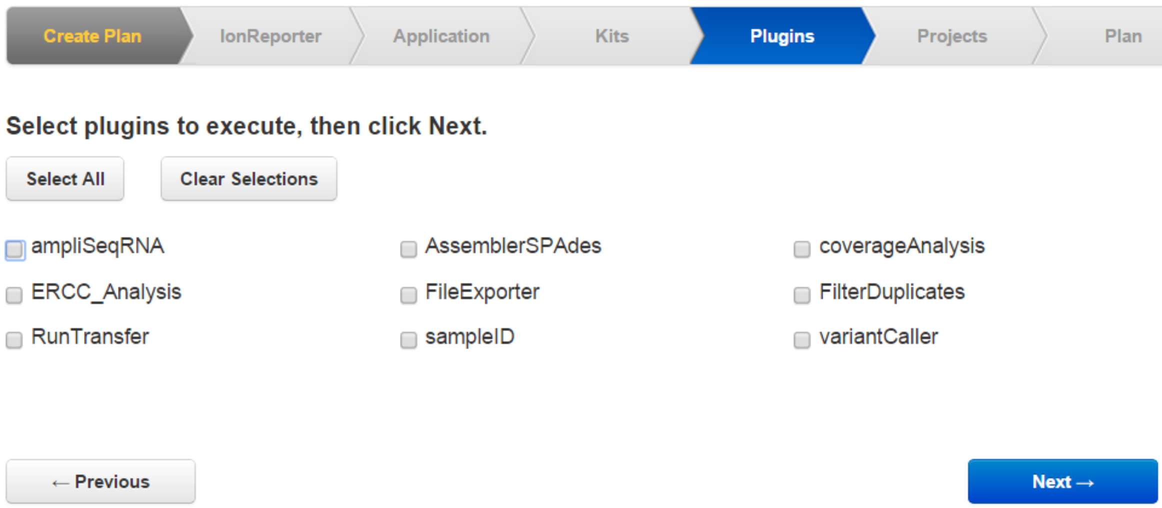
Marking duplicate reads is not appropriate for Ion AmpliSeq™ data, because many independent reads are expected to share the same 5′ alignment position and 3′ adapter flow as each other. Marking duplicates on an Ion AmpliSeq™ run risks inappropriately flagging many reads that are, in fact, independent of one another.

Plugins step in the workflow bar

Use the **Plugins** step in the workflow bar to select plugins to include in a Planned Run or a Planned Run template. Plugins expand the analysis capabilities of Torrent Suite™ Software. Plugins added in this step run automatically during the sequencing run, and plugin results appear in the run report. The plugin results are added to the run report and can be used for various purposes. You can set a plugin to run automatically after every run if you add the plugin to the Planned Run or Planned Run template. You can also configure the plugin, if necessary.

- The list of available plugins depends on which plugins are active on the Ion Torrent™ Server that is connected to the instrument you use. Plugins that are installed, configured, and enabled on your Ion Torrent™ Server are active, and are available in this list.
- The IonReporterUploader plugin does not appear in this list because it is configured in the IonReporterUploader step.
- When you enable some plugins, such as the variantCaller and coverageAnalysis plugins, a **Configure** link appears for that plugin. For information on configuring each plugin, see “Preinstalled plugins” on page 130.
- If you select the variantCaller plugin and the Planned Run does not use a predefined configuration, you must click the **Configure** link to configure the plugin before you can proceed to the next step or save the Planned Run or Planned Run template. For details about how to configure the variantCaller plugin, see Chapter 8, “Variant calls in Torrent Suite™ Software”.

1. In the **Plugins** step in the workflow bar, select the plugins to execute in the Planned Run.



2. Click **Next** in the workflow bar to continue with the Planned Run or with Planned Run template creation.

Projects step in the workflow bar

Use the **Projects** step in the workflow bar to select one or more projects to receive data from the completed run. When you create a Planned Run template, select the projects to receive data from every run that is created from this template.

For more information about projects, see "View result sets in a project" on page 110.

1. In the **Projects** step in the workflow bar, in the scrolling list, select the checkbox next to each project name that is to receive data from the completed run.

   The list includes all projects that are created and available on the Ion Torrent™ Server.

2. (Optional) Search for or add projects.

Item	Selection
Search for projects	To search for projects, enter a case-sensitive search term or partial search term in the search box below the list, then click **Search**.
Add projects	To create a new project or projects, click **Add Project**, then enter a name, or enter multiple names that are separated by commas.
Remove added projects	To remove an added project or projects, click **Remove New Projects**.

3. Click **Next** in the workflow bar to continue with the Planned Run or with Planned Run template creation.

Plan step in the workflow bar

Use the **Plan** step in the workflow bar to review the plan and enter or select additional information to complete the Planned Run.

1. In the **Plan** step in the workflow bar, enter or select the required information. The available options depend on your sequencing application.

Item	Selection
Run Plan Name	Enter a name for the Planned Run.
Analysis Parameters	Select **Default** (recommended) to accept default analysis parameter settings. Advanced users can select **Custom** to edit analysis parameters. For more information, see “Copy an analysis parameter set” on page 57.
Default Reference & Bed Files	
Reference Library	Select the reference library file that is appropriate for the sample. Based on the application, you may have to select separate DNA, RNA, and Fusions reference library files.
Target Regions	Select the target regions BED file that is appropriate for the sample. Based on the application, you may have to select separate DNA and fusions target regions files. Check with your local Field Service Engineer for updates to verify that the most current files are being used. For BED file installation instructions, see “Upload a target regions file” on page 248.
Hotspot Regions	Select the hotspots (BED or VCF) file that is appropriate for your sample. For BED file installation instructions, see “Upload a hotspots file” on page 250.

2. Based on your application, select the **Use same reference & BED files for all chips/barcodes** checkbox if you use the same reference sequences and target regions and hotspots BED files across all chips/barcoded samples that are included in the Planned Run. If you use different reference sequences or BED files for one or more of your chips/barcoded samples, clear the **Use same reference & BED files for all chips/barcodes** checkbox.

3. *(Optional)* For DNA and Fusions application, select the **Same sample for DNA and Fusions** checkbox when you use the same sample for both DNA and Fusions libraries.

4. Based on your application, complete the following.

Item	Selection
Number of barcodes	For barcoded samples, enter the number of barcodes to be used in this run, then click to the right of this option. Alternatively, click the first barcode, then click , and all subsequent barcodes are numbered from the first barcode value.
Number of chips	For non-barcoded samples, enter the number of chips to be used in this run, then click .

The **Samples Table** is populated with barcode information or the chip number for each sample.

5. *(Optional)* Select an option next to the sequencing application (such as **Oncology** or **Pre-implantation Genetic Screening**) to populate required sample information into the **Samples Table**.

6. Complete the **Samples Table**.

   - Save the samples table to a CSV file, fill out required sample information, then upload the samples table file to fill the **Samples Table**.
     a. Click **Save Samples Table** to save the CSV file to your computer.
     b. Edit the CSV file. Enter required sample information into the appropriate columns, then save the CSV file to your computer.
     c. Click **Load Samples Table**, then select an appropriate CSV file that contains sample information specific for this Planned Run.
     d. Click **Load** to fill the **Samples Table** in Torrent Suite™ Software with sample information that is supplied by the CSV file.
   - Alternatively, manually enter sample information into the **Samples Table**. The following table shown an example of what can be shown in the **Samples Table**. The options that are available depend on your sequencing application.

Item	Selection
Barcode	For barcoded samples, select a barcode.
Control Type	Click the **Control Type** column heading to expand the column, then select the control type.
Case ID	Enter an optional case ID for each sample.
Sample ID	Enter an optional sample ID for each sample.
Sample Description	Enter an optional sample description for each sample.
DNA/Fusions	For DNA and Fusions application, select **DNA** or **Fusions** for each sample.
Reference	If you use different reference and BED files for one or more samples, click the **Reference** heading to expand the sections. Then select the reference, target regions, and hotspots files for each sample.
Annotations	Click the **Annotations** column heading to expand the annotation information that is specific for the application (for example, cancer type or Embryo ID), then complete the required information.
Sample Tube Label	Scan or enter the barcode of the Ion Chef™ Library Sample Tubes to be used in the run.
Chip Barcode	Scan or enter the barcode of the Ion Chef™ Library Sample Tubes to be used in the run.
Sample Collection Date	Enter the date that the blood sample was drawn.
Sample Receipt Date	Enter the date that the laboratory received blood sample.

(continued)

Item	Selection
Ion Reporter workflow	Select the Ion Reporter™ Software analysis workflow that is specific for the run. If you do not see the analysis workflow, select the **Show All Workflows** checkbox in the column heading. This option is available if Ion Reporter™ Software is connected.
Relation	Select the sample relationship group. This option is available if Ion Reporter™ Software is connected.
Gender	Select **Male**, **Female**, or **Unknown**. This option is available if Ion Reporter™ Software is connected.
IR Set ID	Set this option to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same **IR Set ID** are considered related samples and are launched in the same analysis (for example, normal sample and its corresponding tumor sample). Do not give unrelated samples the same **IR Set ID** value even if the value is zero or blank. This option is available if Ion Reporter™ Software is connected.

7. *(Optional)* In **Add a note**, add a note about the Planned Run.

8. *(Optional)* If you use LIMS metadata, in **Add LIMS Meta Data**, enter the text that is associated with the Planned Run.

9. *(Optional)* In the **Monitoring Thresholds** pane, adjust **Bead Loading (%)**, **Key Signal (1-100)**, and **Usable Sequence (%)** minimum thresholds for your Planned Run.

   If monitoring thresholds are not met, the run is still processed and an alert message is shown on the run report after analysis is complete.

10. When you are finished with your selections, scroll to the bottom of the screen, then click **Plan Run**.

    The Planned Run is added to the **Planned Runs** list on the **Planned Runs** screen.

Review Planned Run settings

You can review the Planned Run settings of a completed run.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a run of interest.
   The report for that run opens.
2. Click **Report Actions ▸ Review Plan**.
   A Review Plan summary lists the details of the Planned Run. The subcategory details are different based on different runs.

Category	Subcategory
Review Plan	The name of the completed run.
Report	The name of the report that was generated for the completed run.
Application	**Research Application**
	Research Category
	Sample Grouping
	Target Technique
	Sample Set
Kits	**Sample Preparation Kit**
	Library Kit
	Library Key
	3' Adaptor
	Flow Order
	Template Kit
	Templating Protocol
	Sequencing Kit
	Control Sequence
	Library Read Length
	Flows
	Chip Type
	Barcode Set
	Mark as Duplicates Reads
	Base Calibration Mode
	Enable Realignment

(continued)

Category	Subcategory
Kits	Manifold Heater **Note:** A value of **Default** means that this setting is not used. Otherwise, the value for this setting is **Dynamic**.
Monitoring	Bead Loading (%)
	Key Signal (1-100)
	Usable Sequence (%)
Reference	Reference Library
	Target Regions
	Hotspot Regions
	Fusions Reference Library
	Fusions Target Regions
Plugins & Output	Plugins
	Projects
	Uploaders
Notes	
LIMS Meta Data	
Plan Meta Data	TS version when created
	Plan created via
Barcodes & Samples	Sample Tube Label
	Chip Barcode
	A table of information about the barcode used in the run. Table columns may include the following information. Barcode Sample Name Sample ID DNA/RNA Reference Target Regions Hotspot Regions Case ID Control Type
Analysis Parameters Used (Default)	BeadFind Args
	Analysis Args
	Pre-BaseCaller Args for calibration
	Calibration Args

(continued)

Category	Subcategory
Analysis Parameters Used (Default)	BaseCaller Args
	Alignment Args
	IonStats Args
History	A log of system actions taken during the run. Table columns may include the following information. Date User Log Parameter Old Value New Value

3. After reviewing the information, click **Close**.

Import panel files and parameters from AmpliSeq.com

You can import panel files and parameters from **AmpliSeq.com** for an Ion AmpliSeq™ On-Demand Panel, an Ion AmpliSeq™ Made-to-Order Panel, or an Ion AmpliSeq™ Community Panel design. Each package includes a primer pool results panel file, hotspot BED files, target region files, and JSON parameters files that are required to produce your sequencing results. You can import the file package directly to the Ion Torrent™ Server, in which the files are available immediately for use in Torrent Suite™ Software. Before you can import, you must link your Ion Torrent™ Server account with your AmpliSeq.com account. Alternatively, you can manually upload panel files that you have saved to a storage location.

For an Ion AmpliSeq™ Ready-to-Use Panel and an Ion AmpliSeq™ Community Panel, parameter settings that are optimized for the variantCaller plugin are included in your new template. You can configure the variantCaller plugin with these settings when you create the Planned Run, if desired. For details, see "variantCaller plugin configuration manually" on page 186.

Human, animal, and plant reference sequence BED files are also available for import from **AmpliSeq.com**.

For more information about Ion AmpliSeq™ Designer, see the *Ion AmpliSeq™ Designer: Getting Started User Guide* (Pub. No. MAN0010907).

Link your Ion Torrent™ Server account to AmpliSeq.com

To import a Planned Run template from AmpliSeq.com into Torrent Suite™ Software, you must first link your Ion Torrent™ Server account to AmpliSeq.com. Linking your Ion Torrent™ Server account is a one-time requirement.

Before you can link your account, you must generate an access code for AmpliSeq.com.

1. Generate an access code for AmpliSeq.com.
   a. Go to **AmpliSeq.com**, and sign into your account.
   b. Click **My Account ▸ Manage Access Code**
   c. In the **Manage Access Code** dialog box, click **Generate** to receive an access code. If you already have an access code, enter it into the **Access Code** field. Select an **Access Code Expiration** in the dropdown list.
2. In Torrent Suite™ Software, click **(Settings) ▸ Accounts**.
3. Click **Link to AmpliSeq**.
   Scroll down to view the link button, if needed.

4. Enter your AmpliSeq.com username and access code, then click **Save**.

Your Ion Torrent™ Server account is now linked, and import of panel information from AmpliSeq.com is enabled.

To unlink your Ion Torrent™ Server account, click **Unlink username@domain.com**.

Import Planned Run template parameters

You can import Planned Run template parameters directly from **AmpliSeq.com**.

Before you can import files directly from AmpliSeq.com, you must link your Ion Torrent™ Server account to your AmpliSeq.com account. For more information, see "Link your Ion Torrent™ Server account to AmpliSeq.com" on page 55. If you want to import files that are saved to a hard drive or other storage location, see instead "Manually upload panel files in Torrent Suite™ Software" on page 56.

1. In the **Plan** tab, in the **Templates** screen, click **AmpliSeq.com** .
2. In the next screen, select the panel type that you need, then find a panel or panels from the list of available panels.
   - The **DNA** tab includes exome panels and the **RNA** tab includes fusion panels.
   - Some panels do not have optimized variantCaller plugin parameters available for all chips and sequencers. A warning identifies the panels for which optimized variantCaller plugin parameters have not been developed for the selected chip type.
   - If you have ordered Ion AmpliSeq™ Made-to-Order Panel designs, a list of the panel files for import appears on this screen.
   - If you have downloaded a ZIP archive for a panel from AmpliSeq.com to your computer, you can also create a Planned Run template by manually uploading the archive on the right pane of this screen.
3. *(Optional)* Click the columns to sort the rows alphabetically.
4. Click **Import**.
5. In the **Ampliseq Panel Import** dialog box, click **Import Selected**.
6. Refresh your browser to track the progress of the import. When the **Status** column shows **Complete**, return to the **Templates** screen.

The new template is listed on the **Templates** screen.

Manually upload panel files in Torrent Suite™ Software

If you import community or custom Ion AmpliSeq™ Pharmacogenomics Research Panel files from **AmpliSeq.com** and save the files to a storage location, you must manually upload them into Torrent Suite™ Software before you can access them.

Use this procedure only to import files from AmpliSeq.com that were previously saved to a hard drive or other storage location. This procedure is not necessary if you import panel files directly from AmpliSeq.com.

1. Sign in to Torrent Suite™ Software.
2. In the **Plan** tab, click **Templates**.
3. Click **Upload ▸ Upload Design/Panel Zip Archive**.

4. Complete the **Import Panel from Zip Archive** dialog box.
   a. Click **Select File**, then browse to the compressed panel file.
      The instrument type and chip type are automatically selected, based on the configuration file that is included in the panel file.
   b. Click **Upload Panel from Zip Archive**.
   c. Close the upload dialog box.
5. To check the status of the upload, click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Upload History**.
6. Verify that the following templates that are included in the panel files are shown in the **Templates** screen.
   - `PGx_20211109_520_parameter.json`
   - `PGx_20211109_530_parameter.json`
   - `PGx_20211109_540_parameter.json`

Copy an analysis parameter set

You can copy an existing analysis parameter set, and then customize the settings and save it as a new parameter set. Use this procedure if you need a unique analysis parameter set for an analysis that other users do not need to use in their runs. Copying an analysis parameter set allows a parameter set to remain intact for other users.

1. Click ⚙ **(Settings)** ▸ **Analysis Parameters**.
   In the **Analysis Parameters** screen, factory parameters are denoted by "Ion Torrent" in the **Source** column.
2. To filter the parameter sets by chip type, select your chip type from **All Chips**.
3. Identify the parameter set that you want to copy, then in the row for that set, then click ⚙ **(Actions)** ▸ **Copy**.
4. In the **Copy Analysis Parameters** dialog box, enter a parameter name and description, make any changes, then click **Save**.
   Your new analysis parameter set is available in the **Analysis Parameters** table. The **Source** column lists the name of the user who created it.
5. Click ⚙ **(Actions)** ▸ **View** in the row of the analysis parameter set to view the details for that parameter set in the list.

6. You can select the user-defined analysis parameter set for use in a Planned Run or reanalysis of a completed run.

Option	Description
Create a Planned Run or a user-defined Planned Run template	In the **Plan** tab of the workflow bar, under the **Analysis Parameters** section, click **Details+ ▸ Custom**, then select the parameter from the list. You must first specify a chip type for the Planned Run (under **Kits** in the workflow bar) before you can select the parameter set.
Reanalyze a completed run	For details, see “Reanalyze a run” on page 116.

Create Planned Runs

You can create a Planned Run in two different ways.

- Create a Planned Run with sample sets.
- Create a Planned Run with a template.

Plan by sample set

You can define samples and preselect the attributes that you want to associate with each sample before you start to plan templating and sequencing runs. You can then organize samples into sample sets that are available to select and reuse when you create a Planned Run. This approach, which is known as plan by sample set, can save time when you plan your instrument runs if:

- You want to create a Planned Run with samples that use one- or two-pool libraries.
- Your samples include many attributes.
- You want to use the same sample sets for many instrument runs.
- Your laboratory assigns tasks such as planning and defining sample attributes to individuals who have specific areas of expertise.
- Your samples include attributes for use with Ion Reporter™ Software.

  For example, if you select the sample set group type **Trio**, only Trio analysis workflows are available to choose when you configure a Planned Run for use with Ion Reporter™ Software.
- You want to set up multisample analyses in Ion Reporter™ Software.
- Your Ion Torrent™ Server is connected to a LIMS.

Create a Planned Run for a 2-pool sample set

When you use the Oncomine™ Comprehensive Assay Plus panel, you can combine multiple sample sets for use in a single Planned Run. When you combine multiple sample sets, you can extend the number of samples that you use in a sequencing run. Each sample set can contain up to 8 samples. You can use all samples that are included in the sample sets to be combined or you can divide the samples into one or two pools for the sequencing run.

All sample sets that are combined in a Planned Run must use the same library preparation protocol. For example, a planned run that uses 2 pools must use sample sets that are configured to use a multiple pool library preparation protocol.

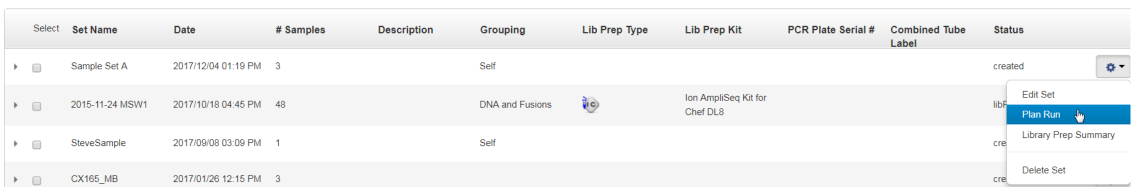
For more information, see *Oncomine™ Comprehensive Assay Plus User Guide*, Pub No. MAN0018490.

Create a Planned Run with sample sets

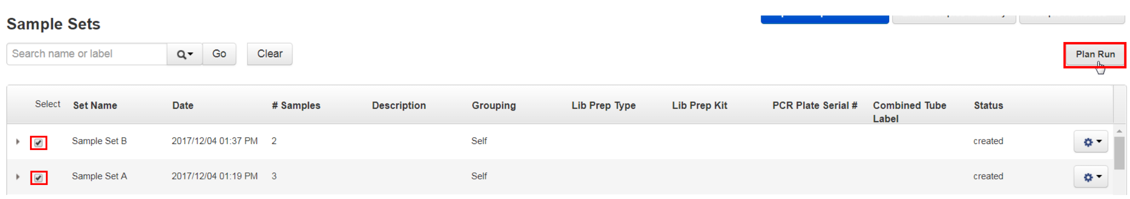
If you set up your samples in sample sets before you plan an instrument run, you can add one or more sample sets to your Planned Run.

Note that a sample set that uses an Ion AmpliSeq™ library preparation kit must also use the corresponding barcode kit that comes with the library preparation kit.

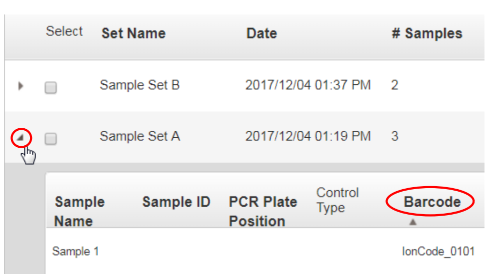
1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** screen, select one or more sample sets to add to the Planned Run.
   - To plan a run that uses a single sample set, click **(Actions)** ▸ **Plan Run** in the row of the sample set.



   - To plan a run that uses multiple sample sets, select the checkboxes next to the sample sets that you want to add to the Planned Run, then click **Plan Run**.



IMPORTANT! Ensure that all selected sample sets use the same barcode kit. To verify the barcode kit that is used, click the **Expand** link to the left of the select box to expand the sample set entry to view the details.



The **Select a Run Template to apply to this experiment** dialog box lists Planned Run templates that support your sample set.

3. In the **Plan Run from Sample Set** dialog box, select a run template to use for the experiment, then click **Plan Run**.

   If you do not see the template that you are looking for, select **Show All Templates**, then look again for the template.

   The **Create Plan** workflow opens to the **Kits** step with the sample sets that you selected:

4. In the **Kits** step, enter or select the required information.

   For more information, see “Kits step in the workflow bar” on page 44.

5. In the **Barcoding** step in the workflow bar, enter or select the required information. Based on your sequencing application, options can vary.

Item	Selection
Analysis Parameters	Select **Default** (recommended) to accept default analysis parameter settings. Advanced users can select **Custom** to edit appropriate analysis options. For more information, see “Command Line Args (Advanced) screen” on page 62.
Reference Library	Select the reference library file that is appropriate for your sample. Based on your application, you may have to select separate DNA, RNA, and fusions reference library files.
Target Regions	Select the target regions BED file that is appropriate for your sample. Based on your application, you may have to select separate DNA and fusions target regions files. Ensure that you use the current BED or VCF files.
Hotspots	Select the hotspots (BED or VCF) file that is appropriate for your sample. Ensure that you use the current BED or VCF files.

6. Select the **Use same reference & BED files for all barcodes** checkbox if you use the same reference, target regions, and hotspots files across all barcoded samples in the Planned Run.

   If you use different reference and/or BED files for one or more of your barcoded samples, deselect the **Use same reference & BED files for all barcodes** checkbox.

7. In **Sample Tube Label**, scan or enter the barcode of the Ion Chef™ Library Sample Tubes to use in the run.

8. In **Chip Barcode**, scan or enter the barcode that is printed on the chip to use for this run.

9. Complete the samples table.

   - You can save the samples table to a CSV file, complete the required sample information, and then upload the samples table to populate the **Samples Table**.
     a. Click **Save Samples Table** to save the CSV file to your computer.
     b. Edit the CSV file by entering required sample information into the appropriate sample information columns, then save the CSV file to your computer.
     c. Click **Load Samples Table**, then select an appropriate CSV file that contains sample information specific for this Planned Run.
     d. Click **Load** to populate the samples table with sample information supplied by the CSV file.
   - Alternatively, you can manually enter sample information into the samples table. Based on your sequencing application, options can vary.

Item	Selection
Barcode	For barcoded samples, select a barcode.
Sample Name	Select a sample that is a part of the selected sample set or sets.
Control Type	Click the **Control Type** column heading to expand the column, then select the control type from the dropdown list.
Sample ID	Review the sample ID information for each sample. To modify **Sample ID**, edit the sample set as described in “Edit a sample set” on page 30.
Sample Description	Review the sample description for each sample. To modify **Sample Description**, edit the sample set as described in “Edit a sample set” on page 30.
DNA/Fusions	For DNA and fusions application, select **DNA** or **Fusions** for each sample.
Reference	If different reference and BED files are used for one or more samples, click the **Reference** column heading to expand the sections, then select reference, target regions, and hotspots files for each sample.
Annotations	Click the **Annotations** column heading to expand the annotations that are specific for your application (for example, cancer type or embryo ID), then complete the required information.
Ion Reporter workflow	Select the Ion Reporter™ Software analysis workflow that is specific for your run. If you do not see your analysis workflow, select the **Show All Workflows** checkbox in the column heading.
Relation	Select sample relationship group.
Gender	Select **Male**, **Female**, or **Unknown**.
IR Set ID	Set the **IR Set ID** to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same **IR Set ID** are considered related samples and are launched in the same analysis (for example, normal sample and its corresponding tumor sample). Do not give unrelated samples the same **IR Set ID** value even if the value is zero or blank.

10. Click **Next** to continue the steps to create the Planned Run.

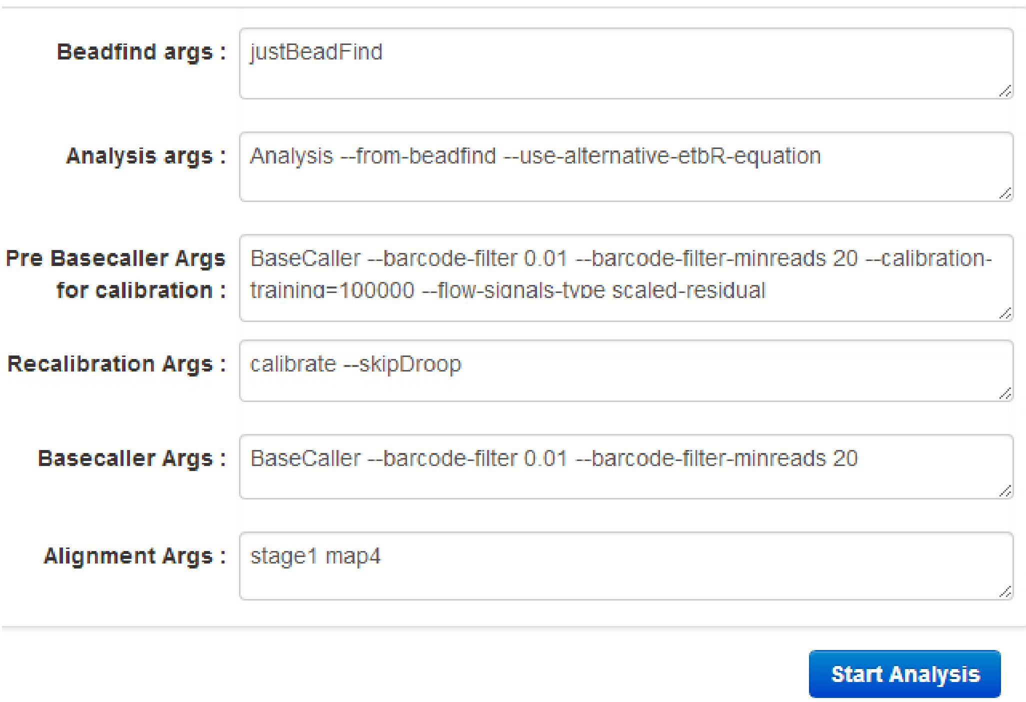
    The software takes you to the next step in the workflow bar.

11. Click **Save & Finish**.

The Planned Run is added to the Planned Runs table and can be used in an instrument run.

Command Line Args (Advanced) screen

When planning a sample set in the **Barcoding** step in the workflow bar, you can click **Custom**, then use the **Advanced Options** screen to customize advanced arguments for analysis parameters.



Argument	Description
Beadfind args	Beadfind module command line arguments. These arguments should not be modified unless you receive instructions from Technical Support.
Analysis args	Analysis command line arguments. These arguments should not be modified unless you receive instructions from Technical Support.

(continued)

Argument	Description
Pre Basecaller args for calibration	BaseCaller command line arguments. For information on `--barcode-mode`, `--barcode-cutoff`, and `--barcode-filter` parameters, see "BaseCaller module arguments" on page 282. This field is used only if a Base Calibration Mode other than 'No Calibration' is used. Other BaseCaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support.
Recalibration Args	Recalibration command line arguments.
Basecaller Args	BaseCaller command line arguments. For information on `--barcode-mode`, `--barcode-cutoff`, and `--barcode-filter`, see "BaseCaller module arguments" on page 282. Other BaseCaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support.
Alignment Args	Arguments for the TMAP aligner. This replaces the TMAP Args setting that appears in previous software releases.

Create a Planned Run for mixed samples with a Planned Run template

To plan a run for DNA and Fusion sample pairs and several individual Fusion or DNA samples, start with a DNA and Fusions Planned Run template. Then alter the template to accommodate single samples on the same chip. The following example is the procedure for creating a mixed sample set consisting of two sample pairs, one DNA-only sample, and two Fusion-only samples.

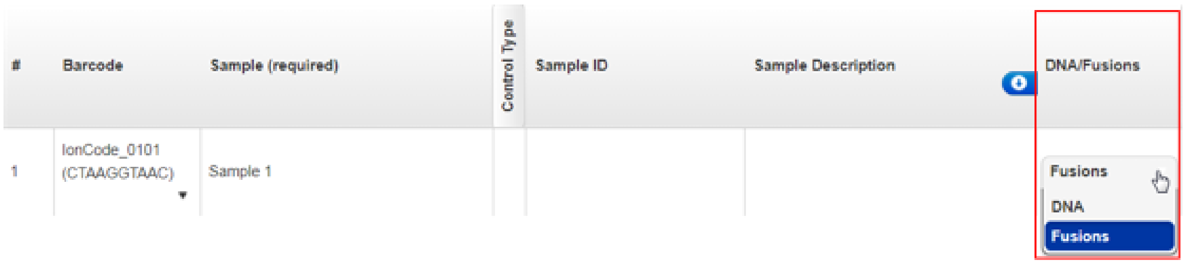
1. In the **Plan** tab, click **Templates**, then click the **DNA and Fusions** research application.
2. Identify a DNA and Fusions template for the instrument system that you use, then in that row click ⚙ **(Actions)** ▸ **Copy**.
3. Enter a **Template Name** and select a DNA Target Regions file, then click **Copy Template**.
4. Return to the **Templates** screen, click the **DNA and Fusions** research application, then select the copied template.
5. In the **Ion Reporter** step of the workflow bar, select an Ion Reporter™ Software account and analysis workflow, ensure that the **DNA and Fusions** Sample Grouping is selected, then click **Next**.
6. Ensure that the **DNA and Fusions (Single Library)** research application and the **AmpliSeq HD DNA and Fusions (Single Library)** target technique is selected, then click **Next**.
7. Click **Plan** in the workflow bar.
8. *(Optional)* The optimal number of barcodes to use with the selected panel/template is set as the default, but you can change it. If necessary, enter the number of barcodes, then click ✓.

9. *(Optional)* Clear the **Same sample for DNA and Fusions** option. This option is available for DNA and fusion applications.

10. Rename the samples if desired. To rename the samples, in the **Samples** table, in the **Sample Name** column, click the sample name, then enter a new name.

11. *(Optional)* Change the DNA/Fusions selections to match samples.

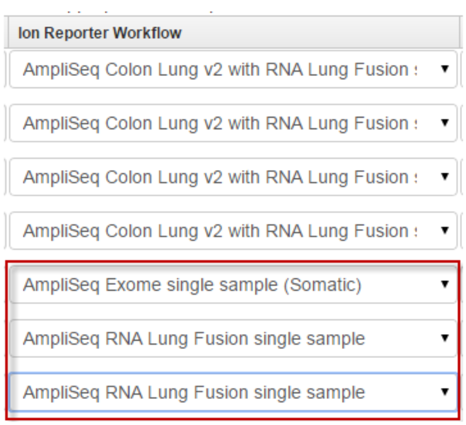
    To change the DNA/Fusions selection for a sample, navigate to the DNA/Fusions column in the **Samples Table**, then select **DNA** or **Fusions** in the row of the sample.



12. *(Optional)* Select a **Cancer Type** for each sample.

13. Select the appropriate Ion Reporter™ Software analysis workflows.

    To select an analysis workflow, click the existing selection in the **Samples Table**, then select an analysis workflow from the list.



14. In the **Samples Table**, select a **Relation** and **Gender** , then click **Plan Run** at the bottom of the screen.

    For more information, see "Create a Planned Run with sample sets" on page 59.

Create a Planned Run with multiple analysis workflows

You can launch multiple Ion Reporter™ Software analysis workflows for use with a single sample when you use preinstalled Planned Run templates for use with the **Immune Repertoire** research application. This topic provides an example of creating a Planned Run with multiple analysis workflows for the **Immune Repertoire** research application.

1. In the **Plan** tab, click **Templates**, then select **Immune Repertoire** from the left navigation menu.
2. In the **Template Name** column of the templates table, click the template name. For example, Ion AmpliSeq™ Immune Repertoire Assay Plus - TCRB for S5.

   Alternatively, you can click **Favorites**, then click the template name, if the template is in the favorites list.

   The **Create Plan** workflow bar opens to the **Plan** step. The **Run Plan Name** is prepopulated with the template name.
3. In **Run Plan Name**, enter a unique name for the Planned Run. Enter other information in the **Plan** step as required.

   For more information, see "Plan step in the workflow bar" on page 49.
4. Click the **Ion Reporter** step in the workflow bar, then select the **Ion Reporter Account** to transfer output files to in Ion Reporter™ Software.

   The Ion Reporter™ Software account that is added to the Planned Run is used to view and further analyze the sequencing run results in Ion Reporter™ Software.

   If the Ion Reporter™ Software account is not configured, click **Configure** to add another account. For more information, see "Set up an account for IonReporterUploader plugin" on page 221.
5. Select a **Sample Grouping** that corresponds to the sample relationship in Ion Reporter™ Software.

   The **Existing Workflow** list updates to show only Ion Reporter™ Software analysis workflows that are appropriate for the sample.
6. Under **Ion Reporter Upload Options**, select **Automatically upload to Ion Reporter after run completion**.

   Run results are automatically uploaded to Ion Reporter™ Software when a run is complete. When you select multiple analysis workflows, you can use multiple analysis workflows with a single immune repertoire sample.

   Note: An analysis that uses the selected analysis workflows is launched immediately after the run. Successful analyses are then available to you when you sign in to Ion Reporter™ Software with the account used in the setup.

   ---
7. In the **Existing Workflow** list, select the Ion Reporter™ Software analysis workflows to use with the sample for automatic launches in Ion Reporter™ Software.

   When a sample and analysis workflows are launched in Ion Reporter™ Software, successful analyses are available for the account and organization that you selected.

8. *(Optional)* To create a new analysis workflow, click **Create New Workflow** to open Ion Reporter™ Software in a new browser window. In Ion Reporter™ Software, create a new analysis workflow, then save it. When you return to Torrent Suite™ Software, refresh the browser. You can then select the newly created analysis workflow in the **Existing Workflow** list.

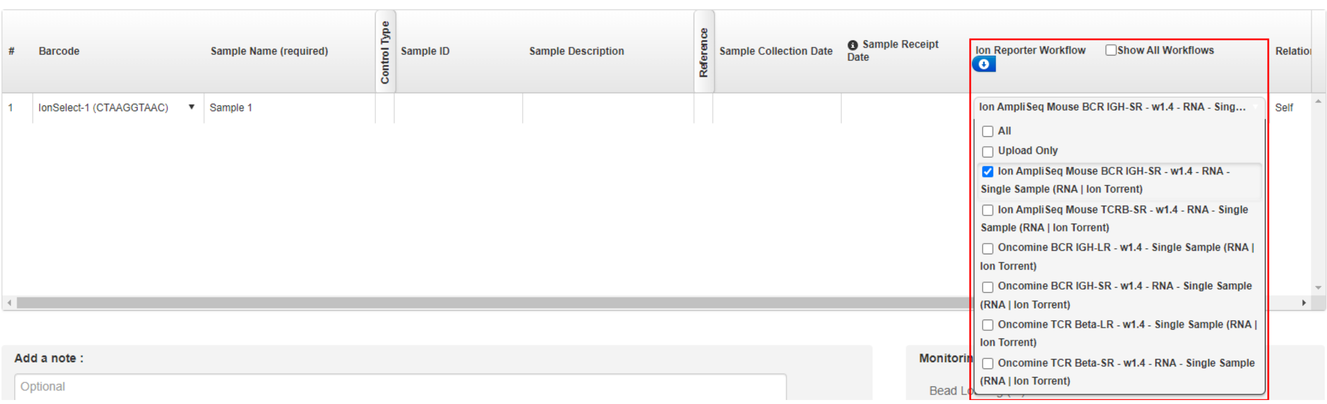
9. Click **Next** to continue with the Planned Run creation.

   The software takes you to the next step in the workflow bar. Use other steps in the workflow bar to enter or change settings if needed. For more information, see "Steps in the workflow bar" on page 39.

10. In the **Plan** step, manually enter sample information into the **Samples Table**. Use the **Ion Reporter workflow** option to select multiple analysis workflows for use with a single immune repertoire sample.

    a. *(Optional)* Deselect the **Show All Workflows** checkbox in the column heading to narrow the list of analysis workflows to only the analysis workflows for use with the nucleic acid type for the sample barcode.

    b. Click the analysis workflow name under **Ion Reporter workflow**.



    Available analysis workflows are listed under **Ion Reporter workflow**.

    c. For each barcode listed in the **Samples Table**, select an option for use to launch multiple analysis workflows with a single immune repertoire sample.

Ion Reporter analysis workflow options for use with immune repertoire Planned Runs

Option	Selection
All	Use this option to select all available analysis workflows for use with the single sample immune repertoire analysis.
Upload Only	*Do not use* this option for Planned Runs that launch multiple analysis workflows.
Analysis workflow	Select one or more analysis workflows for use with the single sample analysis.

The settings are updated in the **Summary** pane. You can also use the information in this pane to review the settings that are predefined by the template.

After you select the analysis workflows for use with the Planned Run, the table lists the number of analysis workflows that are selected.

11. In **Sample Tube Label** and **Chip Barcode**, scan or enter the barcode information.

12. Manually enter other sample information into the **Samples Table**.
The available options are based on the selected research application.

Samples Table options

Item	Selection
Barcode	For barcoded samples, select a barcode.
Sample Name	Select a sample that is a part of the selected sample set or sets.
Control Type	Click the **Control Type** column heading to expand the column, then select the control type.
Sample ID	Enter an optional sample ID for each sample.
Case ID	Enter the option case ID.
Sample Description	Enter an optional sample description for each sample.
DNA/RNA	For DNA and RNA applications, select **DNA** or **RNA** for each sample.
Reference	If you use different reference and BED files for one or more samples, click the **Reference** heading to expand the sections. Then select the reference, target regions, and hotspots files for each sample.
Sample Tube Label	Scan or enter the barcode of the sample tubes to be used in the run.
Sample Receipt Date	Enter the date that the laboratory received blood sample. Click the cell, then select a date in the calendar or enter a date in `YYYY-MM-DD` format.
Ion Reporter workflow[1]	Select the Ion Reporter™ Software analysis workflows for use with the Planned Run. To narrow the list of analysis workflows to only the analysis workflows for use with the immune repertoire analysis, deselect the **Show All Workflows** checkbox in the column heading. This option is available if Ion Reporter™ Software is connected.
Relation	Select sample relationship group. This option is available if Ion Reporter™ Software is connected.
Gender	Select **Male**, **Female**, or **Unknown**. This option is available if Ion Reporter™ Software is connected.
Population	The super population code assignment for a sample, as defined by the human 1000 genomes project (**https://www.internationalgenome.org/faq/which-populations-are-part-your-study/**). The population is relevant to the analysis of samples by the TCRB-LR assay workflow in Ion Reporter™ Software. The TCRB-LR analysis workflow produces a haplotype group assignment for samples that have a population attribute of "European".

Samples Table options *(continued)*

Item	Selection
Mouse Strains	The name of a mouse strain. Choose from a select number of the most common strains. In Ion Reporter™ Software, the selected mouse strain does not affect Ion Reporter™ Software analysis workflows.
IR Set ID	Set this option to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same **IR Set ID** are considered related samples and are launched in the same analysis (for example, normal sample and its corresponding tumor sample). Do not give unrelated samples the same **IR Set ID** value even if the value is zero or blank. This option is available if Ion Reporter™ Software is connected.

[1] See step 10 in this procedure for more information.

13. *(Optional)* Click **Save Samples Table** to save changes to a file that can be manually updated, then imported the file into the software.

14. When you have completed the selections, review the settings in the **Summary** pane, then click **Plan Run** at the bottom of the **Plan** step.
    The Planned Run is added to the list on the **Planned Runs** screen. A **Run Code** is generated as a short cut to identify the Planned Run.

Create multiple Planned Runs

To facilitate running the same sequencing run multiple times, you can use a template CSV file to create multiple Planned Runs at one time. The template CSV file for batch planning is available for download in the Torrent Suite™ Software.

Each column in the CSV file represents an individual Planned Run. Each row contains the run parameters for each individual Planned Run. You can add sample information to Planned Runs that is required for Ion Reporter™ Software analyses, including account, workflow, and workflow-related attributes such as gender, relation, and IR Set ID.

The latest CSV batch planning template file indicates the version of the template in the top row. This version number is required. When you download the template CSV file, the version is automatically included.

Create multiple Planned Runs for nonbarcoded libraries

You can create multiple Planned Runs with a template CSV file. To create multiple Planned Runs for individual nonbarcoded libraries, a single batch planning template CSV file is required.

1. In the **Plan** tab, click **Templates**.

2. In the row for the template that you want to use to create multiple Planned Runs, click ⚙ **(Actions)** ▸ **Plan Multiple** .

3. In the **Plan Runs from Plan Template** dialog box, enter the number of Planned Runs that you want to create, then click **Download Plan Runs File for batch planning**.

4. Save the batch planning template CSV file to your computer, then open the file.

	A	B	C	D	E
1	CSV Version (required)	2			
2	Plan Parameters	Plan 1	Plan 2	Plan 3	Plan 4
3	Template name to plan from (required)	System Generic Seq Template	System Generic Seq Template	System Generic Seq Template	System Generic Seq Template
4	Plan name (required)				
5	Sample (required)				
6	Sample Description				
7	Sample ID				
8	Sample preparation kit name				
9	Library kit name	Ion Xpress Plus Fragment Librar	Ion Xpress Plus Fragment Library	Ion Xpress Plus Fragment Library Kit	Ion Xpress Plus Fragment Library Kit
10	Templating kit name (required)	Ion PGM Template OT2 200 Kit	Ion PGM Template OT2 200 Kit	Ion PGM Template OT2 200 Kit	Ion PGM Template OT2 200 Kit
11	Templating Size				
12	Control sequence name				
13	Sequence kit name	Ion PGM Sequencing 200 Kit v2	Ion PGM Sequencing 200 Kit v2	Ion PGM Sequencing 200 Kit v2	Ion PGM Sequencing 200 Kit v2
14	Chip type (required)				
15	Library Read Length				
16	Flows	500	500	500	500
17	Sample tube label				
18	Bead loading %	30	30	30	30
19	Key signal %	30	30	30	30
20	Usable sequence %	30	30	30	30
21	Reference library	hg19	hg19	hg19	hg19
22	Target regions BED file				
23	Hotspot regions BED file				
24	Plugins	FileExporter;	FileExporter;	FileExporter;	FileExporter;
25	Project names				
26	Export				
27	Notes				
28	LIMS Meta Data				
29	Chip Barcode				
30	IR Account				
31	IR Workflow				
32	IR Relation				
33	IR Gender				
34	IR Set ID				

In this example, the template creates four Planned Runs with non-barcoded libraries.

5. Enter the required plan parameters for each Planned Run.
   - CSV Version
   - Template name to plan from (populated automatically)
   - Plan name
   - Samples CSV file name
   - Templating Kit name (populated automatically)
   - Chip type

6. Name, then save the CSV file.

7. In the **Plan** tab, in the **Templates** screen, click **Upload ▸ Upload Plans**.

8. In the **Upload Plan Runs** dialog box, click **Choose File**, select the edited CSV template, then click **Open**.

9. Click **Upload Plan Runs File for batch planning**.
   The system parses the files, then creates the Planned Runs.

Create multiple Planned Runs for barcoded libraries

To create multiple Planned Runs for multiplex sequencing of barcoded libraries, two batch planning template CSV files are required.

- A master CSV file that is used to specify the plan name, kits, chips, projects, and plugin selections.
- A sample CSV file for each Planned Run.

1. In the **Plan** tab, click **Templates**.
2. In the row for a barcoded template that you want to use to create multiple Planned Runs, click **(Actions)** ▸ **Plan Multiple**.
3. In the **Plan Runs from Plan Template** dialog box, enter the number of Planned Runs that you want to create, then click **Download Plan Runs file (ZIP) for batch planning**.
4. Unzip the downloaded archive file on your drive.
5. Open the tsPlan file appended with *master.csv*, enter the Template name, Plan name, Sample, and Chip type.

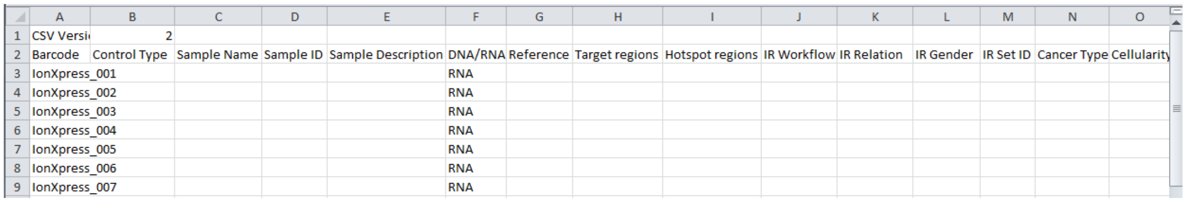
   The master file is prepopulated based on the template that is selected.

A	B	C	D
CSV Version (required)	2.3		
Plan Parameters	Plan 1	Plan 2	Plan 3
Template name to plan from (required)	TS5.22 OCAPlus 550 DNA	TS5.22 OCAPlus 550 DNA	TS5.22 OCAPlus 550 DNA
Plan name (required)			
Samples CSV file name (required)	tsPlan_20250313_102531_plan_01_samples.csv	tsPlan_20250313_102531_plan_02_samples.csv	tsPlan_20250313_102531_plan_03_samples.csv
sampleCollectionDate			
sampleReceiptDate			
Sample preparation kit name			
Library kit name	Ion AmpliSeq Library Kit Plus	Ion AmpliSeq Library Kit Plus	Ion AmpliSeq Library Kit Plus
Templating kit name (required)	Ion 550 Kit-Chef	Ion 550 Kit-Chef	Ion 550 Kit-Chef
Control sequence name			
Sequence kit name	Ion S5 Sequencing Kit	Ion S5 Sequencing Kit	Ion S5 Sequencing Kit
Chip type (required)	550	550	550
Library Read Length	200	200	200
Flows	500	500	500
Sample tube label			
Bead loading %	30	30	30
Key signal %	30	30	30
Usable sequence %	30	30	30
Reference library	hg19	hg19	hg19
Target regions BED file	/hg19/unmerged/detail/xxx.20241022.560.design	/hg19/unmerged/detail/xxx.20241022.560.designed.be	/hg19/unmerged/detail/YYY.20241022.560.designed.bed
Hotspot regions BED file			
DNA/RNA/Fusions	DNA	DNA	DNA
Plugins	coverageAnalysis;rapInit;sampleID;variantCaller;	coverageAnalysis;rapInit;sampleID;variantCaller;	coverageAnalysis;rapInit;sampleID;variantCaller;
Project names			
Export	IonReporterUploader;	IonReporterUploader;	IonReporterUploader;
Notes			
LIMS Meta Data			
Chip Barcode			
IR Account	IR1	IR1	IR1
IR Workflow	Example - 550 - w3.2 - DNA - Single Sample	Example - 550 - w3.2 - DNA - Single Sample	Example - 550 - w3.2 - DNA - Single Sample

   In this example, the template creates four barcoded Planned Runs.

6. Save the CSV file with a unique name.

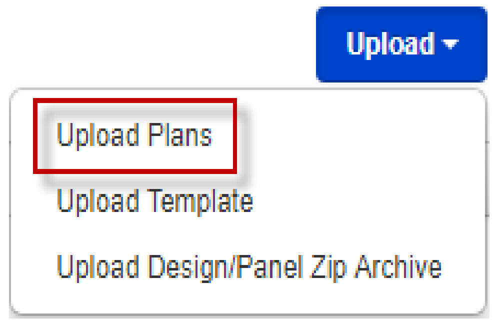
7. Open each tsPlan file appended with *samples.csv*, enter the sample parameter information for each barcoded sample, including Sample Name, Sample ID, Sample Description, and so on, then save each file.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	CSV Versi	2													
2	Barcode	Control Type	Sample Name	Sample ID	Sample Description	DNA/RNA	Reference	Target regions	Hotspot regions	IR Workflow	IR Relation	IR Gender	IR Set ID	Cancer Type	Cellularity
3	IonXpress_001					RNA									
4	IonXpress_002					RNA									
5	IonXpress_003					RNA									
6	IonXpress_004					RNA									
7	IonXpress_005					RNA									
8	IonXpress_006					RNA									
9	IonXpress_007					RNA									

8. Add the Master CSV template and all Sample CSV template files to a compressed (zipped) folder.

9. In the **Plan** tab, in the **Templates** screen, click **Upload ▸ Upload Plans**.



10. In the **Upload Plan Runs** dialog box, click **Choose File**, select the edited CSV template, then click **Open**.

11. Click **Upload Plan Runs File for batch planning**.
The system parses the files, then creates the Planned Runs,. The Planned Runs are then available for use in the **Plan** tab, in **Planned Runs** screen.

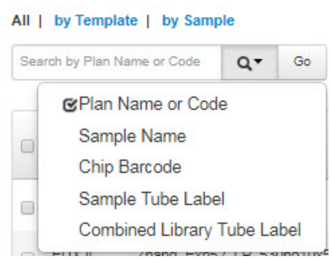
Manage Planned Runs

You can manage Planned Runs in various ways.

On the **Plan** tab, click **Planned Runs** then complete the selections for the following options.

Option	Selection
View all Planned Runs	Click **All** above the list.
View Planned Runs that were created from a Planned Run template	Click **by Template** above the list.
View Planned Runs that were created from a sample set	Click **by Sample** above the list.

(continued)

Option	Selection
Search the list	1. In the search box above the **Planned Runs** list, click (**Search By)**, then select the search type from the options.  2. Enter your search terms in the box, then click **Go**.
Filter the list	Select your filter criteria from the lists above the list of **Planned Runs**.
Clear all search and filter settings and display the complete list	Click **Clear All** above the list.
Sort the list	Click a column heading to sort the list by the information in that column. Only columns with bold headings are sortable.
View multiple pages in the list	Click the page number and scroll buttons below the list.
Display a list of the samples in the Planned Run	In the **Sample** column, place the pointer over the **(Info)** icon.
Display a list of projects that the run results will be transferred to	In the **Project** column, place the pointer over the **(Info)** icon.
Delete Planned Runs	Select the checkbox next to each Planned Run, then click **Delete Selected** below the list. Alternatively, click **(Actions)** in the Planned Run row, then select **Delete.** You cannot delete Planned Runs with a status of **Reserved.**
Edit a Planned Run	1. Click **(Actions)** in the Planned Run row, then select **Edit.** The **Edit Plan** workflow opens. 2. Edit the settings in any of the steps, then click **Update Plan** in the **Save** step.
Review all the settings in a Planned Run	Click **(Actions)** in the Planned Run row, then select **Review.**

(continued)

Option	Selection
Copy a Planned Run	1. Click ⚙ **(Actions)** in the Planned Run row, then select **Copy**. The **Copy Plan** workflow opens. 2. Edit the settings in any of the steps, enter the name of the new plan in **Run Plan Name**, then click **Copy Plan** in the **Save** step.
Transfer a Planned Run to another Ion Torrent™ Server	To transfer a Planned Run, see "Transfer a Planned Run to another Ion Torrent™ Server" on page 75.

Planned Runs list

The **Planned Runs** screen under the **Plan** tab lists Planned Runs that are ready to execute on an Ion Chef™ Instrument or a sequencer. Planned Runs are listed by date, and the list includes basic information about each run, including its status in the sequencing workflow. For more information, see "Planned Run status" on page 73.

The **Planned Runs** screen includes tools for searching, sorting, editing, copying, and deleting Planned Runs, and transferring them to another Ion Torrent™ Server.

Planned Run status

The Planned Run status appears in the **Status** column of the **Planned Runs** list.

When you select a Planned Run on a sequencer and start sequencing, the Planned Run is removed from the **Planned Runs** list.

You can change the status of an Ion Chef™ Instrument run under specific circumstances. For more information, see "Manually change Ion Chef™ Instrument run status" on page 74.

Status	Description
Pending	The Planned Run is available and ready for use by an Ion Chef™ Instrument. It is unavailable for sequencing until the Ion Chef™ Instrument run is complete. The software determines whether a run is **Pending** on an Ion Chef™ Instrument based on the template kit selection when you create the run.
Reserved	The Planned Run is in use by an Ion Chef™ Instrument, and is unavailable for use until the current Ion Chef™ Instrument run completes.
Planned	The Planned Run is available and ready for use by a sequencing instrument.
Voided	The Ion Chef™ Instrument run is canceled through the Ion Chef™ Instrument.

Manually change Ion Chef™ Instrument run status

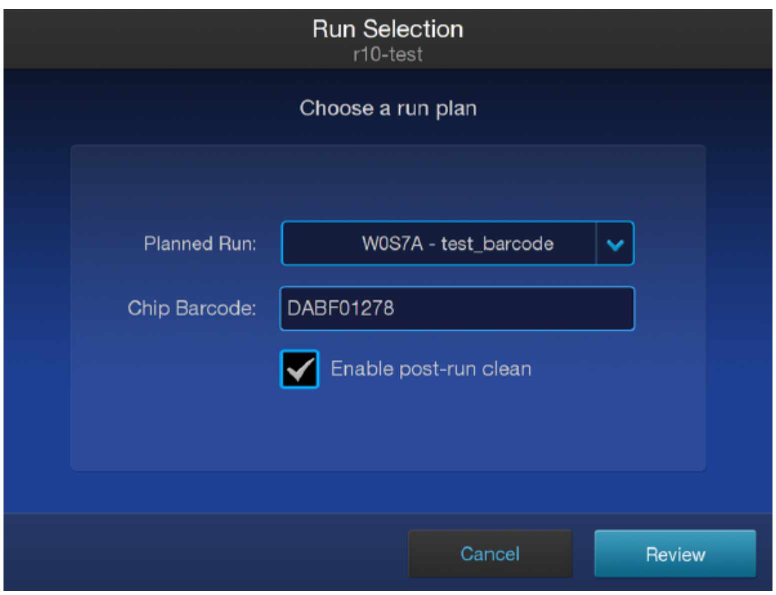
An Ion Chef™ Instrument run must have a status of **Planned** before a sequencer can start a sequencing run. If the connection between an Ion Chef™ Instrument and Torrent Suite™ Software is temporarily lost or interrupted, the status of the Ion Chef™ run may be marked as **Reserved**, even if the run has completed. To resolve this problem, you can manually change the status to **Planned** and enable the run for sequencing.

1. In the **Plan** tab, click **Planned Runs**.
2. Find the Planned Run of interest (with a status of **Reserved**), then click **(Actions)** ▸ **Completed on Chef**.
   The status for the Ion Chef™ Instrument run on the **Planned Runs** screen changes from **Reserved** to **Planned**. The sequencer can now use the Planned Run to start a sequencing run.

Execute a Planned Run on a sequencer

A Planned Run is listed in the **Planned Runs** screen until it is executed on a sequencer. To execute the run, select it on the sequencer touchscreen, after which the run is removed from the **Planned Runs** list.

1. Select the **Planned Run** from the list in the **Run Selection** screen, on the Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, or Ion GeneStudio™ S5 Prime System. Then, tap **Review**.

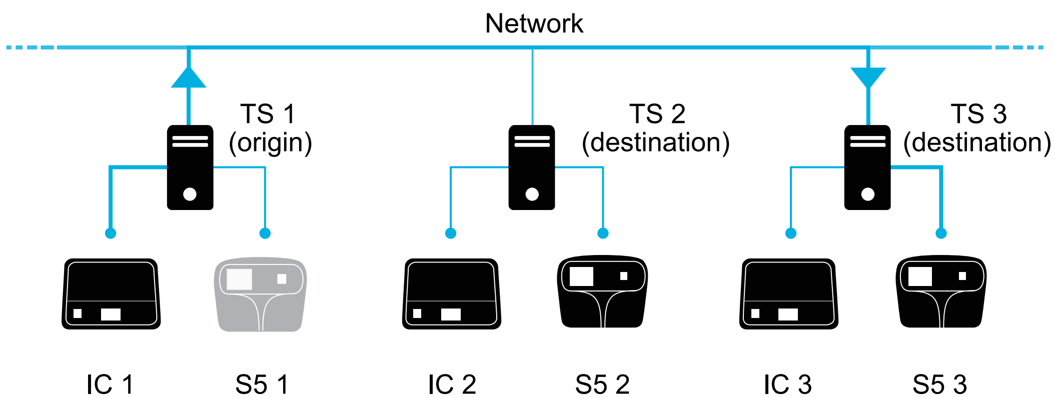


2. On the **Select Run** screen, ensure that the selections are correct, then tap **Start run**.

Transfer a Planned Run to another Ion Torrent™ Server

If you have multiple Ion Torrent™ Servers and sequencers on a network, you can create a Planned Run on one Ion Torrent™ Server, then transfer it to a different Ion Torrent™ Server to perform the run. This is useful if an Ion Chef™ Instrument or sequencer that is associated with a sever that is offline or busy.

A network administrator or Field Service Engineer must first set up this networking capability.

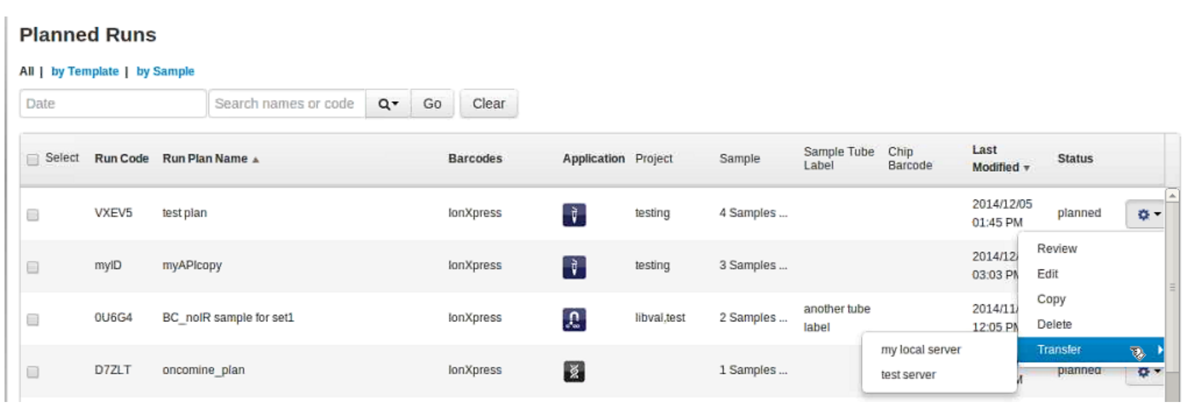


For example, as shown in the diagram, a Planned Run can be set up on the first Ion Torrent™ Server (TS 1) and run on its associated Ion Chef™ Instrument (IC 1). But if the associated Ion GeneStudio™ S5 System (S5 1) is busy or offline, you can transfer the Planned Run to another Ion Torrent™ Server on the network (TS 2 or TS 3) to perform the run on those associated sequencers (S5 2 or S5 3).

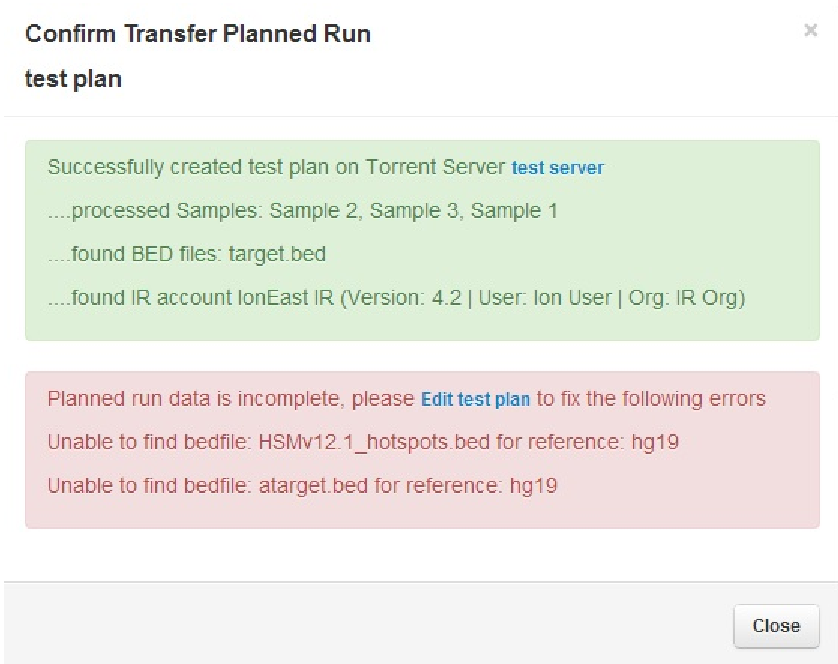
1. In the **Planned Runs** screen, find the Planned Run that you want to transfer.

   The status of the Planned Run listed in the **Status** column must be either **Pending** (for runs to be sent to an Ion Chef™ Instrument) or **Planned** (for runs to be sent to a sequencer). You cannot transfer runs that are in progress on an Ion Chef™ Instrument (status is **Reserved**).

2. Click ⚙ **(Actions)** for the selected Planned Run, select **Transfer**, then select the name of the destination Ion Torrent™ Server on the network.



3. In the confirmation box, click **Transfer** to confirm.
   - If the transfer is successful, a green confirmation message appears.
   - If any files or other settings required for the run are missing on the destination server, a red message reports what is missing. Edit the transferred Planned Run on the destination server to add the files or missing settings to perform the sequencing run.



- You can no longer access the Planned Run on the origin server after the Planned Run is transferred.

5 Monitor runs in progress

In Torrent Suite™ Software, use the **Monitor** tab to monitor information about runs that are in progress on various instruments. You can also review the Planned Run settings for a sequencing run that is currently in progress.

For each run, you can view reported metrics, thumbnail graphs, and other indicators.

Active runs and all runs completed within the previous 7 days are available to view. More data about completed runs is available in the **Data** tab.

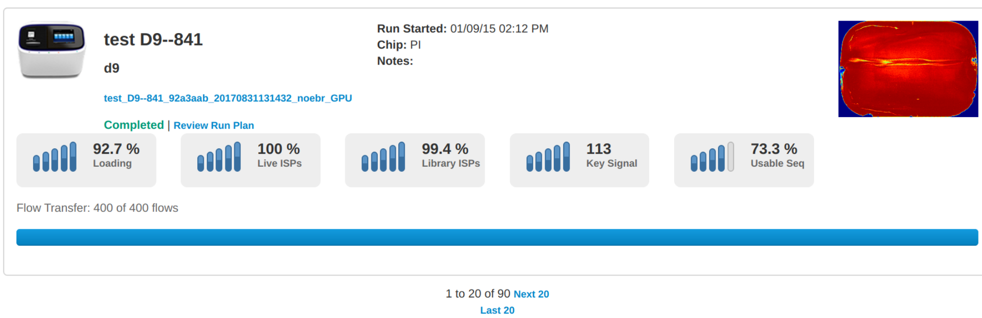
Monitor a sequencing run

Use the **Monitor** tab to view the thumbnail graphs and metrics of a sequencing run that is in progress to determine quickly whether a sequencing run is going well and should continue, or if the run should be aborted.

During a sequencing run, as information becomes available, a temporary thumbnail entry shows run metrics and a heat map image of chip loading. You can see immediately in the heat map image, and in the thumbnail graphs, whether any run quality metrics are flagged with the) icon as falling below the thresholds that are defined in your Planned Run.

1. In the **Monitor** tab, click **Runs in Progress**.

2. In **List View**, review the run metrics.



Metric	Description
Loading	Addressable wells on the chip that have detectable loading.
Live ISPs	Loaded Ion Sphere Particle (ISP) wells that have a live signal.
Library ISPs	Live Ion Sphere Particle (ISP) wells with a library template.
Key Signal	Average 1-mer signal in the library key.
Usable Sequence	Percentage of the sequence available for analysis after filtering.
Flow Transfer	Progress of the sequencing run expressed as number of the total number of flows completed.

If you decide to abort a sequencing run based on the chip loading metrics observed, see "Stop an analysis job" on page 82.

Monitor an Ion Chef™ run

Use the **Monitor** tab to view details about Ion Chef™ runs from the previous 7 days. The **Planned Runs** screen also contains information about the status of Ion Chef™ runs. For more information, see "Planned Run status" on page 73.

1. In the **Monitor** tab, click **Ion Chef**.
2. In the **Ion Chef** screen, view the run information.

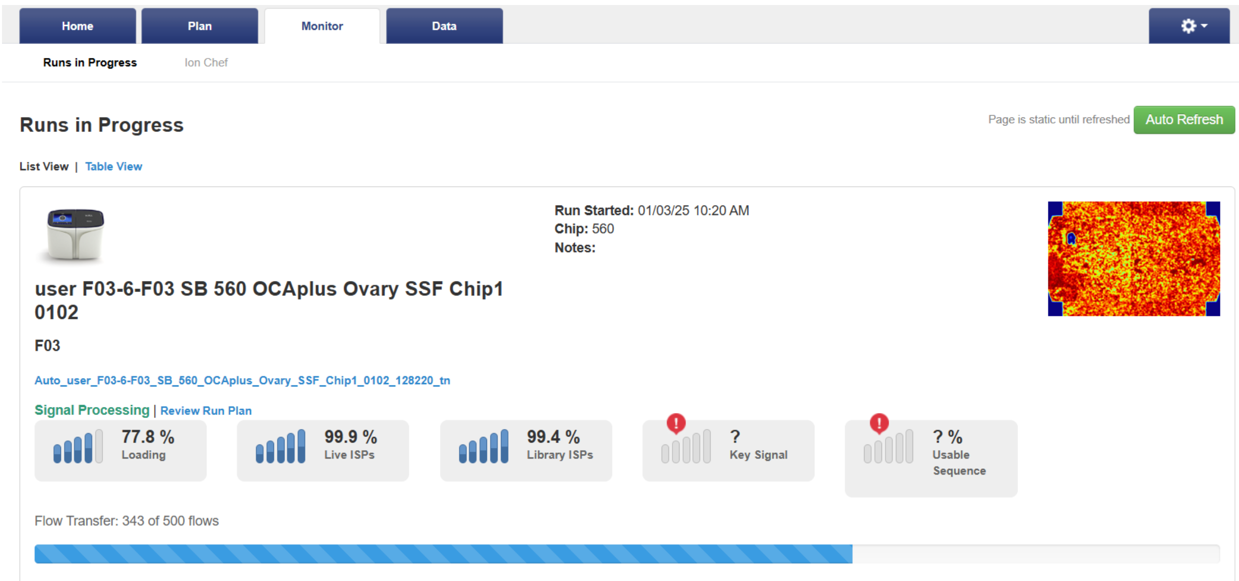
Item	Definition
Last Updated	The date and time of the currently displayed run status.
Sample Set	For more information, see Chapter 3, "Samples and sample sets".
Plan	The Ion Chef™ run plan in progress.
Chef Instrument	The identity of the Ion Chef™ Instrument in use.
Library Prep Progress	The progress of an Ion AmpliSeq™ library preparation run. For an Ion AmpliSeq™ on Ion Chef™ run only.
Library Prep Status	The current stage of the library preparation run. • **Not started** • **In progress** • **Complete**
Template Prep Progress	The progress of a template preparation run.
Template Prep Status	The current stage of the template preparation run. • **Not started** • **In progress** • **Complete**
Estimated Time Remaining	The estimated time remaining until the run is completed.
Estimated Time Until User Intervention	The estimated time and date when the run pauses for QC or is completed.

For an Ion Chef™ run in progress, check the **Estimated Time Remaining** or the **Estimated Time Until User Intervention** to see the time remaining before you can remove Ion Sphere™ Particles (ISPs) samples at the QC pause or remove loaded chips for sequencing.

View data for runs in progress

You can view data for runs that are in progress.

In the **Monitor** tab, click **Runs in Progress**.



The **Runs in Progress** screen has two views.

- Click **List View** to see runs in progress ordered by most recent date.
- Click **Table View** to see runs in progress ordered by row. Click column headings to sort the rows. This view shows only the information that is associated with each report.
- To view more data about a run, click the blue run report name link.
  The run report opens in the **Data** tab. You can use the navigation links to view different types of data.
- Both views show 20 runs at a time. If you have more than 20 runs, multiple screens are available.
- To monitor details about Ion Chef™ Instrument runs, in the **Monitor** tab, click **Ion Chef**.

Automatically refresh the Monitor tab

Use the Auto Refresh feature to update the information on the **Monitor** tab screens every 20 seconds. Without Auto Refresh, the screens are a static display of information at the time you open the screen.

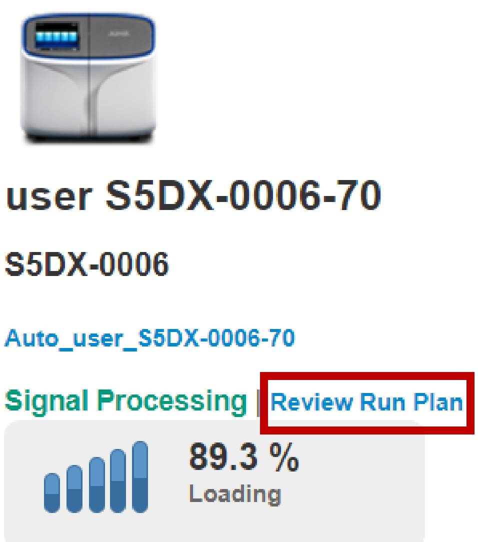
- To set the **Runs in Progress** screen to automatically refresh.
  a. In the **Monitor** tab, select **Runs in Progress**, then click **Auto Refresh**.
  b. Click **Stop Refresh** to turn the Auto Refresh feature off.
- To set the **Ion Chef** screen to automatically refresh.
  a. In the **Monitor** tab, select **Ion Chef**, then click **Auto Refresh**.

b. Click **Stop Refresh** to turn the Auto Refresh feature off.

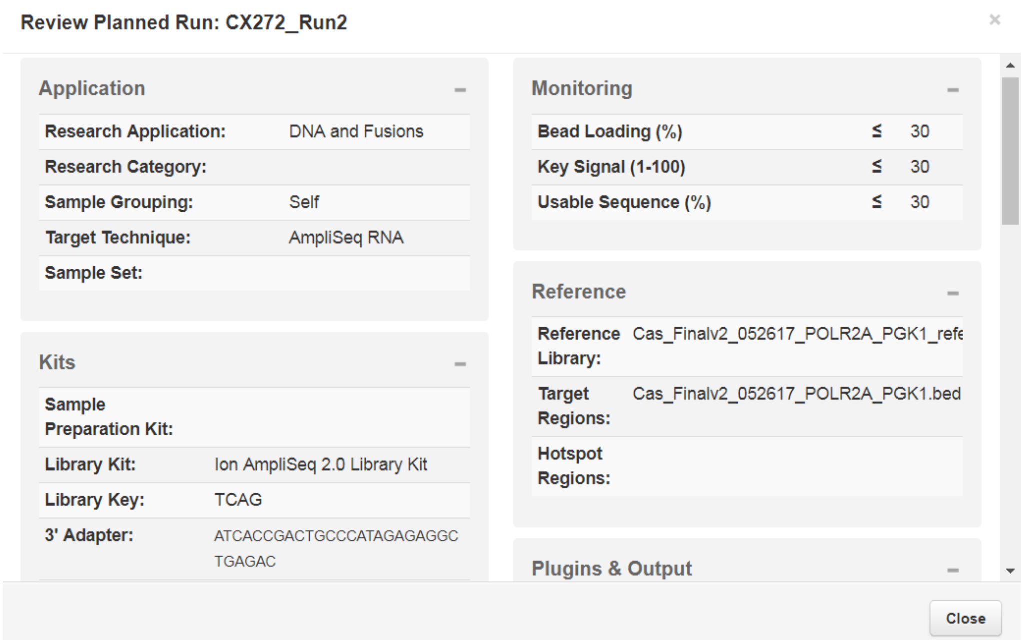
Review the Planned Run settings

In the **Monitor** tab you can review the Planned Run settings for a run that is in progress.

1. In the **Monitor** tab, click **Runs in Progress**.
2. In the **Runs in Progress** screen List View, click **Review Run Plan** for the run of interest.



The **Review Planned Run** dialog box displays the Planned Run information and settings.



3. Click **Close** to return to the **Monitor** tab, **Runs in Progress** screen.

Stop an analysis job

Use the **Services** screen to stop an analysis job for a run that has started but is not complete. Use caution before deciding to stop an analysis job.

You can also stop a plugin run in progress from the **Services** screen.

1. Click ⚙ **(Settings)** ▸ **Services**.
2. In the **Services** screen, scroll down to the **Active Jobs** section, then find the **Name** for the analysis job that you want to stop. The **Status Message** column indicates that the **job is running**.
3. Click **Terminate** for the analysis job that you want to stop.

4. In the confirmation dialog box, click **Yes** to end the job.

5. Refresh your browser to update the information in the **Active Jobs** section.

   The job is removed from the **Active Jobs** list. The list displays **No active jobs** if no other jobs are active.

6. In the **Data** tab, click **Completed Runs & Reports**.
   The status is **TERMINATED** next to the name of the analysis job that you stopped.

View system memory usage

You can view information about how much system memory is being used in the **Services** screen. This information is useful to monitor sequencing runs, and to troubleshoot issues that are related to memory usage.

1. Click ⚙ **(Settings)** ▸ **Services**.

2. Scroll to the **System Memory Usage** section.

The current system memory usage is shown in GB and as a percentage of the total memory that is available.

6 Review and manage run reports

In Torrent Suite™ Software, you can search and review data across all runs, and drill down to see data in the run report. A run report contains statistics and quality metrics from completed sequencing runs. You can use this information to evaluate the run. You can also complete other tasks from the run report.

About run reports

A Torrent Suite™ Software run report contains statistics and quality metrics from completed sequencing runs. You can use this information to evaluate the run. You can also complete other tasks from the run report.

For runs completed on an Ion GeneStudio™ S5 Series sequencer, each run report includes one full chip report and one thumbnail report. If the run has been reanalyzed, more reports are available when you open the run report.

You can complete several tasks from the run report.

- Upload output files from the run report to Ion Reporter™ Software. For more information, see "Run the IonReporterUploader plugin manually" on page 227.
- Review, edit, or copy the Planned Run settings for the sequencing run. For more information, see "Review Planned Run settings" on page 52 and "Create a user-defined Planned Run template" on page 33.
- Rerun the data analysis for the run. For more information, see "Reanalyze a run" on page 116.
- Archive, edit, or delete data for the run. For more information, see Chapter 11, "Data management".
- Toggle between different run reports for the same sample. For more information, see "Open a run report" on page 85.
- Run Torrent Suite™ Software plugins. For more information, see "Run a plugin manually from the sequencing run report" on page 124.
- Download the run report or plugin summary in PDF format. For more information, see "Download a run report summary PDF" on page 103 and "Download a plugin report PDF" on page 124.

For more information about how to evaluate a run, see *How to assess an Ion S5™/Ion GeneStudio™ S5 sequencing run report User Bulletin*, (Pub. No. MAN0017983).

Search for a run report

You can search, sort, or filter the **Completed Runs & Reports** list to find a run report of interest.

1. In the **Data** tab, click **Completed Runs & Reports**, then complete the selections for the following options.

Option	Selection
Search the list	In **Search**, enter a search term, then click **Go**.
Sort the list	Select a sort order from the **Sort** list (**List View** or **Table View**) or click any bolded column heading (List View only) to sort the list. Click the column heading a second time to reverse the sort order.
Limit the list to recent runs	In **Date**, select a preset range, or click **Date Range**, then select a **Start** and **End** date.
Filter the list	Select one or more filters to limit the **Completed Runs & Reports** list. Click **More Filters** to see all available filters. Within a filter, enter text in **Find** to limit the filter choices. To remove a filter, deselect the filter choice or click **Clear** in the filter list.
View favorites	Click the ☆ icon next to **Search** to limit the list to completed runs that are designated as **Favorite**. To set a completed run as a favorite, click the ☆ icon next to the completed run name. The icon color changes to blue.

