ion torrent

# Torrent Suite™ Software 5.14

## USER GUIDE

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ThermoFisher SCIENTIFIC

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| A.0 | 8 July 2020 | Updates for Torrent Suite™ Software 5.14:<br>• Support for Ubuntu 18.04 operating system in "Operating system compatibility" section<br>• Procedures to explain the use of access codes for AmpliSeq Designer in "Link your Ion Torrent Server account to AmpliSeq.com"<br>• Access code procedures added to Set up an account for IonReporterUploader plugin" sections.<br>• Troubleshooting section updates:<br>– Customer Support Archive<br>– Troubleshoot IonReporterUploader plugin account setup<br>– Verify the Ion Torrent™ Server IP address<br>– Troubleshootand configure the time service<br>– Restart services<br>• Updates to reflect changes in the software user interface in "Import panel files and parameters"<br>Updates to Sample Set definitions |

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# 1 Introduction to Torrent Suite™ Software


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## Torrent Suite™ Software product information

Torrent Suite™ Software is used for sequencing data analysis. The software allows you to plan and monitor sequencing runs, view sequencer activity, and review sequencing data that use a variety of sequencer instruments.

Torrent Suite™ Software and hardware provide customizable and automated sample analysis and data storage solutions. You can plan, monitor, and track your sequencing runs all within a web interface, while reviewing the quality and accuracy of your run. In addition, you can perform variant calling and detect SNPs and INDELs.

The *Torrent Suite™ Software 5.14 User Guide* (Pub. No. MAN0019143) and the *Torrent Suite™ Software 5.14 Help* (Pub. No. MAN0019145) provide information for using Torrent Suite™ Software on a Ion PGM™ Sequencer or Ion Proton™, Ion S5™, Ion S5™ XL, and Ion GeneStudio™ S5 Series sequencers.

## Operating system compatibility

Ion Torrent™ Server in Torrent Suite™ Software 5.14 is compatible with Ubuntu operating system 18.04.

## 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

- Ion S5™ Sequencer
- Ion S5™ XL Sequencer
- Ion PGM™ Sequencer
- Ion Proton™ Sequencer
- Ion GeneStudio™ S5 Sequencers
- Ion Chef™ Instrument

# Network and password security requirements

## Network configuration and security

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

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

## Password security

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

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

# 2 Get started with Torrent Suite™ Software


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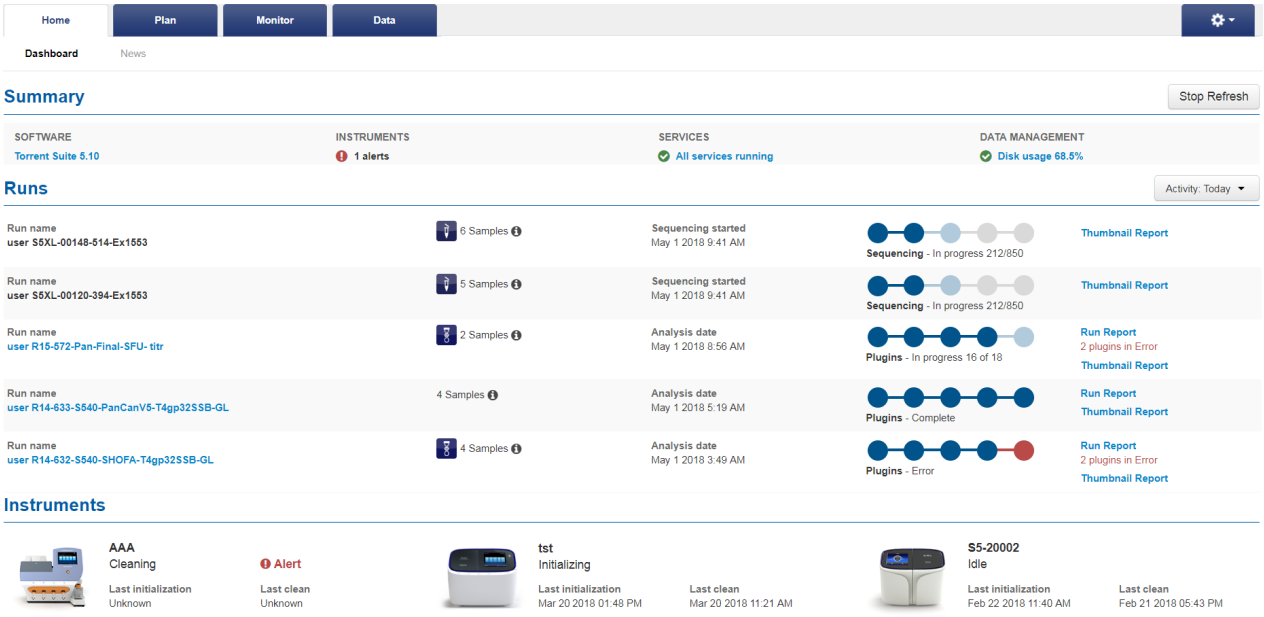
## Dashboard at a glance

Use the Dashboard to see active and recent sequencing runs. The Dashboard provides an overview of activity on all of your sequencers. To view the Dashboard, click the **Home** tab, then click **Dashboard**.



The **Summary** section includes:

- **Software** link—Click the link to open the **Releases** screen, where you can click **Update** to update your software when new versions are released. Scroll down to links to support information.
- **Instruments** column—Shows the number of instruments that are connected or offline, and those that have alerts.
- **Services** links—Reports statuses, such as services that are running, nodes that are down, and RAID storage statuses. Click a link to see details.
- **Data Management** links—Reports disk usage and data management activity. Click a link to see details.
- **Stop Refresh** stops the auto 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 information that is available through the **Data**, **Plan**, and **Monitor** tabs; however, it is a subset of only the most recent information. The **Runs** section includes:

- **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 sequencing runs and plugin use. Blue circles denote normal progress and red circles denote sequencing 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 399 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 in progress.
- The **Activity** list allows you to select a time interval for viewing sequencer 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, which may include:

- **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 Torrent Suite™ Software.

If an instrument has an operational error, hover the mouse over the **Alert** to see details. Then, resolve the issue on the sequencer.

## News screen

Use the **News** screen 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**.

## 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, and also allows 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”.

## 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™ panel).
2. Select a preinstalled Planned Run template with defaults for your application and sequencing product, or create a custom Planned Run template from scratch 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.

# Register for a new user account

When you 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**.
   Upon the approval by an administrator, the new account is created.

# 3 Samples and Sample Sets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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

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. After sample sequencing is complete, software programs, such as Ion Reporter™ Software, use sample information for data analyses.

Sample information can be transferred to Ion Reporter™ Software from the sequencer through Torrent Suite™ Software using the IonReporterUploader plugin, or imported as individual BAM or VCF files. When a 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 preselect the attributes that you want to associate with each sample before you start to plan your templating and sequencing runs. Then you can organize the samples into Sample Sets that you can reuse when you create Planned Runs. Using Sample Sets can save data entry time and reduce the likelihood of errors when you plan your instrument runs by entering the information for the Sample Set only once, then reusing the Sample Set for other runs.

Grouping samples into Sample Sets is also helpful for using barcodes that have attributes that are assigned to each individual barcode. If you create Sample Sets before planning 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 set up samples in Torrent Suite™ 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 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 also edit information in the samples, update Sample Sets, and delete samples and Sample Sets.

## Create a Sample Set manually

Create a Sample Set manually by entering sample information into the Torrent Suite™ Software without the use of an external CSV file. This method of entering sample information 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 your samples to it. Alternatively, you can add your new samples to an existing Sample Set.

For Sample Sets that contain numerous samples, you may want to import samples using a CSV file. For more information, see “Create a Sample Set by importing samples from a CSV file” on page 20.

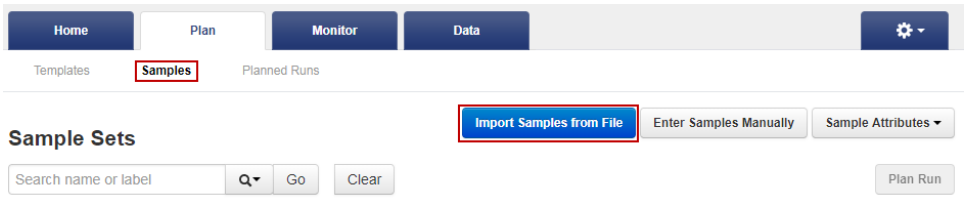
1. In the **Plan** tab, click **Samples**, then click **Add or Update Sample Set/Samples**.
2. Click **Enter New Sample**.
   a. Complete the **Add Sample** dialog box. For information about defining the samples, see “Sample attributes” on page 24.
   b. Click **Done**.
      Your new samples and sample attributes appear in the **Enter Samples** list.
   c. Enter additional samples if needed.
3. Add the samples to a sample set. Do one of the following:
   - 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.
4. Click **Save Sample Set**.
   The set name appears in the Sample Sets list.

You can use the Sample Set to create a Planned Run. The information from the Sample Set and individual samples within 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 64.

# 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 an available CSV template that is available in Torrent Suite™ 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 file. For more information, see “Create a samples file from a template” on page 21.

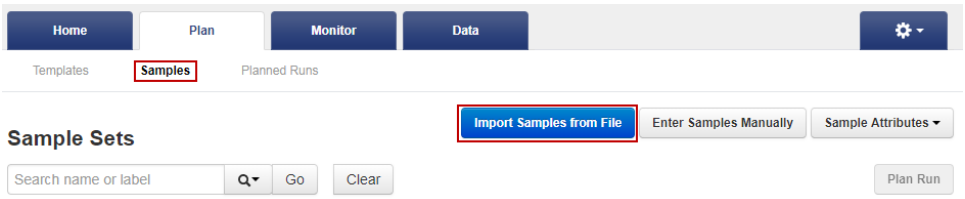
2. Upload the samples file, and optionally add a new Sample Set to receive the samples.
   a. In the **Import Samples** section, click **Select file**, then navigate to sample import file, then upload the sample import file.
   b. Select a Sample Set CSV file, then click **Open**.
   c. Select a Sample Set to receive the samples.
      *(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 system loads, parses, and validates the file, then, if no errors are found, saves the samples and Sample Sets.

# Create a samples file from a template

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

   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 about 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.

# 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.

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 automatically selects a Trio Ion Reporter™ Software analysis workflow when you create a Planned Run.

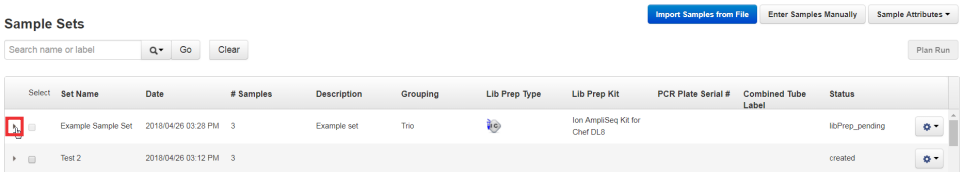
In a Trio Sample Set, you can view attributes that include:

- **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.

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

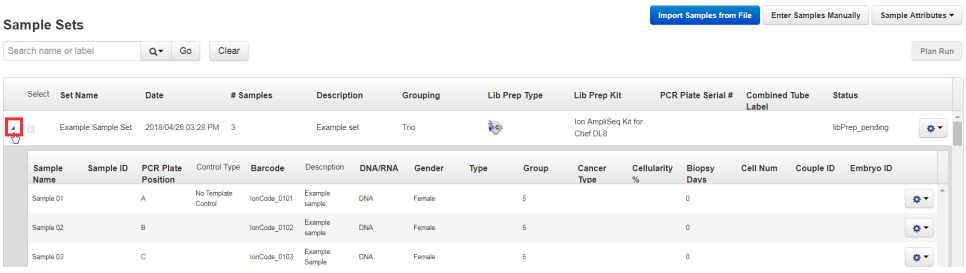
   For more information on locating your sample set, see “Find a Sample Set” on page 29 and “Sort Sample Sets” on page 30.

   In this example, a Trio Sample Set named Example Sample Set is shown




   Each column in the **Sample Sets** table lists a specific attribute for that Sample Set.

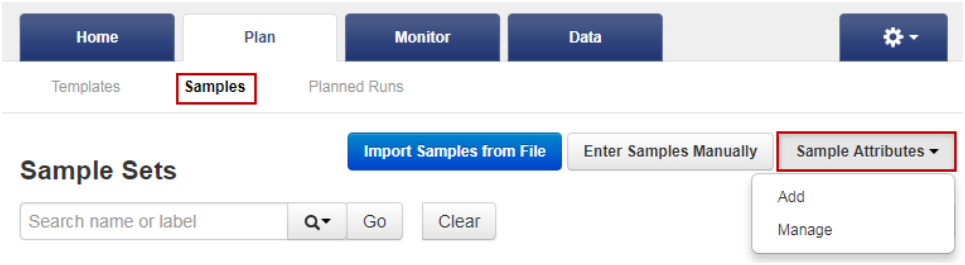
3. Click the arrow in the row of the Sample Set to expand the table and view all samples within the Sample Set.



Each column in the expanded table lists a specific attribute for each sample within the selected Sample Set.

## Add a sample attribute to a sample

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



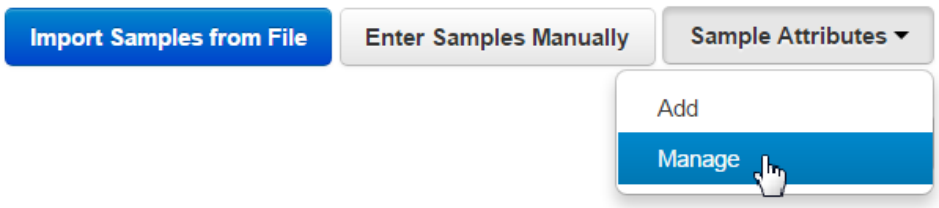

2. In the **Add Attribute** dialog box, enter the following:
   - **Attribute Name**
   - **Attribute Type**
     If the **Attribute Type** is set to Integer, you can enter only numeric characters (whole numbers) for this attribute.
   - *(Optional)* **Description**
3. *(Optional)* Select the **Is Mandatory** checkbox if you want the attribute to be required for every sample.
4. Click **Save**.

## Manage sample attributes

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

- Edit sample attributes.
- Choose whether the attributes are displayed or hidden from the list of samples, Sample Sets, and **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, click ⚙ **(Actions)** in the row of the attribute that you want to manage, then click one of the following:

| Action | Description |
|---|---|
| **Edit** | • In the **Edit Attribute** dialog box, edit one or all of the following:<br>a. **Attribute Name**<br>b. **Attribute Type**<br>c. **Attribute Description**<br>• You can also change whether the attribute is required or optional by selecting or deselecting the **Is Mandatory** checkbox.<br>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. |
| **Show/Hide** | You can hide a user-defined attribute from view without deleting it by using these options.<br>• If you hide an attribute, that attribute no longer appears in the list of samples, Sample Sets, and **Add Sample** dialog box.<br>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.<br>• If you hide a mandatory attribute, that attribute is no longer mandatory. |
| **Delete** | Deleting a sample attribute permanently deletes that attribute from the Ion Torrent™ Server. |

## Sample attributes

When you create a Sample Set, you enter sample attributes into the Torrent Suite™ Software, whether you enter attributes manually or by importing samples using 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**<br>(Required) | The unique name of the sample.<br>Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_).<br>This attribute is used by Ion Reporter™ Software.<br>A 255-character limit must be followed for Ion Reporter™ Software name validation. If you are using 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 External ID** | *(Optional)* If you manage samples in an external system, for example, a Laboratory Information Management System (LIMS), enter the identifier from that system. |
| **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. |
| **Sample ID** | A unique identification code (SampleID) for each barcode in a sample. This helps to track samples or possibly identify misassignment between samples and barcodes in a sequencing run. The SampleID is passed to Ion Reporter™ Software.<br>If you manage samples in an external system (for example, a LIMS), you can use the identifier from that system.<br>This attribute cannot be changed.<br>**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. |
| **Control Type** | The control type used when preparing the sample. If blank, the sample is not a control type sample. |
| **Basic Annotations** | |
| **Description** | Typically one or two sentences that describe the sample. |
| **Nucleotide Type** | • **Unspecified**<br>• **DNA**<br>• **RNA**<br>• **TNA**<br>• **Fusions** |
| **Sample Source** | The source from which the sample is extracted.<br>• **Blood**<br>• **FFPE**<br>• **Other** |
| **Panel Pool Type** | Reserved for future use.<br>• **Dual Pool**<br>• **Single Pool** |

*(continued)*

| Attribute | Description |
|---|---|
| **Gender** | The biological gender of the sample.<br><br>This attribute is used by Ion Reporter™ Software.<br>• **Female**<br>• **Male**<br>• **Unknown**<br><br>**IMPORTANT!** If you are using 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 in conjunction with **Relationship Group**, described below. For example, a relationship group can contain two samples, one with a type **Tumor** and another with a type **Normal**.<br><br>The following sample relationships are supported by Ion Reporter™ Software:<br>• **Control**<br>• **Father**<br>• **Mother**<br>• **Normal**<br>• **Sample**<br>• **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.<br>• **Tumor** |
| **Relationship Group** | Use **Relationship Group** to designate a group of multiple related samples within the same Sample Set. For example, DNA and RNA samples from the same sample would have the same **Relationship Group** number.<br><br>Use a whole number to define a sample as part of a relationship group. It is used in conjunction with **Type**. For example, a Sample Set can contain 6 samples, consisting of 3 groups of 2 related samples each (of types **Tumor** and **Normal**). In this case, you would 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 blood sample was drawn. |
| **Sample Receipt Date** | The date that the laboratory received the blood 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 ( **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 will 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 timepoint post-fertilization at which the biopsy was taken from an embryo. This is a whole number. |
| **Cell Number** | The cell count of the biopsied material. |
| **Couple ID** | An identifier for the couple. For use with the Reproductive research application. |
| **Embryo ID** | An identifier for the embryo. 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

| Item | Description |
|---|---|
| **Sample Set Name**<br>(Required) | Enter a name for this Sample Set.<br>Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_). |
| **Group Type** | Select the Group Type that describes this Sample Set.<br>Valid options are:<br>• **DNA Fusions**<br>• **Other**<br>• **Sample_Control**<br>• **Self**<br>• **Single Fusions**<br>• **Trio**<br>• **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.<br>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.<br>Valid options are:<br>• **Manual**<br>• **AmpliSeq on Chef**<br>• **Ampliseq HD on Chef** |
| **Library Prep Kit** | Select the library preparation kit that is used to prepare your library for this Sample Set.<br>Valid options are:<br>• **Ion AmpliSeq Kit for Chef DL8**<br>• **Precision ID Chef DL8**<br>• **Ion Ampliseq HD DL8** |
| **Library Prep Protocol** | **Myeloid** |

# View Ion AmpliSeq™ library preparation on an Ion Chef™ System 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, of 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**
   - **Solution Part Number**
   - **Solution Expiration**
   - **Chef Script Version**
   - **Chef Package Version**

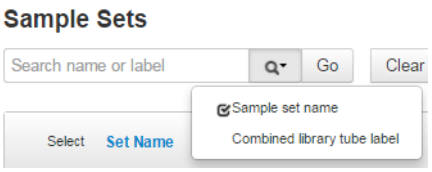
   The **Chef Script Version** lists the version of the software script for the Ion Chef™ Instrument, and the **Chef Package Version** lists the software package that is used by the Ion Torrent™ Server. The release version for these scripts can differ if Torrent Suite™ Software was updated with an off-cycle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

# Find a Sample Set

1. In the **Plan** tab, click **Samples**.
2. In the search box, enter a search term for the Sample Set name, or a Combined Tube Label (Sample Tube Label).

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

3. Click to filter the search, then select **Sample set name** or **Combined library tube label**.



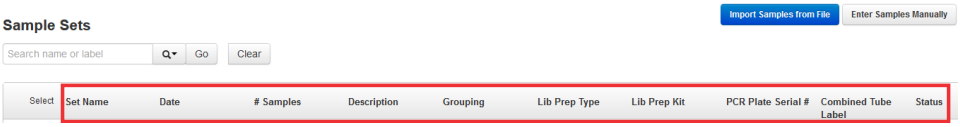

4. Click **Go**.
   The results of the search appear.

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 header to sort the sample rows alphabetically or numerically.