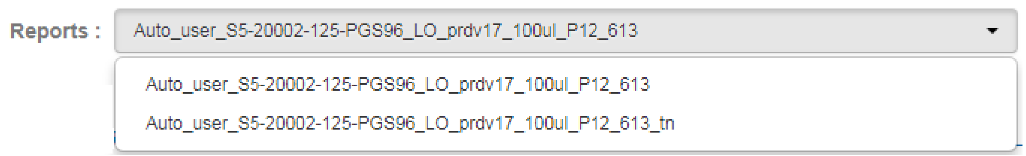
2. Click **Clear All** to remove filters and restore all results.

Open a run report

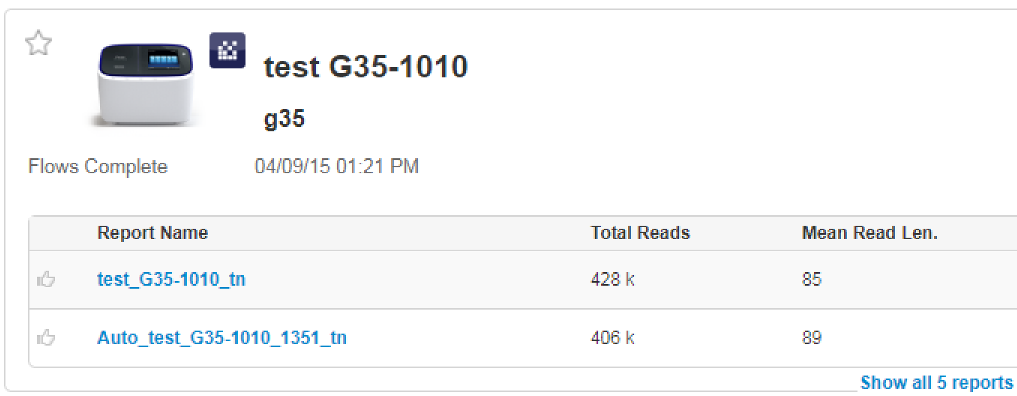
Every sequencing run report includes at least two versions: a full chip report that contains complete details about the run, and a thumbnail version that includes a limited data set. If the run has been reanalyzed, additional reports are available.

The thumbnail reports use a limited set of data points to give a summary of the fully sequenced and analyzed run. Thumbnail reports can be used to determine quickly the success of the run or whether a run should be terminated. Thumbnail reports names are appended with `_tn` and can be opened before the full chip reports are generated. The full chip reports contain all the data points and can be used for your analysis review.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. *(Optional)* Click **Table View** to review **Completed Runs & Reports** details in a table layout.
3. Open an individual run report.
   - In the **Table View**, find the run that you want to view the report for, then click the **Report Name** link to open the report.
     To open another report that is associated with your run of interest, from the **Reports** list, click the down arrow, then select the report that you want to open.



   - In the **List View**, find the pane that contains details about your run of interest, then click the **Report Name** link to view details about any of the available reports for that run.



     If more than two reports are listed, click the down arrow to view all reports associated with your run of interest.

Review unaligned reads

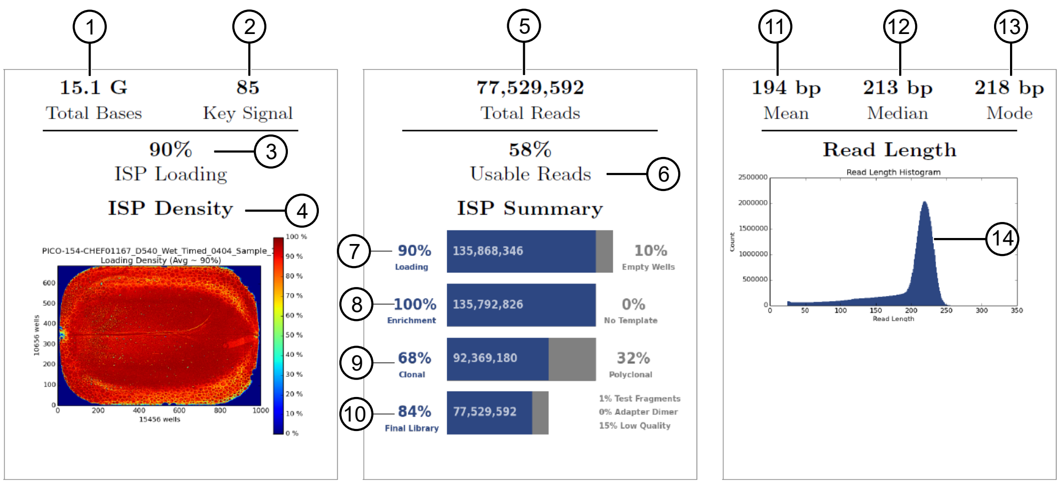
To determine the quality of the sequencing run, you can review the quality metrics for the unaligned reads. Primary pipeline processing, base calling, and signal processing generate these metrics. The quality metrics for unaligned reads are divided into three categories.

- Quality of chip loading, expressed as the density of Ion Sphere™ Particles (ISPs) loaded onto the chip.
- Quality of the ISPs that are loaded onto the chip.
- The length of sequencing reads.

1. In the **Data** tab, in the **Completed Runs & Reports** screen, find the report that you want to review, then click the report name link in the **Report Name** column.
2. In the left navigation menu, click **Unaligned Reads**.
3. Review the unaligned read quality metrics in **ISP Density**, **Chip well details**, and **Read Length** panes.
4. *(Optional)* Click 🔍 to view more details about the information in each pane.

Run report metrics before alignment

To assess the sequencing run, you can review the quality metrics for the unaligned reads. Primary pipeline processing, basecalling, and signal processing generate these metrics. The following prealignment metrics are provided in the run report. Access the run report in the Torrent Suite™ Software using the **Data** tab.



Number	Metric	Description
1	**Total Bases**	The number of filtered and trimmed base pairs reported in the output BAM file.
2	**Key Signal**	The average signal for all library ISPs with library key (TCAG).
3	**ISP Loading**	The percentage of chip wells that contain an Ion Sphere Particle (ISP). The percentage value considers only addressable wells.
4	**ISP Density**	A visual representation of well loading distribution on the chip surface. Red color indicates areas of high loading and blue indicates areas of low loading.
5	**Total Reads**	The total number of filtered and trimmed reads independent of length reported in the output BAM file.
6	**Usable Reads**	The percentage of library ISPs that pass the polyclonal, low quality, and primer-dimer filters. This percentage is calculated by dividing final library ISPs by library ISPs.

(continued)

Number	Metric	Description
7	**Loading**	The percentage of chip wells that contain an ISP. The percentage value considers addressable wells.
8	**Enrichment**	The predicted number of live ISPs that have a key signal identical to the library key signal or test fragment key signal. The Percent Enrichment value reported is the number of loaded wells with live ISPs that are Library ISPs or test fragment ISPs. This number is calculated by dividing wells with live ISPs by the number of wells loaded with ISPs.
9	**Clonal**	The percentage of clonal ISPs (all library and test fragment ISPs that are clonal, not polyclonal). An ISP is clonal if all of its DNA fragments are cloned from a single original template. All the fragments on such an ISP are identical and they respond in unison as each nucleotide is flowed in turn across the chip. This percentage is calculated by dividing the number of ISPs with a single DNA template by the number of live wells.
10	**Final Library**	The percentage of reads, which pass all filters, and which are recorded in the output BAM file. This value can be different from the Total Reads due to technicalities associated with read trimming beyond a minimal requirement that results in Total Reads being slightly less than Final Library.
11	**Mean**	The average length, in base pairs, of called reads.
12	**Median**	The median length, in base pairs, of called reads.
13	**Mode**	The mode length, in base pairs, of called reads.
14	**Read Length Histogram**	The read length histogram is a histogram of the trimmed lengths of all reads present in the output files.

Additional metrics are shown when you click the magnifying glass icon in the run report.

Metric	Description
Empty Wells	Percentage of chip wells that do not contain an ISP. The percentage value considers only potentially addressable wells. The percentage is calculated by the number of potentially addressable wells, minus the number of loaded ISPs, divided by the number of potentially addressable wells.
No Template	Percentage of chip wells that do not contain a DNA template. This percentage is calculated by the number of loaded ISPs minus test fragment ISPs divided by the number of loaded ISPs plus test fragment ISPs.
Polyclonal	Percentage of polyclonal ISPs (ISPs carrying clones from two or more templates). A high polyclonal percentage indicates that library input is too high and should be titrated down. Enrichment does not filter out polyclonal ISPs, it removes only template-negative ISPs. This percentage is calculated by dividing polyclonal ISPs by live ISPs.

Additional metrics are shown when you click the magnifying glass icon in the run report. *(continued)*

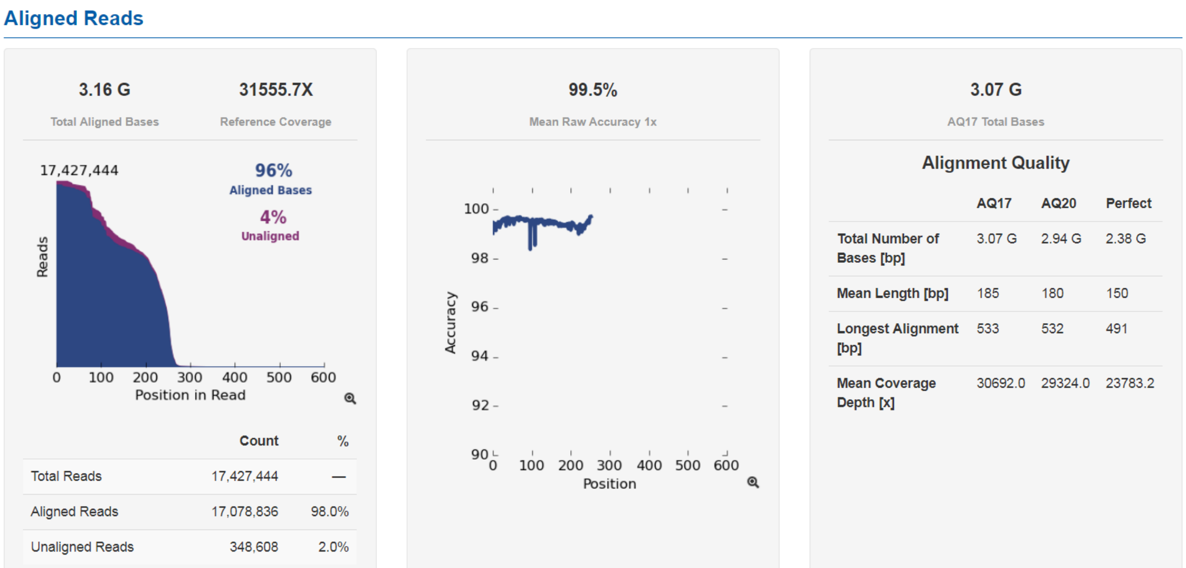
Metric	Description
Final Library	Percentage of reads that pass all filters and are recorded in the output BAM file. This value can be different from the Total Reads due to specifications associated with read trimming beyond a minimal requirement resulting in Total Reads being slightly less than Final Library. This percentage is calculated by dividing final library ISPs by clonal ISPs.
% Test Fragments	Percentage of live ISPs with a key signal that is identical to the test fragment key signal. This percentage is calculated by dividing test fragment ISPs by clonal ISPs.
% Adapter Dimer	Percentage of ISPs with an insert length of less than 8 bp. This percentage is calculated by dividing primer-dimer ISPs by clonal ISPs.
% Low Quality	Percentage of ISPs with a low or unrecognizable signal. This percentage is calculated by dividing low quality ISPs by clonal ISPs.
Addressable Wells	Total number of wells on the chip minus excluded wells.
With ISPs	Number (and percentage) of addressable wells that were determined to be *positive* for the presence of an ISP in the well. Positive is determined by measuring the diffusion rate of a flow with a different pH. Wells containing ISPs have a delayed pH change due to the presence of an ISP slowing the detection of the pH change from the solution. This percentage is calculated by dividing the number of wells with ISPs by total addressable wells.

Review aligned reads

View the aligned reads to determine the accuracy of your sequencing run.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a report of interest.

2. In the left navigation menu, click **Aligned Reads**, then review the metrics to determine the accuracy of your sequencing data.



Click 🔍, or on an image to open an enlarged view.

Quality following alignment

You can use read alignment to evaluate the quality of the sequencing reaction and the quality of the corresponding library when an accurate reference is available. Reads are aligned to a reference genome sequence. Any discrepancy in alignment to a reference, whether biological (actual variant) or technical (sequencing error) is listed as a mismatch. Alignment performance metrics are reported through the alignment quality (AQ) score, which defines the accuracy of sequencing reads when compared to a reference genome sequence.

Torrent Suite™ Software reports alignment quality parameters.

- AQ17—the greatest length at which the error rate is 2% or less.
- AQ20—the greatest length at which the error rate is 1% or less.
- Perfect—the longest perfectly aligned segment.

Aligned read length calculation

The aligned length 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. Accuracy is specified using the Phred -10log10 transformation, where 20 represents an error rate of 1%, and 17 represents an error rate of 2%.

In Torrent Suite™ Software, the alignment quality (AQ) score represents alignment quality for the total number of bases that are sequenced or for consecutive sequencing reads. The AQ20 length is the greatest length at which the error rate is 1% or less, and the AQ17 length is the greatest length at which the error rate is 2% or less. The ideal length is the longest perfectly aligned segment. The AQ score for the total number of bases represents the number of all aligned bases in the sequencing reaction that meet a specific AQ score.

For all of these calculations, the alignment is constrained to start from position 1 in the read - that is, no 5' clipping is allowed. The underlying assumption is that the reference to which the read is aligned represents the true sequence that is seen.

Appropriate caution must be taken when values for the AQ score are interpreted for situations in which the sample that is sequenced has substantial differences relative to the reference used. For example, for alignments to a rough draft genome, or for samples that are expected to have high mutation rates relative to the reference used. In these situations, the AQ20 and AQ17 lengths can be short even when sequencing quality is excellent.

The AQ20 length is calculated using the following steps:

- Every base in the read is classified as being correct or not correct according to the alignment to the reference.
- At every position in the read, the total error rate is calculated up to and including that position.
- The greatest position at which the error rate is one percent or less is identified and that position defines the AQ20 length.

For example, if a 100-bp read consists of 80 perfect bases that are followed by 2 errors that are then followed by 18 more perfect bases, the total error rate at position 80 is zero percent. At position 81 the total error rate is 1.2% (1/81), at position 82 the error rate is 2.4%, continuing up to position 100 where it is 2% (2/100). The greatest length at which the error rate is 1% or less is 80, and the greatest length at which the error rate is 2% or less is 100. Therefore, the AQ20 and AQ17 lengths are 80 and 100 bases, respectively.

Alignment

Torrent Suite™ Software provides a view on alignment that helps determine run and library quality.

Many alignment algorithms are available in the marketplace. You are encouraged to consult with a bioinformatician for the most appropriate alignment algorithm for your downstream analysis requirements.

Alignment in Torrent Suite™ Software is performed using TMAP. TMAP is currently an unpublished alignment algorithm, created by the authors of the BFAST algorithm. For more information, see "TMAP modules" on page 292, or contact your local Field Service Engineer or Technical Support.

Although TMAP is unpublished and a reference is not currently available, the precursor to TMAP, BFAST, is based on the ideas in the following publications:

- Homer N, Merriman B, Nelson SF (2009) BFAST: An Alignment Tool for Large Scale Genome Resequencing. *PLoS ONE* 4(11): e7767. **doi: 10.1371/journal.pone.0007767**
- Homer N, Merriman B, Nelson SF (2009) Local alignment of two-base encoded DNA sequence. *BMC Bioinformatics* 10: 175. **doi: 10.1186/1471-2105-10-175**

Reads used for alignment

The alignment process involves aligning reads produced by the pipeline to a reference sequence and extracting metrics from those alignments. By default, Torrent Suite™ Software aligns all reads to the reference; however, there may be situations, particularly with large reference sequence, when the alignment exceeds the amount of time that you are willing to wait. If so, the software can define on a per-reference basis the maximum number of reads that are aligned from a run.

When the number of reads in a run exceeds a reference-specific maximum, a random sample of reads is taken, and results are extrapolated to represent the full run. By sampling a random pool of reads and extrapolating the alignment quality values to represent all reads, the software provides sufficient information to assess the quality of the sample, library, and sequencing run.

The output of the alignment process is a BAM file. The BAM file includes an alignment of all reads, including the unmapped reads, with exactly one mapping per read. When a read maps to multiple locations, the mapping with the best mapping score is used. If more than one such mapping exists, a random mapping is used and a mapping quality of zero is given.

Predicted quality (Q20)

The number of called bases with a predicted quality of Q20 is reported. The predicted quality values are reported on the Phred scale, defined as -10 log10 (error probability). Q20, therefore, corresponds to a predicted error rate of one percent.

Per-base quality score system

The Ion Torrent™ technology per-base quality score system uses a Phred-like method to predict the probability of correct base calls. The prediction is based on the quality of the base incorporation signal that was used for generating the base calls. The quality score system used by the sequencers uses a set of six predictors whose values are correlated with the probability of a base miscall.

A Phred lookup table is used for converting the values of predictors to error probabilities. The lookup table is generated by training on a representative data set in a customer configuration. The lookup table is retrained for each software release and is shipped as part of the software package. Quality scores are published in the BAM file.

Quality score predictors

Torrent Suite™ Software uses the following six quality score predictors that are correlated with empirical base call quality.

Predictor	Description
P1	**Penalty Residual—**A penalty based on the difference between predicted and actual flow values. Computed by the base caller.
P2	**Local Noise—**Noise (defined as the maximum absolute difference between the flow value and the nearest integer) in the immediate neighborhood (plus/minus 1 base) of the given base.
P3	**High-Residual Events—**Number of high-residual flows in the 20-flow window around the flow that contains the base. A flow has high residual when the normalized difference between the observed and model-predicted signal exceeds 0.4 or falls below –0.4. The more high-residual flows in the window, the lower quality the base call.
P4	**Multiple Incorporations—**Number of incorporated bases in this flow. Length of the homopolymer. For multiple incorporations of the same nucleotide in one flow, the last base in the incorporation order is assigned a value equivalent to the total number of incorporations. All other bases in the sequence of the multiple incorporations are assigned the value 1.
P5	**Environment Noise—**The average signal noise (defined as the absolute difference between the flow value and the nearest integer) in the neighborhood (plus/minus 5 bases) of the given base.
P6	**State Inphase—**Live polymerase in phase.

The six quality predictors are calculated for each base. Other predictors (not described here) are computed from the corrected flow values generated by the base caller.

The corresponding per-base quality value is found by finding the first line in the lookup table for which all six calculated predictors are less than or equal to the predictor values in the table. This process occurs automatically as part of the standard analysis.

The Phred lookup tables are stored in the `/opt/ion/config` directory on Ion Torrent™ Server. The Ion Torrent™ Server supports separate Phred tables for each type of chip.

The per-base quality along with all other read information is written to the unmapped BAM file. The per-base quality scores are reported in the QUAL field, which is part of the SAM/BAM file format specification (see **https://samtools.github.io/hts-specs/SAMv1.pdf**, pages 6 and 8.

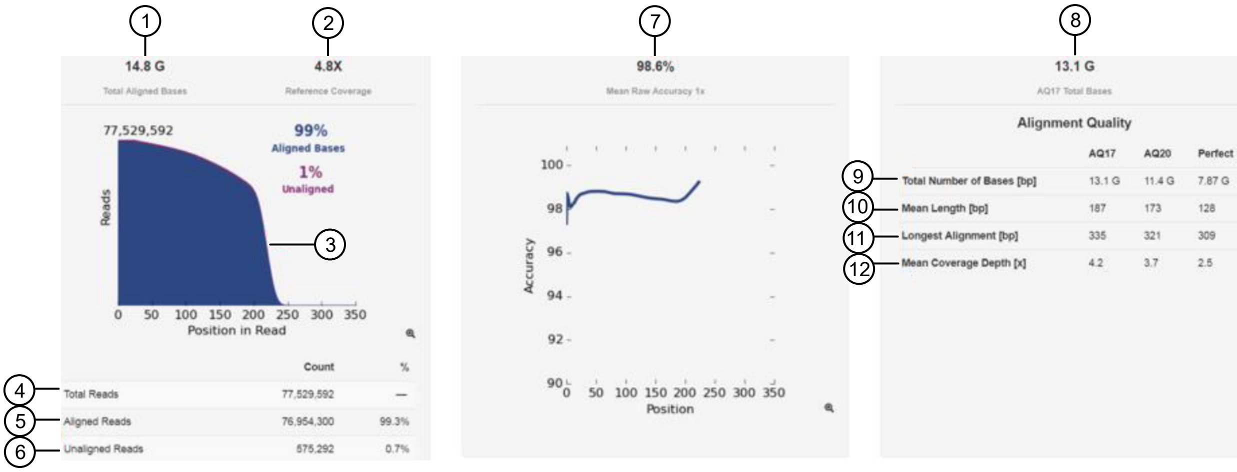
The quality scores are on a phred-10*log_10 (error rate) scale.

References

1. Brockman, et al. (2008): "Quality scores and SNP detection in sequencing-by-synthesis systems." Genome Res. 18: 763-770.References.
2. Ewing B, Hillier L, Wendl MC, Green P. (1998): "Base-calling of automated sequencer traces using phred. I. Accuracy assessment." Genome Res. 8(3):175-185.
3. Ewing B, Green P. (1998): "Base-calling of automated sequencer traces using phred. II. Error probabilities." Genome Res. 8(3):186-194.

Run report metrics after alignment

To assess the sequencing run when an accurate reference is available, you can review the quality metrics for aligned reads. Reads are aligned to a reference genome sequence. The following post-alignment metrics are provided in the run report.



Number	Metric	Description
1	**Total Aligned Bases**	The number of filtered and trimmed aligned base pairs reported in the output BAM file that are aligned to the reference sequence, excluding the library key, barcodes, and 3' adapter sequences.
2	**Reference Coverage**	The ratio of the total aligned bases divided by the number of bases in the reference sequence. Reference coverage does not account for enrichment done to selectively amplify a subset of the reference sequence.
3	**Alignment plot**	A plot of the number of aligned reads (blue) and unaligned (purple) by position in an aligned sequence.
4	**Total Reads**	The total number of reads after filtering.
5	**Aligned Reads**	The number of reads that align to the reference sequence expressed as a total count and percentage of the total aligned reads.
6	**Unaligned Reads**	The number of reads that do not align to the reference sequence expressed as a total count and percentage of the total reads.
7	**Mean Raw Accuracy 1x**	The mean raw accuracy across each individual base position in a read calculated as 1– (total errors in the sequenced base) / total bases sequenced.
8	**AQ17 Total Bases**	The total number of bases over all positions that align with an error rate of 2% or less.
9	**Total Number of Bases (bp)**	The total number of bases over all positions that align with a given error rate. (AQ17 ≤2% error rate, AQ20 ≤1% error rate, Perfect = no measurable error)

(continued)

Number	Metric	Description
10	**Mean Length (bp)**	The average length, in base pairs, for aligned reads at a given error rate. (AQ17 ≤2% error rate, AQ20 ≤1% error rate, Perfect = no measurable error)
11	**Longest Alignment (bp)**	The maximum sequence read length for a given error rate. (AQ17 ≤2% error rate, AQ20 ≤1% error rate, Perfect = no measurable error)
12	**Mean Coverage Depth (x)**	The ratio of the total aligned bases at a given error rate to the size of the target region. (AQ17 ≤2% error rate, AQ20 ≤1% error rate, Perfect = no measurable error)

Run report metrics

You can use the six run report metrics to evaluate the quality of a sequencing run:

- Throughput (or Total Bases)—The number of filtered and trimmed base pairs reported in the output BAM file.
- ISP loading density—The percentage of chip wells that contain Ion Sphere™ Particles (ISPs). The percentage value considers only potentially addressable wells. Bead loading density is calculated as the number of loaded wells divided by the number of potentially addressable wells.
- Total reads—The total number of filtered and trimmed reads independent of length reported in the output BAM file.
- Read length—The length of called reads measured in base pairs.
- Read length histogram—A histogram that reflects the size distribution of the library minus the Ion adapter sequences.
- Key signal—The average signal (post software processing) for all ISPs that identically match the library key (TCAG). When the library key signal is lower than expected, increased 3' quality trimming occurs. Therefore, the higher the starting key signal, the less the impact of signal droop and the less 3' quality trimming is expected to occur.

Throughput considerations

To evaluate a run, review the throughput or total bases. Consider whether these metrics are appropriate for the application and chip type.

The table provides information about the number of wells in each chip type and sequencing throughput specifications for sequencing runs from an Ion GeneStudio™ S5 Series sequencer.

Chip type	Number of addressable wells	Number of reads	Throughput	
			200 Base Read	400 Base Read
Ion 510™ Chip	~6 million	2–3 million	0.3–0.5 Gb	0.6–1 Gb
Ion 520™ Chip	~12 million	4–6 million	0.6–1 Gb	1.2–2 Gb
Ion 530™ Chip	~37 million	15–20 million	3–4 Gb	6–8 Gb
Ion 540™ Chip	~150 million	60–80 million	10–15 Gb	N/A

(continued)

Chip type	Number of addressable wells	Number of reads	Throughput	
			200 Base Read	400 Base Read
Ion 550™ Chip	~260 million	100–130 million	20–25 Gb	N/A
Ion 560™ Chip	~550 million	240-280 million	~45–50 Gb	N/A

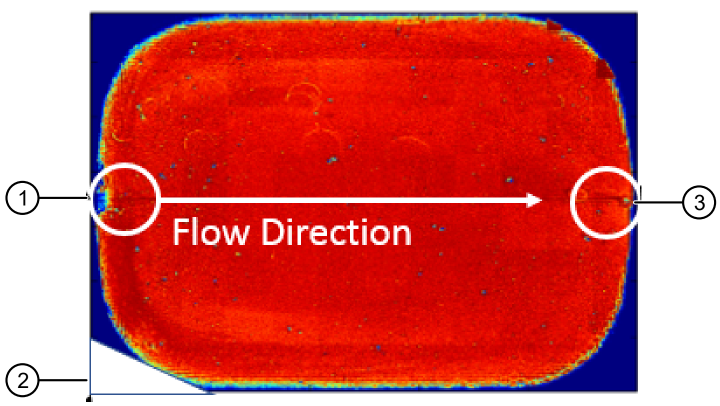
ISP loading density

ISP loading density is the percentage of chip wells that contain an Ion Sphere™ Particle (ISP; templated and non-templated, or live and dud ISPs). This percentage value considers only the potentially addressable wells and is a result of the software well classification step.

For more information, see "Well classification" on page 97.

The ISP Density image is a color image of the Ion Chip™ that shows the percentage of loading across the physical surface. Red indicates adequate ISP density, yellow indicates less-than adequate ISP loading density, and blue indicates an absence of loaded beads.

To open a larger version of the ISP density image, click the chip image in the run report.



ISP density chip image

① Sequencing reagent flow inlet
② Notch position
③ Loading port

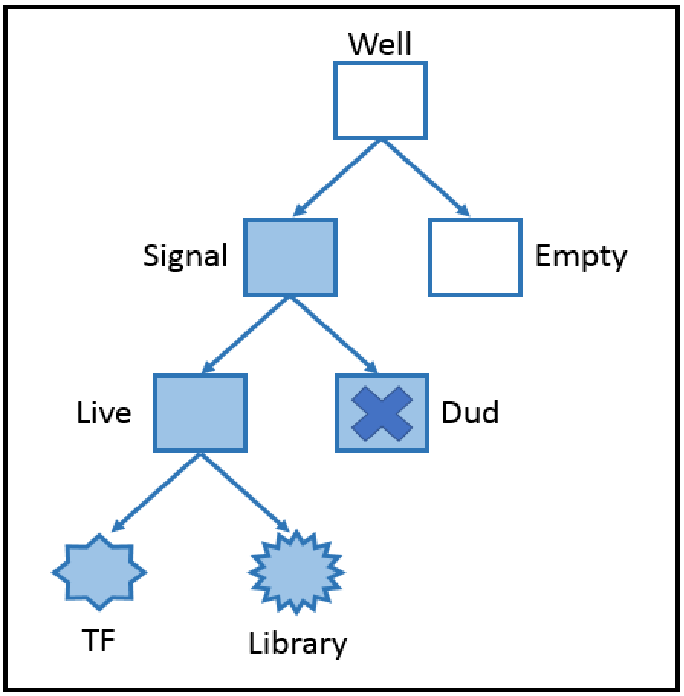
Total reads

The total reads value is the total number of reads that are written to barcode or no-match output BAM files. Filtered reads are not included in this count.

Well classification

Empty and loaded wells are separated by the differences in buffering over the chip during the nucleotide key flows (flows 1-8). Wells that are loaded with ISPs and associated polymerase have greater buffering capacity than empty wells. The software uses this difference to identify and classify loaded versus empty wells.

Live versus dud (non-templated ISPs) and TF versus Library ISPs are also identified based on data from the key flows. Therefore, both sequencing primer and sequencing polymerase are required for proper well classification.



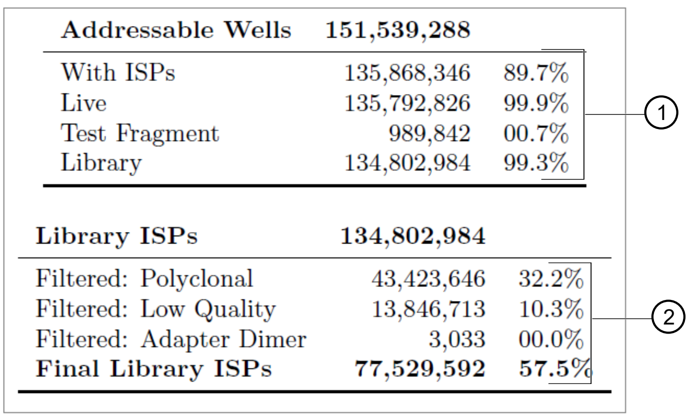
Read filtering and trimming

Empty and loaded wells are separated by the differences in buffering and signal over the chip during the nucleotide key flows (flows 1–8). Wells that are loaded with ISPs and associated polymerase have greater buffering capacity and higher signal than empty wells, and Torrent Suite™ Software uses these differences to identify and classify loaded versus empty wells.

After well classification, the software further processes the identified test fragments and library reads, including read filtering and trimming. This processing affects the total number of library reads and bases. You can see both well classification and library read filtering results that are displayed in the run report. Read trimming operations trim bases off the read. Trimming makes reads shorter, while read filtering operations completely remove them from the output BAM files. By default, reads that have a trimmed read length of <25 bases are being filtered. The different categories of filtered reads shown in the run report are described in the following table.

Filter	Description
Polyclonal	Filters reads from ISPs with >1 unique library template population. Occasionally, low or unexpected signal ISPs can also get caught in this filter.
Low Quality	Filters reads with unrecognizable key signal, low signal quality, and reads trimmed to <25 bases.
Adapter Dimer	Filters reads where no or only a very short sequencing insert is present. Reads that, after P1 adapter trimming, have a trimmed length of <25 bases are considered primer dimers.

You can see both well classification and library read filtering results that are shown in the run report.

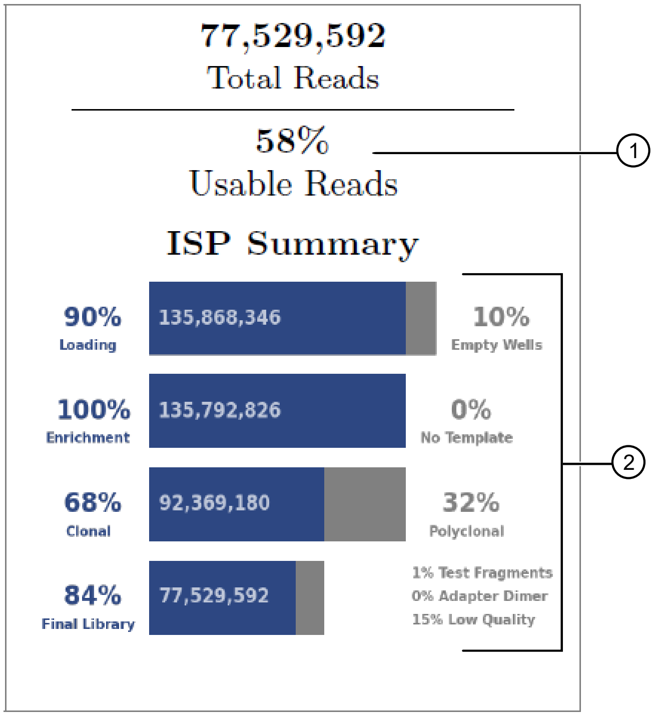


Addressable Wells	**151,539,288**	
With ISPs	135,868,346	89.7%
Live	135,792,826	99.9%
Test Fragment	989,842	00.7%
Library	134,802,984	99.3%

Library ISPs	**134,802,984**	
Filtered: Polyclonal	43,423,646	32.2%
Filtered: Low Quality	13,846,713	10.3%
Filtered: Adapter Dimer	3,033	00.0%
Final Library ISPs	**77,529,592**	**57.5%**

① ISP Summary of well classification results
② Library ISP details of filtering results

The Chip well details panel also presents the ISP Summary of well classification and Library ISP details using slightly different calculations.



① Usable sequence (reads) percentage
② Chip well details (ISP summary) percentages

The usable reads percentage is calculated as follows.

% usable reads = (library reads passing filters × 100) ÷ number of library ISPs identified

Example

% usable reads = (77,529,592 × 100) ÷ 134,802,984

Each ISP summary percentage is calculated by dividing the current value by the previous values.

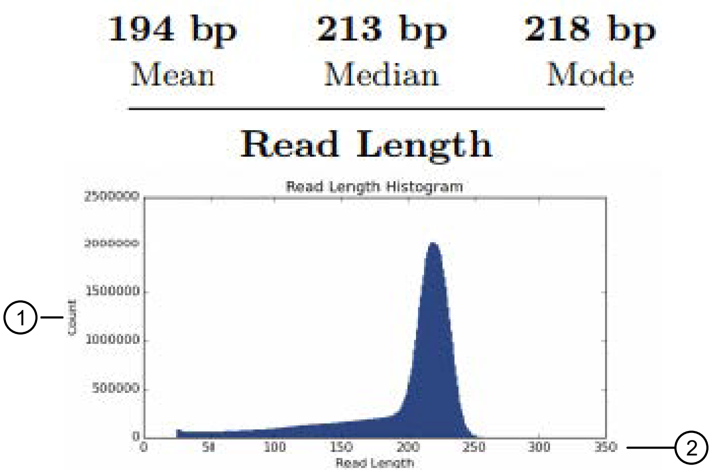
% final library = (library reads passing filters × 100) ÷ number of clonal reads

Example

% final library = (77,529,592 × 100) ÷ 92,369,180

Read length

Read length value is the length of called reads measured in base pairs. The Read Length histogram presents all filtered and trimmed library reads reported in the output BAM file and the mean read length in base pairs. The shape of the histogram should closely resemble the library size distribution trace, without the adapter sequences.



① The y-axis provides the read count.
② The x-axis provides the read length in base pairs.

In addition to the loading density and read filtering and trimming, the average library read length also affects the total sequencing run throughput. Read length is considered in the total sequencing run throughput. For example, a sequencing run on an Ion 530™ chip produces about 15 million final library reads:

- If the average read length is 300 bp, then the approximate throughput is 15,000,000 reads × 300 bp = 4.5 Gbp.
- If the average read length is 400 bp, then the approximate throughput is 15,000,000 × 400 bp = 6 Gbp.

For more information, see *How to assess an Ion S5™/Ion GeneStudio™ S5 sequencing run report User Bulletin* (Pub. No. MAN0017983).

Key signal

The library key signal is determined in the first eight flows of the sequencing run. The library key sequence is TCAG and all four nucleotides (T, C, A, and G) are incorporated during the first eight nucleotide flows.

Note: The library and test fragment (TF) keys, and the flow order can be found under the **Analysis Details** section of the run report.

Analysis detail	Example	Description
Flow Order	TACGTACGTCTGAGGATCGATGTACAGC	The order in which the chip is exposed to each dNTP.
Library Key	TCAG	A short known sequence of bases used to distinguish the library fragment from the test fragment.
TF Key	ATCG	The nucleotide sequence that is used to identify test fragment reads.

The reported key signal is the average signal (post software processing) for all ISPs that identically match the library key (TCAG). Each templated library ISP in a well has many library templates (ideally, clonally amplified during template preparation) all of which contribute to the dNTP incorporation signals reported for that well. The more templates per ISP, the higher the reported incorporation signal is for that well. In simple terms, the key signal essentially measures the number of templates per ISP or the efficiency of the template preparation reaction.

When the library key signal is lower than expected, increased 3’ quality trimming can result, especially for long reads (400-base read sequencing). Over the sequencing run, as with all sequencing by synthesis technologies, the signal that is generated drops due to the reaction conditions (polymerase dissociation) and eventually becomes indistinguishable from the background noise. As the signal generated approaches the background noise, the quality of the read decreases and is subject to 3’ quality trimming by the software. Therefore, the higher the starting key signal, the less the impact of the signal droop and the less 3’ quality trimming is expected to occur.

Output files

You can view and download the output files from your sequencing runs. The files include:

- Library sequences of unaligned and aligned reads
- Barcode reports of performance metrics for each barcode included in the run, if you used barcode adapters during the library preparation for your sample.

Download output files

You can download the library files for unaligned reads in UBAM format or for aligned reads in BAM or BAI file formats.

1. In the **Data** tab, click **Completed Runs & Reports**.

2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.

3. In the left navigation menu, click **Output Files** or scroll to the **Output Files** section, then click a file type to download the files.

File type	Description
Unaligned Reads	Nucleotide bases covered by reads that have not been aligned to the reference. Can be downloaded as an unmapped binary aligned/mapped (UBAM) file.
Aligned Reads	Number of bases covered by reads that have been aligned to the reference. Can be downloaded as a BAM or binary aligned/mapped index (BAI) file.

View and download barcode reports

You can view barcode reports for sequencing runs that use barcode adapters during library preparation for samples. The reports show key performance metrics for each barcode included in the run.

The number of barcodes in the barcode report reflects the barcode set that was used in the run and the barcodes that are present in the sample. Information is included only for barcodes that are present in the run.

1. In the **Data** tab, click **Completed Runs & Reports**.

2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.

3. In the left navigation menu, click **Output Files**, then scroll to the barcode section of the run report.

The barcode section of a run report displays the following information and provides access to downloadable files for each barcode.

Column	Description
Barcode Name	The individual barcode in the barcode set. The row labeled **No barcode** reports on unclassified barcodes, which are reads that could not be classified as a match for one of the expected barcodes in the barcode set.
Sample	Name of the sample that was sequenced on instrument.
Bases	Post-filtering base output for each barcode.
>=Q20 Bases	The percentage of reads that have a predicted quality score of Q20 or better.
Reads	Total number of filtered and trimmed library reads (independent of length). This number is reported in the barcode BAM file.

(continued)

Column	Description
Mean Read Length	The average read length, in base pairs (bp), of all filtered and trimmed library reads reported in the BAM file for the barcoded run.
Read Length Histogram	A thumbnail histogram of the read lengths for this barcode. Click the thumbnail histogram to open a larger image.
UBAM	Click to download a binary file that contains unaligned or unmapped reads. Viewing the file requires an alignment viewer application.
BAM	Click to download a compressed, binary form of the SAM file. The BAM file contains aligned reads sorted by genome reference location.
BAI	Click to download the BAM index (BAI) file. This file speeds up the access time for a coordinate-sorted BAM file.

Download a run report summary PDF

You can download a run report summary in PDF format. The PDF report reflects what is visible in the run report screen.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for your completed sequencing run.
2. Click **Download PDF ▸ Summary PDF**.
   A PDF version of the run report is downloaded through the browser to a directory on your computer, based on the browser settings.

View details about completed runs

You can view various details about a completed sequencing run in Torrent Suite™ Software.

View test fragments for a completed run

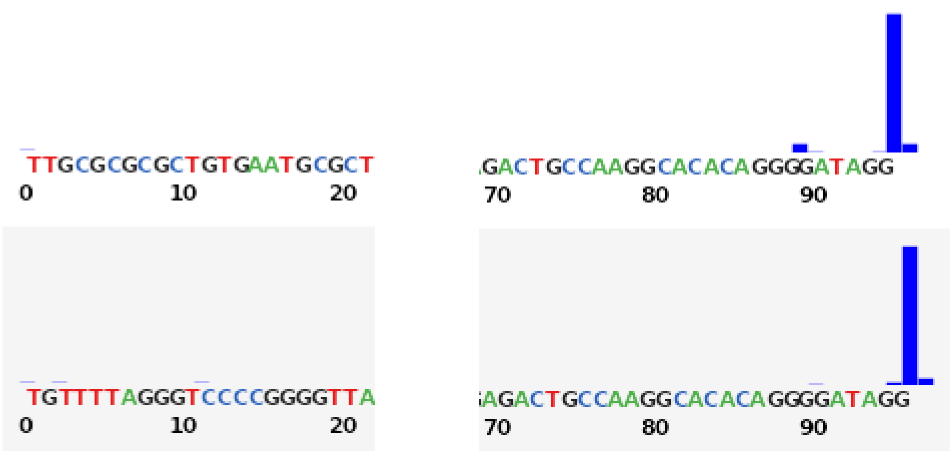
You can view the test fragment results and determine the quality of your sequencing run if you included key signal test fragments in your run.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link to open the report of interest.

2. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click **Test Fragments**.

Parameter	Description
Test Fragment	Test fragment name. For more information, see "Test fragments" on page 266.
Reads	Number of filtered & trimmed reads identified for this test fragment.
Percentage 50AQ17	The percentage of reads for this test fragment with a minimum of 50 base pairs in length and an error rate of 1 in 50, Phred-like 17, or better. Quality is based on alignment, not predicted quality.
Percent 100AQ17	The percentage of reads for this test fragment with a minimum of 100 base pairs in length and an error rate of 1 in 100, Phred-like 17, or better. Quality is based on alignment, not predicted quality.
Read length histogram	A thumbnail histogram of trimmed lengths of all reads present in the test fragment.

3. In the **Read length histogram** column, click the thumbnail histogram to open a larger image and review the histogram details.



The figure shows an example histogram of read lengths, represented in base pairs (bp), that have a Phred-like score of ≥17, or one error in 50 bp (only the ends are shown because of width considerations). Distributions skewed to the right are ideal, showing longer read lengths (test fragments are a discrete length). It is likely that the sequence can extend all the way through the test fragment, if enough flows are run, so that the histogram displays only a maximum size based on the length of the test fragment.

View consumables used in a completed run

You can view a summary of reagent usage in an **S5 Consumable Summary** for sequencing runs on Ion GeneStudio™ S5 Series sequencers.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the name of the report of interest to open the report.

2. In the left navigation menu, click **Details** or scroll down to the **Details** section of the run report, then click the **S5 Consumables Summary** tab.

3. Note the details, including remaining uses for the cleaning solution.

Detail	Description
Chip Type	Chip type and version.
Chip Barcode	Barcode number of the chip.
Ion S5 Cleaning Solution	Row includes Part Number, Lot Number, Expiration Date, and Remaining Uses.
Ion S5 Sequencing Reagent	Row includes Part Number, Lot Number, Expiration Date, and Remaining Uses.
Ion S5 Wash Solution	Row includes Part Number, Lot Number, Expiration Date, and Remaining Uses.

View the summary for an Ion Chef™ Instrument run

You can view a summary for a completed run by an Ion Chef™ Instrument by viewing the run report.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link to open the report of interest.

   If necessary, filter the list to show only Ion Chef™ Instrument runs.

2. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Chef Summary** tab.

The following parameters are listed.

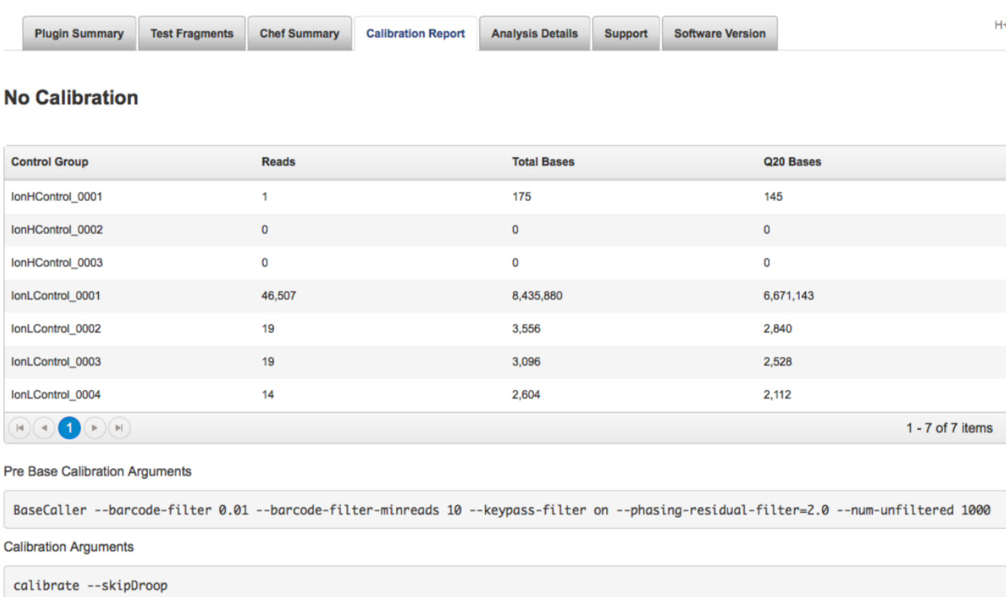
- Chef Last Updated
- Chef Instrument Name
- Chef Operation Mode
- Sample Position
- Tip Rack Barcode
- Chip Type 1
- Chip Type 2
- Chip Expiration 1
- Chip Expiration 2
- Templating Kit Type
- Chef Flexible Workflow
- Reagent Expiration
- Reagent Lot Number
- Reagent Part Number
- Reagent Cartridge Serial Number
- Solution Lot Number
- Solution Part Number
- Templating Protocol Planned
- Solution Cartridge Serial Number
- Solution Expiration
- Templating Protocol Executed
- Chef Script Version
- Chef Package Version
  Start Time
- Completion Time

View the calibration report for a completed run

You can review calibration settings that were applied to a run. Results can be calibrated to TMAP, the reference genome, or the calibration standard. For more information on setting calibration options, see "Advanced Settings—Kits step in the workflow bar" on page 46.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link to open the report of interest.

2. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Calibration Report** tab.
   Calibration details are displayed. If **Default Calibration** was selected for the run, the tab lists the usual Pre Base and Calibration Arguments. If **No Calibration** or the **Calibration Standard** were selected, the tab lists the control reads, total bases, and Q20 bases.



Control Group	Reads	Total Bases	Q20 Bases
IonHControl_0001	1	175	145
IonHControl_0002	0	0	0
IonHControl_0003	0	0	0
IonLControl_0001	46,507	8,435,880	6,671,143
IonLControl_0002	19	3,556	2,840
IonLControl_0003	19	3,096	2,528
IonLControl_0004	14	2,604	2,112

1 - 7 of 7 items

Pre Base Calibration Arguments

```
BaseCaller --barcode-filter 0.01 --barcode-filter-minreads 10 --keypass-filter on --phasing-residual-filter=2.0 --num-unfiltered 1000
```

Calibration Arguments

```
calibrate --skipDroop
```

Another way to access calibration details is to click **Report Actions ▸ Review Plan**, then scroll down to **Advanced Parameters**. For more information, see "Review Planned Run settings" on page 52.

View analysis details for a completed run

You can view analysis details for a completed run report.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link to open the report of interest.

2. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Analysis Details** tab.

3. Review the analysis details.

View software versions used in a run

You can view information about the versions of the software that was used for a completed run.

IMPORTANT! The version numbers that are shown can be different from your current version of the software based on the date of the run. It is possible that the report was analyzed with an older version of the software.

See **(Settings) ▸ About** in Torrent Suite™ Software for a complete list of modules and their version on your Ion Torrent™ Server. See the *Torrent Suite™ Software Release Notes* for details about the versions in a specific release.

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1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link to open the report of interest.

2. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Software Version** tab.

   The **Software Version** tab shows only the software products that were used to analyze the selected run.

Organize run results with projects

Projects are groups of result sets that you can create and to organize results into unique categories that are useful for your organization. A project can contain run results for the same laboratory project or results from completed runs that you want to combine. Projects also allow you to combine run results into a single run report. When you open a list of projects, you can:

- Quickly find and view details for a group of run results.
- Search the list by project name or partial name, or by date (date range, current month, current week, current day, or specific date).
- Click **Edit** to rename a project
- Click **Delete** to delete a project.
- Click **Log** to view a log of the project history.

Search for projects by name

You can search the lists of projects and result sets by name.

1. *(Optional)*To display a result sets list, in the **Projects** table, click the name of the project that contains your result set.

2. To find projects or result sets, search, sort, or filter the **Projects** and **Result Sets** lists.

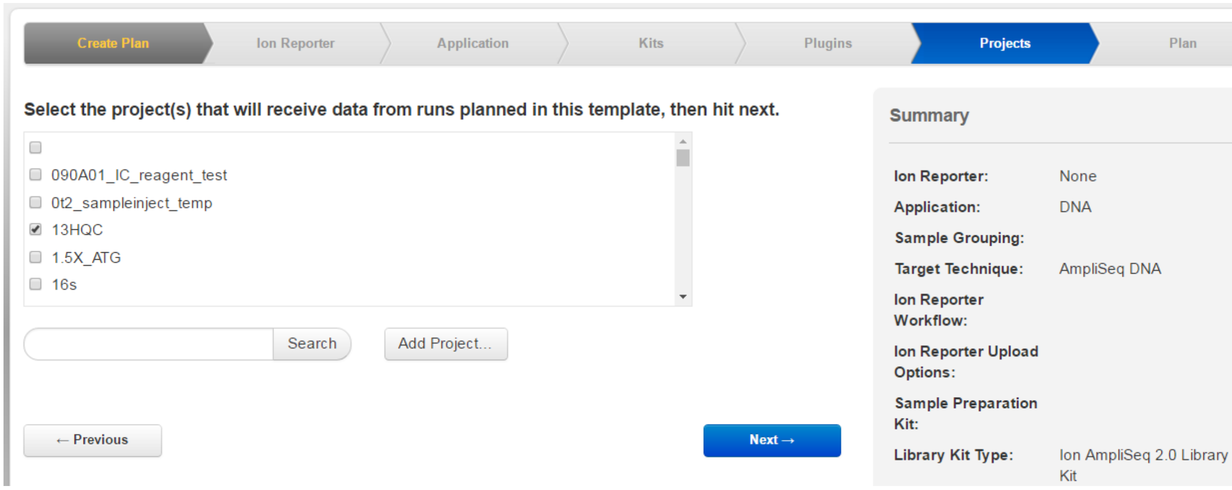
Option	Selection
Search the list	In **Search names**, enter a search term, then click **Go**. The information shown in the table is limited only to the names that match or contain the search string. You can enter a complete or partial name. For example, the following project names match the search string "mpli": amplicon, amplicon33, AmpliSeq, Samplier. The search is not case-sensitive and wildcards are not supported in the search string.
Sort the list	Click any bolded column heading in the table to sort the order in which the projects or result sets are displayed. Click the column heading a second time to reverse the sort order.
Filter the list by date	• In **Last Modified**, select a preset range (for example, last 7 days, or this month). • Click **Date Range**, then select the **Start** and **End** dates to limit your search to projects or result sets that are modified within the selected date range. • Click **Older than Date** or **Newer than Date** to limit your search to project or result sets that are modified before or after the selected date.

3. Click **Clear** to remove all search criteria and to show the unfiltered list of projects or result sets.

Add a project to a Planned Run

You can group specific result sets in a project, then add the project to a Planned Run or Planned Run template. The result sets that are in a project are viewable in the **Data** tab.

When you create a Planned Run, search for, then enter project names in the **Projects** step in the workflow bar.



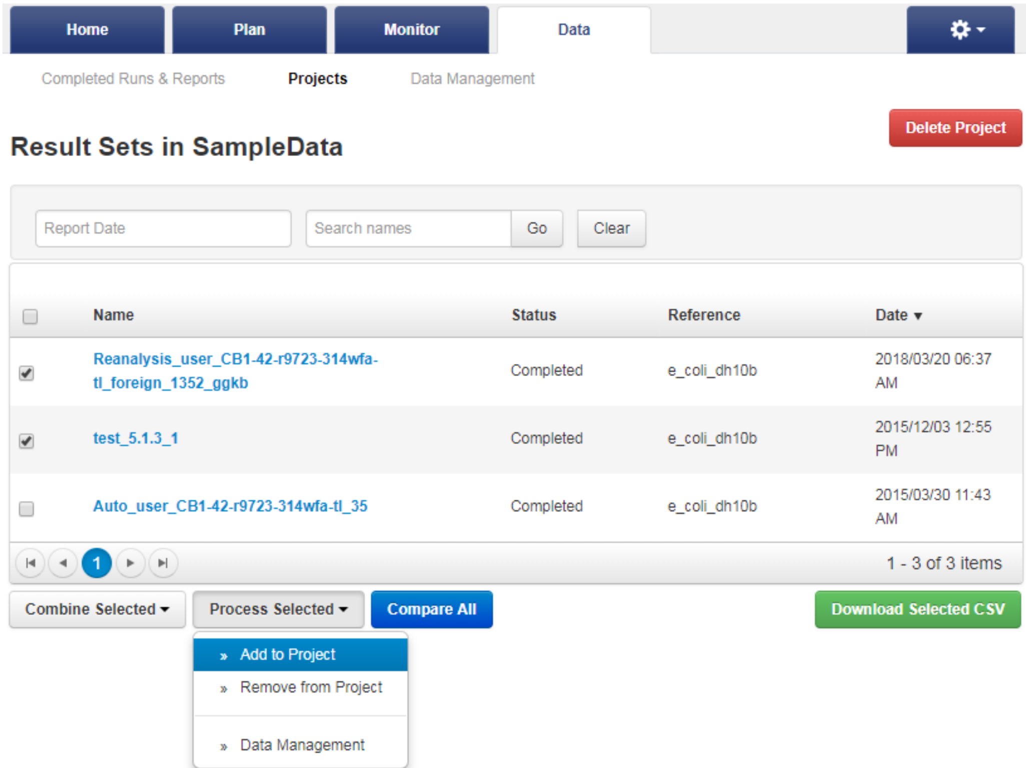
For more information, see Chapter 4, "Plan and execute an instrument run".

Add selected results to another project

1. In the **Data** tab, click **Projects** to see the list of projects.

2. In the **Name** column of the **Projects** table, click the project name to view the list of result sets in the project.

3. Select the checkboxes in the row of the result sets that you want to add to one or more other projects, then click **Process Selected ▸ Add to Project**.



4. Select the checkbox for each project that the result sets are to be copied to, then click **Add projects**.

View result sets in a project

1. In the **Data** tab, click **Projects** to see the list of projects.

2. Select a project in the list to view the result sets that are included in the project.

Compare reports of runs in a project

You can compare report metrics side-by-side for all runs that are assigned to a project.

1. In the **Data** tab, click **Projects**.

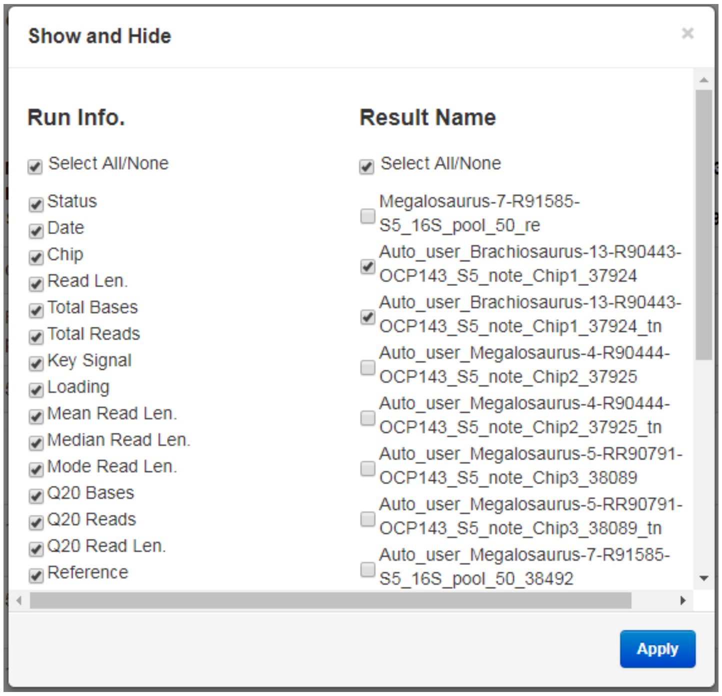
2. Select a project.

3. Click **Compare All** to view all the runs in the project side-by-side.

4. *(Optional)* Click **Customize**.
   The **Show and Hide** dialog box opens.

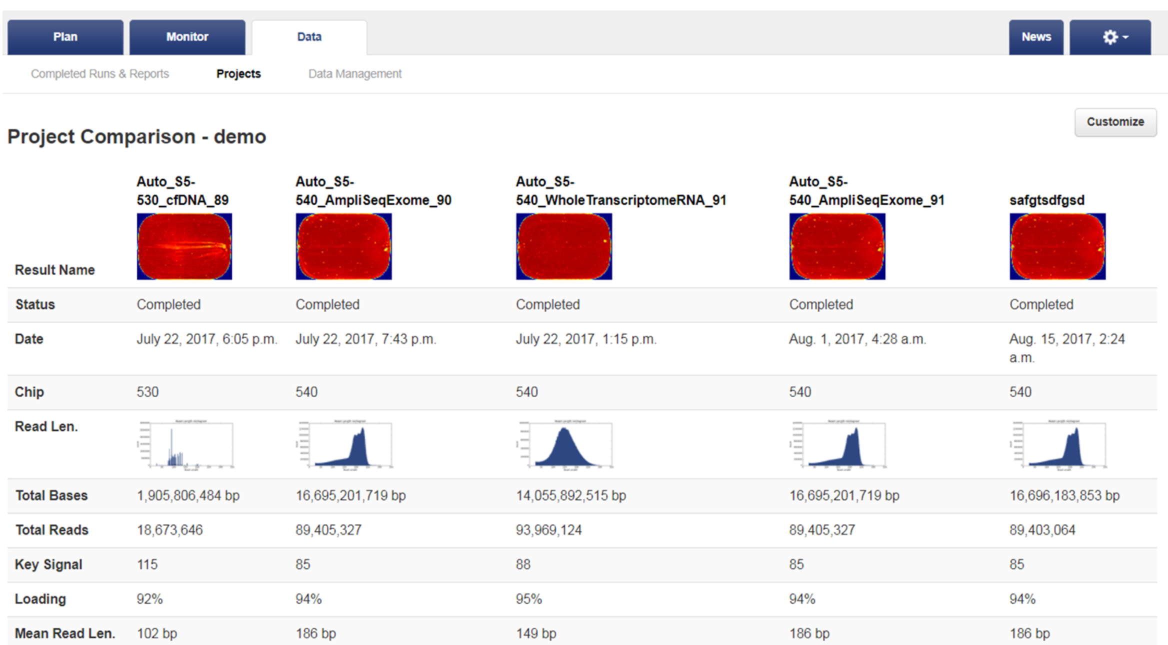
5. *(Optional)* In the **Run Info** column, deselect metadata to remove it from view.

6. *(Optional)* In the **Result Name** column, deselect runs to remove them from view.



7. Click **Apply**.

8. Review the reports for all runs in the project.



9. (Optional) Export the results.

- Click **Download PDF** to create a PDF report.
- Click **Download CSV** to create a CSV file of the results.

Manage data for result sets in projects

You can export, archive, or delete data from results that are included in a project.

1. In the **Data** tab, click **Projects** to see the list of projects.
2. Select a project from the list to view the result sets in the project.
3. Click **Process Selected ▸ Data Management**.

   For details, on how to export, archive, or delete data results in the project, see "Manually export run data" on page 317, "Manually archive run data" on page 317 , and "Manually delete selected data from a run report" on page 318.

Combine aligned reads from multiple run reports

If you are running an application that requires better coverage than the coverage provided by a single chip, you can:

- run multiple chips with the same sample and library
- combine aligned reads from multiple sequencing reports

  The combined aligned reads can be treated the same way as results from a single run. For example, it can be exported or used as an input for a plugin.

1. In the **Data** tab, click **Projects** to see the list of projects.
2. Select a project in the list to view the result sets in the project.
3. Select the result set or sets that you want to combine into a single run result set.
4. Click **Combine Selected ▸ Combine Alignments**.
5. In the **Combine Selected** dialog box, enter a new report name.
6. Make appropriate selections:

Option	Description
Mark as duplicate	Select this to identify duplicate reads and mark them in the BAM file. For some applications, duplicate reads coming from PCR cause problems in downstream analysis. The presence of duplicate reads can create the appearance of multiple independent reads supporting a specific interpretation, when some reads are in fact duplicates of each other with no additional evidence for the interpretation. **Note:** Marking duplicate reads is not appropriate for Ion AmpliSeq™ data, because many independent reads are expected to share 5' alignment position and 3' adapter flow as each other. Marking duplicates on an Ion AmpliSeq™ run risks inappropriately flagging many reads that are in fact independent of one another.
Overwrite sample name	Select this to identify duplicate names in your combined samples so that you can rename them.

7. Click **Launch**.

8. *(Optional)* Click **Report** to open the summary of the report, or **Log** to open the log for the report.

The combined report is added to the project from which the combine action was run.

Download a CSV file of metrics

You can download a CSV file of analysis metrics for one or more result sets, then compare results across analyses.

1. In the **Data** tab, click **Projects**, then click a project name to open the list of result sets for the project.

2. Select the checkboxes for the analyses, then click **Download Selected CSV**.
   The analysis metrics file is downloaded through the browser to a directory on the computer, based on the browser settings.

Analysis metrics file fields

In the analysis metrics file, each line represents a Torrent Suite™ Software analysis run. Within each line, fields are separated by a comma. Each comma-separated field is listed in a separate column. You can open the files with spreadsheet software such as Microsoft™ Excel™ or Apache® OpenOffice™ Calc.

The file has many fields per entry. Rows 4 through 11 (marked by *) contain test fragments metrics. The other rows contain library read metrics.

Field	Description
`Report`	Name of the analysis run report.
`Status`	Status of the analysis, for example, Started, Complete.
`Flows`	Number of flow cycles from the actual sequencing run.
`TF Name`*	Test Fragment Name.
`Q10 Mean`*	Average Q10 read length.
`Q17 Mean`*	Average Q17 read length.
`System SNR`*	System Signal-to-Noise Ratio.
`50Q10 Reads`*	Number of TF Ion Sphere™ Particles (ISP) at 50+ bp at Q10.
`50Q17 Reads`*	Number of TF Ion Sphere™ Particles (ISP) at 50+ bp at Q17.
`Keypass Reads`*	Number of reads that have test fragment keys.
`TF Key Peak Counts`*	Signal strength of the first three bases of the TF key.
`Total_Num_Reads`	Total number of reads.
`Library_50Q10_Reads`	Reads of length at least 50 bp with 90% or greater accuracy.
`Library_100Q10_Reads`	Reads of length at least 100 bp with 90% or greater accuracy.

(continued)

Field	Description
`Library_200Q10_Reads`	Reads of length at least 200 bp with 90% or greater accuracy.
`Library_Mean_Q10_Length`	Average length of reads with 90% or greater accuracy.
`Library_Q10_Coverage`	Average per base coverage that considers reads with 90% or greater accuracy.
`Library_Q10_Longest_Alignment`	Longest read length among reads with 90% or greater accuracy.
`Library_Q10_Mapped Bases`	Total bases from reads with 90% or greater accuracy.
`Library_Q10_Alignments`	Number of alignments from reads with 90% or greater accuracy.
`Library_50Q17_Reads`	Reads of length at least 50 bp with 98% or greater accuracy.
`Library_100Q17_Reads`	Reads of length at least 100 bp with 98% or greater accuracy.
`Library_200Q17_Reads`	Reads of length at least 200 bp with 98% or greater accuracy.
`Library_Mean_Q17_Length`	Average length of reads with 98% or greater accuracy.
`Library_Q17_Coverage`	Average per base coverage that considers reads with 98% or greater accuracy.
`Library_Q17_Longest_Alignment`	Longest read length among reads with 98% or greater accuracy.
`Library_Q17_Mapped Bases`	Total bases from reads with 98% or greater accuracy.
`Library_Q17_Alignments`	Number of alignments from reads with 98% or greater accuracy.
`Library_50Q20_Reads`	Reads of length at least 50 bp with 99% or greater accuracy.
`Library_100Q20_Reads`	Reads of length at least 100 bp with 99% or greater accuracy.
`Library_200Q20_Reads`	Reads of length at least 200 bp with 99% or greater accuracy.
`Library_Mean_Q20_Length`	Average length of reads with 99% or greater accuracy.
`Library_Q20_Coverage`	Average per base coverage that considers reads with 99% or greater accuracy.
`Library_Q20_Longest_Alignment`	Longest read length among reads with 99% or greater accuracy.
`Library_Q20_Mapped_Bases`	Total bases from reads with 99% or greater accuracy.
`Library_Q20_Alignments`	Number of alignments from reads with 99% or greater accuracy.
`Library_Key_Peak_Counts`	Signal strength of the first three bases of the library key.
`Library_50Q47_Reads`	Number of perfect reads of length at least 50 bp.
`Library_100Q47_Reads`	Number of perfect reads of length at least 100 bp.
`Library_200Q47_Reads`	Number of perfect reads of length at least 200 bp.

(continued)

Field	Description
`Library_Mean_Q47_Length`	Average length of perfect reads.
`Library_Q47_Coverage`	Average per-base coverage that considers only perfect reads.
`Library_Q47_Longest_Alignment`	Longest reads length among perfect reads.
`Library_Q47_Mapped_Bases`	Total bases from perfect reads.
`Library_Q47_Alignments`	Number of alignments from perfect reads.
`Library_CF`	CAFIE metric: Carry forward.
`Library_IE`	CAFIE metric: Incomplete extension.
`Library_DR`	CAFIE metric: Signal/polymerase loss (droop).
`Library_SNR`	System signal-to-noise ratio.
`Sample`	Name of the sample.
`Library`	Name of the reference genome.
`Notes`	Any additional user-provided notes.
`Run Name`	Long name of the analysis run.
`Run Date`	Date that the sample was sequenced.
`Run Directory`	Location of the raw DAT files on the Ion Torrent™ Server.
`Num_Washouts`	Deprecated field. Value for this field is 0 in CSV file.
`Num_Dud_Washouts`	Deprecated field. Value for this field is 0 in CSV file.
`Num_Washout_Ambigous`	Deprecated field. Value for this field is 0 in CSV file.
`Num_Washout_Live`	Deprecated field. Value for this field is 0 in CSV file.
`Num_Washout_Test_Fragment`	Deprecated field. Value for this field is 0 in CSV file.
`Num_Washout_Library`	Deprecated field. Value for this field is 0 in CSV file.
`Library_Pass_Basecalling`	Deprecated field. Value for this field is 0 in CSV file.
`Library_pass_Cafie`	Deprecated field. Value for this field is 0 in CSV file.
`Number_Ambiguous`	Deprecated field. Value for this field is 0 in CSV file.
`Number_Live`	Number of wells that produce a signal.
`Number_Dud`	Number of wells with ISPs but no signal.
`Number_TF`	Number of wells that contain test fragment.
`Number_Lib`	Number of wells that contain library.

(continued)

Field	Description
`Number_Bead`	Number of wells that contain beads.
`Library_Live`	Number of wells that contain library ISP with signal.
`Library_Keypass`	Number of wells that contain library ISP with signal and match key.
`TF_Live`	Number of wells that contain test fragment ISP with signal.
`TF_Keypass`	Number of wells that contain test fragment ISP with signal and match key.
`Keypass_All_Beads`	Number of wells that contain ISP with signal and match key.
`P`	Plugin data output in JSON format. The structured text format includes named fields.
`s`	Status of plugin data output in JSON format.

Remove a result set from a project

You can remove a result set from a project. Removing does not delete the selected run reports and their result sets from the system; it removes them only from the current project.

1. In the **Data** tab, click **Projects** to see the list of projects.
2. To view the list of result sets in the project, in the **Name** column of the **Projects** table, click the project name.
3. Select the checkboxes in the row of the result sets that you want to remove from the project, then click **Process Selected ▸ Remove from Project**.

Reanalyze a run

You can reanalyze a run to correct a setup error such as a default reference alignment or an assigned barcode, or to optimize analysis parameters.

Torrent Suite™ Software provides two ways to change the options used to analyze sequencing data. To change the options for the results of a sequencing run once, you can change the options in the run report, then immediately reanalyze the data. To change the run report for all subsequent reanalysis runs, you can edit the run report and save the updated report.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Search, filter, or sort the list to find the run report of interest.

   For more information, see "Search for a run report" on page 85.

3. Do one of the following.
   - In **Table View** mode, in the row of the run of interest, click ⚙ **(Actions)** ▸ **Reanalyze**.
   - Alternatively, select **List View** mode, find the run of interest, then click **Reanalyze**.

4. In the menu on the left, click **Reanalyze Run**, enter a **Report Name** for the new run, then select from the available options.

Option	Description
Thumbnail only	Select to reanalyze only the thumbnail report. This option is available only for data from Ion GeneStudio™ S5 Series sequencers.
Start reanalysis from	• Select **Signal Processing** to reanalyze from DAT files. The analysis does not use the **Use data from the previous result** option but reprocesses from the DAT files. You can also use **Analysis** and **Base Calling** options. • Select **Base Calling** (default) to reanalyze from 1.WELLS files. The analysis uses the **Use data from the previous result** or **Base Calling** options but reprocesses from the 1.WELLS file. The analysis does not use the **Analysis Parameters** option.
Use data from previous result	Select the previous result if more than one result is available. This option applies only when starting reanalysis from the basecalling step.
Analysis Parameters	Use the default analysis parameters or click **Custom** to select the analysis parameters. For more information, see "Copy an analysis parameter set" on page 57.

5. *(Optional)* In the menu on the left, click **Analysis Options**, then set the options as needed.

Option	Description
Library Key	The sequence that is used to identify library reads.
TF Key	The sequence that is used to identify test fragment reads.
3′ Adapter	The sequence of the 3′ Adapter that is used.
Mark as Duplicate Reads	Select to have PCR duplicates flagged in the BAM file.
Base Calibration Mode	Base calibration mode allows for empirical alignments to influence flow signals to achieve better homopolymer calibration to improve overall accuracy.
Enable Realignment	Select to use an optional analysis step to adjust the alignment, primarily in the CIGAR string.

6. *(Optional)* Set the reference and barcoding options.
   a. In the menu on the left, click **Reference & Barcoding**, then click **Edit Run Plan**.
   b. On the **Edit Run Plan** screen, set the default options as needed.
   c. Click **Update Run** to return to the reanalyze setup screen to select plugins to use in the analysis.

   d. If you do not need to return to the setup screen to change the plugins, click **Update Run and Reanalyze**.

7. *(Optional)* In the menu on the left, click **Plugins**.
   a. Select the plugin to include in the reanalysis.
   b. *(If needed)* Click the **Configure** link next to the plugin, scroll down to configure the plugin, then click **Save Plugin Settings**.
      For more information, see "Plugin configuration" on page 121, or the configuration topic specific to the selected plugin.
   c. Repeat steps a and b to include additional plugins in the reanalysis.

8. Click **Start Analysis**.

Change the default alignment reference

You can change the default alignment reference for a completed run, and then analyze the run again.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Use either **Table View** or **List View**, then find the run of interest.
   For help finding an individual run, see "Search for a run report" on page 85.
3. In the row of the run that you want to reanalyze, click ⚙ **(Actions)** ▸ **Reanalyze**.
4. In the next screen, click **Reference & Barcoding**.
5. Click **Edit Run Plan**, change any settings as appropriate, then click either **Update Run** or **Update Run & Reanalyze**.
   For more information, see "Create a user-defined configuration for the variantCaller plugin" on page 189 and "Run the variantCaller plugin manually" on page 185.

Edit a run report

You can edit a completed run report to correct a setup error or optimize parameters for later reanalysis.

Preinstalled Planned Run templates cannot be edited. Create a copy of the preinstalled Plan Run template, then make changes in the copy.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Search, filter, or sort the list to find the run report of interest.
   For more information, see "Search for a run report" on page 85.
3. Open the **Edit Run** workflow bar.
   - In **Table View**, click ⚙ **(Actions)** ▸ **Edit** in the row of the run report that you want to edit.
   - In **List View**, identify the run report of interest, then click **Edit**.

The **Edit Run** workflow bar opens to the **Save** screen.

4. Click a step in the workflow bar to access the screens to make changes.

Workflow step	Description
Ion Reporter	Select the **Ion Reporter Account**, **Sample Grouping**, and **Ion Reporter Upload Options**.
Research Application	Select the **Research Application** and **Target Technique**.
Plugins	Select the plugins to be included in the run.
Projects	Select the Project for the run data.
Save	Enter a **Run Plan Name**, then complete the information.

5. Click **Update Run** to save the edited run for later reanalysis, or click **Update Run & Reanalyze** to save the edited run and start the reanalysis immediately.

Add or change barcoding for a completed run report

You can change barcoding when you set up a reanalysis. You can also change barcoding for all future reanalyses. In either case, you can reanalyze the run after editing the barcode information. Use the **Edit** option to:

- Add barcoding to a completed sequencing run.
- Change the barcode set for a completed sequencing run.
- Remove barcoding from a completed sequencing run.

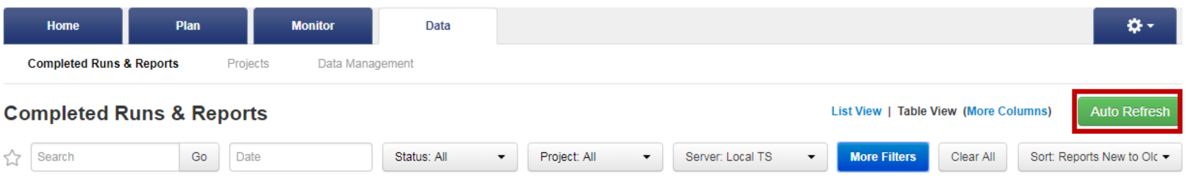
These steps apply only to completed runs.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In table or list view, navigate to your run of interest.
   For help finding an individual run, see "Search for a run report" on page 85.
3. Click ⚙ **(Actions)** ▸ **Edit** in the row of the run.
4. In the **Edit Run** screen, edit the **Run Plan Name**, if desired, make the appropriate barcoding changes, then click **Update Run** to save the edited run for later reanalysis, or click **Update Run & Reanalyze** to save the edited run and start the reanalysis immediately.

Set the Completed Runs & Reports screen to automatically refresh

Use the automatic refresh feature to update the **Completed Runs & Reports** screen. When the automatic refresh feature is turned on (the default), the screen refreshes every 20 seconds. When the automatic refresh feature is turned off, the screen is a static display of information at the time you opened the screen.

1. In the **Data** tab, on the **Completed Runs & Reports** screen, click **Auto Refresh**.



The button changes to **Stop Refresh** indicating that the automatic refresh feature is turned off.

2. Click **Stop Refresh** to turn off the automatic refresh feature.

7 Manage plugins for data analysis

You can expand the analysis capabilities of Torrent Suite™ Software with plugins that are preinstalled with the software. Additional plugins can be downloaded and installed from Thermo Fisher™ Connect Platform. The plugin results are added to the report summary and can be used for a variety of purposes.

Plugin configuration

Some plugins have settings that can be configured by users. These plugins can be configured three different ways.

- **Global configuration:** For plugins that can be configured globally, your administrator can change the settings for all users of the software on a selected server. These default settings can be overridden when setting up a Planned Run or Planned Run template, or when running the plugin manually.

  Some plugins require configuration. Plugins can fail if required settings are not configured. For example, some plugins require that a file directory is configured to receive output files.
- **Planned Run configuration:** Some plugins can be configured when setting up a Planned Run or Planned Run template. These options are available under **Plugins** in the Planned Run workflow bar. Settings that are selected here override the global settings.
- **Manual configuration:** Some plugins can be configured when they are selected to run on the data from a sequencing run after the run is complete. These plugins can be configured and run from the **Run Summary** screen. Settings that are selected here override the global settings or any Planned Run settings.

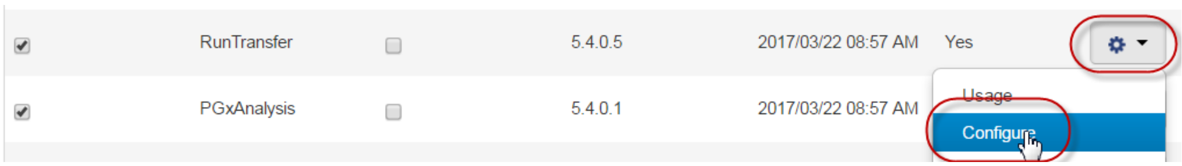
Configure plugins globally

An administrator can change the global configuration of a plugin. The following preinstalled plugins can be configured globally.

- DataExport plugin
- ERCC_Analysis plugin
- FileExporter plugin
- IonReporterUploader plugin

Some plugins that cannot be configured globally can be configured when you set up a Planned Run or a Planned Run template, or if you run the plugin after a sequencing run.

1. Click **(Settings)** ▸ **Plugins**.
2. In the **Manage** column for the plugin of interest, click **(Actions)** ▸ **Configure**.



The settings that are available in the configuration dialog box vary based on the plugin. See the plugin-specific configuration topic for more information.

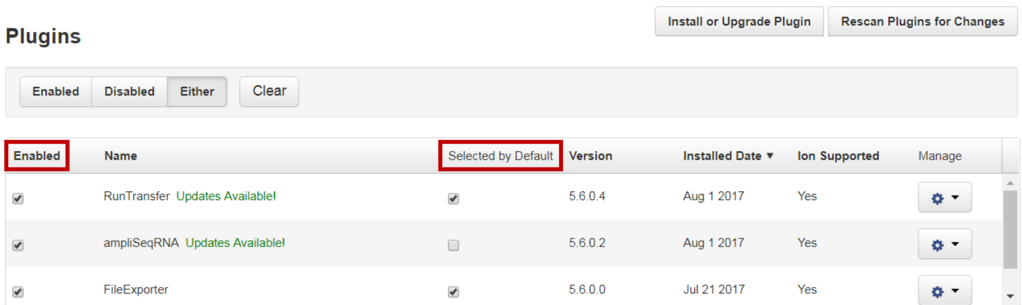
3. To save your changes, click **Submit** or **Save Configuration**.

Configure a plugin to run automatically

You can configure a plugin to run automatically by default. You can configure any plugin that is installed in Torrent Suite™ Software, whether it is preinstalled or is downloaded from Thermo Fisher™ Connect Platform. If a plugin runs automatically, you can still rerun the plugin manually after a sequencing run is completed. For details, see "Run a plugin manually from the sequencing run report" on page 124.

- To set a plugin to run automatically after every run:
  a. Click **(Settings)** ▸ **Plugins**.

b. Ensure that the **Enabled** checkbox is selected next to the plugin name that you want to run automatically by default.



You can click **Enabled**, **Disabled**, or **Either** to filter the list of plugins that is shown.

c. Select the **Selected by Default** checkbox next to the plugin name.

The plugin is now set to run after every sequencing run. You can deselect the **Selected by Default** checkbox to disable automatic execution of the plugin.

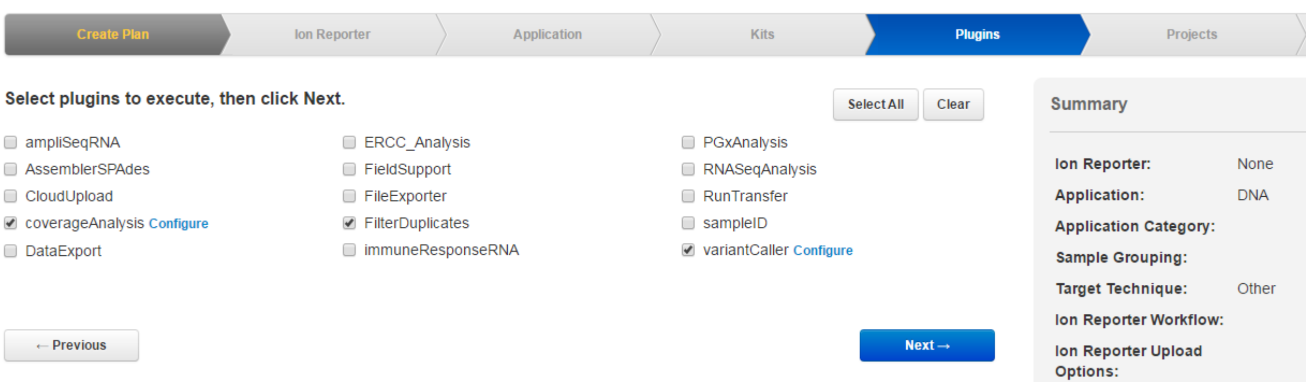
- To set a plugin to run automatically as part of a Planned Run or Planned Run template (not required if you previously set the plugin to run by default after every run):

a. Under the **Plan** tab, in the **Templates** screen, select an application in the left navigation menu.

b. Select an existing Planned Run template from the list. Alternatively, select **Add New Template**, or **Plan New Run** to create a new Planned Run template or Planned Run.

c. Click **Plugins** in the workflow bar.

d. Select the plugins that you want to run automatically after a run.



Note: If **Configure** appears after selecting the plugin, be sure to click the link and configure the plugin before starting the run. For detailed plugin configuration information for available plugins, see "Preinstalled plugins" on page 130.

e. Click **Next**, or another tab in the workflow bar to make further changes to your Planned Run.

f. When all changes to the Planned Run have been made, click **Plan** in the workflow bar, then click **Plan Run**.

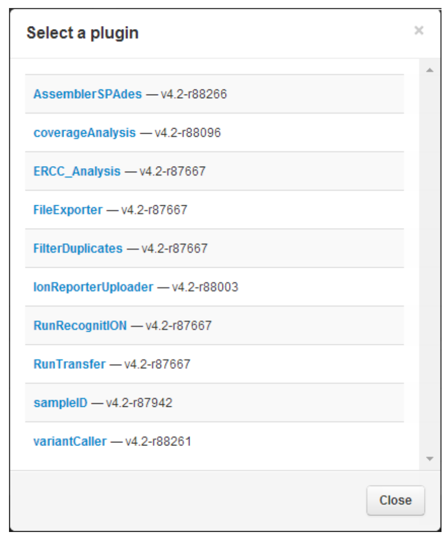
The plugin is now set to run after every sequencing run that uses the Planned Run or Planned Run template.

Run a plugin manually from the sequencing run report

You can manually run a plugin to perform an analysis after a sequencing run is complete. You might need do this if you did not configure the plugin in the run plan, to run an updated version of a plugin, or to run a previously used plugin with a different configuration.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for a completed sequencing run.
2. Click **Plugins** ▸ **Select Plugins to Run**.
3. In the **Select a plugin** window, , click the name of the plugin that you want to run.
4. In the **Configure Plugin** screen, configure the plugin if needed. If prompted, select the desired plugin options, then click **Submit** to start the analysis.

   If the plugin does not require configuration, analysis starts immediately without a confirmation screen.



Download a plugin report PDF

You can download a PDF file of a plugin report for a completed sequencing run.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for your completed sequencing run.
2. Click **Download PDF** ▸ **Plugins PDF**.

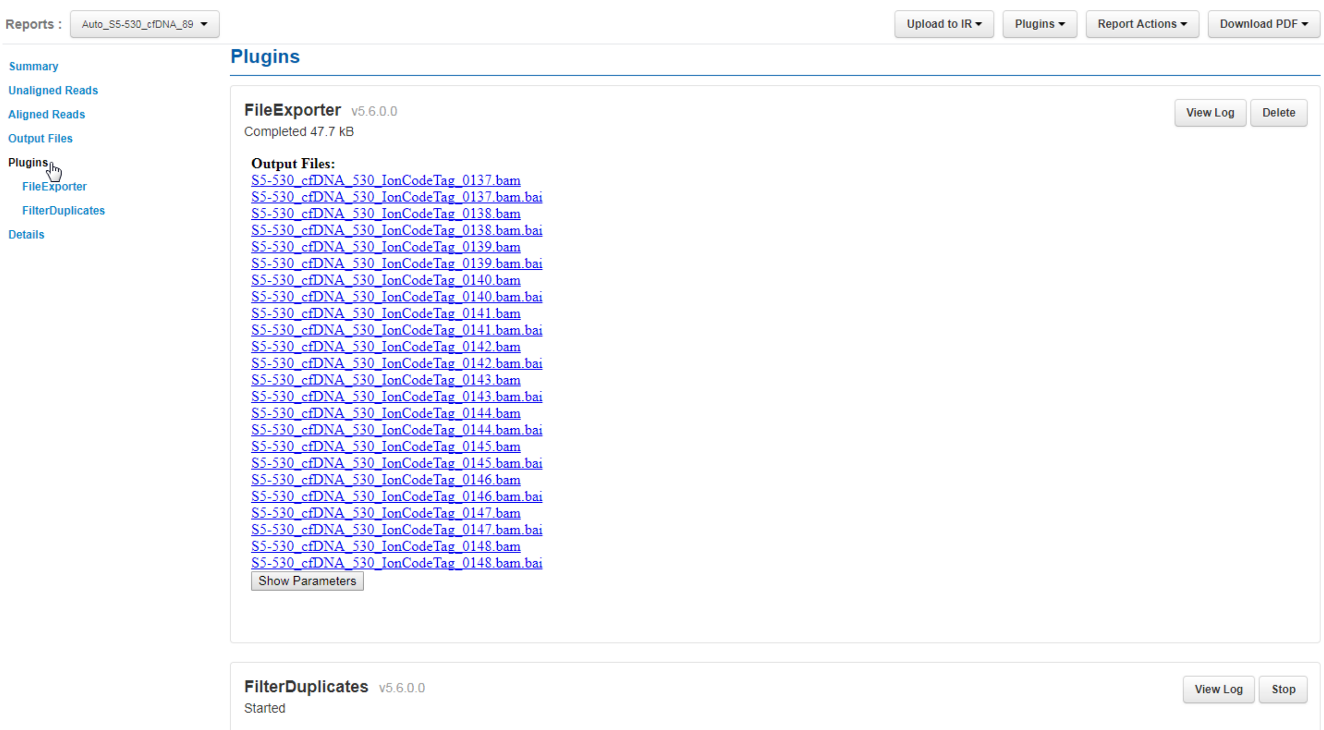
   A detailed report of the plugins that are included the run report is downloaded through the browser to a directory on your computer, based on the browser settings.

View plugin run status

After a plugin run is started, it is listed in the Plugin section of the run report. You can view the status of a plugin run to determine whether the run has completed. You can also stop a plugin run in progress, view a log for the plugin run, or delete the completed plugin report.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.

2. In the left navigation menu, click the plugin name, or scroll to the **Plugins** section of the run report.



The plugin run status (Queued, Started, or Completed) is listed under the name of each plugin.

Stop a plugin run

Use the **Data** tab to stop a plugin run that is in progress. You can also stop a plugin run in progress from the **Services** screen.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for a completed sequencing run.

2. In the left navigation menu, click the name of the plugin you want to stop, or scroll to the appropriate plugin section in the run report.

3. Click **Stop** to cancel a plugin run that has started.

Open a plugin log

If a Plugin report indicates that an error occurred during a plugin run, you can view a log that contains details about the plugin run.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
2. In the left navigation menu, click **Plugins**, or the name of the plugin that has the log you want to view.
3. Click **View Log** to the right of the plugin name.
   The log for the plugin run opens.

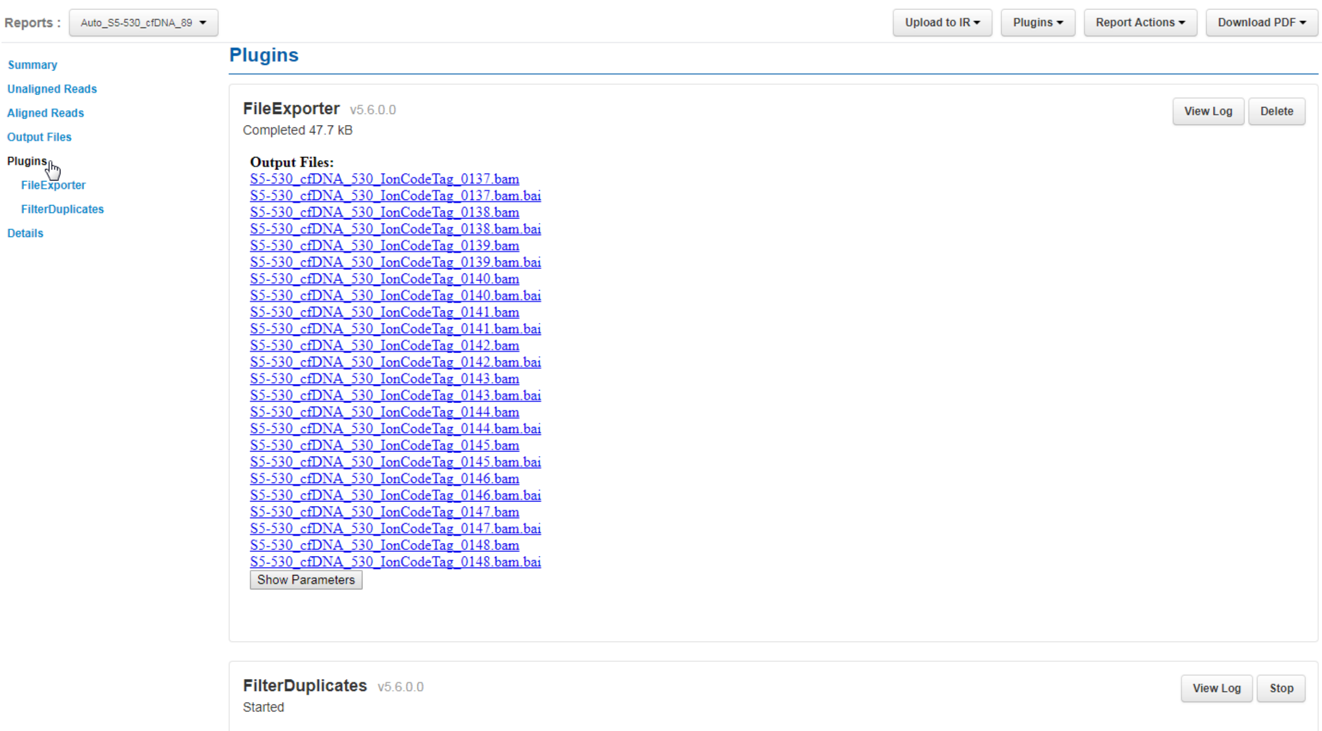
```
Plugin: coverageAnalysis - 0
Host: ts-docs
SGE: /var/lib/gridengine/iontorrent Home: /home/ionian Job: 497 - 'ion_plugin_coverageAnalysis_launch.sh'
SGE User:  Host: ts-docs Work Dir: '/'
INFO:ion-plugin-status:Updated PluginResult '7':True to status 'Started'
version=0
start time=Mon Mar 27 19:52:56 UTC 2017
command line=
coverageAnalysis: starting execution of plugin code
start time=2017-03-27 19:52:56.745368873
===============================================================================
DEBUG:ion.plugin.commands:Called with: Namespace(bctable_columns=False, block=None, dry_run=False, inspect=False, runmode='launch', verbose=2)
INFO:ion.plugin.runtime:Plugin Launch: 'coverageAnalysis' v5.4.0.5

(Mon Mar 27 19:52:57 UTC 2017) Started coverageAnalysis

Run configuration:
  Plugin version:   5.4.0.5
  Launch mode:      Manual
  Parameters:       startplugin.json
  Barcodes:         barcodes.json
  Output folder:    /results/analysis/output/Home/Auto_S5-540_WholeTranscriptomeRNA_91_003/plugin_out/coverageAnalysis_out.7
  Output file stem: S5-540_WholeTranscriptomeRNA_Auto_S5-540_WholeTranscriptomeRNA_91
Run parameters:
  Library Type:     RNA Sequencing
  Reference Name:   None
  Target Regions:   None
  Target Padding:   0
  Sample Tracking:  No
  Uniquely Mapped:  No
  Non-duplicate:    No
  Min Align Length: 0
  Min Map Quality:  0

Processing 0 barcodes...

Skipping IonXpressRNA_004:
ERROR: Analysis requires alignment to a reference

(Mon Mar 27 19:52:57 UTC 2017) Collating barcodes summary data...
coverageAnalysis_plugin.py: ERROR: No valid barcode alignment files were found for this barcoded run.
===============================================================================
ERROR: Plugin exited unexpectedly with error: 1 - Plugin Execution Error
INFO:ion-plugin-status:Updated PluginResult '7':True to status 'Error'
SGE exit_status: 1
```

Delete plugin results from a run report

You can delete plugin results from the **Plugins** section of the run report.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for your completed sequencing run.

2. In the left navigation menu, click **Plugins**, or the name of the plugin results that you want to delete.



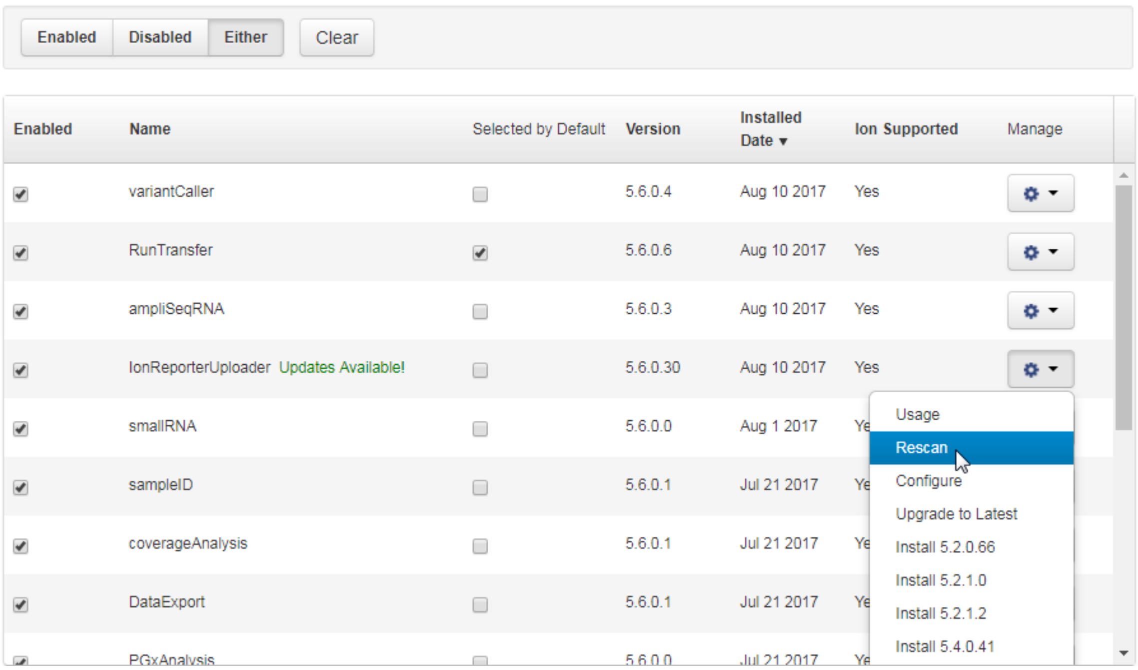
3. Click **Delete** to the right of the plugin name.
   The plugin results are deleted from the run report.

Rescan a plugin

If you uninstalled and reinstalled a plugin, you can rescan the plugin to ensure that all files from the previous installation were removed. When you rescan a plugin, the files for the plugin are updated with any changes.

You can also rescan the output files from the list of reports when you view the usage for a plugin.

1. Click ⚙ **(Settings)** ▸ **Plugins**. The installed plugins are listed.



2. Click ⚙ **Actions** ▸ **Rescan** in the row of the plugin.

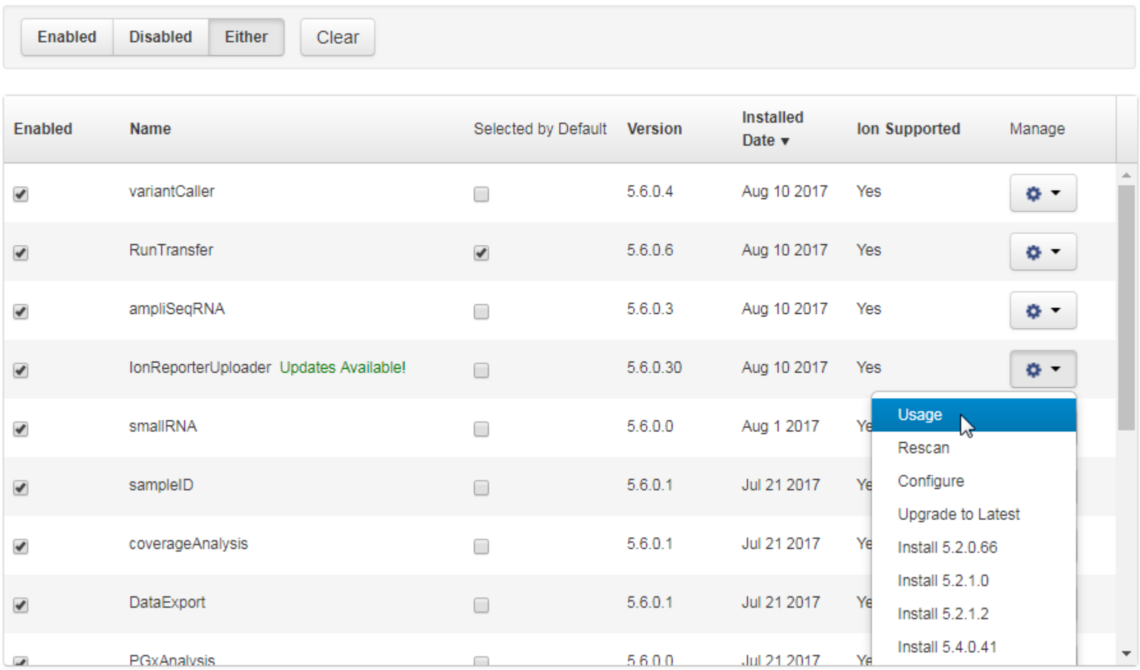
   You cannot complete other operations in Torrent Suite™ Software until the rescan is complete.

View plugin usage

You can view a list of the run reports on which a plugin has been run, the plugin completion status, and the size of the plugin outputs.

1. Click ⚙ **(Settings)** ▸ **Plugins**.
   The installed plugins are listed.

2. In the row of a selected plugin, click ⚙ **(Actions)** ▸ **Usage**.



3. In the **Plugin Results** section, view the following information from the list of run reports:
   - Date and time that a plugin run started and ended.
   - Status of the plugin run.
   - Size of the plugin run result output files.

Preinstalled plugins

This table lists and describes the plugins that are preinstalled in Torrent Suite™ Software.

Plugin name	Description
"ampliSeqRNA plugin" on page 131	Generates statistics, downloadable data files, and interactive visualizations that represent targeted RNA transcripts for sequencing runs that use the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit or Ion AmpliSeq™ RNA panels.
"CustomerSupportArchive" on page 135	Generates a customer support archive file during sequencing runs.
"coverageAnalysis plugin" on page 135	Generates statistics and graphs to describe the level of sequence coverage that is produced for targeted genomic regions.
"DataExport plugin" on page 145	Exports data from a sequencing run to an external hard drive or a removable media, such as a USB drive.
"ERCC_Analysis plugin" on page 146	Indicates whether a problem exists with library preparation or sequencing for runs that use the ERCC RNA Spike-In Mix.
"FieldSupport plugin" on page 150	Provides help with technical support. Enable and run this plugin only under the guidance of Thermo Fisher Scientific Technical Support. If you have questions about this plugin, contact your local Field Service Engineer or Technical Support.
"FileExporter plugin" on page 150	Customizes the output file names of an analysis run. This plugin allows you to rename output files. This plugin also generates a FASTQ format file of the analysis output, renames variantCaller plugin output files (when available), and compresses output files.
"FilterDuplicates plugin" on page 153	Removes duplicate reads and creates BAM files that do not include the duplicate reads.
"immuneResponseRNA plugin" on page 154	Quantifies gene expression levels for the Oncomine™ Immune Response Research Assay.
"IonReporterUploader plugin" on page 156	Transfers run results files to Ion Reporter™ Software. For details, see Chapter 9, "Integration with Ion Reporter™ Software".
"molecularCoverageAnalysis plugin" on page 157	Generates statistics, downloadable data files, and interactive visualization of molecular coverage over targeted regions of the reference genome.
"PGxAnalysis plugin" on page 163	For use with the Ion AmpliSeq™ Pharmacogenomics Research Panel, which is a targeted gene panel that allows the interrogation of pharmacogenomics variants in samples for genotyping and CYP2D6 copy number detection. For more information, see *Ion AmpliSeq™ Pharmacogenomics Research Panel User Guide* (Pub. No. MAN0014300).

(continued)

Plugin name	Description
"sampleID plugin" on page 166	This plugin produces an identification code (sampleID) for each barcode in a sample.
"variantCaller plugin" on page 167	Calls single-nucleotide polymorphisms (SNPs), multinucleotide polymorphisms (MNPs), insertions, deletions (INDELs), and block substitutions in a sample across a reference or in a targeted subset of that reference.

ampliSeqRNA plugin

The ampliSeqRNA plugin is used with the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit, Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit, or Ion AmpliSeq™ RNA panels. The plugin generates statistics, downloadable data files, and interactive visualizations that represent targeted RNA transcripts.

Use the ampliSeqRNA plugin on runs that are aligned to the human or mouse transcriptome references and appropriate target regions files listed below.

Reference	Target Regions file	Notes
hg19_AmpliSeq_Transcriptome_V1.2 [1]	hg19_AmpliSeq_Transcriptome_v1.2	The most up-to-date annotations are included in the panel files available from **AmpliSeq.com**. For more information, see the *Ion AmpliSeq™ Transcriptome Human Gene Expression Kit User Guide* (Pub. No. MAN0010742).
AmpliSeq_Mouse_Transcriptome_V1	AmpliSeq_Mouse_Transcriptome_v1	The most up-to-date annotations are included in the panel files available from **AmpliSeq.com**. For more information, see the *Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit User Guide* (Pub. No. MAN0017343).

[1] Previous versions (hg19_AmpliSeq_Transcriptome_ERCC_V1 and hg19_AmpliSeq_Transcriptome_V1.1) are still supported for those users who have already performed experiments with the older reference files.

ampliSeqRNA plugin configuration

The configuration options for the ampliSeqRNA plugin are described in the following table. This plugin cannot be configured globally.

You can change the reference genome that is used in the plugin run, for example from hg19 to mm10, if you edit the run report, then reanalyze the raw reads. For details, see “Edit a run report” on page 118.

Alternatively, you can reanalyze the run. For details, see “Reanalyze a run” on page 116.

Setting	Description
The following settings can be configured when you select the ampliSeqRNA plugin as part of a Planned Run or Planned Run template.	
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses if they have a relatively low number of reads, such as those that can result from barcode contamination. A warning appears in the barcode summary report if any barcodes were discounted from the analysis. This setting is ignored for runs not employing barcodes. Typically, this option is not needed if your Planned Run specifies which samples to associate with specific barcodes.
ERCC Tracking	Select this checkbox if your Ion AmpliSeq™ RNA targets (amplicons) were spiked with ERCC tracking targets.
The following settings can be configured when you run the ampliSeqRNA plugin manually.	
Library Type	ampliSeqRNA is selected by default and is the only library type that the ampliSeqRNA plugin is designed to work with. If the Planned Run specified a different application, a message warns you that the plugin may not be appropriate for the run.
Targeted Regions	This is set by default to the target regions file used in the Planned Run. You can override the default setting that each barcode uses. This might be useful to specify a subset of genes of interest, or to correct the original Planned Run.
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses. Typically, this option is not needed if your Planned Run specifies which samples to associate with specific barcodes.
ERCC Tracking	Select this checkbox if your Ion AmpliSeq™ RNA targets (amplicons) include a spike-in with ERCC tracking targets.

Review ampliSeqRNA plugin results

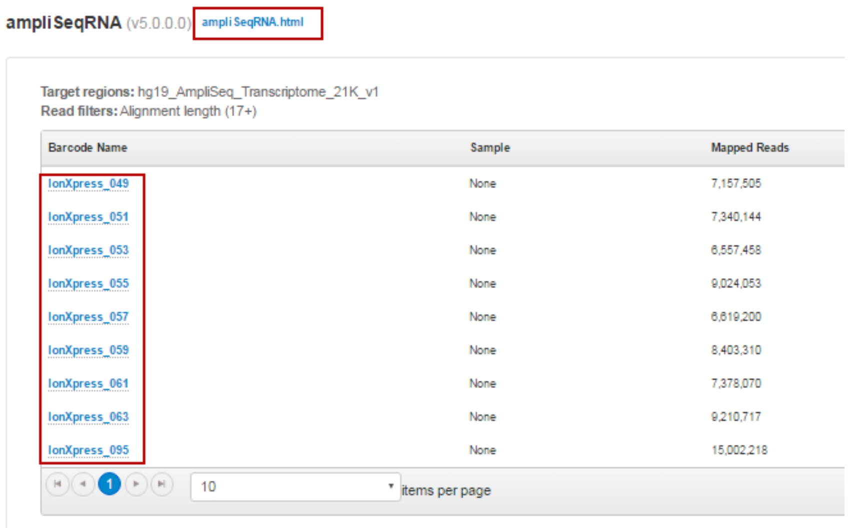
The ampliSeqRNA plugin generates an initial summary report that lists the samples, the number of mapped reads, the percent of valid reads, and the percent of targets detected. A series of log2 reads-per-million (RPM) pair correlation plots are included for rapid correlation analysis. Microsoft™ Excel™-compatible reports are also generated, including differential expression tables. Additional details about read coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

After the sequencing run completes, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.

2. In the list of runs, locate the run of interest, then click the link in the **Report Name** column.

3. In the left navigation menu, click **ampliSeqRNA** to view the plugin results.



- Click the **ampliSeqRNA.html** link to open the **ampliSeqRNA Report – Barcode Summary** for all barcodes.
- In the barcode table, click individual barcode names to see the results for an individual barcode.
- Click the **Distribution Plots**, **Correlation Heatmap**, **Correlation Plot**, and **Gene Heatmap** tabs to review the data graphically.

Graphical report	Description
Distribution Plots	
Reads Alignment Summary	A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the **Barcode Summary** table.
Distribution of Gene Reads	Distribution of genes across barcodes showing the frequency of numbers of genes having similar log10 read counts. All curves are plotted on the same axis scale. The counts data are fitted to a Gaussian kernel using the default R 'density' function.
Correlation Heatmap	A heatmap of Spearman correlation r-values for comparing log2 RPM reads pair correlation barcodes, with dendrogram reflecting ordering of barcodes as being most similar by these values.

(continued)

Graphical report	Description
Correlation Plot	
Barcode read pair correlation plot	Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show the frequency density plot for the individual log(RPM+1) values for each barcode. (If only one barcode has reads, a density plot is displayed.) Click the plot to open an expanded view.
Gene Heatmap	
Gene Representation Heatmap	Displays 250 genes showing the most variation in representation across barcodes as measured by the coefficient of variation (CV) of normalized read counts for genes that have at least one barcode with at least 100 RPM reads, plotted using log10 of those counts. For this plot, barcodes are omitted if they have $<10^5$ total reads.

- Click the links at the bottom of the report to download associated report files.

ampliSeqRNA plugin reports

The following ampliSeqRNA plugin reports are available for download from the results screen as tab-delimited text files, which are compatible with Microsoft™ Excel™ or similar applications.

Report	Description
Barcode Summary Report	A table listing each barcode sample name, total reads, aligned reads, and percent aligned.
Absolute Reads Matrix	A table listing absolute reads for the genes found on each barcode.
Absolute Normalized Reads Matrix	A table listing absolute normalized reads for the genes found on each barcode.
CHP files normalized by RPM	A file format designed for use with Applied Biosystems™ microarray software applications to produce additional reports.
Differential Expression for Barcode Pair	A pop-up window that allows you to compare two barcodes. You can set a threshold for minimum read count and exclude targets from the differential expression table. Differential expression for each target that is represented as the log2 of the ratio of RPM reads of the experiment barcode to the control barcode.

CustomerSupportArchive

The CustomerSupportArchive plugin generates a customer support archive file during sequencing runs.

A customer support archive file is a downloadable compressed file that a support representative can use to diagnose issues with sequencing runs or with Torrent Suite™ Software. The compressed file contains log files and other technical data about the software and other files from sequencing runs.

You can download the customer support archive file from the **Data** tab from the **Completed Runs & Reports** screen. For more information, see "Download a customer support archive file" on page 343.

Under some circumstances, you can use the FieldSupport plugin to generate an archive for use by Technical Support. For details, see "FieldSupport plugin" on page 150.

coverageAnalysis plugin

Use the coverageAnalysis plugin to view statistics and graphs that describe the level of sequence coverage produced for targeted genomic regions. The results in the **Summary** screen for a run analyzed with the plugin vary based on the library type that you select when you configure the plugin. You can export some charts as graphics, such as the **Amplicon** and **Reference Coverage** charts.

coverageAnalysis plugin configuration

The coverageAnalysis plugin uses the following settings.

Setting	Description
The following settings are available for all library types.	
Reference Genome	The reference genome selected in the Planned Run.
Library Type	The default value is the library type selected in the Planned Run and can be changed only if the plugin is run manually. If you change the library type, a different report is generated.
Targeted Regions	The targeted regions are selected in the Planned Run, and can be changed only after the run is complete if the plugin is run manually. Target regions can be overwritten by the specific barcode targets. Select the targeted regions file from the list. For whole genome and Ion Total RNA-Seq sequencing runs, you typically select None.

(continued)

Setting	Description
Barcode-specific Targets	This option is available only when the coverageAnalysis plugin is run manually. Select the checkbox to assign specific target region files to individual barcodes. **1.** Select a specific barcode. **2.** Select the specific Target Regions file to associate with the selected barcode. **3.** Click **Add**. **4.** Repeat steps 1 through 3 to associate additional barcodes with specific Target Region files. Alternatively, you can copy and paste the barcode/target file pairs manually. Barcodes without a Target Region specified above assume the default target specified by the **Target Regions** option. For targeted applications, any barcode targets specifically set to **None**, or defaulting to **Target Regions** set to **None**, are omitted from subsequent analysis. When the **Barcode-specific Targets** option is deselected, all barcodes use the targets that are specified by the Target Regions, even if barcode-specific targets are listed.
The following settings are advanced options.	
Minimum Aligned Length	Specify the minimum aligned length that is required to ensure that the read is included in an analysis.
Minimum Mapping Quality	Specify a minimum value that reads must exceed to be included in the analysis.
Tier 1 Coverage Depth	Specify the first-tier coverage depth at which percentage of target coverage is reported. This value must be at least 2, because the coverage depth output is always specified at 1*x* read depth. The default value of 20 means that the percentage of targets, total base targets, and/or individual target bases with at least 20 reads is reported.
Tier 2 Coverage Depth	Specify the second-tier coverage depth at which percentage of target coverage is reported. This value must be greater than the value used for the first-tier coverage. The default value of 100 means that the percentage of targets, total target bases, and/or individual target bases with at least 100 reads is reported.
Tier 3 Coverage Depth	Specify the third-tier coverage depth at which percentage of target coverage is reported. This value must be greater that the value used for the second-tier coverage. The default value of 500 means that the percentage of targets, total target bases, and/or individual target bases with at least 500 reads is reported.
The following settings are available only with specific library types.	
Uniquely Mapped Reads	Select this option to analyze only reads that are mapped to a unique location in the reference. 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.

(continued)

Setting	Description
Sample Tracking	The Ion AmpliSeq™ Sample ID Panel is a companion panel of 9 primer pairs that can be added to any Ion AmpliSeq™ human gDNA panel during target amplification to generate a unique identification tag for research samples. Select this checkbox if you added the Ion AmpliSeq™ Sample ID Panel to your library.
Target Padding	Enter a number to pad the target by the number of bases entered. If you do not enter a number, the default of 0 is used.
Non-duplicate Reads	Select the checkbox to avoid duplicate reads. The analysis must have included alignments with **Mark Duplicates** enabled.

Review coverageAnalysis plugin results

The coverageAnalysis plugin generates a Coverage Analysis Report. This report includes read statistics and several charts. The statistics and charts that are presented depend on the library type for the analysis.

The report summary lists the barcodes, the samples, the number of mapped reads, the percentage of on target reads, mean base coverage depth, and base coverage uniformity. Microsoft™ Excel™-compatible reports are also generated, including differential expression tables. Additional details about read coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

You can download statistics files and the aligned reads BAM file from the file links at the bottom of the Coverage Analysis Report. After the sequencing run completes, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **coverageAnalysis** to view the plugin summary.
   A summary table of the coverage analysis, by barcode, is included in the coverageAnalysis summary pane.
4. In the coverageAnalysis barcode summary, in the **Barcode Name** column, click a link to open a detailed **Coverage Analysis Report** for that barcoded sample.
   Alternatively, click the **coverageAnalysis.html** link to open the summary table for all barcodes in a new window.
5. In the **Coverage Analysis Report**, review the plugin results. Click the links at the bottom of the **Coverage Analysis Report** to download associated statistics and summary files for each barcoded sample in the run.

Reads statistics

General statistics

Statistic	Description
Number of mapped reads	The total number of reads mapped to the reference genome.
Percent reads on target	The percentage of filtered reads mapped to any targeted region relative to all reads mapped to the reference. If no target regions file is specified, this value is the percentage of reads passing uniquely mapped and/or nonduplicate filters, or 100% if no filters were specified. A read is considered on target if at least one aligned base overlaps at least one target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5' bases of the read, is not counted.

Amplicon read coverage statistics

The following statistics describe the reads that are assigned to specific amplicons. Each sequence read is assigned to exactly one amplicon specified by the targets file. If a read spans multiple amplicon targets, the target region that the reads covers most is assigned. If there is a tie, the target that is the closest to the 3' end is assigned.

Statistic	Description
Number of amplicons	The number of amplicons that is specified in the target regions file.
Percent assigned amplicon reads	The percentage of reads that were assigned to individual amplicons relative to all reads mapped to the reference. A read is assigned to a particular (inner) amplicon region if any aligned bases overlap that region. If a read might be associated with multiple amplicons, it is assigned to the amplicon region that has the greatest overlap of aligned sequence.
Average reads per amplicon	The average number of reads assigned to amplicons.
Uniformity of amplicon coverage	The percentage of amplicons that had at least 20% of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts.
Amplicons with at least *N* reads	The percentage of all amplicons that had at least *N* reads.
Amplicons with no strand bias	The percentage of all amplicons that did not show a bias towards forward or reverse strand read alignments. An individual amplicon has read bias if it has ≥10 reads and the percentage of forward or reverse reads to total reads is >70%. Amplicons with <10 reads are considered to have no strand bias.

(continued)

Statistic	Description
Amplicons reading end-to-end	The percentage of all amplicons that were considered to have a sufficient proportion of assigned reads (70%) that covered the whole amplicon target from 'end-to-end'. To allow for error, the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.
Amplicon base composition bias	A number that represents the proportion of amplicons showing low representation (<0.2x mean reads) in the lower and/or upper quartiles of amplicons ordered by increasing GC base pair content of their insert sequences. The value is relative to that in the center 50th percentile of amplicons and weighted by the standard deviation of representation over all amplicons. An RMS (root mean square) value is used so that a bias greater in either upper or lower quartiles produces a larger value than a mean bias seen more equally in both outer quartiles. The value is 0 if the uniformity of amplicon coverage metric is 100%, however, the value is not necessarily high at lower amplicon uniformity.

Target base coverage statistics

The following statistics describe the targeted base reads of the reference. A base covered by multiple target regions is counted only once per sequencing read.

Statistic	Description
Bases in target regions	The total number of bases in all specified target regions of the reference.
Percent base reads on target	The percent of all bases covered by reads aligned to the reference that covered bases in target regions. Clipped bases, deletions, and insertions (relative to the reference) are not included in this percentage. If no specific target regions were specified, the whole genome is the targeted regions.
Average base coverage depth	The average number of reads of all targeted reference bases. This is the total number of base reads on target divided by the number of targeted bases, and therefore includes any bases that had no coverage.
Uniformity of base coverage	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.
Target base coverage at *N*x	The percentage of target bases covered by at least *N* reads.
Target bases with no strand bias	The percentage of all target bases that did not show a bias toward forward or reverse strand read alignments. An individual target base is considered to have read bias if it has ≥10 reads and the percentage of forward or reverse reads to total reads is >70%. Target bases with <10 reads are considered to have no strand bias.
Percent end-to-end reads	The percentage of on-target reads that fully cover their assigned amplicon (insert) from 'end-to-end'. To allow for error, the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.

Example charts generated by the coverageAnalysis plugin

Many of the charts in the detailed **Coverage Analysis Report** include a **Plot** menu where you can change characteristics of the chart.

Use the button (in the top right corner of a chart) to open the chart **Viewing Options** panel. The button opens a description of the chart.

In most charts, you can click a data point to open a detail pane for that data. For example, in the Depth of Coverage Chart, click an individual orange bar to open the detail pane for bases within a specific range of base read depths.

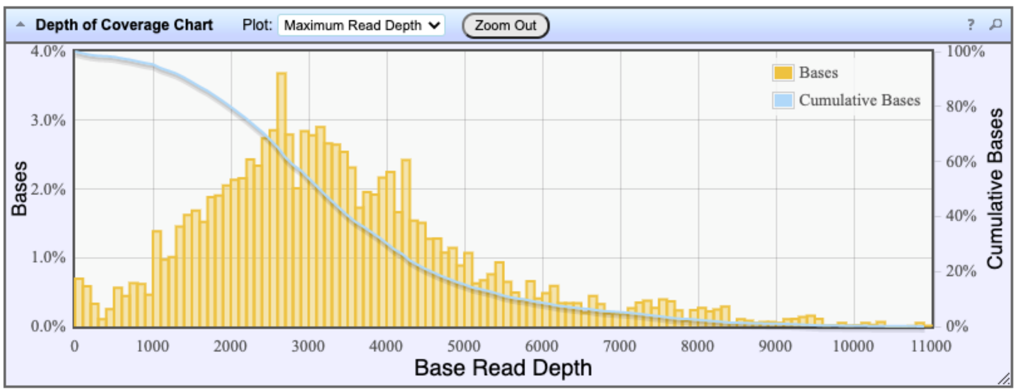


Figure 1 Representative Depth of Coverage Chart The Depth of Coverage Chart shows the distribution of targeted base coverage. The X-axis represents the base read depth. The left Y-axis represents the number of reads at a given base read depth or a range (bin) of base read depths, as a percentage of the total number of base reads. The right Y-axis represents the cumulative count of the number of reads at a given read depth or greater, as a percentage of the total number of reads. The individual orange bars represent the percentage of reads in the specific range of base read depths. The blue curve measures the cumulative reads at a given base read depth or greater. If your analysis includes a regions of interest file, this chart reflects only target regions (reads that fall within a region of interest).

Click a point on the blue curve to open the detail pane for cumulative bases at that base read depth or greater.

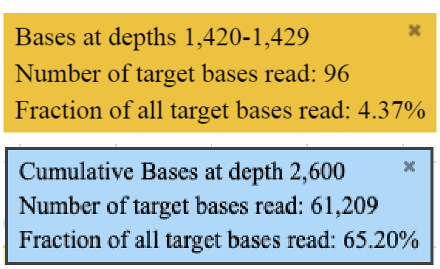


Figure 2 Example detail panes

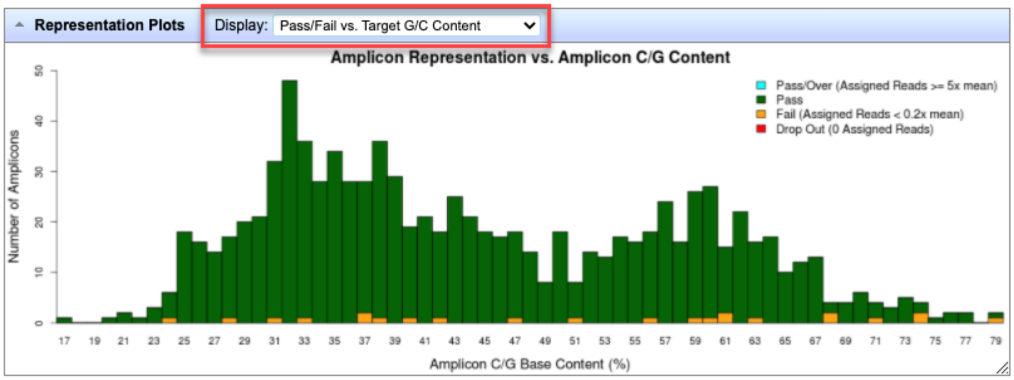


Figure 3 **Representation Plots** Use the **Display** list to view different amplicon representation plots. This figure shows an example Pass/Fail vs. Target G/C Content plot.

Similarly, you can click any point or bar within the Amplicon Coverage Chart to view details for each amplicon coverage bin.

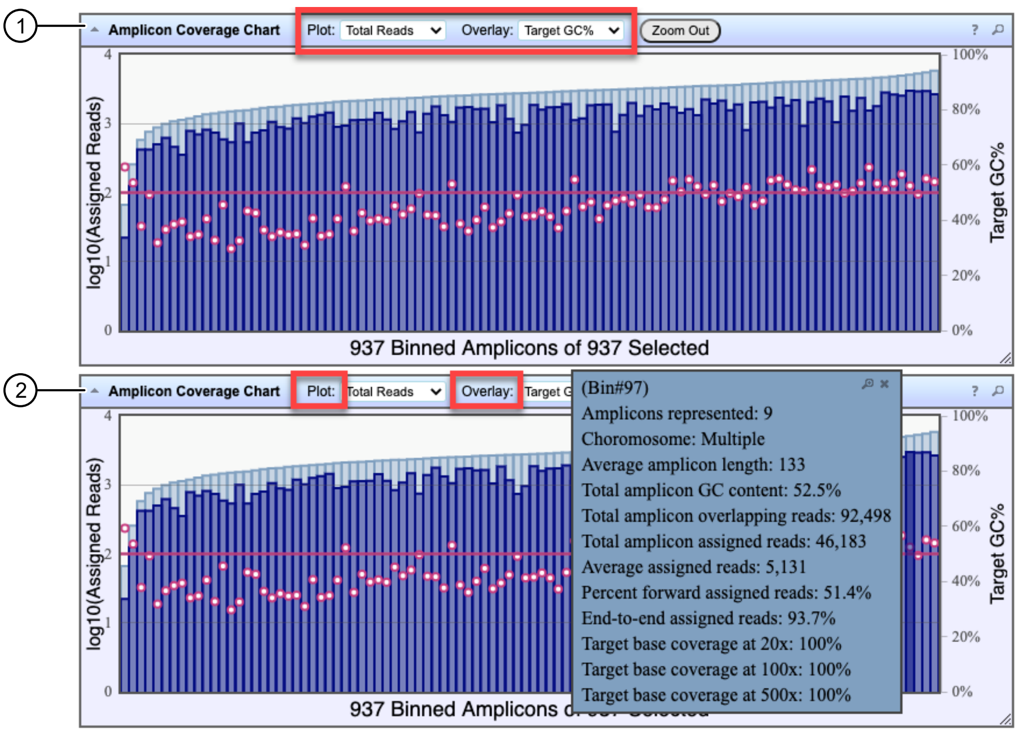


Figure 4 **Example Amplicon Coverage Chart** The Amplicon Coverage Chart summarize the amplicon coverage results. Several plots views are available. The X-axis in all plots represents individual amplicons. The left Y-axis in all plots represents assigned reads (on a base 10 log scale). Use the **Plot** and **Overlay** lists to customize the chart view. The right Y-axis varies based on the plot view that is selected from the **Overlay** dropdown list. Click any bar or any point on the chart to view the detail pane for that data.

① Example Total Reads plot with a Target GC percentage overlay

② Click a bar to open the detail pane for the specific amplicon. Click **View in Reference Coverage Chart** to zoom in on the amplicon in the **Reference Coverage Chart** (see callout 3 in Figure 5).

In the Reference Coverage Chart, you can show both strands.

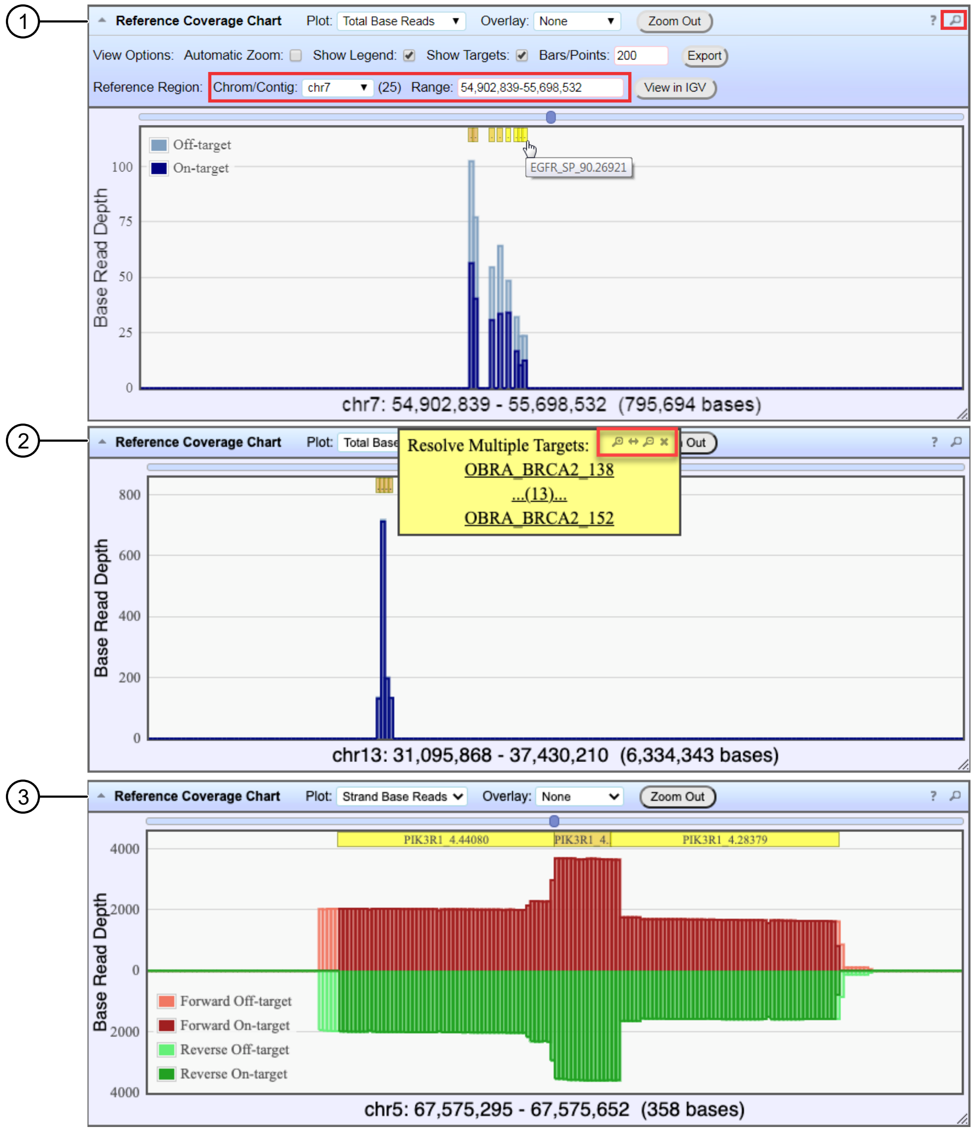


Figure 5 Example Reference Coverage Chart The Reference Coverage Chart is an overlay of where target regions are defined and overlap on the reference. The X-axis represents the target region chromosomal position. The Y-axis represents the Base Read Depth.

① Example Total Base Reads plot with Viewing Options panel expanded. The plot is zoomed in on a specific chromosomal region. Click a yellow bar (top of the chart) to open the detail pane for a specific amplicon in the Amplicon Coverage Chart (see callout 2 in Figure 4).

② Example Total Base Reads plot where a chromosomal region has multiple targets. In the Resolve Multiple Targets pane, click 🔍 to zoom in on the region, or click one of the links to open the detail pane for the specific amplicon in the Amplicon Coverage Chart.

③ Example Strand Base Reads plot that is zoomed in on one specific amplicon. Click the yellow box to open the detail pane for the specific amplicon in the Amplicon Coverage Chart.

Output files generated by the coverageAnalysis plugin

You can download coverageAnalysis plugin results files from links that are contained in the **File Links** section.

Sometimes the file name can be too long to open in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file and click **Save As** to rename the downloaded file.

Click ⓘ **(Help)** next to the file to open a description of the file.

The following is an example of the content of a results file that is generated by the coverageAnalysis plugin.

The list of files depends on the application type selected.

File	Description
Coverage statistics summary	A summary of the statistics presented in the tables at the top of the plugin report. The first line is the title. Each subsequent line is either blank or contains a statistic title followed by a colon (:) and its value.
Base depth of coverage	Coverage summary data used to create the Depth of Coverage Chart. This file contains the following fields. • `read_depth`—The depth at which a (targeted) reference base has been read. • `base_cov`—The number of times any base was read (covered) at this depth. • `base_cum_cov`—The cumulative number of reads (coverage) at this read depth or greater. • `norm_read_depth`—The normalized read depth (depth divided by average base read depth). • `pc_base_cum_cov`—Same as `base_cum_cov`, but represented as a percentage of the total base reads.

(continued)

File	Description
Amplicon coverage summary	Coverage summary data used to create the **Amplicon Coverage Chart**. This file contains these fields. • `contig_id`—The name of the chromosome or contig of the reference for this amplicon. • `contig_srt`—The start location of the amplicon target region. This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file. • `contig_end`—The last base coordinate of this amplicon target region. **Note:** The length of the amplicon target is given as `tlen` = (`contig_end` - `contig_srt` + 1). • `region_id`—The ID for this amplicon as given as the 4th column of the targets BED file. • `gene_id`—The gene symbol as given as the last field of the targets BED file. • `gc_count`—The number of G and C bases in the target region. `%GC` = 100% * `gc` / `tlen`. • `overlaps`—The number of times this target was overlapped by any read by at least one base. Individual reads might overlap multiple amplicons where the amplicon regions themselves overlap. • `fwd_e2e`—The number of assigned forward strand reads that read from one end of the amplicon region to the other end. • `rev_e2e`—The number of assigned reverse strand reads that read from one end of the amplicon region to the other end. • `total_reads`—The total number of reads assigned to this amplicon. This value is the sum of `fwd_reads` and `rev_reads` and is the field that rows of this file are ordered by (then by `contig id`, `srt` and `end`). • `fwd_reads`—The number of forward strand reads assigned to this amplicon. • `rev_reads`—The number of reverse strand reads assigned to this amplicon. • `cov20x`—The number of bases of the amplicon target that had at least 20 reads. • `cov100x`—The number of bases of the amplicon target that had at least 100 reads. • `cov500x`—The number of bases of the amplicon target that had at least 500 reads.

(continued)

File	Description
Chromosome base coverage summary	Base reads per chromosome summary data used to create the default view of the **Reference Coverage Chart**. This file contains these fields. • `chrom`—The name of the chromosome or contig of the reference. • `start`—The coordinate of the first base in this chromosome. This is always 1. • `end`—The coordinate of the last base of this chromosome. Also, its length in bases. • `fwd_reads`—The total number of forward strand base reads for the chromosome. • `rev_reads`—The total number reverse strand base reads for the chromosome. • `fwd_ontrg` (if present)—The total number of forward strand base reads that were in at least one target region. • `seq_reads`—The total sequencing (whole) reads that are mapped to individual contigs.
Aligned reads BAM file	Contains all aligned reads that are used to generate this report, in BAM format. This is the same file that can be downloaded from the main report (for the specific barcode). See the current SAM tools documentation for more file format information.
Aligned reads BAI file	Binary BAM index file as required by some analysis tools and alignment viewers. This is the same file that can be downloaded from the main report (for the specific barcode).

DataExport plugin

Use the DataExport plugin to export data from a sequencing run to a network drive, an external hard drive, or a removable media device, such as a USB drive. The exported data can be used to create backups, or to quickly transfer files to another system. When you configure the plugin, you select which file categories from the run are included in the export.

Before you use the DataExport plugin, a software administrator must configure the path to the directory that is used for the export. The **Destination Path** to the external drive is then available in the global settings for the plugin.

DataExport plugin configuration

The DataExport plugin can be configured to set the destination path of the exported files, and to specify the file types to be exported.

The configuration options for the DataExport plugin are described in the following table:

Setting	Description
Destination Path	Designates the location of the network drive, external hard drive or removable media device where the files are exported to.
Signal Processing Input	Exports DAT files.
Basecalling Input	Exports WELLS files.
Output Files	Exports all output files, including BAM files, reports, and analysis files.
Intermediate Files	Exports files used for troubleshooting by qualified system engineers.

Review DataExport plugin results

After the sequencing run completes, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the **Summary**, click **DataExport** to view the plugin summary.

After the export is complete, the report is available in the **DataExport** pane. The following parameters are shown.

Parameter	Description
FILE CATEGORIES	Lists the categories for the file types that are included in the export.
DESTINATION	Location where the files are exported to after the plugin is run.
STATUS	Shows the status of the file transfer.

ERCC_Analysis plugin

Use the ERCC_Analysis plugin to determine if a problem exists with either the library preparation or the sequencing instrument run. The ERCC_Analysis plugin determines the relative abundance of the actual versus expected number of ERCC transcript reads for sequencing runs that include ERCC RNA Spike-in Controls.

The ERCC_Analysis plugin takes approximately 2–3 minutes to complete for sequencing runs with ≤1,000,000 total reads, and 1–2 minutes longer for each additional million total reads. For example, a run with 5 million total reads can take 10–15 minutes. If Torrent Suite™ Software is busy performing additional processing functions, plugin run times are longer.

You can configure the ERCC_Analysis plugin to run automatically. However, automatic execution is not recommended, unless most analyses on the Ion Torrent™ Server include ERCC controls.

ERCC_Analysis plugin configuration

The configuration options for the ERCC_Analysis plugin are described in the following table.

Setting	Description
Use only forward strand reads	Available when manually running the plugin.
Passing R-squared value	To change the R-squared value to set a default value for the summary report screen, enter a value between 0 and 1 as your minimum acceptable R-squared value (a lower value is indicated by a red light in the summary report). The value you enter on the **ERCC Plugin Configuration** screen is used when the plugin is run automatically and when the plugin is manually launched without entering a value. You can override this value on a per-run basis when the plugin is manually launched.
Minimum transcript counts	The minimum number of reads that an ERCC transcript must have to be included in the analysis.
ERCC pool used	Select the ERCC transcript pool used when preparing the library.
Barcodes of interest	**IMPORTANT!** If you configure a Planned Run or Planned Run template to execute the ERCC_Analysis plugin, and your experiment uses the Ion Total RNA-Seq Kit v2, you must select a barcode option. • Select **IonXpressRNA** if your experiment uses this kit. • Select **RNA_Barcode_None** if your experiment does not use a barcode kit

Review the ERCC_Analysis plugin results

After the sequencing run completes, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, locate the run of interest, then click the link **Completed Runs & Reports** screen, click the report name to open.
3. In the left navigation menu of the **EERC_Analysis** screen, click **ERCC_Analysis** to view a summary of the plugin analysis results.

4. (Optional) Click the **ERCC_Analysis.html** link to open the ERCC Analysis Report and view the barcode summary and plugin analysis results.

Setting	Description
Use only forward strand reads	Indicates whether forward strand reads were used in the analysis.
Passing R-squared value	The value entered on the **ERCC Plugin Configuration** screen.
Minimum transcript counts	The minimum number of reads included in the analysis.
ERCC pool used	The ERCC transcript pool used when preparing the library.

5. In **Barcode Name**, click the barcode name to open the ERCC Report to see the results for an individual barcode.

Interpret the ERCC Dose Response plot

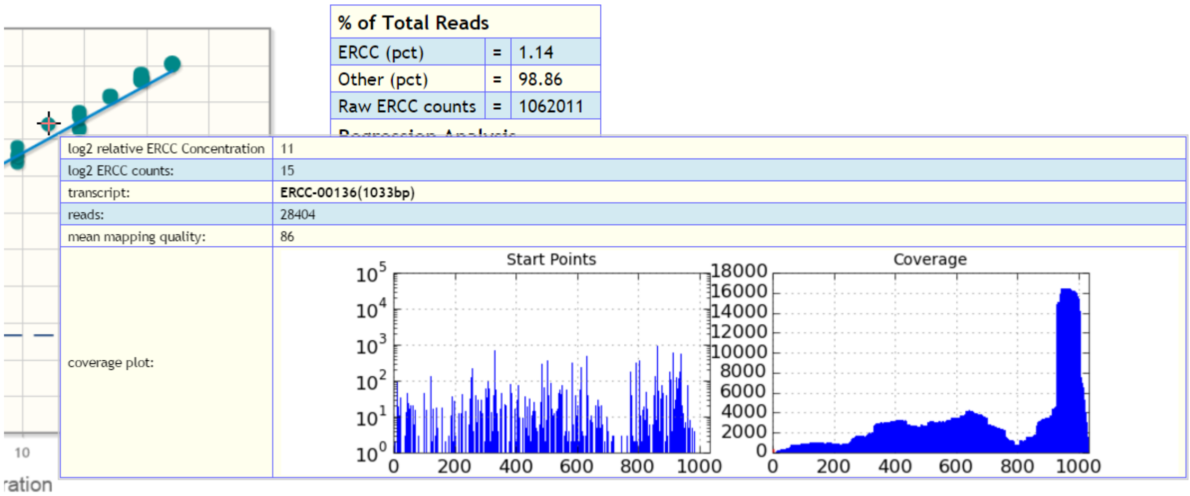
The axes of the ERCC Dose Response plot are log (base 2), with the raw read counts for each ERCC transcript on the y-axis and the known relative concentration of the ERCC transcripts on the x-axis. In the plot, the points are color-coded based on mapping quality. There is also a line of best fit, the parameters (slope, y-intercept, and R-squared value) of which are shown in tabular form to the right of the graph (N = the number of points (ERCC transcripts) included in the regression analysis). Ideally, the points all fall on a straight line. However, the raw counts and relative concentration should at least correlate with a high R-squared (for example, ≥0.9) value. Although 92 transcripts are in the ERCC mix, it is not expected that all 92 are detected. The number of transcripts detected depends on the sequencing depth.

View ERCC transcript details

You can look at the details of an ERCC transcript in a plot or a transcript. To view all details of a ERCC transcript, use both the plot and transcript.

- View the plot—Place the pointer over a point in the ERCC Dose Response plot to open a popup window that shows details about that transcript. Zoom in on the points that overlap on the plot to more easily distinguish the points.
    a. To zoom in on area of interest, click, then drag the cursor over the area. Release the cursor when the area of interest is highlighted.

b. Double-click in the plot, or click **Reset Zoom** to zoom out to the full view of the **ERCC Dose Response** plot.



Parameter	Description
log2 relative ERCC concentration	The log (base 2) of the relative ERCC transcript concentration.
log2 ERCC counts	The log (base 2) of the mapped reads to an ERCC transcript.
transcript	The ERCC transcript identifier. Includes length in base pairs (bp).
reads	The number of reads that map to the particular transcript.
mean mapping quality	A Phred score of the probability that this read was aligned specifically versus any other place in the reference. In practice it is based on the absolute and relative alignment scores for the first two alternative mapping locations. The mapping quality that is reported is based on mean base read quality (after trimming). The points in the display are color coded based on the mapping quality.

- View the transcript—Scroll to the transcript, then click the [+] next to the transcript name.

Parameter	Description
Reads	The number of reads that map to the transcript.
Coverage Depth	The minimum and maximum number of reads covering bases in the transcript. If coverage is 100%, the minimum value is >0.
Coverage	The number of base positions covered by at least one read. Also expressed as a percentage of the full length.
Start Sites	The number of base positions that are the start site for a read.
Unique Start Sites	The number of base positions that have only one read starting at the position.
Coverage CV	The coefficient of variation for coverage equals average coverage divided by standard deviation coverage for the entire transcript.

Definitions

This section defines terms used in the plugin output.

- **Coverage Depth**—The minimum and maximum number of reads covering bases in the transcript. If coverage is 100%, the minimum value is >0.
- **Coverage**—The number of base positions covered by at least one read.
- **Start Sites**—The number of base positions that are the start site for a read.
- **Unique Start Sites**—The number of start sites that have only one read starting at the site.
- **Coverage CV**—The coefficient of variation for coverage = average coverage / stddev coverage for the entire transcript.

ERCC resources

The **External RNA Controls Consortium (ERCC)** is hosted by the U.S. National Institute of Standards and Technology.

For more information on ERCC RNA Spike-In Control Mixes (Cat. Nos. 4456739 and 4456740), see the *ERCC RNA Spike-In Control Mixes User Guide* (Pub. No. 4455352).

For more information on ERCC analysis, see the *ERCC_Analysis Plugin User Bulletin* (Pub. No. 4479068).

FieldSupport plugin

The FieldSupport plugin is used for technical support purposes only. For details, contact Technical Support or your Field Application Scientist.

IMPORTANT! Enable and run this plugin only when directed by Thermo Fisher Scientific Technical Support or your local Field Service Engineer.

FileExporter plugin

Use the FileExporter plugin to rename the output files from the Torrent Suite™ Software runs.

The plugin also offers the following options:

- Generates files of the analysis results that use BAM, VCF, XLS, or FASTQ formats.
- Renames variantCaller plugin output files (when available).
- Compresses the analysis results files.
- Provides links that allow you to download the results files.

Configure the FileExporter plugin

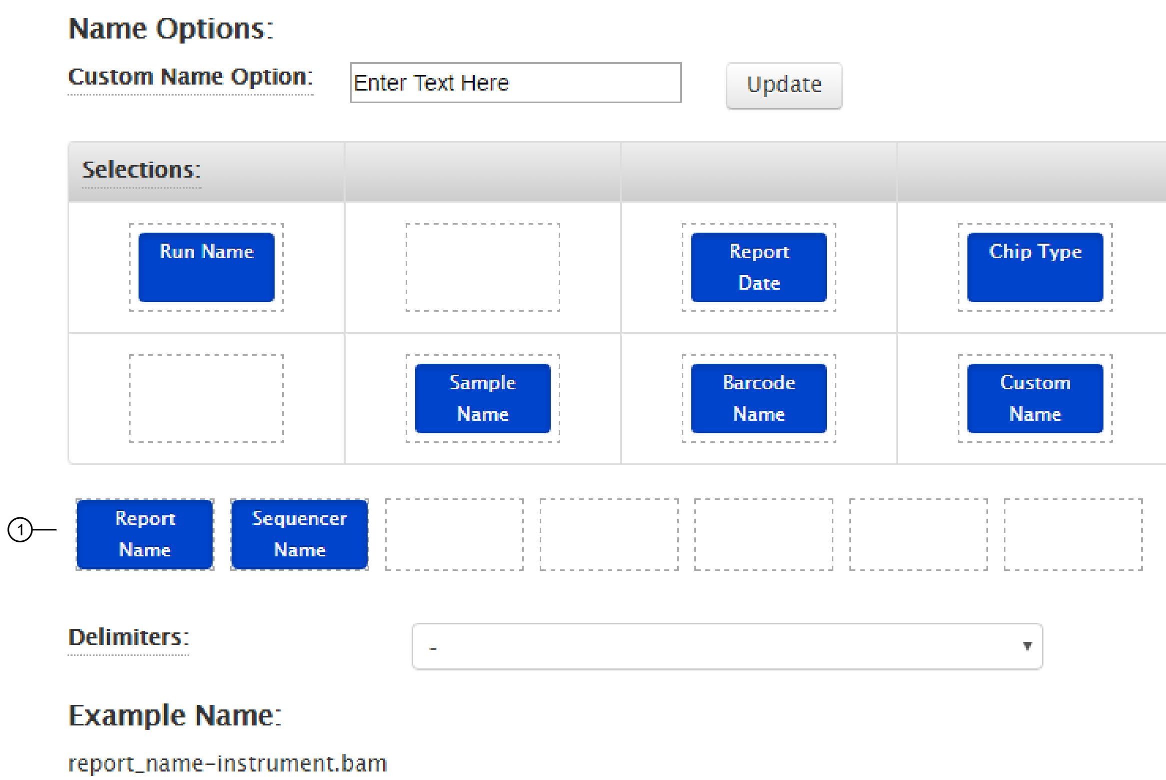
1. Select from the following options to choose the file types that you export.

Option	Description
Include	Select to generate a separate link for the file in the plugin results.
Archive	Select **Archive** for each file type that you want to include in a compressed file. You can export a standard compressed directory in a ZIP or tar.bz2 format.

For each option, choose to include or archive the following file types.

File types	Description
BAM	Native file format for data generated by Ion instruments.
Variant Call Format (VCF)	File that contains only the differences between the BAM file and a reference file.
Variant Caller File (XLS)	Microsoft™ Excel™ format of VCF.
FASTQ	Text format of the nucleotides.

2. Name the file. Select one of the following.
    - Select a unique file name by entering the desired name in the **Custom Name** text box.
    - Create a name using parameters of the run. Drag and drop components from the selections pane onto the name pane row. The naming options are in the blue boxes. The name appears under **Example Name**.
    - Select the delimiter that is used between metadata fields. Supported delimiters are dot, dash, and underscore (a naming pattern uses only one delimiter).



① Name pane row

3. Click **Save Configuration**.

Review FileExporter plugin results

After the sequencing run completes, you can download the following files after you run the FileExporter plugin from the report summary.

- Any of the Torrent Suite™ Software analysis output files that use BAM, VCF, XLS, or FASTQ formats.
- A compressed file that contains the analysis output files.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.

3. In the left navigation menu, click **FileExporter** to view the plugin summary.

   BAM files load quickly, and you may see these files first in the list of links. The other file formats take longer to download, so you may have to wait for the links to the VCF, XLS, and FASTQ formats to appear.

4. Ensure that the status of the plugin run is **Completed**. If the status is not **Completed** or the list of files does not include all the files that you selected when you configured the plugin, click **Plugins ▸ Refresh plugins** at the top of the **Summary** screen.

5. When the list contains the files that you want to download, click a file name link under **Output Files** to download.

6. To review the parameters that were used for the files, click **Show Parameters**.

FilterDuplicates plugin

Use the FilterDuplicates plugin to remove duplicate reads from merged data after a run is completed. The removed BAM files are saved in the FilterDuplicates directory. The original BAM files in the main analysis directory are not modified.

The **Mark as Duplicate Reads** feature in the main analysis pipeline marks reads as duplicates but does not remove them from the BAM files. Enable this feature in the **Kits** step of Planned Run creation.

Review FilterDuplicates plugin results

After the sequencing run completes, review the FilterDuplicates plugin results, and download the BAM files with duplicate reads removed.

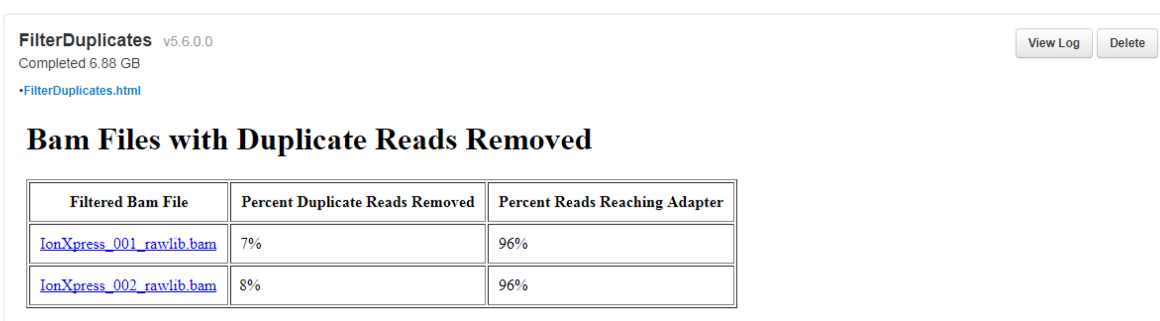
1. In the **Data** tab, click **Completed Runs & Reports**.

2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.

3. In the left navigation menu, click **FilterDuplicates** to view the plugin summary.

4. In the **FilterDuplicates** section, click **FilterDuplicates.html** to open the **BAM Files with Duplicate Reads Removed** report in the browser.

   BAM files load quickly, and you may see these files first in the list of links. The other file formats take longer to download, so you may have to wait for the links to the VCF, XLS, and FASTQ formats to appear.



The plugin output contains links to the BAM files that have duplicate reads that are removed. This table also shows the percentage of reads that were removed and the percentage of all reads that reached the adapter.

5. To download the filtered BAM Files, click the link for each file listed that you want to download.
   The BAM files are downloaded through the browser to a folder on the computer, based on the browser settings.

immuneResponseRNA plugin

Use the immuneResponseRNA plugin to quantify gene expression levels for the Oncomine™ Immune Response Research Assay. This plugin produces gene transcript quantification from sequence read data. The plugin summary includes gene expression counts (number of aligned reads to a given gene target), a data analysis summary, and QC plots. The normalized, gene-level count data from the run are available to download for further analyses with Transcriptome Analysis Console (TAC) v3.1 software.

The immuneResponseRNA plugin requires a target regions BED file and an associated reference sequence library FASTA file. For more information on installing these files, see Chapter 10, "References management".

The plugin also accepts a second (optional) BED file that specifies a subset of target genes allowing sample clustering.

immuneResponseRNA plugin configuration

The configuration options for the immuneResponseRNA plugin are described in the following table.

Setting	Value
Library Type	AmpliSeqRNA
Targeted Regions	`ImmuneResponse_v3.1_target_designed_20160908.bed`
Add new gene list	In the **Add genes of interest** list, select an optional target gene subset BED file.

Review immuneResponseRNA plugin results

After the sequencing run completes, review the plugin results in the report summary.

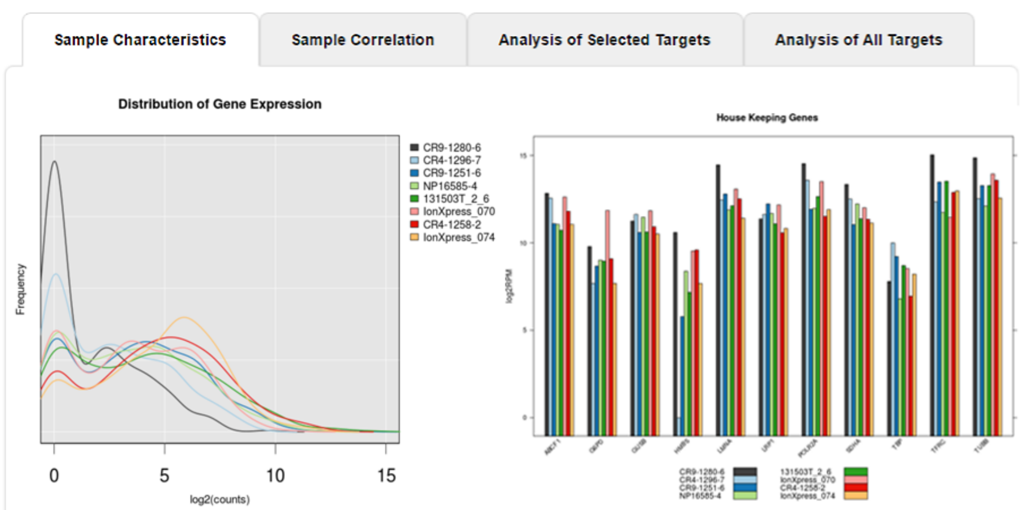
1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **immuneResponseRNA** to view the plugin summary.
4. In the **immuneResponseRNA** section, click the **immuneResponseRNA.html** link to open the **immuneResponseRNA Report** for all barcodes.
   BAM files load quickly, and you may see these files first in the list of links. The other file formats take longer to download, so you may have to wait for the links to the VCF, XLS, and FASTQ formats to appear.

5. In the **Analysis Summary** window, review your **Mapped Reads**, **Valid Reads**, and the **Targets** that are detected by barcode.

Column	Description
Barcode Name	The barcode used for the sample.
Sample	The sample name as it was entered in the sequencing Run Plan.
Mapped Reads	The number of reads that map to the reference sequences.
Valid Reads	The percentage of mapped reads ≥50% amplicon length.
Targets ≥1 reads	The number of targets/genes with at least 1 read.
Targets ≥2 reads	The number of targets/genes with at least 2 reads.
Targets ≥10 reads	The number of targets/genes with at least 10 reads.

6. Click an individual barcode name to view the results for that barcode.

7. Scroll down, then click the **Sample Characteristics**, **Sample Correlation**, **Analysis of Selected Targets** (available only if a Genes of interest subset BED file was selected), or **Analysis of All Targets** tabs to review the data in graphic format.



Downloadable reports

The following reports are available for download as tab-delimited text files, compatible with Microsoft™ Excel™, or similar applications.

At the bottom of the screen are links for downloading raw analysis output files:

Report hyperlink	Description
Download Barcode Summary Report	A table listing the sample name for each barcode, total reads, aligned reads on targets, and number of targets detected.
Download absolute read counts data	A table listing read counts for each barcoded sample along with gene annotations.

(continued)

Report hyperlink	Description
Download RPM data (normalized by total read counts)	A table listing RPM (Read count per million mapped reads) for each barcoded sample along with gene annotations. RPM is calculated as: (read count) × 10^6 / total number of mapped reads
Download mean housekeeping scaled log2 RPM data	A table listing housekeeping-gene normalized, log2-transformed read counts for each barcoded sample along with gene annotations. Conceptually, these values are read count normalized by the average expression of housekeeping (*hk*) genes rather than by the total number of mapped reads as described above. The values are calculated as: $log2(\text{count}+1) - \frac{\sum(log2(hk\ \text{counts}+1))}{\text{number of } hk \text{ gene}} + log2(10^6)$ These values are useful for differential analysis when a large proportion of the target genes (non-housekeeping genes) are expected to be differentially expressed or when the expression levels of the housekeeping genes in the 2 groups differ significantly.
Download CHP files normalized by RPM	The RPM data is converted to CHP file format for use with Transcriptome Analysis Console (TAC) software. The downloaded ZIP file contains all the CHP files from the sequencing run. Each barcoded sample has one CHP file.
Download CHP files normalized by mean housekeeping genes	The housekeeping genes data is converted to CHP file format for use with Transcriptome Analysis Console (TAC) software. The downloaded ZIP file contains all the CHP files from the sequencing run. Each barcoded sample has one CHP file.
Download background expression from genomic DNA and H_2O neg_control	A table that contains background expression (in absolute read count) from four experiments using genomic DNA and H_2O as negative control samples.

IonReporterUploader plugin

Analysis files that are generated in Torrent Suite™ Software can be directly transferred to an Ion Reporter™ Software account in Ion Reporter™ Software with the IonReporterUploader plugin.