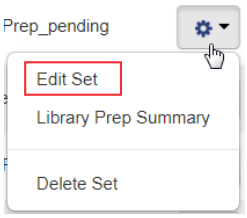

3. Click the column header again to reverse the order of the column contents.

4. Click **Clear** to undo the sort.

## Edit a Sample Set

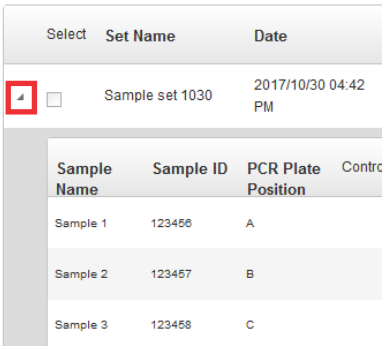
1. In the **Plan** tab, click **Samples**.

2. In the row of the Sample Set that you want to edit, click **(Actions)** ▸ **Edit Sample Set**.




3. In the **Edit Sample Set** dialog box, make any desired changes, then click **Done**.

4. To edit an individual sample within a Sample Set:




   a. Click the triangle to the left of the Sample Set name to expand the Sample Set, then click ⚙ **(Actions)** ▸ **Edit Sample in Set** in the row of the sample that you want to edit.

   b. Complete the **Edit Sample** dialog box, if needed, then click **Done**.

   c. 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!**.

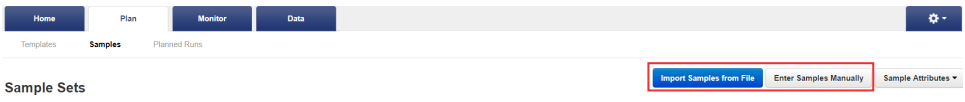
To delete a sample from a Sample Set, see “Edit a Sample Set”

## Create an Ion AmpliSeq™ on Ion Chef™ Sample Set

Samples that are in the Ion AmpliSeq™ on Ion Chef™ Sample Set samples can be automatically 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 automatically transferred to the Planned Run when templating and sequencing the combined library.

To create a Ion AmpliSeq™ on Ion Chef™ Sample Set, you can either import samples from a CSV file, or enter them manually. The following is an example of importing samples from a CSV file.

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



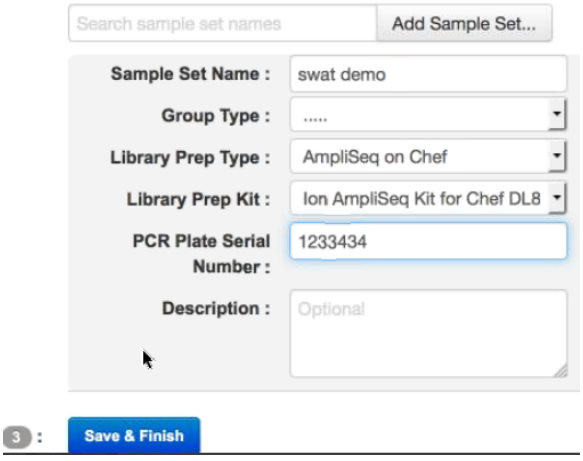
2. In the **Import Samples** screen, click **Sample File Format**.
   A CSV template file downloads to your computer in the Downloads folder.

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. Click **Select 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. Type or scan the PCR plate serial number.
   f. In **Description**, enter additional information.

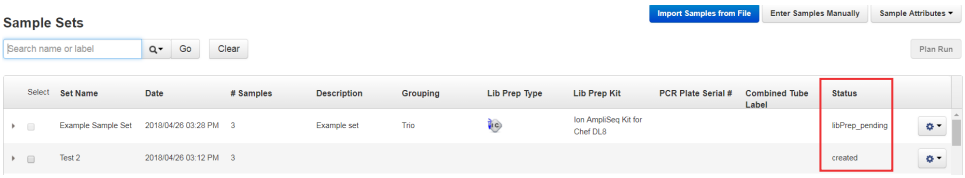



   g. Click **Save & Finish**

A new Sample Set is created.

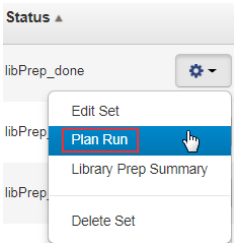
7. In the **Plan** tab, click **Samples**.

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

# 4 Plan and execute an instrument run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## Planned Runs

Planned Runs are the digital instructions for the sequencing instrument that contain specifications for sample preparation, sequencing, data export, and post-sequencing data analysis. A Planned Run contains all of the 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 Plan Run template specifies a default chip type, but you can change the chip based on your throughput needs. Changing the chip triggers 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 system-installed 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 you can create your own unique Planned Run template. Custom templates are for advanced users. 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 category.

| 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 of the 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. |

*(continued)*

| Research application | Description |
|---|---|
| **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. |
| **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, which do not assume enrichment and do not require a target regions file. |
| **16S rRNA Sequencing** | Ion 16S™ Metagenomics Kit. |
| **16S Target Sequencing** | Ion 16S™ Metagenomics Kit. |

## Create a custom Planned Run template

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

**IMPORTANT!** Before you create a custom Planned Run 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.

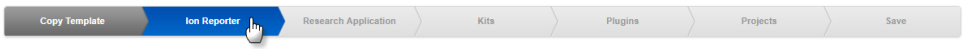
1. In the **Plan** tab, click **Templates**, then select the desired type of research application from the left navigation menu.
2. In the list of templates, find the system Planned Run template from which you want to create your custom Planned Run, then click **(Actions)** ▸ **Copy**.
   The **Copy Template** workflow bar opens to the **Save** step.

3. Enter or select the required information in the **Save** step.

   As you make your selections, your settings are updated in the **Summary** pane.

| Item | Description |
|---|---|
| **Template Name** (Required) | Enter a name for your custom Planned Run template. |
| **Set as Favorite** | Select the **Set as Favorite** checkbox to add your custom template to the **Favorites** list. |
| **Analysis Parameters** | Select **Default** to accept default analysis parameter settings (recommended). Advanced users can customize analysis parameters by selecting **Custom** and editing appropriate analysis information.<br>For more information, see "Copy an analysis parameter set" on page 62. |
| **Reference Library** | Select the reference library file appropriate for your sample. Depending on your application, you may have to select separate DNA, RNA, and fusions reference library files. |
| **Target Regions** | Select the target regions BED file appropriate for your sample. Depending on your application, you may have to select separate DNA and Fusions Target Regions file.<br>Check with your local Field Service Engineer for updates to ensure that the most current files are being used.<br>For BED file installation instructions, see "Upload a target regions file" on page 270. |
| **Hotspots** | Select the hotspots (BED or VCF) file appropriate for your sample.<br>For BED file installation instructions, see "Upload a hotspots file" on page 272. |

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, then click **Next**.




   For more information, see "Ion Reporter step in the workflow bar" on page 45.

5. 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 47.

6. 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 49.

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

   For more information on configuring plugins, see “Plugin configuration” on page 129.

   ( For more information on the **Plugins** step, see “Plugins step in the workflow bar” on page 52.

8. 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 53.

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

The customized template is now available in the **Templates** screen in the **Research Application** group from which you copied the preinstalled Planned Run template. If you set your customized template to favorites, it also appears in the **Favorites** list.

## Create a Planned Run from a Planned Run template

You can create Planned Runs from a preinstalled or custom 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. Custom Planned Run templates can be created as described in “Create a custom Planned Run template” on page 36

When creating a Planned Run from a preinstalled Planned Run template, some settings are predefined by the template and some remain to be selected or completed. The predefined settings can vary between Planned Run 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 in the last screen. If you accept all of the Planned Run template settings, you need only supply a run plan name and sample names to create a Planned Run.

1. In the **Plan** tab, click **Templates**, then select the desired research application from the left navigation menu. Instead, you may click **Favorites** if you have added the template to your favorites list.

2. In the **Template Name** column of the templates table, click the template name.

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 54.

   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.

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.

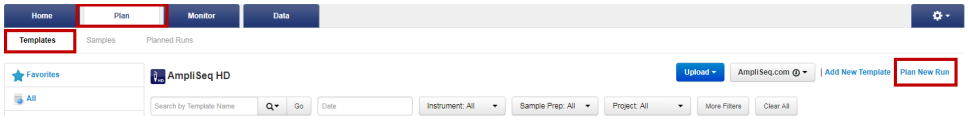
   The Planned Run is added to the list on the **Planned Runs** screen.

## Create a Planned Run without a Planned Run template

If you do not create a Planned Run from a preinstalled or custom Planned Run template, you can create a Planned Run without a Planned Run template and predefined settings.

Best practice is to create a Planned Run from preinstalled or custom Planned Run templates. Planned Run templates contain predefined settings for preparing multiple runs of the same type (for example, if you are performing the same type of sequencing analysis using the same instruments and reagents on multiple samples). For more information, see “Create a custom Planned Run template” on page 36 and “Create a Planned Run from a Planned Run template” on page 38.

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




   The **Create Plan** wizard 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 45.

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 47.

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 49.

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

   For more information on configuring plugins, see “Plugin configuration” on page 129.

   For more information on the Plugins step, see “Plugins step in the workflow bar” on page 52.

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 53.

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 54.

9. 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 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**.

| To… | Actions… |
|---|---|
| Search the list | 1. 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 within a specific research application.<br>2. In **Search by Template Name**, enter a search term, then click **Go**.<br>The templates that contain your search term in the template name are listed. |
| Sort the list | Click any bolded column header in the list of templates to sort the order in which the templates appear. Click the column header 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. Within 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.<br>In some cases, you can select more than one choice within a single filter category. |

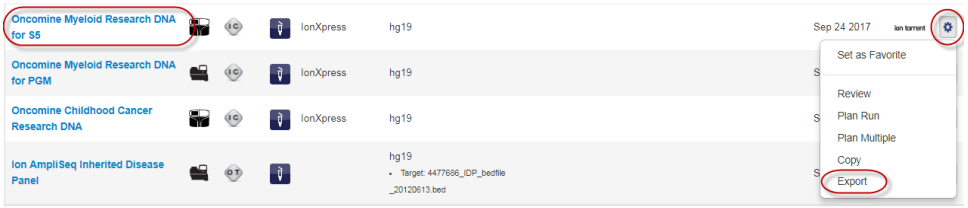
2. Click **Clear All** to remove filters and restore all results.

## 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 42.

You can also open the file in a spreadsheet application such as Microsoft™ Excel™ and edit the settings before import, but be careful to preserve the column headings and layout.

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**.




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

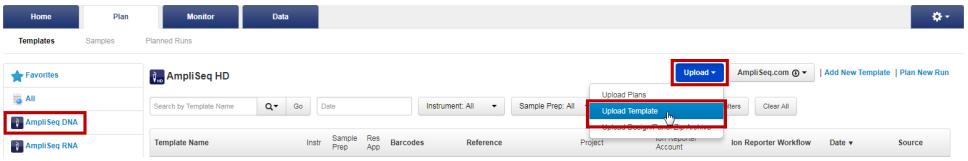
Exported Planned Run templates have "exported" appended to the front of the original template name, unless you edit the name in the CSV file.

## 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, but be careful to preserve the column headings and layout.

To export Planned Run template settings to a CSV file, see “Export a Planned Run template” on page 42.

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.

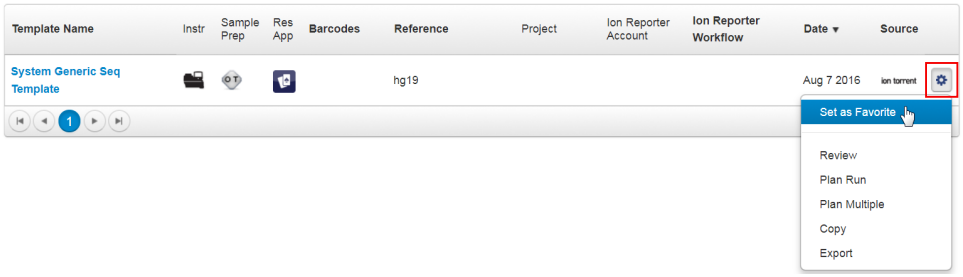
4. *(Optional)* Edit the template name, by clicking ⚙ **(Actions)** ▸ **Edit**.

## 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 41.

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

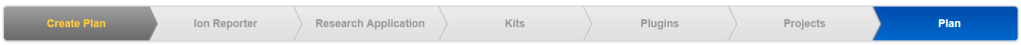



3. To see the list of saved favorites, in the **Plan** tab, click **Templates**, then click **Favorites** at the top of the research applications list.

# Steps in the workflow bar

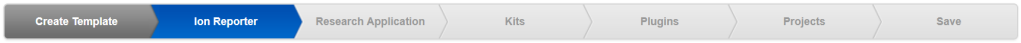
Torrent Suite™ Software guides you through steps to provide the information that is required to create Planned Runs and Planned Run templates, and then execute a Planned Run. You can work through the steps in workflow order, or you can use only the steps that you need.

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 38.

Use the steps in the Create Template workflow bar to:




- Create custom Planned Run templates that fit your sequencing needs, and save the custom Planned Run templates to reuse for future Planned Run creation. For more information, see “Create a custom Planned Run template” on page 36.
- 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 39.

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

You can also download a CSV file and customize it to create multiple Planned Runs without using the workflow bar. For more information, see “Create multiple Planned Runs” on page 71.

## 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 this 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 is launched 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. In this case, you can launch the analysis manually in Ion Reporter™ Software. This approach is commonly used to annotate the VCF files by using the Annotation-only analysis workflow in Ion Reporter™ Software. For details, see *Ion Reporter™ Software 5.14 Help* (Pub. No. MAN0018033.

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 your Planned Run or template. For details, see “Plan by Sample Set” on page 64.

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 239.

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** | 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 your browser. You can then select the newly created analysis workflow in the **Existing Workflow** list.

5. Select an **Ion Reporter Upload 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.<br><br>**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** | 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 using Ion AmpliSeq™ HD technology. This method is compatible with DNA 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 using Ion AmpliSeq™ HD technology. This method is compatible with DNA and RNA 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 using Ion AmpliSeq™ HD technology. This method is compatible with DNA and RNA purified together from tumor or liquid biopsy research samples. |
| **Fusions** | Detects and annotates gene fusions from targeted RNA libraries using Ion AmpliSeq™ HD technology. This method is compatible with RNA 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 Ion AmpliSeq™ Pharmacogenomics Research Panel that is imported from**AmpliSeq.com** . |
| **RNA** | For use with RNA (fusions) libraries. |
| **Typing** | For use when performing 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**<br>**AmpliSeq Exome**<br>**AmpliSeq HD - DNA**<br>**Other**<br>**TargetSeq**<br>**Whole Genome** |
| **DNA and Fusions (Separate Libraries)** | **AmpliSeq DNA and Fusions**<br>**AmpliSeq HD - DNA and Fusions (Separate Libraries)**<br>**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**<br>**Mixed Samples (DNA/RNA)** |
| **Metagenomics** | **16S Targeted Sequencing**<br>**Other** |
| **Mutation Load** | **AmpliSeq DNA** |
| **Oncology - Liquid Biology** | **Tag Sequencing** |
| **Pharmacogenomics** | **AmpliSeq DNA** |
| **RNA** | **AmpliSeq RNA**<br>**RNA Sequencing** |
| **Typing** | **Other**<br>**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 kits used. Options that are available in the **Kits** step depend on the instrument used. Selections in this step influence how the data are analyzed after sequencing.

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

| Item | Selection |
|---|---|
| **Instrument** | Select the sequencing instrument system being used, for example, Ion PGM™ System. |
| **Sample Preparation Kit** | *(Optional)* Select the sample preparation kit used. |
| **Library Kit Type** | Select the kit used to prepare the library (for example, Ion AmpliSeq™ Library Kit Plus). |
| **Template Kit** | 1. Select the instrument system used: **OneTouch**, **IonChef**, or **IA**.<br>2. Select the templating kit used. |
| **Sequencing Kit** | Select the sequencing kit used (for example, Ion S5™ Sequencing Kit). |
| **Chip Type** | Select the sequencing chip type to be used (for example, Ion 540™ Chip). |
| **Control Sequence** | *(Optional)* Select the control sequence added to the library preparation. Leave blank if not used. |
| **Barcode Set** | Select the barcode set used (for example, IonXpress). |
| **Flows** | Enter the number of nucleotide reagent flows required to complete the sequencing run (for example, 400). |
| **Mark as Duplicate Reads** | Select this option to mark duplicate reads in the BAM file after a run is completed. Do not use with Ion AmpliSeq™ data.<br>For more information, see “About the Mark as Duplicates Reads option” on page 52.<br>To remove marked duplicates from the BAM file, select the FilterDuplicates plugin in the **Plugins** step in the workflow bar.<br>For more information, see “Plugins step in the workflow bar” on page 52. |
| **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.<br>For more information, see “TMAP modules” on page 325. |

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

   For more information, see "Advanced Settings—Kits step in workflow bar" on page 51.

   **IMPORTANT!** We recommend using the default settings. Consult your local Field Service Engineer before modifying advanced settings.

| Setting | Description |
|---|---|
| **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:<br>• **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.<br>• **Enable Calibration Standard**—allows wells belonging to the Calibration Standard to be selected as the training subset.<br>• **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.<br>• **No Calibration**<br>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 424. |

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

## Advanced Settings—Kits step in workflow bar

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

**IMPORTANT!** We recommend using the default settings. Consult your local Field Service Engineer before modifying 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.

| Setting | Description |
|---|---|
| **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:<br>• **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.<br>• **Enable Calibration Standard**—allows wells belonging to the Calibration Standard to be selected as the training subset.<br>• **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.<br>• **No Calibration**<br>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 424. |

### About the Mark as Duplicates Reads option

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 particular 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 by using 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. Use of this method is recommended over other approaches, which consider only the 5′ alignment start site.

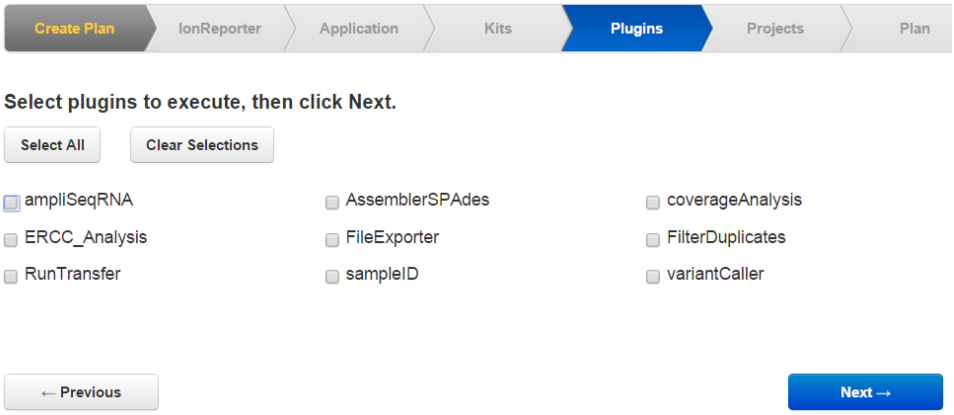
Marking duplicate reads is not appropriate for Ion AmpliSeq™ data, because many independent reads are expected to share the same 5′ alignment position and 3′ adapter flow as each other. Marking duplicates on an Ion AmpliSeq™ run risks inappropriately flagging many reads that are in fact independent of 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 135.
- 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 116.

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

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

2. You can also search for or add projects.

| Option | Description |
|---|---|
| **Search for projects** | *(Optional)* 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** | *(Optional)* 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** | *(Optional)* 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 are different depending on your sequencing application.

| Option | Description |
|---|---|
| **Run Plan Name** (Required) | Enter a name for the Planned Run. |
| **Analysis Parameters** | Select **Default** to accept default analysis parameter settings *(recommended)*.<br>Advanced users can customize analysis parameters by selecting **Custom** and editing appropriate analysis parameters. For more information, see "Copy an analysis parameter set" on page 62. |
| **Default Reference & Bed Files** | |
| **Reference Library** | Select the reference library file appropriate for your sample. Depending on your application, you may have to select separate DNA, RNA, and Fusions reference library files. |
| **Target Regions** | Select the target regions BED file appropriate for your sample. Depending on your application, you may have to select separate DNA and fusions target regions files.<br>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 270. |
| **Hotspot Regions** | Select the hotspots (BED or VCF) file that is appropriate for your sample.<br>For BED file installation instructions, see "Upload a hotspots file" on page 272. |

2. Depending on your application, select the **Use same reference & BED files for all chips/barcodes** checkbox if you are using the same reference sequences and target regions and hotspots BED files across all of your chips/barcoded samples in the Planned Run. If you are using 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 using the same sample for both DNA and Fusions libraries.

4. Depending on your application, complete the following:

| Option | Description |
|---|---|
| **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.<br>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 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 to populate the **Samples Table** automatically.
     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 containing sample information specific for this Planned Run.
     d. Click **Load** to populate 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** using the software. The available options are different depending on your sequencing application.

| Option | Description |
|---|---|
| **Barcode** | For barcoded samples, select a barcode. |
| **Control Type** | *(Optional)* Click the **Control Type** column heading to expand the column, then select the control type. |
| **Sample ID** | *(Optional)* Enter a sample ID for each sample. |
| **Sample Description** | *(Optional)* Enter a sample description for each sample. |
| **DNA/Fusions** | For DNA and Fusions application, select **DNA** or **Fusions** for each sample. |

*(continued)*

| Option | Description |
|---|---|
| **Reference** | If using 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 your 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™ sample tubes to be used in the run. |
| **Chip Barcode** | Scan or enter the barcode of the Ion Chef™ 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. |
| **Ion Reporter workflow** | Select the Ion Reporter™ Software analysis workflow specific for your run. If you do not see your analysis workflow, select the **Show All Worklows** checkbox in the column heading.<br>This option is available if Ion Reporter™ Software is connected. |
| **Relation** | Select sample relationship group.<br>This option is available if Ion Reporter™ Software is connected. |

*(continued)*

| Option | Description |
|---|---|
| **Gender** | Select **Male**, **Female**, or **Unknown**.<br>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).<br>Do not give unrelated samples the same **IR Set ID** value even if the value is zero or blank.<br>This option is available if Ion Reporter™ Software is connected. |

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

8. *(Optional)* If using 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. In the **Summary** section, 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 |
| Monitoring | Bead Loading (%) |
| | Key Signal (1-100) |

*(continued)*

| Category | Subcategory |
|---|---|
| Monitoring | Usable Sequence (%) |
| Reference | Reference Library |
| | Target Regions |
| | Hotspot 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 include:<br>Barcode<br>Sample Name<br>Sample ID<br>DNA/RNA<br>Reference<br>Target Regions<br>Hotspot Regions |
| Analysis Parameters Used (Default) | Thumbnail BeadFind Args |
| | Thumbnail Analysis Args |
| | Thumbnail Pre-BaseCaller Args for calibration |
| | Thumbnail Calibration Args |
| | Thumbnail BaseCaller Args |
| | Thumbnail Alignment Args |
| | Thumbnail IonStats Args |
| History | A log of system actions taken during the run. Table columns include:<br>Date<br>User<br>Log |

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” on page 202.

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 on **AmpliSeq.com**.

1. To 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 menu.
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**.
   **Note:** Enter the access code that you generated on **AmpliSeq.com** into the **Access Code** field.

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 60. If you want to import files that are saved to a hard drive or other storage location, see instead "Manually upload panel files" on page 62.

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 select 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. 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

If you import individual panel files from **AmpliSeq.com** and save the files to a storage location, you must manually upload them before you can access them in Torrent Suite™ Software.

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** as described in "Import Planned Run template parameters" on page 61.

1. In the **Plan** tab, click **Templates**.
2. Click **Upload** ▸ **Upload Design/Panel Zip Archive**.
3. In the **Import Panel from Zip Archive** dialog box:
   a. Click **Select File**, then browse to your compressed panel file.
   b. Select the instrument type and chip type.

      **IMPORTANT!** If you do not select the correct chip type, a validation error occurs during the upload.

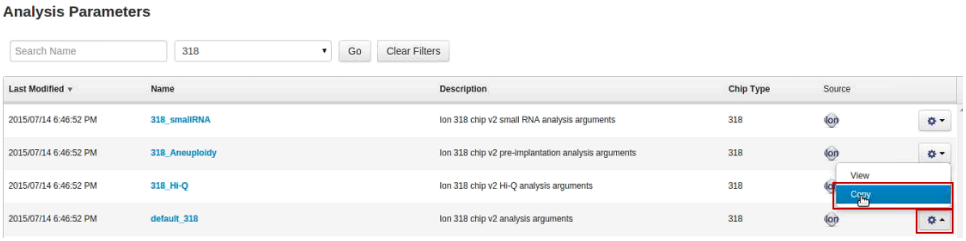
   c. Click **Upload Panel from Zip Archive**.
   d. Close the upload dialog box.
4. To check the status of the upload, click **(Settings)** ▸ **Reference Sequences**, then click **Upload History**.

## Copy an analysis parameter set

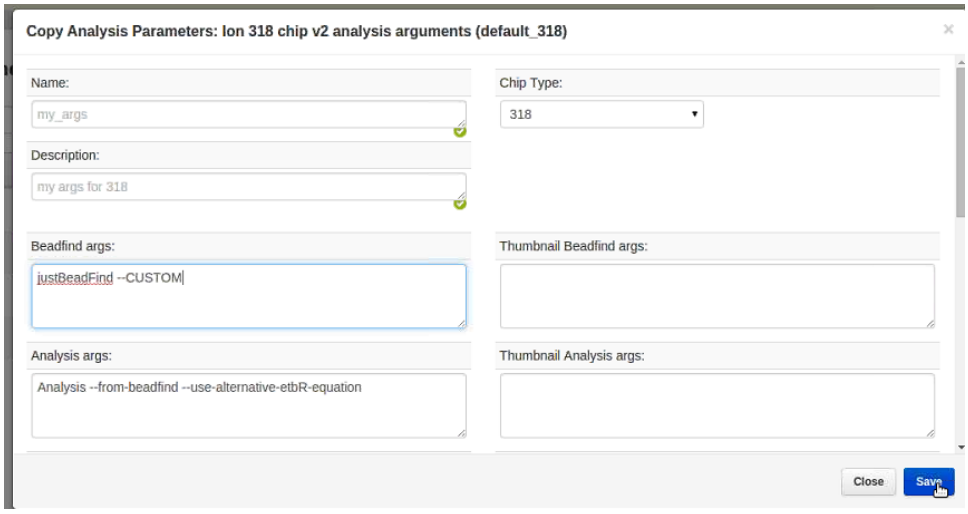
You can copy an existing analysis parameter set, and then customize the settings and save it as a new parameter set. We recommend this procedure if you need a unique analysis parameter set for a custom analysis that other users do not need to use in their runs. Copying an analysis parameter set ensures that the uploaded parameter set remains 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 click ⚙ **(Actions)** ▸ **Copy** in the row for that set.




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 custom analysis parameter set for use in a Planned Run or reanalysis of a completed run.

| Option | Description |
|---|---|
| Create a Planned Run or a custom 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.<br>You must first specify a chip type for the Planned Run (under **Kits** in the workflow bar) before you can select the custom parameter set. |
| Reanalyze a completed run | For details, see "Reanalyze a run" on page 124. |

# 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:

- 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.

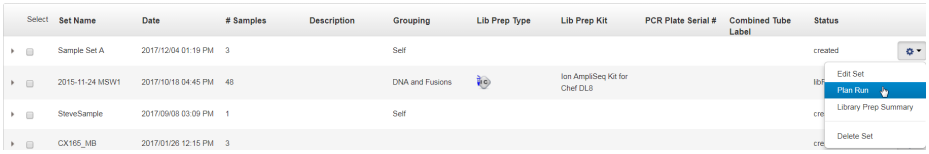
  For example, if you select the Sample Set group type **Trio**, only Trio analysis workflows are available to choose when you configure a Planned Run for use with Ion Reporter™ Software.
- You want to set up multisample analyses in Ion Reporter™ Software.
- Your Ion Torrent™ Server is connected to a LIMS.

# Create a Planned Run with Sample Sets

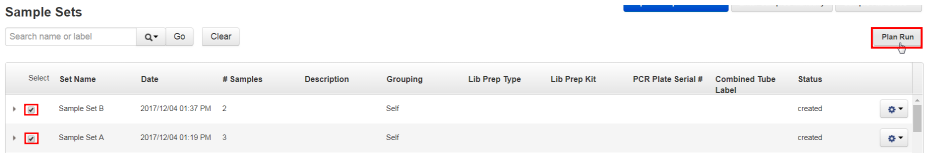
If you set up your samples in Sample Sets before you plan an instrument run, you can add one or more Sample Sets to your Planned Run.

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.

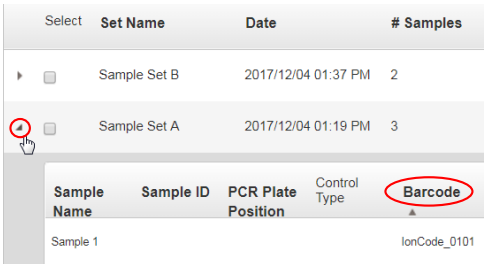
1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** screen, select one or more Sample Sets to add to the Planned Run.
   - To plan a run using a single Sample Set, click ⚙ **(Actions)** ▸ **Plan Run** in the row of the Sample Set.




   - To plan a run using 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 of the selected Sample Sets used in the Planned Run use the same barcode kit. To verify the barcode kit that is used, expand the Sample Set entry to view its 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 **Barcoding** step with the Sample Sets that you selected:

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

| Option | Description |
|---|---|
| **Analysis Parameters** | Select **Default** to accept default analysis parameter settings *(recommended)*.<br>Advanced users can customize analysis parameters by selecting **Custom**, then editing appropriate analysis options. For more information, see "Command Line Args (Advanced) screen" on page 68. |
| **Reference Library** | Select the reference library file that is appropriate for your sample. Depending on your application, you may have to select separate DNA, RNA, and fusions reference library files. |
| **Target Regions** | Select the target regions BED file appropriate for your sample. Depending on your application, you may have to select separate DNA and fusions target regions files.<br>Ensure that you are using the current BED or VCF files. |
| **Hotspots** | Select the hotspots (BED or VCF) file appropriate for your sample.<br>Ensure that you are using the current BED or VCF files. |

5. Select the **Use same reference & BED files for all barcodes** checkbox if you are using the same reference, target regions, and hotspots files across all of your barcoded samples in the Planned Run.

   If you are using 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.

6. In **Sample Tube Label**, scan or enter the barcode of the Ion Chef™ sample tubes to be used in the run.

7. In **Chip Barcode**, scan or enter the barcode that is printed on the chip that is used for this run.

8. Complete the samples table.

   - You can save the samples table to a CSV file, fill out all required sample information, and then upload the samples table to populate the **Samples Table** automatically.
     a. Click **Save Samples Table** to save the CSV file to your computer.
     b. Edit the CSV file by entering all 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 containing 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. Depending on your sequencing application, options can vary.

| Options | Description |
|---|---|
| **Barcode** | For barcoded samples, select a barcode. |
| **Sample Name** (Required) | 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** | *(Optional)* Review sample ID information for each sample. To edit **Sample ID**, you must edit the Sample Set as described in “Edit a Sample Set” on page 30. |
| **Sample Description** | *(Optional)* Review sample description for each sample. To modify **Sample Description**, you must 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 using different reference and BED files 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 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 specific for your run. If you do not see your analysis workflow, select the **Show All Worklows** checkbox in the column heading. |
| **Relation** | Select sample relationship group. |

*(continued)*

| Options | Description |
|---|---|
| **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. |

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

   The software takes you to the next step in the workflow bar. For more information, see “Steps in the workflow bar” on page 44.

10. 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

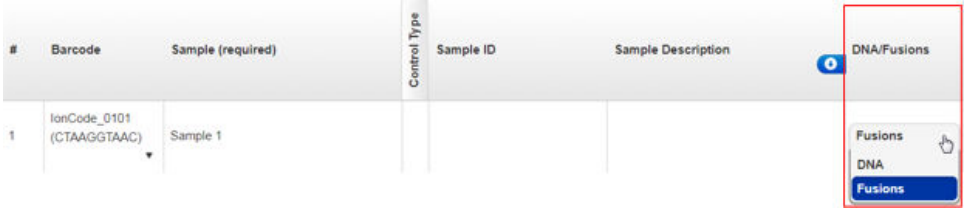
When planning a Sample Set in the **Barcoding** step in the workflow bar, you can click **Custom**, then use the **Advanced Options** screen to customize advanced arguments for analysis parameters.

| Argument | Description |
|---|---|
| **Beadfind args** | Beadfind module command line arguments.<br>These arguments should not be modified unless instructed by Ion Torrent™ Technical Support. |
| **Analysis args** | Analysis command line arguments.<br>These arguments should not be modified unless instructed by Ion Torrent™ Technical Support. |
| **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 313.<br>This field is used only if a Base Calibration Mode other than 'No Calibration' is used.<br>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 313.<br>Other BaseCaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support. |
| **Alignment Args** | Arguments for the TMAP aligner.<br>This replaces the TMAP Args setting that appears in previous software releases. |

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

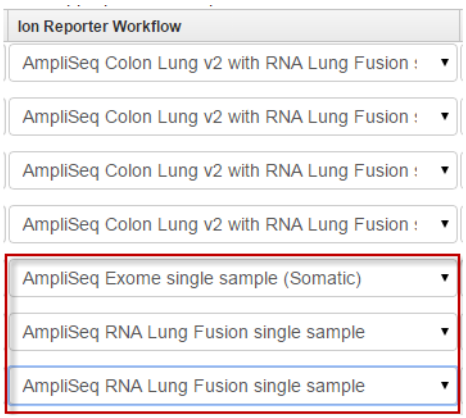
To plan a run for DNA and Fusion sample pairs and several individual Fusion or DNA samples, start with a DNA and Fusions Planned Run template. Then alter the template to accommodate single samples on the same chip. The following example is the procedure for creating a mixed Sample Set consisting of two 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 65.

# 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.

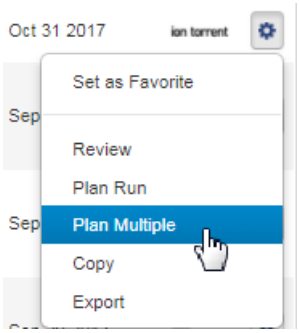
The latest CSV batch planning template file indicates the version of the template in the top row. This version number is required. When you download the template CSV file, the version is automatically included.

# Create multiple Planned Runs for non-barcoded libraries

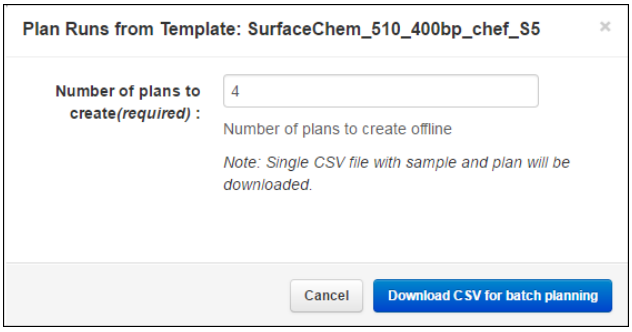
You can create multiple Planned Runs with a template CSV file. To create multiple Planned Runs for individual non-barcoded libraries, a single batch planning template CSV file is required.

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 Template** dialog box, enter the number of Planned Runs that you want to create, then click **Download CSV for batch planning**.

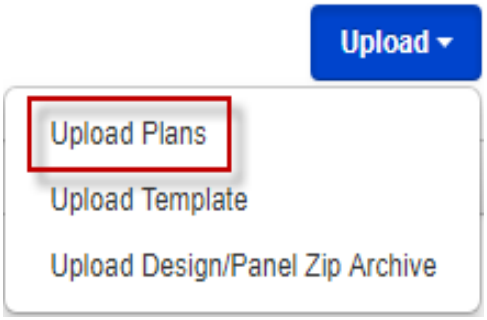



4. Save the batch planning template CSV file to your drive, then open the file.

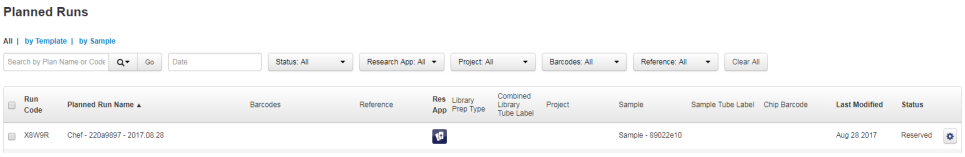
| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | CSV Version (required) | 2 | | | |
| 2 | Plan Parameters | Plan 1 | Plan 2 | Plan 3 | Plan 4 |
| 3 | Template name to plan from (required) | System Generic Seq Template | System Generic Seq Template | System Generic Seq Template | System Generic 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 plan parameters for each Planned Run. The following parameters are required:
   - Template name (auto-populated)
   - Plan name
   - Sample
   - Templating Kit name (auto-populated)
   - 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 CSV for batch planning**.



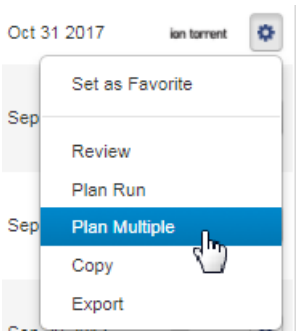

The system parses the files, then creates the Planned Runs.

## Create multiple Planned Runs for barcoded libraries

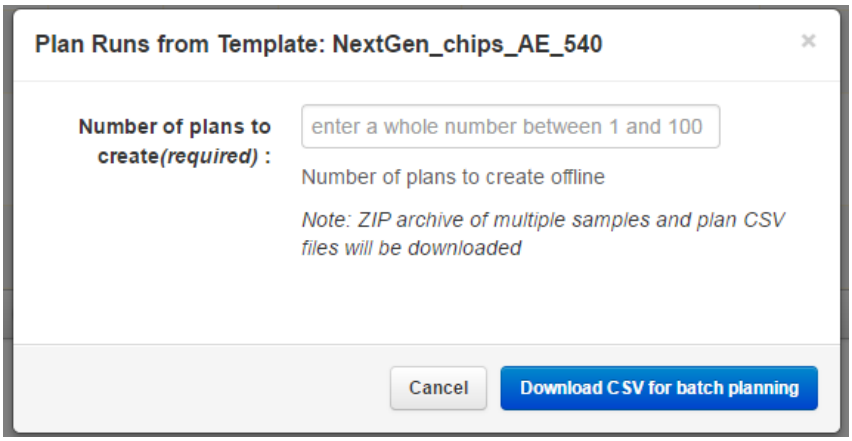
To create multiple Planned Runs for multiplex sequencing of barcoded libraries, two batch planning template CSV files are required:

- 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 Template** dialog box, enter the number of Planned Runs that you want to create, then click **Download CSV for batch planning**.




4. Unzip the downloaded archive file, then save the files to your drive.

5. Open the tsPlan file appended with *master.csv*, enter the Template name, Plan name, Sample, and Chip type.

| | 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 barcoded Planned Runs.

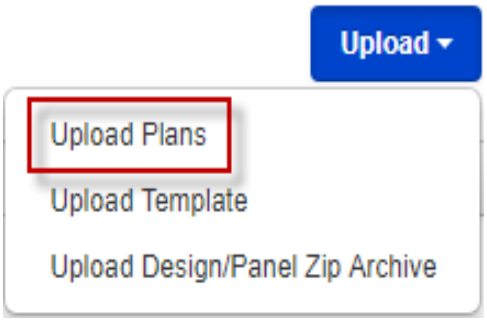
6. Save the CSV file.

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 CSV for batch planning**.
    The system parses the files, then creates the Planned Runs, that are then available for use in the **Plan** tab, in **Planned Runs** screen.