Ion Reporter™ Software uses Torrent Suite™ Software output BAM file for analysis. The Ion Reporter™ Software annotation-only analysis workflow also accepts the VCF output file of the variantCaller plugin. Use the IonReporterUploader plugin to transfer these BAM and VCF output files to Ion Reporter™ Software.

For details about the IonReporterUploader plugin, see Chapter 9, "Integration with Ion Reporter™ Software"

molecularCoverageAnalysis plugin

Use the molecularCoverageAnalysis plugin to view statistics and graphs that describe the level of sequence molecular coverage produced for targeted genomic regions. The plugin generates statistics, downloadable data files, and interactive visualization of molecular coverage over targeted regions of the reference genome.

The plugin is only compatible with libraries prepared using Ion AmpliSeq™ HD or Taq Sequencing chemistries.

molecularCoverageAnalysis plugin configuration

The molecularCoverageAnalysis plugin uses the following settings.

Setting	Description
Reference Genome	The reference genome selected in the Planned Run.
Library Type	The library type selected in the Planned Run.
Targeted Regions	Select the appropriate target region for this run. The targeted regions are selected in the Planned Run and can be changed only after the run is complete if the plugin is run manually. Target regions can be overwritten by the specific barcode targets.
Barcode-specific Targets	Select this checkbox to assign specific target region files to individual barcodes. This option is available only when the plugin is run manually. **1.** Select a specific barcode. **2.** Select the specific Target Regions file to associate with the selected barcode. **3.** Click **Add**. **4.** Repeat above steps to associate additional barcodes with specific Target Region files. Alternatively, you can copy and paste the barcode/target file pairs manually. When the **Barcode-specific Targets** option is deselected, all barcodes use the targets specified by the target regions, even if barcode-specific targets are listed.
Parameter file	Click to load the external parameter file that pertains to this plugin.

Review molecularCoverageAnalysis plugin results

The molecularCoverageAnalysis plugin generates a Molecular Coverage Analysis Report. This report includes molecule statistics and several charts. The report summary lists the barcodes, the samples, the median molecular coverage, the molecular uniformity, and the median reads per functional molecule, and the median percentage of functional reads. Additional details about molecular coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

You can download statistics files at the bottom of Molecular Coverage Analysis Report. After the sequencing run is complete, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the left navigation pane, click **molecularCoverageAnalysis** to view the plugin summary.
   A summary table of the molecular coverage analysis, by barcode, is included in the **Summary** screen.
4. In the **molecularCoverageAnalysis barcode** summary, in the **Barcode Name** column, click a link to open a detailed **Molecular Coverage Analysis Report** for that barcoded sample.
   Alternatively, click the **molecularCoverageAnalysis.html** link to open the summary table for all barcodes in a new window.
5. In the **Molecular Coverage Analysis Report**, review the plugin results.
6. Click the links at the bottom of the **Molecular Coverage Analysis Report** to download associated statistics and summary files for each barcoded sample in the run.

Molecule statistics

Statistic	Description
Median Functional Molecular Coverage per Amplicon	A functional molecule is a collection of reads that covers the amplicon and satisfies all criteria associated with the parameters (for example, `min_tag_fam_size` and `min_fam_per_strand_cov`) in the parameter file. Molecular coverage is the number of functional molecules. The median is calculated across all amplicons.
Uniformity of Molecular Coverage for all Amplicons	The percentage of amplicons having molecular coverage between 0.5x and 2x of the median molecular coverage.
Percentage of Amplicons larger than 0.8x Median Functional Molecular Coverage	The percentage of amplicons having molecular coverage more than 0.8x of the median functional molecular coverage
Median Total Molecular Coverage per Amplicon	The number of molecules that satisfies size criteria in the parameters file. The median is calculated across all amplicons.
Percentage of Reads with Perfect Molecular Tags	The percentage of reads whose molecular tags are exactly the same with design.
Median Functional Molecular Loss due to Strand Bias per Amplicon	The metric is calculated by 1 - (`Functional Molecular Coverage`) / (`Molecular Coverage without Strand Constraint`), which measures the loss of functional molecules due to strand constraint for molecular functionality. The median is calculated across all amplicons.

(continued)

Statistic	Description
Median Percentage of Functional Molecules out of Total Molecules per Amplicon	The percentage of functional molecules out of the number of molecules that satisfies size criteria. The median is calculated across all amplicons.
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 Reads Contributed to Functional Molecules per Amplicon	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.
Percentage of Amplicons below (around) n% LOD	LOD is calculated based on the number of functional molecules for each amplicon and variant calling parameters in the parameter file. Around x n% means in the range from 0.5x n% to 2x n%.

Example charts generated by the molecularCoverageAnalysis plugin

The charts in the detailed **Molecular Coverage Analysis Report** include **Plot** and **Overlay** menus that allow you to customize the data that is displayed in each chart.

Click **(Search)** (in the top right corner of a chart) to open the chart **Viewing Options** panel. Click **(Help)** to open a description of the chart.

The Depth of Coverage Chart summarizes the amplicon depth of coverage results. The X-axis represents the amplicon molecular depth. The left Y-axis represents the number of amplicons at a given molecular depth or a range (bin) of molecular depths, as a percentage of the total number of amplicons. The right Y-axis represents the cumulative count of the number of amplicons, at a given molecular depth or greater, as a percentage of the total number of amplicons. The individual orange bars represent the percentage of amplicons in the specific range of molecular depths. The blue curve measures the cumulative amplicons at a given molecular depth or greater. Use the **Plot** dropdown list to switch between **Maximum Read Depth**, **99.9% of All Reads**, and **Normalized Coverage** plots.

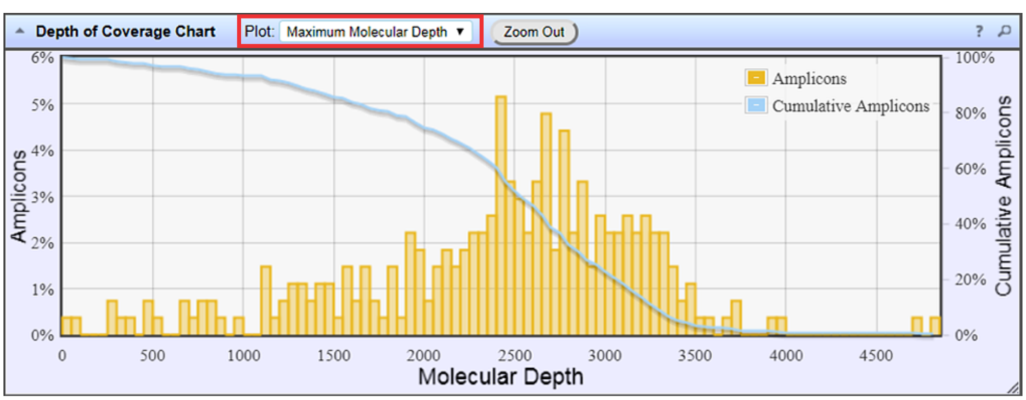


Figure 6 Representative Depth of Coverage Chart

The Amplicon Coverage charts summarize the amplicon molecular coverage results. The X-axis in all plots represents amplicons that are grouped into bins, where each bin contains amplicons that have the same molecular coverage. Based on the plot selection, the left Y-axis represents either the average number of molecules (on a base 10 log scale) or the percentage of functional molecules in a given bin. The right Y-axis represents the percentage of functional reads in a given bin. Use the **Plot** and **Overlay** dropdown lists to customize the chart view. To zoom in on a subset of amplicons, use the pointer to draw a rectangle that contains the region of interest. Use **Zoom Out** to revert to the initial view.

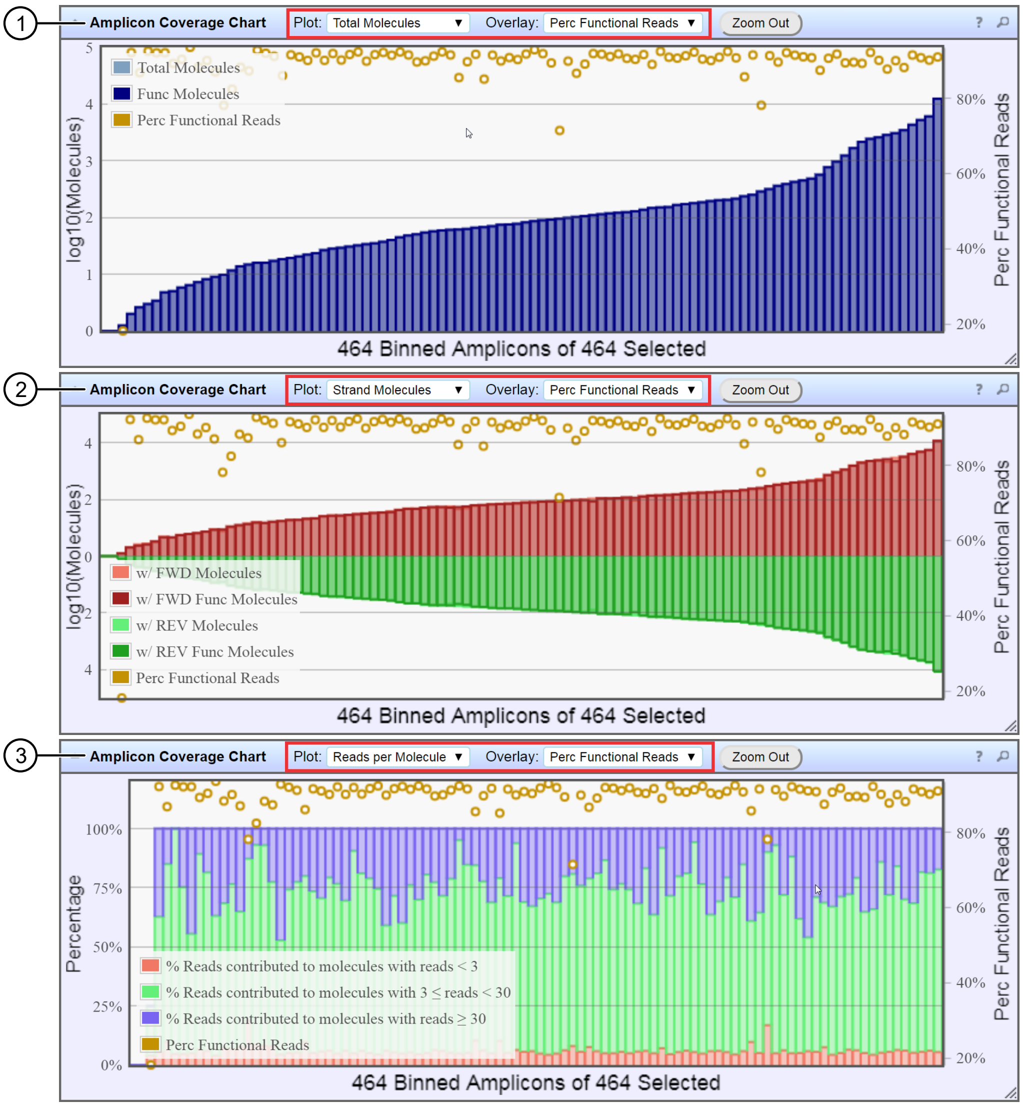


Figure 7 Representative Amplicon Coverage charts

1. Representative **Total Molecules** plot with a **Perc Functional Reads** overlay.
2. Representative **Strand Molecules** plot with a **Perc Functional Reads** overlay.
3. Representative **Reads per Molecule** plot with a **Perc Functional Reads** overlay.

In most plots, you can click a data point to open a detail pane for that data (see Figure 8). For example, in the Depth of Coverage Chart in Figure 6, click an individual orange bar to open the detail pane for amplicons in a specific range of molecular depths, or click a point on the blue curve to open the detail pane for cumulative amplicons at that molecular depth or greater.

Similarly, you can click any point or bar within each Amplicon Coverage chart to view details for each amplicon coverage bin.

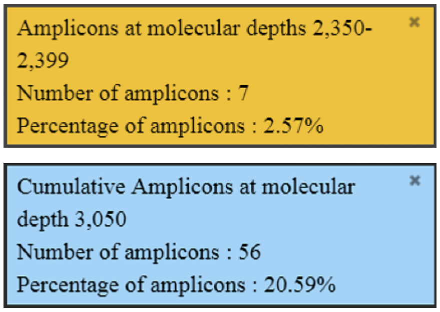


Figure 8 Example detail panes

Output files generated by the molecularCoverageAnalysis plugin

You can download plugin results files from links that are contained in the **File Links** section of the **Molecular Coverage Analysis Report**. The output is a tab-separated text file with an XLS file extension.

Click ⓘ **(Help)** next to the file to open a description of the file.

The following is an example of the content of a results file that is generated by the molecularCoverage Analysis plugin.

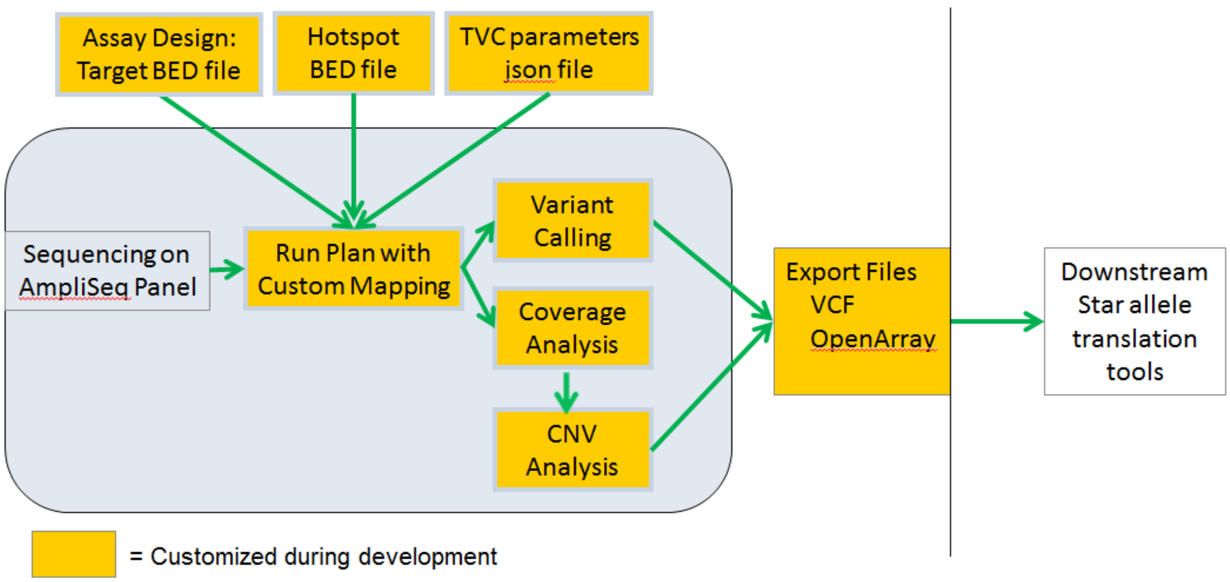
Setting	Description
Coverage statistics summary	This section of the file is a summary of the statistics presented in the tables at the top of the **Molecular Coverage Analysis Report** plugin report. The first line is the title. Each subsequent line is either blank or a particular statistic title followed by a colon (:) and its value.
Amplicon molecular coverage summary	This section of the file contains the following fields. • `contig_id`—The name of the chromosome or contig (from contiguous) of the reference for this amplicon. • `contig_srt`—The start location of the amplicon target region. This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file. • `contig_end`—The last base coordinate of this amplicon target region. The length of the amplicon target is given as (contig_end - contig_srt + 1). • `region_id`—The ID for this amplicon as given as the 4th column of the targets BED file. • `gene_id or attributes`—The gene symbol or attributes field as provided in the targets BED file. • `func_mol_cov`—The number of molecules (functional molecules) which are available for the variantCaller plugin. • `lod`—LOD (limitation of detection) calculated from the number of functional molecules. • `strict_func_umt_rate`—The percentage of functional molecules used with strict molecular tags. • `func_mol_cov_loss_due_to_strand`—The percentage of functional molecules loss due to strand bias. • `fwd_only_mol_cov`—The number of molecules that contain forward strand only. • `rev_only_mol_cov`—The number of molecules that contain reverse strand only. • `both_strands_mol_cov`—The number of molecules that contain both forward strand and reverse strand. • `r2m_conv_rate_all`—The percentage of reads contributed to functional molecules. • `reads_per_func_mol`—The average reads per functional molecules. • `perc_to_mol_(<3_reads)`—The percentage of reads contributed to small size molecules(size<3). • `perc_to_mol_(>=3&<30_reads)`—The percentage of reads contributed to median size molecules (size ≥3 && size <30). • `perc_to_mol_(>=30_reads)`—The percentage of reads contributed to large size molecules (size ≥30).

PGxAnalysis plugin

The PGxAnalysis plugin is designed to be used with the Ion AmpliSeq™ Pharmacogenomics Research Panel, a targeted gene panel that allows the interrogation of Pharmacogenomics variants in samples for genotyping and CYP2D6 copy number detection. The plugin analysis incorporates optimized variant calling for the Ion AmpliSeq™ Pharmacogenomics Research Panel, export of results that are compatible with downstream reporting software, and detection of gene and exon-level CNV for CYP2D6.

Analysis by the PGxAnalysis plugin requires two other Torrent Suite™ Software plugins: the variantCaller plugin for genotyping and the coverageAnalysis plugin for CYP2D6 copy number detection.

The figure shows a summary of the analysis pipeline.



The run plan template that is used with the Ion AmpliSeq™ Pharmacogenomics Research Panel must be imported from **AmpliSeq.com**. The template includes optimized parameters. The variantCaller plugin that includes pharmacogenomics parameters settings must be run before the PGxAnalysis plugin to complete the analysis.

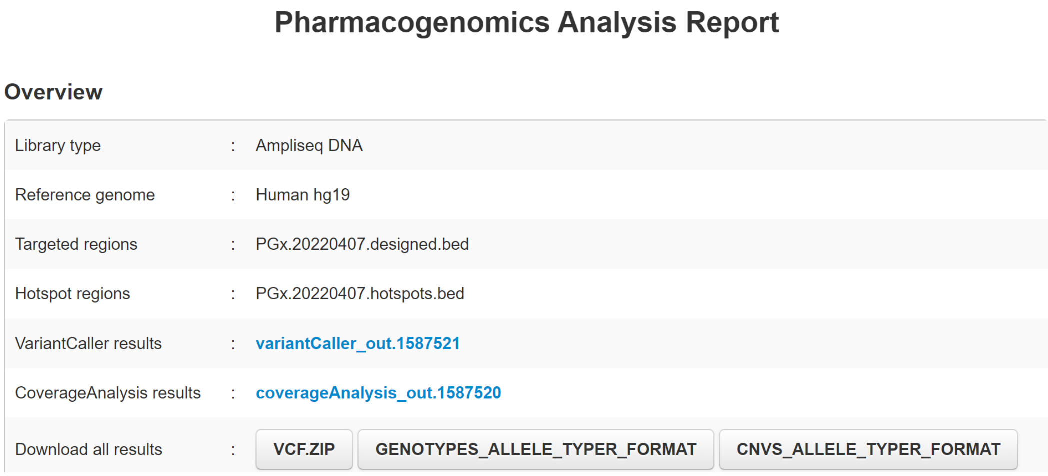
For details about how to set up Torrent Suite™ Software Planned Runs that incorporate the Ion AmpliSeq™ Pharmacogenomics Research Panel Planned Run template and the PGxAnalysis plugin, see the *Ion AmpliSeq™ Pharmacogenomics Research Panel User Guide* (Pub. No. MAN0014300.

Review PGxAnalysis plugin results

After the sequencing run completes, review the plugin results in the report summary.

1. Sign in to Torrent Suite™ Software
2. In the **Data** tab, click **Completed Runs & Reports**.
3. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
4. In the left navigation menu, click **PGxAnalysis** to view the plugin summary.

5. Click the **PGxAnalysis.html** link to open the **Pharmacogenomics Analysis Report**.



6. Download one or more results files in the following formats.

File name	Description
`VCF.ZIP`	Results in VCF format, which is the standard format used by downstream analysis tools, such as Translational Software.
`GENOTYPES_ALLELE_TYPER__FORMAT`	Results in the Allele Typer format. The format matches the output from the TaqMan™ Genotyping application assays.
`CNVS_ALLELE_TYPER_FORMAT`	Results in the format that is used by CopyCaller™ software for cross-compatibility with TaqMan™ Genotyping application assays.

For more information, see *Ion AmpliSeq™ Pharmacogenomics Research Panel User Guide* (Pub No. MAN0014300).

7. In the **CYP2D6 CNV QC Analysis** table, review the following metrics for the CYP2D6 CNV calling algorithm.
   - Total Number of Valid Samples
   - Number of Samples that passed QC for CNV Calling
   - Number of Samples that did not pass QC metric 'Average coverage >= 100'
   - Number of Samples that did not pass QC metric 'Uniformity Rate>=80%'

8. Review the **Variant Summary**.

The **Variant Summary** table lists all CNV variants, including hotspots, novel variants, and CYP2D6 gene-level and exon 9-level CNVs and their confidence levels. Click barcode name links to see plugin results for individual samples. The Confidence Level is measured by a Phred-like quality score $Q = -10 \log_{10} P$, where P is the probability of incorrect call.

Column	Description
Hotspot Variants Detected	Reports the genotype for each sample at each hotspots position, whether that is heterozygous, homozygous non-reference, or homozygous reference.
CYP2D6 Gene CNV	Copy number (gene copies, deletions, duplications, and so on) for CYP2D6 Gene (excluding exon 9).
CYP2D6 Exon 9 CNV	Copy number (gene copies, deletions, duplications, and so on) for exon 9 of CYP2D6.
CYP2D6 Gene CNV Confidence	Phred quality score for CYP2D6 Gene level CNV call. A score of lower than 50 results in a no-call, with probability of incorrect call <0.00001.
CYP2D6 Exon 9 CNV Confidence	Phred quality score for CYP2D6 Exon 9 CNV call. A score of lower than 15 results in a no-call, with probability of incorrect call <0.032.

Note: If you see **Novel Variants Detected**, the results are not correct. if novel variants are called, It is likely that the optimized variantCaller plugin settings were not used. Rerun the variantCaller plugin on the run results to see if results are improved.

Possible copy number states reported by the plugin are as follows.

CYP2D6 Gene CNV	CYP2D6 Exon 9 CNV	Explanation
0	0	homozygous deletion (CYP2D6*5/5)
1	1	single copy (carries one CYP2D6*5 deletion allele)
2	2	two copies
3	3	three copies (carries one CYP2D6 gene duplication allele)
3	2	two normal and one CYP2D6/CYP2D7 hybrid allele (for example, CYP2D6*36 allele)
4	4	four copies (carries 2 CYP2D6 duplication or a multiplication)
NOCALL	NOCALL	undetermined

In most cases, CYP2D6 gene and exon 9 have the same copy number. However, some samples may contain hybrid alleles that formed by recombination between CYP2D6 and the highly homologous CYP2D7 pseudogene; for example, CYP2D6*36 allele contains a gene conversion to CYP2D7 sequences in exon 9 and is associated with negligible CYP2D6 enzyme activity. The CYP2D6*36 allele is most common in East Asian populations, and is more rare in other populations. It is most typically found in tandem with a CYP2D6*10 allele. A sample that carries a normal CYP2D6 gene on one chromosome and a CYP2D6*36+*10 arrangement on the other will have copy number 2 at the exon 9-level and copy number 3 at the gene-level.

There are two circumstances where NOCALLs may happen, either at gene-level or exon 9-level. First, the samples did not pass one of the basic QC metrics – 'Average coverage>=100' or 'Uniformity Rate >=80%'. Second, as the plugin utilizes the coverage of CYP2D6 amplicons to infer the copy number status via a clustering approach, it can be sometimes difficult to make a reliable prediction due to unbalanced sample coverage or suboptimal sample preparation. Gene-level CNV tend to be more robust than the exon 9-level CNV, due to different numbers of amplicons used for the clustering algorithm (9 at gene-level versus 1 at exon 9-level). It is highly recommended to have a rerun including the samples with NOCALL, or verify those with orthogonal platforms (ex: qPCR TaqMan™ assay). This is especially important when a CYP2D6*10 that contains sample is reported with copy number 3 at gene-level and NOCALL at exon 9-level, in order to distinguish between CYP2D6 duplication and CYP2D6*36 alleles.

Note: Allele-specific CNVs are not supported by the plugin.

9. View the other plugin analysis reports that are available in the **Pharmacogenomics Analysis Report**.

   For more information, see the Torrent Suite™ Software help system.

   - Click the **variantCaller_out** link to view the **Variant Caller Report**.
   - Click the **coverageAnalysis_out** link to view the **Coverage Analysis Report.**

sampleID plugin

Use the sampleID plugin to track samples or possibly identify misassignment or mix up between samples and barcodes in a sequencing run. The sampleID plugin produces a unique identification code (**SampleID**) for each barcode in a sample.

The sampleID plugin can be run on sequencing results from any panel that includes the 9 primer pairs of the Ion AmpliSeq™ Sample ID Panel.

For example, the Ion AmpliSeq™ Sample ID Panel contains 9 primer pairs that can be combined with any Ion AmpliSeq™ Ready-to-Use Panel or Custom Panel. The sampleID plugin can be used with the Ion AmpliSeq™ Sample ID Panel, which is a human SNP genotyping panel, to verify the accuracy of samples and increase confidence in sample data management. The Ion AmpliSeq™ Sample ID Panel is composed of the identified human sample gender and IUPAC base letters for eight high-frequency noncoding SNPs.

For the samples to work with this plugin, the Ion AmpliSeq™ library must have been prepared with Ion AmpliSeq™ sample tracking amplicons.

The sampleID plugin is preconfigured and does not require user input.

Review sampleID plugin results

After the sequencing run completes, review the plugin results in the report summary.

Note: If the sampleID is undetermined, a `?` can appear in the report summary. The sampleID can be undetermined due to low coverage. For example, `F-?` is reported if only the female gender can be called due to low coverage and `F-GCTYR??A` is reported if specific alleles (2 out of the 8 called) could not be called due to low coverage or ambiguous allele frequency.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **sampleID** to view the plugin summary.
4. Click **sampleID.html** to open the sampleID report in the browser tab. Then, you can open a detailed report and other data files.
5. *(Optional)* Scroll to the File Links table, then click a link to:
   - Download a PDF image of the report.
   - Download all variant calls as a table file.
   - Download the tracking target regions file.
   - Download the tracking loci regions (SNPs) file.
   - Download the aligned tracking reads (BAM) file.
   - Download the aligned tracking reads index (BAI) file.
6. *(Optional)* Click **Download Barcode Summary Report** to open the data in a downloadable tab-separated spreadsheet, or PDF report.
7. To return to Torrent Suite™ Software, click back in the browser.

variantCaller plugin

The variantCaller plugin calls single-nucleotide polymorphisms (SNPs), multiple nucleotide polymorphisms (MNPs), insertions, deletions, and block substitutions in a sample across a reference or within a targeted subset of that reference.

This plugin provides optimized preset parameters for many experiment types. It can also be customized. After you find a parameter combination that works well on your data and that has the balance of specificity and sensitivity that you want, you can save that parameter set and reuse it in your research. Customization is supported when you run the plugin after a sequencing run and when the plugin is run through a Planned Run.

For details about the variantCaller plugin, see Chapter 8, "Variant calls in Torrent Suite™ Software".

Plugin available on Connect Platform

The following plugin is supported by Thermo Fisher Scientific and is available on Thermo Fisher™ Connect Platform at **https://apps.thermofisher.com/apps/publiclib/#/plugins**. This plugin is not preinstalled in the Torrent Suite™ Software.

For details about plugins that are included with the software, see "Preinstalled plugins" on page 130.

Plugin name	Description
"RNASeqAnalysis plugin" on page 168	Analyzes cDNA reads. This plugin is an RNA transcript alignment and analysis tool for use with the reference genomes hg19 and mm10.

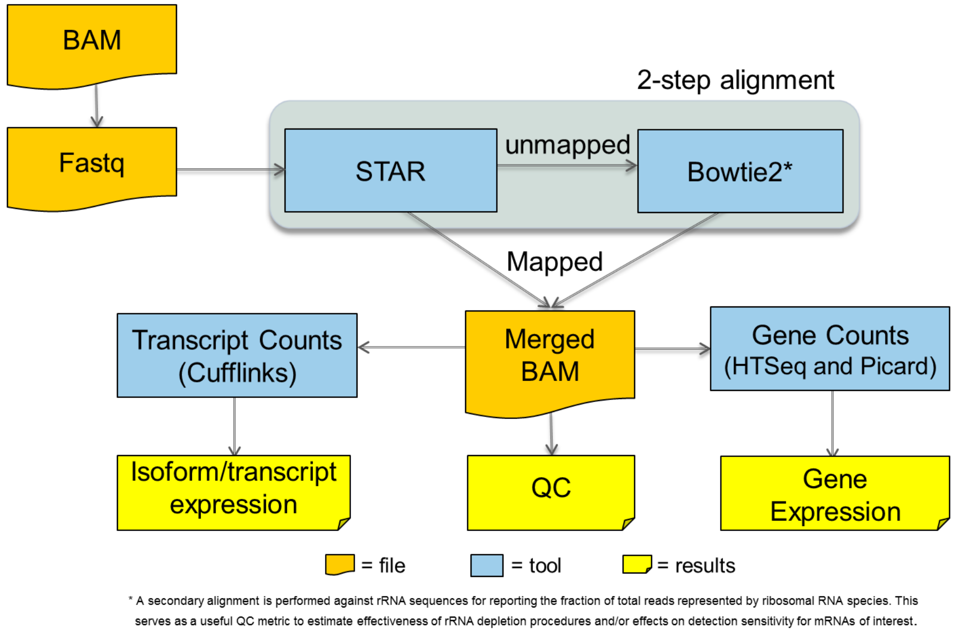
RNASeqAnalysis plugin

The RNASeqAnalysis plugin is an RNA Transcript Alignment and Analysis tool for use with reference genomes hg19 and mm10.

To use the hg19 or mm10 genomes with this plugin, the reference genomes must first be imported from the preloaded references screen. Also, annotation files for the human and mouse references are available for import. For more information on downloading references and annotation files, see "Import a preloaded reference sequence file" on page 240.

Annotation file name	Description
Human	
`hg19_annotation_v1.gtf`	Human genome annotation (hg19)
`XrRNA.fasta`	Human auxiliary small RNA sequences (cDNA)
Mouse	
`mm10_annotation_v1.gtf`	Mouse genome annotation (mm10)
`XrRNA.fasta`	Mouse auxiliary small RNA sequences (cDNA)

Use this plugin to analyze cDNA reads, as produced by RNA-Seq. Reads are aligned to the reference genome using STAR and bowtie2 aligners to find full and partial mappings. The alignments are analyzed by HTSeq and Picard tools to collect assigned read counts and cufflinks to extract gene isoform representation. For barcoded data, comparative representation plots across barcodes are created in addition to individual reports for each barcode. All alignment, detail, and summary report files are available for download.



RNASeqAnalysis plugin configuration

The RNASeqAnalysis plugin can be configured with either the hg19 or mm10 reference genome when you plan a run. You can also add hg19 and mm10 annotation files.

This plugin requires the use of the RNA Seq Planned Run templates for sequencing runs: Ion RNA–Small or Ion RNA–Whole Transcriptome. If the RNA Seq Planned Run templates are not used when you run the plugin manually, you receive an error.

Setting	Description
Reference Genome	In the **Configure Plugin** dialog box, select a reference genome. • **hg19** • **mm10**
Regenerate indices	Ensure that any indexing and annotation files previously generated for the specified reference are deleted before the analysis starts. New files are generated as needed, which may add several hours to the plugin run time (about 3 hours for human-sized genomes). Using this option is necessary if the plugin previously failed during reference index generation or the reference sequence was updated.

To use the mouse mm10 Reference Genome with this plugin, first import the preloaded Ion reference genome using Torrent Suite™ Software. For more information, see “Import a preloaded reference sequence file” on page 240.

Review RNASeqAnalysis plugin run results

After a sequencing run completes, you can review plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
   Reports for any plugins that have completed analysis are included in the **Summary**.
3. In the left navigation menu, click **RNASeqAnalysis** to view the plugin results.
4. In the **RNASeqAnalysis** section, view the **Barcode Summary** for the RNASeqAnalysis plugin.
   The summary includes columns for Barcode Name, Sample, Total Reads, Aligned Reads, Percent Aligned, Mean Read Length, Genes Detected, and Isoforms Detected.
   - Click the **RNASeqAnalysis.html** link to open the report in the browser tab.
   - Click the **RNASeqAnalysis.html** link to view the **RNASeqAnalysis Report** for all barcodes.
   - Click the links at the bottom of the report to download associated report files:

Link name	Download description
Barcode Summary Report	A table that lists the sample name for each barcode, total reads, aligned reads and percent aligned.
absolute reads table	A table that lists absolute reads for the genes found for each barcode.
absolute normalized reads table	A table that lists absolute normalized reads for the genes found for each barcode.
aligned reads distribution table	A table that lists the distribution of genes across barcodes to show the frequency of numbers of genes having similar log10 read counts.
isoform FPKM values table	The isoform gene heatmap in a table format.

   - Click the links at the bottom of the **RNASeqAnalysis Report** to download raw analysis output files for the selected barcode. For examples, see "Individual barcode view" on page 174.

Link name	Raw analysis output file description
Download the Statistics Summary	An overview of the individual barcodes from the RNASeqAnalysis plugin results.
Gene Read Counts	A table that lists the number of times a gene was counted for the individual barcodes.
Output Files	A directory for various output files for the selected barcode.
Cufflinks Output Files	A list of links to Cufflinks output files.

- Click individual barcode names to see graphs for the selected barcode. For examples, see "Downloadable reports for individual RNASeqAnalysis plugin barcodes" on page 172.

Link name	Download description
Reference table	A plot that shows the number of genes that have reads in log10 counting bins.
Gene Mapping Summary	A summary of reads mapped to genes of the annotated reference.
Base Mapping Summary	A summary of base reads aligned to genetic features of an annotated reference.
Normalized Transcript Coverage	A plot of normalized transcript coverage that shows the frequency of base reads with respect to the length of individual transcripts as they are aligned to in the 3" to 5" orientation.
Gene Isoform Expression	Box plots showing variation of isoforms expressed at FPKM ≥0.3 for each set of genes grouped by the number of anticipated (annotated) isoforms. Whiskers are defined by points within Q1-1.5xIQR to Q3+1.5xIQR. Only genes with 25 or less isoforms are represented in this plot. The data and a plot for all genes are available for download using the download reports links at the bottom of the screen.

- Click the **Distribution Plots**, **Correlation Heatmap**, **Correlation Plot**, and **Gene Heatmap** tabs to review the following data graphically.

Graphical report	Description
Distribution Plots	For details, see "Distribution plots" on page 176.
Correlation Heatmap	For details, see "Correlation heatmap" on page 178.
Correlation Plot	For details, see "Correlation plot" on page 179.
Gene Heatmap	For details, see "Gene heatmap" on page 180.
Isoform Heatmap	For details, see "Isoform heatmap" on page 180.

Downloadable reports for individual RNASeqAnalysis plugin barcodes

You can download raw analysis output files for individual barcodes if you click the links at the bottom of the **RNASeq Analysis Report**.

- **Download the Statistics Summary**—Provides an overview of the individual barcodes RNA Seq Analysis results.

```
RNASeqAnalysis Summary Report

Sample Name: None
Reference Genome: hg19
Adapter Sequence: None
Reads Sampled: 100.0%
Alignments: IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes

Total Reads:      11283208
Aligned Reads:    10997469
Pct Aligned:      97.47%
Mean Read Length: 102.4
Strand Balance:   0.4980

Reference Genes:  55765
Reads Mapped to Genes: 7390706
Genes with 1+ reads: 26969
Genes with 10+ reads: 16626
Genes with 100+ reads: 9531
Genes with 1000+ reads: 1429
Genes with 10000+ reads: 35

Total Base Reads:     1155834791
  Pct Aligned Bases:  79.14%
  Pct Usable Bases:   63.01%
Total Aligned Bases:  914778477
Pct mRNA Bases:       79.61%
  Pct Coding Bases:   39.68%
  Pct UTR Bases:      39.93%
Pct Ribosomal Bases:  0.94%
Pct Intronic Bases:   15.65%
Pct Intergenic Bases: 3.98%

Isoforms Annotated: 230756
Isoforms Detected:  58457
```

- **Download the Gene Read Counts**—Lists the number of times a gene was counted for the individual barcode.

	A	B
1	Gene	Reads
2	5S_rRNA	3
3	7SK	547
4	A1BG	3
5	A1BG-AS1	34
6	A1CF	0
7	A2M	14
8	A2M-AS1	16
9	A2ML1	45
10	A2ML1-AS	0
11	A2ML1-AS	0
12	A2MP1	0
13	A3GALT2	0
14	A4GALT	45
15	A4GNT	0
16	AAAS	492

- **Download Output Files (page)**—Provides a directory for various output files for this barcode.

File Size	Date	File
871M	2015-06-02	alignedSTAR.bam
72M	2015-06-02	Chimeric.out.junction
495M	2015-06-02	Chimeric.out.sam
90	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.bam
27K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.geneisoexp_all.png
19K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.geneisoexp.png
1.3M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.geneisoexp.xls
660K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genereads.xls
4.4K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.generep.png
129	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genes.fpkm_tracking
132	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.isoforms.fpkm_tracking
19K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.rnareads.png
107	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.rnareads.xls
121	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.skipped.gtf
1.3G	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.STARBowtie2.bam
3.5M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.STARBowtie2.bam.bai
660K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.STARBowtie2.gene.count
20K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.STARBowtie2.RNAmetrics.png
2.9K	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.STARBowtie2.RNAmetrics.txt
897	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.stats.txt
125	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.transcripts.gtf
1.7K	2015-06-02	Log.final.out
12K	2015-06-02	Log.out
32K	2015-06-02	output_cufflinks
19K	2015-06-02	rnaseq.log
5.0M	2015-06-02	SJ.out.tab
92	2015-06-02	xrRNA.bam
2	2015-06-02	xrRNA.basereads

- **Download Cufflinks Output Files (page)**—Provides a list of links to Cufflinks output files.

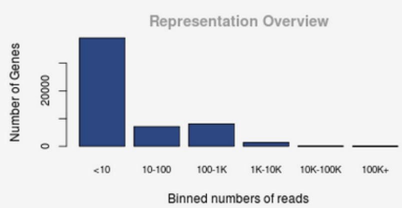
File Size	Date	File
5.5M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genes.fpkm_tracking
24M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.isoforms.fpkm_tracking
0	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.skipped.gtf
305M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.transcripts.gtf

Individual barcode view

Click any barcode of interest in the **RNASeqAnalysis Report** to see graphs for the selected barcode.

Reference table—A plot showing the number of genes with reads in log10 counting bins.

Reference: hg19



Total reads	11,283,208
Aligned reads	10,997,469
Percent aligned reads	97.47%
Mean read length	102.4
Genes Detected	16,626
Isoforms Detected	58,457

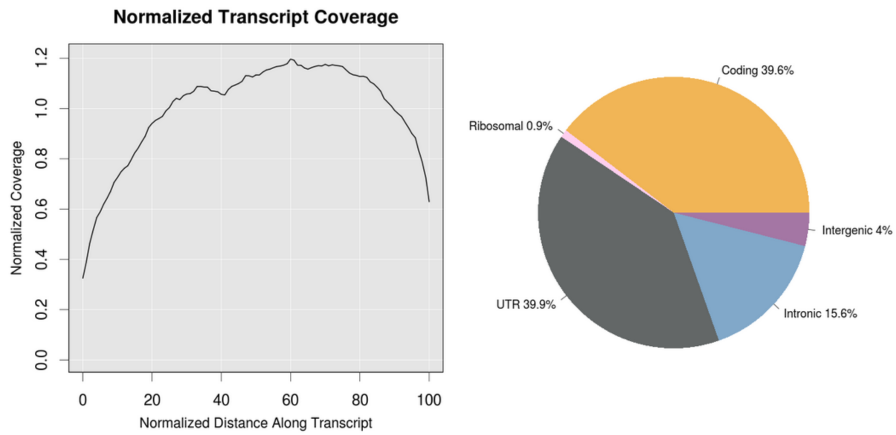
Gene Mapping Summary—A summary of reads mapped to genes of annotated reference.

Gene Mapping Summary	
Reference genes	55,765
Reads mapped to genes	7,390,706
Genes with 1+ reads	26,969
Genes with 10+ reads	16,626
Genes with 100+ reads	9,531
Genes with 10,00+ reads	1,429
Genes with 10,000+ reads	35
Isoforms Annotated	230,756
Isoforms Detected	58,457

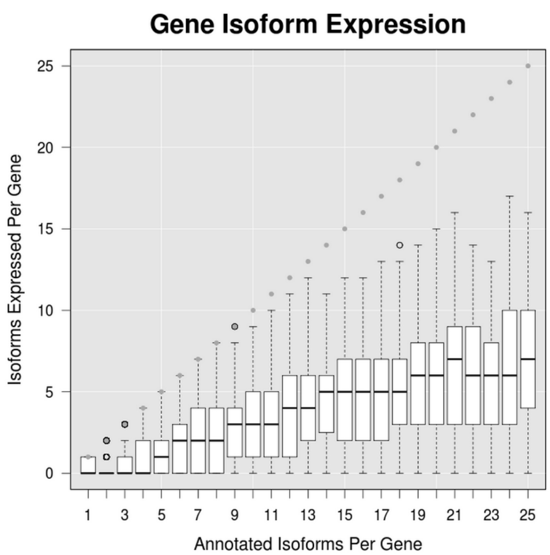
Base Mapping Summary—A summary of base reads aligned to genetic features of an annotated reference.

Base Mapping Summary	
Total base reads	1,155,834,791
Total aligned bases	914,778,477
Percent aligned bases	79.14%
Percent coding bases	39.68%
Percent UTR bases	39.93%
Percent ribosomal bases	0.94%
Percent intronic bases	15.65%
Percent intergenic bases	3.98%
Strand balance	0.4980

Transcript Coverage—A plot of normalized transcript coverage the frequency of base reads the length of individual transcripts they are aligned to in the 3' to 5' .

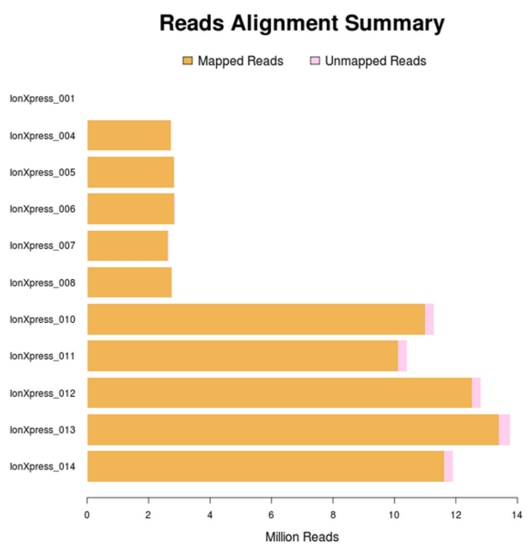


Isoform Expression— plots showing the variation of isoforms that are expressed at FPKM ≥ 0.3 for each set of genes that are grouped by the number of anticipated (annotated) . Whiskers points Q1-1.5xIQR to Q3+1.5xIQR. Only genes with 25 or less isoforms are represented in this plot. The data and a plot for all genes are available for download using the download reports links at the bottom of the screen.

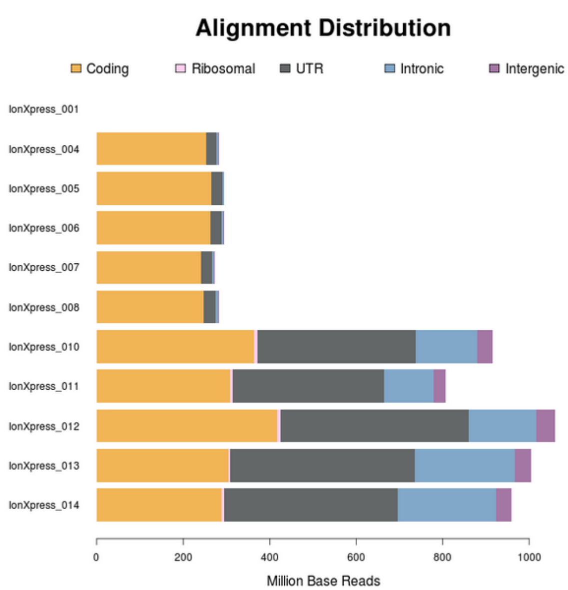


Distribution plots

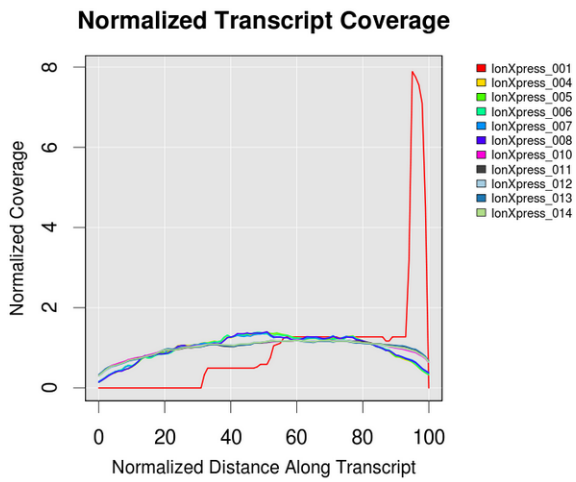
Reads Alignment Summary—A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the barcode summary table.



Alignment Distribution—A graphical summary of the distribution of reads to genomic features.

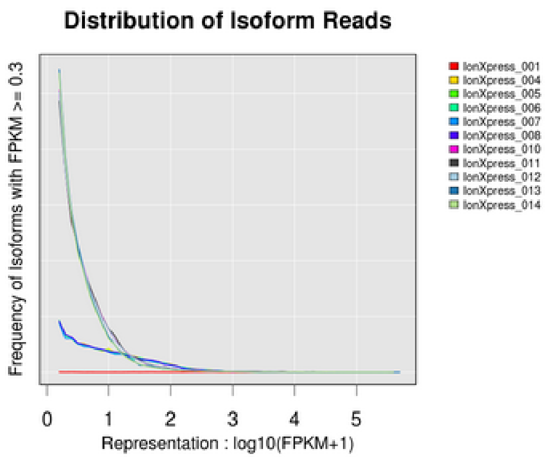


Normalized Transcript Coverage—An overlay of individual normalized transcript coverage plots for each barcode.



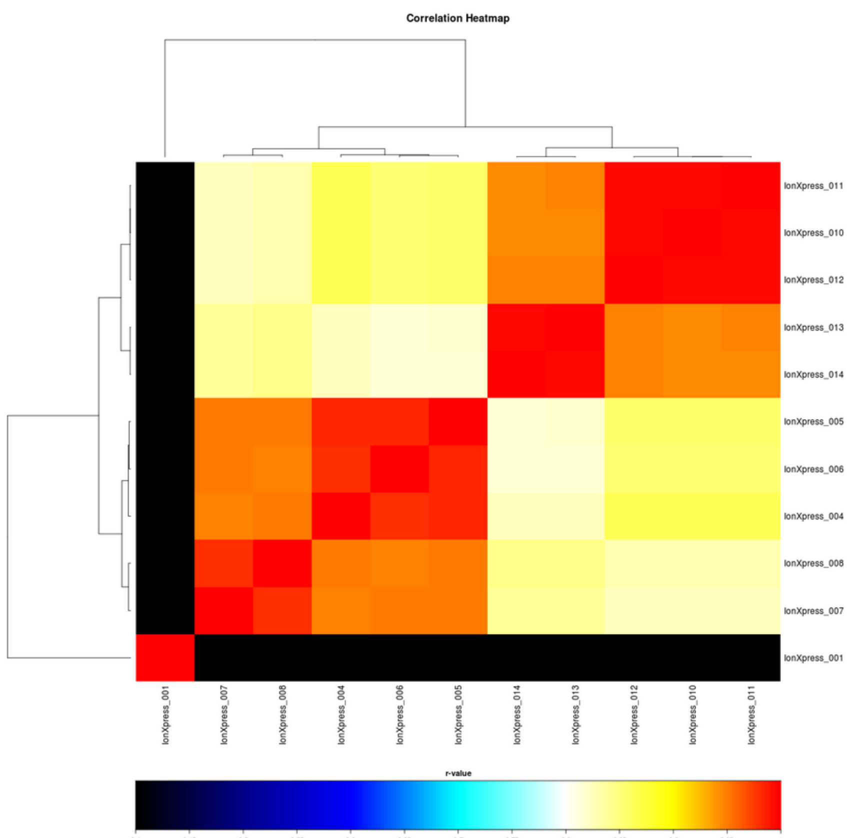
Distribution of Gene Reads—Distribution of genes across barcodes showing the frequency of numbers of genes having similar log10 read counts. All curves are plotted on the same axis scale. The counts data are fitted to a Gaussian kernel using the default R 'density' function.

Distribution of Isoform Reads—Distribution of transcript isoforms across barcodes showing the counts of isoforms having similar FPKM values. All curves are plotted on the same y-axis, normalized to the highest count, and scaled for FPKM values ≥0.3.



Correlation heatmap

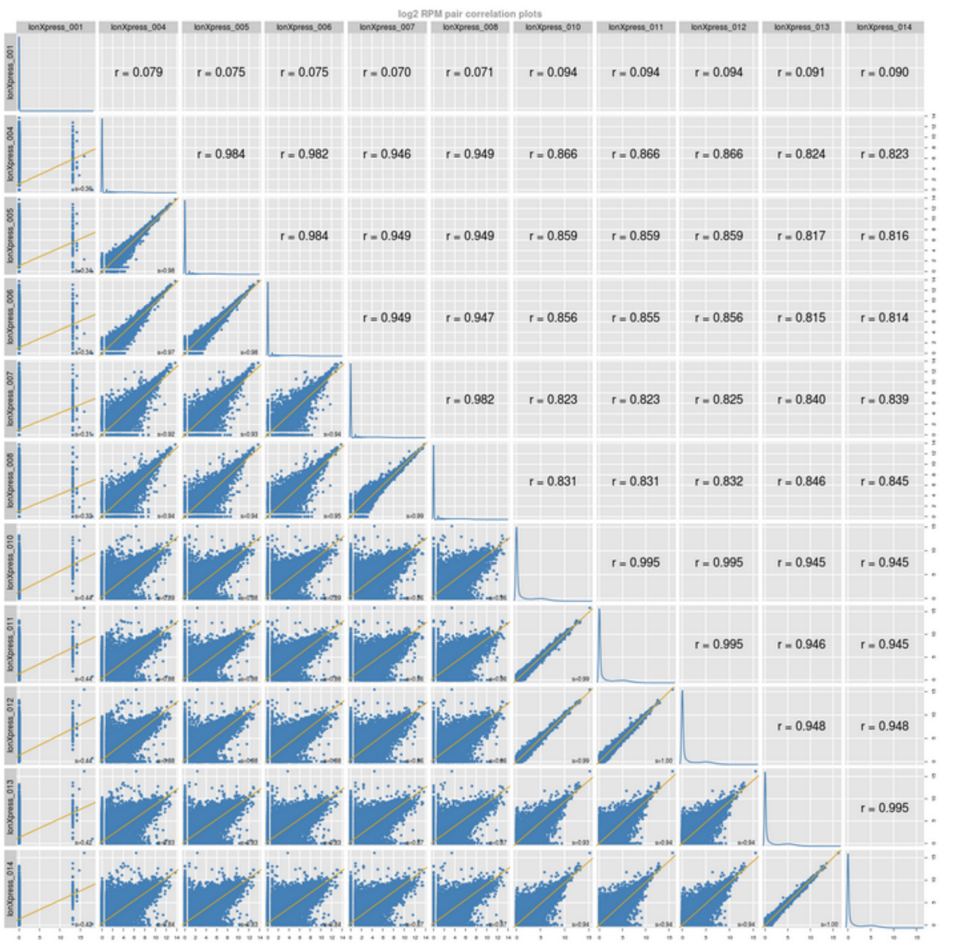
The correlation heatmap is a heatmap of Spearman correlation r-values for comparing log2 RPM reads pair correlation barcodes. The dendrogram (lines on the top and the left side of the heatmap) reflects the ordering of barcodes as being most similar by these values.



Correlation plot

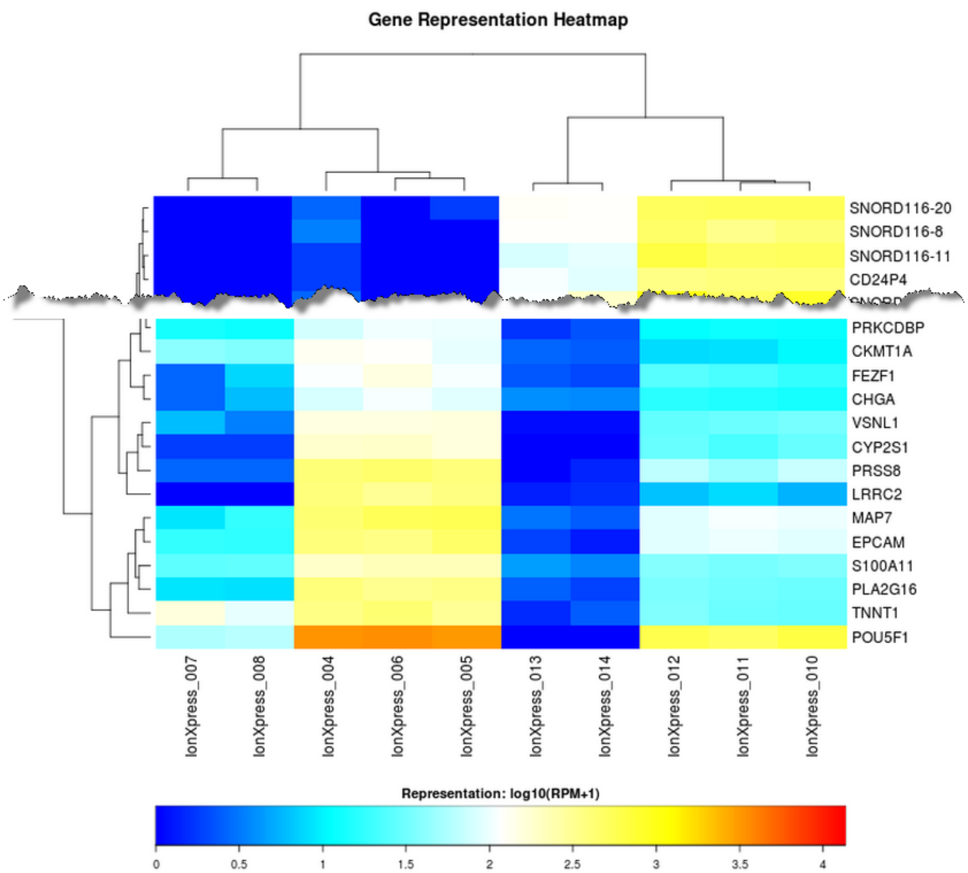
The correlation plot is a barcode read pair correlation plot. Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show the frequency density plot for the individual log(RPM+1) values for each barcode. (If only one barcode has reads, a density plot is displayed.)

Click the plot to open an expanded view in a new window.



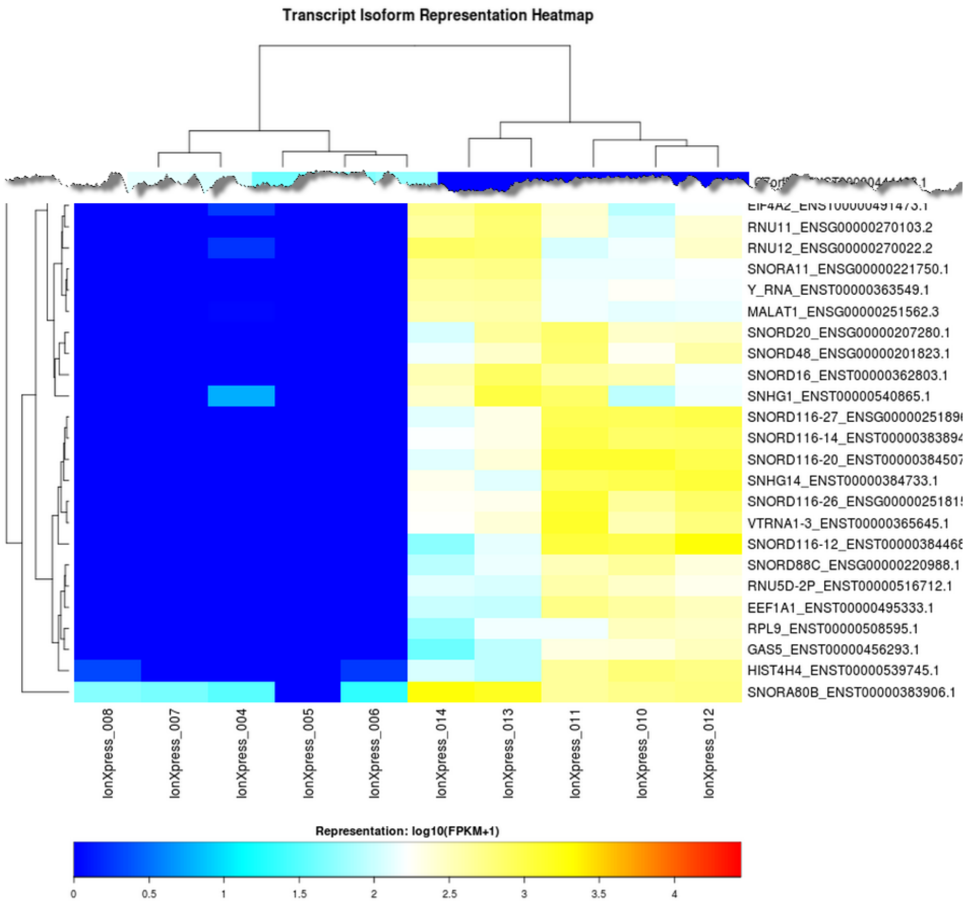
Gene heatmap

The following is a gene representation heatmap of 250 genes showing the most variation in representation across barcodes as measured by the coefficient of variant (CV) of normalized read counts for genes that have at least one barcode with at least 100 RPM reads. The heatmap is plotted using log10 of those counts. For this plot, barcodes are omitted if they have less than 100,000 total reads.



Isoform heatmap

A transcript isoform representation heatmap of up to 250 gene transcript isoforms showing the most variation in representation across barcodes as measured by the coefficient of variation (CV) of FPKM values for isoforms that have an FKPM value ≥ 100 for at least one barcode, plotted using log10 of FKPM+1. Barcodes are excluded if they have less than 1,000 isoforms detected at FPKM values ≥ 0.3.



8 Variant calls in Torrent Suite™ Software

About the variantCaller plugin

The variantCaller plugin calls single-nucleotide polymorphisms (SNPs), multinucleotide polymorphisms (MNPs), insertions, deletions (INDELs), and complex variants in a sample across a reference genome or in a targeted subset of that reference in Torrent Suite™ Software.

You can set the plugin to run automatically after a sequencing run is complete, or you can run the plugin manually for runs that are complete.

When you configure the variantCaller plugin, you can adjust parameter settings to affect the stringency of the variant calls that are made on your data. Adjustments to the parameters balance the specificity (that is, false positive calls) and sensitivity (that is, true positive calls). For more information, see "variantCaller plugin configuration manually" on page 186.

These parameter settings, when combined with the reference genome that you select, a target regions file, and a hotspots file, comprise a configuration that you can save, then use for subsequent runs of the variantCaller plugin. You can also select a predefined configuration that is included with Torrent Suite™ Software.

Preconfigured plugin settings are available as predefined or user-defined configurations that are available when you configure the variantCaller plugin. Use these configurations to save significant setup time. Predefined configurations for use with the Ion AmpliSeq™ and Ion AmpliSeq™ HD research applications can also be downloaded from **AmpliSeq.com**. For more information, see "Import panel files and parameters from AmpliSeq.com" on page 54.

Run the variantCaller plugin

The variantCaller plugin identifies variants and adds a VCF file to the output that is available for completed sequencing runs. You can run the variantCaller plugin for sequencing results in Torrent Suite™ Software.

- To see variant calls immediately after a sequencing run, configure the variantCaller plugin as part of the Planned Run.
- You can also manually configure and run the variantCaller plugin after the sequencing run from a completed run report. You must run the plugin manually after the run to use a reference genome, target regions file, or hotspots file for one or more barcodes that are is specified in the Planned Run.

To get variantCaller plugin results as quickly as possible, set up the plugin to run automatically.

Configure the variantCaller plugin to run by default after every run

To run the variantCaller plugin automatically after the Torrent Suite™ Software analysis is complete, configure the variantCaller plugin to run by default.

If the variantCaller plugin runs automatically, you can run the plugin again manually after a sequencing run is complete. For more information, see "Run the variantCaller plugin manually" on page 185.

IMPORTANT! Parameter changes that you make in a Planned Run affect only that specific run. If you change variantCaller plugin parameter settings in a Planned Run template, the changes affect all users who create Planned Runs from that template.

---

1. Click ⚙ **(Settings) Plugins**.
2. Ensure that the **Enabled** checkbox in the row of the variantCaller plugin is selected.
3. Select the **Selected by Default** checkbox in the row of the variantCaller plugin name.
   The plugin is now set to perform its function after every sequencing run.
4. (*Optional*) To disable automatic execution of the plugin, deselect the **Selected by Default** checkbox in the row of the plugin.
5. To use a reference genome, target regions file, or hotspots file for one or more barcodes that are specified in the Planned Run, run the variantCaller plugin manually after the run.

Configure the variantCaller plugin to run as part of a Planned Run

Variants are called for each barcode that is configured to use the specific reference genome, target regions file, and hotspot file that is included in the Planned Run. For more information, see "Plan step in the workflow bar" on page 49. When you configure the variantCaller plugin in a Planned Run, you cannot change the reference genome, target regions file, or hotspots file for any barcoded sample. The Planned Run uses the same parameter file for all barcodes.

You can configure the barcodes and set the variantCaller plugin to run automatically as part of a Planned Run in Torrent Suite™ Software.

If the variantCaller plugin runs automatically, you can run the plugin again manually after a sequencing run is completed.

IMPORTANT! The variantCaller plugin parameter settings are saved in Planned Run templates but *are not saved* in Planned Runs. Parameter changes that you make in a Planned Run affect only that specific run. When you change variantCaller plugin parameter settings in a Planned Run template, your changes affect all users who create Planned Runs from that template.

---

1. In the **Plan** tab, click **Templates**, then select a research application in the left navigation menu.
2. Select an existing Planned Run template from the list. Alternatively, select **Add New Template**, or **Plan New Run** to create a new Planned Run template or Planned Run.
3. Click **Plugins** in the workflow bar.
4. Select **variantCaller** plugin, then click **Configure**.

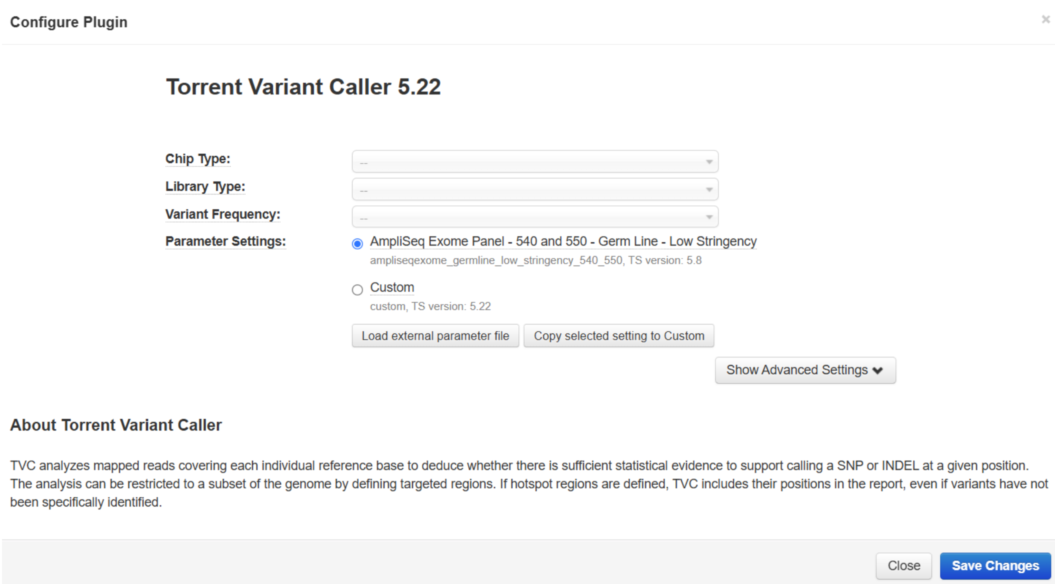
   The variantCaller plugin parameter settings change according to the selections you make. Default settings can be different based on the sequencer in use.
5. Select settings that are appropriate for the sequencing instrument that is used, the experiment, and the frequency of the variants of interest. For more information, see "variantCaller plugin configuration manually" on page 186.
6. Click **Next**, or another tab in the workflow bar to make further changes to your Planned Run.
7. When all changes to the Planned Run have been made, click **Plan** in the workflow bar, then click **Plan Run**.

The plugin is now set to run after every sequencing run that uses the Planned Run template or new Planned Run.

variantCaller plugin configuration from a planned run

Use the **Torrent Variant Caller** screen to configure the variantCaller plugin to run as part of a Planned Run.

For information about how to get to this screen, see "Configure the variantCaller plugin to run as part of a Planned Run" on page 183



Run the variantCaller plugin manually

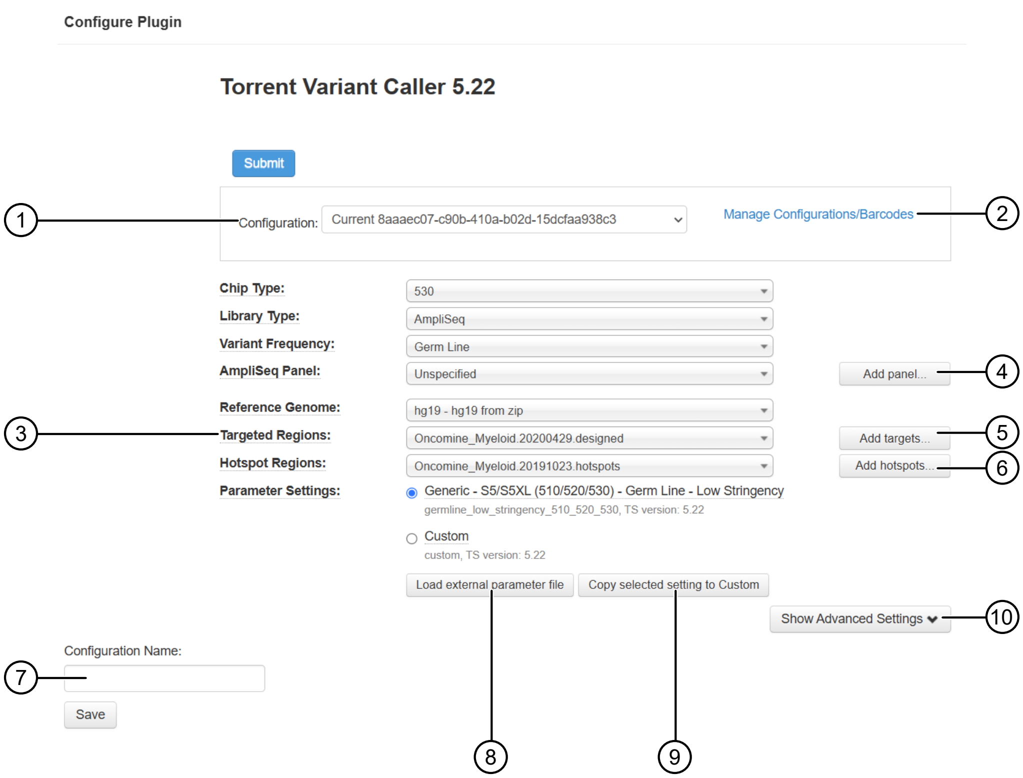
You can run the variantCaller plugin on a completed run report in Torrent Suite™ Software. If the sequencing run includes barcodes, you can apply one configuration that you saved previously to all barcodes. You can also apply a different configuration for individual barcodes. For more information, see "Create a user-defined configuration for the variantCaller plugin" on page 189.

1. In the **Data** tab, click **Completed Runs & Reports** screen, then click the **Report Name** link for the completed sequencing run of interest.
2. Click **Plugins** ▸ **Select Plugins to Run**, then click the name of the plugin that you want to run.
3. In the **Select a Plugin to Run** dialog box, select the variantCaller plugin, then click **Configure**.
   The **Configure Plugin** window opens. The variantCaller plugin parameter settings change according to the selections you make. Default settings can be different based on the sequencer in use.
4. Select settings that are appropriate for the sequencing instrument that is used, the experiment, and the frequency of the variants of interest.
   For more information, see "variantCaller plugin configuration manually" on page 186.
5. When your changes are complete, click **Submit**.
   The variantCaller plugin reruns, then applies the changes that you made.

variantCaller plugin configuration manually

Use the **Torrent Variant Caller** screen to configure the variantCaller plugin to run on the data from a sequencing run after the run is complete.

For information about how to get to this screen, see "variantCaller plugin configuration from a planned run" on page 185



1. Configuration
2. Manage Configurations/Barcodes
3. Settings included in a predefined or user-defined configuration
4. Add panel
5. Add targets
6. Add hotspots
7. Configuration Name
8. Load external parameter file
9. Copy selected setting to Custom
10. Show Advanced Settings

Setting	Details
Configuration [1]	A reusable predefined configuration or a user-defined configuration for the variantCaller plugin that includes settings for a reference genome, targeted regions, hotspots, and parameter settings. For more information, see "Create a user-defined configuration for the variantCaller plugin" on page 189.
Manage Configurations/Barcodes [1]	Manage and apply configurations for barcodes when you run the variantCaller plugin manually. (*Optional*) To run the variantCaller plugin manually for a few barcodes, select **Skip this barcode** for the barcodes that you do not want to include in the plugin run. For more information, see "Apply configuration settings to specific barcodes" on page 190.
Chip Type [1]	The chip type that is used in the sequencing run. If you change this option, it affects only the options that are selected by default in the **variantCaller Configuration** screen. It does not affect the sequencing run.
Library Type [1]	Library type options include the following. • **Whole Genome** • **AmpliSeq** • **AmpliSeq HD** • **TargetSeq** When **Library Type** is set to **AmpliSeq**, read trimming is applied to remove the primers from reads.
Variant Frequency [1]	Variant frequency options include the following. • **Somatic**—Detects somatic variants at low allele frequencies. • **Germline**—Detects germline variants that are expected to be present at low allele frequencies. • **Rare Somatic**—Detects rare somatic variants in FFPE or cfDNA samples. The option is available only if **AmpliSeq HD** is selected as the **Target Technique** in the Planned Run.
AmpliSeq Panel [1]	Panels ordered from **AmpliSeq.com** have predefined variantCaller plugin parameter settings. For details, see "Import panel files and parameters from AmpliSeq.com" on page 54. When an Ion AmpliSeq™ panel is selected, the plugin configuration screen selects default target regions, hotspots, and parameter settings files that are for use with the panel.
Add panel [1]	Upload a panel.

(continued)

Setting	Details
Reference Genome [1]	The reference genome that is for use with variant calling. To configure barcodes in the run to use the same genome reference that was used for the current run report, select the **As Specified in the Plan for Each Barcode** option. If the selected reference genome differs from the reference genome that is included in the Planned Run, the software must realign the data, which requires more time for the plugin run.
Targeted Regions	Target Regions are the regions of interest for which you want to call variants. If a target regions file is not provided, the variantCaller plugin analyzes every position of the reference genome, which typically takes longer. To configure barcodes to use the same target regions file that is included in the current Planned Run, select the **As Specified in the Plan for Each Barcode** option. Before a targeted regions file can be selected, it must be uploaded from in Torrent Suite™ Software and associated with a specific reference genome. For more information, see "Upload a target regions file" on page 248.
Add targets	Upload a target regions file.
Hotspots	Hotspots files are BED or VCF files that define variant alleles of interest. Hotspots files instruct the variantCaller plugin to include these variant alleles in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspots file affects only the variantCaller plugin, not other parts of the analysis pipeline. If you do not specify a hotspots file, the software reports only the de novo variants that show as present. In contrast, if a hotspots file is used, the variant calls and the filtering metrics for each hotspot allele are reported in the output VCF file, including data for absent or NOCALL variants. To configure hotspots files to use the same hotspots file that is included in the current Planned Run, select the **As Specified in the Plan for Each Barcode** option. Before a hotspots file can be selected, it must be uploaded from in Torrent Suite™ Software and associated with a specific reference genome. For more information, see "Upload a hotspots file" on page 250. **IMPORTANT!** A carefully designed hotspot file is recommended to optimize the overall performance of variant calling. For help with designing a hotspots file, contact your local Field Service Engineer.
Add Hotspots	Upload a hotspots file.

(continued)

Setting	Details
Parameter Settings	Option to use parameter settings that are predefined in the variantCaller plugin, or to use user-defined parameter settings. For Ion AmpliSeq™ experiments, panel templates from **AmpliSeq.com** can contain parameter settings that are optimized for the variantCaller plugin and are available for use during variantCaller plugin configuration. • The variantCaller plugin provides generic parameter settings that are optimized for the selected **Chip Type**, **Library Type**, and **Variant Frequency**, and for the parameters settings that are downloaded from **AmpliSeq.com** . • Select **Custom** to change the advanced parameter settings. For more information, see “variantCaller plugin advanced parameters” on page 207.
Load external parameter file	Imports a file that contains parameter settings.
Copy selected settings to Custom	Creates a user-defined parameter setting that is based on a generic parameter setting. For more information, see “Create and use a user-defined parameters setting” on page 192.
Configuration Name [1]	Names and saves a user-defined configuration when changes are made to the predefined settings.
Show Advanced Settings	Configure advanced parameter settings. For more information, see “variantCaller plugin advanced parameters” on page 207. Place the pointer over the field to see tooltips with descriptions of the advanced settings.

[1] Hidden in sequencing runs that use Tag Sequencing as the target technique.

Create a user-defined configuration for the variantCaller plugin

You can create a user-defined configuration for the variantCaller plugin in Torrent Suite™ Software. Later, when you run the plugin manually, you can apply the user-defined configuration to the variantCaller plugin run, or to individual barcodes that are included in the run.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for the completed sequencing run.
2. Click **Plugins ▸ Select Plugins to Run**.
3. In the **Select a Plugin to Run** dialog box, select the variantCaller plugin, then click **Configure**.

4. In the **variantCaller plugin configuration** screen, specify plugin configuration parameters.
   For more information, see "variantCaller plugin configuration manually" on page 186 and "Create and use a user-defined parameters setting" on page 192.

5. In **Configuration Name**, enter a name for the configuration, then click **Save**.

The new configuration is included in the **Configuration** list in the **variantCaller plugin configuration** screen. You can apply the configuration when you run the variantCaller plugin manually.

Apply configuration settings to specific barcodes

You can save and retrieve user-defined configurations, for the variantCaller plugin and apply these configurations to specific barcodes when you run the variantCaller plugin manually from a completed Torrent Suite™ Software run report. By applying specific user-defined configurations to individual barcodes, you can refine your analysis results.

Use configurations for barcoded runs to do the following.

- Save a variantCaller plugin configuration that applies to all barcodes.
- Apply one or more saved configurations to individual barcodes.
  When you apply saved configurations to specific individual barcodes, you must run the plugin immediately after you apply the configurations. You can apply a saved configuration to an individual sample on a chip for each barcode. Configurations applied to individual barcodes cannot be saved.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** for the completed sequencing run of interest.

2. Click **Plugins** ▸ **Select Plugins to Run**, then in the **Select a Plugin to Run** dialog box, click **variantCaller**.

3. In the **Configure Plugin** dialog box, click **Manage Configurations/Barcodes**, then click **OK**.

4. In the **Torrent Variant Caller** dialog box, in the **Setup** tab, apply configurations to all barcodes or specific configurations to individual barcodes.

Option	Selection
Apply the same configuration to all barcodes in a sequencing run.	In **Set All**, select the configuration to apply to all barcodes, then click **Submit**.
Apply a specific configuration to an individual barcode.	In the **Configuration** column, select the configuration from the list of available configurations for each individual barcode.

5. *(Optional)* In the **Torrent Variant Caller** dialog box, in the **Configuration** tab, create a new configuration or edit an existing one.

Option	Selection
Edit a configuration.	a. Click **Edit**. The **Configure Plugin** dialog box becomes specific to the configuration selected. b. Change the existing selections as desired. To add a new panel, target regions, or hotspots file, you are redirected to the appropriate **Settings** screen. Close the **Configure Plugin** dialog box, then reopen the dialog box to access the new file. c. Click **Show Advanced Settings**, then edit the parameters if needed. d. Click **Save**.
Delete a configuration.	a. Click **Delete**. b. Click **OK** to confirm.
Create a new configuration.	a. Click **Add**. b. Enter a configuration name. c. Make selections from the available lists. d. Click **Save**.

Create and use a user-defined parameters setting

You can create a user-defined parameters setting that you can save and reuse. The parameters setting that you create can be used with a manual run of the variantCaller plugin. To use a user-defined parameter file that is designed for your customized panel or other special requirements, you must create a parameters setting, then run the variantCaller plugin manually with the user-defined parameters setting.

The parameters file in the variantCaller plugin is a JSON text file that contains the variant calling parameters that are listed in "variantCaller plugin advanced parameters" on page 207.