# Planned Runs list

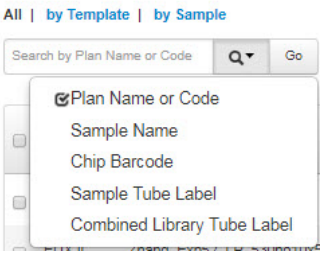
The **Planned Runs** screen under the **Plan** tab lists Planned Runs that are ready to execute on an Ion Chef™ Instrument or a sequencer. Planned Runs are listed by date, and the list includes basic information about each run, including its status in the sequencing workflow. For more information, see “Planned Run status” on page 78.

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

# Manage Planned Runs

You can manage Planned Runs in various ways.

On the **Plan** tab, click **Planned Runs** to access various functions to manage Planned Runs.

| To... | Do the following... |
|---|---|
| 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. |
| Search the list | 1. In the search box above the **Planned Runs** list, click **(Search By)**, then select the search type from the options.<br>  <br>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**.<br> |
| Clear all search and filter settings and display the complete list | Click **Clear All** above the list. |
| Sort the list | Click a column header to sort the list by the information in that column. Only columns with bold headers 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, hover over the **(Info)** icon. |

*(continued)*

| To... | Do the following... |
|---|---|
| Display a list of projects that the run results will be transferred to | In the **Project** column, hover over the **(Info)** icon. |
| Delete Planned Runs | Select the checkbox next to each Planned Run, then click **Delete Selected** below the list.<br>Alternatively, click **(Actions)** in the Planned Run row, then select **Delete**.<br>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.<br>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**. |
| Copy a Planned Run | 1. Click **(Actions)** in the Planned Run row, then select **Copy**. The **Copy Plan** workflow opens.<br>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 81. |

# 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 78.

| 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.<br><br>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.

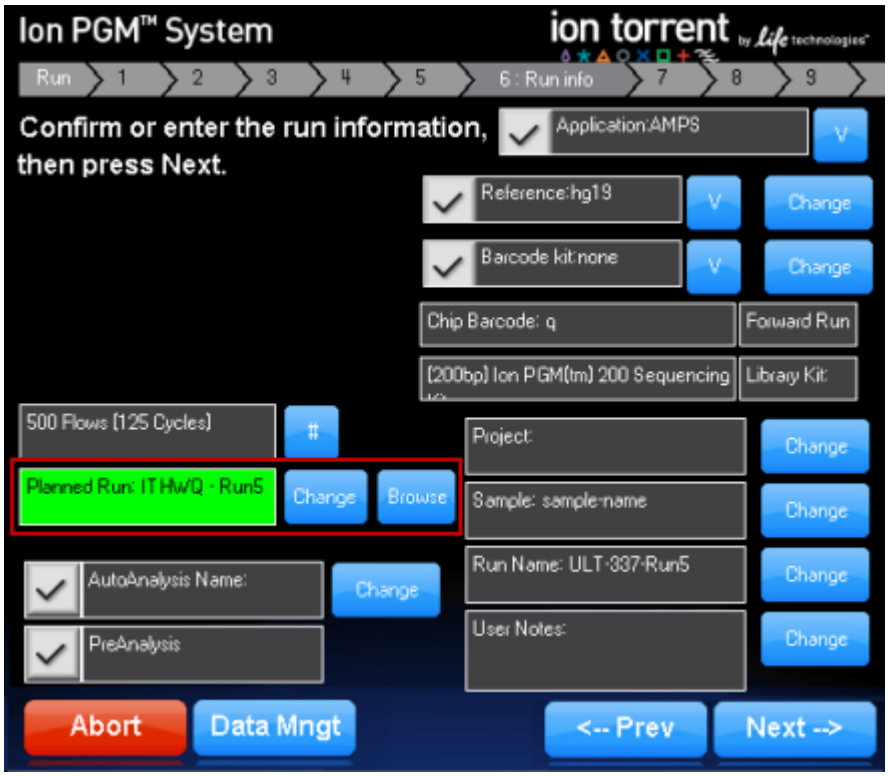
1. In the **Plan** tab, click **Planned Runs**.
2. Find the Planned Run of interest (with a status of **Reserved**), then click **(Actions) ▸ Completed on Chef**.
   The status for the Ion Chef™ Instrument run on the **Planned Runs** screen changes from **Reserved** to **Planned**. The sequencer can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

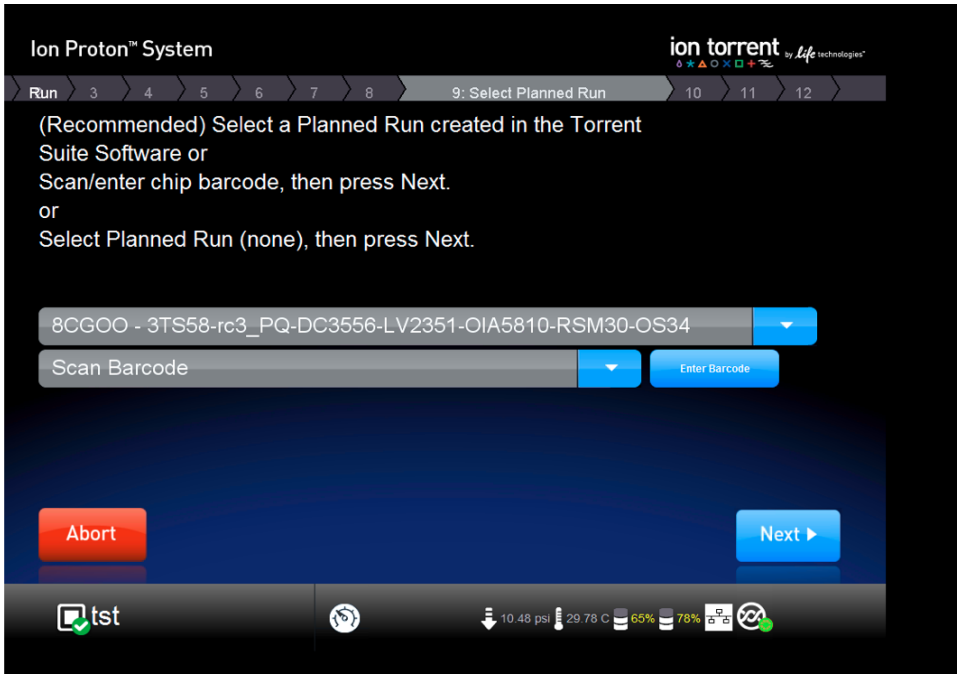
Depending on your sequencing system, use one of these methods to execute a Planned Run.

- On the Ion PGM™ System: Press **Run** in the home screen, then follow the on-screen instructions. Pending run information appears in the **Run info** screen. If needed, press **Browse** to select a different Planned Run from the Planned Runs list. Press **Next -->** when you are ready to start your sequencing run.

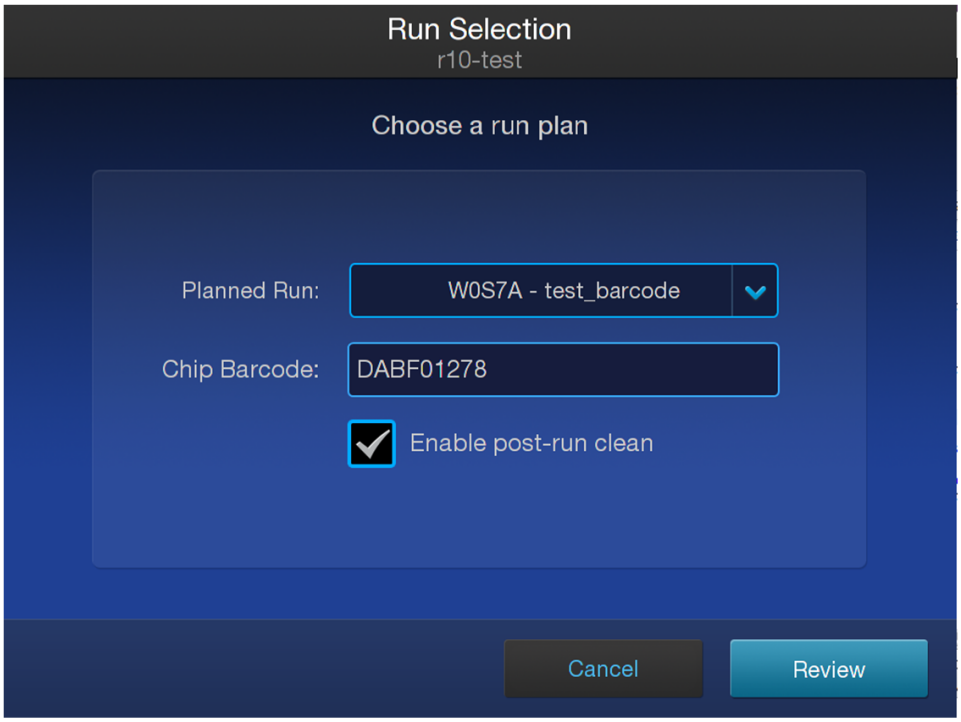



You can also type the Planned Run short code (for example, ITHWQ) into the **Planned Run:** text box on the run information dialog box.

- On the Ion Proton™ System: Press **Run** in the home screen, then follow the on-screen instructions. In step 9 of the workflow, select a Planned Run from the list, scan or enter the chip barcode, then press **Next ►**. Pending run information appears in the next screen. Follow the on-screen instructions to confirm the run information, load and calibrate the chip, and start the sequencing run.



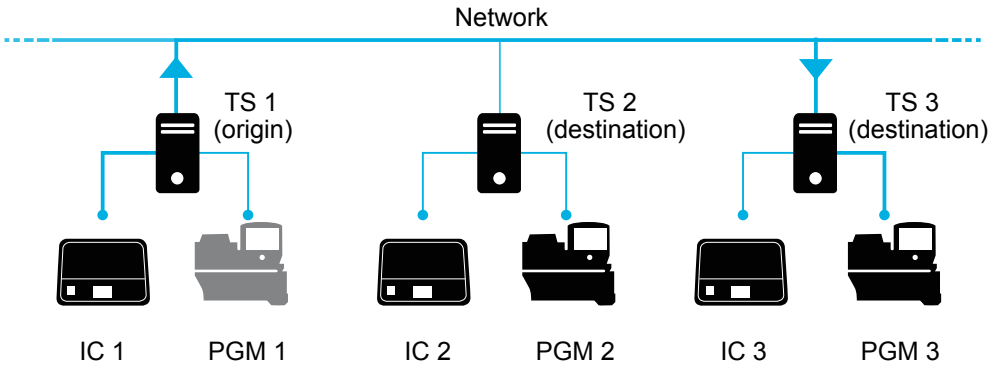

- On the Ion GeneStudio™ S5 System and Ion S5™ System: Select your run from the **Run Selection** screen, then press **Review**. On the **Select Run** screen, ensure that the run selections are correct, then press **Start run**.



# 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 a Ion Chef™ Instrument or sequencer associated with a particular sever is offline or busy.

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

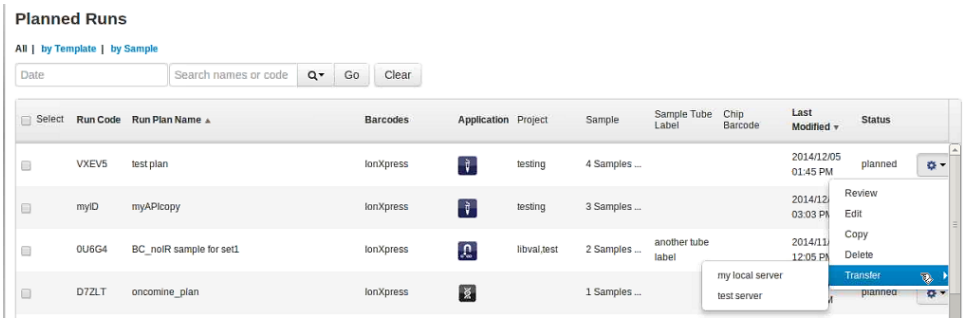



For example, as shown in the diagram, a Planned Run can be set up on the first Ion Torrent™ Server (TS 1) and run on its associated Ion Chef™ Instrument (IC 1). But if the associated Ion PGM™ Sequencer (PGM 1) is busy or offline, you can transfer the Planned Run to TS 3 or TS 2 to perform the run on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

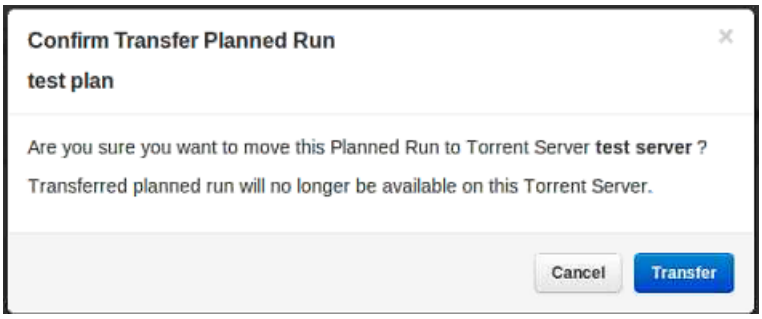
1. In the **Planned Runs** screen, find the Planned Run that you want to transfer.

   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**).

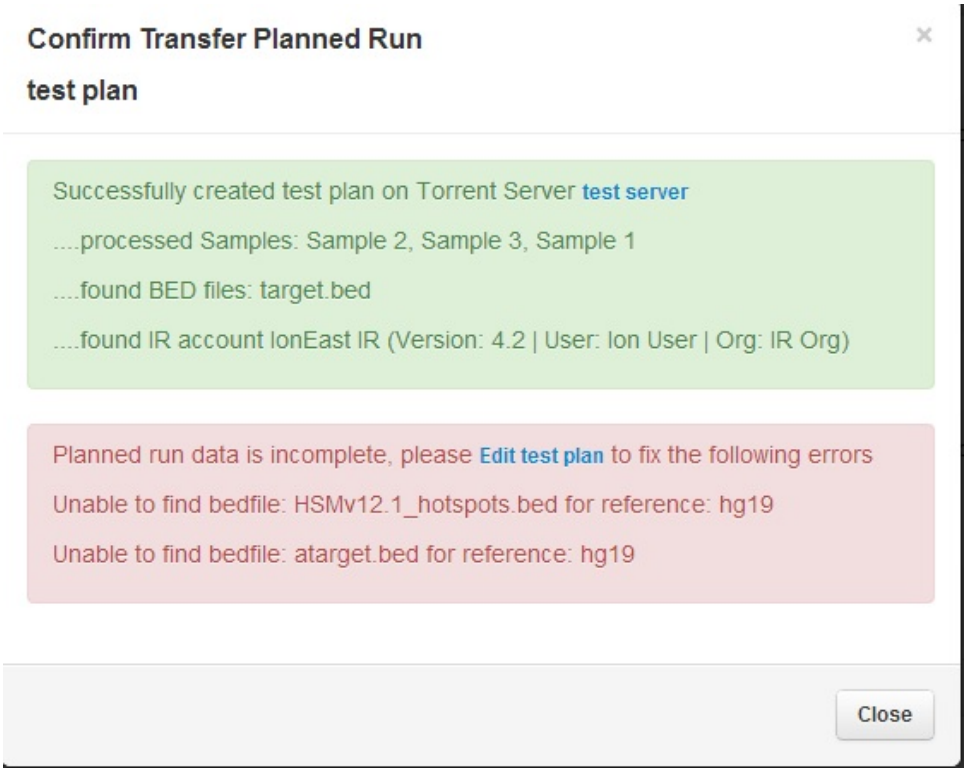
2. Click ⚙ **(Actions)** for the selected Planned Run, select **Transfer**, then select the name of the destination Ion Torrent™ Server on the network.



3. 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 it has transferred.
- To move the results of a sequencing run to a different Ion Torrent™ Server, use the **RunTransfer** plugin. For more information, see “RunTransfer plugin” on page 179.

# 5 Monitor runs in progress


- Monitor a sequencing run . . . . . 83
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- View data for runs in progress . . . . . 85
- Auto Refresh the Monitor tab . . . . . 86
- Review the Planned Run settings . . . . . 86
- Stop an analysis job . . . . . 87
- View system memory usage . . . . . 88


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.

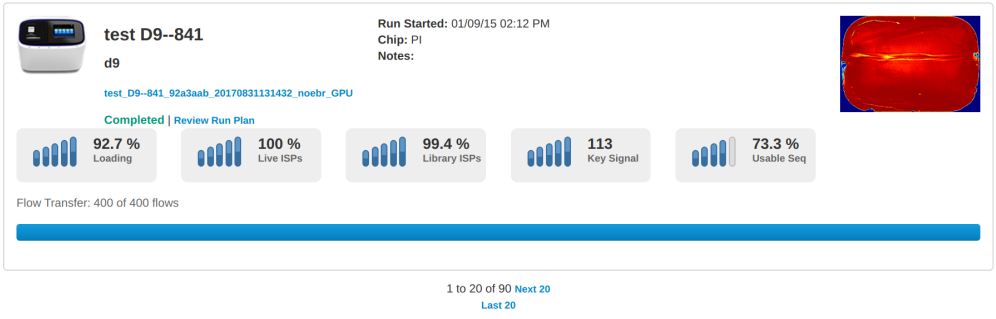
For each run, you can view reported metrics, thumbnail graphs, and other indicators.

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 in progress to determine quickly whether to abort or continue the sequencing run.

In the **Monitor** tab, click **Runs in Progress**, then view run metrics in **List View**.




During a sequencing run, a temporary thumbnail entry shows run metrics and a heatmap image of chip loading as the information becomes available. You can see at a glance in the heatmap image, and in the thumbnail graphs, whether any run quality

metrics are flagged (❗) as falling below the thresholds that are defined in your Planned Run.

| Metric | Description |
|---|---|
| Loading | Addressable wells on the chip that have detectable loading. |
| Live ISPs | Loaded wells that have a live signal. |
| Library ISPs | Live wells with a library template. |
| Key Signal | Average 1-mer signal in the library key. |
| Usable Seq | 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 87.

# 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 78.

1. In the **Monitor** tab, click **Ion Chef**.
2. 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.<br>• **Not started**<br>• **In progress**<br>• **Complete** |
| **Template Prep Progress** | The progress of a template preparation run. |

*(continued)*

| Item | Definition |
|---|---|
| **Template Prep Status** | The current stage of the template preparation run.<br>• **Not started**<br>• **In progress**<br>• **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

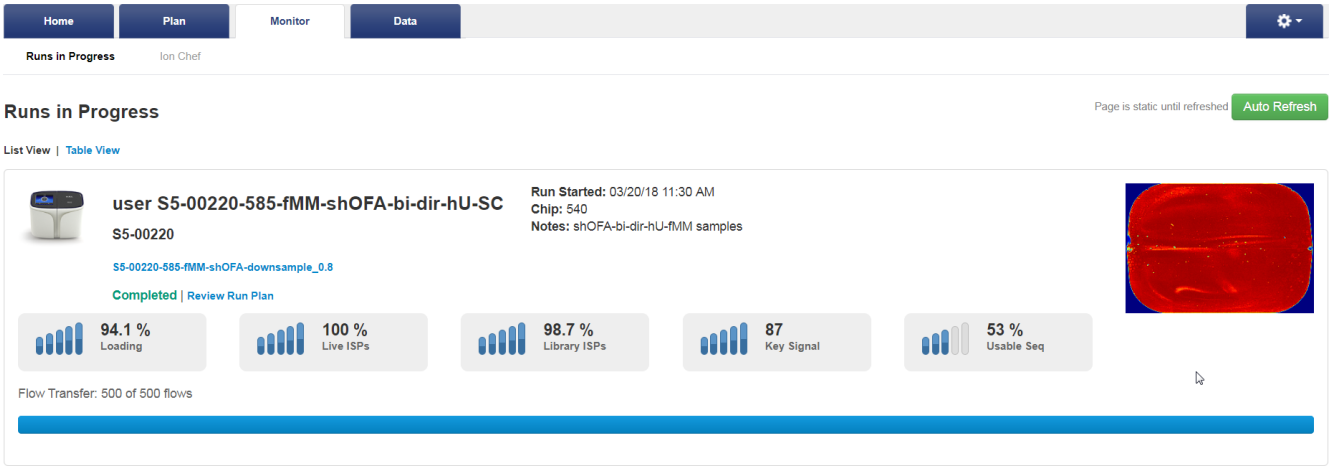
You can view data for runs that are in progress.

The **Runs in Progress** screen on the **Monitor** tab has two views:

- **List View** shows 20 runs per screen, with details for each run.
- **Table View** shows one run per row in columns that you can sort by clicking a column head. This view shows only the information that is associated with each report.

Both views show 20 runs at a time. If you have more than 20 runs, multiple screens are shown.

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



2. To monitor details about Ion Chef™ Instrument runs, click **Ion Chef**.
3. To view more data about a run, click the run report name link.
   The run report is opened in the **Data** tab. You can use the navigation links to view different types of data.

## Auto Refresh the Monitor tab

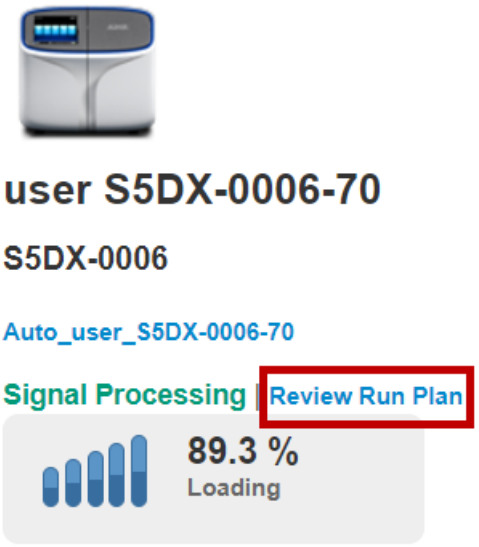
Use the Auto Refresh feature to update the information on the **Monitor** tab screens every 20 seconds. Without Auto Refresh, the screens are a static display of information at the time you open the screen.

- 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.

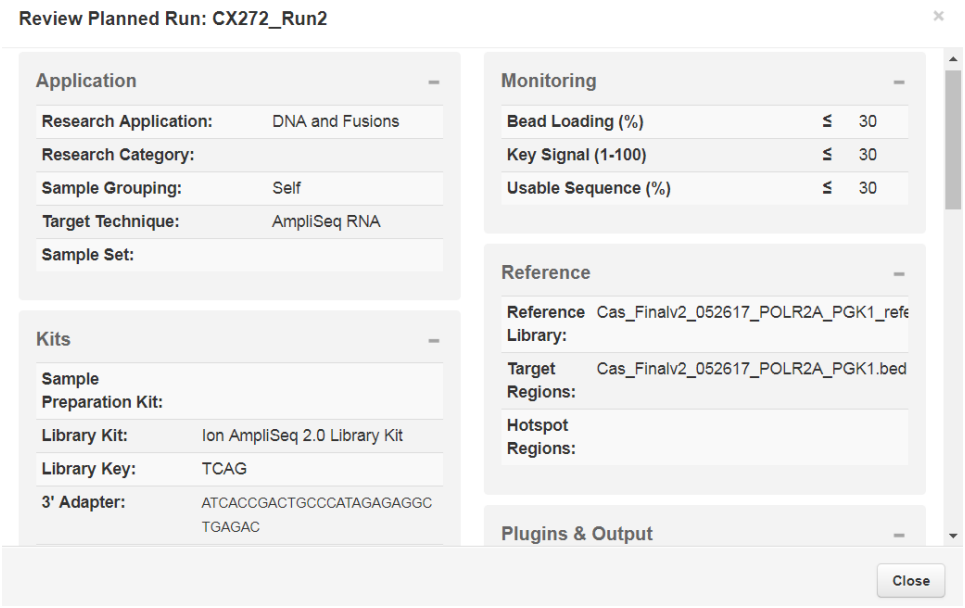
## Review the Planned Run settings

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

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



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

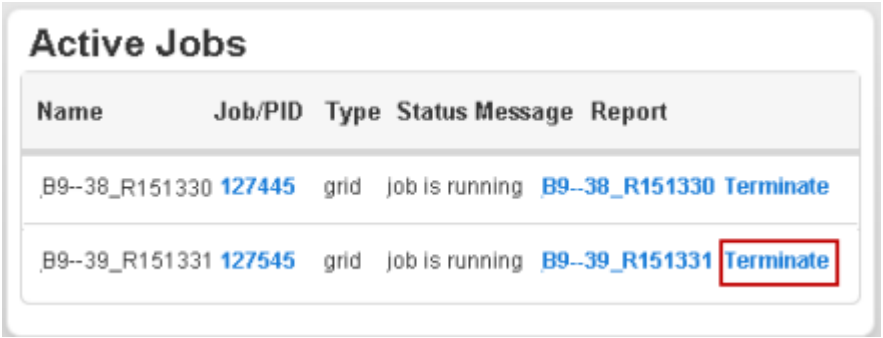



2. Click **Close** to return to the **Monitor** tab, **Runs in Progress** screen.

## Stop an analysis job

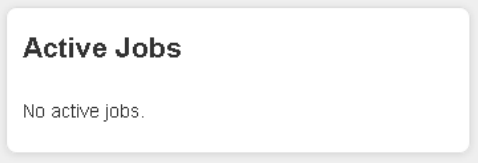
You can stop an analysis job for a run that has started but is not completed.

1. Click ⚙ **(Settings)** ▸ **Services**.
2. Scroll down to the **Active Jobs** section, find the **Name** for the sequencing run that you want to stop. The **Status Message** column indicates **job is running**.



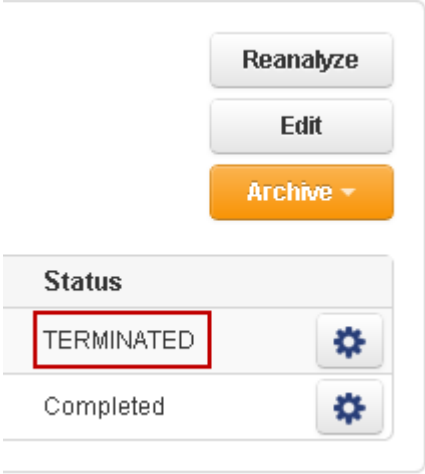

3. Click **Terminate** for the analysis job that you want to stop.
4. In the confirmation dialog box, click **Yes** to end the job.

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.




You can always start a new analysis job.

## View system memory usage

You can view information about how much system memory is being used in the **System 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


- About run reports . . . . . 89
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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.

## About run reports

For runs completed on a Ion Proton™, Ion S5™, Ion S5™ XL, or Ion GeneStudio™ S5 Series system, each run report includes one full chip report and one thumbnail report. If the run has been reanalyzed, additional reports are available when you open the run report. If the run was completed on an Ion PGM™ System, only a full chip report is available.

You can complete several tasks from the run report, including:

- Upload output files from the run report to Ion Reporter™ Software. For more information, see “Run the IonReporterUploader plugin manually” on page 245.
- Review, edit, or copy the Planned Run settings for the sequencing run. For more information, see “Review Planned Run settings” on page 58 and “Create a custom Planned Run template” on page 36.
- Rerun the data analysis for the run. For more information, see “Reanalyze a run” on page 124.
- 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 91.

- Run Torrent Suite™ Software plugins. For more information, see “Run a plugin manually from the sequencing run report” on page 132.
- Download the run report or plugin summary in PDF format. For more information, see “Download a run report summary PDF” on page 108 and “Download a plugin report PDF” on page 132.

For further details 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**.

| To… | Steps… |
|---|---|
| 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 header (List View only) to sort the list. Click the column header 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.<br>Click **More Filters** to see all available filters. Within a filter, enter text in **Find** to limit the filter choices.<br>To remove a filter, deselect the filter choice or click **Clear** in the filter list. |
| View favorites | Click ☆ next to **Search** to limit the list to completed runs that are designated as Favorite.<br>To set a completed run as a Favorite, click ☆ beside the completed run name. The Favorite indicator turns 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.

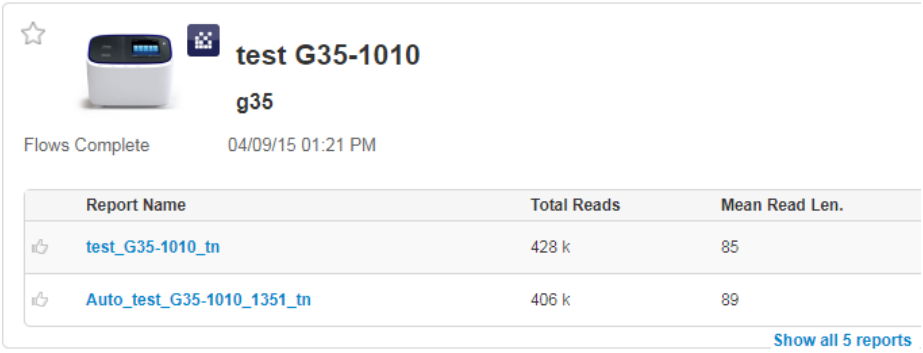
The thumbnail reports use a limited set of data points to give a summary of the fully sequenced and analyzed run. Thumbnail reports can be used to determine quickly the success of the run or whether a run should be terminated. Thumbnail reports are appended with `_tn` at the end of the report name and 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**.
   **Completed Runs & Reports** are displayed in **List View** layout.

2. *(Optional)* Click **Table View** to review **Completed Runs & Reports** details in a table layout.

3. To open an individual run report:

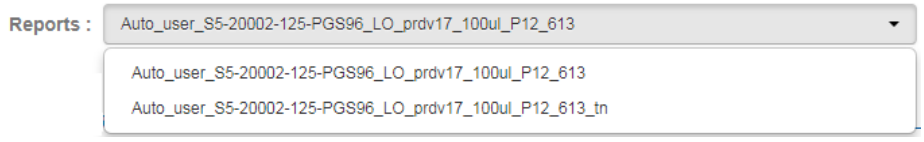
   - In the **List View**, find the pane that contains details about your run of interest, then click the link in the **Report Name** column to view details about any of the available reports for that run.

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

   - In the **Table View**, find the run that you want to view the report for, then click the link in the **Report Name** column.

     To open another report that is associated with your run of interest, click the **Reports** list, then select the report that you want to open.

## Review unaligned reads

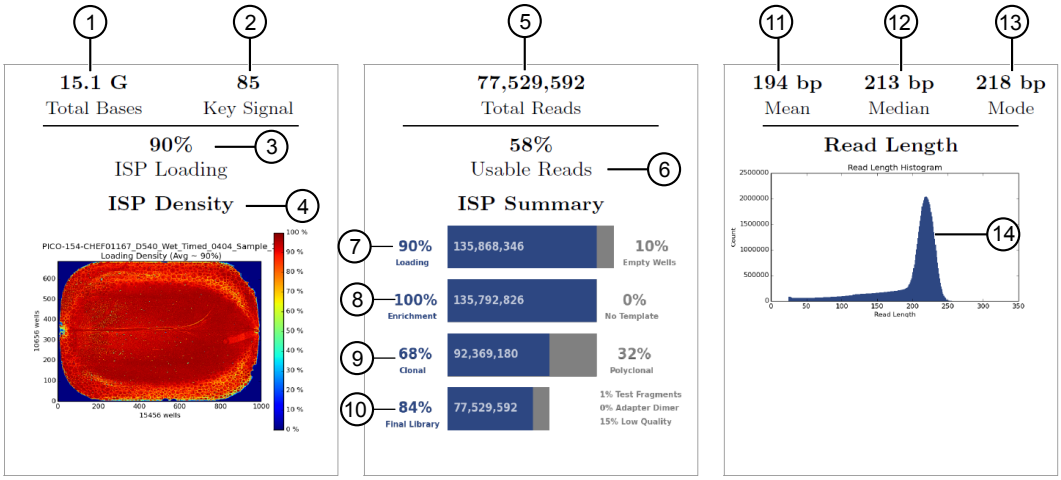
To determine the quality of the sequencing run, you can review the quality metrics for the unaligned reads. Primary pipeline processing, base calling, and signal processing generate these metrics. The quality metrics for unaligned reads are divided into three categories:

- 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**, **ISP Summary**, 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 Loading 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. |
| 7 | **ISP Summary-Loading** | The percentage of chip wells that contain an ISP. The percentage value considers addressable wells. |

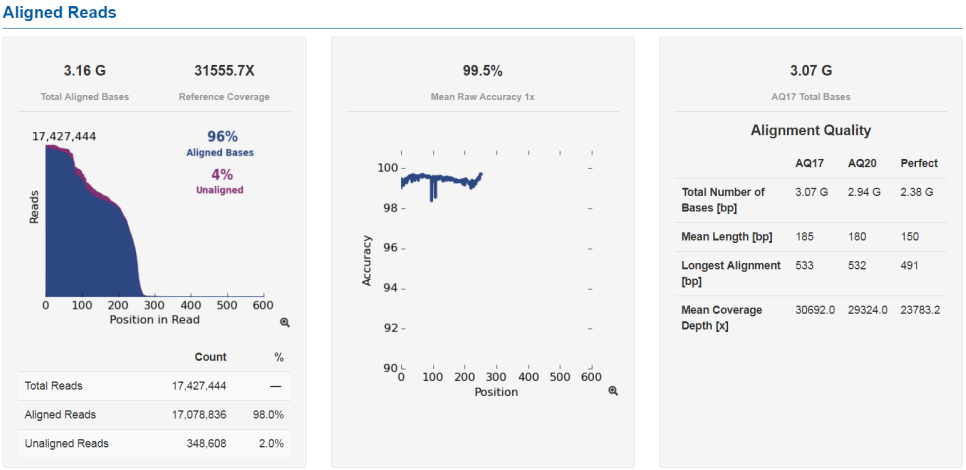
*(continued)*

| Number | Metric | Description |
|---|---|---|
| 8 | **ISP Summary-Enrichment** | The predicted number of live ISPs that have a key signal identical to the library key signal or test fragment (TF) key signal. The Percent Enrichment value reported is the number of loaded wells with live ISPs that are Library ISPs or TF ISPs. This number is calculated by dividing wells with live ISPs by the number of wells loaded with ISPs. |
| 9 | **ISP Summary-Clonality** | The percentage of clonal ISPs (all library and TF ISPs that are clonal, not polyclonal).<br>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 | **ISP Summary-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 Read Length** | The average length, in base pairs, of called reads. |
| 12 | **Median Read Length** | The median length, in base pairs, of called reads. |
| 13 | **Mode Read Length** | 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. |

# 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 using 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 using three 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 325, 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 containing 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. |

*(continued)*

| Predictor | Description |
|---|---|
| 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.

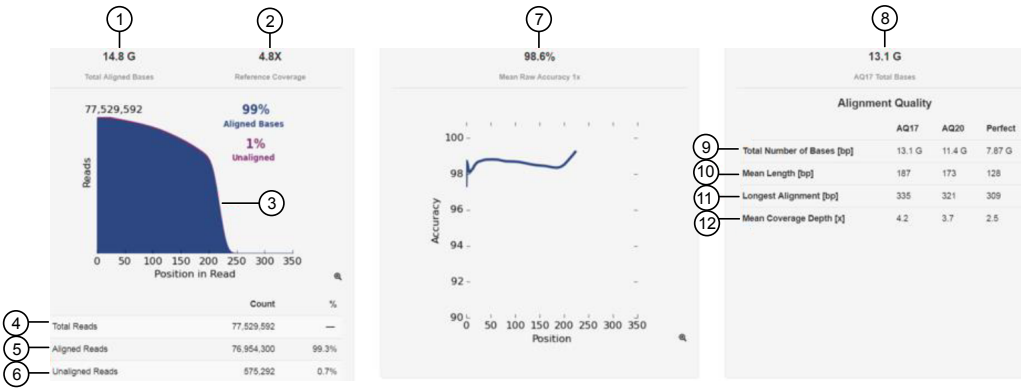
The quality scores are on a phred-10*log_10 (error rate) scale.

**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. |

*(continued)*

| Number | Metric | Description |
|---|---|---|
| 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) |
| 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 assess a run, examine the throughput or total bases. Consider whether these numbers make sense for the application and chip type.

| 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 |
| Ion 550™ Chip | ~260 million | 100–130 million | 20–25 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.

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 loading density, yellow indicates less-than adequate loading density, and blue indicates an absence of loaded beads.

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

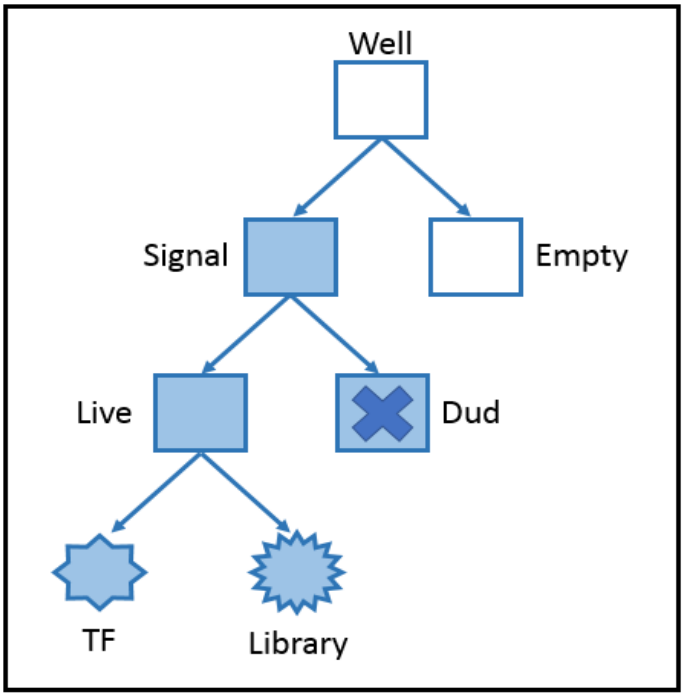
## Total reads

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 and 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, thereby making reads shorter, while read filtering operations completely remove them from the output BAM files. By default, reads that have a trimmed read length of less than 25 bases are being filtered. The different categories of filtered reads shown in the run report are in the table below.

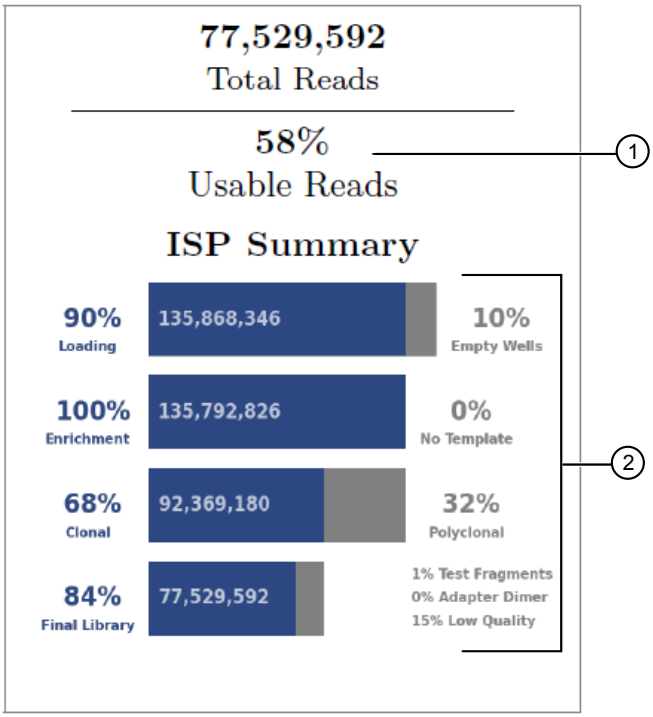
| Filter | Description |
|---|---|
| Polyclonal | Filters reads from ISPs with >1 unique library template population. Occasionally, low or unexpected signal ISPs can also get caught in this filter. |
| Low Quality | Filters reads with unrecognizable key signal, low signal quality, and reads trimmed to <25 bases. |
| Primer 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 displayed 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 Well classification results
② Library ISP filtering results

The ISP summary panel also presents the well classification and library ISP summary table using slightly different calculations.