1. In the **Data** tab, in the **Completed Runs & Reports** screen, click the report name of the run that you want to apply configuration settings to.
2. Click **Plugins ▸ Select Plugins to Run**, then in the **Select a Plugin to Run** dialog box, click **variantCaller**.
3. In the **Parameter Settings** section, click **Custom**, then complete the selections for one of the following options.

Option	Selection
Use a JSON parameters file that you have saved on your computer.	a. Click **Load external parameter file**. b. Browse to the JSON file, then click **Open**.
Edit a predefined parameters setting.	a. In **Configuration**, select the predefined parameter setting that you want to edit. b. Click **Copy selected to Custom**. c. If you agree to save the new parameter setting, click **OK**. d. Click **Show Advanced Settings** to enter changes for the user-defined parameter settings.

   You can edit the values of the advanced parameter settings. Predefined parameter settings are in read-only mode and cannot be edited.

The user-defined parameters setting is now ready to be applied to run results when you run the variantCaller plugin manually.

Review variantCaller plugin run results

After a variantCaller plugin run completes, you can access variantCaller run results from the run report screen in Torrent Suite™ Software. On the run report screen, in the variantCaller section you can do the following.

- Review the summary of the variantCaller plugin run for each barcode used.
- Review the library type, reference genome, targeted regions, hotspots file, and parameter settings that were used in the run.
- Download data files for all barcodes and each individual barcode or sample.

You can also access the detailed variantCaller plugin summary report for each barcode or sample from the variantCaller section on the run report screen. In the detailed variantCaller plugin summary report screen, you can do the following.

- View variant call information by allele for the specific barcode, such as allele location on the chromosome, allele annotations, coverage metrics, and quality metrics.
- Review the library type, reference genome, target regions, hotspots, and parameter settings that were used in the run.
- Download BED files and the parameters file that are used for the specific barcode.
- Download BAM and BAI files for the mapped and processed reads.
- Download data files for variant calls and coverage for the specific barcode.
- View variant calls in a visualization tool that is installed on your computer.

The variantCaller plugin supports SNPs, MNPs, INDELs, and complex alleles as input candidates at genomic positions with the target regions file. If the variant is outside of the target regions, then the variant is not generated as a candidate and is not further evaluated, even if the variant is specified in the hotspots file.

1. In the **Data** tab, click **Completed Runs & Reports**.

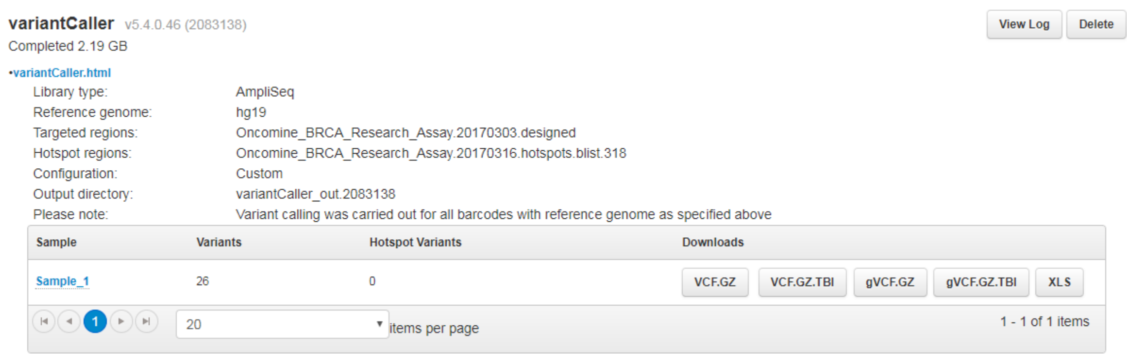
2. In the **Completed Runs & Reports** list, find the run of interest, then click the report link in the **Report Name** column in the row of the run.

3. In the left navigation menu, click **variantCaller** to navigate to the variantCaller results section.
   - If the sequencing run contains barcodes, the plugin report includes a list of the barcodes that were used and file download options for all barcodes and each individual barcode.

Download options for all barcodes that were used in the plugin run.

Download option	Description
VCF.ZIP	A compressed directory that contains separate VCF files for each barcode.
XLS.ZIP	A compressed directory that contains separate XLS files for each barcode.
XLS	A file that contains a list of alleles for all barcodes in a tab-separated file that can be opened by Microsoft™ Excel™.
COV	A file that contains the coverage of the variant call results for all barcodes in tab-separated file format, which can be opened in Microsoft™ Excel™.

- If the sequencing run does not contain barcodes, the plugin report contains information for the sample that is used in the run and the file download options for the sample.

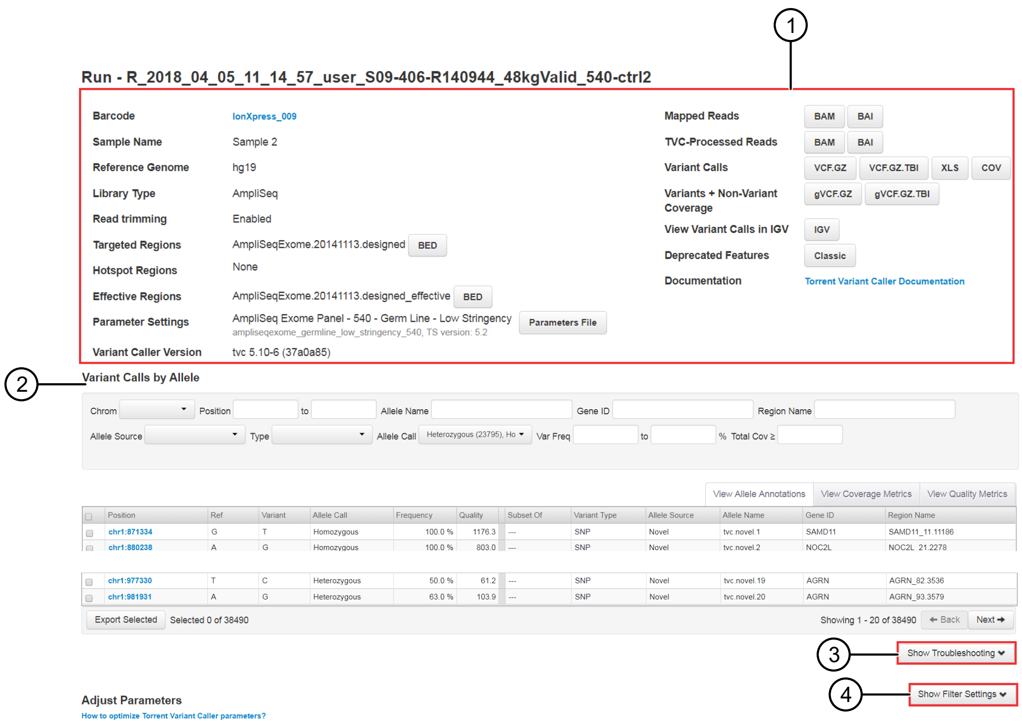


4. To open the detailed variantCaller plugin report for a specific barcode or sample, do one of the following in the variantCaller run summary table:
   - In the **Barcode name** column in a barcoded sequencing run report, click the barcode name link.
   - In the **Sample** column in a report from a sequencing run that does not contain barcodes, click the sample name link.

Detailed variantCaller plugin report

The detailed variantCaller plugin report contains variantCaller run information, results, and the associated files for download that are specific for an individual barcode or sample.

To access the report, click the sample name link in the **Sample** column in a report from a sequencing run.



1. Review run information for a specific barcode or sample and download the associated files.
2. View variants called and their associated allele annotation information, coverage metrics, and quality metrics. For more information, see "Variant Calls by Allele table" on page 196.
3. Export the variant data files for troubleshooting. For more information, see "Export files for troubleshooting" on page 204.
4. Adjust variantCaller plugin filter settings that were used for the specific barcode or sample, then save the adjusted parameters to a new configuration. For more information, see "Save adjusted parameters to a variantCaller plugin configuration" on page 206.

The following table lists and describes the download options for an individual barcode, or the sample used in the run. The available options in the table depend on the run type.

Download option	Description
Target Regions	**BED**—The BED file that specifies the genomic positions of interest.
Hotspot Regions	**BED**—The BED file that specifies the variant alleles of interest.
Effective Regions	**BED**—The BED file that specifies the regions that are processed in the variantCaller run for the sample or barcode (available if read trimming is enabled).
Parameter Settings	**JSON**—The JSON file that contains the parameter settings used in the variantCaller run for the sample or barcode.

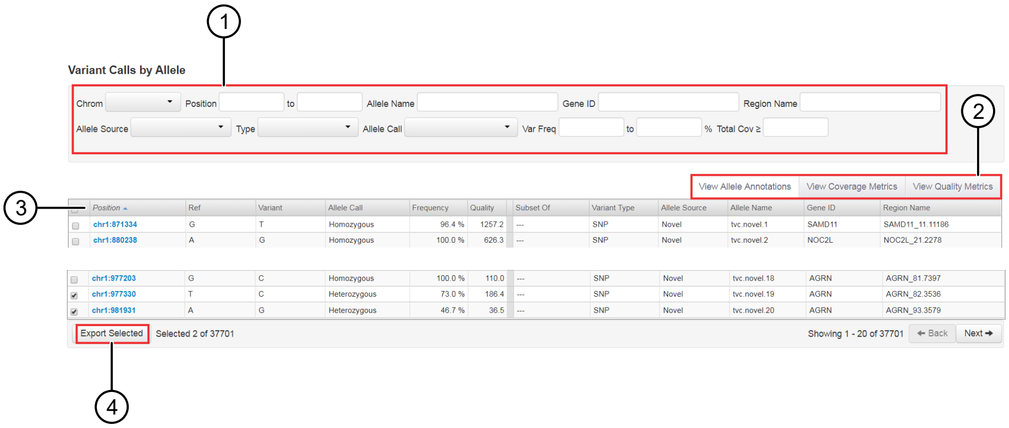
(continued)

Download option	Description
Mapped Reads	• **BAM**—The BAM file that is input to the Torrent Variant Caller (TVC) module Pipeline for discovering and evaluating variants. Note that realignment may be applied. • **BAI**—The BAI file that contains the index information for the corresponding BAM file.
Torrent Variant Caller-Processed Reads	• **BAM**—The BAM file that is processed by Torrent Variant Caller (TVC) module . Note that read trimming and read filtering may be applied. In Tag Sequencing and Ion AmpliSeq™ HD runs, the BAM file may contain consensus reads that are obtained by compressing the reads in the mapped BAM that originate from the same DNA molecule. • **BAI**—The BAI file that contains the index information for the corresponding BAM file.
Variants Calls	• **VCF.GZ**—The compressed VCF file that contains the variant calls. • **VCF.GZ.TBI**—The index file for VCF.GZ. • **XLS**—The file that contains a list of variant alleles in the tab-separated file format, which can be opened in Microsoft™ Excel™. • **COV**—The file that contains coverage analysis for each variant call, which can be opened in Microsoft™ Excel™.
Variants + Non-Variant Coverage	• **gVCF.GZ**—The bgzip-compressed genome-VCF file that contains the variant calls. • **gVCF.GZ.TBI**—The tabix index file for gVCF.GZ.
View Variant Calls in IGV	**IGV**—The Integrative Genomics Viewer is not supported in Torrent Suite™ Software 5.22.

Variant Calls by Allele table

The detailed variantCaller plugin report contains the **Variant Calls by Allele** table. The table lists the details about each variant that is called, including the allele locus, allele annotation, coverage metrics, and quality metrics for the specific barcode or sample. You can use the table to find the variant alleles of interest and information about those alleles. You can also export the information to be saved to your local storage.

To access the report, click the sample name link in the **Sample** column in a report from a sequencing run.



① Find the variants of interest by applying filters to the table to narrow down the list of variants called.

② Change the display of the table to view allele annotation, coverage metrics, or quality metrics for each variant. To switch between different displays, select one of the following tabs.

- **View Allele Annotation**. For more information, see "View allele annotations " on page 198.
- **View Coverage Metrics**. For more information, see "View coverage metrics " on page 199.
- **View Quality Metrics**. For more information, see "View quality metrics" on page 200.

③ Click the column heading to sort variant alleles by the values in the column.

④ Export the information that is associated with the selected variant alleles to an XLS file. The exported XLS file contains all the information about the selected variants, including the information listed in the **View Allele Annotation**, **View Coverage Metrics**, and **View Quality Metrics** tabs. The tabs that are available depend on the run type. For information about how to export the information, see "Export variant calls to a file" on page 201.

Column	Description
Position	The chromosome (or contig) name in the reference genome, and the position of the chromosome (or contig) in the one-based coordinate.
Ref	The reference base or bases.
Variant	The variant allele base or bases.
Allele Call	The zygosity (homozygous or heterozygous) or type (Absent or No Call) of the allele that is called by the zygosity (homozygous or heterozygous) or type (Absent or No Call) of the allele all by the variantCaller plugin.
Frequency	The frequency, in %, of the variant allele.
LOD	The limit of detection (LOD) at the genome location, estimated based on the number of detected molecules. This column is available only for sequencing runs that use the tag sequencing or Ion AmpliSeq™ HD as the target technique in the Planned Run. For more information, see "Research Application step in the workflow bar" on page 42.

(continued)

Column	Description
Quality	The Phred-scored quality. For variants found by the Long INDEL Assembler, this value is always set to 50. Larger values mean higher confidence in the call. Quality is calculated by posterior probability that the variant allele frequency is greater than the cutoff (`min_allele_freq` in the parameter file), if a variant call is made, or posterior probability that the variant allele frequency is below this cutoff (if a reference call). The posterior probability that is computed as conditional on the reads observed includes sampling variability. Quality score is typically very large for reads strongly distinguishing variants with good depth, that is, under the model assumed, evidence is overwhelming for the variant or for the reference. Marginal values can mean that either the reads do not distinguish the variant well, there is insufficient depth to resolve, or the observed allele frequency is near the cutoff.
PPA	An indication (0 or 1) of whether the variant allele is a possible polyploidy allele (PPA). Only absent alleles can be labeled as PPA; heterozygous and homozygous alleles are not treated as PPA. This column is available if the `report_ppa` parameter is set to 1. For more information on how to set the `report_ppa` parameter, see "Torrent Variant Caller module advanced settings" on page 207.

View allele annotations

You can view the following information in the **View Allele Annotations** tab of the **Variant Calls by Allele** table.

Column	Description
Allele Call	Decision whether the allele is detected (Heterozygous or Homozygous), not detected (Absent), or filtered (No Call). No Call and Absent are for only hotspot calls.
Subset Of	The name of the called allele that is a strict superset of the two SNPs. For example, if a called (homozygous or heterozygous) MNP is composed of two SNPs, then the MNP is considered to be a strict superset of the two SNPs.
Variant Type	The type of the variant called. • SNP—single nucleotide polymorphism. • IND—insertion. • DEL—deletion. • MNP—multiple nucleotide polymorphism or the substitution of a block sequence by the block of another length. • COMPLEX—Block substitution of sequence by a block of unequal length.
Allele Source	Allele source is called as: • Hotspot—for alleles included in the hotspots file. • Novel—for all other alleles.

(continued)

Column	Description
Allele Name	The allele name as defined in the hotspots file. For novel alleles, the name is defined as `tvc.novel.#`.
Gene ID	The Gene ID as defined in the target regions file.
Region Name	The region name as defined in the target regions file.

View coverage metrics

You can view the following information in the **View Coverage Metrics** tab of the **Variant Calls by Allele** table. The columns that are available on this tab vary according to the run type.

Column	Description
Total Read Cov	Total read coverage at this position, after downsampling. Variants calls are made on a sample of reads when coverage is higher than specified in the parameter settings file. This is referred to as "downsampling". For more information, see `downsample_to_coverage` in “variantCaller plugin advanced parameters” on page 207.
Read Cov +[1]	Total read coverage on the forward strand, after downsampling.
Read Cov -[1]	Total read coverage on the reverse strand, after downsampling.
Allele Read Cov	The number of reads that contain this allele, after downsampling.
Allele Read Freq[2]	The frequency of this allele across all reads.
Total Mol Cov[2]	The number of molecules that cover this location.
Allele Mol Cov[2]	The number of detected molecules that contain this allele.
Allele Mol Freq[2]	The frequency of molecules that contain this allele.
Allele Cov +[3]	Allele coverage on the forward strand, after downsampling.
Allele Cov -[3]	Allele coverage on the reverse strand, after downsampling.
Strand bias[3]	The discrepancy between allele frequencies on the forward and reverse strands.

[1] This column is unavailable for sequencing runs that use Tag Sequencing as the target technique.
[2] This column is only shown for sequencing runs that use Tag Sequencing or Ion AmpliSeq™ HD as the target technique.
[3] This column is unavailable for sequencing runs that use Tag Sequencing or Ion AmpliSeq™ HD as the target technique.

View quality metrics

You can view the following information in the **View Quality Metrics** tab of the **Variant Calls by Allele** table.

This tab does not appear in Tag Sequencing runs.

Column	Description
Common Signal Shift	The distance between predicted and observed signal at the allele locus. [RBI]
Reference Signal Shift	The distance between predicted and observed signal in the reference allele. [REFB]
Variant Signal Shift	The distance between predicted and observed signal in the variant allele. [VARB]
Relative Read Quality	The Phred-scaled mean log-likelihood difference between the prediction under reference and variant hypothesis. [MLLD]
HP Length	Homopolymer length.
Context Error +	The probability of sequence-specific error on the forward strand (reported only for deletion variants).
Context Error -	The probability of sequence-specific error on the reverse strand (reported only for deletion variants).
Context Strand Bias	Basespace strand bias (reported only for deletion variants).

Values that cause a candidate to be filtered out are shown in colored cells. For candidates that are filtered out, the column name shows the filtering reason.

lele coverage	allele coverage +	allele coverage -	strand bias
29	21	8	0.5897
23	15	8	0.5522
15	15	0	0.5000
15	15	0	0.5000
288	133	155	0.5000
95	88	7	0.5028
20	20	0	0.5000
5	0	5	0.5000
259	102	157	0.5000
187	80	107	0.5000
239	91	148	0.5000

Export variant calls to a file

You can export variant calls to a tab-separated file that can be opened using spreadsheet software such as Microsoft™ Excel™. The exported file is named `subtable.xls` and has the same columns that are included in the **Variant Calls by Allele** table. The file includes columns for all three display options: **View Allele Annotations**, **View Coverage Metrics**, and **View Quality Metrics**.

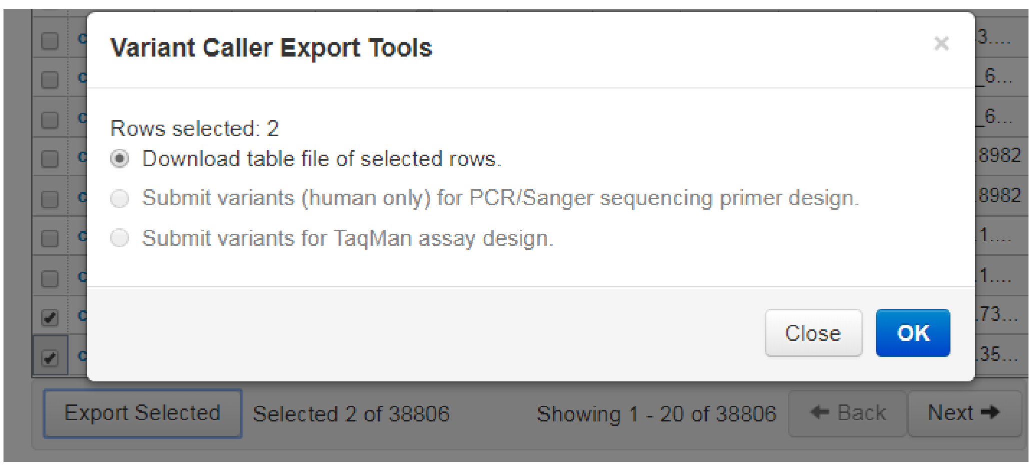
1. In the **Data** tab, in the **Completed Runs & Reports** screen, click the report name of the run from which you want to export variant calls to a file.

2. In the left navigation menu, click **variantCaller**, then open the detailed variantCaller plugin report for a specific barcode or sample.

   - In the **Barcode name** column in a barcoded sequencing run report, click the barcode name link.
   - In the **Sample** column in a report from a sequencing run that does not contain barcodes, click the sample name link.

3. In the **Variant Calls by Allele** table, select the checkbox in the row of each variant that you want to export, then click **Export Selected**.

4. In the **Variant Caller Export Tools** dialog box, select **Download table file of selected rows**, then click **OK**.

   The `subtable.xls` file is created and downloaded to your computer.

5. *(Optional)* View the file, then save it to your local storage using a descriptive file name.

Troubleshoot variantCaller plugin results

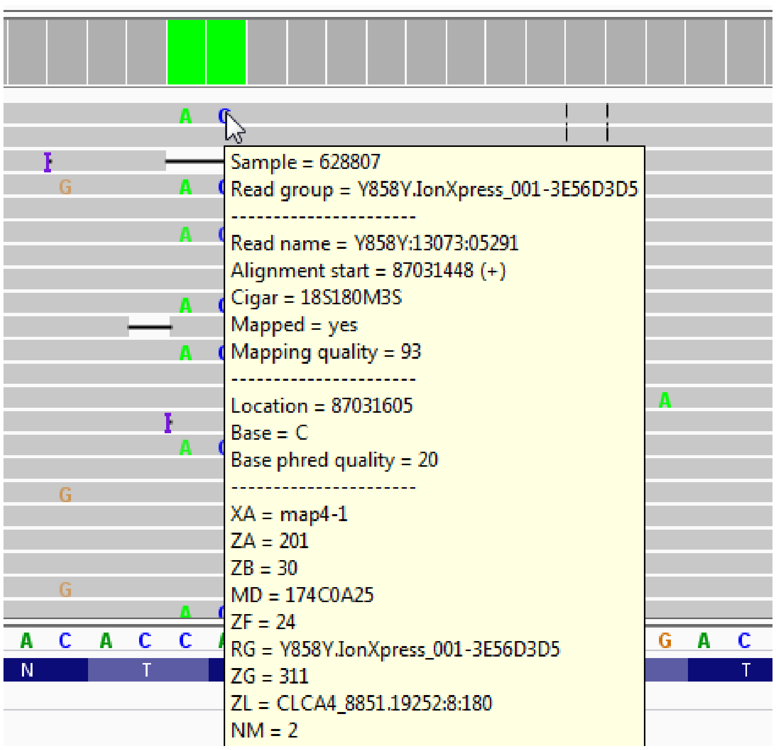
You can troubleshoot results in the variantCaller plugin detailed report to analyze why expected variants were missed (false negatives) or to examine false positives. Plugin parameters can be adjusted and the plugin can be rerun. You can also export troubleshooting results to share them with a field bioinformatics specialist (FBS).

Find false negatives with an alignment viewer

When an expected variant is not called by the variantCaller plugin, an alignment viewer, such as Ion Reporter™ Genomic Viewer (IRGV) can help you confirm the absence of the variant in the sample, or help you understand how to adjust the plugin parameters to enable the plugin to call the variant. Using IRGV can reveal problems such as mismapping or low coverage. In particular, an alignment viewer lets you visually inspect the coverage of the region where the variant is expected, and focus attention on the depth of coverage and the quality of the bases covering the position of the variant. Low coverage or low base quality can explain a no-call. A genomic viewer can also reveal that variant is slightly misplaced (especially for INDELs) and therefore not called.

1. In the viewer, select the chromosome where the variant of interest is located, scroll to the chromosomal position of the variant, then zoom in until you can read the nucleotide sequence surrounding the variant allele.

2. Place the pointer over a variant read to view read analytics.



3. Adjust plugin parameters.

4. Rerun the variantCaller plugin.

Find false negatives by using the variantCaller plugin report

You can use built-in variantCaller plugin tools to display and examine call details.

- If a hotspots file was used:
  a. Check that the position of the variant is included in the hotspots file.
  b. Check the Variant Calls output table. Values that cause a candidate to be filtered out are shown in colored cells. For candidates that are filtered out, the column name shows the filtering reason.

allele coverage	allele coverage +	allele coverage -	strand bias
29	21	8	0.5897
5	0	5	0.5000
259	102	157	0.5000
187	80	107	0.5000

  c. Adjust plugin parameters.
  d. Rerun the variantCaller plugin.
- If a hotspots file was not used:
  a. Navigate to the variantCaller plugin results directory on the Ion Torrent™ Server and open the `small_variants_filtered.vcf` file.
     – Open the detailed report in Torrent Suite™ Software, then click the **Barcode Name** link in a report from a barcoded sequencing run. Or, click the **Sample Name** link in a report from a non-barcoded sequencing run. Scroll to, or search for the **small_variants_filtered.vcf** link. You can click the link to view the file in the browser or download the file to your computer.
     – On Linux backend, the variantCaller plugin results directory can be found at `/results/analysis/output/Home/{analysis_report_name}/plugins/variantCaller/` for non-barcoded runs or `/results/analysis/output/Home/{analysis_report_name}/plugins/variantCaller/{bar code}/` for barcoded runs.
  b. Find the location of the variant, then examine the FR (filtered reason) field.
  c. Relate the reason to plugin run parameters using the parameter definitions in “variantCaller plugin advanced parameters” on page 207.
  d. Adjust plugin parameters.
  e. Rerun the variantCaller plugin.

Fix false positives

False positives are usually related to artifacts that create unexpected amplification, such as a primer-dimer or contamination problems. Some false positives are reported because of the difficulties inherent with the handling of homopolymer regions.

Use one of the following methods to resolve these issues:

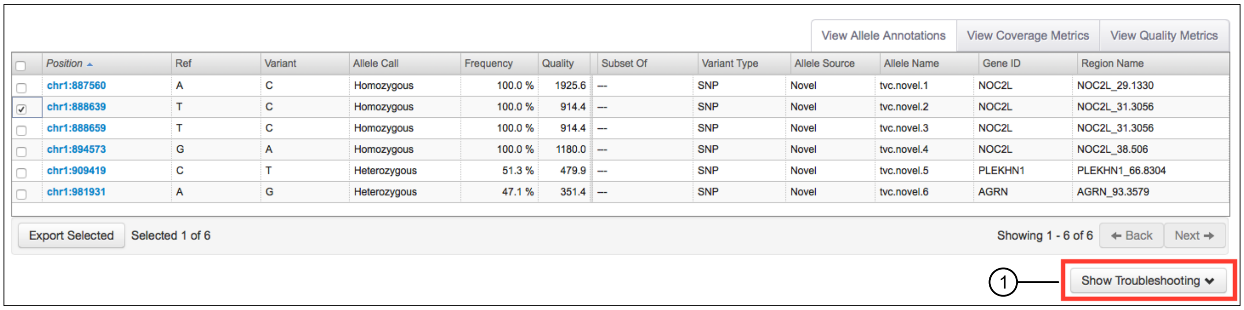
- Adjust parameters that control the homopolymer calls. This can increase the report of false negatives.
- If you are repeatedly running a panel, manually curate specific sites (positions), since the false positive tends to occur in the same positions.

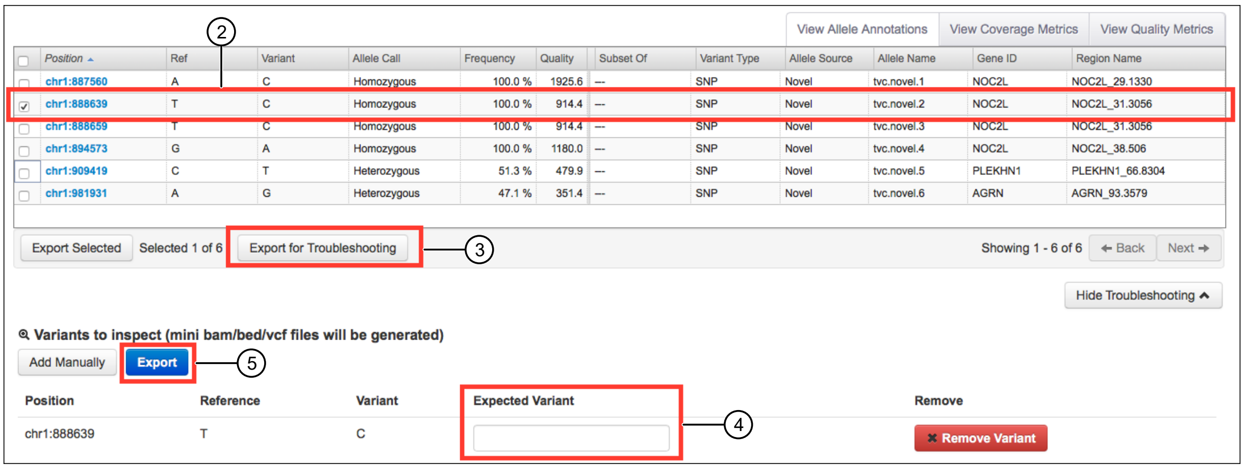
The variantCaller plugin does not support manual curation. Manual curation is available in the command-line version.

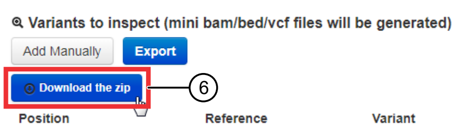
Export files for troubleshooting

Torrent Suite™ Software includes a tool that helps you determine why variant calls are unclear in analyses. You can use the Slicer tool to select one or more variant calls, then export the related data as miniature BAM, BED, and VCF files. You can then share these files with a field bioinformatics specialist for further review.

Navigate to the variantCaller plugin output table, then follow the steps listed below.







1. Click **Show Troubleshooting**.
2. Select the variant or variants of interest.
3. Click **Export for Troubleshooting**.
4. Enter the **Expectant Variant**.
5. Click **Export**.
6. Wait for the export process to complete, then click **Download the zip**.

The compressed folder of miniature BAM, BED, and VCF files is downloaded to a folder on the computer, based on the browser settings.

Save adjusted parameters to a variantCaller plugin configuration

You can adjust the variantCaller plugin parameters that are used for the barcode, then save the adjusted parameters to a configuration.

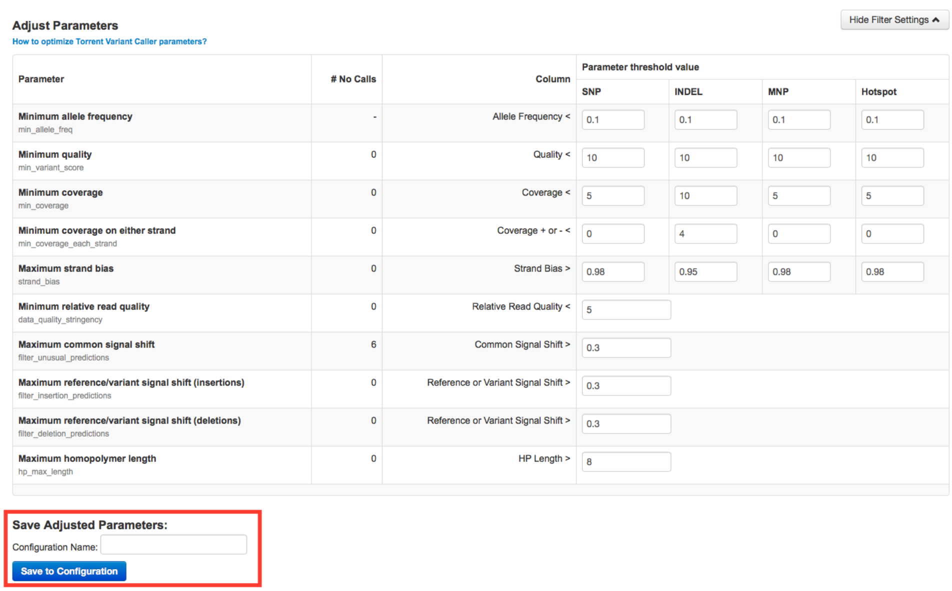
The reference genome, target regions, and hotspots files in the saved configuration inherit the files that are used to obtain the variantCaller plugin results for this barcode.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** for the completed sequencing run of interest.

2. In the left navigation menu click **variantCaller**, or scroll to the **variantCaller** results section.

3. Click the barcode name link to open the detailed **variantCaller** plugin summary.

4. Scroll down the page, click **Show Filter Settings**, then adjust the parameter threshold values.



5. Enter the **Configuration Name** that stored the adjusted parameters, then click **Save to Configuration**.

6. To apply the updated configuration to additional barcodes, run the plugin manually, then assign the saved configuration with the adjusted parameters to the desired barcodes.

variantCaller plugin advanced parameters

Advanced parameter settings for the variantCaller plugin allow additional customization of the variant calling algorithm and are for use only by advanced users.

In general, you can safely customize parameters for SNP calling. For INDEL calling, changes to the parameters tend to have a significant impact on the number of INDELs called. As a result, consider the tradeoff between sensitivity and specificity for INDEL calls when you use customized parameters.

The variant calling pipeline in the variantCaller plugin contains three modules.

- Torrent Variant Caller module—The Torrent Variant Caller module evaluates the list of candidates and examines evidence for the variants in flow space.
- FreeBayes module—The FreeBayes module, as a candidate generator, generates lists of variant candidates.
- Long INDEL Assembler module—Some long INDELs, if improperly aligned to the reference genome, may not be discovered and evaluated by the FreeBayes and the Torrent Variant Caller modules. The purpose of the Long INDEL Assembler module is to call these long INDELs. A unification step in the pipeline combines the variants that are called by the Torrent Variant Caller and the Long INDEL Assembler module into one VCF file. The VCF file is available to the software.

Torrent Variant Caller module advanced settings

The following table lists and describes some of the Torrent Variant Caller module advanced settings.

IMPORTANT! The advanced parameters and settings are recommended for advanced users only. If you need help setting advanced parameters, contact your local Field Service Engineer.

Torrent Variant Caller advanced parameters and settings

Parameter	Description
`X_min_allele_freq`	Minimum allele frequency, where X is one of the allele types in {indel, snp, mnp, hotspot}. A variant evaluation parameter: The presence of the allele of the type is defined by which allele frequency is greater than this value. **Allowed values**—Decimal between 0 and 1 **Suggested trial value**—Between 0.01 (somatic) and 0.2 (germline)
`X_min_variant_score`	Minimum quality, where X is one of the allele types in {indel, snp, mnp, hotspot}. A filter parameter: A called allele of the type needs to have a QUAL score greater than this Phred-scaled value. **Filter reason**—quality score **Related VCF fields**—QUAL **Allowed values**—Decimal values ≥0 **Suggested trial value**— >10

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`X_min_coverage`	Minimum coverage, where X is one of the allele types in {indel, snp, mnp, hotspot}. A filter parameter: The location of a called allele of the type needs to have a coverage greater than this value. **Filter reason—**MINCOV **Related VCF fields—**FRO, FAO **Allowed values—**Integers ≥0 **Suggested trial value—**between 5 and 20
`X_min_cov_each_strand`	Minimum coverage on either strand, where X is one of the allele types in {indel, snp, mnp, hotspot}. A filter parameter: Minimum coverage required on each strand for the type of allele to be called. **Filter reason—**PosCov or NegCov **Related VCF fields—**FSRF, FSRR, FSAF, FSAR **Allowed values—**Integers ≥0 **Suggested trial value—** ≥3
`X_strand_bias`	Maximum strand bias, where X is one of the allele types in {indel, snp, mnp, hotspot}. A filter parameter: A candidate allele of the type is filtered out if its strand bias p-value is less than `X_strand_bias_pval`, and its strand bias is greater than `X_strand_bias`. The parameter is critical for filtering out the false positive calls due to the strand-specific sequencing error. **Filter reason—**STDBIAS and STDBIASPVAL **Related VCF field—**STB **Allowed values—**Decimal numbers between 0.5 (requires perfect balance on both strands) and 1.0 (tolerates extremely strong strand bias) **Suggested trial value—**0.95

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`X_strand_bias_pval`	Maximum strand bias p-value, where X is one of the allele types in {indel, snp, mnp, hotspot}. A filter parameter: A candidate allele of the type is filtered out if its strand bias p-value is less than `X_strand_bias_pval` and its strand bias is greater than `X_strand_bias`. **Filter reason**—STDBIAS and STDBIASPVAL **Related VCF field**—STBP **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.01 for strand bias filter, 1 for no strand bias filter
`data_quality_stringency`	A filter parameter for the minimum relative read quality. A called variant needs to have a mean log-likelihood difference per read greater than this Phred-scaled value. **Filter reason**—STRINGENCY **Related VCF field**—MLLD **Allowed values**—Decimal numbers ≥0 **Suggested trial value**— ≥6.5
`filter_unusual_predictions`	A filter parameter for the maximum common signal shift. A called variant needs to have RBI less than this value. The parameter is critical for filtering out the false positive calls due to the strand-specific sequencing error. **Filter reason**—PREDICTIONSHIFTx **Related VCF fields**—RBI = sqrt(FWDB ^ 2 + REVB ^ 2) **Allowed values**—Decimal numbers ≥0 **Suggested trial value**—0.3
`X_min_var_coverage`	Minimum number of variant coverage, where X is one of the allele types in {indel, snp, mnp, and hotspot}. A filter parameter: Minimum number of variant coverage after flow-evaluation required to make the call. **Filter reason**—VarCov **Related VCF field**—FAO **Allowed values**—Integer ≥0 **Suggested trial value**—3 (TagSequencing or AmpliSeq HD), 0 (other)

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`use_fd_param`	(experimental in Torrent Suite™ Software 5.4) A filtering parameter: Use Flow Disruptiveness (FD) instead of allele types (INDEL, SNP, MNP) as the criterion to select the parameter set. If turned on, the (non-FD, weak FD, strong FD) allele applies the (INDEL, SNP, MNP) parameters, respectively. If powered on, the (non-FD, weak FD, strong FD) allele applies the (INDEL, SNP, MNP) parameters, respectively. **Allowed values**—0: do not use FD parameters, 1: use FD parameters.
`filter_insertion_predictions`	A filter parameter for the maximum reference/variant signal shit (insertions). Filter out an insertion if the observed clusters deviate from predictions more than this amount. **Filter reason**—PREDICTIONVarSHIFTx or PREDICTIONRefSHIFTx **Related VCF fields**—VARB, REFB **Allowed values**—Decimal numbers ≥0 **Suggested trial value**—0.2
`filter_deletion_predictions`	A filter parameter for the maximum reference/variant signal shift (deletions). Filter out a deletion if the observed clusters deviate from predictions more than this amount. **Filter reason**—PREDICTIONVarSHIFTx or PREDICTIONRefSHIFTx **Related VCF fields**—VARB, REFB **Allowed values**—Decimal numbers ≥0 **Suggested trial value**—0.2
`downsample_to_coverage`	Down sample to coverage. Reduce coverage in over-sampled locations to this value to save computational time. **Allowed values**—Integers ≥1 **Suggested trial value**—400 (germline), 2000 (somatic)
`min_callable_prob`	A reporting parameter Tag Sequencing and Ion AmpliSeq™ HD runs only. The minimum callable probability for the calculation of Limit Of Detection (LOD). LOD is defined to be the lowest possible allele frequency in the sample such that the variant is callable with probability greater than this value, when the molecular depth is given. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.98

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`min_fam_per_strand_cov`	An evaluation parameter for Tag Sequencing and Ion AmpliSeq™ HD runs only. Minimum required coverage of reads on each strand in a bi-directional functional molecular tag family. **Allowed values**—Integers ≥0 **Suggested trial value**—1
`min_tag_fam_size`	An evaluation parameter for Tag Sequencing and Ion AmpliSeq™ HD runs only. Minimum number of reads with same molecular tag required to form a functional molecular family. **Allowed values**—Integer ≥1 **Suggested trial value**—3
`tag_trim_method`	An evaluation parameter for Tag Sequencing and Ion AmpliSeq™ HD only. Requirement of the molecular tag of the read must match the format specified in the Planned Run. **Allowed values**—Strict-trim (requires match), sloppy-trim (does not require match) **Suggested trial value**—Sloppy-trim
`indel_func_size_offset`	An evaluation parameter for Tag Sequencing and Ion AmpliSeq™ HD runs only. requires a family size ≥ (`min_tag_fam_size` + this value) to be functional for calling HP-INDEL. **Allowed values**—Integers ≥0 **Suggested trial value**—0
`heavy_tailed`	A variant evaluation parameter: (2*heavy_tailed-1) is the degree of freedom of the t-distribution for modeling the heavy tail in signal residual distribution. **Allowed values**—Integers ≥1 **Suggested trial value**—3
`outlier_probability`	A variant evaluation parameter: probability that a read comes from none of the models under consideration. The variantCaller plugin makes NOCALL with filter reason REJECTION if FXX is too high. **Related VCF field**—FXX **Allowed values**—Decimal numbers between 0 and 1.0 **Suggested trial value**—Between 0.005 and 0.01
`prediction_precision`	A variant evaluation parameter: The number of pseudo data points suggesting our predictions match the measurements without bias. **Allowed values**—Decimal numbers ≥0.1 **Suggested trial value**—1.0

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`max_flows_to_test`	A variant candidate evaluating parameter: The maximum number of scoring flows being used. **Allowed values**—Integers >0 **Suggested trial value**—10
`suppress_recalibration`	A variant evaluation parameter: Homopolymer recalibration values should not be used when set. **Allowed values**— 0 = enable recalibration, 1 = disable recalibration **Suggested trial value**—0
`do_snp_realignment`	A variant candidate evaluating parameter: Realign reads in the vicinity of SNP candidates when set. **Related VCF content**—REALIGNEDx **Allowed values**— 0 = do not realign, 1 = realign **Suggested trial value**—0
`do_mnp_realignment`	A variant candidate evaluating parameter: Realign reads in the vicinity of MNP candidates when set. **Related VCF content**—REALIGNEDx **Allowed values**—0 = do not realign, 1 = realign **Suggested trial value**—0
`realignment_threshold`	A variant candidate evaluating parameter: Maximum allowed fraction of reads where realignment causes an alignment change. **Related VCF content**—SKIPREALIGNx **Allowed values**—Decimals between 0 and 1 **Suggested trial value**—1
`min_ratio_for_fd`	A filter parameter: Claim flow-disruption if the portion of reads that are flow-disrupted greater than or equal to this value. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.1
`indel_as_hpindel`	A filter parameter: A flag indicating whether INDEL filters or SNP filters should be applied to non-HP INDELs. **Allowed values**—0 (false), 1 (true)

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`hp_max_length`	A filter parameter: HP indels of more than this length is filtered out. **Filter reason—**HPLEN **Related VCF field—**HRUN **Allowed values—**Integers ≥1 **Suggested trial value—**8
`hp_indel_hrun`	A filter parameter: Define the HRUN for filtering HP-INDEL variants with lengths specified by `hp_del_len` and `hp_ins_len`. **Filter reason—**HPINSLEN, HPDELLEN **Related VCF field—**HRUN **Allowed values—**Vector of positive integers (for example, [1,2,3]) with size matches `hp_del_len` and `hp_ins_len` **Suggested trial value—** []
`hp_ins_len`	A filter parameter: Filter out HP-INS variants whose INS length is less than or equal to the corresponding entry of this vector if the HRUN is defined in `hp_indel_hrun`. **Filter reason—**HPINSLEN **Related VCF field—**HRUN **Allowed values—**Vector of non-negative integers (for example, [1,2,3]) with size matches `hp_del_len` and `hp_indel_hrun`. **Suggested trial value—** []
`hp_del_len`	A filter parameter: Filter out HP-DEL variants whose DEL length is less than or equal to the corresponding entry of this vector if the HRUN is defined in `hp_indel_hrun`. **Filter reason—**HPDELLEN **Related VCF field—**HRUN **Allowed values—**Vector of nonnegative integers (for example, [1,2,3]) with size matches `hp_ins_len` and `hp_indel_hrun`. **Suggested trial value—** []
`use_position_bias`	A filter parameter: Enable the position bias filter when set. **Filter reason—**POSBIAS, POSBIASPVAL **Allowed values—** 0 = disable, 1= enable **Suggested trial value—** (AmpliSeq) 1, (other) 0

Torrent Variant Caller advanced parameters and settings *(continued)*

Parameter	Description
`position_bias`	A filter parameter: Filter out a variant if the position bias is greater than `position_bias` and the position bias p-value is less than `position_bias_pval`. **Filter reason—**POSBIAS, POSBIASPVAL Related VCF field: POSBIAS **Allowed values—**Decimal numbers between 0 and 1 **Suggested trial value—**0.75
`position_bias_pval`	A filter parameter: Filter out a variant if the position bias is greater than `position_bias` and the position bias p-value is less than `position_bias_pval`. **Filter reason—**POSBIAS, POSBIASPVAL Related VCF field: POSBIASPVAL **Allowed values—**Decimal numbers between 0 and 1 **Suggested trial value—**0.05
`position_bias_ref_fraction`	A filter parameter: Skip the position bias filter if (reference read count) / (reference and alt read count) ≤ this value. **Filter reason—**POSBIAS, POSBIAS-PVAL **Allowed values—**Decimal numbers between 0 and 1 **Suggested trial value—**0.05
`error_motifs`	The file name of the error motif file
`sse_prob_threshold`	A filter parameter: Filter threshold for motif-predicted error probability. **Filter reason—**NOCALLxPredictedSSE, NOCALLxPositiveSSE, NOCALLxNegativeSSE **Related VCF fields—**SSEP, SSEN **Allowed values—**Decimal numbers between 0 and 1 **Suggested trial value—**0.02
`report_ppa`	A reporting parameter: Report Possible Polyploidy Alleles (PPA) in the VCF file and the variant calls in the XLS files. **Related VCF field—**PPA **Allowed values—** 1 = report PPA, 0 = do not report **Note:** This feature is experimental and by default is set to `report_ppa` = 0 (do not report).

FreeBayes module advanced settings

The FreeBays module advanced parameter settings control the behavior of the FreeBayes module, a module within the variantCaller plugin, which performs read filtering and generates lists of variant candidates.

IMPORTANT! The advanced parameters settings are recommended for advanced users only. If you need help setting advanced parameters, contact your local Field Service Engineer.

Parameter	Description
`allow_indels`	Candidate generation parameter: Allow INDEL candidates to be generated when set. **Allowed values**—0 = does not generate indel candidates, 1 = generates INDEL candidates **Suggested trial value**—1
`allow_snps`	Candidate generation parameter: Allow SNP candidates to be generated when set. **Allowed values**—0 = does not generate SNP candidates, 1 = generates SNP candidates **Suggested trial value**—1
`allow_mnps`	Candidate generation parameter: Allow MNP candidates to be generated when set. **Allowed values**—0 = does not generate MNP candidates, 1 = generates MNP candidates **Suggested trial value**—1
`allow_complex`	Candidate generation parameter: Allow complex variant candidates to be generated when set. **Allowed values**—0 = does not generate complex candidates, 1 = generates complex candidates **Suggested trial value**—1
`gen_min_alt_allele_freq`	A candidate generation parameter: A non-HP-INDEL candidate needs to have an allele frequency greater than this value in the pileup. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.02 to 0.15
`gen_min_indel_alt_allele_freq`	A candidate generation parameter: An HP-INDEL candidate needs to have an allele frequency greater than this value in the pileup. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.02 to 0.15

(continued)

Parameter	Description
`gen_min_coverage`	A candidate generation parameter: A variant candidate location needs to have coverage depth greater than this value. **Allowed values**—Integers ≥0 **Suggested trial value**—6
`min_mapping_qv`	A read filtering parameter: Minimum mapping quality value required for a read to be counted (for both candidate generation and variant evaluation). **Allowed values**—≥0 **Suggested trial value**—4
`read_snp_limit`	A read filtering parameter: Do not use reads that have number of SNPs above this. **Allowed values**—Integers ≥0 **Suggested trial value**—10
`read_max_mismatch_fraction`	A read filtering parameter: Ignore reads that have fraction of mismatch greater than this value. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—1.0
`min_cov_fraction`	A read filtering parameter: Do not use reads that have fraction of covering the best assigned unmerged target region below this. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.9 (TagSequending and Ion AmpliSeq™ HD), 0 (otherwise)
`read_mismatch_limit`	A read filtering parameter: Do not use reads that have number of mismatches (where 1 gap open counts 1) above this value. **Allowed values**—Integers ≥0 (0 disables the filter) **Suggested trial value**—5 (Tag Sequencing and Ion AmpliSeq™ HD), 0 (other)

Long INDEL Assembler module advanced settings

The Long INDEL Assembler module advanced parameter settings control the behavior of the Long Indel Assembler module, a module within the variantCaller plugin.

IMPORTANT! The advanced parameters settings are recommended for advanced users only. If you need help setting advanced parameters, contact your local Field Service Engineer.

Parameter	Description
`kmer_len`	Size of the smallest K-mer used in assembly. Impact—Increasing values make INDEL calls less sensitive but more specific. **Allowed values**—Integers ≥5 **Suggested trial value**—11 and 30
`min_var_freq`	Minimum frequency of the variant to be reported. Impact—Increasing values make INDEL calls less sensitive but more specific. **Allowed values**—Decimal numbers between 0 and 1 **Suggested trial value**—0.1 and 0.4
`min_var_count`	Minimum support for a variant to be evaluated. Impact—Increasing values make INDEL calls less sensitive but more specific. **Allowed values**—Integers >1 **Suggested trial value**—3 and 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**—Integers >2 **Suggested trial value**—4 and kmer_len
`min_indel_size`	Minimum size INDEL reported by assembly. Impact—Increasing values make INDEL calls less sensitive but more specific. **Allowed values**—Integers >0 **Suggested trial value**—2 and 30
`max_hp_length`	Variants that contain HP larger than this are not reported. Impact—Increasing values make INDEL calls more sensitive but less specific. **Allowed values**—Integers >1 **Suggested trial value**—2 and 11

(continued)

Parameter	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**—Decimal numbers between 0 and 1 **Suggested trial value**—0.6 and 1.0
`output_mnv`	Enables reporting of complex variants. **Allowed values**—1 = report complex variants, 0 = do not report **Suggested trial value**—0

Advanced argument settings

Parameter	Description
Torrent Variant Caller arguments	Advanced arguments for Torrent Variant Caller.
Alignment arguments	Desirable alignment arguments for variant calling purpose. Realignment of the BAM file may be triggered in the plugin if the entry is different from the alignment arguments specified in the run plan.
Unify vcf arguments	Advanced manipulation of the variant calling VCF file.
SVB BED file	Full path to a previously uploaded sequence variant baseline (SVB) BED file. For example, `/results/uploads/BED/65536/foo/unmerged`.

9 Integration with Ion Reporter™ Software

Torrent Suite™ Software can group data and transfer files from a completed run report to Ion Reporter™ Software.

About Ion Reporter™ Software

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, if an annotation-only analysis workflow is used to process the files in Ion Reporter™ Software.

To transfer these BAM and VCF output files to Ion Reporter™ Software, you must add one or more Ion Reporter™ Software accounts. You can add accounts at any time, or when you configure the IonReporterUploader plugin. After an account is configured, there are several ways that you can transfer files to an organization in Ion Reporter™ Software.

- Torrent Suite™ Software can automatically transfer files from a completed run report to Ion Reporter™ Software and either make them available:
  - As analyses in Ion Reporter™ Software. In this case, the output files are transferred to Ion Reporter™ Software and the analysis workflow of your choice for use in Ion Reporter™ Software is automatically launched on your newly transferred samples.
  - In Ion Reporter™ Software as BAM and VCF files that can be later defined as samples in Ion Reporter™ Software.
- You can optionally choose to upload data manually to Ion Reporter™ Software Use this option if, for example, you want to upload data to Ion Reporter™ Software multiple servers. To upload data manually from Torrent Suite™ Software to Ion Reporter™ Software, do one of the following:
  - Run the IonReporterUploader plugin manually from a completed run report.
  - Select the option to review results from a completed run in Torrent Suite™ Software when you create a Planned Run.

When the IonReporterUploader plugin defines samples from the newly transferred samples for Ion Reporter™ Software, sample relationships for paired and trio samples and sample attributes are also defined. For details, see “Sample gender” on page 227.

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

Install the IonReporterUploader plugin on an Ion Torrent™ Server

The IonReporterUploader plugin is installed automatically on an Ion Torrent™ Server when you update to a new release of Ion Reporter™ Software.

To update the IonReporterUploader plugin on Ion Reporter™ Software that is connected to the Internet, you can use the off-cycle plugin upgrade process. For details, see “Enable off-cycle product updates” on page 310 and “Update off-cycle release plugins” on page 312.

If you do not have an internet connection, then download and install the latest version that is named `IonReporterUploader_<version>.deb` from **http://iru.ionreporter.thermofisher.com/**.

1. Click **(Settings)** ▸ **Plugins**.
2. In the list of plugins, click **Install or Upgrade Plugin**.
3. Click **Upload a Plugin file**, then browse to and select the **IonReporterUploader.zip** file that you downloaded.
4. Click **Open**, then click **Upload and Install**.

The new IonReporterUploader plugin is added to the list of plugins in Torrent Suite™ Software.

Set up an account for IonReporterUploader plugin

Before you can transfer files to Ion Reporter™ Software with the IonReporterUploader plugin, you must configure a valid Ion Reporter™ Software account. Torrent Suite™ Software uses the account information to transfer output files to an Ion Reporter™ Software organization.

You can add more than one account for the IonReporterUploader plugin. When you add multiple accounts, any available account can be selected when you run the plugin, or when you manually upload output files to Ion Reporter™ Software. You can upload the Torrent Suite™ Software output files to any of the Ion Reporter™ Software accounts that are available in Torrent Suite™ Software.

IMPORTANT! When you upgrade to a new version of Ion Reporter™ Software, you must reconfigure the IonReporterUploader plugin with an Ion Reporter™ Software account that is set up for the new version of Ion Reporter™ Software. This account must be set up before you can access the IonReporterUploader plugin from the updated software.

When an account is set up, two email notifications are sent each time that an IonReporterUploader plugin finishes a run. The first email is sent when the plugin run starts. Another email is sent when the upload to Ion Reporter™ Software is complete. The notifications are sent to the email address of the Ion Reporter™ Software user who is signed in when the IonReporterUploader plugin is launched.

If you use Ion Reporter™ Software on Connect you must have an access code to complete this procedure. An access code is not required for Ion Reporter™ Server.

1. If you do not already have an access code for use with Ion Reporter™ Software on Connect, create an access code for use with the IonReporterUploader plugin setup. If you use Ion Reporter™ Server, proceed to Step 2.
   a. Sign in to Ion Reporter™ Software.
   b. Click **(Settings)** ▸ **Manage Tokens**.
   c. Click **Set New Access Code**, then enter an access code in the **New Access code** field.
      The access code must contain at least 6 characters. The maximum length of the access code is 50 characters.
   d. Select an expiration time in the **Access code Age** list, then click **Save and Generate**.
      The **IRU token** is used for the IonReporterUploader command-line utility. The **API token** is used internally for the Ion Reporter™ Software Web services API. Neither token is required for this procedure. For more information, see Ion Reporter™ Software system help.

   The access code that you must use to set up the IonReporterUploader plugin is shown in the **Manage Tokens** dialog box. Save this access code for use in future account setups. Alternatively, you can reset the access code.

2. In Torrent Suite™ Software, click **(Settings)** ▸ **Ion Reporter Configure**:
3. In the **Ion Reporter Uploader Account Configuration** screen, click **Add Account**, then select an account type.

Option	Selection
Ion Reporter™ Software on Connect	Select **Ion Reporter Cloud**
Ion Reporter™ Software on an Ion Reporter™ Server	Select **Ion Reporter**

4. In the **Add Ion Reporter account** screen, enter the appropriate account information.
   Ask your system administrator for values for a local Ion Reporter™ Server.

Item	Selection
Server Type	Enable HTTPS.
Display Name	Enter a name of your choice for the account. This name can be selected when you configure a Planned Run template or run the IonReporterUploader plugin manually. Use only the alphanumeric, dash, underscore, and space characters.
Server	Enter: 40.dataloader.ionreporter.iontorrent.com, or the address for your local Ion Reporter™ Server.
Port	443 is the port number that is automatically populated.
Username	Enter your Ion Reporter™ Software username (your email address).
Password	For Ion Reporter™ Software on Connect. Enter the access code. For Ion Reporter™ Server, enter the password.

5. Select one of the following account options.
    - **Default**—The account that is configured by default in the Planned Run templates and Planned Runs. If the main account is for file transfers, select the **Default** checkbox. You can change the default account after you set up a Planned Run template, or through the **Upload to IR** menu on the completed run report.
    - **Get Versions**—Select an available version of the software.

      This option is available if multiple versions of Ion Reporter™ Software are available and multiple accounts are configured.
6. Click **Add**.
7. (*Optional*) The IonReporterUploader plugin can manage multiple configurations. To add another configuration, repeat the procedure.

When at least one account is successfully configured, the IonReporterUploader plugin is ready to transfer output files to Ion Reporter™ Software. If you set up multiple accounts, the accounts are listed in the **Upload to IR** menu in the completed run report that you can use to upload output files manually to Ion Reporter™ Software. The accounts are also listed when you configure an Ion Reporter™ Software Planned Run or Planned Run template.

Automatically transfer Torrent Suite™ Software output to Ion Reporter™ Software

To transfer output files from a Torrent Suite™ Software analysis to Ion Reporter™ Software automatically, configure the IonReporterUploader plugin when you create a Planned Run.

The results files that are transferred to Ion Reporter™ Software can be:

- Automatically defined as samples Ion Reporter™ Software that are launched in an analysis workflow immediately after the instrument run is complete. Successful analyses are then available in Ion Reporter™ Software when you sign in with the account that is included in the setup.

  IMPORTANT! To set up automatic transfer, select the IonReporterUploader plugin *and* select the Ion Reporter™ Software analysis workflow in the Planned Run.
- Made available in Ion Reporter™ Software as output files (BAM and VCF files) that can later be defined as samples in Ion Reporter™ Software. In this case, you can define your samples and then launch the analysis manually in Ion Reporter™ Software. This approach is commonly used to annotate the VCF files, using the annotation-only analysis workflow in Ion Reporter™ Software.

  VCF files are available as output files when you configure the IonReporterUploader plugin. For details, see Chapter 8, "Variant calls in Torrent Suite™ Software".

For sequencing runs that use barcoded data, select the correct barcode kit under **Kits** in the workflow bar. When you select a barcode kit, a sample name field for each barcode is generated.

Use the plan by Sample Set feature when you configure Ion Reporter™ Software in your Planned Run or template. For details, see "Plan by sample set" on page 58.

1. In the **Plan** tab, click **Templates**, then select a **Research Application**, for example, **AmpliSeq DNA**.
2. Select a template that matches your panel.

   For example, if you are using an Ion AmpliSeq™ Exome Panel, select the Ion AmpliSeq™ DNA template with the same name.
3. Add samples, confirm the default settings, enter a plan name, then select **Ion Reporter** in the workflow bar.
4. Select the Ion Reporter™ Software account that you want to use by default for the transfer of output files to Ion Reporter™ Software.

   Data files are transferred to the default Ion Reporter™ Software account, unless you change it during run planning.

   IMPORTANT! If you select **None**, you must transfer files from Torrent Suite™ Software manually to Ion Reporter™ Software.
5. If the Ion Reporter™ Software account is not configured, click **Configure** to add an account.

   For more information, see "Set up an account for IonReporterUploader plugin" on page 221.
6. Select a **Sample Grouping** that corresponds to the sample relationship in Ion Reporter™ Software.

   When you select a **Sample Grouping**, the workflow bar in Ion Reporter™ Software displays only analysis workflows that match the type of analysis workflows selected.
7. Select an option in the **Existing Workflow** menu.

Option	Description
Ion Reporter workflow for your sample type	Use this option to launch automatically the analysis workflow in Ion Reporter™ Software with the sample data from the run. Successful analyses are available in Ion Reporter™ Software with the account and organization that you selected.
Upload Only	Use this option to transfer only the output files from the sequencing run to Ion Reporter™ Software. If you use this option, you can access the samples in Ion Reporter™ Software. VCF files are also available if you ran the variantCaller plugin.

8. *(Optional)* Click **Create New Workflow** to open Ion Reporter™ Software in a new browser window. In Ion Reporter™ Software, create your new analysis workflow, then save it.

   When you return to Torrent Suite™ Software, refresh the browser. You can select the newly created analysis workflow in the **Existing Workflow** menu.

9. Under **Ion Reporter Upload Options**, select an option.

Option	Description
Review results after run completion, then upload to Ion Reporter	Use this option to review the completed run report and then manually transfer the data files to Ion Reporter™ Software. IMPORTANT! After you review the results in **Completed Runs & Results**, you must click **Upload to IR ▸ Upload as Planned** to upload the data to Ion Reporter™ Software.
Automatically upload to Ion Reporter after run completion	Use this option to upload automatically results to Ion Reporter™ Software. If you select an analysis workflow, an Ion Reporter™ Software analysis is launched immediately after the run. Successful analyses are then available in Ion Reporter™ Software when you sign in with the account that is included in the setup.

10. Continue with the steps to create the Planned Run.

    For more information, see "Steps in the workflow bar" on page 39.

11. If appropriate, enter the gender of a sample.

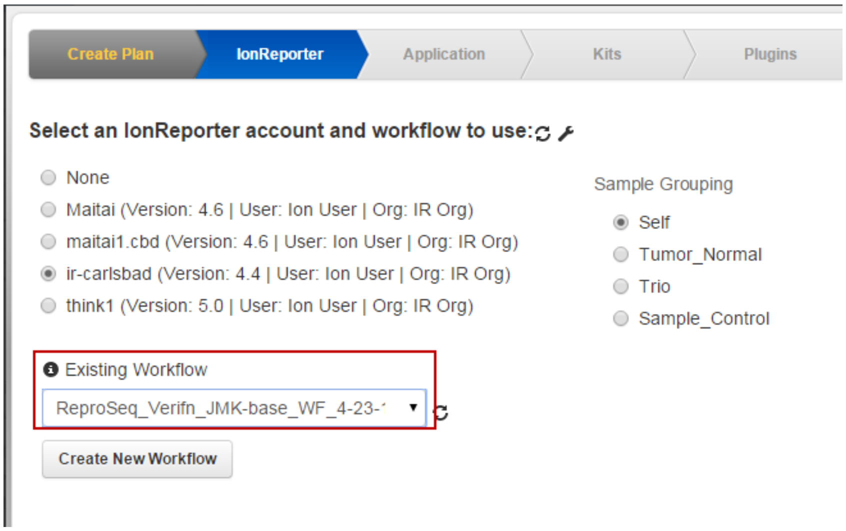
    For more information, see "Sample gender" on page 227.

12. To save the Planned Run or Planned Run template, do one of the following actions in the workflow bar:
    - Click **Save** for a new Planned Run template, enter the new template name, and optionally mark it as a favorite.
    - Click **Save & Finish** if you used Plan by Sample Set, then enter the new Planned Run name.
    - Click **Plan Run** for a new Planned Run, then enter the new run plan name and sample information.

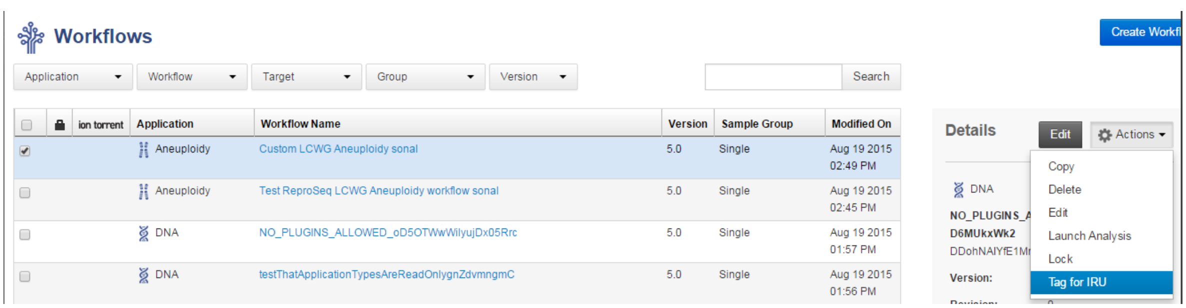
13. The Planned Run is added to the **Planned Runs** table and can be used in an instrument run.

Manage the Ion Reporter™ Software analysis workflow list

You can reduce the number of Ion Reporter™ Software analysis workflows that are listed in Torrent Suite™ Software in the IonReporter step in the workflow bar when you create a Planned Run or Planned Run template. To do so, use the **Tag for IRU** label in Ion Reporter™ Software. Only workflows that use this tag are listed when you plan instrument runs in Torrent Suite™ Software.



1. Sign in to Ion Reporter™ Software.
2. In the **Workflows** tab, click **Overview**.
3. Select an analysis workflow, then click **Actions ▸ Tag for IRU**.



The **Tag for IRU** in the **Details** section for the analysis workflow is changed to **Yes**. Only analysis workflows that include the **Tag for IRU** label are listed in Torrent Suite™ Software when you plan instrument runs.

4. To undo, select **Untag for IRU**.

Sample gender

Several analysis workflows in Ion Reporter™ Software, especially copy number variation detection and Ion AmpliSeq™ IDP trio, are limited when the sample gender is unknown, and they return unexpected results when the 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.

If a sample with no gender is transferred from Torrent Suite™ Software to Ion Reporter™ Software, you can go to the **Define Samples** step in Ion Reporter™ Software and edit the sample to specify the gender attribute.

Note: You cannot edit samples that have been launched in an Ion Reporter™ Software analysis. Instead, define new samples from the BAM or VCF data files, and add the correct gender metadata to the new samples.

Run the IonReporterUploader plugin manually

You can run the IonReporterUploader plugin manually from a completed run report in Torrent Suite™ Software. This process transfers data from a completed sequencing run to Ion Reporter™ Software. You might want to run IonReporterUploader plugin manually if after a sequencing run is completed, for example, you want to annotate only variants, and therefore upload only VCF files. This option is not available when the plugin is run from the Planned Run or run template. Instead, both BAM and VCF files are uploaded to Ion Reporter™ Software.

For barcoded runs, you can select the barcodes that you want to include in your plugin results. You can also select the barcode kit on the instrument before the run, then run the plugin manually when the run is complete. In this case, the barcode kit that you enter on the sequencer is used in the run. The barcode kit that you select on the instrument overwrites the barcode kit that is selected in the Planned Run.

When you run the plugin manually, you can select whether to upload only VCF files, only BAM files, or both VCF and BAM files.

You can also see the barcoded samples that were used in the sequencing run. You can upload any barcoded sample that includes a sample name.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
2. Click **Plugins ▸ Select Plugins to Run** link, then click **IonReporterUploader**.
   The **Configure Plugin** dialog box opens.

3. For runs that include barcoded samples, click **Barcode Sample Settings**.

   You can select one or more samples to upload to Torrent Suite™ Software. Select the checkbox for barcodes for the sample or samples that you want to upload. By default, all samples that include sample names are selected for upload.

   IMPORTANT! To upload a sample with a barcode, the barcode must include a sample name. If you select a barcode for a sample that is not named, the IonReporterUploader plugin does not upload the sample.

4. *(Optional)* To adjust speed parameters in IonReporterUploader plugin that change the rate at which files are uploaded, click **Advanced Settings**.
   a. Set the Number of Parallel Streams to **Default** (the recommended optimal speed) or select **1-5** to slow down upload.
   b. Set File Segment Size to **Default** (recommended), or **16MB**, **32MB**, **64MB**, or **128MB**.

5. In the **Upload Options** section of the **Configure Plugin** dialog box, select the file types that you want to upload: BAM, VCF, or BAM and VCF. Then click **Launch IRU** in the row next to the Ion Reporter™ Software account that you want to use for the upload.

6. Click **Yes** to confirm that you want to upload the data.

   Your upload starts. Upload times vary based on the speed of your internet connection and the size of the dataset being transferred. An email notification is sent when the upload is complete. When the upload completes, you can sign in to Ion Reporter™ Software, then launch an analysis on the new datasets.

IonReporterUploader plugin configuration

The following settings are configured when you set up accounts for the IonReporterUploader plugin. For more information, see "Set up an account for IonReporterUploader plugin" on page 221.

Item	Selection
Server Type	Enable HTTPS.
Display Name	Enter a name of your choice. This name can be selected when a run plan template is created or edited and is visible to other Torrent Suite™ Software users. Use only the alphanumeric, dash, underscore, and space characters.
Server	Enter 40.dataloader.ionreporter.iontorrent.com.
Port	Enter 443.
Username	Enter your Ion Reporter™ Software user name (your email address).
Password	For Ion Reporter™ Software on Connect. Enter the access code for use with the IonReporterUploader plugin setup. For Ion Reporter™ Server, enter the password.

(continued)

Item	Selection
Default	Enable if this account is for automatic analyses in Ion Reporter™ Software.
Version	Select the version for use with each account.

The following settings can be configured when you run the IonReporterUploader plugin manually.

You can select barcodes for the samples or samples that were used in the sequencing run. By selecting these barcodes, you can select the samples that you want to upload to Ion Reporter™ Software. For details, see "Run the IonReporterUploader plugin manually" on page 227.

Setting	Description
Barcode Sample Settings	Select the barcodes for the sample or samples used in the sequencing run that you want to upload to Ion Reporter™ Software.
Select Ion Reporter™ Software account	Select the Ion Reporter™ Software account to use to upload files to Ion Reporter™ Software
Upload Options	
BAM	Select this option to upload only BAM files.
VCF	Select this option to upload only VCF files.
BAM and VCF	Select this option to upload both BAM and VCF files.
Advanced Settings For details on these settings, see "Tune IonReporterUploader plugin speed parameters" on page 231.	
Number of Parallel Streams	Set the Number of Parallel Streams to **Default** (the recommended optimal speed) or select **1-5** to slow down upload.
File Segment Size	Set File Segment Size to **Default** (recommended), or to **16MB**, **32MB**, **64MB**, or **128MB**.

IonReporterUploader plugin file transfer progress

You can monitor the progress of the transfer of analysis results files from Torrent Suite™ Software to Ion Reporter™ Software.

Monitor file transfer progress	Details
Email	Email notifications sent for each plugin run: • When the plugin starts to transfer your files. • When the upload to Ion Reporter™ Software is complete. Email notifications are sent to the email address of the Ion Reporter™ Software user whose authentication token was used to configure the plugin.

(continued)

Monitor file transfer progress	Details
Torrent Suite™ Software	"View plugin run status" on page 125.
Log files	"Open a plugin log" on page 126.

View IonReporterUploader plugin status details

You can view a list of the run reports on which the IonReporterUploader plugin has been run, the plugin completion status, and the size of the plugin outputs.

1. Click ⚙ **(Settings)** ▸ **Plugins**.
   The installed plugins are listed.
2. In the row of the IonReporterUploader plugin, click ⚙ **(Actions)** ▸ **Usage**.
3. In the **Plugin Results** section, view the information from the list of run reports.
   - Date and time that a plugin run started and ended.
   - Status of the plugin run.
   - Size of the plugin run result output files.

Delete IonReporterUploader plugin report files

You can delete IonReporterUploader plugin report files from Torrent Suite™ Software.

IMPORTANT! This action permanently deletes the IonReporterUploader plugin report for a run and cannot be undone.

1. Click ⚙ **(Settings** ▸ **Plugins**.
   The installed plugins are listed.
2. For the IonReporterUploader plugin, click ⚙ **(Settings)** ▸ **Usage**.
3. Click **Delete** on the row for the plugin report that contains the files that you want to delete.

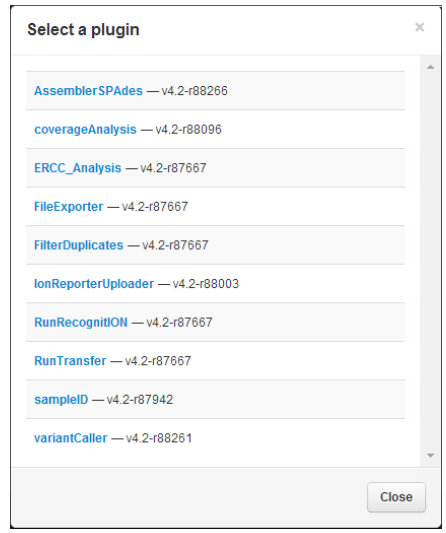
The output files for the plugin report are deleted.

Tune IonReporterUploader plugin speed parameters

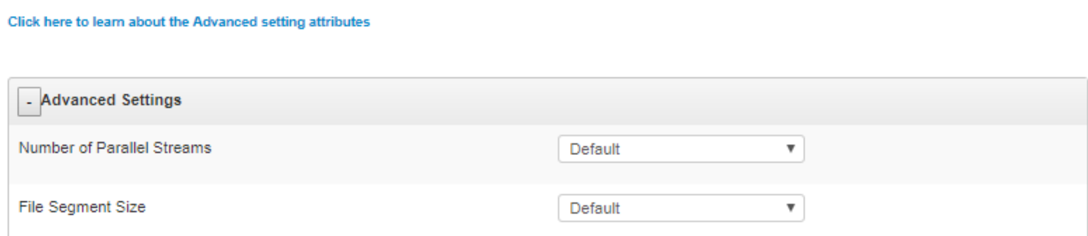
You can adjust speed parameters for the IonReporterUploader plugin to change the rate at which files are uploaded.

Update these settings only if file transfers from IonReporterUploader plugin are difficult or slow with the default settings.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Click **Plugins ▸ Select Plugins to Run**, then select **IonReporterUploader**.



3. Click **Advanced Settings**.
    a. Set the Number of Parallel Streams to **Default** (the recommended optimal speed) or select **1-5** to slow down upload.

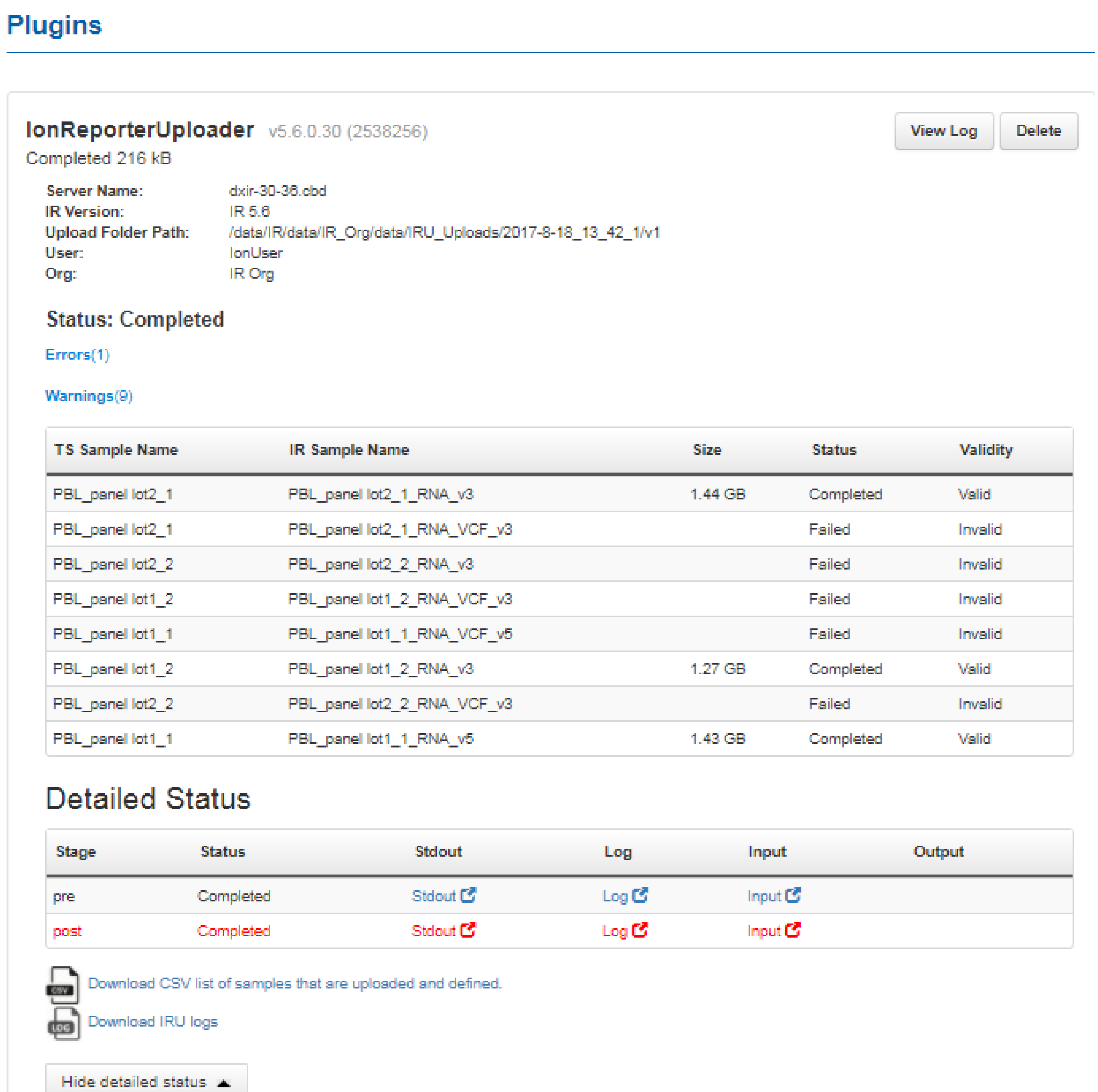


    b. Set **File Segment Size** to **Default** (recommended), or **16MB**, **32MB**, **64MB**, or **128MB**.

Review IonReporterUploader plugin results

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **IonReporterUploader** to view the plugin results.

4. In the IonReporterUploader plugin report, view information that is related to the data transfer. Information includes the name of the Ion Reporter™ Server used, the version of Ion Reporter™ Software that is on the server, the server directory that contains the uploaded files, and the Ion Reporter™ Software organization and user account that was used. You can also review details about barcoded samples that were uploaded with the IonReporterUploader plugin, or details about barcoded samples that failed to upload.



5. *(Optional)* Click an active link to see more information or to download files.

To do this	Click
View error messages associated with the plugin run.	**Errors**
View warnings that contain details about the barcoded samples that are used in the plugin run.	**Warnings**
Show or hide a detailed status of the pre- and post-processing of the data transfer.	**Show detailed status/Hide detailed status**
Open a report about the data transfer in a separate browser tab.	**Stdout**
Open the plugin log files in a separate browser tab.	**Log**
Open a `startplugin.json` file that contains metadata used by the plugin.	**Input**
Download a CSV file that contains a list of the uploaded and defined samples.	**Download CSV list of samples that are uploaded and defined**
Download a compressed directory of the IonReporterUploader plugin log files and other plugin files	**Download IRU logs**

Torrent Suite™ Software output and Ion Reporter™ Software analysis phases

The BAM file output of your Torrent Suite™ Software analysis is typically uploaded to Ion Reporter™ Software, and then the software runs through major analysis phases.

1. Mapping
2. Variant calling
3. Annotation

The following table describes how Torrent Suite™ Software output files are used in Ion Reporter™ Software analyses.

Torrent Suite™ Software output file	Output from this Torrent Suite™ Software analysis phase	Input to this Torrent Suite™ Software workflow
BAM file	Torrent Suite™ Software analysis pipeline	Any except annotation-only
VCF file	Torrent Suite™ Software variantCaller plugin	Annotation-only

The IonReporterUploader plugin by default uploads both the BAM file and the VCF file from the Ion Torrent™ Server to Torrent Suite™ Software.

The following table describes the input and output file types for the analysis phases.

Analysis phase	Input file type	Output file type
Mapping	BAM file (mapped or unmapped)	Mapped BAM file
Variant calling	Mapped BAM file	VCF file
Annotation	VCF file (with or without annotations)	Annotated VCF file

Each output file type is required as input to the next analysis phase. In almost all cases, the Ion Reporter™ Software analysis phases are performed in order.

The exception is the annotation phase. The annotation-only analysis workflow runs this phase by itself. (All other analysis workflows include the annotation phase as their last analysis phase.) The annotation-only analysis workflow requires as input a VCF file, which can be generated from either an Ion Reporter™ Software analysis, a variantCaller plugin analysis, or a different source.

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 use the IonReporterUploader command-line utility to transfer results files to Ion Reporter™ Software and to define samples, based on the transferred files. The IonReporterUploader command-line utility uses your sign in 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
- 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. You can enter IonReporterUploader command-line utility options through command line arguments, or through a properties file.

CAUTION! 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.

The IonReporterUploader command-line utility can be run on any of the following:

- An Ion Torrent™ Server.
- A standard Linux™ computer.
- A standard Windows™ computer that uses the Windows™ XP operating system or later.
- A standard Macintosh™ computer.

IonReporterUploader command-line utility supports the upload of combined Ion Reporter™ Software analysis results that are output by the **Combine Alignments** option in the Torrent Suite™ Software Projects tab. The IonReporterUploader plugin does not support uploading these files.

Download IonReporterUploader command-line utility

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

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

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.

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1. Download the IonReporterUploader command-line utility at: **http://iru.ionreporter.thermofisher.com/**.

   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.

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` folder to a convenient location on the target computer.

Run IonReporterUploader command-line utility

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

Instructions to use the IonReporterUploader command-line utility are downloaded with the utility. For more information, see the *IonReporterUploader Command-Line Utility User Guide* (Pub. No MAN0017648.

10 References management

About references

Torrent Suite™ Software provides access to references for use in your sequencing runs. References are available for reference genome sequences, barcode sets, test fragments, and other files that filter or restrict genomic sequencing and analysis to the regions of interest. Before you can use some of these files in sequencing runs, you must upload or import the files to the Ion Torrent™ Server that is connected to the sequencing instrument.

References are accessed in Torrent Suite™ Software from **(Settings)** ▸ **Reference Sequences**. Options are listed in the left navigation menu.

Reference type	Description
Reference sequences	Import preloaded Torrent Suite™ Software validated reference genome sequences, or a custom reference genome to add them to Planned Runs and Planned Run templates.
Obsolete reference sequences	Reference sequences become obsolete and are listed in the **Obsolete References Sequences** screen after Torrent Suite™ Software is updated with a release that includes a new TMAP index.
Target regions	Upload these files to add them to your Planned Runs.
Hotspot Sets	Upload hotspots files to add them to your Planned Runs. The variantCaller plugin generates output files that include these positions regardless of whether a variant is called, and include evidence for a variant and the filtering thresholds that disqualified a variant candidate.
Test fragments	Use these known sequences to monitor system characteristics.
Barcodes	Upload Ion barcode sets or your own custom barcodes sets for use in sequencing runs.
Upload history	Review records of recent uploads of target regions, hotspots, and ZIP files from **AmpliSeq.com**.

Reference sequences

To identify genetic variations within a nucleic acid sample, sequencing reads are aligned to a reference genome sequence. Torrent Suite™ Software comes preloaded with reference genome files that contain genomic sequences that represent several species, including two commonly used human genome references—hg19 and GRCh38. You can also import a custom reference file to use for your sequence analysis.

As part of the standard analysis process in Torrent Suite™ Software, reads are aligned to a genomic reference that uses the TMAP aligner that comes preinstalled on the Ion Torrent™ Server. For more information, see "TMAP modules" on page 292.

hg19 reference

The Human Genome version 19 (hg19) reference is a human genome reference that is based on the Genome Reference Consortium's human genome assembly version 37 with patch 5 (GRCh37.p5), and its equivalent UCSC hg19 reference. For more information, see the Genome Reference Consortium's website: **ncbi.nlm.nih.gov/grc/human/data**. Several notable differences exist between the hg19 reference in Torrent Suite™ Software and the GRCh37.p5/UCSC hg19 reference sequences:

- The Y chromosome PAR regions in the hg19 reference are hard masked with `'N'`. The GRCh37.p5 reference Y chromosome PAR regions are unmasked.
- Three nucleotide positions that are masked with `'N'` in the GRCh37/UCSC hg19 reference have IUPAC ambiguity codes in hg19 reference sequence.
- The hg19 reference in Torrent Suite™ Software uses the Revised Cambridge Reference Sequence (rCRS) for chromosome M. UCSC hg19 uses the original chromosome M sequence.

For more information, see **genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**. To download sequence and annotation data, go to the Genome Browser FTP server at **hgdownload.soe.ucsc.edu/goldenPath/hg19** or go to the **Downloads** screen at **hgdownload.soe.ucsc.edu/downloads.html#human**.

Hard masked PAR regions in chromosome Y

The mammalian Y chromosome contains regions that are identical to the X chromosome called pseudoautosomal regions (PARs). These regions allow for recombination between the sex chromosomes. When the human Y chromosome was sequenced and assembled, the PAR regions were not sequenced, and therefore were not included in the assembly. Instead, the corresponding sections from the X chromosome sequence were copied onto the Y chromosome. This sequence duplication must be considered when sequence analysis is performed by the software so that allelic duplication can be distinguished from other types of duplications such as repeats and segmental duplication.

When the female DNA sample is sequenced, reads from the PAR regions align to both the X and the Y PAR sequences. This alignment affects the mapping quality of the reads in these regions and creates problems with variant calling on the gender chromosomes. For this reason, the PAR sequence on the Y chromosome is replaced with `'N'`, or "hard masked", in the hg19 reference. In the GRCh37 reference, the PAR sequence is unmasked. Hard masking the PAR sequence on the Y chromosome preserves the PAR coordinates on the Y chromosome and eliminates the duplication at this locus. The Y chromosome in the hg19 assembly contains two PAR regions that are taken from the corresponding regions in the X chromosome and have identical DNA sequences.

Chromosome Y PAR coordinates	Corresponding chromosome X PAR coordinates
10,001–2,649,520	60,001–2,699,520
59,034,050–59,363,566	154,931,044–155,260,560

Three positions with ambiguity codes

Three positions on chromosome 3 are masked with 'N' in the UCSC version of the hg19/GRCh37 reference genome. These positions have IUPAC ambiguity codes in our hg19 version:

- M—IUPAC code for A or C nucleotides
- P—IUPAC code for A or G nucleotides

Position	IUPAC ambiguity code in hg19 reference	Hard masked character in UCSC hg19
60830534	M	N
60830763	R	N
60830764	R	N

Chromosome M reference sequence

The hg19 reference in Torrent Suite™ Software uses the Revised Cambridge Reference Sequence (rCRS) for the Homo sapiens mitochondrial sequence (chromosome M or chrM) – GenBank accession number NC_012920. UCSC hg19 reference uses the original chromosome M sequence – GenBank accession number NC_001807. UCSC begins using the rCRS for chromosome M in the GRCh38 (hg38) assembly.

The following background information is from the UCSC site **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**.

"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."*

GRCh38 reference

The Genome Reference Consortium human genome assembly version 38 (GRCh38) is the latest version of the GRC human genome reference. The GRCh38 assembly is referred to as "hg38" in the UCSC Genome Browser and includes the following updates to the GRCh37/UCSC hg19 version:

- Alternate sequences for highly variable genes
- Centromere representation
- Sequence updates such as fixed errors, filled gaps, and changes to chromosome coordinates
- Updated mitochondrial genome sequence (GenBank accession number NC_012920.1)

- Hard masked PAR regions in chromosome Y

Chromosome Y PAR coordinates	Corresponding chromosome X PAR coordinates
10,000–2,781,479	10,000–2,781,479
56,887,902–57,217,415	155,701,382–156,030,895

For more information, see **genome.ucsc.edu/cgi-bin/hgGateway?db=hg38**.

To download sequence and annotation data, go to Genome Browser FTP server at **hgdownload.soe.ucsc.edu/goldenPath/hg38/** or go to the **Downloads** screen at **hgdownload.soe.ucsc.edu/downloads.html#human**.

Import reference sequence files

Torrent Suite™ Software includes validated reference sequence files that you can download onto your Ion Torrent™ Server. You can also import custom reference sequence files from your local storage or a remote server. After you import the reference sequence files onto a Ion Torrent™ Server, the reference sequences are available for use when you set up Planned Runs and when the software performs data analysis.

Guidelines for importing a custom reference sequence file

The following guidelines are for preparing a new custom reference genome sequence file to be imported into Torrent Suite™ Software.

- Download a FASTA format reference genome file and save it to your local storage. FASTA files can be found at **ncbi.nlm.nih.gov/genome**.
- The file selected for import must have a `.fasta` extension.
- When working with larger genomes, performance improves if you compress the FASTA file. Torrent Suite™ Software supports ZIP and GZIP archive file formats, provided each archive file contains one FASTA file.
- The variantCaller plugin does not support IUPAC base codes other than A, C, T, G, and N. When the software uploads a genome that contains other IUPAC characters, each such character is replaced with N.
- Prepare any related target regions, hotspots, and reference annotation files to upload with the reference genome file, then save them to your local storage. For more information, see “Target regions and hotspots files” on page 245 and “Import reference sequence files” on page 239.

Import a preloaded reference sequence file

Before you can use the reference files in Planned Runs, you must import the reference files into the software.

Validated reference sequence genome files suitable for use with Torrent Suite™ Software are preloaded on the Ion Torrent™ Server.

You can also edit the reference genome information, or permanently delete the reference file from the software. For more information, see "View a reference sequence file" on page 241.

IMPORTANT! If you edit or delete a reference sequence file, the change affects all users.

1. Click (Settings) ▸ **Reference Sequences**.
2. Click **Import Preloaded Reference Sequences**.
   The **Ion References** and **Downloads** lists are displayed.
3. In the **Ion References** list, find the file of interest, then complete the selections based on the reference type.

Option	Selection
Reference file	Click **Import** in the row of the reference file to import.
BED file	a. Click **Import BED Files**. b. In the **Import BED Files** dialog box, select the items that you want to import, then click **Import Selected**.

   The file import status appears in the row of the selected file in the **Ion References** list. When the status is **complete**, a compressed folder in ZIP file format is added to the **Downloads** list and the reference genome is added to the **Reference Sequences** table. You can now use the reference genome in a Planned Run.

Import a custom reference sequence file

You can use a custom reference genome sequence in a Planned Run and data analysis. Import a custom reference FASTA file into the Torrent Suite™ Software from your local storage or from a remote server location.

IMPORTANT! First-time users should review custom reference file rules and restrictions to avoid errors. For more information, see "Guidelines for importing a custom reference sequence file" on page 239.

1. Click (Settings) ▸ **Reference Sequences**.
2. Click **Import Custom Reference**.

3. In the **Add New Reference Genome** dialog box, upload a FASTA file using one of these methods.
   - Upload a FASTA file from your local storage.
     a. Select the **Upload File** tab.
     b. Click **Select File**.
     c. Navigate to and select the file from your local storage, then click **Open**.
   - Upload a FASTA file from a remote server.
     a. Select the **Install via URL** tab.
     b. In **Reference URL**, enter the file path to the reference file.
4. Complete the required information. This information is used in various report outputs and in the **Reference Sequences** table.

Item	Description
Short name	Enter a recognizable short form of the genome name. Make sure that the short name does not repeat with other reference genome files. You can delete any reference genome that you do not use to allow the short name to be used again for a new reference genome file. Use any alphanumeric character and underscore (_).
Description	Enter a longer, more descriptive reference genome name. The description usually includes the genus, species, version, and other identifying reference genome information.
Version	Enter the genome version number and the accession number, if there is one (for example, "hg19", "gi|39933080|NC_005296.1").
Notes	Record optional notes about the reference genome.

5. Click **Import Reference**.
   The reference genome and associated information is added to the **Reference Sequences** table.

After the reference genome file is imported, you can view and edit the reference genome information, or delete the reference genome file. For more information, see "View a reference sequence file" on page 241. If you encounter errors during file upload, see "Troubleshooting file import/upload errors" on page 348.

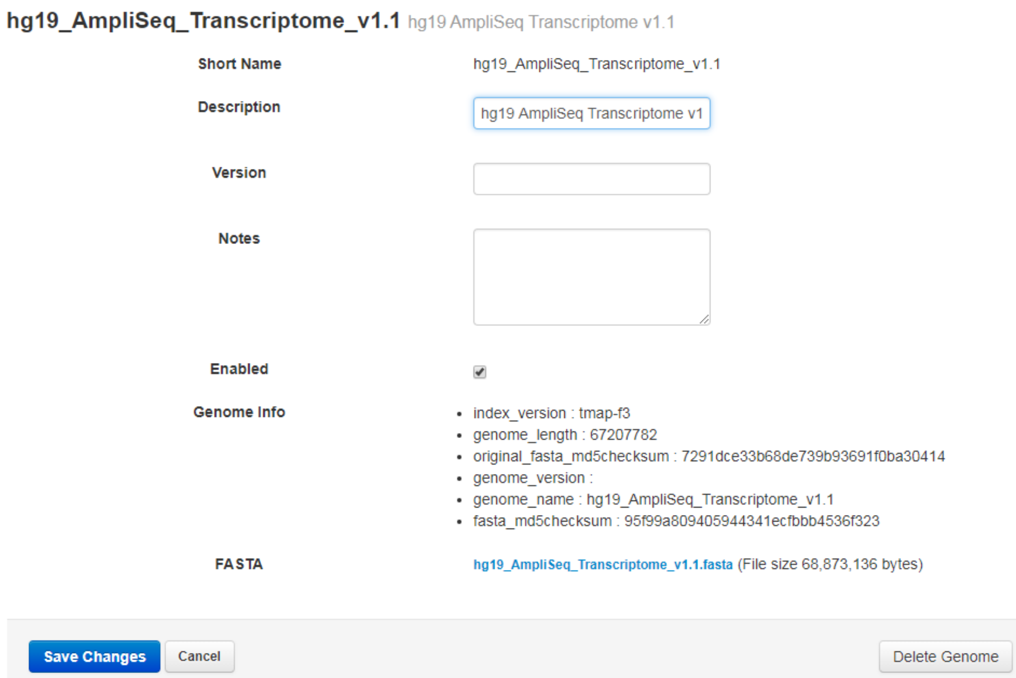
View a reference sequence file

After you import a reference sequence file into Torrent Suite™ Software, you can view the file properties and review the reference sequence in the FASTA format.

For instructions about how to import the file into Torrent Suite™ Software , see "Import reference sequence files" on page 239.

1. Click **(Settings)** ▸ **Reference Sequences**.
2. In the **Reference Sequences** screen, do one of the following to access the reference sequence file properties screen.
   - In the **Reference Sequences** table, in the **Short Name** column, click the reference sequence file name.
   - Click **Import Preloaded Reference Sequences**, then click **complete** in the row of the imported Ion reference file.

3. In the file properties screen, click the FASTA file link to view the FASTA format reference sequence in the browser window.



Edit reference sequence file properties

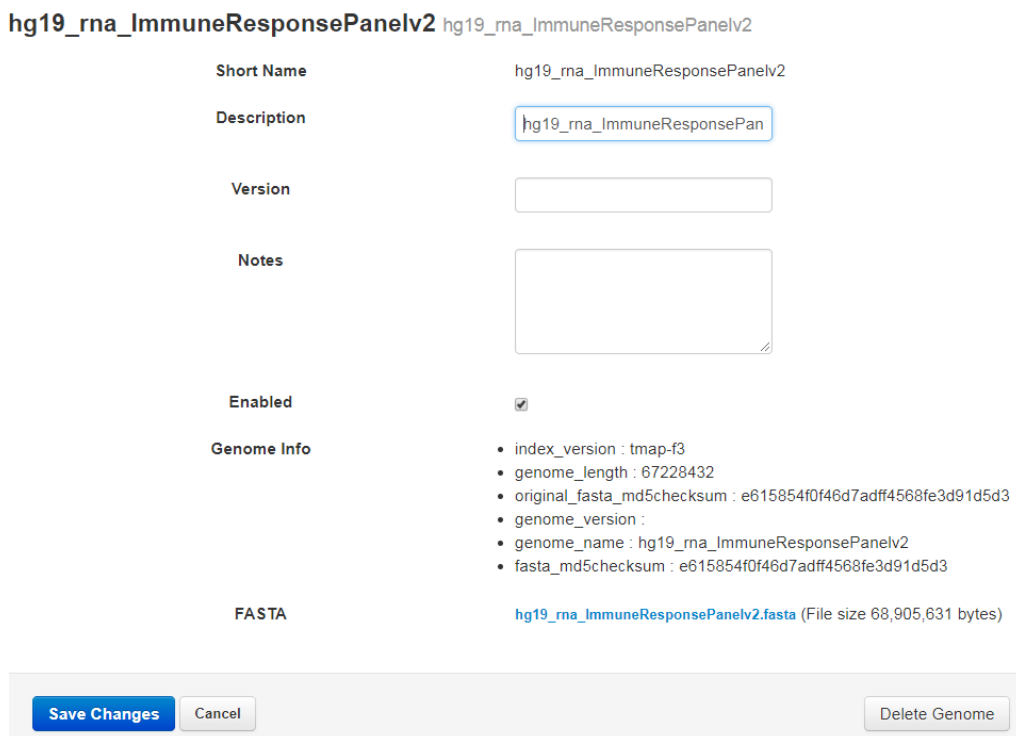
After you import a reference sequence file into Torrent Suite™ Software, you can edit the file properties.

To edit the reference sequence file properties, you must first import the file into Torrent Suite™ Software. For more information, see “Import reference sequence files” on page 239.

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**.

2. In the **Reference Sequences** table, in the **Short Name** column, click the reference sequence file name.

3. In the file properties screen, edit the file properties.



Only these file properties can be edited.

Property	Description
Description	Description of the reference.
Version	Version number or letter of the reference.
Notes	Any meaningful details about the reference.
Enabled	References are enabled by default. To disable a reference that is not being used anymore, deselect **Enabled**.

You cannot edit **Short Name**. To change or reuse the reference sequence file short name, click **Delete Genome** to delete the existing reference sequence file from the Ion Torrent™ Server, then upload a new file.

4. Click **Save**.

Permanently delete a reference sequence file

After you import a reference sequence file using Torrent Suite™ Software, you can permanently delete the file from the Ion Torrent™ Server. Deleting a reference sequence file is useful if you want to upload a new file using the same **Short Name** as an existing file, or if you no longer require the reference for your Planned Runs.

IMPORTANT! Recovery of a deleted reference sequence is not possible.

1. Click **(Settings)** ▸ **Reference Sequences**.
2. In the **Reference Sequences** table, in the **Short Name** column, click the reference sequence file name.
3. In the file properties screen, click **Delete Genome** ▸ **Yes, Delete It**.
   The reference is removed from the **Reference Sequences** table and is permanently deleted from the Ion Torrent™ Server.

Obsolete reference sequences

View obsolete reference sequences in the **Reference Sequences** screen. A reference sequence becomes obsolete and is listed in the **Obsolete References Sequences** table after Torrent Suite™ Software is upgraded with a release that includes a new TMAP index.

The software records the libraries that are installed before the upgrade, then creates the list of obsolete reference sequences. The list is based on the reference sequences that you use.

Obsolete Reference Sequences

Name	Description	Notes	Date ▾	Index version	Status
S_aureus	S_aureus	Added by Steven Lee	1969/12/31 04:00 PM		
Pseudomonas_aerugin...	P_aeruginosa	Added by Steven Lee	1969/12/31 04:00 PM		
hg19_old	Homo sapiens		1969/12/31 04:00 PM	tmap-f2	complete
CFTR_38amp_v2	CFTR_38amp_v2		1969/12/31 04:00 PM	tmap-f2	complete
e_coli_k12	E. coli K-12 MG1655		1969/12/31 04:00 PM	tmap-f2	complete
rhodopalu	Rhodopseudomonas palustris CGA009 chromosome		1969/12/31 04:00 PM	tmap-f2	complete
e_coli_dh10b	E. coli DH10B		1969/12/31 04:00 PM	tmap-f2	error

Target regions and hotspots files

Target regions and hotspots Browser Extensible Data (BED) files supply chromosome positions or regions as reference information during the analysis of a sequencing run. These BED files, when applied to a reference sequence in a Planned Run, or in a variantCaller plugin configuration, perform two functions:

- Target regions files narrow the analysis to your regions of interest, for example, regions that are amplified with primer panels in targeted sequencing. When a target regions file is selected in a Planned Run, the complete Torrent Suite™ Software analysis pipeline, including plugins, is restricted to the regions of the reference sequence specified in that file. Target regions files use only BED file format.
- Hotspots files instruct the variantCaller plugin to include loci known to be frequently altered in its output files, including evidence for a variant and the filtering thresholds that disqualify a variant candidate. A hotspots file affects only the variantCaller plugin, not other parts of the analysis pipeline. A hotspots file is usually a BED file, or it can be a Variant Call Format (VCF) file that is generated from a BED file by Torrent Suite™ Software during a sequencing run.

Target regions and hotspot files are listed on screens that are accessed from the **Reference Sequences** link. When a target regions or hotspots file is uploaded to Torrent Suite™ Software, it is assigned a specific reference sequence, and is then available for use when that reference sequence is selected during run planning.

Guidelines for using target regions and hotspots files

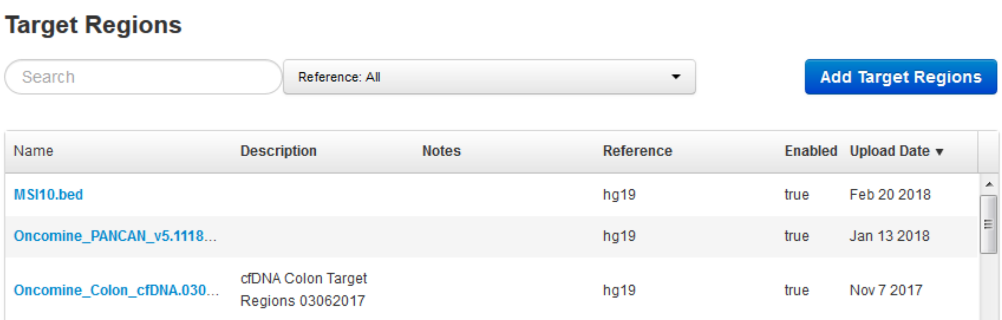
- Target regions BED files provide an option to restrict analysis to regions of interest. Do not specify a target regions BED file in the **Plan** step of the Planned Run workflow if you want variant analysis to span an entire genome. Use the whole-genome application with a reference sequence to support whole-genome analysis.
- All regions that are included in the target regions BED file that you select are analyzed. Before you upload your target regions BED file, follow the instructions in "Modify a BED file" on page 248 to delete lines representing regions containing variants that you do not want to call.
- The BED file coordinates (for example: chr2 29443689 29443741) use zero-based indexing and a half-open interval. The start position is included, and the range extends up to, but not including, the end position.
- BED files that are used with Ion AmpliSeq™ panels define only the internal segment, and do not include the primer sequence.
- A BED or VCF file is tied to a specific reference sequence. The coordinates in a BED or VCF file must match coordinates and the coordinate sorting in the reference sequence. Torrent Suite™ Software reference sequences are sorted alpha-numerically (not by a chromosome sort). The BED files and VCF files that you use with references must also use an alpha-numeric sort. If you upload your own reference genome sequence, the BED and VCF files that you use with that reference must be sorted by the same method as your reference file.
- The variantCaller plugin calls variant candidates at hotspot positions with higher sensitivity than candidates at other positions. You can customize specific variantCaller parameters separately for hotspot candidates.
- Torrent Suite™ Software accepts VCF files as hotspots files, but the VCF file must be generated by the variantCaller plugin from a hotspots BED file.

- Target regions and hotspots files for use with Ion AmpliSeq™ panels can be downloaded with panel files from **AmpliSeq.com**.
- If you need Oncomine™ panel target regions and hotspots files, contact your local Field Service Engineer.

View and manage target regions files

You can upload target regions files for use with a specific reference sequence. After target regions files are uploaded, the files are available when the related reference sequence is selected for use in a Planned Run. When you select a target regions file in run planning, the sequencing run results are restricted to the regions of the reference sequence that is specified in the target regions file.

1. To view the target regions files that are available on the Ion Torrent™ Server, click **(Settings)** ▸ **Reference Sequences**.
2. In the left navigation pane, click **Target Regions**.
   The target regions files that are available are listed in the **Target Regions** table.



3. In **Search**, enter a search term, or select a target region from **Reference** to filter the list.
4. Click a target regions file name to open a **Target Regions Details** screen to view information about the selected target region.
   The information that is available depends on the selected target region. Scroll to view original upload information.

File information	Target regions detail
Processed File	The name of the file. Click to download the BED file to view on your computer.
Reference	The reference genome species.
Number of Targets	The number of target regions included in the file.
Number of Genes	The number of genes included in the target regions file.
Covered Bases	The number of bases covered by the target regions file.
Description	A short description of the file.
Notes	Additional information about the target regions file.
Enabled	Select the checkbox to enable use of the file in a Planned Run. Deselect the checkbox to prevent use of the file in a Planned Run.

5. Enter additional information, if needed, then click **Save**.

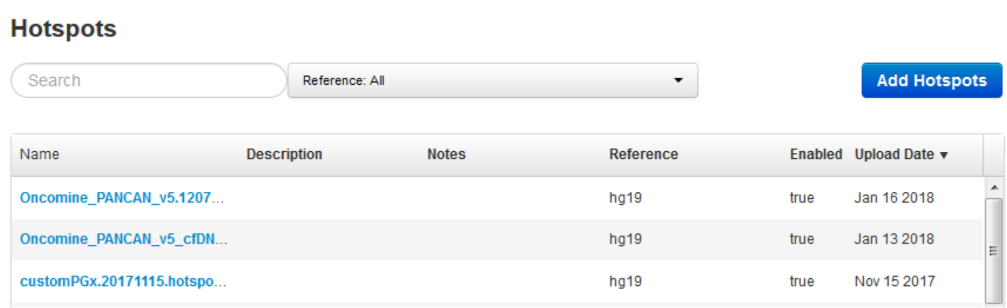
6. *(Optional)* To delete a target regions file from the Ion Torrent™ Server, click **Delete**.

View and manage hotspots files

You can upload hotspots files for use with a specific reference sequence. After hotspot files are uploaded, the files are available when that reference sequence is selected for use in a Planned Run or variantCaller plugin configuration. When you select a hotspots file in run planning, the file instructs the variantCaller plugin to report on loci, which is known to be frequently altered, in its output files.

1. To view the hotspots files that are available on the Ion Torrent™ Server, click **(Settings)** ▸ **Reference Sequences**.

2. In the left navigation menu, click **Hotspots**. Available hotspots files are listed in the **Hotspots** table.



3. In **Search**, enter a search term, or select a hotspot from **Reference** to filter the list.

4. Click the name of a hotspots file to open a **Hotspots Details** screen to view information about the selected Hotspot.

   The information that is available depends on the selected target region. Scroll to view original upload information.

File information	Hotspots detail
Processed File	The file name. Click to download the BED file to view on your computer.
Reference	The reference genome species.
Number of Loci	The number of hotspot alleles included in the hotspot file.
Description	A short description of the file.
Notes	Additional information about the hotspot file.
Enabled	Select the checkbox to enable use of the file in a Planned Run. Deselect the checkbox to prevent use of the file in a Planned Run.

5. Enter any additional information if needed, then click **Save**.

6. *(Optional)* To delete a hotspots file from Torrent Suite™ Software, click **Delete**.

Modify a BED file

You can modify an existing target regions or hotspots BED file, if necessary, to remove regions from the file for which you do not want variants called.

If you modify a BED file, you must modify it *before* it is uploaded to Torrent Suite™ Software. A target regions or hotspots BED file cannot be modified after the file is uploaded.

To modify a BED file:

1. Copy the BED file, then rename the new file in a way that reflects changes you make to the regions being analyzed.
2. Open the BED file with a text editor.
3. Delete the lines for regions you do not want.
4. Save the file.
5. Upload the modified file as described in "Upload a target regions file" or "Upload a hotspots file".

Note:
- If the region (or regions) appears in both your target regions BED file and in your hotspots BED file, you must delete the line for those regions from both types of BED file.
- Do not modify a VCF hotspots file. We recommend that you upload it first to convert it to a BED file before modifying it.

Upload a target regions file

You can upload a target regions BED file from your computer to Torrent Suite™ Software to use with a reference sequence.

Target regions files are only BED files. Supported file extensions are .bed, .zip, and .bed.gz. During file upload, the software validates the BED file, then confirms that the coordinate regions of the BED file are valid for the selected reference genome. The new BED file is then available as an option when you create a Planned Run.

IMPORTANT! You must upload target regions files that match both the reference sequence and the reference sequence version. The uploader cannot always detect mismatch errors. It is your responsibility to avoid the following uploading errors:

- Uploading a BED file for a reference sequence of a different version (for example, an hg18 BED with an hg19 reference).
- Uploading a BED file for a different species.
- Uploading a hotspots BED file as a target regions BED file.

---

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Target Regions** in the left navigation menu.

2. In the **Target Regions** screen, click **Add Target Regions**.

3. In the **New Target Regions** screen, click **Select File**, then navigate to the file to be uploaded.

4. Select the reference sequence from the **Reference** list.

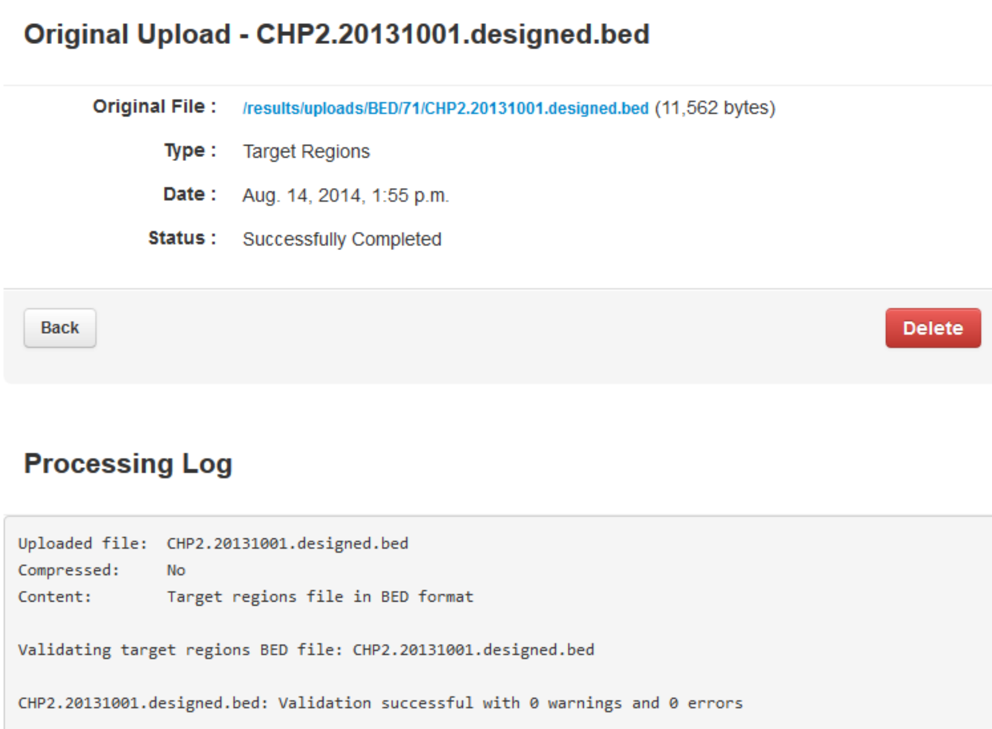
   IMPORTANT!
   - Be careful to select the correct reference sequence because the new target regions file can be used only with this reference.
   - The reference sequence must be uploaded and available for selection before a target regions file can be uploaded.

   ---

5. *(Optional)* Add a description and notes.

6. Click **Upload Target Regions File**.

   Wait while the file is validated. The status updates to **Successfully Completed** after the upload finishes. Errors are reported in the **Processing Log** pane.



   For large files, validation can take several minutes. Refresh your browser to check that validation is complete.

The new file appears in the **Target Regions** list in the **Plan** step of the Planned Run workflow bar, and in the **Target Regions** table in the **References** tab.

Upload a hotspots file

You can upload a hotspots file from your computer to Torrent Suite™ Software to use with a reference sequence.

Hotspots files can be BED or VCF files. Supported file extensions are .bed, .vcf.gz, .zip, .bed.gz, and .vcf.gz. During file upload, the software validates the BED or VCF file, then confirms that the coordinate regions of the file are valid for the selected reference sequence. The new BED file is then available as an option when you create a Planned Run or configure the variantCaller plugin.

IMPORTANT! Upload BED or VCF files that match both the reference sequence and the reference sequence version. The uploader cannot always detect mismatch errors. It is your responsibility to avoid the following uploading errors:

- Uploading a BED or VCF file to a reference sequence of a different version (for example, an hg18 BED file with an hg19 reference).
- Uploading a BED or VCF file for a different species.
- Uploading a target regions BED file as a hotspots BED file.
- Uploading a hotspots file listing loci not included in a target regions file.

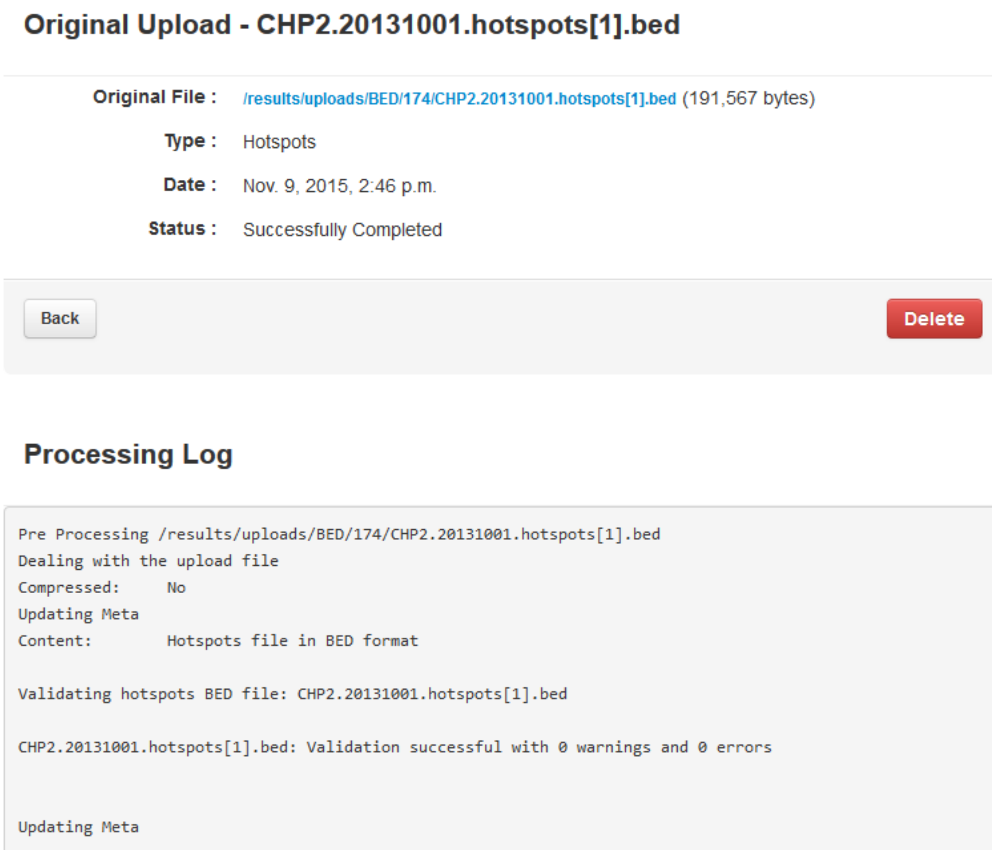
---

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Hotspots** in the left navigation menu.
2. In the **Hotspots** screen, click **Add Hotspots**.
3. In the **New Hotspots** screen, click **Select File**, then navigate to the file to be uploaded.
4. Select the reference sequence from the **Reference** list.

   IMPORTANT!
   - Ensure that you select the correct reference sequence because the new hotspots file can be used only with this reference.
   - The reference sequence must be uploaded and available for selection before a hotspots file can be uploaded.

   ---
5. *(Optional)* Add a description and notes.
6. Click **Upload Hotspots File**.

   Wait while the file is validated. The status updates to **Successfully Completed** after the upload finishes. Errors are reported in the **Processing Log** pane.



Note:
- For large files, validation can take a few minutes. Refresh your browser to check that validation is complete.
- If you selected a VCF file for upload, the software validates it and converts it to a BED file.

The new file appears in the **Hotspots** list in the **Plan** step of the Planned Run workflow bar, and in the **Hotspots** table in the **References** tab.

BED file formats and examples

The Browser Extensible Display (BED) file format is used for both target regions files and hotspot files. BED files are text files with tab-separated fields.

Torrent Suite™ Software also accepts the Variant Call Format (VCF) for hotspot files.

Target regions BED file formats

Target regions files use a BED file format in 3-column, 4-column, 6-column, and 8-column formats.

3-column target regions BED file format

The 3-column target regions BED file format is used when amplicon IDs and gene names are not known.

The track line is optional. If present, it includes these tab-separated fields.

Field	Type	Description
`name`	String	A unique design identifier. Optional.
`description`	String	A description of the design. Optional.

The following is an example of how to enter an optional track line.

```
track name="ASD270245" description="AmpliSeq Pool ASD270245"
```

In a 3-column target regions BED file, the coordinates lines require the following tab-separated fields.

Field	Type	Description
`chrom`	String (chars ≥0 × 20, other than \tab)	The name of the chromosome. This name must be an exact match with a chromosome in the reference.
`chromStart`	Unsigned int64	The starting position of the feature (zero-based).
`chromEnd`	Unsigned int64	The ending position of the feature (not inclusive). Must be greater than `chromStart`.

The following is a partial example of a 3-column target regions BED file.

```
chr9 133738312 133738379
chr9 133747484 133747542
chr9 133748242 133748296
chr9 133748388 133748452
chr9 133750331 133750405
chr9 133738312 133738379
chr9 133747484 133747542
chr9 133748242 133748296
chr9 133748388 133748452
chr9 133750331 133750405
chr14 105246407 105246502
chr14 105246407 105246502
chr14 105246407 105246502
chr2 29432658 29432711
```

4-column target regions BED file format

The 4-column target regions BED file format is used when gene names are not known and some or all amplicon IDs are known.

The track line is optional. If present, it includes these tab-separated fields.

Field	Type	Description
`name`	String	A unique design identifier. Optional.
`description`	String	A description of the design. Optional.

The following is an example of how to enter an optional track line.

```
track name="ASD270245" description="AmpliSeq Pool ASD270245"
```

In a 4-column target regions BED file, the coordinates lines require the following tab-separated fields.

Field	Type	Description
`chrom`	String (chars ≥0 × 20, other than \tab)	The name of the chromosome. This name must be an exact match with a chromosome in the reference.
`chromStart`	Unsigned int64	The starting position of the feature (zero-based).
`chromEnd`	Unsigned int64	The ending position of the feature (not inclusive). Must be greater than `chromStart`.
`AmpliconID`	String	The amplicon ID. If missing, the following string is used "`chrom`" + ":" + "`chromStart`" + "-" + "`chromEnd`"

The following is a partial example of a 4-column target regions BED file.

```
chr9 133738312 133738379 amplID73150
chr9 133747484 133747542 amplID73075
chr9 133748242 133748296 amplID73104
chr9 133748388 133748452 491413
chr9 133750331 133750405 74743
chr9 133738312 133738379 73150
chr9 133747484 133747542 73075
chr9 133748242 133748296 73104
chr9 133748388 133748452 491413
chr9 133750331 133750405 74743
chr14 105246407 105246502 329410
chr2 29432658 29432711 34014
```

6-column target regions BED file format

The 6-column target regions BED file format is used when some or all gene names are known. BED files that are generated by **AmpliSeq.com** use this 6-column format.

The track line is required in a 6-column target regions BED file. The track line includes the following information.

Field	Type	Description
`name`	String	A unique design identifier.
`description`	String	A description of the design.
`type`	String	Must be `bedDetail` (without quotes).
`ionVersion`	String	The version of Torrent Suite™ Software to which you are importing.

The following is an example track line.

```
track name="ASD270245" description="AmpliSeq Pool ASD270245" type=bedDetail
```

In a 6-column target regions BED file, the coordinates lines require the following fields.

Field	Type	Description
`chrom`	String (chars ≥0 × 20, other than \tab)	The name of the chromosome. This name must be an exact match with a chromosome in the reference.
`chromStart`	Unsigned int64	The starting position of the feature (zero-based).
`chromEnd`	Unsigned int64	The ending position of the feature (not inclusive). Must be greater than chromStart.
`AmpliconID`	String	Amplicon ID. If missing, the following string is used "`chrom`" + ":" + "`chromStart`" + "-" + "`chromEnd`"
ID	String	A customer-specified ID. If missing, set to '.'. This field is not used.
`GeneSymbol`	String	The gene name. If missing, set to '.'.

Partial example of a 6-column target regions BED file:

```
track name="ASD270249_v1" description="AmpliSeq Pool ASD270249"
type=bedDetail
chr9 133738312 133738379 AM73150 NM_005157 ABL1
chr9 133747484 133747542 AM73075 NM_005157 ABL1
chr9 133748242 133748296 AM73104 NM_005157 ABL1
chr9 133748388 133748452 AM491413 NM_005157 ABL1
chr9 133750331 133750405 74743 NM_005157 ABL1
chr9 133738312 133738379 73150 NM_007313 ABL1
chr9 133747484 133747542 73075 NM_007313 ABL1
chr9 133748242 133748296 73104 NM_007313 ABL1
chr9 133748388 133748452 491413 NM_007313 ABL1
chr9 133750331 133750405 74743 NM_007313 ABL1
chr14 105246407 105246502 329410 NM_001014431 AKT1
chr14 105246407 105246502 329410 NM_001014432 AKT1
```

```
chr14 105246407 105246502 329410 NM_005163 AKT1
chr2 29432658 29432711 34014 NM_004304 ALK
```

8-column target regions BED file format

An 8-column target regions BED file format is for fusion panels.

In an 8-column target regions BED file, the coordinates lines require the following tab-separated fields. The format is similar to the 6-column target regions BED file, with two additional columns. The additional columns are `score` and `strand`.

Field	Type	Description
`chrom`	String (chars ≥0 × 20, other than \tab)	The name of the chromosome. This name must be an exact match with a chromosome in the reference.
`chromStart`	Unsigned int64	The starting position of the feature (zero-based).
`chromEnd`	Unsigned int64	The ending position of the feature (not inclusive). Must be greater than `chromStart`.
`AmpliconID`	String	Amplicon ID. If missing, the following string is used "`chrom`" + ":" + "`chromStart`" + "-" + "`chromEnd`"
ID	String	A customer-specified ID. If missing, set to '.'. This field is not used.
`GeneSymbol`	String	The gene name. If missing, set to '.'.
`score`	Unsigned int64	Score. If missing, set to "."
`strand`	String (+ or -)	Strand. If unknown, set to "+".

BED files generated by Ion AmpliSeq™ Designer custom designs

The track line for BED files generated by Ion AmpliSeq™ Designer custom designs follow the 6-column BED format, but with two additional fields. These additional fields are not used by Torrent Suite™ Software.

Field	Type	Description
`name`	String	A unique design identifier.
`description`	String	A description of the design.
`type`	String	Must be `bedDetail` (without quotes).
`ionVersion`	String	Introduced in Torrent Suite™ Software 4.0. When set to "4.0" or later, indicates that the BED file supports the Extended BED Detail format.
`db`	String	The UCSC Assembly ID.
`reference`	String	The Ion Torrent™ Server reference ID. Present for Ion AmpliSeq™ Designer 5.2 and higher.

(continued)

Field	Type	Description
`color`	String	The code for color track in the UCSC Genome Browser when the file is uploaded from **AmpliSeq.com**.
`priority`	String	Sets the order for color track in the UCSC Genome Browser when the file is uploaded from **AmpliSeq.com**.

Hotspots file format

The track line is required in a hotspots BED file.

The following is an example track line.

```
track name="ASD270245" description="HotSpots locations for AmpliSeq
ASD270245" type=bedDetail db=hg38 reference=GRCh38.p2
```

The track line includes these tab-separated fields.

Field	Type	Description
`track name`	String	A unique design identifier.
`description`	String	A description of the design.
`type`	String	Must be `bedDetail` (without quotes).
`db`	String	The UCSC Assembly identifier.
`reference`	String	The Ion Torrent™ Server reference identifier. (This field is optional for hg19, and is required for GRCh38.)

In hotspots BED files, the coordinates lines require these tab-separated fields.

Field	Type	Description
`chrom`	String (chars ≥0x20, other than \tab)	The name of the chromosome. This name must be an exact match with a chromosome in the reference.
`chromStart`	Unsigned int64	The starting position of the feature (zero-based).
`chromEnd`	Unsigned int64	The ending position of the feature (not inclusive). Must be greater than `chromStart`.
`HotSpotName`	String	The name is either the COSMIC identifier, dbSNP identifier, or is user-defined. If missing, the following string is used "`chrom`" + ":" + "`chromStart`" + "-" + "`chromEnd`"

(continued)

Field	Type	Description
`HotSpotAlleles`	String	A string that describes the variant using the following format: `REF=reference_allele`;`OBS=observed_allele`;`ANCHOR=base_before_allele`
`AmpliconID`	String	The Amplicon identifier. If missing, the following string is used `"chrom"` + ":" + `"chromStart"` + "-" + `"chromEnd"`

`HotSpotAlleles` field

The `HotSpotAlleles` field in the hotspots file specifies the alleles that are involved in variant calls, using this format:

`REF= reference_allele; OBS= observed_allele`

Examples:

- A TT insertion with 1-base prior at reference C: `REF=;OBS=TT`
- A TT deletion with 1-base prior at reference G: `REF=TT;OBS=`

Notes

- 6-column format
  - The elements can be empty: `REF=;` or `OBS=;`. Empty means deletion.
  - An additional element `ANCHOR=base_before_allele` can be provided for backward compatibility, but is optional. It is recommended that the ANCHOR key not be provided for Torrent Suite™ Software 4.2 or later.
  - Insertion alleles should have the same start and end position, and that position corresponds to a region between two bases. SNV, MNV, deletion, and complex variants should correspond to the reference bases that are spanned by the event.
  - The REF and OBS should be on the forward genomic strand. There should be one alternative allele per line.

  8-column format
  - The +/- strand notation in the hotspots file refers to the orientation of the Ion AmpliSeq™ Designer design input sequence, not to the reference sequence. REF and OBS alleles must always be reported on the forward strand of the reference sequence.
  - HotSpotAlleles are always reported based on the allele information from the positive strand of the reference sequence. Even if the allele strand is negative, the REF and OBS bases still report the alleles on the positive strand.

  For example, if there is a hotspot either on the positive strand or on the negative strand on a genomic coordinate, the strand information makes no difference as to what is reported on the HotSpotAlleles column. The HotSpotAlleles column always reports the alleles on the positive strand. In the following example, the strands are different, but the reported alleles are always from the positive strand:

```
chr 143815007 43815009 ID1 0 - REF=TG;OBS=AA AMPL1
chr 143815007 43815009 ID2 0 + REF=TG;OBS=AA AMPL2
```

Partial example of a hotspots BED file

```
track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" type=bedDetail
```

```
chr1 43815007 43815009 COSM19193 REF=TG;OBS=AA AMPL495041
chr1 43815008 43815009 COSM18918 REF=G;OBS=T AMPL495041
chr1 115256527 115256528 COSM585 REF=T;OBS=A AMPL30014
chr1 115256527 115256528 COSM586 REF=T;OBS=G AMPL30014
chr1 115256527 115256529 COSM33693 REF=TT;OBS=CC AMPL30014
chr1 115256527 115256529 COSM30646 REF=TT;OBS=CA AMPL30014
chr1 115256527 115256530 COSM53223 REF=TTG;OBS=CTT AMPL30014
chr1 115256528 115256529 COSM583 REF=T;OBS=A AMPL30014
chr1 115256528 115256529 COSM584 REF=T;OBS=C AMPL30014
chr1 115256528 115256529 COSM582 REF=T;OBS=G AMPL30014
chr1 115256528 115256530 COSM12725 REF=TG;OBS=AA AMPL30014
chr1 115256528 115256530 COSM579 REF=TG;OBS=CT AMPL30014
```

Note: The `REF=;OBS=` field is required, as is the track line.

Extended BED Detail format

Ion AmpliSeq™ Designer 3.0 and later uses Extended BED Detail format for the following fixed panels.

- CCP
- CFTR
- CHP v2
- Ion AmpliSeq™ Exome

New fixed panels that are introduced for Ion AmpliSeq™ Designer 3.0 and later also follow this format. Other panels, and all panels from previous releases, do not use this format.

The Extended BED Detail format contains two additional fields (at the end of each line).

Name	Values	Description
`Id`	Any string supplied by the user or '.'	A user-supplied name or identification for the region.
`Description`	The key-value pairs separated by semicolon, or '.', if empty	Contains a '.' or one or more of the following key-value pairs. • `GENE_ID=` • `Pool=` • `SUBMITTED_REGION=` These key-value pairs are described in the next table.

The following table lists the keys and describes the key-value pairs that are supported.

Key	Description
GENE_ID	A gene symbol or comma-separated list of gene symbols. If no gene symbol is available, this key is absent. Example: `GENE_ID=brca1` Example: `GENE_ID=brca1, ret`
Pool	The Ion AmpliSeq™ Designer pool or pools that contains this amplicon. Example: `Pool=2` If an amplicon is present in multiple pools, the pools are delimited with "," (comma), with the primary pool listed first. For example, if an amplicon is present in pools 1 and 3, and 1 is the primary pool, the entry is `Pool=1,3`. Single-pool designs do not include the `Pool=` key-value pair.
SUBMITTED_REGION	The region name provided during the Ion AmpliSeq™ Designer design process. If a region name is not provided, this key is absent. Example: `SUBMITTED_REGION=Q1`
CNV_ID	A gene symbol used to specify a copy number region for the cnv pca algorithm. This takes precedence over the `GENE_ID` and once CNV_ID can span multiple `GENE_ID`s.
CNV_HS	A CNV region hotspot. This can be a value of either 0 or 1. • 1 reports as a hotspot (HS) in the output VCF file from the CNV PCA algorithm. • 0 is not reported as HS.

The Extended BED Detail format requires a track line with both `type=bedDetail` and `ionVersion=4.0`. The Torrent Suite™ Software BED validator treats the `Id` and `Descriptor` fields as optional.

Examples from BED files in the Extended BED Detail format

This example shows the `GENE_ID=` and `Pool=` keys.

```
track name="4477685_CCP" description="Amplicon_Insert_4477685_CCP"
type=bedDetail ionVersion=4.0
chr1 2488068 2488201 242431688 . GENE_ID=TNFRSF14;Pool=2
chr1 2489144 2489273 262048751 . GENE_ID=TNFRSF14;Pool=4
chr1 2489772 2489907 241330530 . GENE_ID=TNFRSF14;Pool=1
chr1 2491241 2491331 242158034 . GENE_ID=TNFRSF14;Pool=3
```

This example is from the CFTR designed.bed file.

```
track type=bedDetail ionVersion=4.0 name="CFTRexon0313_Designed"
description="Amplicon_Insert_CFTRexon0313"
chr7 117119916 117120070 CFTR_1.91108 .
GENE_ID=CFTR;Pool=1;SUBMITTED_REGION=1,31
chr7 117120062 117120193 CFTR_1.38466 .
GENE_ID=CFTR;Pool=2;SUBMITTED_REGION=1
chr7 117120186 117120304 AMPL244371551 .
GENE_ID=CFTR;Pool=1;SUBMITTED_REGION=1,32
```

Merged Extended BED Detail format files

In the case of two overlapping records, those records are merged during upload into Torrent Suite™ Software. An ampersand (&) is the delimiter between multiple values in merged files.

Example 1

When these two `GENE_ID` fields appear in overlapping records:

`GENE_ID`=raf

`GENE_ID`=brca1

The merged G`ENE_ID` field is:

`GENE_ID`=raf&brca1

Example 2

When these two `GENE_ID` fields appear in overlapping records:

`GENE_ID`=raf

`GENE_ID`=brca1,ret

The merged `GENE_ID` field is:

`GENE_ID`=raf&brca1,ret

Score and strand fields in uploaded hotspot BED files

Uploaded BED files are converted to add score and strand columns, with the default values `0` and `+`. You see these values in BED files that you download from Torrent Suite™ Software:

```
track type=bedDetail name="BRCA1.BRCA2_HotSpots"
description="BRCA_HOTSPOT_ALLELES" allowBlockSubstitutions=true
chr13 32890649 32890650 COSM35423 0 + REF=G;OBS=A AMPL223487194
chr13 32893206 32893207 COSM23930 0 + REF=T;OBS= AMPL223519297
chr13 32893221 32893221 COSM23939 0 + REF=;OBS=CCAATGA AMPL223519297
chr13 32893290 32893291 COSM172578 0 + REF=G;OBS=T AMPL223521074
```

RNA fusions BED file formats and examples

The following information describes the target regions Browser Extensible Display (BED) format used with Ion AmpliSeq™ RNA fusion designs. BED files are text files with tab-separated fields.

Track line

The track line is required in the Target Regions BED file. The following is an example track line.

```
track name="Fusions 2.6" description="AmpliSeq RNA" type=bedDetail
ionversion="4.0"
```

The track line includes these tab-separated fields.

Field	Type	Description
`name`	String	A unique design identifier.
`description`	String	The description of the design.
`type`	String	Must be `bedDetail` (without quotes).
`ionversion`	String	Introduced in the Torrent Suite™ Software 4.0 (Ion AmpliSeq™ Designer 3.0 and higher fixed panels). When set to "4.0", indicates that the BED file supports the Extended BED Detail format. This optional field relates to only the BED file format version, not the version of panel designs.

Columns

This format includes eight required columns that are separated by a tab (`\t`) character.

Field	Type	Description
`chrom`	String (chars ≥0 × 20, other than \tab)	The name of the chromosome. This name must be an exact match with a chromosome in the reference.
`chromStart`	Unsigned int64	The starting position of the feature (Insert Start not the Amplicon Start). Must be zero-based.
`chromEnd`	Unsigned int64	The ending position of the feature (not inclusive) (Insert End not the Amplicon End). Must be greater than `chromStart`.
`AmpliconID`	String	The Amplicon ID. If missing, the following string is used "`chrom`" + ":" + "`chromStart`" + "-" + "`chromEnd`"
`Score`	Unsigned int64	Score. If missing, set to '.'. This field is not used.
`Strand`	String (+ or -)	Strand. If unknown, set to '+'.

(continued)

Field	Type	Description
`ID`	String	A customer-specified ID. If missing, set to '.'. This field is not used.
`Key-value pairs`	String	Multiple attributes specified as semicolon separated key-value pairs. See below for specific key-value pairs. All of these KVPs are required for fusions designs files, but most of these are optional for other White Glove designs.

The genomic (hg19) coordinates provided in the key-value pairs must represent the entire amplicon sequence. If you want to generate the fusions mapping reference FASTA file from the BED file, all the information that is needed should be available in the BED file.

The following key-value pairs are supported.

Key	Value	Example
`TYPE`	The type of the event. Allowed values are the following. • `Fusion` • `CONTROL` or `ExpressionControl` • `Driver_Gene` or `5p3pAssay` • `GeneExpression` • `RNA_Hotspot`	`TYPE=Fusion`
`FP_TRANSCRIPT_ID`	The transcript ID for the 5′ gene partner. This key value pair is only for fusion type targets.	`FP_TRANSCRIPT_ID=ENSG00000156735`
`TP_TRANSCRIPT_ID`	The transcript ID for the 3′ gene partner. This field is absent for CONTROL type amplicons. This key value pair is only for fusion type targets.	`TP_TRANSCRIPT_ID=ENSG00000077782`
`BREAKPOINT`	The position in the sequence for the breakpoint. Applicable to only fusion type amplicons. This position is number of bases from the Insert start, not the Amplicon Start.	`BREAKPOINT=56`
`FP_GENE_ID`	The name of the 5′ gene partner in the fusion. This key value pair is only for fusion type targets.	`FP_GENE_ID=BAG4`

(continued)

Key	Value	Example
`FP_GENE_STRAND`	The strand for the 5′ gene partner. Allowed values are '+' and '-'. This key value pair is only for fusion type targets.	`FP_GENE_STRAND=+`
`FP_EXON_NUMBER`	The exon number in the 5′ gene. Use comma-separated values if the amplicon spans multiple exons. This key value pair is only for fusion type targets.	`FP_EXON_NUMBER=2`
`TP_GENE_ID`	The name of the 3′ gene partner in the fusion. This field is absent for CONTROL type amplicons. This key value pair is only for fusion type targets.	`TP_GENE_ID=FGFR1`
`TP_GENE_STRAND`	The strand for the 3′ gene partner. Allowed values are '+' and '-'. This field is absent for CONTROL type amplicons. This key value pair is only for fusion type targets.	`TP_GENE_STRAND=-`
`TP_EXON_NUMBER`	The Exon number in the 3′ gene. Use comma-separated values if the amplicon spans multiple exons. This key value pair is only for fusion type targets.	`TP_EXON_NUMBER=6`
`FP_CHROM`	The chromosome of the 5′ gene. This key value pair is only for fusion type targets.	`FP_CHROM=chr8`
`FP_START`	The start position for the 5′ segments. Use comma-separated values if there are multiple segment Starts. This key value pair is only for fusion type targets.	`FP_START=38050257`
`FP_END`	The end position for the 5′ segments. Use comma-separated values if there are multiple segment Ends. This key value pair is only for fusion type targets.	`FP_END=38050313`

(continued)

Key	Value	Example
TP_CHROM	The chromosome of the 3′ gene. This field is absent for CONTROL type amplicons. This key value pair is only for fusion type targets.	TP_CHROM=chr8
TP_START	The start position for the 3′ segments. Use comma-separated values if there are multiple segment Starts. This key value pair is only for fusion type targets.	TP_START=38283673
TP_END	The end position for the 3′ segments. Use comma-separated values if there are multiple segment Ends.	jTP_END=38283763
HOTSPOT_POSITION	The genomic coordinate of the hotspot snp covered by the amplicon. Use comma-separated values if multiple hotspots are covered by the amplicon.	HOTSPOT_POSITION=38283769
CHROM	The chromosome name of the target region. This key is for all non-fusion type targets. For fusion targets, we have FP_CHROM and TP_CHROM.	CHROM=chr8
GENE_ID	The name of the gene for nonfusion type targets. For fusion targets, we have FP_GENE_ID and TP_GENE_ID.	GENE_ID=LMNA
TRANSCRIPT_ID	The transcript Id for nonfusion type targets. For fusion targets, we have FP_TRANSCRIPT_ID and TP_TRANSCRIPT_ID.	TRANSCRIPT_ID=ENST00000389048
GENE_STRAND	The strand of the gene. This key is for all nonfusion type targets. For fusion targets, we have FP_GENE_STRAND and TP_GENE_STRAND.	GENE_STRAND=+

(continued)

Key	Value	Example
`EXON_NUM`	The exon number or numbers in the gene. Use comma-separated values if the amplicon spans multiple exons. For fusion targets, we have FP_EXON_NUM and TP_EXON_NUM.	`EXON_NUM=3,4`
`START`	The start position of the target segment. Use comma-separated values if there are multiple segment starts in genomic space. This key is for all nonfusion type targets. For fusion targets, we have FP_START and TP_START.	`START=53586113,53585786`
`END`	The end position of the target segment. Use comma-separated values if there are multiple segments in genomic space. This key is for all nonfusion type targets. For fusion targets, we have FP_END and TP_END.	`END=53586228,53585803`
`MIN_READ_COUNT`	The minimum number of reads needed to call the particular target as present/absent. This value is optional and, if present, overrides the universal minimum read count threshold (for example, 20). Example usage—For EGFR deletion assay, use a higher read count threshold (>20).	`MIN_READ_COUNT=100`

Example BED file entries

```
BAG4-FGFR1.B2F6 1 156 AMP1 . + . TYPE=Fusion;BREAKPOINT=36;
FP_GENE_ID=BAG4;
FP_ GENE_STRAND=+;
FP_EXON_NUM=2;
TP_GENE_ID=FGFR1;
TP_GENE_STRAND=-;
TP_EXON_NUM=6;
FP_CHROM=chr8;
FP_START=3805025 7;
FP_END=38050313;
TP_CHROM=chr8;
TP_START=38283673;
TP_END=38283763;
FP_TRANSCRIPT_ID=ENSG00000156735;
TP_TRANSCRIPT_ID=ENSG00000077782 ITGB7.ENCTRL.E14E15 1 132  AMP99 . + .
TYPE=CONTROL;
```

```
FP_GENE_ ID=ITGB7;
FP_CHROM=chr12;
FP_EXON_NUM=14,15;
FP_START=53586113,53585786;
FP_END=53586228,53585803;
FP_GENE_STRA ND=-;
FP_TRANSCRIPT_ID=ENSG00000139626
```

Test fragments

Test fragments are known genetic sequences that are used to measure the quality of your chip loading and sequencing run. You can include test fragments in your sequencing run and, after the run completes, review the **Details** section of the run report to evaluate the quality of your loading and sequencing run. For example, TF_1 is a single known sequence fragment that is added along with the customer library at the beginning of templating and is processed through sequencing. TF_C is already templated on ISPs and added after enrichment so goes through the loading and sequencing portions of the workflow.

Test fragments are displayed when there are at least 500 high-quality reads, with an 85% match against the appropriate template in the **Test Fragment** list.

View test fragment details

You can view existing test fragment nucleotide sequences used for quality metrics.

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Test Fragments** in the left navigation menu.
2. In the **Test Fragments** table, in the **Name** column, click a test fragment name to view the test fragment details such as the complete test fragment sequence.
3. *(Optional)* In the **Edit Test Fragment** dialog box, add comments about the test fragment.

Add a test fragment

You can add a new test fragment to Torrent Suite™ Software.

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Test Fragments** in the left navigation menu.
2. In the **Test Fragments** screen, click **Add Test Fragment**.

3. Complete the **Add New Test Fragment** dialog box.

Item	Selection
Name	Enter a unique name for your test fragment.
Key	Enter the test fragment key using only the uppercase letters A, T, C, and G.
Sequence	Enter the sequence using only the uppercase letters A, T, C, and G.
Enabled	Select to enable.
Comments	Enter optional comments about the test fragment.

   If you enter an invalid character or a duplicate test fragment, you cannot save changes.

4. Click **Save**.

The new test fragment and the details are listed in the test fragment table.

Edit or delete a user-defined test fragment

You can edit or delete a user-defined test fragment in Torrent Suite™ Software.

IMPORTANT! The TF_1 default test fragment is used to evaluate templating, bead loading, and sequencing quality. Do not modify the default test fragment sequence that is supplied in Torrent Suite™ Software.

You can make the following edits to a user-defined test fragment.

- Change the test fragment name, key, or comments.
- Change the test fragment nucleotide sequence.
- Change whether the test fragment is enabled.

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Test Fragments** in the left navigation menu.

2. In the **Test Fragments** table, click a link in the **Name** column.

3. To manage the test fragments, in the **Edit Test Fragment** dialog box, do one of the following.

   - Edit **Name**, **Key**, **Sequence**, or **Comments**, or select/deselect the **Enabled** checkbox, then click **Save**. Changes appear in the **Test Fragments** table.
   - Click **Delete** to permanently remove the test fragment from Torrent Suite™ Software. Deleted test fragments are removed from the **Test Fragments** table.

Barcodes and barcode sets

Torrent Suite™ Software supports sequencing runs in which multiple samples can be processed, or multiplexed. A DNA barcode adapter molecule is used to tag a sample library when it is prepared. The sample is then identified and tracked based on the barcode.

Barcode adapter sets are used to associate multiple adapter barcodes with each individual sample when Planned Runs are set up, or when manual runs of Torrent Suite™ Software plugins are configured. For more information, see "Plan step in the workflow bar" on page 49 and "Run a plugin manually from the sequencing run report" on page 124.

During a sequencing run that uses barcodes, Torrent Suite™ Software generates output files with reads that are associated with the barcodes. The barcoded reads are aligned against the reference genome, and results are stored in BAM and BAM index (BAI) files for each barcode. Reads that cannot be classified as a barcode in the designated set are grouped into a no-match group; alignment against the reference is also performed on the no-match group.

Alignment metrics for each barcoded read are available in the run reports for completed sequencing runs. For more information, see "Output files" on page 101.

A sequencing run on an Ion GeneStudio™ S5 Series sequencer that uses barcodes requires a sample preparation kit that includes a barcode set or kit, such as the Ion Xpress™ barcode set or Ion Torrent™ Dual Barcode Kit 1–96. Torrent Suite™ Software includes barcode sets for the latest available barcode kits.

You can create new sets of barcode adapters with comma-separated value (CSV) files, then upload these files onto the Ion Torrent™ Server as barcode sets for use during sequencing runs. To create the barcode set, you can download a sample DNA barcode set CSV file or an existing DNA barcode set CSV file, customize the DNA barcode set in the file, then import the DNA barcode set to the Ion Torrent™ Server.

You can also transfer DNA barcode sets between two different Ion Torrent™ Servers.

IMPORTANT! The creation of new barcode sets is for advanced users only. For help with creating barcode sets, contact your local Field Service Engineer.

View a DNA barcode sequence

You can view barcode ID, barcode and adapter sequences, notes, and barcode type for individual barcodes in a barcode set.

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Barcode Sets** in the left navigation menu.
2. In the **Barcode Sets** table in the **Name** column, click the barcode name.
   A table appears listing all the individual barcode IDs in the barcode set and their associated information.
3. Click any column heading to sort the column.

To edit the existing barcode set, download the barcode set CSV file, edit the file, then import the revised CSV file into Torrent Suite™ Software. For more information, see "Download a DNA barcode set CSV file" on page 269.

Download a DNA barcode set CSV file

You can download a DNA barcode set CSV file and save it to your local storage. Use this feature to transfer DNA barcode sets between two different Ion Torrent™ Servers.

Alternatively, you can download an existing DNA barcode set CSV file, customize the DNA barcode set, then import the new DNA barcode set onto your Ion Torrent™ Server.

1. Click **(Settings)** ▸ **Reference Sequences**, then click **Barcode Sets** in the left navigation menu.
2. In the **Barcode Sets** table, in the **Action** column, click **(Actions)** ▸ **Download**.
   A CSV file that contains DNA barcode information is downloaded.

Save the CSV file to your local storage. You can now transfer this file to another Ion Torrent™ Server or edit the file and import the new DNA barcode set into Torrent Suite™ Software. For more information, see "Add a new DNA barcode set" on page 269.

Add a new DNA barcode set

You can create a DNA barcode set that fits your needs and add it to Torrent Suite™ Software for use in Planned Runs.

To add a barcode set, you must generate a Comma-Separated Value (CSV) file, save the file to your local storage, then import the file into Torrent Suite™ Software. All user-defined DNA barcode set files must have a `.csv` extension.

1. Click **(Settings)** ▸ **Reference Sequences**, then in the left navigation menu click **Barcode Sets**.
   The **Barcode Sets** table lists all the available user-defined and preinstalled DNA barcode sets.
2. *(Optional)* Create a new DNA barcode set CSV file using an existing DNA barcode set.
   a. In the row of the existing DNA barcode set, In the **Action** column, click **(Actions)** ▸ **Download**.
      A CSV file is downloaded to your computer.
   b. Edit the CSV file as described in "DNA barcode set CSV file setup" on page 270, then save it to your local storage.
3. In the **Barcode Sets** screen, click **Add new DNA Barcodes**.
4. In the **Add New DNA Barcodes** dialog box, in **Barcode Set Name**, enter a descriptive name for the DNA barcode set.
5. *(Optional)* Create a new DNA barcode set CSV file using the example CSV file.
   a. Click **Download the example Barcode CSV file**.
      A CSV file is downloaded to your computer.
   b. For each individual barcode in the barcode set, enter the information in each column as described in "DNA barcode set CSV file setup" on page 270.
   c. Save the CSV file to your local storage.

6. Click **Choose File**, select a DNA barcode set CSV file from your local storage, then click **Open**.

7. Click **Upload File**.
   The new DNA barcode set is added to the **DNA Barcodes** table in the **Barcode Sets** screen and is now available to be used in a Planned Run.

DNA barcode set CSV file setup

Each row in the CSV file contains information for an individual barcode in the barcode set.

Column name	Type	Description
`index`	Integer	A unique index for an individual barcode (for example, 1, 2, 3,...) Only numerical characters are accepted.
`id_str`	String	A unique name for an individual barcode entry. Typically, this is the name of the barcode kit followed by a 3′– to 4′– digit unique identifier, such as IonXpress_001 or IonCode_0101.
`sequence`	String	The 5′– barcode sequence. Upper-case G, C, A, and T are allowed.
`adapter`	String	The portion of the 5′– barcode adapter not used to identify this barcode. Often referred to as the "stuffer sequence". Upper-case G, C, A, and T are allowed.
`annotation`	--	Use for any barcode-specific notes.
`end_sequence`	String	The 3′– barcode sequence. **IMPORTANT!** This sequence is required for libraries that were prepared using dual barcode technology. Upper-case G, C, A, and T are allowed.
`end_adapter`	String	The portion of the 3′– barcode adapter not used to identify this barcode. Often referred to as the "stuffer sequence". This sequence is available only for libraries that were prepared using dual barcode technology. Upper-case G, C, A, and T are allowed.

Delete a user-defined DNA barcode set

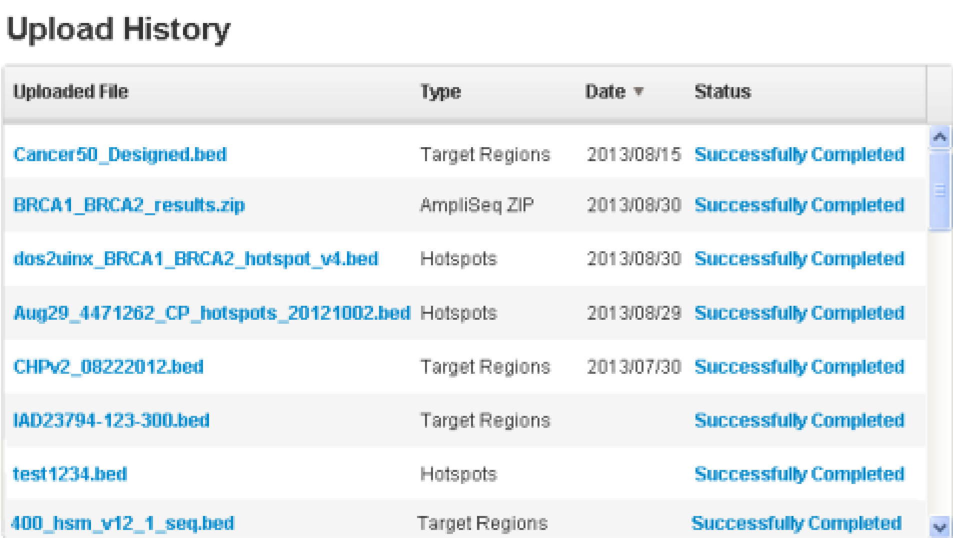
After you import a user-defined DNA barcode set into Torrent Suite™ Software, if you no longer want to use the set in your Planned Runs, you can delete it from the Ion Torrent™ Server.

IMPORTANT! Do not delete any preinstalled barcode set.

1. Click **(Settings)** ▸ **Reference Sequences**, then click **Barcode Sets** in the left navigation menu.
2. In the **Barcodes** table, in the row of the barcode set to be deleted, click **(Actions)** ▸ **Delete**.
3. In the **Confirm Delete Barcode Set** dialog box, click **Delete Barcode Set**, then click **Yes, Delete!** to confirm.
   The DNA barcode set is permanently deleted from your Ion Torrent™ Server.

Upload history

The **Upload History** table in the **References Sequences** screen provides a list of the recent uploads of compressed file directories from **AmpliSeq.com**, target regions files, and hotspots files.



The following information is displayed.

Column	Description
Uploaded File	The file name of the uploaded file.
Type	Indicates whether the file is a target regions, hotspots, or ZIP file.
Date	The date that the file was uploaded.
Status	A report of whether the file was successfully uploaded. Errors that occur during upload are shown in this column.

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Data management

Use the **Data** tab to view information about completed runs. You can also import data for data transfers or restoration.

Data management actions to archive, export, or delete files require administrator rights, except for import; a regular user can import or restore data back to a local drive.

View disk usage parameters

1. In the **Data** tab, click **Data Management**.

2. Scroll to the **Disk Usage** section to review the disk usage parameters.

3. Review the disk usage information

Parameters in the Disk Usage section.

Parameter	Definition
Keep	File space devoted to files that are to be kept.
Used	File space currently used by data files.
Free	Space available for storing data files.
Threshold I	Threshold above which intermediate files are deleted or archived, based on the automatic configuration settings.
Threshold S	Threshold above which signal processing input files are deleted or archived, based on the automatic configuration settings.
Threshold O	Threshold above which output files are deleted or archived, based on the automatic configuration settings.
Threshold B	Threshold above which basecaller input files are deleted or archived, based on the automatic configuration settings.

For details about automatic deletion and archive creation, see “Archive or delete data automatically” on page 314.

View category statistics

1. In the **Data** tab, click **Data Management**, then scroll to the **Category Statistics** section.

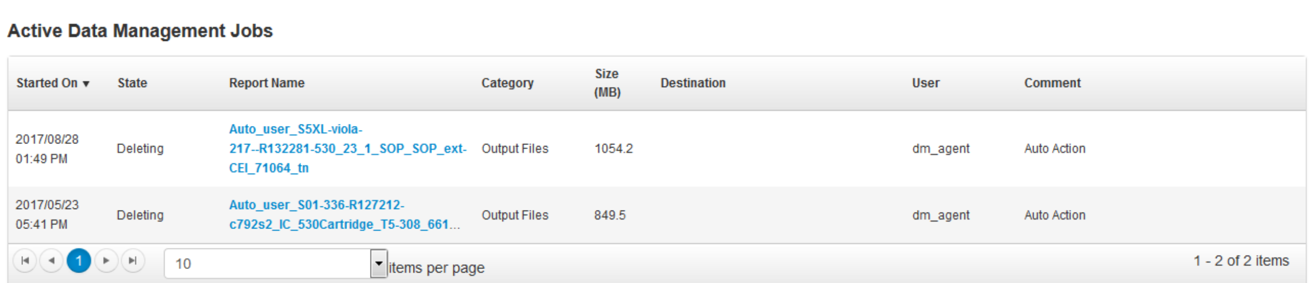
2. View the **Category Statistics** section.

Column	Definition
File Category Group	File type. For details, see “Ion instrument data types” on page 313.
Total	The total number of data sets in each file category.
Keep	The number of data sets in each file category that are exempt from data management actions.
Archived	The number of data sets in each file category that have been removed from your Ion Torrent™ Server by data management archival.
Deleted	The number of data sets in each file category that have been removed from your Ion Torrent™ Server by data management deletion.
In Process	The data sets that are currently archiving/deleting/importing.
Error	Error column displays the count of file categories that are currently in an error state. If a data management action is rerun on one of these file categories and completes successfully, then that file category no longer appears in the error count.
Disk Usage (GB)	Gigabytes of storage used by each file category.

View active data management jobs

You can view runs that are in progress on the Torrent Server.

1. Click the **Data** tab, click **Data Management**, then scroll to the **Active Data Management Jobs** section.



Active Data Management Jobs

Started On ▾	State	Report Name	Category	Size (MB)	Destination	User	Comment
2017/08/28 01:49 PM	Deleting	Auto_user_S5XL-viola-217--R132281-530_23_1_SOP_SOP_ext-CEI_71064_tn	Output Files	1054.2		dm_agent	Auto Action
2017/05/23 05:41 PM	Deleting	Auto_user_S01-336-R127212-c792s2_IC_530Cartridge_T5-308_661...	Output Files	849.5		dm_agent	Auto Action

1 10 items per page 1 - 2 of 2 items

2. *(Optional)* Click a report to see the status of that report.