① Usable reads percentage
② ISP summary percentages

The usable reads percentage is calculated by

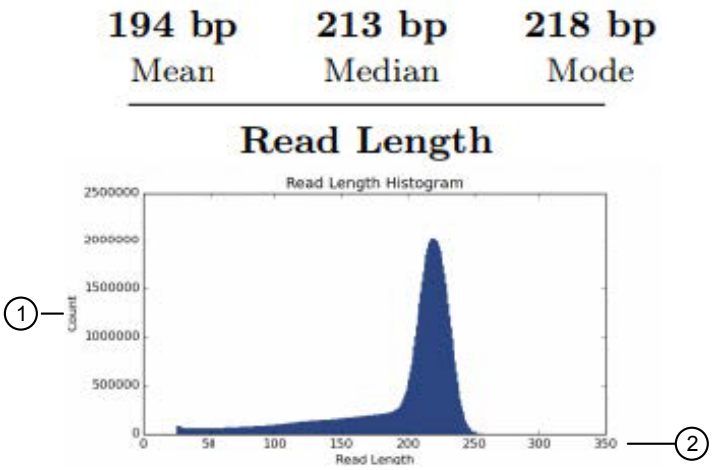
$$\% \text{ usable reads} = \frac{\text{library reads passing filters (77,529,592) x 100}}{\text{number of library ISPs identified (134,802,984)}}$$

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

$$\%\ \text{final library} = \frac{\text{library reads passing filters (77,529,592) x 100}}{\text{number of clonal reads (92, 369,180)}}$$

## 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.

### 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 | TACGTACGTCTGAGGATCGATGT ACAGC | The order in which the chip is exposed to each particular 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 and 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 following files:

| Option | Description |
|---|---|
| Unaligned Reads | Nucleotide bases covered by reads that have not been aligned to the reference. Can be downloaded as a binary aligned/mapped (BAM) 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. Data 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.<br>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** | 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. |
| **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** | Download a binary file that contains unaligned or unmapped reads. Viewing the file requires a viewer application, such as the Integrative Genomics Viewer from the Broad Institute. |
| **BAM** | Download a compressed, binary form of the SAM file. The BAM file contains aligned reads sorted by genome reference location. |
| **BAI** | 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 that is indicated in the browser settings.

# View details about completed runs

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

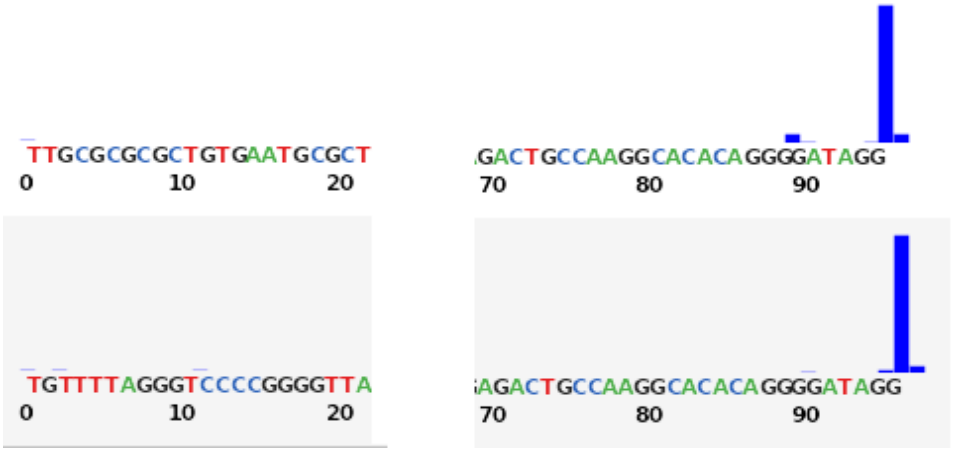
## View test fragments for a completed run

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 294. |
| **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 S5™ Systems and Ion GeneStudio™ S5 Systems.

**Note:** A summary of consumables that are used during a sequencing run is not reported for sequencing runs performed on the Ion PGM™ or Ion Proton™ systems.

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 Reagents** | 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

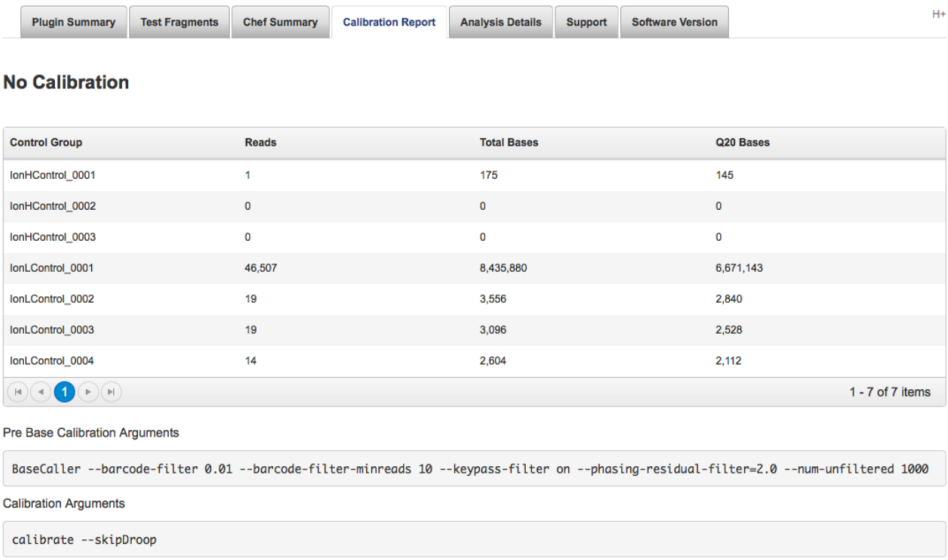
## View the calibration report for a completed run

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 workflow bar” on page 51.

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.




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 58.

## 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 depending 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 for projects and result sets lists by name.

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

2. To find the projects or result sets or of your interest, you can search, sort, and filter the **Projects** and **Result Sets** lists:

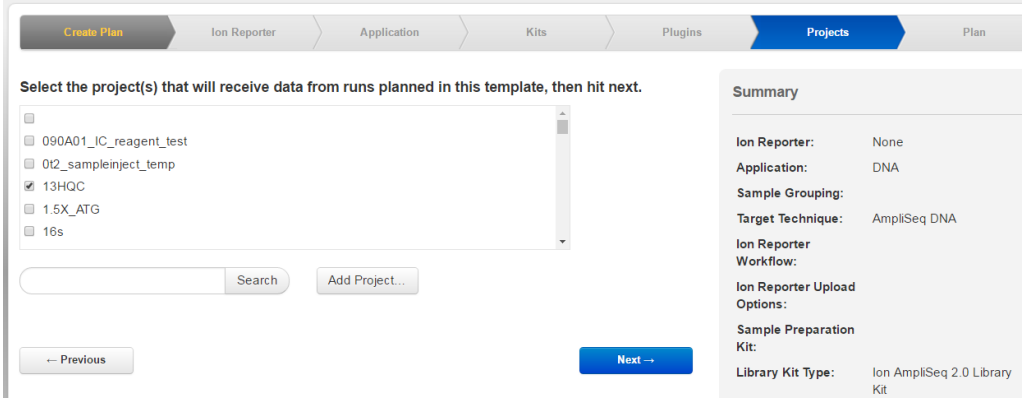
| To... | Steps... |
|---|---|
| Search the list | Enter your search term in **Search names**, then click **Go**. The information displayed in the table is limited only to the names that match or contain the search string.<br>In **Search names**, you can enter a complete or partial name. For example, 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 header in the table to sort the order in which the projects or result sets are displayed. Click the column header 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).<br>• 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.<br>• 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 display 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 results sets 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:

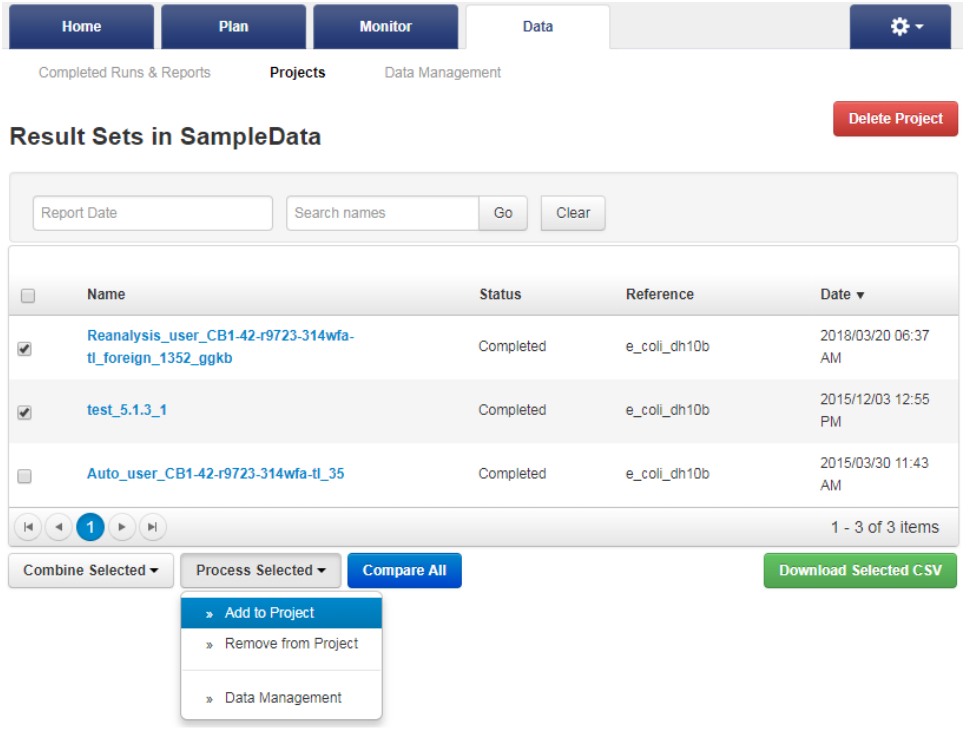



For more details, see Chapter 4, “Plan and execute an instrument run”.

## 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

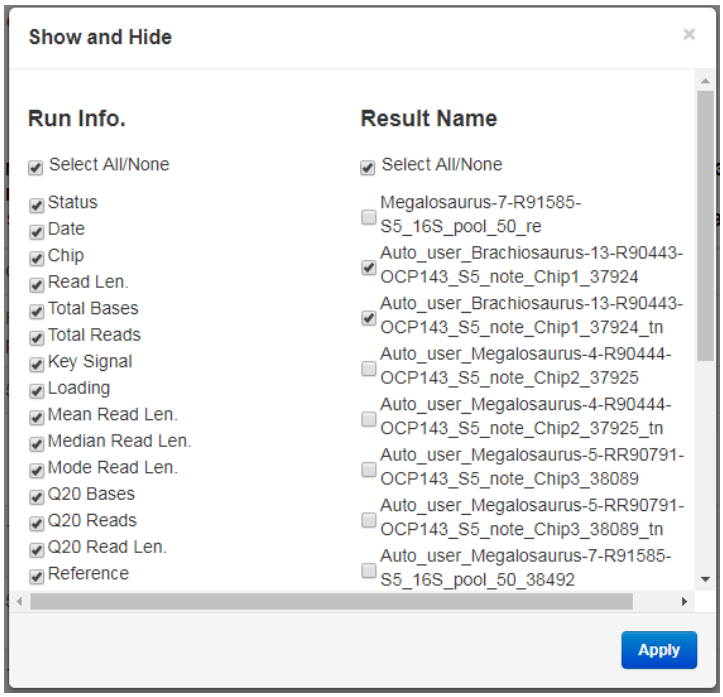
1. In the **Data** tab, click **Projects** to see the list of projects.
2. Select a project in the list to view the result sets that are included in the project.

## Compare reports of runs in a project

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

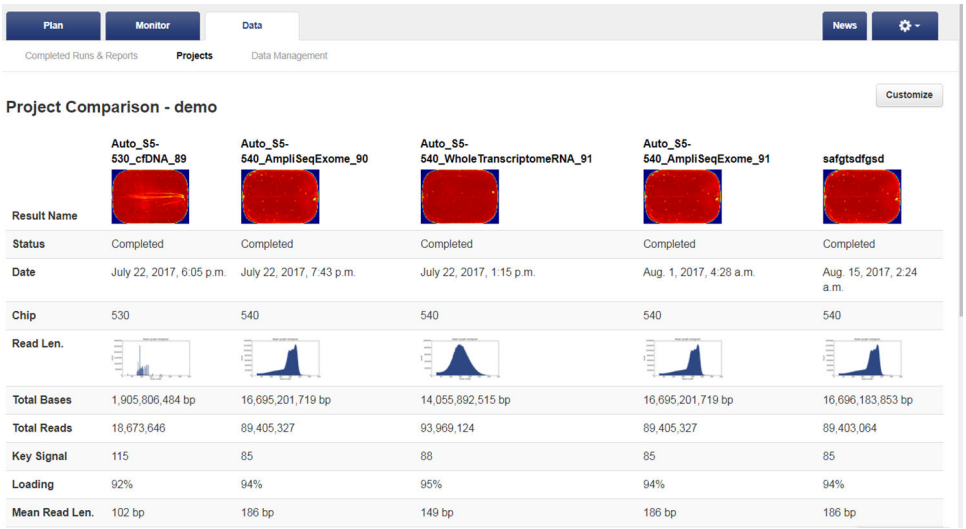
1. In the **Data** tab, click **Projects**.
2. Select a project.
3. Click **Compare All** to view all the runs in the project side-by-side.
4. *(Optional)* Click **Customize**.
   The **Show and Hide** dialog box opens.
5. *(Optional)* In the **Run Info** column, deselect metadata to remove it from view.

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




7. Click **Apply**.

8. Review the reports for all of the 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 356, “Manually archive run data” on page 357 , and “Manually delete selected data from a run report” on page 357.

## 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.<br>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 particular interpretation, when some reads are in fact duplicates of each other with no additional evidence for the interpretation.<br>**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 your browser to a directory on your computer, based on your 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 50bp with 90% or greater accuracy. |
| Library_100Q10_Reads | Reads of length at least 100bp with 90% or greater accuracy. |
| Library_200Q10_Reads | Reads of length at least 200bp 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 considering reads with 90% or greater accuracy. |

*(continued)*

| Field | Description |
|---|---|
| Library_Q10_Longest_Alignment | Longest read length amongst 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 50bp with 98% or greater accuracy. |
| Library_100Q17_Reads | Reads of length at least 100bp with 98% or greater accuracy. |
| Library_200Q17_Reads | Reads of length at least 200bp 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 considering reads with 98% or greater accuracy. |
| Library_Q17_Longest_Alignment | Longest read length amongst 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 considering reads with 99% or greater accuracy. |
| Library_Q20_Longest_Alignment | Longest read length amongst reads with 99% or greater accuracy. |
| Library_Q20_Mapped_Bases | Total bases from reads with 99% or greater accuracy. |

*(continued)*

| Field | Description |
|---|---|
| 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. |
| Library_Mean_Q47_Length | Average length of perfect reads. |
| Library_Q47_Coverage | Average per-base coverage considering only perfect reads. |
| Library_Q47_Longest_Alignment | Longest reads length amongst 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. |
| PGM Name | Name of the Ion PGM™ or Ion Proton™ instrument where the sample was sequenced. |
| Run Date | Date that the sample was sequenced. |
| Run Directory | Location of the raw DAT files on the Ion Torrent™ Server. |
| Num_Washouts | NA |

*(continued)*

| Field | Description |
|---|---|
| Num_Dud_Washouts | NA |
| Num_Washout_Ambigous | NA |
| Num_Washout_Live | NA |
| Num_Washout_Test_Fragment | NA |
| Num_Washout_Library | NA |
| Library_Pass_Basecalling | NA |
| Library_pass_Cafie | NA |
| Number_Ambiguous | NA |
| Number_Live | Number of wells producing a signal. |
| Number_Dud | Number of wells with ISPs but no signal. |
| Number_TF | Number of wells containing test fragment. |
| Number_Lib | Number of wells containing library. |
| Number_Bead | Number of wells containing beads. |
| Library_Live | Number of wells containing library ISP with signal. |
| Library_Keypass | Number of wells containing library ISP with signal and match key. |
| TF_Live | Number of wells containing test fragment ISP with signal. |
| TF_Keypass | Number of wells containing test fragment ISP with signal and match key. |
| Keypass_All_Beads | Number of wells containing ISP with signal and match key. |
| P | JSON string of plugin data. |
| s | JSON string of plugin data. |

## 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. If you want to change the options for the results of a sequencing run one time, you can change the options in the run report, then immediately reanalyze the data. If you want to change the run report for all future reanalyses of the data, 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 your run report of interest. For more information, see “Search for a run report” on page 90.
3. Do one of the following:
   - In **Table View** mode, in the row of the run of interest, click **(Actions) ▸ Reanalyze**.
   - Alternatively, switch to **List View** mode, find your 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 Ion Proton™ System, Ion S5™ System, and Ion GeneStudio™ S5 Systems data. |
| **Start reanalysis from** | • Select **Signal Processing** to reanalyze from DAT files.<br>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.<br>• Select **Base Calling** (default) to reanalyze from 1.WELLS files.<br>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.<br>For more information, see “Copy an analysis parameter set” on page 62. |

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 129, 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 90.
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 custom configuration for the variantCaller plugin" on page 205 and "Run the variantCaller plugin manually" on page 201.

# Edit a run report

You can edit a completed run report to correct a setup error or optimize parameters for all future reanalyses.

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 90.

3. Open the **Edit Run** wizard.
   - Use **Table View**, then click **(Actions)** ▸ **Edit** in the row of the run report that you want to edit.
   - Use **List View**, then identify the run report of interest, then click **Edit**.

   The **Edit Run** wizard 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 as needed. |

5. Click **Update Run**, or **Update Run & Reanalyze** to start the reanalysis immediately.

## Add or change barcoding for a completed run report

You can change barcoding when you set up a reanalysis, but you can also change barcoding for all future reanalyses. 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.

In each case, you must reanalyze the run after editing the barcode information. These steps apply only to completed runs.

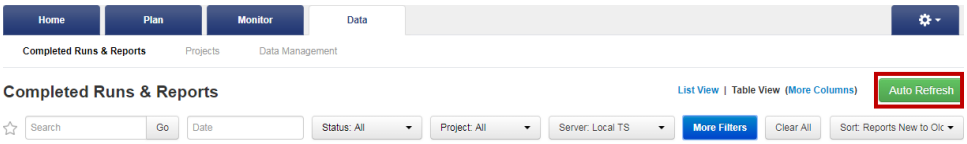
1. In the **Data** tab, click **Completed Runs & Reports**.
2. In table or list view, navigate to your run of interest.

   For help finding an individual run, see “Search for a run report” on page 90.
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 either **Update Run**, or **Update Run & Reanalyze**.

# 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 function is turned on (the default), the screen refreshes every 20 seconds. When the automatic refresh function 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 function is turned off.

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

# 7 Manage plugins for data analysis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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 Connect. 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 particular 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, and fail unless certain settings are 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.

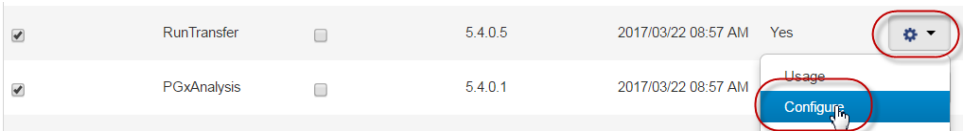
## Configure plugins globally

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
- RunTransfer 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**.



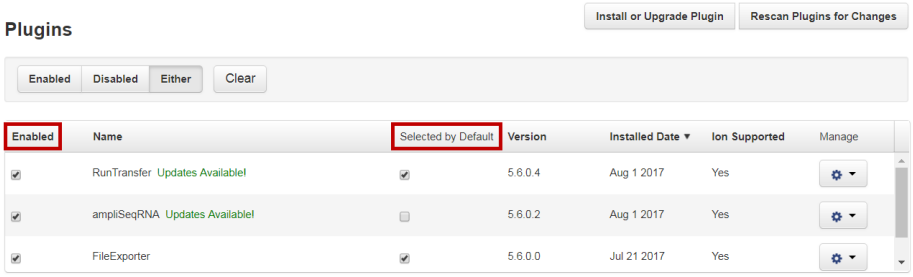

   The settings in the configuration dialog box vary depending on the plugin. See the plugin-specific configuration topic for more information.