Details about active data management jobs.

Parameter	Definition
Started On	The start date and time of the job.
State	The status of the job or file.
Report Name	The identifier of job.
Category	Identifies the file as one of the following file types. • Signal processing input—Required input files for signal processing. • Basecalling input—Required input files for base calling. • Output files—Files for data processing. • Intermediate files—Files used for debugging.
Size	The file size, in MB, of the report.
Destination	The folder location for archive or export action on the report.
User	The user who started the data management action. For auto-actions, "dm-agent" is the user.
Comment	Text box for optional notes.

Error messages

Monitor the **Disk Space Management** section for messages that require administrator action.

Error message	Action
Backup drive is full or missing	Replace the backup drive.
Error	Check the file `/var/log/ion/data_management.log` for information about the specific error condition. If appropriate, report the error to Ion technical support.

Disk full message

Torrent Suite™ Software performance is affected when a disk partition is more than 95% full. When an Ion Torrent™ Server or a mounted storage device reaches 95% full (and again at 99%), a warning is displayed at the top of the Torrent Suite™ Software screen.

```
*** CRITICAL! /results/: Partition is getting very full - 95% ***
```

Search for run reports with disk usage status

You can find run reports with searches that are based on disk usage status, such as whether the data type is archived, or is stored in a local directory. You can also use other search criteria, including name and report date.

1. In the **Data** tab, click **Data Management**, then scroll to the **Disk Space Management** section.
2. Enter a search term or select for the following criteria:
   - **Search names**
   - **Report date**
   - File type settings that are configured as **Keep**, are stored in the **Local** directory, **Archived**, **Deleted**, **In-process**, or contain an **Error** for each file type:
     - SigProc (Signal processing)
     - Basecalling (Basecalling input)
     - Output
     - Intermediate
3. After you select the filters, click **Go**.

Run reports that match the criteria that you use in the search are listed in the **Disk Space Management** table.

Keep run report data

You can prevent data from being deleted for individual run reports by applying the **Keep** attribute to the types of files that are associated with the run report that were created during the run. These file types are:

- Signal processing input files
- Basecalling input files
- Output files
- Intermediate files

If the **Keep** is applied to a data file, the data file cannot be deleted by any user. Instead, an error occurs if any user tries to confirm deletion of run report data.

1. In the **Data** tab, click **Data Management**, then scroll to the **Disk Space Management** section.
2. Find the report, then select the checkbox under the **Keep** column that appears on the *left* of each file category in that row.

Import data for data transfers or restoration

You can import data to an Ion Torrent™ Server from a mounted storage drive such as an external server or a USB drive. The import function can be used to transfer data between servers or restore data that has been archived.

Data can be imported only from storage drives that have been mounted on your Ion Torrent™ Server. For information about mounting a storage drive, see "Increase file storage and available disk space" on page 319.

The Import function can retrieve only the data files that were previously exported or archived. For example, if you try to import files from an archive that does not include signal processing input or basecalling input files, these files are not retrieved.

Note:

- Exported and archived files on a mounted drive can be viewed and analyzed directly in Torrent Suite™ Software under **Completed Runs & Reports**. If you unmount the storage device, the data is no longer available. Import files before unmounting a drive to continue using them.
- If you import data that has been previously archived, the original archive remains. After an import, data exists in two locations. The data are copied to Ion Torrent™ Server and remains in the original archive location. If you later delete the file manually, the imported files remain in the archive.

---

1. Under the **Data** tab, click **Data Management**.
2. In the **Data Import** section, click **Import**.
3. Select a mounted Archive Directory, or click **Browse** to navigate to a particular subdirectory, then click **Select**.
4. Use the checkboxes to select the file categories that you want to import, then click **Import**.

Imported files appear as standard data files under **Completed Runs & Reports**. The State of the data files on the server is **Local**.

View the data management actions log

You can view a log for each run report that describes each change that is made to data management settings.

1. In the **Data** tab, click **Data Management**.
2. Scroll to the **Disk Space Management** section.
3. Find the report for which you want to view the data management actions log.

   For details about how to search for a run in the **Disk Space Management** section, see "Search for run reports with disk usage status" on page 275.

4. Click ⚙ **(Actions)** ▸ **View Log**.
   A new screen opens that shows a chronological list of actions that are taken for this run report. The date of the action, name of the user, and any comments are displayed.

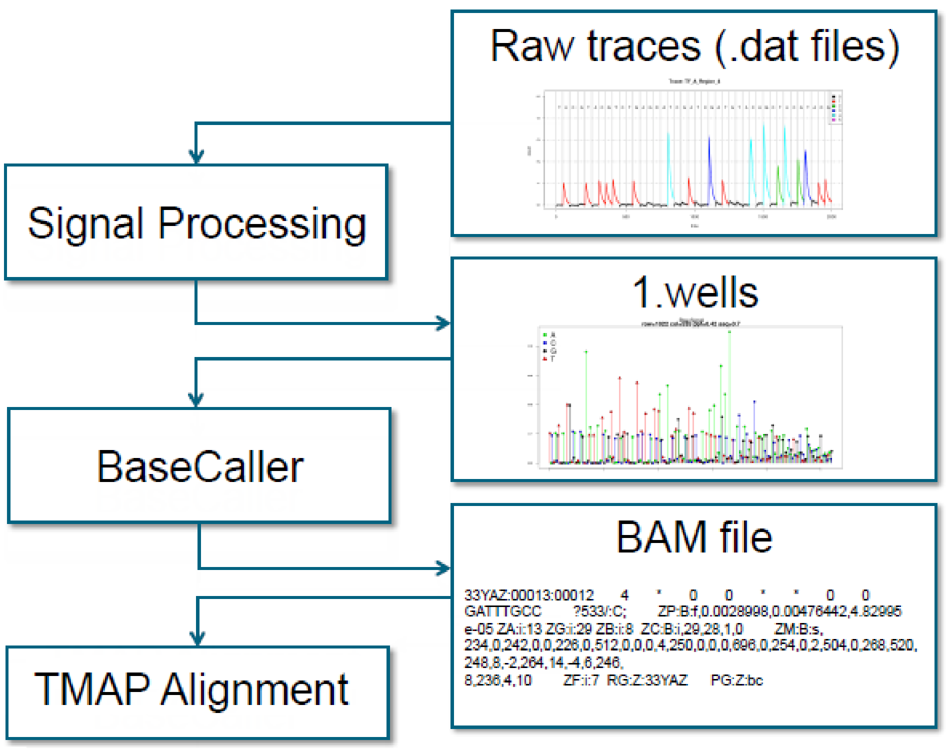
5. Click **Close** to return to the **Data Management** screen.

12 Supported software modules

Torrent Suite™ Software supports additional software modules in the analysis pipeline. These are the Signal Processing module, the BaseCaller module, and the TMAP modules.

Analysis pipeline overview

The following depicts and describes the beginning steps of the Torrent Suite™ Software analysis pipeline.



Steps:

1. The sequencing instrument generates DAT files of raw traces of electrical signals.
2. The signal processing step converts the raw traces into a single number per flow per well, in the 1.WELLS file.
3. The BaseCaller module converts the 1.WELLS file information into a sequence of bases, and writes the sequence into an unaligned BAM file.
4. The BAM file is passed to the TMAP module for alignment.

The signal processing step also marks several types of low-quality reads:

- Polyclonal reads (reads with two template beads instead of one).
- Reads with high signal processing residual (indicating an ambiguous signal value).
- Reads that do not contain a valid library key.

The signal processing step marks these problematic reads but does not remove them.

BaseCaller module and barcode classification overview

The BaseCaller module controls barcode classification and filtering and trimming.

Overview of the BaseCaller module functionality

In addition to creating a sequence of bases from the 1.WELLS file information, the BaseCaller module also performs read filtering and read trimming.

Notes on read filtering

- Filters out low-quality reads that were marked during signal processing.
- Filters out reads that fail basecalling filters.
- The removed reads *do not* appear in the BAM file. The BaseCaller module keeps counts of these reads but there is no record of specific reads that are filtered out.

Notes on read trimming

- Removes specific bases from the read for quality reasons.
- The read appears in the BAM file.
- The removed bases do not appear in the BAM file.

The BaseCaller module performs these functions:

1. Removes low-quality reads that were marked during the signal processing step.
2. Performs base calling:
    a. From the signal values, creates the sequence of bases.
    b. Estimates the base quality value for each base.
3. Performs 5′ barcode classification:
    a. Assigns each read to a barcode.
    b. Trims the barcode sequence away if the parameter `--trim-barcodes=on` is specified. The default is 'on'.

4. Trims 5′ PCR handle.
5. Trims 5' unique molecular tag.
6. Trims extra bases at the 5' end. Trimming is controlled by the parameter `--extra-trim-left`. The default is 0, meaning no extra trimming.
7. Filters out reads that are too short. Filtering is controlled by the parameters`--min-read-length` and `-- trim-min-read-len`.
8. Filters out reads that do not have the correct library key. Filtering is turned off by the parameter `--keypass-filter`.
9. Trims the P1 adapter at the 3' end.
10. Classifies and trims the 3′ barcode.
11. Trims the 3′ PCR handle.
12. Trims the 3’ unique molecular tag.
13. Trims extra bases on the 3’ end. Trimming is controlled by the parameter `--extra-trim-right` . The default is 0, meaning no extra trimming.
14. Performs quality trimming. Trimming is affected by the parameters `--trim-qual-window-size` and `-- trim-qual-cutoff`.

The BaseCaller module classifies and trims read elements from the outside inwards to obtain the query sequence. If an outer element cannot be identified, the BaseCaller module does not attempt to identify and trim an inner one. If trimming the barcode is disabled using the `-trim-barcodes` parameter, PCR handles, UMTs, or extra-bases will not be trimmed. Read elements on the 5' end will only be trimmed if the P1 adapter was found.

Notes about quality trimming

- The purpose of quality trimming is to identify where quality problems start at the end of a read. We try to identify when bases fall below a quality threshold and trim both of those bases and a bit before those bases.
- The parameter `--trim-qual-window-size` sets the window size for quality trimming. The algorithm slides through the sequence of bases and, each time the window shifts, computes the mean Base QV value for all bases in the window.
- If the mean Base QV value for all bases in the window falls below a threshold (set by the parameter `--trim-qual-cutoff` where the default 16), then trims all bases from the center of the window at that time to the 5' end.

Notes about barcode classification and barcode filtering

Barcode classification determines which barcode group a read is assigned to. Barcode classification is done for each read immediately after base calling.

Barcode filtering determines if a specific barcode is included in the run report or is filtered out. Barcode filtering works on the barcode groups as a whole

Ion Torrent™ BAM format

Ion Torrent™ BAM files follow the conventions of the SAM/BAM Format Specification Working Group. SAM stands for Sequence Alignment/Map.

The following highlights specific Ion Torrent™ conventions and the meaning of custom tags.

- Run ID: Every TS analysis assigns a run ID, a 5-character string consisting of upper-case letters and numbers. A reanalysis of a specific run assigns a different run ID. Example: 0JU8V.
- Read Group ID: For non-barcoded runs the read group ID is equal to the run ID. For barcoded runs it is a combination of the run ID and the barcode name, which is separated by a dot. Example: 0JU8V.IonXpress_001.
- Key Sequence (KS): For non-barcoded runs, the Key Sequence tag is the Ion Torrent™ library key (TCAG). For barcoded runs the KS tag entry includes the barcode sequence and the barcode adapter sequence if barcode trimming is enabled.
- Reverse Key Sequence (sk): For dual-barcoded runs, the Reverse Key Sequence tag includes the end barcode adapter sequence and the end barcode sequence.
- SAM record (read) names: Read names are a combination of the run ID and the chip coordinates of the well that produced the read. The coordinate values are 5-digit numbers and are given in the order row and the column, which is separated by a colon. Example: 0JU8V:01308:00107.
- BAM header comment lines (CO): Comment lines in the BAM header are used to store base calibration information, or information about the 3' adapter sequences.

Custom BAM recorder tags

Ion Torrent™ technology uses a collection of tags to store sequencing and alignment information useful for downstream processing. In general, these BAM tags starting with Z or Y are written by the BaseCaller module, and BAM tags starting with X stem from TMAP. As a consequence, tags starting with Z or Y are present both in aligned and unaligned BAM files, whereas tags starting with X appear only in aligned BAM files.

BAM tag	Type	Description
XA	Z	The algorithm that produced this mapping and from what stage. The format is the algorithm name and the zero-based stage (separated by a dash).
XM	i	The target length, that is, the number of reference bases spanned by the alignment.
XS	i	The alignment score of the next-best suboptimal mapping.
ZA	i	The number of library insert bases, where the library insert is defined as the sequence after the 5' trimmed region (sequence in tags KS, ZK, ZT, ZE), and before the 3' trimmed region (sequence belonging to the 3' adapter, and the content of the tags SK, YK, YT, YE). Present only if a 3' adapter was found.
ZB	i	The number of overlapping adapter bases. Present only if a 3' adapter was found.)

(continued)

BAM tag	Type	Description
ZC	B:i	A vector of the following four values (present only if a 3' adapter was found): • Field 1: The zero-based flow during which the first base of the adapter was incorporated (same as ZG). • Field 2: The zero-based flow corresponding to the last insert base. • Field 3: Length of the last insert homopolymer. • Field 4: Zero-based index of adapter type found.
ZF	i	The zero-indexed flow position corresponding to the first template base after 5' trimmed region.
ZG	i	The zero-based flow during which the first base of the adapter was incorporated. (Present only if a 3' adapter was found.)
ZM	B:s	Normalized signals, which include phasing effects. Stored as floor(256*value).
ZP	B:f	The estimated phase parameters for the read. The values are stored in the order CF (carry forward), IE (incomplete extension), and DR (droop).
ZT	Z	The trimmed 5’ unique molecular tag sequence. Written only if a tag was trimmed.
YT	Z	The trimmed 3’ unique molecular tag sequence. Written only if a tag was trimmed.
ZE	Z	The 5’ trimmed sequence removed by the `extra-trim-left` command. Written only if a sequence was trimmed.
YE	Z	The 3’ trimmed sequence removed by the `extra-trim-right` command. Written only if a sequence was trimmed.
ZK	Z	The trimmed 5' portion of read group specific identifiers that can vary within a read group. Written only if a tag was trimmed.
YK	Z	The trimmed 3' portion of read group specific identifiers that can vary within a read group. Written only if a sequence was trimmed.

BaseCaller module arguments

The following select arguments are used with the BaseCaller module.

About BaseCaller module parameters

BaseCaller module parameters are provided to reanalyze a completed run. The default BaseCaller module parameters are tuned for Ion Torrent™ sequencing data. In most cases, you do not need to modify these settings.

Modifying these parameters is recommended for advanced users only. If you need to use a user-defined barcode set, contact your Field Service Engineer for assistance. Correct parameter settings require knowledge of the barcodes distances in signal space. The BaseCaller module parameter defaults are optimized for the Ion Xpress™ barcode set, and likely are not correct for a user-defined barcode set.

When you reanalyze a run, other parameters are also listed in the BaseCaller arguments field. These parameters are for internal use; do not change or remove these fields.

Note: Barcode classification is the process by which reads are assigned to one of the barcodes present in one analysis run. Correct barcode classification is important because a classification error results in a read being assigned to the wrong barcode, which in turn leads to the read being analyzed as belonging to a wrong sample.

Barcode classification determines which barcode group a read is assigned to. Barcode classification is done for each read immediately after base calling.

Barcode filtering determines if a specific barcode is included in the run report or is filtered out. Barcode filtering works on the barcode groups as a whole.

Barcode classification parameters

The more common BaseCaller module parameters related to barcode classification are listed and described in the following table. All parameters listed in this table are barcode classification parameters.

Parameter	Default	Description
`--barcode-cutoff`	1.0 (Float)	The maximum distance allowed in barcode matches. A threshold that sets the stringency for barcode matches. Lower values require more exact matches when assigning reads to barcodes. Higher values allow less exact matches. Reads that have a distance greater than this value are counted as barcode no-matches.
`--barcode-mode`	2 (Integer)	The barcode mode. • 0—Classification based on exact barcode base match. • 1—A barcode is scored by comparing each read sequence to each barcode sequence in a flow space alignment. Errors in each flow are summed over the length of the barcode flows. Then any barcode with a number of errors equal to or less than the `--barcode-cutoff` value can be considered, and the barcode with the fewest errors with respect to the input sequence is the matching barcode. • 2—The barcode classification is based on signal information, specifically on the squared distance between the measured signal and the predicted barcode signal.
`--barcode-separation`	2.5 (Float)	This setting controls how much ambiguity in barcode assignment you want to tolerate, by investigating the distances to both the closest barcode and to the next closest barcode. A read is rejected if the difference in these two distances is less than the `--barcode-separation` setting. `--barcode-separation` has no effect when `--barcode-mode` is set to 1.

(continued)

Parameter	Default	Description
`--barcode-filter-postpone`	1 (Integer)	• 0—Keeps the 4.0 behavior: barcode filtering is done independently on each block. • 1—The BaseCaller module does barcode prefiltering at a 10x lower frequency threshold (10 times more lenient). Barcode filtering is done on the full information of the whole chip, after the 96 blocks are merged into one. • 2—The BaseCaller module does not do any barcode prefiltering. All barcode classification happens after the 96 blocks are merged into one. The "2" setting is slower, creates more files, and involves more processing than the "1" setting.
`--barcode-filter`	0.01 (Float)	Barcode frequency threshold to be reported in the user interface. The relative frequency of a barcode is the number of assigned reads divided by the number of reads assigned to the most frequent barcode. 0.0—Off. The setting 0.0 causes all barcodes in the barcode set to be reported in the user interface, including barcodes with no or very few reads, provided that the barcode group has at least `--barcode-filter-minreads` number of reads. Barcodes with no or very few reads typically are not relevant to your analysis and should be filtered out.
`--barcode-filter-minreads`	20 (Integer)	The threshold for the minimum number of reads in a barcode group for that group to be reported in the user interface.

(continued)

Parameter	Default	Description
`--end-barcodes`	on (Boolean)	For dual barcoding runs, specifying "off" disables end barcode classification and uses the start barcodes for read classification instead of both barcodes. For example, for DNA germline or somatic variant calling, dual barcodes are generally not needed. You may also do hybrid analyses with varying limits of detection, that account for fluctuations in read coverage. When the parameter is set to "off": • End barcodes are still searched for and trimmed off the read, if found. • No sk tag is written in the BAM read group heading. • End barcodes, end barcode adapters, or PCR handles are trimmed if they are found and all stored in the YK tag. • Reads not having a YK tag are those that are normally filtered by a dual barcode analysis, such as read where no bead adapter was found. • Using Ion AmpliSeq™ HD chemistry, the tag trimmer options have to be adjusted to keep reads where no end barcode was identified. Use `--tag-filter-method=need-prefix`. • Using Ion AmpliSeq™ HD chemistry, variant caller options have to be adjusted to emulate a normal Ion AmpliSeq™ analysis. Specifically, variant caller needs to be configured to ignore molecular tag information. • End barcode classification is not completely off. Reads where an end barcode was identified that does not match read group expectations, that is, reads that are “certified contamination” are still being filtered and pushed to no-match.
`--trim-barcodes`	on (Boolean)	Trim the barcode and barcode adapter. If off, disables all other 5’ trimming.
`--barcode-adapter-check`	0.15 (Float)	Validate the barcode adapter sequence. The parameter given is the maximum allowed squared residual per flow. This feature reduces cross-contamination, for example, between the Ion Xpress™ Barcode Adapters and IonCode™ Barcode Adapters barcode sets. 0—Off.

Cutoff setting

Notes about setting the `--barcode-cutoff` parameter with `--barcode-mode` set to 1:

- The setting 0 is the most restrictive setting. `--barcode-cutoff` set to 0 allows only reads that perfectly match a barcode in base space.
- The setting 0 works with any barcode set (for both Ion Torrent™ sets and user-defined barcode sets).
- Do not set `--barcode-cutoff` >2 with the Ion Xpress™ Barcode Adapters barcode set. Values >2 relax the classification rules and allow incorrect barcode assignments.

A guideline for the maximum `--barcode-cutoff` setting is based on the minimum distance of the barcode set in flow space.

$$\text{barcode-cutoff} \leq (d_{min} - 1) / 2$$

The minimum distance for the Ion Xpress™ Barcode Adapters barcode set is 5. The maximum recommended value for `--barcode-cutoff` is 2 for analyses that use the Ion Xpress™ Barcode Adapters barcode set.

Separation setting

Notes about setting the `--barcode-separation` parameter.

- Larger values (close to the minimum distance of the code) require more strict matching of the predicted signal for a read to be assigned to a barcode.
- Smaller values (for example, 0.2 and below) allow barcode assignment with an expanded tolerance for errors. For example in the extreme case of separation = 0, the measured signal may be in between two predicted barcode signals.
- If `--barcode-separation` is set at or above the minimum distance of the barcodes in flow space, no reads at all are assigned to a barcode.
- If `--barcode-separation` is set near the minimum distance of the barcodes in flow space, very few reads are assigned to a barcode.
- If `--barcode-separation` is too small, the risk of cross-contamination increases. More ambiguous reads are forced into a barcode assignment (with a higher rate of error in these assignments).

A guideline for a good `--barcode-separation` setting is one half of the minimum distance of the barcode set in flow space:

$$\text{barcode-separation} \approx d_{min} / 2$$

Other public parameters

The BaseCaller module and its parameter settings control these types of filtering:

- Keypass
- Quality trimming
- Adapter trimming

The public BaseCaller module parameters are listed and described in the following table. The defaults for these parameters are optimized for most scenarios and in most cases the default settings are recommended.

Parameter	Default	Description
`-d`, or `--disable-all-filters`	off	When on, disables all filtering and trimming and overrides other filtering and trimming settings.
`-k`, or `--keypass-filter`	on	When on, filters out reads that do not both produce a signal and match the library key (or the test fragment key).

(continued)

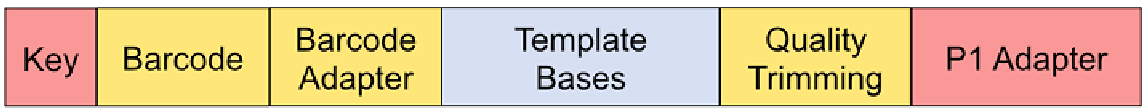
Parameter	Default	Description
`--min-read-length`	25 (integer)	Filters out reads less than this minimum read length. This filter screens out poor reads early on to avoid wasting processing time on them. See also `--trim-min-read-len`, which sets the minimum length threshold that is applied after trimming.
`--prefix-mol-tag`	Empty	Base structure of 5’ unique molecular tag (ACGTN bases) to be trimmed after the barcode adapter. In Torrent Suite™ Software 5.10 and 5.12, specify the read structure via a read structure input file. While this option still exists in the code base and could potentially be used, none of our run plans do so.
`--suffix-mol-tag`	Empty	Base structure of 5’ unique molecular tag (ACGTN bases) to be trimmed before P1 adapter. In Torrent Suite™ Software 5.10 and 5.12, specify the read structure via a read structure input file. While this option still exists in the code base and could potentially be used, none of our run plans do so.
`--extra-trim-left`	0 (integer)	Trims this number of bases beyond the barcode adapter and the 5' unique molecular tag (if applicable).
`--extra-trim-right`	0	Trims this number of bases at the 3' end of the template before the 3' unique molecular tag (if applicable) and the P1 adapter. Only done if the P1 adapter was found.
`--trim-adapter-cutoff`	16 (float)	A score cutoff value. Smaller values correspond to more stringent adapter search and larger values to less stringent adapter search. Set to 0 to turn off.
`--trim-adapter-min-match`	6 (integer)	The minimum number of P1 adapter bases required in order to trim the P1 adapter.
`--trim-qual-window-size`	30 (integer)	Window size for quality trimming.
`--trim-qual-cutoff`	16 (float)	Cutoff for quality trimming. Set to 100 to turn off. When set to 100, no reads are filtered out due to this parameter.
`--trim-min-read-len`	25 (integer)	Filters out any reads that fall below this minimum read length after any trimming step. By default it is initialized with the value of `min-read-length`.

About barcodes

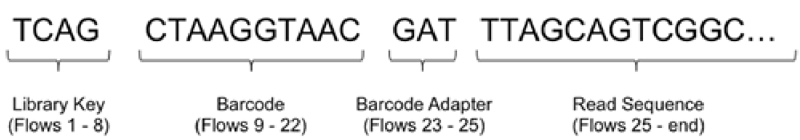
Barcodes are short base sequences that during library preparation are placed between the library key and the read. The barcode sequences provide a mechanism to distinguish and identify reads from different samples during data analysis.

The use of barcodes allows multiple samples to be sequenced together on one chip during a sequencing run, and still have the read data for the run be analyzed separately afterward as distinct samples.

This diagram shows the placement of the barcode sequence, and the library key and adapters, with the read sequence (labeled "Template Bases"). The key is on the 5' end.



This example shows the location of the barcode sequence in both base space and flow space, using barcode IonPress_001 as an example:



Troubleshooting barcode classification issues

Use the BaseCaller JSON output file to troubleshoot barcode classification issues. Barcode classification metrics are available in the `basecaller_results/datasets_basecaller.json` file in the Torrent Suite™ Software analysis directory. This file contains information about all barcodes, whether they appear in the run report or are filtered out.

A sample of this file is shown here, and is used in later examples.


```
{
    "0NCWC.IonHDdual_0101": {
        "Q20_bases": 25,
        "barcode": {
           "barcode_adapter": "",
            "barcode_adapter_filtered": 0,
            "barcode_bias": [
                0.209209978580475,
                -0.0654730796813965,
                -0.0002632737159729,
                0.0154760638251901,
                0.177288889884949,
                0.2724369764328,
                0.00381212681531906,
                0.105516441166401,
                -0.292163729667664,
                0.0684881433844566,
                -0.000637046992778778,
                -0.0821478366851807,
                -0.0467215031385422,
                0.0904117226600647
```

```
            ],
            "barcode_distance_hist": [
                0,
                1,
                0,
                0,
                0
            ],
            "barcode_errors_hist": [
                1,
                0,
                0
            ],
            "barcode_match_filtered": 0,
            "barcode_name": "IonHDdual_0101",
            "barcode_sequence": "CTAAGGTAAC"
        },
        "barcode_name": "IonHDdual_0101",
        "description": "KHAresPanV5bidirhUnewdesignMETCNVladder3",
        "end_barcode": {
            "adapter_filtered": 0,
            "barcode_adapter": "",
            "barcode_errors_hist": [
                1,
                0,
                0,
                0
            ],
            "barcode_filtered": 757,
            "barcode_name": "IonHDdual_0101",
            "barcode_sequence": "TGACTCTATTCG",
            "no_bead_adapter": 17
        },
        "filtered": true,
        "handle": {
            "bc_handle_distribution": [
                0,
                1,
                0
            ],
            "bc_handle_errors_hist": [
                1,
                0,
                0,
                0
            ],
            "bc_handle_filtered": 82,
            "end_handle_distribution": [
                0,
                0,
                1
            ],
            "end_handle_errors_hist": [
                1,
                0,
                0,
                0
            ],
            "end_handle_filtered": 0
        },
        "index": 1,
```

```
        "mol_tag_prefix": "TNNNACTNNNTGAT",
        "mol_tag_suffix": "ATCANNNAGTNNNA",
        "num_blocks_filtered": 96,
        "platform_unit": "s5/540/QPT035/18/DADL00693/IonHDdual_0101",
        "read_count": 1,
        "reference": "hg19",
        "sample": "none",
        "total_bases": 25
    }
}
```


Explanation of fields in the BaseCaller JSON file

The information that follows describes the numbers of barcodes that would be included or discarded if you reanalyze a run with certain changed BaseCaller module settings.

`barcode_bias` field

The `barcode_bias` values show the mean signal deviation by flow, that is, how much of the observed signal is off from the expected signal. Low bias values, for example with the value shown here, are indications of good signal.

Bias values around 0.33 indicate a signal that is about a third of a base off. Values near 0.5 indicate a signal that is half a base off. Values in this range indicate a problem with the sequencing run or with the barcode classification.


```
"barcode_bias": [ 0.026, -0.028, -0.034, 0.011, -0.019, -0.001, 0.072,?
-0.061, 0.103, -0.008, -0.062, 0.110, -0.021, 0.001],
```


Barcode distance histogram

The barcode distance histogram shows, *in signal space*, the number of reads at various squared residual distances between the predicted signal and the observed signal.

The distance fields are given in 0.2 increments:

- The first field gives the number of reads with a squared residual distance of between 0 and 0.2.
- The second field gives the number of reads with a squared residual distance of between 0.2 and 0.4.
- The third field gives the number of reads with a squared residual distance of between 0.4 and 0.6, and so on.

Smaller distances reflect better matches of the read to barcode. Larger distances reflect poorer matches.

This example reflects the pattern that is typical of a real barcode:

- The most reads have shorted distance residuals.
- Fewer reads have larger distance residuals.
- The entry 5342 in the fifth field tells us that reducing `--barcode-cutoff` to 0.8 would cause those 5342 reads not to be assigned to a barcode.


```
"barcode_distance_hist": [ 907546, 50122, 10793, 4498, 5342 ],
```

Barcode errors histogram

The barcode errors histogram shows the number of reads with difference levels of basecalling errors in this barcode:

- The first field gives the number of reads that have 0 basecalling errors (949782 in this example). This is the number of reads that perfectly match this barcode (in base space).
- The second field gives the number of reads that have one basecalling error (24584 in this example).
- The third field gives the number of reads that have two basecalling errors (3935 in this example).

From the 3935 value with 2 basecalling errors, we know that if we reanalyze with the number of allowed errors set to 1 instead of 2, then 3935 fewer reads are assigned to this barcode.

```
"barcode_errors_hist": [ 949782, 24584, 3935 ],
```

This histogram is typical of a real barcode. A large majority of reads are perfect matches, a few have one error, and a smaller number have two errors.

If the pattern is reversed (with very few perfect matches, some reads with one error, and many reads with 2 errors), we suspect that this is probably a fake barcode.

`barcode_match_filtered` field

The `barcode_match_filtered` field gives the number of reads that perfectly match the barcode *in base space*, and also are filtered out because they do not meet the separation criteria *in signal space*. The signal for these reads is in between two barcodes and is not close enough to either barcode to be assigned.

```
"barcode_match_filtered": 162,
```

`filtered` field

The `filtered` field is `true` if this barcode is filtered out and `false` if the barcode appears on the run report.

```
"filtered": false,
```

`read_count` field

The `read_count` field shows how many reads were assigned to this barcode.

```
"read_count": 978301,
```

TMAP modules

The Torrent Mapping Alignment Program (TMAP) is a sequence alignment software program that is optimized specifically for Ion Torrent™ data.

TMAP generally operates in two phases:

- Initial mapping, when the read sequences are located within the reference genome.
- Alignment refinement, when each particular position of the read is aligned to the corresponding position in the reference.

TMAP can run mapping/alignment cycles iteratively, applying different algorithms and parameters to the reads that were not aligned at earlier iterations. In a typical workflow, just one mapping/alignment iteration is used.

TMAP provides several mapping algorithms by using the map1, map2, map3, map4, and mapvsw modules, each with its own best application. The default algorithm is the map4 module.

The alignment refinement phase includes initial alignment using Smith-Waterman or Needleman-Wunch algorithms, and some optional alignment refinement stages that are designed to compensate for specific systematic biases of the sequencing process. Thus:

- The reads can be realigned for better homopolymer alignment (the `--context` option).
- The portions with likely phasing errors can be realigned with low indel scores (the `--do realign` option).
- The long indels at the edges of amplicons can be salvaged (the `--end-repair` option).
- The tandem repeats can be clipped from the read 3' tail (the `--do-repeat-clip` option).
- The alignment can be performed in flowspace instead of basespace (the `--final-flowspace` option). In this case the flow signal obtained from the sequencer is aligned with the estimated flow signal from corresponding zone in the reference genome.

When you reanalyze a run, you can change both the TMAP module and the module parameters. Usually, you do not need to modify the default parameter settings. The default TMAP parameters are tuned for Ion Torrent™ data. Modifying these parameters is for advanced users.

Mapping modules

The mapping alternatives supported by TMAP are listed and described in the following table. The map4 module is the default. Other modules are not run unless specifically called, for instance on the **Reanalyze** screen.

Click the module name link to see the options supported for that module.

Module	Description
"TMAP map1 options" on page 294	BWA-short reads mapping. • Very fast at finding perfect matches. • Very slow at finding a set of matches with up to two mismatches.
"TMAP map2 options" on page 296	BWA-long / BWASW reads mapping.

(continued)

Module	Description
"TMAP map3 options" on page 296	Simplified SSAHA, based on a K-mer lookup table.
"TMAP map4 options" on page 297	Based on the BWA fastmap routine. Searches for the maximum exact matches between the reads and reference.
"TMAP mapvsw options" on page 298	A vectorized implementation of Smith-Waterman. • A single mapping strategy that is twice as fast as the other modules. • Modified to improve specificity.
`mapall`	A tool that allows algorithms from the map1, map2, map3, map4, and mapvsw modules to be combined into read processing pipelines. The tool allows the read mapping process to be organized into a sequence of up to 9 stages that are sequentially applied to each read. If the read is successfully mapped at one stage, the further stages are not applied to it. At each stage, one or more algorithms can be applied. All hits found by any mapping algorithm are processed together. The syntax for `mapall` invocation is: `tmap <global options> mapall stage1 <stage 1 spec> [stage2 <stage2_spec> [stage3 ....]]]` where `stageX_spec` takes the form: `"<stage parameters> mapping_method1 <mapping_method1_params> [mapping_method2 <mapping_method2_params>...]"`.

Find the TMAP command for a specific analysis

For steps to open the run report log and search for the TMAP command, see "TMAP examples" on page 293. The analysis must be completed before you can find the command.

TMAP examples

This example uses TMAP map4 mapping algorithm with default settings.

```
tmap mapall -f /results/referenceLibrary/tmap-f3/hg19/hg19.fasta
-r /<server_path>/results/analysis/output/Home/Auto_user_G35-685--
R65832-110mM_K2SO4-OT_salts-0630_24057_58335/IonXpress_057_rawlib.bam
-v
-Y
-u --prefix-exclude 5
-o 2 stage1 map4
```

The next example is the previous TMAP default setting. This example uses the map1, map2, and map3 modules in that order. Progressively more reads are mapped by each module.

```
tmap mapall -f <FASTA_file>
-v
```

```
-Y
-u --prefix-exclude 5 stage1 map1 map2 map3
```

Global options used by all TMAP modules

Option	Alternate option	Type	Default	Description
`-f`	`--fn-fasta`	File	[no default]	FASTA reference file.
`-r`	`--fn-reads`	File	Standardinput (stdin)	The reads file name.
`-i`	`--reads-format`	String	Unknown	The reads file format (fastq | fq | fasta | fa |sam | bam).
`-s`	`---fn-sam`	File	Standardoutput (stdout)	The SAM file name.
`--bam-start-vfo`	none	Integer	0	Sets the starting virtual file offsets that limit the range of BAM reads to process.
`-A`	`--score-match`	Integer	1	Score for a match.
`-M`	`--pen-mismatch`	Integer	3	Mismatch penalty.
`-O`	`--pen-gap-open`	Integer	5	INDEL start penalty.
`-E`	`--pen-gap-extension`	Integer	2	INDEL extension penalty.
`-G`	`--pen-gap-long`	Integer	-1	Long INDEL penalty.

Global pairing options

Option	Alternate option	Type	Default	Description
`-Q`	`--pairing`	Integer	0	The insert pairing. • 0 = Do not perform pairing. • 1 = Mate pairs (-S 0 -P 1). • 2= Paired end (-S 1 -P 0).

TMAP map1 options

The TMAP map1 module implements BWA-short reads mapping and has these characteristics.

- map1 is very fast at finding perfect matches.
- map1 is very slow at finding a set of matches with up to two mismatches.

The map1 module is not the default for TMAP, map4 is the default.

The following options are supported with the TMAP map1 module (all are optional).

Option	Type	Default	Description
`--seed-length`	Integer	32	The K-mer length to seed CALs. -1 to disable.
`--seed-max-diff`	Integer	2	The maximum number of edits in the seed.
`--seed2-length`	Integer	48	The secondary seed length. -1 to disable
`--max-diff`	Float	0.04	The maximum number of edits or false-negative probability assuming the maximum error rate.
`--max-error-rate`	Float	0.02	The assumed per-base maximum error rate.
`--max-mismatches`	Integer	3	The maximum number of or (read length) fraction of mismatches.
`--max-gap-opens`	Integer	1	The maximum number of or (read length) fraction of INDEL starts.
`--max-gap-extensions`	Integer	6	The maximum number of or (read length) fraction of INDEL extensions.
`--max-cals-deletion`	Integer	10	The maximum number of CALs to extend a deletion.
`--indel-ends-bound`	Integer	5	The number of bps from the end of the read.
`--max-best-cals`	Integer	32	Optimal CALs have been found.
`--max-nodes`	Integer	2,000,000	The maximum number of alignment nodes.
`--min-seq-length`	Integer	-1	The minimum sequence length to examine. -1 to disable
`--max-seq-length`	Integer	-1	The maximum sequence length to examine. -1 to disable

TMAP map2 options

The TMAP map2 module implements BWA-long / BWASW reads mapping.

The map2 module is not the default for TMAP, map4 is the default.

The following options are supported with the TMAP map2 module (all are optional).

Option	Type	Default	Description
`--max-seed-hits`	Integer	1,024	The maximum number of hits returned by a seed.
`--length-coef`	Float	5.5	The coefficient of length-threshold adjustment.
`--max-seed-intv`	Integer	6	The maximum seeding interval size.
`--z-best`	Integer	1	The maximum number of top-scoring nodes to keep on each iteration.
`--seeds-rev`	Integer	5	The number of seeds to trigger reverse alignment.
`--narrow-rmdup`	Integer	false	Remove duplicates for narrow SA hits.
`--max-chain-gap`	Integer	10,000	The maximum gap size during chaining.
`--min-seq-length`	Integer	-1	The minimum sequence length to examine. -1 to disable.
`--max-seq-length`	Integer	-1	The maximum sequence length to examine. -1 to disable

TMAP map3 options

The TMAP map3 module implements a simplified SSAHA, based on a K-mer lookup table.

The map3 module is not the default for TMAP, map4 is the default.

The following options are supported with the TMAP map3 module (all are optional).

Option	Type	Default	Description
`--seed-length`	Integer	-1	The K-mer length to seed CALs. -1 to disable
`--max-seed-hits`	Integer	20	The maximum number of hits returned by a seed.
`--hit-frac`	Float	0.2	The fraction of seed positions that are under the maximum.
`--seed-step`	Integer	8	The number of bases to increase the seed for each seed increase iteration. -1 to disable
`--hp-diff`	Integer	0	The single homopolymer error difference for enumeration.
`--fwd-search`	Boolean	False	Use forward search instead of a reverse search.

(continued)

Option	Type	Default	Description
`--skip-seed-frac`	Float	0.2	The fraction of a seed to skip when a lookup succeeds.
`--min-seq-length`	Integer	-1	The minimum sequence length to examine. -1 to disable
`--max-seq-length`	Integer	-1	The maximum sequence length to examine. -1 to disable

TMAP map4 options

The TMAP map 4 module is based on the BWA fastmap routine, and searches for the maximum exact matches between the reads and reference.

The map4 module is the default for TMAP.

The following options are supported with the TMAP map4 module (all are optional).

Option	Type	Default	Description
`--seed-step`	Integer	8	The number of bases to increase the seed for each seed increase iteration. -1 to disable.
`--hit-frac`	Float	0.2	The fraction of seed positions that are under the maximum.
`--min-seed-length`	Integer	-1	The minimum seed length to accept hits. -1 to disable
`--max-seed-length`	Integer	48	The maximum seed length to accept hits.
`--max-seed-length-adj-coef`	Float	2.0	maximum seed length adjustment coefficient. -1 to disable
`--max-iwidth`	Integer	20	The maximum interval size to accept a hit.
`--max-repr`	Integer	3	The maximum representative hits for repetitive hits.
`--rand-repr`	Integer	false	Choose the representative hits randomly. Otherwise uniformly.
`--use-min`	Boolean	false	When seed stepping, try seeding when at least the minimum seed length is present. Otherwise, use the maximum seed length.
--min-seq-length	Integer	-1	The minimum sequence length to examine. -1 to disable.
--max-seq-length	Integer	-1	The maximum sequence length to examine. -1 to disable

TMAP mapvsw options

The TMAP mapvsw module implements a vectorized implementation of Smith-Waterman.

The mapvsw module is not the default for TMAP, map4 is the default.

The following options are supported with the TMAP mapvsw module (all are optional)

Option	Type	Default	Description
`--min-seq-length`	Integer	-1	The minimum sequence length to examine. -1 to disable
`--max-seq-length`	Integer	-1	The maximum sequence length to examine. -1 to disable

TMAP alignment refinement

The TMAP alignment refinement phase is organized as a pipeline of optional processing stages, each controlled by a specific set of options. The following stages are available (given in the order of their optional invocation).

Stage	Description
Flow space realignment	Performs alignment of the sequence of read flow signals to simulated reference flow signal. Turned on with the `--final flowspace` flag.
Context-based realignment	Realigns read with reduced cost of INDELs within homopolymers. Turned on with the `--context` flag.
Local realignment with reduced gap cost	Realigns specific zones that are likely to contain errors associated with signal phasing using reduced gap cost. This helps to reduce false positive variants in low complexity zones. Turned on with the `--do-realign` flag.
Long INDEL salvage	Realigns zones adjacent to the read ends while allowing for longer indels. Helps to find long indels continued beyond the ends of the amplicons. Turned on by specifying long INDEL gap penalty using the (`-G` option.
End repair	Extends the alignment beyond the amplicon edges, where suitable. Also trims alignment tails below given mismatch ratio. This is a simplified and often better performing version of the `long indel salvage` option. It is turned on by specifying the `--end-repair <MM>` option on the TMAP command line, where `<MM>` is the maximum allowed mismatch percentage at the alignment edge. End repair is typically used together with the `--J` option (max adapter bases for soft clipping), where recommended usage is `--end-repair 25-J 15`).
Bed file	The BED file specification, given with the `--bed-file file_name` option, provides TMAP with the amplicon boundary coordinates. This is used by end-repair to extend gaps over amplicon edges. It also alters the way mapping scores are calculated: the reads mapped within amplicon boundaries are given a 12-point boot in MAPQ, and thus are preferred over the alternative mapping locations outside of the amplicon set.

(continued)

Stage	Description
5' softclip removal	Removes soft clips from the 5' end of the alignment if introduced by end-repair. This option is on by default. Turned off with the `--er-5clip` flag.
3' tandem repeat clipping	Clips tandem repeats from 3' end of the reads. Helps reduce variant detection errors in tandem repeat zones. Turned on with the `--do-repeat-clip` flag.

13 Administer Torrent Suite™ Software

Administrators use the **Admin Interface** application to configure Torrent Suite™ Software, administer Ion Torrent™ Server databases, manage user accounts, back up and restore data, and enable remote monitoring.

An administrator account is required to perform the procedures in this section. A regular user account does not have sufficient privileges to perform these procedures.

Use the Admin Interface application

An administrator can use tools in the **Admin Interface** application to perform software and database administration functions, to monitor the Torrent Suite™ Software, and to configure and modify settings for the software features. The **Admin Interface** application opens in a separate browser window.

IMPORTANT! Use extreme caution when you modify any of the settings in this screen. Settings with incorrect values can corrupt the database or produce unpredictable results. Check with your Field Service Engineer if you want to change any of the settings or complete any of the procedures that are available through this administrative tool.

---

1. In Torrent Suite™ Software, click **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section.
3. Click the **Admin Interface** link to access the database administration application.
4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.
5. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

Install Torrent Suite™ Software

An administrator can use these steps to install Torrent Suite™ Software for the first time on an Ion Torrent™ Server or to reimage a server.

IMPORTANT! To upgrade to a new version of Torrent Suite™ Software, see instead "Update Torrent Suite™ Software" on page 308. During an installation, you must use the same user account for both the Ion Torrent™ Server and the Ion Torrent™ instrument (Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, Ion GeneStudio™ S5 Prime System, or Ion Chef™ Instrument.)

IMPORTANT! Torrent Suite™ Software 5.14 is the last software release that can be used with an Ion PGM™ System or Ion Proton™ System.

---

1. Ensure that there are no active jobs running.
2. In Torrent Suite™ Software, click **(Settings)** ▸ **Configure**.
3. In the **Configure** screen, scroll down to the **Database Administration** section.
4. Click the **Admin Interface** link to access the database administration application.
5. In the **Admin Interface** application, scroll down to the **MANAGEMENT ACTIONS** section, then click **Update Server**.
6. Click **Check**.

7. When the **Available** message displays, click **Update Server** to start the update process.

8. On the **User Agreement (EULA)** screen, scroll down to read and then accept terms to begin the update.

9. When finished, ensure that the ***Upgrade completed Successfully!*** message appears.

10. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

Manage Torrent Suite™ Software user accounts

An administrator can manage Torrent Suite™ Software user accounts from the **Site Administration** screen.

1. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Configure**.

2. In the **Configure** screen, scroll down to the **Database Administration** section.

3. Click the **Admin Interface** link to access the database administration application.

4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.

5. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

User (Staff) versus Administrator (Superuser) roles

In Torrent Suite™ Software, the User (Staff) role allows the creation and execution of Planned Runs on a sequencing instrument.

The Administrator (Superuser) role also allows the creation and execution of Planned Runs, server configuration, user configuration, base caller configuration, reference management, and data management. For more information on Administrator functions, see Chapter 13, “Administer Torrent Suite™ Software”.

Create a new user account

New users can request a user account in the Torrent Suite™ Software sign-in screen. An administrator must approve each request before the new account is active. An administrator account is required to approve or reject each request for a new user account.

When the administrator signs in to Torrent Suite™ Software, a message alerts the administrator that requests are pending for new user accounts.

IMPORTANT! Use extreme caution when you modify any of the settings in this screen. Settings with incorrect values can corrupt the database or produce unpredictable results. Check with your Field Service Engineer if you want to change any of the settings or complete any of the procedures that are available through this administrative tool.

---

1. In the message for the new pending account registration, click **Account Management**.

   Alternatively you can check for new user registrations if you click **(Settings)** ▸ **Accounts**, then go to the **User Registrations** section in the **User Profile/Account Information** screen.

   The **User Registrations** section shows the pending requests for new user accounts.

2. Review the new user registration request, then click **Approve**.

3. In the confirmation message, click **Yes, Approve!**

The new user account is added to the list of user accounts in the **Admin Interface** application.

Modify a user account

An administrator can modify the access level for an existing user using tools in the **Admin Interface** application.

IMPORTANT! Use extreme caution when you modify any of the settings in this screen. Settings with incorrect values can corrupt the database or produce unpredictable results. Check with your Field Service Engineer if you want to change any of the settings or complete any of the procedures that are available through this administrative tool.

---

1. In Torrent Suite™ Software, click **(Settings)** ▸ **Configure**.

2. In the **Configure** screen, scroll down to the **Database Administration** section.

3. Click the **Admin Interface** link to access the database administration application.

4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.

5. In the **Admin Interface** application, in the **AUTHENTICATION AND AUTHORIZATION** section, in the **Users** row, click **Change**.

6. In the **Select user to change** screen, click the **Username** link of the user that you want to change.

   User names can be filtered, selected to the right, according to: **By staff status**, **By superuser status** or **By active** status.

7. In the **Change user** dialog box, modify the user information, such as user name, password, first and last name, email address, or active/inactive status.

8. In the **Permissions** section, confirm that permissions are selected as follows for Staff and Superusers.

Option	Description
Staff (User)	Select the **Active** option.
Superuser (Administrator)	Select the **Active**, **Staff** and **Superuser** options.

WARNING! Do not modify **Groups** or **User permissions** categories. Do not click **Staff** or **Superuser** individually. **Staff** and **Superuser** must both be unchecked for Staff user, but both must be checked for Superuser.

9. Select one of the **Save** options at the bottom of the screen to save your changes.

   The user account is modified.

10. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

Delete a single user account

An administrator can delete a user account, using tools in the **Site administration** screen.

IMPORTANT! Use extreme caution when you modify any of the settings in this screen. Settings with incorrect values can corrupt the database or produce unpredictable results. Check with your Field Service Engineer if you want to change any of the settings or complete any of the procedures that are available through this administrative tool.

1. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section.
3. Click the **Admin Interface** link to access the database administration application.
4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.
5. In the **Users** line of the main **Site administration** menu, click **Change**.
6. On the **Select user to change** screen, click the **Username** of the user to be deleted.
7. At the bottom-left of the **Change user** screen, click **Delete**.

8. In the confirmation message, click **Yes, I'm sure**.

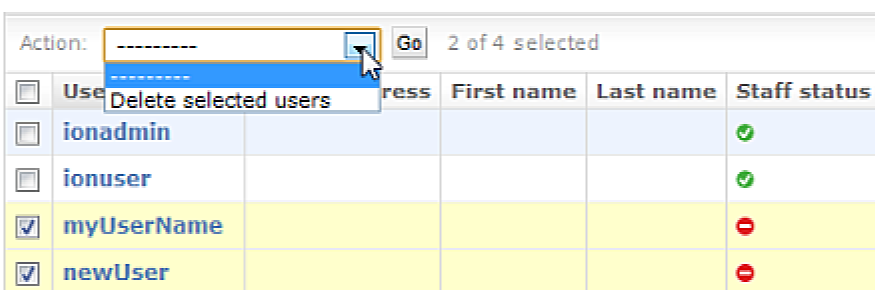
9. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

Delete multiple user accounts

An administrator can delete multiple users using tools in the **Admin Interface** application.

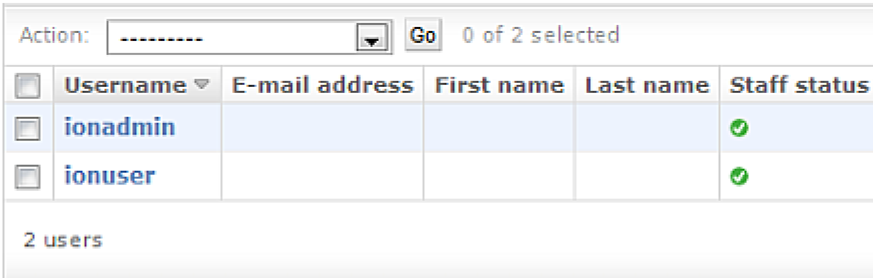
IMPORTANT! Use extreme caution when you modify any of the settings in this screen. Settings with incorrect values can corrupt the database or produce unpredictable results. Check with your Field Service Engineer if you want to change any of the settings or complete any of the procedures that are available through this administrative tool.

1. In Torrent Suite™ Software, click **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section.
3. Click the **Admin Interface** link to access the database administration application.
4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.
5. In the **AUTHENTICATION AND AUTHORIZATION** section, in the **Users** row, click **Change**.
6. On the **Select user to change** screen, check the checkbox for each user that you want to delete.
7. In **Action**, select **Delete selected users**, then click **Go**.



8. Ensure that the list of users that you want to delete is correct by clicking **Yes, I'm sure**.

9. Review the **Select user to change** screen to confirm that the selected users no longer appear on the list.



The accounts are deleted.

10. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

Add support contacts

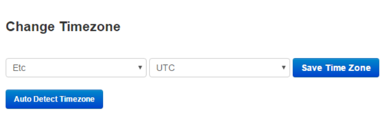
When an administrator is setting up the Torrent Suite™ Software, the administrator can complete the **Customer Support Contact** dialog box with helpful information about how a user can get technical support within the organization.

1. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Configure**.
2. In the **Customer Support Contact** section, add the information for a customer support contact in your organization.
3. In the **IT Contact** section, add the information for an IT contact in your organization
4. Click **Save Contacts**.

Change the time zone for the Ion Torrent™ Server

An administrator can change the time zone for the Ion Torrent™ Server.

1. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Configure**.
2. Scroll to **Change Timezone** section, select a region and a time zone, then click **Save Time Zone**.



Alternatively, you can click **Auto Detect Timezone**, then click **Save Time Zone**.

The new time zone takes effect immediately on the Ion Torrent™ Server.

Change the displayed server name

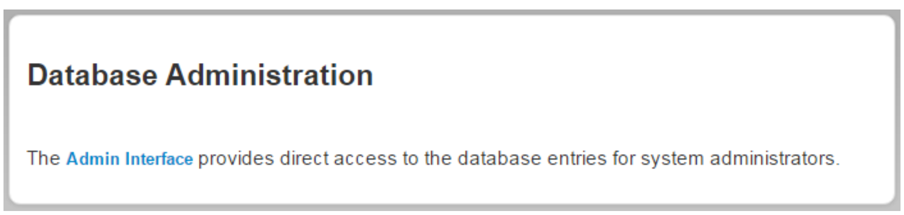
An administrator can change the server name that appears in the Torrent Suite™ Software. By default, this name is Torrent Server. This change affects only the server name that is shown in the Torrent Suite™ Software, and the default bookmark name that appears in the browser when a bookmark is created.

1. In Torrent Suite™ Software, click **(Settings)** ▸ **Configure**.
2. Scroll to the **Customize Site Name** section.
3. Enter the name of your choice, then click **Save Name**.
   The server name that is displayed in the browser is changed.

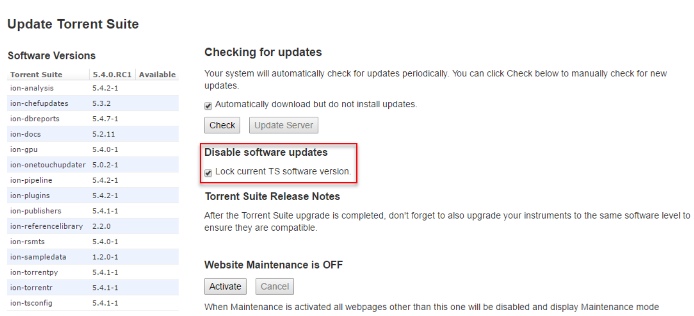
Lock the current Torrent Suite™ Software version

An administrator can prevent other users from installing updates to Torrent Suite™ Software. Use this procedure to lock the current version of software and to prevent accidental software updates.

1. In Torrent Suite™ Software, click **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section.
3. Click the **Admin Interface** link to access the database administration application.
4. Scroll to the **Database Administration** section, then click **Admin Interface**.



5. Scroll to the **MANAGEMENT ACTIONS** section, then click **Update Server**.
   In the **Software Versions** list, the software versions that are currently available are listed. You can click **Check** to see whether updates are available before you lock the current version. For example, **No updates** indicates that updates are not available.



6. Under **Disable software updates**, select the **Lock current TS software version** checkbox.

The software is locked at the current version.

Update Torrent Suite™ Software

The instructions in this section describe how to update Torrent Suite™ Software to a new version.

IMPORTANT! Additional steps and procedures might be required, based on the type of Torrent Suite™ Software upgrade. For complete instructions, see the latest Release Notes on the software product.

IMPORTANT! For compatibility between the software and your instruments, you must also upgrade your instruments after the upgrade is complete.

Update Torrent Suite™ Software

An administrator can use this procedure to update the Torrent Suite™ Software on the Ion Torrent™ Server. Software updates cause the software web services to restart.

IMPORTANT! You cannot roll back to a previous version of Torrent Suite™ Software and Ion Torrent™ instrument software after you perform the upgrade.

In order for the software to remain compatible with instrument control software, ensure that you upgrade sequencing instruments after the Torrent Suite™ Software upgrade is complete. During an upgrade, you must use the same user account for both the Ion Torrent™ Server and the Ion Torrent™ instrument (Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, Ion GeneStudio™ S5 Prime System, or Ion Chef™ Instrument.)

1. In Torrent Suite™ Software, click **(Settings)** ▶ **Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section.
3. Click the **Admin Interface** link to access the database administration application.
4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.
5. In the **Admin Interface** scroll to the **MANAGEMENT ACTIONS** section, then click **Update Server**.

   The **Update Torrent Suite** screen opens with information about available software versions, including whether updates are available.
6. Click **Activate** to power on website maintenance.
7. Click **Check** to check for updates.
8. Click **Update Server** to update software on the server.
9. When you finish with the changes, click **LOG OUT** to close the **Admin Interface** application and return to the Torrent Suite™ Software.

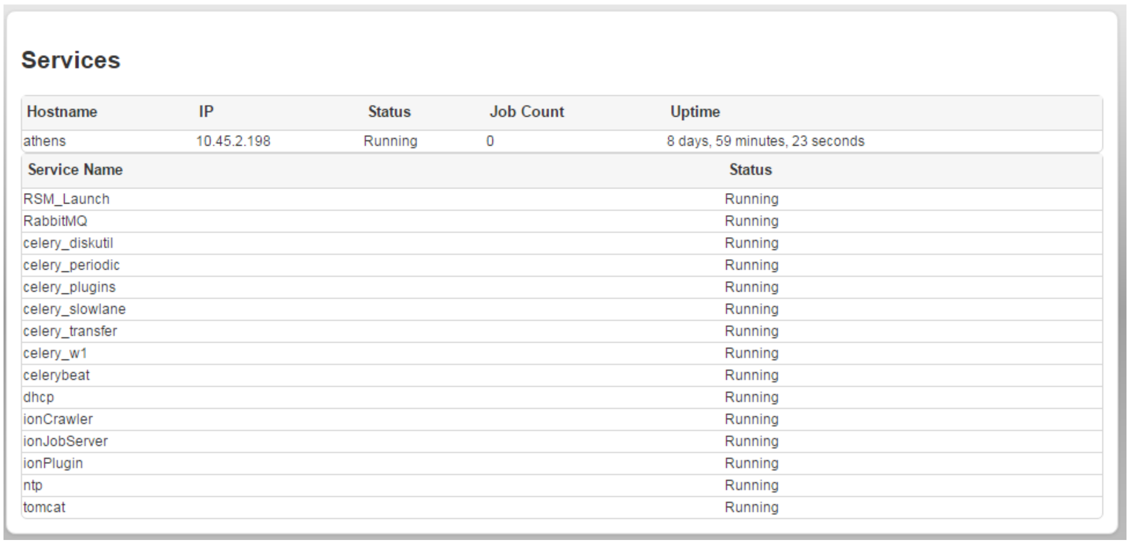
10. Click **(Settings)** ▸ **About**.

11. In the Releases list, review the software version number to verify that the number reflects the version of the update.

12. Review the software upgrade version of services to help ensure that the software is ready to run analysis programs.

    a. Click **(Settings)** ▸ **Services**.

    b. In **Services** screen in the **Status** column, review all services to ensure that each is running.



Services

Hostname	IP	Status	Job Count	Uptime
athens	10.45.2.198	Running	0	8 days, 59 minutes, 23 seconds

Service Name	Status
RSM_Launch	Running
RabbitMQ	Running
celery_diskutil	Running
celery_periodic	Running
celery_plugins	Running
celery_slowlane	Running
celery_transfer	Running
celery_w1	Running
celerybeat	Running
dhcp	Running
ionCrawler	Running
ionJobServer	Running
ionPlugin	Running
ntp	Running
tomcat	Running

Ensure that you upgrade sequencing instruments after the Torrent Suite™ Software upgrade is complete so that the software remains compatible with instrument control software.

IMPORTANT! The instruments cannot obtain the correct on-instrument analysis if both systems are not updated.

Check for off-cycle updates

To check if there are any newer updates for your products, plugins, or instrument, use the **Updates** screen.

To update Torrent Suite™ Software, see "Update Torrent Suite™ Software" on page 308.

1. Click ⚙ **(Settings)** ▸ **Updates**.
2. On the **Updates** screen, compare your installed versions with the available versions to determine if any updates listed are relevant to your work.
3. Perform the desired updates.
   - To update products, see "Enable off-cycle product updates" on page 310.
   - To update plugins, see "Update off-cycle release plugins" on page 312.
   - To manually install update files, see "Install off-cycle bundles without Internet access" on page 310.

Enable off-cycle product updates

An administrator can add new kits, chips, templates, plugins and Ion Chef™ scripts that are released outside of the regular software release cycle.

When you learn of a new product that you would like to use, check to see if a software update is available.

1. Click ⚙ **(Settings)** ▸ **Updates**.
2. In the **Update Products** section, select the desired new product, then click **Update**.
   Your installed version of Torrent Suite™ Software is updated to include the new products that you selected.

Install off-cycle bundles without Internet access

If the site Ion Torrent™ Server is not connected to the Internet, an administrator can manually install updates.

1. Request a USB device that contains the updates from your local Field Service Engineer or Technical Support.
2. Insert the USB device into your Ion Torrent™ Server.
3. In Torrent Suite™ Software, click ⚙ **(Settings)** ▸ **Updates**.
4. On the **Updates** screen, under **Manual Upload**, click **Click to Upload and Install**.
5. Follow the prompts to upload the compressed folder.

Install or upgrade plugins

On Thermo Fisher™ Connect Platform, an administrator can install or upgrade the following plugins.

- Upgrades for plugins that are preinstalled in Torrent Suite™ Software. For details about plugins that are preinstalled with the software, see "Preinstalled plugins" on page 130.
- The RNASeqAnalysis plugin. This plugin is supported by Thermo Fisher Scientific but is not preinstalled in the Torrent Suite™ Software. You can download the Ion-supported RNASeqAnalysis plugin if you click **View all apps**, then click the **Plugins** resource library when you are signed into an account on Connect Platform.

1. Navigate to Connect Platform at **thermofisher.com/connect**.
2. Click **Sign in** the upper right corner.
3. Click the **Apps** icon ().
4. In **AppConnect**, under **Resource Libraries**, click **Plugins**.
5. *(Optional)* Click a category at the top of screen.
   The list of plugins is narrowed to include only the plugins that are in the selected category.
6. Click to download the plugin. Select the checkbox to indicate that you agree to the terms and conditions, then click **Download Plugin**.
   Either a compressed directory or a debian file that contains the plugin is downloaded to your local machine.
7. In Torrent Suite™ Software, click **(Settings)** ▸ **Plugins** ▸ **Install or Upgrade Plugin**.
8. Click **Select File**, browse to the location where you downloaded the plugin file, select the file, then click **Open**.
9. In the **Install or Upgrade Plugin** dialog box, click **Upload and Install**.

The plugin is now visible in Torrent Suite™ Software.

Enable an installed plugin

After a plugin is installed, an administrator must enable the plugin to make the plugin available in Torrent Suite™ Software.

1. Click **(Settings)** ▸ **Plugins**.
2. In the **Plugins** list, in the **Enabled** column, select the checkbox next to any installed plugin to enable the plugin in the software.
   The plugin is immediately available in the software.

Update off-cycle release plugins

An administrator can add new plugins that are released outside of the regular software release cycle.

When you learn of an updated plugin that you would like to use, check to see if the update is available to install.

1. Click ⚙ **(Settings)** ▸ **Updates**.
2. Scroll to the **Update Plugins** section.
3. Select the new Torrent Suite™ Software plugin that you want to install, then click **Update**.
   Your installed version of Torrent Suite™ Software is updated to include the new plugin that you selected.

Uninstall a plugin

An administrator can uninstall a plugin from Torrent Suite™ Software.

1. Click ⚙ **(Settings)** ▸ **Plugins**.
2. In the row of the plugin that you want to remove, click ⚙ **(Actions)** ▸ **Uninstall**.
3. Click **Yes, Uninstall!** to confirm that you want to uninstall the selected plugin.

Manage disk usage

It is critical that sufficient disk space is available on the server to store data files and avoid data loss. It is important to have a strategy that periodically monitors disk space and archives or deletes data to allow for storage of new data files.

To avoid data loss and to ensure that sufficient disk space is available on the server, an administrator can configure Torrent Suite™ Software to automatically archive and delete sequencing data that are no longer needed. An administrator can also manually archive or delete data from individual run reports or groups of reports, or export selected data to a mounted external drive. To understand how disk space is allocated and how files are managed, you can view disk usage, active data management jobs, statistics, and detailed logs on each of these activities.

View disk usage parameters

1. In the **Data** tab, click **Data Management**.
2. Scroll to the **Disk Usage** section to review the disk usage parameters.
3. Review the disk usage information

   Parameters in the Disk Usage section.

Parameter	Definition
Keep	File space devoted to files that are to be kept.
Used	File space currently used by data files.
Free	Space available for storing data files.
Threshold I	Threshold above which intermediate files are deleted or archived, based on the automatic configuration settings.
Threshold S	Threshold above which signal processing input files are deleted or archived, based on the automatic configuration settings.
Threshold O	Threshold above which output files are deleted or archived, based on the automatic configuration settings.
Threshold B	Threshold above which basecaller input files are deleted or archived, based on the automatic configuration settings.

   For details about automatic deletion and archive creation, see "Archive or delete data automatically" on page 314.

Ion instrument data types

Data that are generated from Ion Torrent™ sequencers consists of the following types of files:

- Signal processing input files (DAT)
- Basecalling input files (1.WELLS)
- Output files (BAM, plugin output, and so forth)
- Intermediate files

For more information about these file types, see "Analysis pipeline overview" on page 278.

Recommendations on when to archive each file type are listed in the following table.

Data type	Details
Signal processing input files	Signal processing input files (4 files per cycle) consist of the raw voltage measurement data collected during the sequencing run. Signal processing input data are deleted from the Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, and Ion GeneStudio™ S5 Prime System. These files are available in Torrent Suite™ Software only as thumbnails. **IMPORTANT!** If you plan to reanalyze a run starting from raw signal processing data, keep the signal processing input files.
Basecalling input files	Signal processing input files are converted to a single condensed basecalling input file representing the processed signal. Keep or archive these basecalling input data to reanalyze the run. This can save time and resources because reanalysis use the basecalling input data, rather than the raw signal processing input data. Basecalling input data are transferred to the Torrent Suite™ Software and are available for use in reanalysis.
Output files	Output files consist of all BAM files, run reports, and plugin results. It is important to keep and archive these files. Delete output files only if you are sure that you no longer need the files.
Intermediate files	Intermediate files contain information used for debugging runs. You can delete these files immediately after instrument runs, without affecting data.

Archive or delete data automatically

To avoid data loss, it is critical that sufficient disk space is available on the Ion Torrent™ Server. Therefore, it is important to have a strategy to monitor disk space and archive or delete data.

An administrator can configure the Ion Torrent™ Server to archive data to a mounted drive automatically after a data age threshold is met. Data that is assigned to be automatically archived are copied to the designated location, then deleted from the Ion Torrent™ Server. Automatic archiving helps to maintain available disk space and simplifies management of data that you want to save to another volume.

An administrator can also configure the Ion Torrent™ Server to delete data automatically when thresholds are met for filled disk space and data age. Automatic deletion of files is important to maintain available disk space and simplifies removal of data that are no longer necessary to keep.

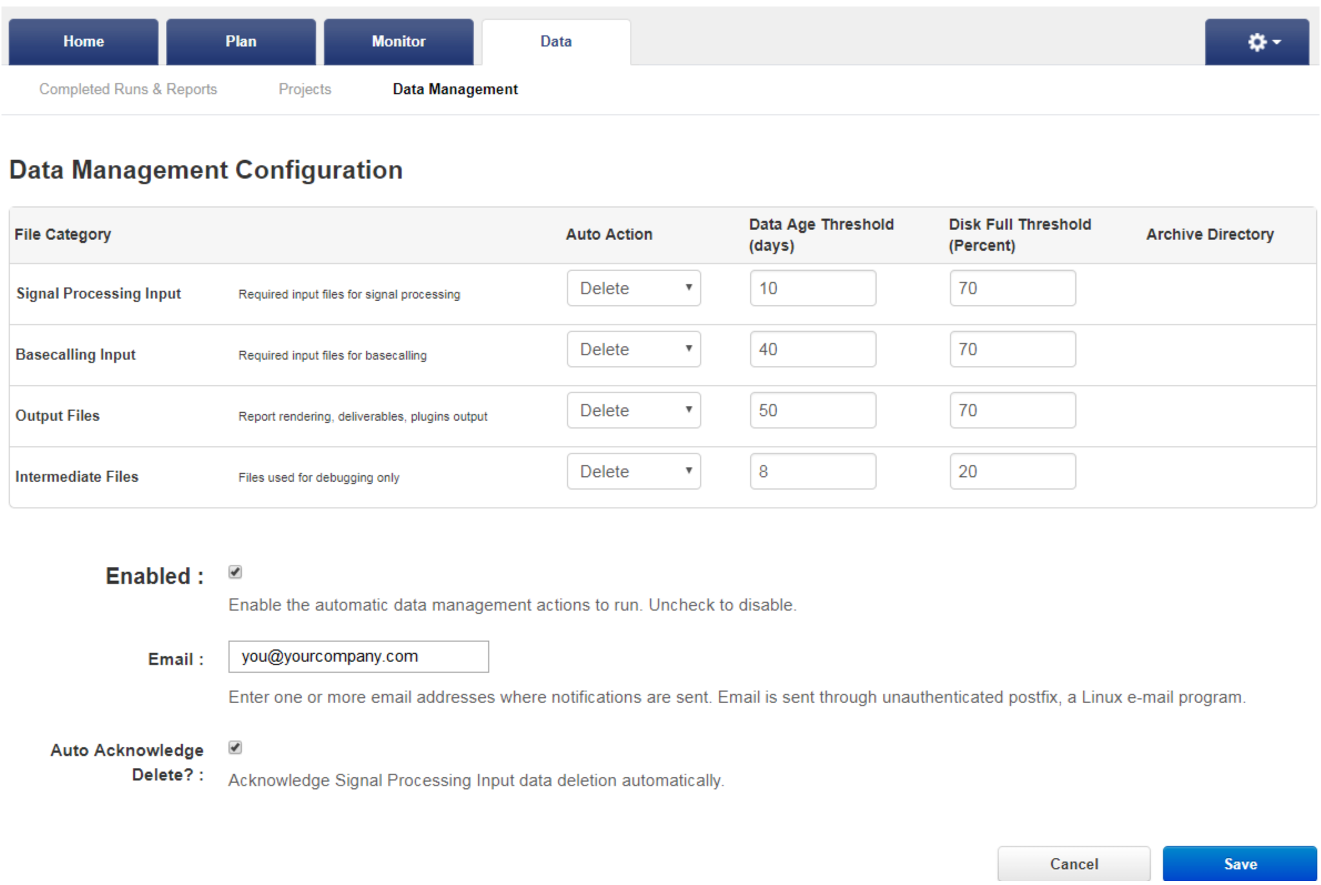
An administrator can also assign automated archive or delete actions to each data file category independently of the others.

IMPORTANT! When you configure the Ion Torrent™ Server to delete data automatically, the data are permanently deleted. You cannot restore data after deletion.

---

1. In the **Data** tab, click **Data Management**, go the **Configuration** section, then click **Configure**.

2. On the **Data Management Configuration** screen, select an auto-action, or select **Disabled** for each file category.



3. Configure the options.

If you select this auto action	Select these options
Disabled	No selections are necessary. Data in the file category must be archived or deleted manually.
Archive	• **Data Age Threshold (days)**—Set the number of days that you want data to be stored on the server before data are archived. • **Archive Directory**—Select the mounted volume where you want to store the archive, or click **Browse**, then navigate to the mounted volume where you want the archive to be stored.
Delete	• **Data Age Threshold (days)**—Set the number of days that data are stored on the server before data are deleted. • **Disk Full Threshold (Percent)**—Set the percentage of disk space that is filled on the server before data are deleted.

4. Select the **Enabled** checkbox to enable the automatic data management that you have configured. Deselect the checkbox to suspend automatic action.

5. In **Email**, enter an email address for who should receive notifications for automatic data management actions.

   If you use a Linux™ mail server, you might have access to Postfix, an open-source Linux™ mail server. Postfix has many configuration options that IT administrators can use to adjust mail routing parameters. You can find Postfix documentation at **http://www.postfix.org/documentation.html**.

6. *(Optional)* To enable auto-acknowledgement of deletion of signal processing input data, select the **Auto Acknowledge Delete?** checkbox. Action is not required for data deletion to occur.

   If you deselect **Auto Acknowledge Delete**, notifications are sent for each signal processing input deletion. A reviewer must manually acknowledge each deletion action before the signal processing input data are deleted.

7. After you have completed the configuration, click **Save**.

8. *(Optional)* On the **Data Management** screen, in the **Configuration** section, click **Configuration Log** to view a record of configuration changes.

9. *(Optional)* To view a record of data management actions (archiving and deleting), click **History**.

Manually export run data

An administrator can manually export run data to a storage device that is mounted on the Ion Torrent™ Server. When you export the data, it is copied from the Ion Torrent™ Server to the archive location. The data remains on the Ion Torrent™ Server, and the run results listed in the **Completed Runs & Results** screen continue to link to the data on the Ion Torrent™ Server. For more information, see “Increase file storage and available disk space” on page 319.

1. In the **Data** tab, click **Data Management**, then scroll to the **Disk Space Management** section.
2. Select the checkboxes to the left of the report names that contain the run data that you want to export, then click **Process Selected**.

   To export data from only one run report, click **(Settings)** ▸ **Actions** to the right of the report name.
3. In the dialog box, click the checkbox to the left of each **File Category** for the type of data that you want to export, then click **Export Selected**.
4. Click **Browse** to select an export directory from the list of mounted storage devices.
5. *(Optional)* Enter a comment.
6. Click **Confirm**.

   The data for the file categories of the selected run reports are copied to the external hard drive. The data are also available in the local hard drive run results directory.

Manually archive run data

An administrator can manually archive run data from the local drive on the Ion Torrent™ Server to a connected storage device. When you archive the data, it is moved from the Ion Torrent™ Server to the archive location. The run results listed in the **Completed Runs & Results** screen link to the data on the archive storage device as long as that device remains mounted on the Ion Torrent™ Server. For details, see “Increase file storage and available disk space” on page 319.

1. In the **Data** tab, click **Data Management**, then scroll to the **Disk Space Management** section.
2. Select the checkboxes to the left of the report names that contain data that you want to archive, then click **Process Selected**.

   To archive data from only one run report, you can alternatively click **(Settings)** ▸ **Actions** to the right of the report name.
3. In the dialog box, click the checkbox to the left of each **File Category** for the type of data that you want to archive, then click **Archive Selected**.
4. Click **Browse** to select an archive directory from the list of mounted storage devices.
5. *(Optional)* Enter a comment.
6. Click **Confirm**.

   The data in the file categories of the selected run reports are moved to the archive location.

Manually delete selected data from a run report

An administrator can manually delete selected data from a run report to free up storage space on the Ion Torrent™ Server. This procedures deletes the files that are associated with the run report that were created during the run. These file types are as follows.

- Signal processing input files
- Basecalling input files
- Output files
- Intermediate files

For details about automatic deletion of selected run data, see "Archive or delete data automatically" on page 314.

IMPORTANT! Use this procedure only if you are sure that you no longer require access to the selected run data. If a run is archived, deleting the data removes the archived copy when the archived drive is mounted. If a run was previously imported or restored, deleting removes only the local copy."

---

1. In the **Data** tab, click **Data Management**, then scroll to the **Disk Space Management** section.
2. Do one of the following:
   - To delete selected data from more than one run report, select the checkboxes to the left of the report names that contain the run data that you want to delete, then click **Process Selected**.
   - To delete data from only one run report, click **(Settings)** ▸ **Actions** to the right of the report name.

   If **Keep** is enabled, the data cannot be deleted and are kept on the local hard drive. If you try to delete this data, an error occurs.
3. In the dialog box, select the checkbox to the left of each **File Category** for the type of data that you want to delete, then click **Delete Selected**.
4. *(Optional)* Enter a comment.
5. Click **Confirm**.
   The data for the file categories of the selected run reports is permanently deleted from the Ion Torrent™ Server.

Increase file storage and available disk space

An administrator can increase file storage space for data and results files with a network access storage (NAS) device or with a USB drive.

After one or more of these storage drives has been installed and configured, an administrator can use the drive to save data locally in the laboratory, transfer data between servers, store data with disk failure tolerance, and expand storage space.

Storage method	Description
NAS device	A system administrator can install a NAS device directly on an Ion Torrent™ Server, an Ion GeneStudio™ S5 System, or an Ion GeneStudio™ S5 Prime System. The administrator can also connect over a network to the Ion Torrent™ Server. After installation, the device must be mounted on the local server as described in "Connect to a NAS device" on page 319.
USB Drive	A USB drive attaches directly to an Ion Torrent™ Server, an Ion GeneStudio™ S5 System, or an Ion GeneStudio™ S5 Prime System. After installation, the device must be mounted on the local server as described in "Mount a USB drive" on page 321.

Connect to a NAS device

Initial setup of a NAS device is provided by your system administrator. If the device is moved or disconnected for any reason (for example, a power outage), an administrator can reconnect the device to Ion Torrent™ Server.

- If the NAS device connects directly to the Ion Torrent™ Server, see "Connect directly to a NAS device" on page 319.
- If the NAS device connects over a network to the Ion Torrent™ Server, see "Connect over a network to a NAS device" on page 320.

Connect directly to a NAS device

If the Ion Torrent™ Server, Ion GeneStudio™ S5 System, or Ion GeneStudio™ S5 Prime System is connected directly to a NAS device, an administrator can use the following steps to mount the device.

1. Click **(Settings)** ▸ **About**, then confirm that the Torrent Suite™ Software version is 5.2 or later.

   To update your software, see "Update Torrent Suite™ Software" on page 308.

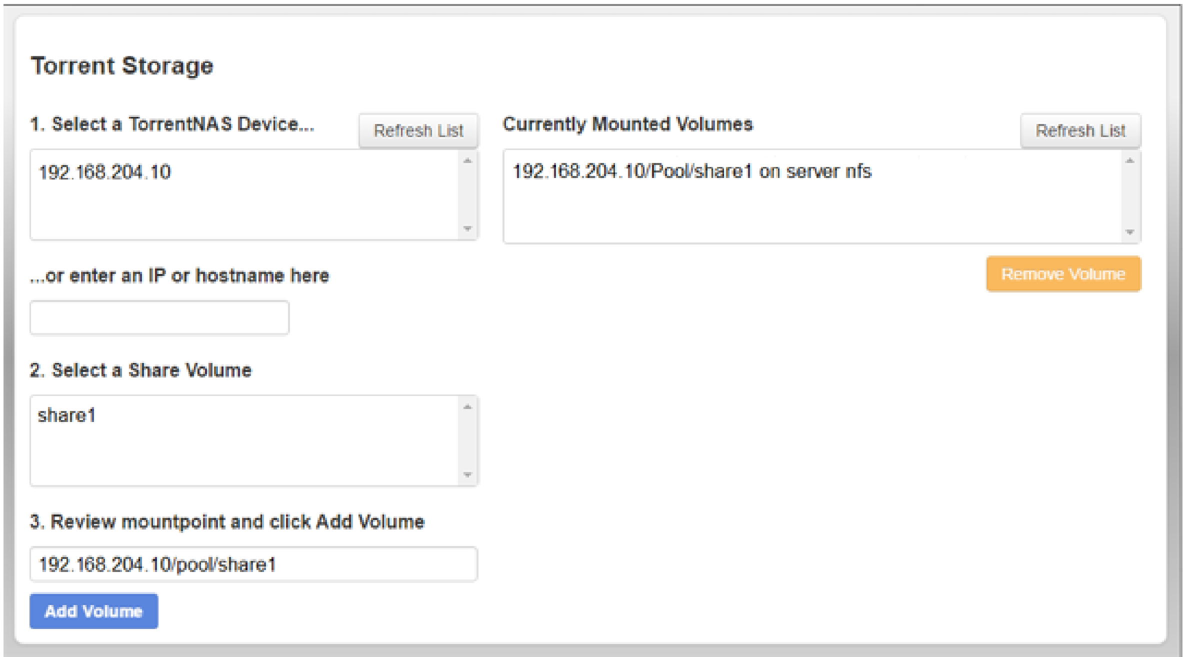
2. Click **(Settings)** ▸ **Configure**, then scroll to **Torrent Storage**.

3. Find the IP address of the NAS device in the **Select a TorrentNAS Device** list. It can take several seconds for the list to populate.

   If the NAS device is not automatically detected in <1 minute, confirm that the correct network ports are connected, then click **Refresh List**.

4. In the **Select a TorrentNAS Device** list, select the IP address of the device.

5. Under **Select a Share Volume**, select a volume on the device, then click **Add Volume**.



The storage volume is connected to the server and is listed in the **Currently Mounted Volumes** list.

Connect over a network to a NAS device

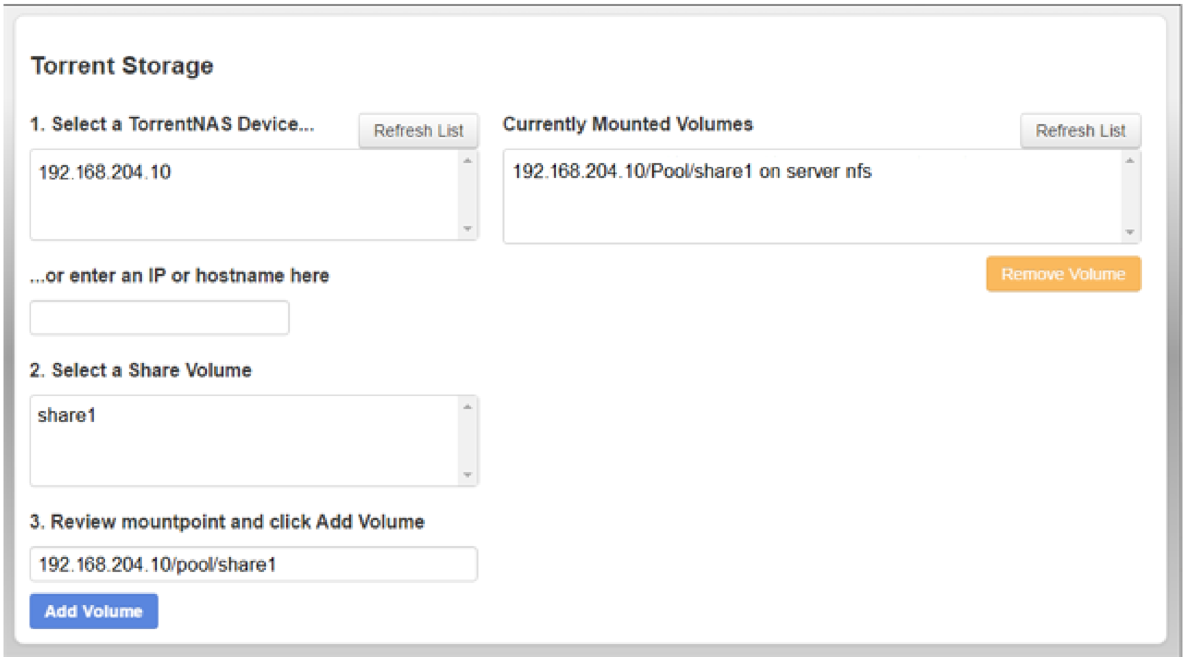
If the Ion Torrent™ Server, Ion GeneStudio™ S5 System, or Ion GeneStudio™ S5 Prime System is installed on the same network as the NAS device, an administrator can use the following steps to mount the device.

1. Click **(Settings)** ▸ **About**, then confirm that the Torrent Suite™ Software version is 5.2 or later.
   To update your software, see "Update Torrent Suite™ Software" on page 308.

2. Click **(Settings)** ▸ **Configure**, then scroll to **Torrent Storage**.

3. In **or enter an IP or hostname here**, enter the IP address of the NAS device, then press the **Enter** key.

4. In **Select a Share Volume**, select a volume on the device, then click **Add Volume**.



The storage volume is connected to the server and is listed in **Currently Mounted Volumes**.

Mount a USB drive

To mount a USB drive (either an external hard drive or large flash drive), a working knowledge of Linux™ command line interface and a basic understanding of disk drives and partitions are necessary.

Ion Torrent™ Server is an Ubuntu™ server, which does not mount external hard drives automatically. To address this need, the `ion-usbmount` utility is included with Torrent Suite™ Software. This utility automatically mounts attached USB drives in the `/media` directory. If `ion-usbmount` does not mount a particular USB drive automatically, follow these steps to mount the drive manually.

These instructions provide only an overview of the required steps, and can be a helpful reminder if you are new to the Linux™ operating system. For more detailed instructions and background information, see the Ubuntu™ documentation at **https://help.ubuntu.com/community/Mount/USB**.

We recommend that a system administrator perform the Linux™ mount and unmount procedures.

1. Before connecting a USB drive, enter the following command to see a list of the drives in the system: `sudo fdisk -l`
   The local hard drive usually has a name such as `/dev/sda`, as in the following example:

```
<yourusername>@itw-test01: ~$ sudo fdisk -l

Disk /dev/sda: 500.1 GB,
500107862016 bytes
255 heads, 63 sectors/track, 60801
cylinders
Units = cylinders of 16065 * 512 =
8225280 bytes
Sector size (logical/physical) : 512
bytes / 512 bytes
```

```
Disk identifier: 0x0004366b

Device Boot Start End
Blocks Id System
/dev/sdal * 1 37
291840 83 Linux
Partition 1 does not end on
cylinder boundary.
/dev/sda2 37 60802
488092673 5 Extended
/dev/sda5 37 60802
488092672 8e Linux LVM
```

2. Connect the USB drive.

3. Wait approximately 10 seconds, then reenter: `sudo fdisk -l`.
The new USB drive appears in the list. The name of the USB drive is usually `/dev/sdb` or `/dev/sdc`, based on the number of drives installed. The partition is a number that is added to the name of the physical drive. For example, the first partition on drive `/dev/sdc` would be called `/dev/sdc1`. In the following example, there is a 2-GB partition (1953512001 blocks) attached to the system that is named `/dev/sdb1`. It is configured with a Linux™ partition. (If the drive was formatted on Windows™, it is either a FAT or an NTFS partition).

```
<yourusername>@itw-test01:/$ sudo fdisk -l

Disk /dev/sda: 500.1 GB,
500107862016 bytes
255 heads, 63 sectors/track,
60801 cylinders
Units = cylinders of 16065 *
512 = 8225280 bytes
Sector size
(logical/physical): 512 bytes
/ 512 bytes
I/O size (minimum/optimal) :
512 bytes / 512 bytes
Disk identifier: 0x0004366b

Device Boot Start End
Blocks Id System
/dev/sdal * 1 37
291840 83 Linux
Partition 1 does not end on
cylinder boundary.
/dev/sda2 37 60802
488092673 5 Extended
/dev/sda5 37 60802
488092672 8e Linux LVM

Disk /dev/sdb: 2000.4 GB,
2000398934016 bytes
255 heads, 63 sectors/track,
243201 cylinders
Units = cylinders of 16065 *
512 = 8225280 bytes
Sector size
(logical/physical): 512 bytes
/ 512 bytes
```

```
I/O size (minimum/ optimal) :
512 bytes / 512 bytes
Disk identifier: 0x5786fcfb

Device Boot Start End
Blocks Id System
/dev/sdb1 1 243201
1953512001 83 Linux
```

4. If the drive has a Windows™ FAT or NTFS partition, reformat the drive as an ext3 partition to preserve the Linux™ file information.

   IMPORTANT! Be careful to format the correct hard drive.

   a. Enter `sudo mkfs.ext3 <your_device>`. For example:

      ```
      sudo mkfs.ext3 /dev/sde5
      ```

   b. Label the partition on the external USB drive. To label the partition, enter the following:

      ```
      sudo e2label <your_device_place> <partition_label>.?
      ```

      For example, the external drive that is connected in /dev/sdbl is labeled as `TS_Backup1`:

      ```
      sudo e2label /dev/sdb1 TS_Backup1
      ```

      It is important to provide a different label name to each partition to avoid error when multiple external USB drives are connected to the Ion Torrent™ Server at the same time.

5. Ensure that the external USB drive mounts automatically. Disconnect the external USB drive, then reconnect it. Wait approximately 10 seconds.

   The external USB drive appears under the **Services** tab in Torrent Suite™ Software

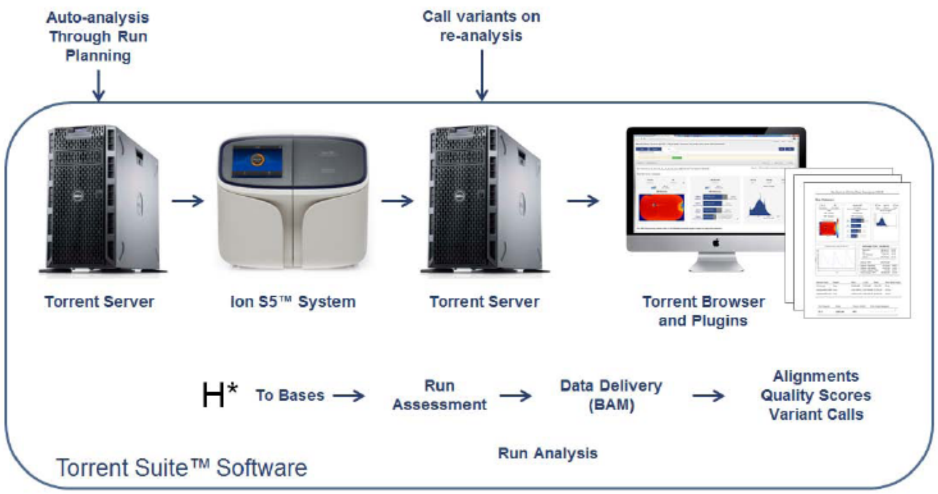
Unmount a USB drive

IMPORTANT! Before disconnecting a USB drive, unmount it first to ensure that all data have been written to disk. If you pull out the USB connection without unmounting the USB drive first, there is a high risk of data loss.

To unmount a USB drive, enter the following command in the command line of your Ion Torrent™ Server: `sudo umount /dev/sdb1 /media/external`.

Dataflow file sizes

The Ion Torrent™ dataflow involves the transfer of raw sequencing data from the Ion Chef™ Instrument or Ion GeneStudio™ S5 Series sequencer to the Ion Torrent™ Server for analysis and reporting.



The following tables show high-level views of the dataflow from different devices.

Dataflow using the 200-bp kit on Ion GeneStudio™ S5 Series sequencers

The following table shows a high-level view of the dataflow using the 200-bp kit on the Ion GeneStudio™ S5 Plus System, Ion GeneStudio™ S5 Prime System, and Ion GeneStudio™ S5 System. File sizes for Ion Torrent™ data depend on the Torrent Suite™ Software version, chip type, and kit type.

Step	Resulting file type	Ion 510™ Chip	Ion 520™ Chip	Ion 530™ Chip	Ion 540™ Chip	Ion 550™ Chip	Ion 560™ Chip
Read capacity	—	2.5 M	5 M	15–20 M	60–80 M	100–130 M	240–280 M
Signal processing input[1]	DAT	65 GB	125 GB	310 GB	2 TB	1 TB	1.8 TB
Signal processing output	WELLS	9 GB	18 GB	45 GB	180 GB	330 GB	900 GB
Base calling output	Unaligned BAM	3 GB	8 GB	23 GB	85 GB	100 GB	200 GB
Aligned output	Aligned BAM	3 GB	7 GB	20 GB	55 GB	90 GB	150 GB

[1] Signal Processing input files for Ion GeneStudio™ S5 Series sequencers are not transferred from the instrument and are not part of Ion Torrent™ Server data management.

Dataflow using the 400-bp kit on Ion GeneStudio™ S5 Series sequencers

The following table shows a high-level view of the dataflow using the 400-bp kit on the Ion GeneStudio™ S5 Plus System, Ion GeneStudio™ S5 Prime System, and Ion GeneStudio™ S5 System. File sizes for Ion Torrent™ data depend on the Torrent Suite™ Software version, chip type, and kit type.

Step	Resulting file type	Ion 510™ Chip	Ion 520™ Chip	Ion 530™ Chip
Read capacity	—	2.5 M	5 M	15–20 M
Signal processing input[1]	DAT	110 GB	210 GB	530 GB
Signal processing output	WELLS	15 GB	30 GB	75 GB
Base calling output	Unaligned BAM	6 GB	12 GB	30 GB
Aligned output	Aligned BAM	5 GB	10 GB	25 GB

[1] Signal Processing input files for Ion GeneStudio™ S5 Series sequencers are not transferred from the instrument and are not part of Ion Torrent™ Server data management.

Set up flexible workflows

An administrator can set up flexible workflows by forming a connection between two or more Ion Torrent™ Servers that are running the same software version.

With flexible workflows, you can:

- View all runs of interest across multiple Ion Torrent™ Servers on the **Completed Runs and Reports** screen.
- Transfer Planned Runs between sequencers that are connected to different Ion Torrent™ Servers.
- Track reagent and cartridge usage across Ion Chef™ Instrument flexible workflows from different Ion Chef™ Instruments connected to different Ion Torrent™ Servers.

1. In any tab, click ⚙ **(Settings)**, then click **Ion Mesh**.
2. Select one of the following options.

Option	Description
Link Selected TS	Select an Ion Torrent™ Server, then link the selected server.
Link TS Manually	Manually link an Ion Torrent™ Server.

3. Enter the following information in the **Setup Mesh Computer** dialog box.

Option	Definition
Hostname/Address	Host name or address of the server. If linking a selected Ion Torrent™ Server, this option is automatically populated and cannot be edited.
Nickname	A common name that is assigned to the Ion Torrent™ Server.
Username	Your username.
Password	Your password.

4. Click **Setup**.
   The linked and enabled Ion Torrent™ Servers are available to be used in the Planned Runs, and the data from the linked and enabled Ion Torrent™ Servers can be viewed on the same **Data** screen.

View sequencing runs on multiple sequencers with Ion Mesh

Before viewing runs on multiple servers, you first connect the servers using Ion Mesh. For more information, see "Set up flexible workflows" on page 325.

1. In the **Data** tab, click **Completed Runs and Reports**.
2. In **Server**, select one or more Ion Torrent™ Servers for which you want to view data.
   The server name is now reflected in the **Run Name** column. The results across multiple sequencers are ready for review.

Transfer a Planned Run to an Ion Torrent™ Server with Ion Mesh

You can transfer Planned Runs that are created on one Ion Torrent™ Server and transfer them to another Ion Torrent™ Server by using Ion Mesh. This is useful if the sequencer connected directly to the original Ion Torrent™ Server is offline or busy.

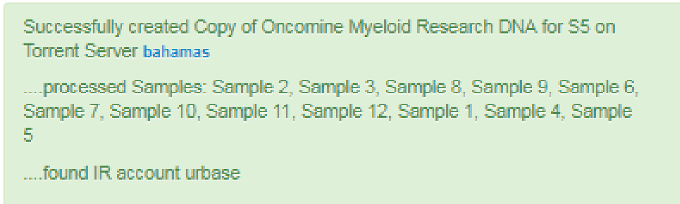
Before Planned Run transfer, first connect the Ion Torrent™ Server with Ion Mesh. For more information, see "Set up flexible workflows" on page 325.

IMPORTANT! The Ion Torrent™ Server must have the same version of Torrent Suite™ Software installed in order for a Planned Run transfer to be successful.

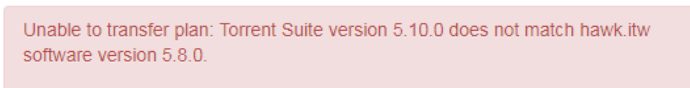
1. Sign in to Torrent Suite™ Software on the original Ion Torrent™ Server.
2. In the **Plan** tab, click **Planned Runs**.
3. Find the row of the Planned Run that you want to transfer, then click ⚙ **(Actions)** ▸ **Transfer**.
4. Select the Ion Torrent™ Server that you want to receive the Planned Run.
5. In the confirmation dialog box, confirm the information, click **Transfer**, then select the server to receive the run.
   You can no longer access this Planned Run on the original server after it has transferred.

A status message indicates the results of the transfer.

- A green message lists the Planned Runs that are successfully transferred.



- A red message lists any failed Planned Run transfers.



Track Ion Chef™ Instrument flexible workflows for Ion 550™ or Ion 560™ chips with Ion Mesh

To use the flexible workflow feature and to enable cartridge use tracking between Ion Torrent™ Servers across multiple Ion Chef™ Instruments, Ion Torrent™ Servers must be linked in an Ion Mesh configuration.

For more information on how to link Ion Torrent™ Servers in an Ion Mesh configuration, see "Set up flexible workflows" on page 325. For more information on configuring a flexible workflow on the Ion Chef™ Instrument , see the *Ion 550™ Kit – Chef User Guide* (Pub No. MAN0017275) or *Ion 560™ Kit – Chef User Guide* (Pub No. MAN1000018).

IMPORTANT! If you have Ion Torrent™ Servers that are connected in an Ion Mesh configuration, and the connection to one Ion Torrent™ Server is disrupted or lost, cartridge use tracking between servers is disabled. In this situation, an error message appears if you attempt to start a run. The run is not allowed to start because the cartridge use status is not trackable. Ion Mesh communication must be restored to verify cartridge use status before the run can start.

---

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Click a completed report.
3. In the run report, scroll down to the **Details** section, then select the **Chef Summary** and **S5 Consumable Summary** tabs to track the cartridge use.

Data backup and restore locations

The Ion Torrent™ Server maintains the following types of data in separate locations.

Data type	Storage location
Ion GeneStudio™ S5 System and Ion GeneStudio™ S5 Plus System data	/results/<instrument_name> folder, by default.
Report data	`/results/analysis/output/Home` folder, by default.
Database records	PostgreSQL® database

The nightly backup of the database is created automatically, then stored for 30 days.

Restore the PostgreSQL® Database

To restore the PostgreSQL® database, you need a complete working Ion Torrent™ Server installation.

There are two scenarios for restoring a database.

- Install a new Ion Torrent™ Server from the Ion Torrent™ Server installation disk due to migrating the database to a new server or needing to reinstall the server.
- Replace the database on an existing Ion Torrent™ Server, possibly because the database is corrupted, and you want to restore a previous version.

- To restore the database from the backup file, execute these commands on the Ion Torrent™ Server.

```
{{# copy the backup file to the server and decompress it
gzip -d iondb.20100711_142442.backup.gz

# stop the Torrent Server background processes
sudo /etc/init.d/ionCrawler stop
sudo /etc/init.d/ionJobServer stop
sudo /etc/init.d/ionPlugin stop
sudo /etc/init.d/celeryd stop

# login as user postgres
sudo su postgres

# restart the service to clear database connections
 /etc/init.d/postgresql restart

# drop the existing iondb database
dropdb iondb

# create a new empty database
psql <<-EOFdb CREATE DATABASE iondb;
GRANT ALL PRIVILEGES ON DATABASE iondb to ion;
\q EOFdb

# import data
psql -e iondb < iondb.20100711_142442.backup

# logout of user postgres
exit
```

```
# start the Torrent Server background processes
sudo /etc/init.d/ionCrawler start
sudo /etc/init.d/ionJobServer start
sudo /etc/init.d/ionPlugin start
sudo /etc/init.d/celeryd start}}
```

Occasionally, a Django error occurs after completing the import data step. If this error occurs, repeat the following steps.

1. Drop database.
2. Create database.
3. Import data.

Manage telemetry services

Torrent Suite™ Software uses telemetry services to provide connectivity to the software that allows Thermo Fisher Scientific Technical Support personnel to perform remote troubleshooting on request. Telemetry services also report anonymous usage statistics to Thermo Fisher Scientific.

The telemetry services used with the software are:

- Axeda™ Remote System Monitoring agent (Axeda™ RSM agent)
- Deep Laser

To enable or disable these telemetry services, click **(Settings)** ▸ **Services**, then scroll down to the **Telemetry Services** section.

Disabling telemetry services stops all Thermo Fisher™ Connect Platform integration and makes remote support more difficult.

Axeda™ Remote System Monitoring (RSM)

Remote access for troubleshooting

When there is a problem with a sequencer or the Ion Torrent™ Server, the Axeda™ RSM agent allows Thermo Fisher Scientific Technical Support personnel to remotely:

- Collect log files from the system for review.
- Restart the device.
- Upgrade software.
- Provide a remote sign in connection to the device for further diagnostic work.

When a problem with an Ion Chef™ Instrument and Ion GeneStudio™ S5 Series sequencer or Torrent Suite™ Software is reported, Thermo Fisher Scientific Technical Support tries to solve the problem by telephone or email. If remote access is required for additional troubleshooting, a member of Thermo Fisher Scientific Technical Support requires authorization from the technical contact to initialize a remote connection. Only after getting authorization does Thermo Fisher Scientific Technical Support proceed with remote troubleshooting. After the problem is resolved, you are notified. Additional authorization is required before starting any further remote help.

Axeda™ RSM agent overview

The Axeda™ Remote System Monitoring agent (Axeda™ RSM agent) is a software component that is installed by default on the Ion Torrent™ Server, and on an Ion GeneStudio™ S5 Series sequencer through the software update process.

Approximately every 60 seconds, the Axeda™ RSM agent sends a heartbeat message to Thermo Fisher Scientific. This information is used to track the deployment and software configuration of machines in the field.

Data collected in the Axeda™ monitoring database can be reviewed by Thermo Fisher Scientific Technical Support personnel. Because the heartbeat message is sent many times an hour, Technical Support can quickly see the following information.

- Whether an instrument is online.
- The software versions that are in use.
- Some technical details about the instrument, such as temperature and hard drive status.

The agent also allows Thermo Fisher Scientific personnel to remotely access the sequencers and the Torrent Suite™ Software, which is required for system support. Without remote access, Thermo Fisher Scientific Technical Support personnel cannot access, view, and diagnose problems with machine performance.

Port assignments

To support fully the Ion Torrent™ Server and multiple Ion Torrent™ sequencers, remote monitoring must be provided using Axeda™ Remote System Monitoring software enabled, and must be able to reverse SSH into the boxes. This requirement means that the multiple sequencers and the Ion Torrent™ Server must be connected to the Internet with outbound connections that are permitted on the following ports.

Port	Required	Use
22	Yes	Start reverse SSH tunnel for remote troubleshooting.
80	Yes	Download updates from **http://updates.iontorrent.com** and **http://us.archive.ubuntu.com.**.
123	Yes	(UDP) NTP access to the Internet, incoming and outgoing.
443	Yes	Enable sending of basic status information to the remote monitoring server. The IonReporterUploader plugin also requires port 443 to transfer data to Ion Reporter™ Software.
5432	No	Remote access to the PostgreSQL® database.

Deep Laser

When there is a problem with a sequencer or the Ion Torrent™ Server or Torrent Suite™ Software, the Deep Laser telemetry service allows Thermo Fisher Scientific Technical Support personnel to remotely troubleshoot the problem.

Telemetry services are enabled by default. When enabled, connectivity to Thermo Fisher™ Connect Platform is allowed in order to transfer support archived files to Thermo Fisher Scientific technical support staff to perform remote troubleshooting. Anonymous usage statistics are also reported to Thermo Fisher Scientific.

Disabling telemetry services stops all Connect Platform integration and may make remote support more difficult. You can disable telemetry services on the **Services** screen in the **Telemetry Services** section. Disabling telemetry services disables both Deep Laser and Axeda™ RSM agent.

For assistance, contact your local Field Service Engineer.

Data automatically collected by the telemetry services

Event names, data types, and sample values of the data being collected by Axeda™ RSM agent and the Deep Laser telemetry service are described in the following tables. This information is sent automatically to Thermo Fisher Scientific from the Ion Torrent™ Server, or an Ion GeneStudio™ S5 Series sequencer.

Ion Torrent™ Server data

The following table lists and describes the Ion Torrent™ Server events that are collected by the Axeda™ RSM agent and the Deep Laser telemetry service. The event information is automatically sent to Thermo Fisher Scientific.

Event name	Data type	Sample value
`TS.Config.biosversion`	String	6.00
`TS.Config.configuration`	String	standalone
`TS.Config.hostname`	String	Ion-torrent-server
`TS.Config.ipaddress`	String	10.45.3.246
`TS.Config.mode`	String	Master
`TS.Config.serialnumber`	String	1SMJFP1 (Dell™ service tag)
`TS.Contact.IT Contact`	String	email, phone
`TS.Contact.Lab Contact`	String	email, phone
`TS.Experiment`	String	chip type, flow count, run type, bedfile, barcode count, seq s/n
`TS.GPU`	String	No problems
`TS.host`	String	Ion-torrent-server
`TS.HW.HD./results`	String	58.99

(continued)

Event name	Data type	Sample value
`TS.Location.City`	String	Rockville
`TS.Location.Org-Name`	String	Unknown
`TS.Location.Postal-Code`	String	Unknown
`TS.Location.State`	String	Unknown
`TS.Location.Street-Address`	String	Unknown
`TS.Nexenta<n>_lic_days_left`	String	180
`TS.Nexenta<n>_lic_status`	String	license status
`TS.Nexenta<n>_machine_sig`	String	5EDI8L9NA
`TS.Nexenta<n>_UUID`	String	44454c4c-5900-1046-8048-b2c04f533532
`TS.Nexenta<n>_vol<v>`	String	`pool1 size=32.5T allocated=860G free=31.7T capacity=2% health=ONLINE`
`TS.Nexenta<n>_vol<v>_d<d>`	String	`c0t5d1 health=ONLINE vendor=SEAGATE product=ST6000NM0034 serial=Z4D1XT26 size=6TB`
`TS.Server.celerybeat`	String	ok/offline/error
`TS.Server.celery_diskutil`	String	ok/offline/error
`TS.Server.celery_periodic`	String	ok/offline/error
`TS.Server.celery_plugins`	String	ok/offline/error
`TS.Server.celery_slowlane`	String	ok/offline/error
`TS.Server.celery_transfer`	String	ok/offline/error
`TS.Server.celery_w1`	String	ok/offline/error
`TS.Server.dhcp`	String	ok/offline/error
`TS.Server.ionCrawler`	String	ok/offline/error
`TS.Server.ionJobServer`	String	ok/offline/error
`TS.Server.ionPlugin`	String	ok/offline/error
`TS.Server.ntp`	String	ok/offline/error
`TS.Server.RabbitMQ`	String	ok/offline/error
`TS.Server.RSM_Launch`	String	ok/offline/error
`TS.Server.tomcat`	String	ok/offline/error

(continued)

Event name	Data type	Sample value
`TS.TYPE`	String	TS1
`TS.Version.alignment`	String	1.42-0
`TS.Version.analysis`	String	1.40-0
`TS.Version.dbreports`	String	1.95-3
`TS.Version.docs`	String	1.15-1
`TS.Version.referenceLibrary`	String	1.6-1
`TS.Version.tmap`	String	0.0.19-1
`TS.Version.tsconfig`	String	1.3-9

Ion GeneStudio™ S5 Series sequencer data

Data from Ion GeneStudio™ S5 Series sequencers are divided into categories.

- DataCollect—These items come from the instrument configuration file.
- RunData—These items reflect parameters from the last Auto pH or sequencing run.
- Status—These items reflect the current instrument parameters.
- System—These items provide parameters related to the operating system supporting the instrument.
- Version—These items provide the version numbers for the various software packages installed on the instrument.

In addition, two items (InstrumentState, Type) are not placed in any category.

The following table lists and describes the events from Ion GeneStudio™ S5 Series sequencers that are collected by Axeda™ RSM agent and the Deep Laser telemetry service and automatically sent to Thermo Fisher Scientific. The number and names of these entries are subject to change across software releases.

Data item name	Data type	Sample value
`Alarm.*`	String	Various hardware alarm messages
`BIOS.BIOS`	Analog	5350
`DataCollect.FlowsSinceClean`	Analog	400
`DataCollect.RunsSinceClean`	Analog	1
`Event.CleanCompleted`	String	Clean completed
`Event.DatacollectStarted`	String	Datacollect Started
`Event.InstrumentMustBeInitialized`	String	Instrument must be initialized
`Event.PostRunCleanHasNotBeenRun`	String	Post Run Clean has not been run

(continued)

Data item name	Data type	Sample value
`InstrumentState`	String	Idle
`RunData.a1a2`	String	R_2016_02_17_13_01_08_user_F4--145 W1.dat dffffe cntArry 9 0 0 9
`RunData.AutoPhFinal`	Analog	7.660635
`RunData.AutoPhInitial`	Analog	6.321023
`RunData.AutoPhIterations`	Analog	4
`RunData.AutoPhResult`	String	Pass
`RunData.AutoPhTotalW1Volume`	Analog	1.0
`RunData.ChipGain`	Analog	1.066389
`RunData.ChipPixelAverage`	Analog	8241
`RunData.ChipPixelsInRange`	Analog	164698460
`RunData.ChipPixelsPinnedHigh`	Analog	0
`RunData.ChipPixelsPinnedLow`	Analog	676
`RunData.ChipTemp`	Analog	81.826172
`RunData.CpuTemp0`	Analog	53
`RunData.CpuTemp1`	Analog	74
`RunData.efuse`	String	**********L:Q6C841,W:4,J:WC2012C00086-C00272,P:16,C:PT4,F:F6,Y:4,X:0,B:3,SB:31,B:1P,N:343**************************************
`RunData.FpgaMasterTemp`	Analog	113
`RunData.FpgaSlaveTemp`	Analog	118.4
`RunData.GpuTempC`	Analog	82
`RunData.LastAutoPhRealPh`	Analog	766
`RunData.LastAutoPhRef`	Analog	745
`RunData.LastAutoPhTarget`	Analog	770
`RunData.R1pH`	Analog	7.00
`RunData.R2pH`	Analog	7.00
`RunData.R3pH`	Analog	7.00
`RunData.R4pH`	Analog	7.00

(continued)

Data item name	Data type	Sample value
`RunData.W1pH`	Analog	8762
`RunData.W2pH`	Analog	7619
`RunData.W3RefpH`	Analog	7.45
`Status.HDPctFull`	Analog	0.823612
`Status.SsdPctFull`	Analog	6.220454
`System.CpuUsagePct`	Analog	7
`System.Date`	String	2013-01-0
`System.FreeMemoryKB`	Analog	129951948
`System.Hostname`	String	d1.ite
`System.IpAddress`	String	10.25.3.150
`System.PhysMemTotalGB`	Analog	128
`System.Time`	String	03:42:58 PM GMT
`TYPE`	String	Proton1
`Version.Datacollect`	String	3371
`Version.DiskImage`	String	2015_06_04
`Version.Graphics`	String	80
`Version.KernelRelease`	String	3.13.9-ionrt1
`Version.LiveView`	String	2166
`Version.OIA`	String	5203
`Version.OS`	String	17
`Version.Reader FPGA`	String	3d400109
`Version.Reader FPGA1`	Analog	33400109
`Version.Reader Woddr FPGA`	String	3400043
`Version.Reader Woddr FPGA1`	String	340004b
`Version.RSM`	String	24
`Version.Scripts`	String	2.0.63
`Version.S5 Release`	Analog	5.2
`Version.S5 Script`	String	0.1.13

(continued)

Data item name	Data type	Sample value
`Version.TSLink`	String	1.0.2r5
`Version.Valve FPGA`	String	c010

Administration with command-line utilities

You can use command-line utilities to perform some administrative tasks.

Monitor disk space

You can use a command-line utility to monitor disk space if the Torrent Suite™ Software is not available.

You can also monitor disk space through the Ion Reporter™ Software. For details, see "View disk usage parameters" on page 272.

1. Sign in to the Ion Torrent™ Server using an `ssh` client.

   ```
   $ ssh <yourpassword>@ion-torrent-server
   $ password: <yourPassword>
   ```

2. Type the `$ df -h` command to show the partitions and disk utilization information.

Change the hostname

You can use a command-line utility to change the host name.
Use the following command to change the host name.

```
sudo TSconfig --change-hostname
```

You must restart the server after the host name is changed. This command automatically restarts the server.

Change the time zone

You can use a command-line utility to change the time zone.
Use the following command to change the time zone.

```
sudo TSconfig --configure-timezone
```

Add an HTTP proxy

You can use a command-line utility to add an HTTP proxy.

Use the following command to add an HTTP proxy.

```
sudo TSsetproxy
```

Set the proxy address and authentication according to the following prompts.

1. `Enter http proxy address`: Enter the proxy address. (If no address is entered, you are prompted to exit the program.)
2. `Enter http proxy port number [3128]`: Enter a port number or carriage return to accept the default, 3128, port number.
3. `Enter the username for proxy authentication`: Enter a username. If you do not enter a username, no authentication is set.
4. `Enter the password for proxy authentication`: Enter a password. If you do not enter a password, no authentication is set.

A proxy address confirmation message is displayed.

```
http_proxy is set to http://username:password@proxyAddress
```

The recommended usage is to enter the command sudo TSsetproxy, as shown above, and be prompted for each value. You can however use the TSsetproxy arguments instead.

```
Usage: TSsetproxy [option]... --address Proxy address (example:
                'http://proxy.net') --port Proxy port number (default: 3128)
--username
                Username for authentication --password Password for
authentication --remove Removes
                proxy setting --debug,-d Prints script commands when
executing (set -x) --demo
                Prints what changes would be executed only. No changes are
made --help,-h Prints
                command line args --version,-v Prints version
```

Verify services are running

You can use a command-line utility to verify that services are running.

1. Connect to your Ion Torrent™ Server host, using `ssh`, and verify that the Crawler and Job Server services are running:

   ```
   ps -aux | grep py
   ```

   This should show active `crawler.py` and `serve.py` processes.

2. Run a test analysis of the provided cropped data set and review the resulting report.

A Troubleshooting

This appendix includes information on troubleshooting tips, resources, and where to find more information or Technical Support. It also includes known recommended actions to observed problems, such as low loading density, low percentage of live ISPs, and missing test fragments.

Troubleshooting resources

For more troubleshooting information, see the instrument user guides on the product pages for the Ion Chef™ Instrument or Ion GeneStudio™ S5 Series sequencers. You can also search for manuals on the **Services & Support** tab at **www.thermofisher.com**.

- Customer support archive (CSA)—For problems that require technical help, obtain the CSA file. The sequencing CSA file is used by Technical Support to diagnose problems. Due to the large size of the full analysis CSA file, it is necessary to use the thumbnail version for troubleshooting purposes. To download a CSA file, see "Download a customer support archive file" on page 343:
- Instrument log files—Instrument log files from Ion Chef™ instruments can also be sent to Technical Support for troubleshooting help. Contact Technical Support for instructions to download instrument log files. The CSA includes instrument log files.
- Turn off 3' quality trimming—In cases of shorter-than-expected library reads, the 3' quality trimming can be turned off to help distinguish short reads that originate from the library preparation and short reads results that can result from the 3' quality trimming by the software. To turn off the 3' quality trimming:
  a. In the **Data** tab, click **Completed Runs & Reports**.
  b. Search, filter, or sort the list to find a run report of interest.
  c. In **Table View** mode, in the row of the run of interest, click **(Actions)** ▸ **Reanalzye**.
  d. Under the **Advanced** options, on the **Start reanalysis from line**, select **Base Calling**.
  e. In the **Basecaller args** field, change the `--trim-qual-cutoff 15` value to 100. The field should now read, `BaseCaller --trim-qual-cutoff 100 --trim-qual-window-size 30 --trim-adapter-cutoff 16`.
  f. Give the reanalysis report a new name and select **Start Analysis**.

Troubleshoot Torrent Suite™ Software

Troubleshoot a sequencing run

Low loading density, low live ISPs, missing test fragments, primer-dimers, and other issues can affect sequencing runs.

Observation	Possible cause	Recommended action
Failed status for a run	It is not clear whether the sequencing run data transfer completed, and if you can access the sequencer.	Go to the sequencer **Data Management** screen to confirm complete data transfer. If you are not sure the data set was transmitted, you can re-transfer it.
	The run report was successful, but you are not sure whether the data was transferred.	In Torrent Suite™ Software, under the **Data** tab, click **Completed Runs & Reports** to view the run report and ensure that the file transfer was complete. Also, check if there are any error messages, such as **User Aborted** on the report itself. Look for a status of **Error** or **Pending**.
	You cannot determine the cause of the failed run status.	Reanalyze the run. For more information, see “Reanalyze a run” on page 116.
		Send the customer support archive to your local Field Service Engineer or Technical Support for review. For more information, see “Get technical support files for a completed run” on page 342.
Low loading density from Ion Chef™ Instrument runs	Too little library input into template preparation. Low enriched ISP recovery is caused by unenriched ISPs with <5% templated ISPs.	Review Qubit™ QC assay results to determine if % templated ISPs was low. • If % templated ISPs was <10%, increase library input to target 15–25% templated ISPs. • Requantify the library. If the library yield was below recommended, rebuild the library.

Observation	Possible cause	Recommended action
Low loading density from Ion Chef™ Instrument runs *(continued)*	Too much library input into template preparation.	Review Qubit™ QC assay results to determine if % templated ISPs was too high after template preparation. • If library is >25% but <70% templated ISPs, rebuild the library. Decrease library input to target 10-25% templated ISPs, or continue with sequencing and expect lower throughput. • If library is >70% templated ISPs, check Agilent™ 2100 Bioanalyzer™ traces for adapter dimer peak (amplicon library or Ion AmpliSeq™ library peak near 70 bp; miRNA library peak near 60 bp). Repurify (reduce) library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in appropriate user guides.
	Chip adapter placement or removal was improper.	Inspect chip adapters for any obvious defects before installation.
		The best practice for removing chip adapters for Ion S5™ chips is to squeeze the adapter ends at the same time and remove the adapter by keeping it parallel to the chip.
	Bubbles were present during chip loading or during the chip run.	Air bubbles in the reagent tubes can affect various steps through the sequencing run, including loading density. Let reagents settle before using.
Low percentage of live ISPs. **Details:** The Live (% enrichment) metric includes both live TF and library ISPs. When evaluating the percent enrichment metric, also consider the ratio of test fragment ISPs to library ISPs. If the ratio of TF ISPs is higher than expected, the reported percent of enriched library ISPs is not accurate. Instead, calculate the library enrichment from the values in the run report table: [Library ISPs/(Live ISPs - TF ISPs)] x 100 = Library ISP % Enrichment.	Improper addition of sequencing primer and polymerase.	Review Test Fragment (TF) metrics. If TFs are missing or the signal is low: primer or polymerase can have been added incorrectly.
		Ensure proper addition of sequencing primer and polymerase.
	Poor library amplification onto the ISPs (template preparation).	Review Test Fragment (TF) metrics. If TFs are present and have the expected key signal and quality, check the Qubit™ QC assay. If results indicate <10% templated unenriched ISPs, then too little library was added.
	Too little library input into template preparation.	Review Test Fragment (TF) metrics. If TFs are present and have the expected key signal and quality, check the Qubit™ QC assay. If results indicate <10% templated unenriched ISPs, then too little library was added.

Observation	Possible cause	Recommended action
Low percentage of live ISPs. **Details:** The Live (% enrichment) metric includes both live TF and library ISPs. When evaluating the percent enrichment metric, also consider the ratio of test fragment ISPs to library ISPs. If the ratio of TF ISPs is higher than expected, the reported percent of enriched library ISPs is not accurate. Instead, calculate the library enrichment from the values in the run report table: [Library ISPs/(Live ISPs - TF ISPs)] x 100 = Library ISP % Enrichment. *(continued)*	Enrichment failure.	Check the ES enrichment process to ensure that fresh melt-off and the correct enrichment beads (Dynabeads™ MyOne™ beads) and solutions are used.
		If >5-µL residual volume is left in wells 1 through 8, perform a residual volume test.
Test Fragment-1 is not present or conversion is low **Details:** There are two test fragment controls—TF-C is a sequencing control and TF-1 is an amplification control.	Poor amplification onto the ISPs (template preparation).	Review Qubit™ QC assay results to determine if % templated ISPs was low after template preparation.
		Ensure that reagents were set up correctly.
	Sequencing or consumables problem occurred.	Ensure that the sequencing primer was annealed, and the sample was incubated with polymerase.
		Ensure that all reagents are from the same sequencing kit type. Do not swap reagents between kit types.
		Ensure that reagents are set up correctly.
		Send the Customer Support Archive (CSA) file to Technical Support.
Lower than expected **Details:** The number of live library ISPs with a key sequencing matching the library key (TCAG).	Low loading density reported.	See recommendations for low loading density.
	Bad library key used.	Ensure that the library was properly prepared. This is less likely to be the problem if an Ion library kit was used for library construction.
		Ensure that the template preparation was successful. Check Qubit QC assay results. Send Customer Support Archive (CSA) to Technical Support.
	Poor library quality or templating efficiency.	Ensure that the library was properly prepared. This is less likely to be the problem if an Ion library kit was used for library construction.
		Ensure that the template preparation was successful. Check Qubit QC assay results. Send Customer Support Archive (CSA) to Technical Support.

Observation	Possible cause	Recommended action
High polyclonal count	Too much library input into template preparation.	Verify library quantification and template preparation with QC (Qubit assay). If the library key signal is lower than expected, % polyclonal estimate might be inaccurate.
High number of low-quality reads. **Details:** Reads are too short after quality trimming.	Poor library quality or templating efficiency: bad, or low, key signal.	Check Agilent™ Bioanalyzer™ Instrument trace to ensure that library is within the recommended size range (amplified, non-equalizer libraries only).
		Ensure that library was properly prepared (correct adapter sequences, adapter ligation).
		Review the library ISP QC data Qubit™ data.
High relative primer dimer count	Library preparation resulted in high number of primer dimers.	Check Agilent™ Bioanalyzer™ Instrument trace. Non-barcoded adapter dimers ~70 bp. Barcoded adapter dimers ~80 bp.
		Repurify library to remove adapter dimers.
Low Final Library ISPs count	Low ISP loading density reported.	Review well classification and read filtering results to narrow troubleshooting focus.
	Poor library read filtering reported.	Send a Customer Support Archive (CSA) to Technical Support.

Get technical support files for a completed run

You can view the report log when troubleshooting a completed run. If you need further assistance after viewing the report log, you can generate a customer support archive that you can share with technical support for assistance.

1. In the **Data** tab, click **Completed Runs & Reports**, then click a **Report Name** link to open the report of interest.

2. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Support** tab.

3. Click **View the report log** to see a list of errors.

4. If you are unable to resolve an issue using the report log, you can download a customer support archive file.

   This is a compressed file that contains a PDF and HTML version of the run report, and logs that can be used for troubleshooting. See "Download a customer support archive file" on page 343.

5. (Optional) If you download a customer support archive file, send the compressed file in an email message to your local Field Service Engineer or Technical Support representative.

   If you would like to be able to upload the customer support archive directly, ask your Field Service Engineer how to enable the customer support archive upload function.

Download a customer support archive file

An administrator can download a customer support archive file for a completed run. Use the thumbnail version of the report that is appended with _tn (thumbnail). The customer support archive file contains a PDF and HTML version of a run report with run logs. You send the customer support archive file to Customer Support in an email message if you need help to diagnose a problem with a run.

Ion Torrent™ Server cannot access the customer support server automatically. If you would like to upload files directly to your Ion Torrent™ Server, contact your local Field Service Engineer about how to enable the customer support archive function.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a report name.
2. In the report screen, in the **Reports** list, select the thumbnail version of the Report Name link that ends in "_tn" .
3. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Support** tab.
4. Click **Download** to download a compressed archive file.
   A compressed archive file is downloaded to the directory location that you specified to download files from the browser. The location depends on your browser settings. Attach this file to an email to send to Customer Support.
5. Attach the downloaded file to an email message and send the compressed file to Technical Support.

Customer support archive contents

The tables in this section describe the files and locations of the files that are included in a customer support archive. Files for optional modules (such as recalibration) appear only if the optional module is run.

Files in the `top level` folder

File	Description
`Report.pdf`	A PDF file of the analysis report and plugin results (similar to the output of the **Download as PDF** button on a run report).
`DefaultTFs.conf`	Contains a list of known test fragment sequences and their bases.
`drmaa_stderr_block.txt`	The analysis pipeline error log for the block being executed by Oracle™ Grid Engine.
`drmaa_stdout.txt`	The log of events after primary analysis.
`drmaa_stdout_block.txt`	The analysis pipeline output log for the block being executed by Oracle™ Grid Engine.

Files in the top level folder *(continued)*

File	Description
`explog.txt`	The initial run settings needed for Torrent Suite™ Software analysis when being exported from an instrument.
`explog_final.txt`	The final run settings needed for Torrent Suite™ Software analysis when being exported from an instrument.
`InitLog.txt`	The instrument auto pH log.
`InitValsW2.txt`	The pH log of the W2 solution.
`InitValsW3.txt`	The pH log of the W3 solution.
`ion_params_00.json`	A JSON-format file that contains the **Planned Run** related metadata.
`<barcode>_rawlib.ionstats_alignment.json`	A JSON-format file with Quality Control metrics.
`ionstats_alignment.json`	A JSON-format file with Quality Control metrics.
`iontrace_Test_Fragment.png`	Graphic that shows the peak signal per well in the first key flow.
`RawInit.txt`	Contains initialization data output.
`sysinfo.txt`	Contains Torrent Suite™ Software system settings.
`uploadStatus`	The log of metrics being uploaded to the Torrent Suite™ Software.
`version.txt`	Lists the Torrent Suite™ Software versions used for the analysis report.

Files in the `basecaller_results` folder

File	Description
`basecaller.log`	A log file for the BaseCaller analysis module.
`BaseCaller.json`	A JSON-format file with Quality Control metrics.
`datasets_basecaller.json`	A JSON-format file of the settings needed for the BaseCaller module to analyze the sample data.
`datasets_pipeline.json`	A JSON-format file of the settings needed by the pipeline to run the BaseCaller module.
`datasets_tf.json`	A JSON-format file of the settings needed for the BaseCaller module to analyze the Test Fragments.

Files in the basecaller_results folder *(continued)*

File	Description
`<barcode>_rawlib.ionstats_basecaller.json`	A JSON-format file with Quality Control metrics for the barcode.
`<barcode>_rawlib.sparkline.png`	A graphics file with the thumbnail histogram that shows the read lengths for the barcode.
`ionstats_basecaller.json`	A JSON-format file with Quality Control metrics.
`ionstats_tf.json`	A JSON-format file with Quality Control metrics.
`nomatch_rawlib.ionstats_basecaller.json`	A JSON-format file with Quality Control metrics for the reads that cannot be classified as into one of the barcodes (the no-match group).
`nomatch_rawlib.sparkline.png`	A graphics file with the thumbnail histogram that shows the read lengths for the no match group.
`TFStats.json`	A JSON-format file of test fragments results statistics.

Files in the `basecaller_results/recalibration` folder

File	Description
`BaseCaller.json`	A log file for the BaseCaller analysis module during base recalibration.
`basecaller.log`	A JSON-format file with Quality Control metrics for base recalibration.
`datasets_basecaller.json`	A JSON-format file of the settings needed for the BaseCaller module to analyze the sample data for base recalibration.
`datasets_pipeline.json`	A JSON-format file of the settings needed by the pipeline to run the BaseCaller module for base recalibration.

Files in the `basecaller_results/unfiltered.trimmed` folder

File	Description
`datasets_basecaller.json`	A JSON-format file of the settings needed for the BaseCaller module to analyze the sample data, when generating the raw BAM file.

Files in the `basecaller_results/unfiltered.untrimmed` folder

File	Description
`datasets_basecaller.json`	A JSON-format file of the settings needed for the BaseCaller module to analyze the sample data, when generating the raw BAM file.

Files in the `sigpror_results` folder

File	Description
`analysis.bfmask.stats`	Contains analysis statistics of wells in the bead find stage (the bfmask is a set of bit flags for each well, indicating the contents of each well).
`avgNukeTrace_ATCG.txt`	Contains ATCG key signal measurements.
`avgNukeTrace_TCAG.txt`	Contains TCAG key signal measurements.
`bfmask.stats`	Contains summary statistics of wells in the bead find stage.
`BkgModelFilterData.h5`	Contains debug information for the polyclonal filter. In particular, this file contains PPF (percent positive flows) and SSQ (residual) values for each read.
`pinsPerFlow.txt`	Contains the number of pixels that are detected as pinned in each flow. Pinned pixels either contain zero or a very high signal (above the threshold) in the flow.
`processParameters.txt`	Contains parameter settings for analysis signal processing.
`sigproc.log`	A log file for the analysis module.

Files in the `sigpror_results/dcOffset` folder

File	Description
`dcOffset.txt`	The background model parameter values of `dcOffset`.

The files in the `sigpror_results/NucStep` folder contain background model parameter values based on the location of the well in the chip.

Files in the `sigpror_results/NucStep` folder

File
`NucStep_frametime.txt`
`NucStep_inlet_bead.txt`
`NucStep_inlet_empty.txt`
`NucStep_inlet_empty_sd.txt`
`NucStep_inlet_step.txt`
`NucStep_middle_bead.txt`
`NucStep_middle_empty.txt`
`NucStep_middle_empty_sd.txt`
`NucStep_middle_step.txt`
`NucStep_outlet_bead.txt`

Files in the sigpror_results/NucStep folder *(continued)*

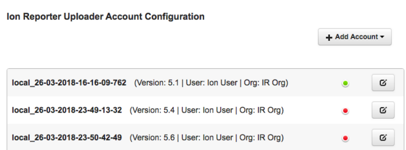
File
NucStep_outlet_empty.txt
NucStep_outlet_empty_sd.txt
NucStep_outlet_step.txt

The files in the `sigpror_results/NucStepFromBeadfind` folder contain background model parameter values based on the location of the well in the chip during the bead find stage.

Files in the `sigpror_results/NucStepFromBeadfind` folder

File
dcOffset.txt
NucStep_frametime.txt
NucStep_inlet_bead.txt
NucStep_inlet_empty.txt
NucStep_inlet_empty_sd.txt
NucStep_inlet_step.txt
NucStep_middle_bead.txt
NucStep_middle_empty.txt
NucStep_middle_empty_sd.txt
NucStep_middle_step.txt
NucStep_outlet_bead.txt
NucStep_outlet_empty.txt
NucStep_outlet_empty_sd.txt
NucStep_outlet_step.txt

Troubleshoot IonReporterUploader plugin account setup

Observation	Possible cause	Recommended action
IonReporterUploader plugin account is not configured. **Details:** Red status appears on the Ion Reporter™ Software account configuration screen. 	If you change your account password, you see a red dot in the status column of the IonReporterUploader plugin account.	When you change your account password, click **Edit** and enter the access code for the account. For more information, see "Set up an account for IonReporterUploader plugin" on page 221.
	If you upgrade your account, you see a red dot in the status column of the IonReporterUploader plugin account.	When you upgrade your account to a new version, click **Edit**, then delete your old account and create a new account for the new version.

Troubleshooting file import/upload errors

Some types of errors do not appear in the **Processing Log** section (see the log entry under **Upload History** in **Reference Sequence**). Major problems with files can prevent file uploading.

Observation	Possible cause	Recommended action
File upload errors. **Details:** File validation cannot be attempted.	• Incorrect file format • Incorrect file extension • The ZIP file contains no files or multiple files • A corrupted ZIP or GZ file	Check for possible errors in the source BED or VCF file. Fix the errors, or replace the file, then reattempt the upload.
Reference genome file cannot be opened	• An invalid FASTA file was uploaded. • No FASTA header found at line 1. • Genome information text file may have been deleted.	1. Delete the existing reference sequence entry. 2. Identify and correct formatting errors in the FASTA file. 3. Upload the corrected file.

Troubleshoot Ion Torrent™ Server

These troubleshooting recommendations apply to system level issues such as networking, disk space, and system load.

Check crawler and job server status

Use the **Crawler and Jobs Server** screen to view information about the background processes that are running on Torrent Suite™ Software.

If these processes are not running, run information is not updated and analysis reports are not generated. If this occurs, there is no risk of data loss but the Crawler and Jobs Server processes should always be running. The Archive process runs only if archiving has been configured.

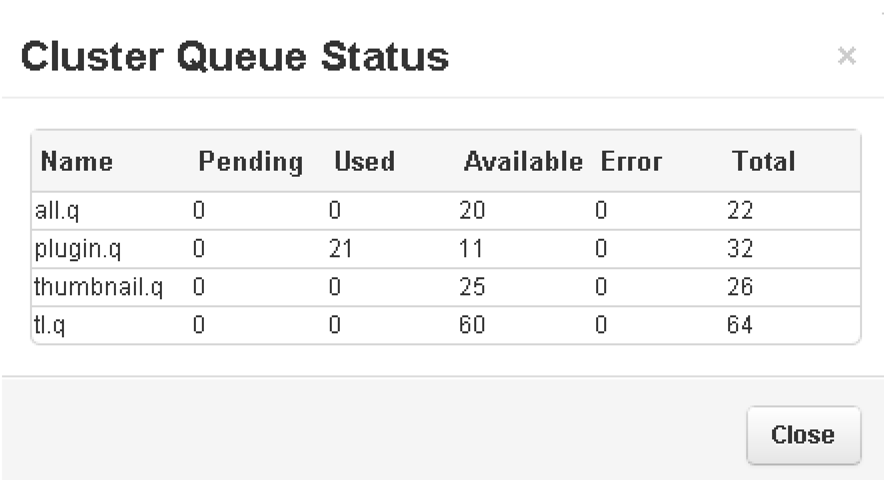
If a process is not running, a Down or Offline reason displays in the **Services** screen. An example message is ***The crawler is offline***.

1. Click **(Settings) ▸ Services**.
2. In the **Crawler and Jobs Server** screen, review the information about the background processes described in the following table.

Process	Program	Startup script	Description
Crawler	`crawler.py`	ionCrawler	Searches for new runs from the sequencers and puts run information into the database so that it appears in the **Data > Completed Runs & Reports** screen.
Job Server	`serve.py`	ionJobServer	Sends analysis jobs to the Oracle™ Grid Engine.
Plugin Server	`ionPlugin.py`	ionPlugin	Sends plugin jobs to the Oracle™ Grid Engine.
Celeryd	`manage.py`	celeryd	A background job processor for Django (the web application framework).

Queue status

Click the **Queue Status** link in the Active Jobs section to open a table of SGE queue activity:


Cluster Queue Status

Name	Pending	Used	Available	Error	Total
all.q	0	0	20	0	22
plugin.q	0	21	11	0	32
thumbnail.q	0	0	25	0	26
tl.q	0	0	60	0	64

Close

Verify network connectivity and name resolution

There can be many reasons for network connectivity or name resolution to fail. Use this procedure to try to resolve connectivity and name resolution problems.

If you cannot reach the Ion Torrent™ Server by using an IP address, you are likely to need help from the site IT administrator who understands how the local network is configured.

1. Click **(Settings) Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section, then click **Admin interface** link to access the database administration functions.
3. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.
4. In the **Site administration** screen, scroll down to **Management Actions**, then select the **Network Settings** line.

   The software performs several network checks.

   Ethernet 0 Detected ✓
   IP Address Detected ✓
   Default route Detected ✓

updates.iontorrent.com:80	**Detected** ✓
us.archive.ubuntu.com:80	**Detected** ✓
drm.appliedbiosystems.com:443	**Detected** ✓
security.ubuntu.com:80	**Detected** ✓
rssh.iontorrent.net:22	**Detected** ✓

5. Verify that the Ion Torrent™ Server is configured correctly by reviewing the Ion Torrent™ Server deployment instructions.
6. Find the IP address of the Ion Torrent™ Server as described in "Verify the Ion Torrent™ Server IP address" on page 350.

After you are finished, click **Back to Main Site** at the top of the screen to return to the software.

Verify the Ion Torrent™ Server IP address

The Ion Torrent™ Server is configured out-of-the-box to automatically get an IP address from the DHCP server on the network. Unless the local IT administrator has specifically assigned an IP address in advance, you may not know the current IP address.

The Ion Torrent™ Server has several Ethernet ports on the back. Make sure that your site network is connected to the port labeled **LAN**, called **eth0** in Linux™ terminology. The Ethernet ports are identified as **eth0**, **eth1**, ..., for as many ports as are available. On Ion Torrent™ Server, **eth0** is the only port connected to your network and is configured by DHCP.

If the port is set to DHCP the IP address should be permanently assigned to that port after the server IP address setting is configured.

If you are still concerned about network connectivity, you can test whether different desktops are able to successfully `ping` the server IP address. If you are not able to `ping` the server from the desktops that need to access the Torrent Suite™ Software running on the server, contact your site IT administrator.

1. To determine the IP address assigned to **eth0**, sign in and type: `ifconfig eth0`.

   This displays the following output.

```
<yourusername>@ion-torrent-server:~$ ifconfig eth0
eth0 Link encap:Ethernet HWaddr 00:1b:21:5b:bb:44
inet addr:192.168.1.123 Bcast:192.169.4.255 Mask:255.255.255.0
inet6 addr: fe80::21b:21ff:fe5b:bb44/64 Scope:Link
UP BROADCAST RUNNING MULTICAST MTU:1500 Metric:1
RX packets:209970726 errors:0 dropped:0 overruns:0 frame:0
TX packets:419252947 errors:0 dropped:0 overruns:0 carrier:0
collisions:0 txqueuelen:1000
RX bytes:14131928595 (14.1 GB) TX bytes:607398487997 (607.3 GB)
Memory:fbea0000-fbec0000
```

   Your IP address is the inet addr.

```
inet addr:192.168.1.1 Bcast:192.169.4.255 Mask:255.255.255.0
```

2. You can configure a static IP address in the **Network Settings** screen.
   a. Click **(Settings)** ▸ **Configure**.
   b. Scroll to the **Database Administration** section, then click the **Admin Interface** link.
   c. In the **Admin Interface** screen, scroll to the **Management Actions** section, then click **Network Settings**.
   d. Select **Static**, enter the IP address into the **IP address** field, then click **Update**.

3. Another useful check is the line beginning with **UP**, which indicates the interface is active and working.

```
UP BROADCAST RUNNING MULTICAST MTU:1500 Metric:1
```

   If the **eth0** port is not available, it is possible that the Ethernet cable is not connected to a network, so you may not see the word **UP**.

```
BROADCAST MULTICAST MTU:1500 Metric:1
```

   If an IP address is assigned, the interface is likely to work. If no IP address is assigned and the interface is not UP, you may need to get help from your site IT administrator.

Troubleshoot and configure the time service

The Ion Torrent™ Server uses the Linux™ Network Time Protocol (NTP) program to synchronize its time with another time server. By default, the Ion Torrent™ Server is configured to synchronize its time service to a trusted time service on the Internet. This configuration requires that the network configuration allows the NTP network protocol to connect to that time service on the Internet.

The Ion Torrent™ Server can also act as a time server for an Ion Chef™ Instrument, or an Ion GeneStudio™ S5 Series sequencer.

To help ensure that the server is synchronized with connected instruments, Ion Torrent™ Server will serve as authoritative time server for directly connected instruments when primary time servers cannot be reached. Ion Torrent™ Server does not forward information that is potentially not correct to other machines.

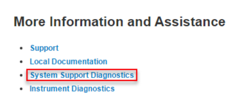
If the network configuration is blocking the NTP protocol from reaching the Internet, the Ion Chef™ Instrument, or Ion GeneStudio™ S5 Series sequencer cannot synchronize time. If the server is restricted from reaching to the external time server, edit the NTP settings to point to the local NTP server.

Your site network administrator is probably aware of this connectivity restriction, and it is likely that a time server is available on the site network.

View system support diagnostics

System diagnostics information can help in troubleshooting network, disk space, and system status problems.

1. To access system diagnostics information, click **(Settings)** ▸ **About**
2. Scroll down to the **More Information and Assistance** section, then click **System Support Diagnostics**.

   The diagnostics screen provides **Network**, **System**, and **Data** sections. A small section of each screen is shown.

Network

```
==================================================================
-----Looking up the MAC address for the server-----
MAC Address = 00:10:18:a2:3d:00

==================================================================
-----Checking that that server has acquired an IP Address-----
GOOD - this server has an IP address: 167.116.6.195

==================================================================
-----Checking network connection-----
GOOD - the 'eth0' ethernet port is UP
```

System

```
=================================================================
Date Collected:
Wed Sep  5 20:45:26 PDT 2012

=================================================================
Server Uptime:
20:45:26 up 14 days,  8:05,  7 users,  load average: 0.15, 0.17, 0.17

=================================================================
Ion Software Package Status:
Desired=Unknown/Install/Remove/Purge/Hold
| Status=Not/Inst/Cfg-files/Unpacked/Failed-cfg/Half-inst/trig-aWait/
|/ Err?=(none)/Reinst-required (Status,Err: uppercase=bad)
||/ Name                                 Version
+++-=================================-===========================
ii  ion-alignment                        3.0.2-1
```

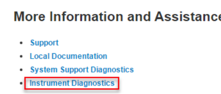
Data

```
Raw Data Storage Report
Runs Total               :           15
Runs Deleted             :            0
Runs Archived            :            0
Runs Live                :           15
Runs to Keep             :            0
Runs to Archive Raw      :           14
Runs to Delete Raw       :            1
Runs in Grace Period     :            2

Disk Space Allocation Report: /results/ (/dev/mapper/ion--torrent--ser

Total Disk Space         :       10286 GBytes
Used Disk Space          :        2082 GBytes 20.2%
Free Disk Space          :        8204 GBytes 79.8%

File servers and PGMs writing to them:
192.168.201.1: (not mounted)
default
PGM_test
ts: (not mounted)
import
```

View instrument diagnostics

Use **Instrument Diagnostics** to investigate chip and sequencing instrument problems, such as pH levels.

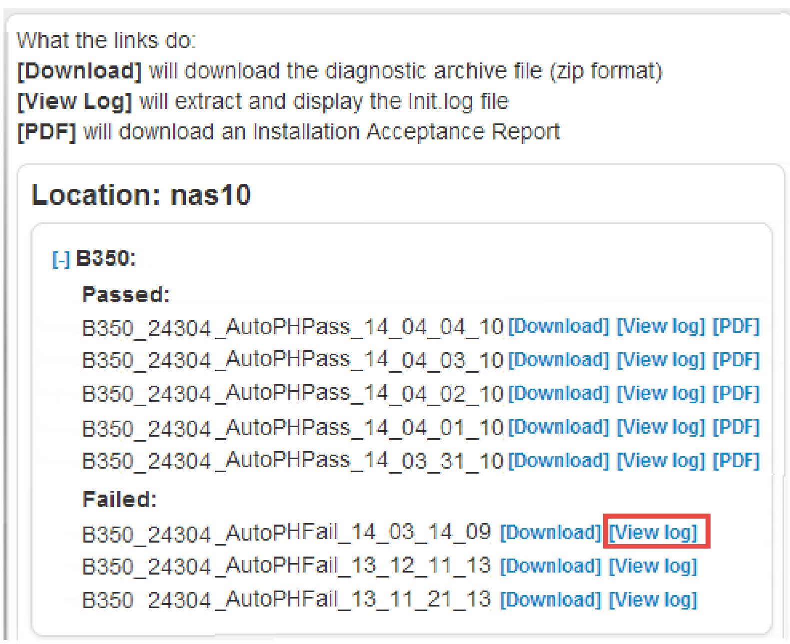
1. To access the **Instrument Diagnostics** information, click ⚙ **(Settings)** ▸ **About**.
2. Scroll down to the **More Information and Assistance** section, then click the **Instrument Diagnostics** link.



   The **Instrument Diagnostics** screen lists the sequencing instruments that are associated with each of your results partitions. Passed and failed analysis runs are shown for each instrument.

   The `InitLog.txt` file includes diagnostic measurements and, if possible, presents a probable cause and suggests next steps.

3. To investigate a failed run, click the **View log** link for that run.



   The `InitLog.txt` file opens for that run on the instrument.

InitLog.txt

```
Fri Mar 14 09:23:17 2014
serial=24304
Name: B350
Sequencing Kit Used: IonPGM400Kit
ChipChecking...
Prepping for Chip Calibrate
Calibrating Chip
Started
Optimizing Reference Electrode
Optimizing Channel Dacs
Optimizing Reference Dacs
Measuring Noise
Chip Noise 2.54/2.85, Avg Vout 1.22
Generating LS Row Image
VREF=38108 Chan dacs=<24304 24111 24112 24037>
RefV=<16828 16832>
Chip Noise 2.54/2.85, Avg Vout 1.22
Passed gain:0.711542
Chip Type 314R
Starting AutoPH (PH:7.70 < 7.75 < 7.85)
-145 < target=63 < 167
ADC counts/pH = 2090
surface=TiN mv/pH=42.310000 TiNGainCutoff=0.660000
PHShift(pH)=0.330000, PHShift(counts)=689 PHRef=7.450000
phTotalAdded=0.000000
stddev = 67
W1 Step 8865 counts.
W2 Avg=9078 StdDev = 1
stddev = 973
Chip Reading Inconsistent.
Run Flow Check to confirm no waste line blockages and/or
replace chip. Press start to try again.
Fri Mar 14 09:28:10 2014
```

Close

Restart services

If you need to restart a service, you cannot restart the service in Torrent Suite™ Software. The easiest approach is to shut down and restart the Ion Torrent™ Server.

After you shut down and restart the server, all services that were running before the shutdown continue from the point where they were interrupted, and no more user interaction is needed.

1. Shut down and restart the Ion Torrent™ Server.
2. (*Optional*) Restart the services.

```
sudo service <name> start
```

3. Verify that the services are running using the `ps ax | grep py` command or the Torrent Suite™ Software user interface.

   If the services do not continue to run after the restart, contact your local Field Service Engineer for assistance.

Further investigation and problem resolution

After the root cause of a major problem is identified, the following more intrusive action may be needed:

- Replace a failed hard disk drive
- Downgrade software packages
- Reinstall software
- Modify configuration files
- Add, modify, or delete database information

Contact your local Field Service Engineer for assistance before you attempt any of these actions.

B Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Related documentation

Related software documentation is available at **http://www.thermofisher.com/ngssoftwaresupport**.

Document	Publication number
Torrent Suite™ Software 5.22 User Guide	MAN1000552
Ion 560™ Kit – Chef User Guide	MAN1000018
Ion Reporter™ Software 5.20 User Guide or later	MAN0028231
Ion AmpliSeq™ Designer Help	MAN0018937
Oncomine™ Comprehensive Assay Plus User Guide	MAN0018490
How to assess an Ion S5™/Ion GeneStudio™ S5 sequencing run report User Bulletin	MAN0017983

(continued)

Document	Publication number
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit	MAN0017648
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit User Guide	MAN0017343
Ion 550™ Kit – Chef User Guide	MAN0017275
Ion AmpliSeq™ Pharmacogenomics Research Panel User Guide	MAN0014300
Ion AmpliSeq™ Designer: Getting Started User Guide	MAN0010907
Ion AmpliSeq™ Transcriptome Human Gene Expression Kit	MAN0010742

Limited product warranty

Life Technologies Corporation and its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.

Glossary

50Q10 Reads

Number of Ion Sphere™ Particles at 50+ bp at Q10.

50Q17 Reads

Number of Ion Sphere™ Particles at 50+ base pairs at Q17.

adapter dimer (ISPs)

Primer and adapter sequences that anneal during library preparation or templating to form a short fragment that can be sequenced. These adapter dimer ISPs generate unusable reads that the software filters out of the final sequencing data. High levels of adapter dimer ISPs can reduce the number of valid reads.

aligned read length

The aligned length 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. For example, the AQ17 length of a read is the greatest length at which the read error rate is 2% or less. The "perfect" length is the longest perfectly aligned segment. For all of these calculations, the alignment is constrained to start from position 1 in the read—that is, no 5′ clipping is allowed.

aligned reads

The number of bases covered by reads aligned to the reference sequence.

Ion Torrent™ Server API

Ion Torrent™ Server API (Application Programming Interface) can be used to access database records on an Ion Torrent™ Server. Ion Torrent™ Server APIs are compliant with REST (Representational State Transfer) architectural constraints and can be used to retrieve all available information about sequencing run results and plugin data, and create Planned Runs using third-party software solutions.

AQ score

An alignment quality (AQ) score defines read accuracy when compared to the reference sequence. The discrepancy can be biological (real variant) or technical (sequencing error). For example, an AQ score of 17 represents 2% aligned read error rate, while an AQ score of 20 represents 1% aligned read error rate when compared to the reference sequence.

average base coverage depth

The average number of reads of all targeted reference bases.

average base read depth

The average number of reads of all targeted reference bases that were read at least once.

BAM file

A BAM (binary alignment map) file (.bam) is the binary version of a SAM (sequence alignment map) file (.sam). A SAM file is a tab-delimited text file that contains sequence alignment data. A BAM file contains aligned reads sorted by reference location.

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.

base calling

Base calling is the process by which raw data from the sequencing instrument is converted to nucleotide sequences. This is performed by base calling software that is usually run from the instrument itself.

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.

bead loading

The percentage of chip wells that contain live ISPs. The percentage value considers only potentially addressable wells. Bead loading is calculated as number of loaded ISPs divided by the number of potentially addressable wells.

bp

Abbreviation for "base pair(s)".

cellularity (%)

The percentage of tumor cells in a given sample.

BED file

Browser Extensible Data file—BED file—defines chromosome positions or regions.

clonal ISP

An ISP is clonal if all of its DNA fragments are cloned from a single original template. All fragments on such a bead are identical and respond in unison during a nucleotide flow across the chip.

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.

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.

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.

user-defined template

A Planned Run template that is created by a user to meet specific needs. A Planned Run template can be created based on a preinstalled Planned Run template, and then modified to fit specific needs.

DAT file

A DAT file is a generic data file. It contains data that may be opened or referenced by a specific application. While some DAT files can be opened directly, most contain program data that is referenced the program when it is running. Therefore, most DAT files are not meant to be opened manually.

de novo assembly

Nucleic acid sequence data that is assembled from sequencing reads without the aid of a reference genome library sequence.

empty wells

Wells on a chip that do not contain an ISP.

enrichment (%)

Predicted number of live ISPs that have a key signal identical to the library key signal. The percent enrichment value reported is the number of loaded ISPs that are library ISPs, after taking out the test fragment ISPs.

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.

final library reads

Number (and percentage) of reads, passing all filters that are recorded in the unmapped BAM file. This value may be different from the total number of reads located in the Library Summary Section due to specifications associated with read trimming beyond a minimal requirement resulting in total number of reads being slightly less than Final Library Reads.

flow

A flow is the event of exposing a chip to one particular dNTP (deoxyribonucleotide triphosphate), followed by a washing step.

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.

flow transfer

Progress of the sequencing run expressed as number of total flows completed. For example, a sequencing run set to 500 flows shows 250/500 flows in the status column of the **Runs in Progress** table when the sequencing run is half completed.

fusions

A targeted sequencing technique used for detection and annotation of gene fusions (or translocation of genetic material) in samples.

hotspots file

A BED or a VCF file that defines variants. Specifying a hotspots file to use in a run enables the Torrent Variant Caller (TVC) module to identify variants that may be present in sample DNA. A hotspots file instructs the Torrent Variant Caller (TVC) module to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspots file does not affect other parts of the analysis pipeline.

If you do not specify a hotspots file, the software tells only the difference between your sequence and the reference genome.

IA

Isothermal application (IA) is a template preparation technique that uses nonemulsion isothermal reaction to clonally amplify DNA onto an ISP surface.

INDEL

INDEL is an abbreviation used to designate an insertion or deletion of bases in the genome of an organism.

intermediate files

Intermediate files contain information used for debugging runs.

ISPs

Ion Sphere™ Particles (ISPs) are particles that contain bound copies of a single (ideally) DNA fragment amplified during template preparation.

key signal

Average 1-mer signal in the library key.

library ISPs

Live ISPs that have a key signal identical to the library key signal.

LIMS metadata

Laboratory Information Management System (LIMS) software is used for recording sequencing metadata. Entered text is associated with the Planned Run and can be extracted using APIs for LIMS consumption.

live ISP

An ISP with a signal of sufficient strength and composition to be associated with the library or test fragment key.

low quality ISP

An ISP with a low or unrecognizable signal.

library key

A short known sequence of bases used to distinguish a library fragment from a test fragment (for example, "TCAG").

mapped reads

Sequencing reads that have been mapped to the reference sequence.

mean raw accuracy

Average raw accuracy of 1-mers at a specific position in the read.

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.

on target reads

Sequencing reads mapped to any target region of the reference. A read is considered to be on target if at least one aligned base overlaps a target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5′ bases of the read, is not counted.

output files

Output files consist of all BAM files, run reports, and plugin results.

preinstalled Planned Run template

A Planned Run template that comes preinstalled with Torrent Suite™ Software on the Ion Torrent™ Server. Preinstalled Planned Run templates are designed to help users create a Planned Run or a customized Planned Run template for a specific research application. Preinstalled Planned Run templates cannot be deleted from the server.

Planned Run

A Planned Run is a file that contains executable instructions for individual runs. The file contains all the specifications, settings, and parameters for template preparation and chip loading on the Ion Chef™ Instrument, and sequencing on an Ion GeneStudio™ S5 Series sequencer.

Planned Run template

A reusable experimental design (digital protocol) for the sequencing instrument that holds specifications for sample preparation, sequencing, data export, and post-sequencing data analysis.

polyclonal ISP

An ISP that carries clones from two or more library sequences.

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.

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.

Q10

Predicted error rate of 10%.

Q17

Predicted error rate of 2%.

Q17 bases

The number of bases that have a Q Score of ≥17 in a given sequencing output.

Q20

Predicted error rate of 1%.

Q20 bases

The number of bases that have a Q Score of ≥20 in a given sequencing output.

read length

The length of called reads measured in base pairs.

read

The sequence of a section of a unique fragment obtained after the end of the sequencing process.

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.

SAM file

A SAM (sequence alignment map) file (.sam) is a tab-delimited text file that contains sequence alignment data. It stores biological sequences aligned to a reference sequence.

sample

Genetic material from one source (for example, DNA from one individual).

signal processing input files

Signal processing input files consist of the raw voltage measurement data collected during the sequencing run. These files are required to reanalyze the run from signal processing.

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.

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.

system SNR

System signal-to-noise ratio.

target base coverage

Summary statistics for targeted base reads of the reference. A base covered by multiple target regions is counted only once per sequencing read.

target regions file

A BED file that specifies 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 include only these specified regions instead of the entire reference library.

test fragment

A test fragment (TF) is a known nucleotide sequence that is used to monitor system characteristics.

test fragment ISPs

Live ISPs with a key signal that is identical to the test fragment key signal.

test fragment key

A test fragment key (TF key) is the nucleotide sequence that is used to identify test fragment reads.

TF key peak counts

Signal strength of the first three bases of the TF key.

total reads

Total number of filtered and trimmed reads independent of length reported in the output BAM file.

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*.

uBAM file

An unmapped BAM (uBAM) file is a variant form of the BAM file format in which the read data does not contain mapping information. This is basically an "off-label" use of the BAM format (which was specifically designed to contain mapping information) that is used for data management reasons. It allows you to attach metadata to the reads from as early on in the analysis process as possible.

unaligned reads

Nucleotide bases covered by reads that are not aligned to the reference.

uniformity of base coverage

The percentage of bases in all targeted regions (or whole genome) covered by at least 0.2X the average base coverage depth.

usable sequence / usable reads

Usable reads consist of library ISPs that pass the polyclonal, low quality, and primer dimer filters.

user-defined template

A Planned Run template that is created by a user to meet specific needs. A Planned Run template can be created based on a preinstalled Planned Run template, and then modified to fit specific needs.

VCF file

A variant call format (VCF) file specifies a variant of interest and its location. This file stores the differences between the BAM file and the reference file.

wells with ISPs

Number of wells that were determined to be positive for the presence of an ISP within the well. Wells that contain ISPs have a delayed pH change due to the presence of an ISP that slow the detection of the pH change from the solution.

XLS file

Files that use the .xls extension. XLS files can be created by Microsoft™ Excel™ and other similar spreadsheet programs.