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 it is downloaded from the Connect. 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 132.

- 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.



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 135.

  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

After a sequencing run is complete, you may want to manually run a plugin to perform an analysis, regardless of whether a plugin was run automatically after a run. You may 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 the completed sequencing run.
2. Click **Plugins** ▸ **Select Plugins to Run**, then click the name of the plugin that you want to run.
3. In the **Configure Plugin** screen, click the screen to configure the plugin if needed. If prompted, select the desired plugin options, then click **Submit** to start the analysis.

   For detailed plugin configuration information for the available plugins, see the configuration topic for each plugin in “Preinstalled plugins” on page 135.

   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

You can stop a plugin run that is in progress.

1. 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.
2. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
3. In the left navigation menu, click **Plugins**, or the name of the plugin results to be deleted.
4. 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.

## Delete plugin results from a run report

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

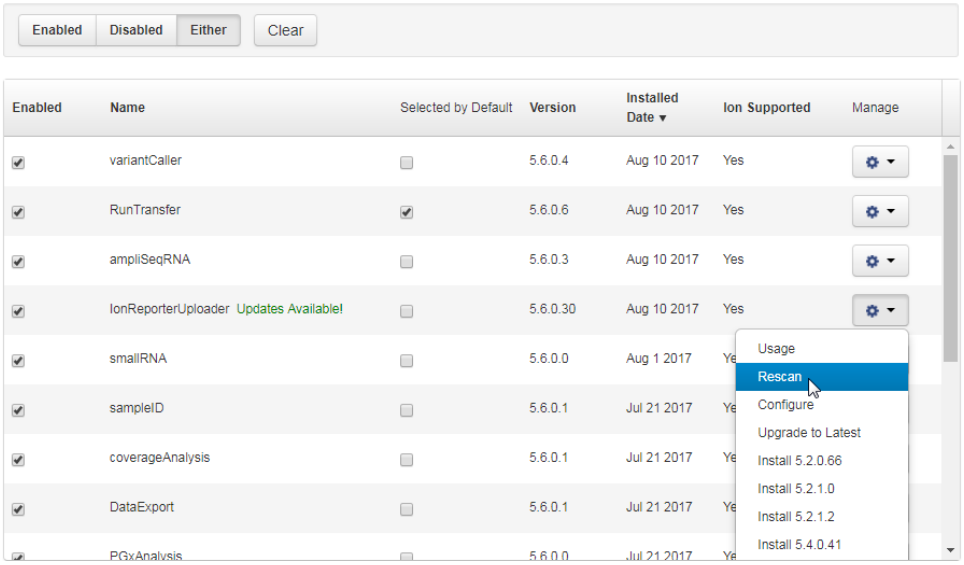
1. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for your completed sequencing run.
2. In the left navigation menu, click **Plugins**, or the name of the plugin results that you want to delete.
3. Click **Delete** to the right of the plugin name.
   The plugin 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.

# Preinstalled plugins

The following table lists and describes the plugins that are preinstalled with Torrent Suite™ Software.

| Plugin name | Description |
|---|---|
| ampliSeqRNA plugin | 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. |
| AssemblerSPAdes plugin | Performs an initial level analysis on assembly and provides metrics. The plugin is ideal for genomes less than 50 megabases in size. |
| coverageAnalysis plugin | Generates statistics and graphs to describe the level of sequence coverage that is produced for targeted genomic regions. |
| DataExport plugin | Exports data from a sequencing run to an external hard drive or a removable media, such as a USB drive. |
| ERCC_Analysis plugin | Indicates whether a problem exists with library preparation or sequencing for runs that use the ERCC RNA Spike-In Mix. |
| FieldSupport plugin | Provides assistance 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 | Customizes the output file names of an analysis run. This plugin allows you to rename output files. Also generates a FASTQ format file of the analysis output, renames variantCaller plugin output files (when available), and compresses output files. |
| FilterDuplicates plugin | Removes duplicate reads and creates BAM files that do not contain the duplicate reads. |
| immuneResponseRNA plugin | Used to quantify gene expression levels for the Oncomine™ Immune Response Research Assay. |
| IonReporterUploader plugin | Transfers run results files to Ion Reporter™ Software.<br>For details, see Chapter 9, “Integration with Ion Reporter™ Software”. |
| molecularCoverageAnalysis plugin | Generates statistics, downloadable data files, and interactive visualization of molecular coverage over targeted regions of the reference genome. |

*(continued)*

| Plugin name | Description |
|---|---|
| PGxAnalysis plugin | 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. |
| RunTransfer plugin | Transfers the signal processing output of a completed sequencing run from one Ion Torrent™ Server to another Ion Torrent™ Server, then runs an analysis of the transferred files on the receiving Ion Torrent™ Server. |
| sampleID plugin | Uses sample fingerprinting to identify any cross-contamination between samples or between barcodes in a run. |
| variantCaller plugin | Calls single-nucleotide polymorphisms (SNPs), multinucleotide polymorphisms (MNPs), insertions, deletions, and block substitutions in a sample across a reference or within 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. For more information on the references, see “Import a preloaded reference sequence file” on

page 260 and for more information on target regions files, see "View and manage target regions files" on page 267.

| Reference | Target Regions file | Description |
|---|---|---|
| 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**.<br>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**.<br>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 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 126.

Alternatively, you can reanalyze the run. For details, see "Reanalyze a run" on page 124.

| 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.<br><br>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. |

| Setting | Description |
|---|---|
| **The following settings can be configured when you run the ampliSeqRNA plugin manually.** | |
| **Library Type** | ampliSeqRNA is selected automatically and is currently the only library type that the ampliSeqRNA plugin is designed to work with.<br><br>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.<br><br>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. |

*(continued)*

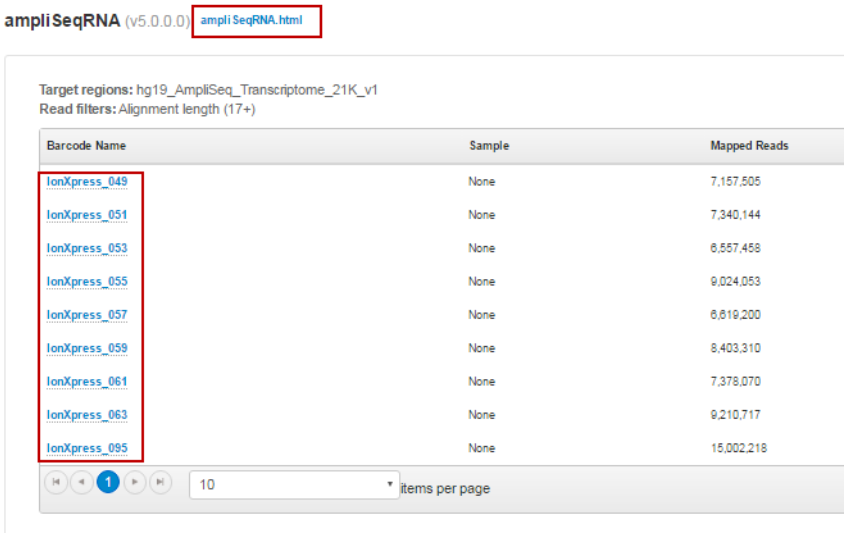
| Setting | Description |
|---|---|
| **Filter Barcodes** | Select this checkbox to remove whole barcodes from subsequent analyses.<br>Typically, this option is not needed if your Planned Run specifies which samples to associate with specific barcodes. |
| **ERCC Tracking** | Select this checkbox if your Ion AmpliSeq™ RNA targets (amplicons) are spiked 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. |

# AssemblerSPAdes plugin

The AssemblerSPAdes plugin is a de Bruijn graph assembler. The plugin breaks sequence reads into kmers of defined length, makes a connected graph, and traverses through that graph to produce contigs. The plugin report includes basic analysis metrics such as number of contigs, N50, length of the longest contig, and a downloadable FASTA file of the assembled sequences. The plugin assumes a haploid genome, and is ideal for genomes under 50 megabases in size. For multiploid genomes, reads from different copies of a chromosome tend to assemble into different contigs.

For de novo assembly, use a Generic Sequencing application Planned Run template for the Torrent Suite™ Software analysis.

## AssemblerSPAdes plugin configuration

The configuration options for the AssemblerSPAdes plugin are described in the following table. These settings can be configured only when you select this plugin to run manually from the **Run Summary** screen. After you select the plugin, click **Advanced Settings +** to display these options.

| Setting | Description |
|---|---|
| **Fraction of reads to use** | The default setting of 100% is recommended, and handles most changes in coverage. If you enter a value of less than 100%, the reads are randomly sub-sampled. |
| **Only process barcodes** | By default, the plugin processes all barcodes in the analysis and produces a separate set of contigs for each barcode. To limit plugin analysis to only specific barcodes, list those barcodes here (separated by commas and no spaces. For example, IonXpress_001,IonXpress_002,IonXpress_003). |
| **Skip barcodes with fewer than __ reads** | The software ignores barcodes whose number of reads do not meet the threshold specified here. The default threshold is 500 reads. This setting is intended to filter out barcode classification problems with noisy data. |
| **RAM to allocate** | The plugin attempts to allocate the specified amount of RAM when it runs. The default is 32 GB. With larger amounts of memory, the plugin runs faster. With less memory, the plugin takes longer to complete.<br>The plugin crashes if the memory allocation fails. |
| **SPAdes version** | Select the version that you prefer. Select the default of **3.1.0** if you are not sure. |

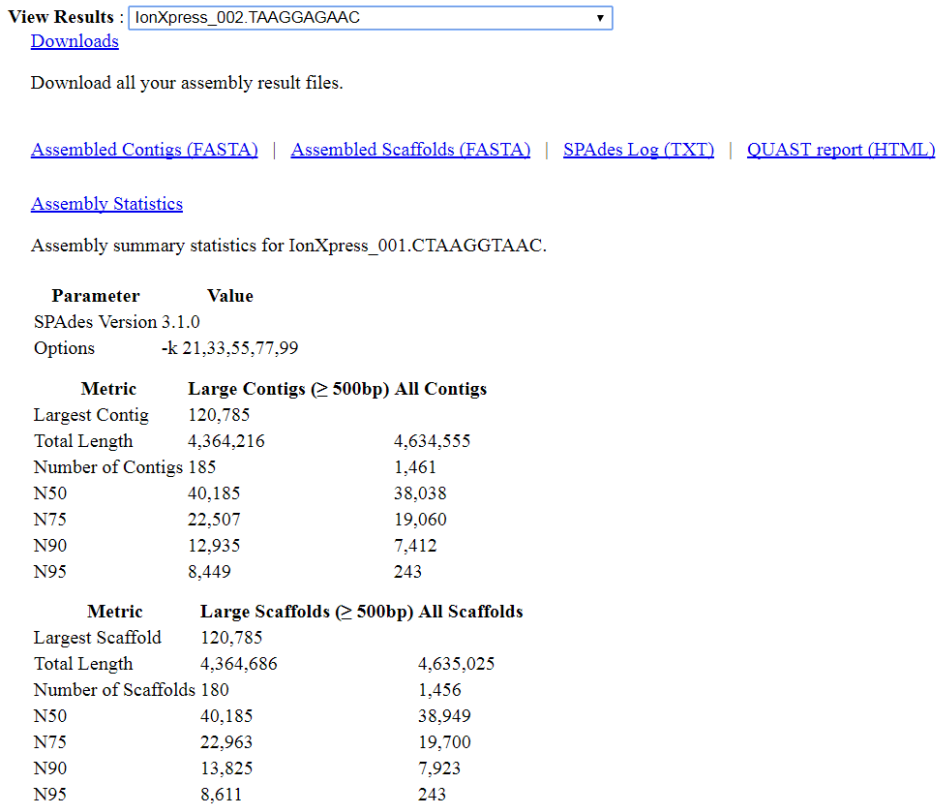
*(continued)*

| Setting | Description |
|---|---|
| **Assembly settings** | Set this menu as follows:<br>• **Uniform coverage** (default setting)—This is used for data with average GC (35–68%) content. This setting uses the default kmers.<br>• **Non-uniform coverage**—choose this setting for data with low GC (<35%) content. This setting uses the default kmers.<br>• **Highly non-uniform coverage**—choose this setting for data with high GC (>68%) content. This setting uses a different set of kmers.<br>• **Custom**—choose this setting to enter user-defined **K** and **Mode** settings.<br>– **K**—enter values (separated by commas, no spaces) to determine the size and number of kmers to be used in the analysis. Enter short kmer values to improve error-prone or low-coverage regions, long kmers to resolve repetitive regions, or a combination of kmer values to account for both situations.<br>**Note:** Each additional kmer adds a fixed amount to the processing time (for example, using 2 kmers takes twice as long as 1 kmer).<br>– **Mode**—Select **Multi-cell** (default) for data with average or low GC content. Select **Single-cell** for data with high GC (>68%) content. |
| **Run read correction before doing assembly** | This setting is enabled by default, which is recommended. |
| **Skip assembly if previous results exist** | Select this checkbox to detect whether assembly results already exist and you do not want to overwrite the results. |

## Review AssemblerSPAdes 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 in the **Report Name** column.
3. In the left navigation menu, click **AssemblerSPAdes** to view the plugin results.



**View Results :** IonXpress_002.TAAGGAGAAC

Downloads

Download all your assembly result files.

Assembled Contigs (FASTA) | Assembled Scaffolds (FASTA) | SPAdes Log (TXT) | QUAST report (HTML)

Assembly Statistics

Assembly summary statistics for IonXpress_001.CTAAGGTAAC.

| Parameter | Value |
|---|---|
| SPAdes Version | 3.1.0 |
| Options | -k 21,33,55,77,99 |

| Metric | Large Contigs (≥ 500bp) | All Contigs |
|---|---|---|
| Largest Contig | 120,785 | |
| Total Length | 4,364,216 | 4,634,555 |
| Number of Contigs | 185 | 1,461 |
| N50 | 40,185 | 38,038 |
| N75 | 22,507 | 19,060 |
| N90 | 12,935 | 7,412 |
| N95 | 8,449 | 243 |

| Metric | Large Scaffolds (≥ 500bp) | All Scaffolds |
|---|---|---|
| Largest Scaffold | 120,785 | |
| Total Length | 4,364,686 | 4,635,025 |
| Number of Scaffolds | 180 | 1,456 |
| N50 | 40,185 | 38,949 |
| N75 | 22,963 | 19,700 |
| N90 | 13,825 | 7,923 |
| N95 | 8,611 | 243 |

The plugin results show assembly statistics for the selected barcode.

- To show assembly statistics for an individual barcode, select a barcode in the **View Results** menu.
- To download results for all barcodes used in the run, click **Downloads**.
- To download a FASTA file of the assembled contigs, click **Assembled Contigs (FASTA)**.
- To download a FASTA file of the assembled scaffolds, click **Assembled Scaffolds (FASTA)**.
- To download a copy of the assembly summary statistics, click **Assembly Statistics**.

You can also click **SPAdes Log (TXT)** to view the execution file for the AssemblerSPAdes plugin, or click **QUAST report (HTML)** to view a QUAST report.

## 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 50bp with 90% or greater accuracy. |
| `Library_100Q10_Reads` | Reads of length at least 100bp with 90% or greater accuracy. |
| `Library_200Q10_Reads` | Reads of length at least 200bp with 90% or greater accuracy. |

*(continued)*

| Field | Description |
|---|---|
| Library_Mean_Q10_Length | Average length of reads with 90% or greater accuracy. |
| Library_Q10_Coverage | Average per base coverage considering reads with 90% or greater accuracy. |
| Library_Q10_Longest_Alignment | Longest read length amongst 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 50bp with 98% or greater accuracy. |
| Library_100Q17_Reads | Reads of length at least 100bp with 98% or greater accuracy. |
| Library_200Q17_Reads | Reads of length at least 200bp 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 considering reads with 98% or greater accuracy. |
| Library_Q17_Longest_Alignment | Longest read length amongst 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 considering reads with 99% or greater accuracy. |

*(continued)*

| Field | Description |
|---|---|
| Library_Q20_Longest_Alignment | Longest read length amongst 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. |
| Library_Mean_Q47_Length | Average length of perfect reads. |
| Library_Q47_Coverage | Average per-base coverage considering only perfect reads. |
| Library_Q47_Longest_Alignment | Longest reads length amongst 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. |
| PGM Name | Name of the Ion PGM™ or Ion Proton™ instrument where the sample was sequenced. |

*(continued)*

| Field | Description |
|---|---|
| Run Date | Date that the sample was sequenced. |
| Run Directory | Location of the raw DAT files on the Ion Torrent™ Server. |
| Num_Washouts | NA |
| Num_Dud_Washouts | NA |
| Num_Washout_Ambigous | NA |
| Num_Washout_Live | NA |
| Num_Washout_Test_Fragment | NA |
| Num_Washout_Library | NA |
| Library_Pass_Basecalling | NA |
| Library_pass_Cafie | NA |
| Number_Ambiguous | NA |
| Number_Live | Number of wells producing a signal. |
| Number_Dud | Number of wells with ISPs but no signal. |
| Number_TF | Number of wells containing test fragment. |
| Number_Lib | Number of wells containing library. |
| Number_Bead | Number of wells containing beads. |
| Library_Live | Number of wells containing library ISP with signal. |
| Library_Keypass | Number of wells containing library ISP with signal and match key. |
| TF_Live | Number of wells containing test fragment ISP with signal. |
| TF_Keypass | Number of wells containing test fragment ISP with signal and match key. |
| Keypass_All_Beads | Number of wells containing ISP with signal and match key. |
| P | JSON string of plugin data. |
| s | JSON string of plugin data. |

# 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 it 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.<br>Select the targeted regions file from the list. For whole genome and Ion Total RNA-Seq sequencing runs, you typically select **None**. |
| **Barcode-specific Targets** | This option is available only when the coverageAnalysis plugin is run manually.<br>Select the checkbox to assign specific target region files to individual barcodes.<br>**1.** Select a specific barcode.<br>**2.** Select the specific Target Regions file to associate with the selected barcode.<br>**3.** Click **Add**.<br>**4.** Repeat steps 1 through 3 to associate additional barcodes with specific Target Region files.<br>Alternatively, you can copy and paste the barcode/target file pairs manually.<br>Barcodes without a Target Region specified above assume the default target specified by the **Target Regions** option.<br>For targeted applications, any barcode targets specifically set to **None**, or defaulting to **Target Regions** set to **None**, are omitted from subsequent analysis.<br>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. |
| **The following 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. |

*(continued)*

| Setting | Description |
|---|---|
| **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. |
| **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 duplicates. 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 regarding 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 will be the percentage of reads passing uniquely mapped and/or non-duplicate 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 of the amplicons specified by the targets file. If a read spans multiple amplicon targets, the target region that the reads covers most is assigned. In the event of 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. |

**Amplicon read coverage statistics** *(continued)*

| Statistic | Description |
|---|---|
| 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 greater than 70%. Amplicons with <10 reads are considered to have no strand bias. |
| 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 G/C 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.<br>If no specific target regions was specified, the targeted regions is the whole genome. |
| 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 statistics** *(continued)*

| Statistic | Description |
|---|---|
| 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 greater than 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 that allows you to change characteristics of the chart. For example, you can show both strands (see callout 3 in Figure 5).

The 🔍 button (in the top right corner of a chart) opens the chart **Viewing Options** panel. The ? button opens a description of the chart.

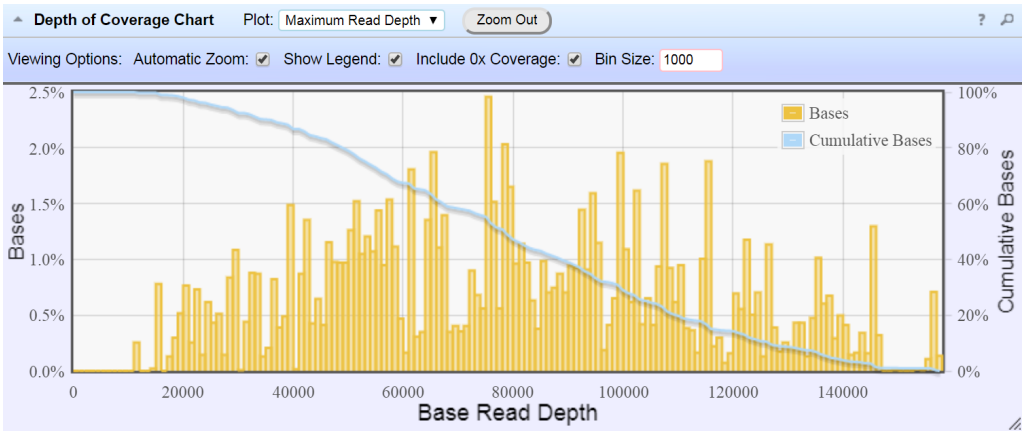



**Figure 1 Representative Depth of Coverage Chart**

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).

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 in Figure 1, click an individual orange bar to open the detail pane for bases within a specific range of base read depths. Click a point on the blue curve to open the detail pane for cumulative bases at that base read depth or greater. For example detail panes, see Figure 2. Similarly, you can click

any point or bar within the Amplicon Coverage chart to view details for each amplicon coverage bin (see Figure 4).

Bases at depths 1,420-1,429
Number of target bases read: 96
Fraction of all target bases read: 4.37%

Cumulative Bases at depth 960
Number of target bases read: 1,863
Fraction of all target bases read: 84.87%

**Figure 2 Example detail panes**

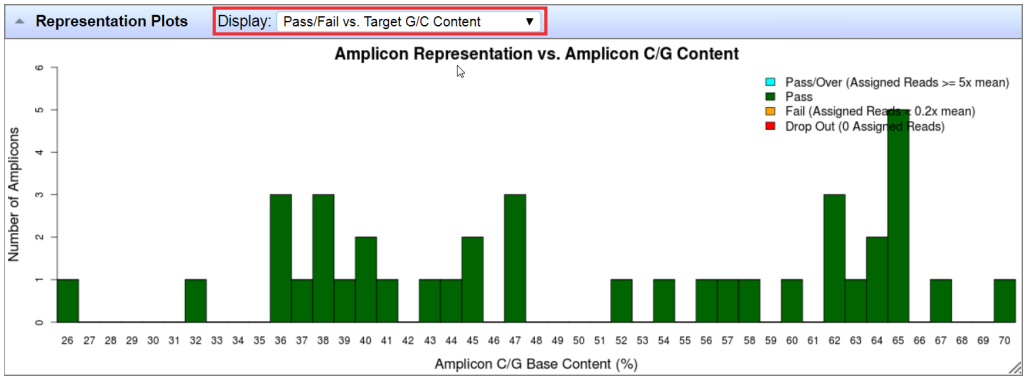



**Figure 3 Representation Plots**

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

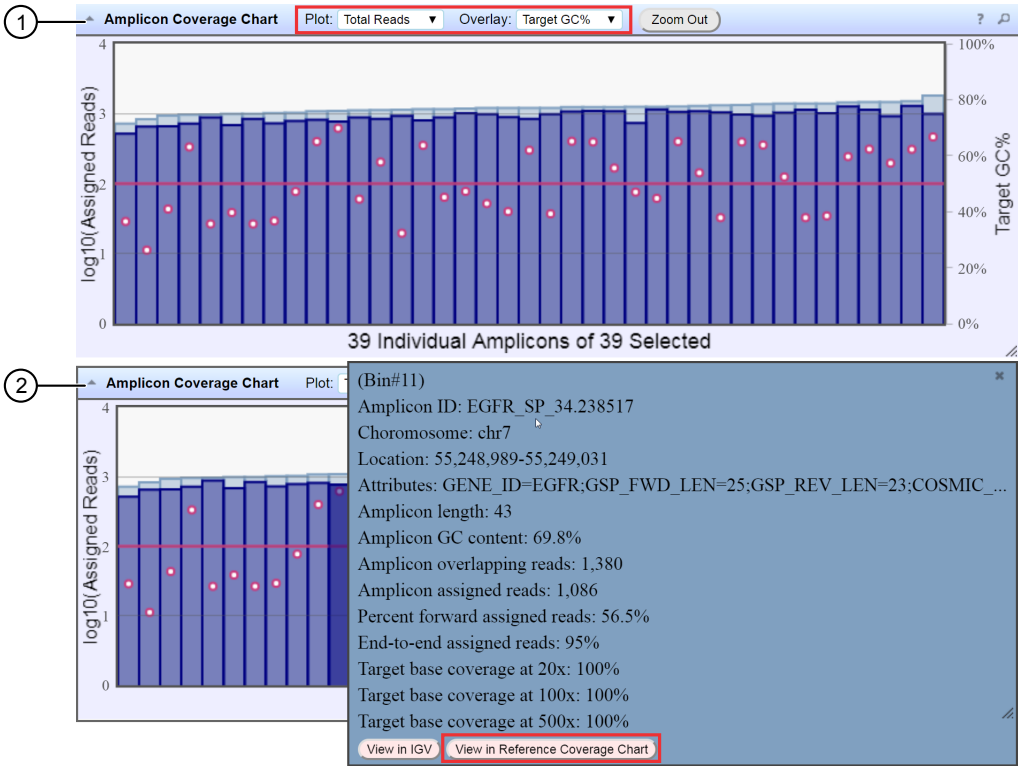



**Figure 4 Representative Amplicon Coverage Chart**

The Amplicon Coverage charts 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 depending on the plot view that is selected from the **Overlay** dropdown list. Click on 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 on the 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).

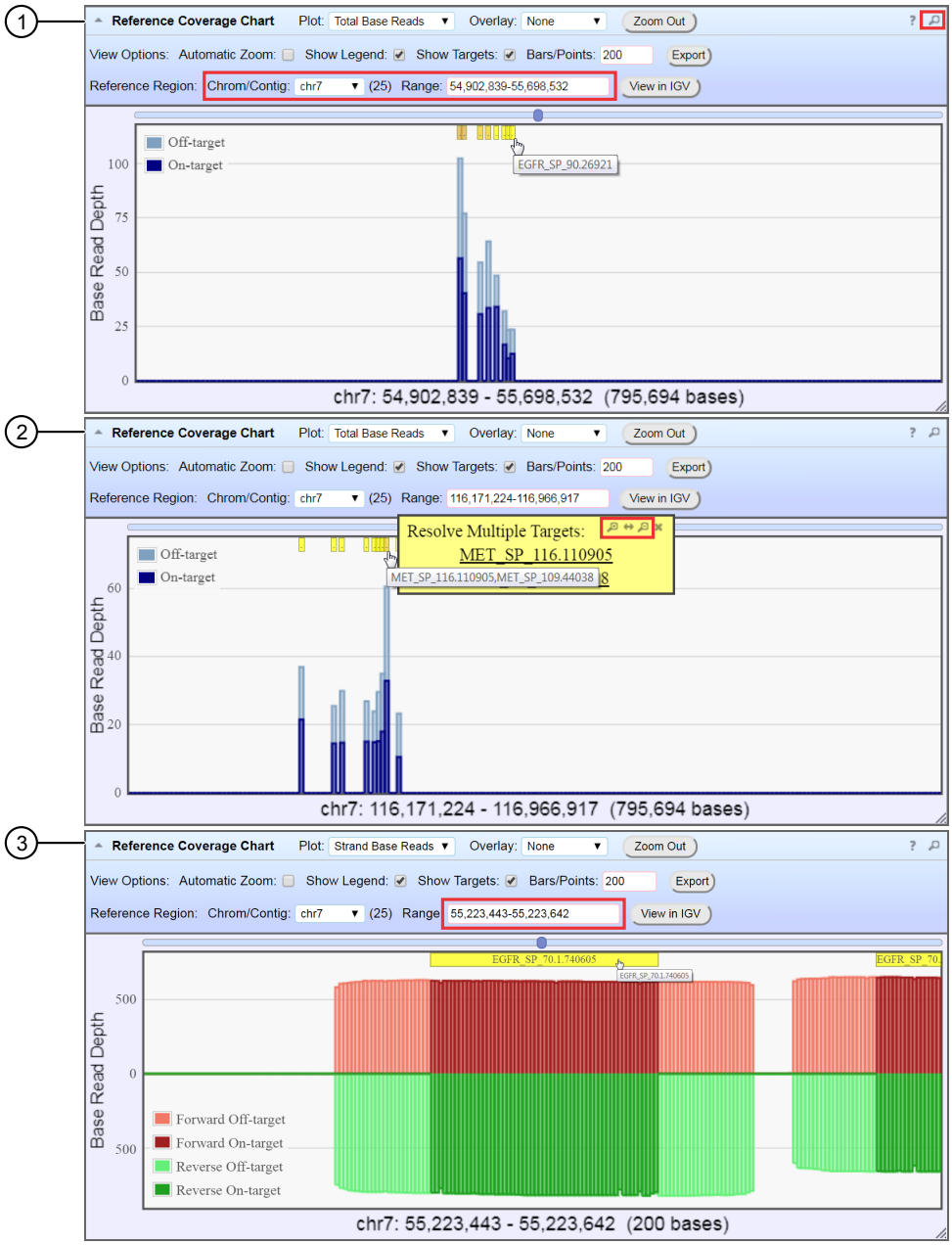



**Figure 5 Representative Reference Coverage Chart**

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 a particular 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:<br>• `read_depth`: the depth at which a (targeted) reference base has been read.<br>• `base_cov`: the number of times any base was read (covered) at this depth.<br>• `base_cum_cov`: the cumulative number of reads (coverage) at this read depth or greater.<br>• `norm_read_depth`: the normalized read depth (depth divided by average base read depth).<br>• `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:<br>• `contig_id`: the name of the chromosome or contig of the reference for this amplicon.<br>• `contig_srt`: the start location of the amplicon target region.<br>This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file.<br>• `contig_end`: the last base coordinate of this amplicon target region.<br>**Note:** The length of the amplicon target is given as tlen = (contig_end - contig_srt + 1).<br>• `region_id`: the ID for this amplicon as given as the 4th column of the targets BED file.<br>• `gene_id`: the gene symbol as given as the last field of the targets BED file.<br>• `gc_count`: the number of G and C bases in the target region. %GC = 100% * gc / tlen.<br>• `overlaps`: the number of times this target was overlapped by any read by at least one base.<br>Individual reads might overlap multiple amplicons where the amplicon regions themselves overlap.<br>• `fwd_e2e`: the number of assigned forward strand reads that read from one end of the amplicon region to the other end.<br>• `rev_e2e`: the number of assigned reverse strand reads that read from one end of the amplicon region to the other end.<br>• `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`).<br>• `fwd_reads`: the number of forward strand reads assigned to this amplicon.<br>• `rev_reads`: the number of reverse strand reads assigned to this amplicon.<br>• `cov20x`: the number of bases of the amplicon target that had at least 20 reads.<br>• `cov100x`: the number of bases of the amplicon target that had at least 100 reads.<br>• `cov500x`: the number of bases of the amplicon target that had at least 500 reads. |
| 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:<br>• `chrom`: the name of the chromosome or contig of the reference.<br>• `start`: the coordinate of the first base in this chromosome. This is always 1.<br>• `end`: the coordinate of the last base of this chromosome. Also its length in bases.<br>• `fwd_reads`: the total number of forward strand base reads for the chromosome.<br>• `rev_reads`: the total number reverse strand base reads for the chromosome.<br>• `fwd_ontrg` (if present): the total number of forward strand base reads that were in at least one target region.<br>• `seq_reads`: the total sequencing (whole) reads that are mapped to individual contigs. |

*(continued)*

| File | Description |
|---|---|
| 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 such as IGV. 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** | *(Optional)* 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).<br>The value you enter on the **ERCC Plugin Configuration** screen is used when the plugin is auto-run 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:<br>• Select **IonXpressRNA** if your experiment uses this kit.<br>• 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

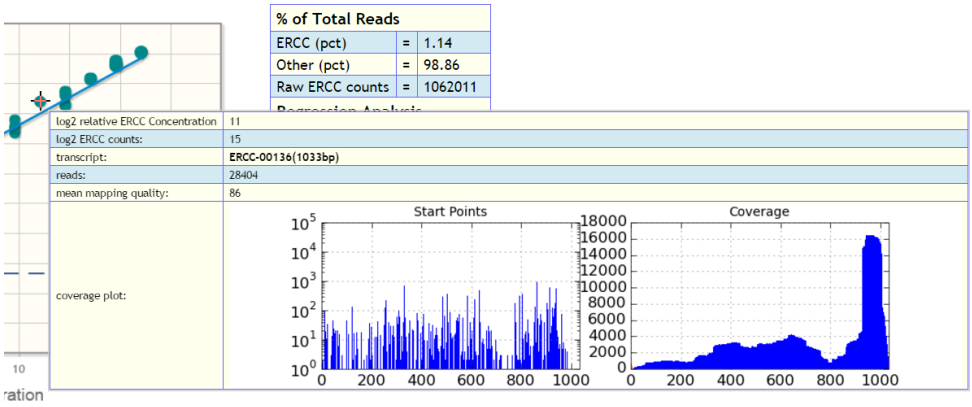
The axes of the ERCC Dose Response plot are log (base 2), with the raw read counts for each ERCC transcript on the y-axis and the known relative concentration of the ERCC transcripts on the x-axis. In the plot, the points are color-coded based on mapping quality. There is also a line of best fit, the 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 a particular ERCC transcript in two ways: by using the plot or by using the transcript. To view all of the details regarding a particular ERCC transcript, you can use both ways.

- Using the plot—Hover your mouse-cursor over a point in the ERCC Dose Response plot to display a popup window that shows details regarding that transcript. Zoom in on the overlapping points on the plot to more easily distinguish the points.
    a. To zoom in on a selected area, click-drag your mouse to highlight the area of interest.

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, including 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).<br>The points in the display are color coded based on the mapping quality. |

- Using the transcript—Scroll to the particular transcript, then click the [+] next to the transcript name.

| Parameter | Description |
|---|---|
| **Reads** | The number of reads that map to the particular 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. |

*(continued)*

| Parameter | Description |
|---|---|
| **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 = average coverage / 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 4453740), 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

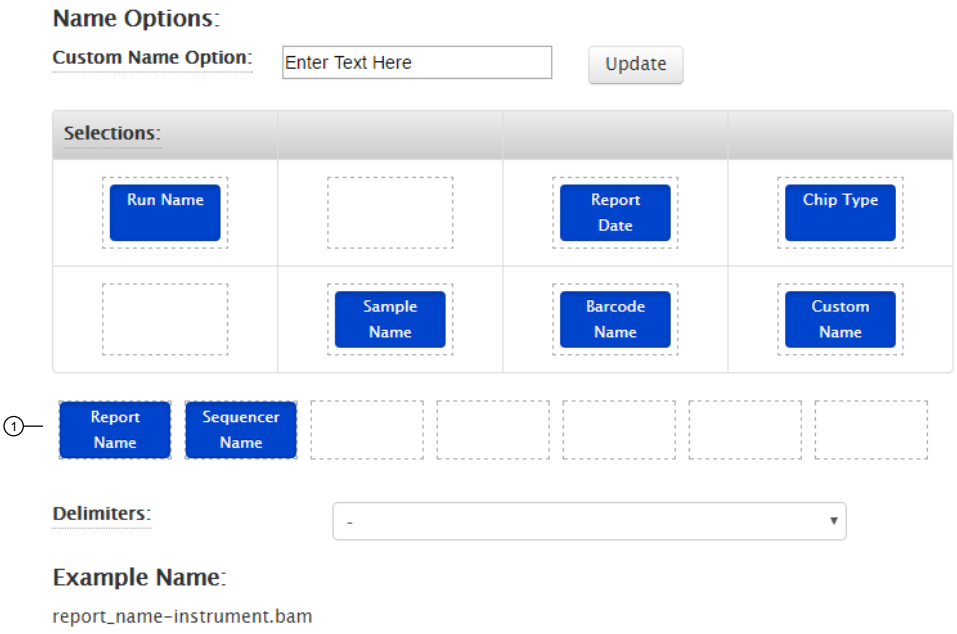
1. Select from the following options to choose the file types that you export:

| 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, you can 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 containing 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. Support 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 all of 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.

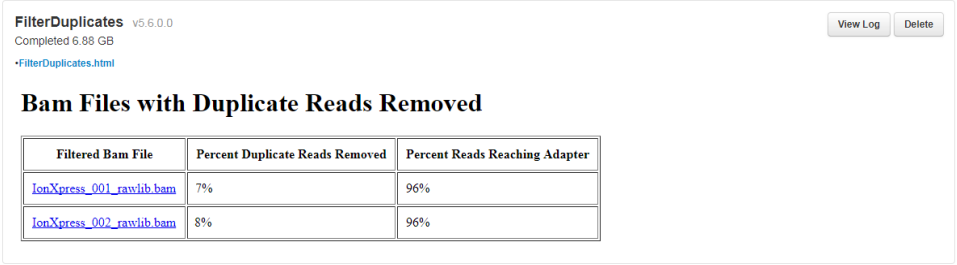
1. In the **Data** tab, click **Completed Runs & Reports**.

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 to the directory that you use to download files from the browser. This location depends on your 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** | *(Optional)* Select your target gene subset BED file in the **Add genes of interest** list. |

### 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.

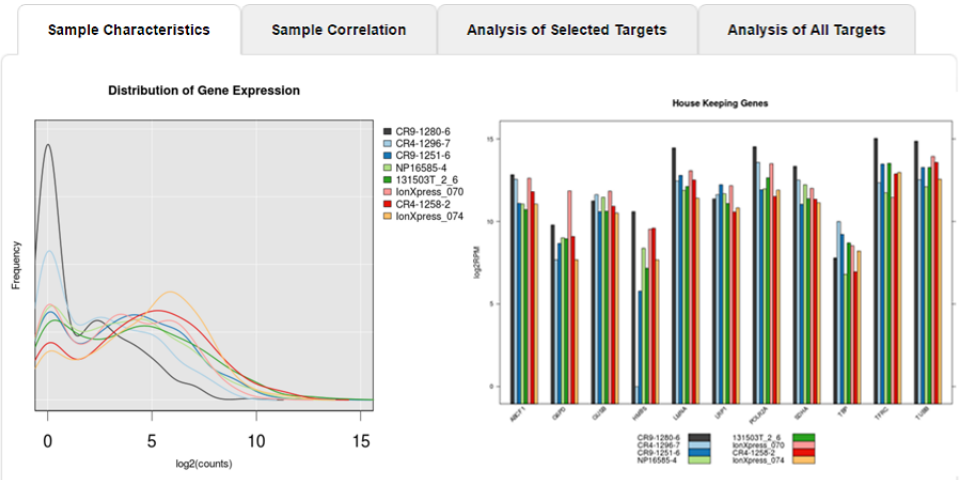
   BAM files load quickly, and you may see these files first in the list of links. The other file formats take longer to download, so you may have to wait for the links to the VCF, XLS, and FASTQ formats to appear.

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. |
| 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:<br>(read count) $\times 10^6$ / 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 samples 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:<br>$log2(\text{count}+1) - \frac{\sum(log2(hk\ \text{counts}+1))}{\text{number of } hk \text{ gene}} + log2(10^6)$<br>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 | Similar to the above CHP file, but data in these CHP files are normalized by housekeeping genes. |
| Download background expression from genomic DNA and $H_2O$ neg_control | A table containing 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.<br>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.<br>1. Select a specific barcode.<br>2. Select the specific Target Regions file to associate with the selected barcode.<br>3. Click **Add**.<br>4. Repeat above steps to associate additional barcodes with specific Target Region files.<br>Alternatively, you can copy and paste the barcode/target file pairs manually.<br>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 regarding 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 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 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 | Percentage of amplicons having molecular coverage between 0.5x and 2x of the median molecular coverage. |

*(continued)*

| Statistic | Description |
|---|---|
| Percentage of Amplicons larger than 0.8x Median Functional Molecular Coverage | 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 | 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. |
| 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.

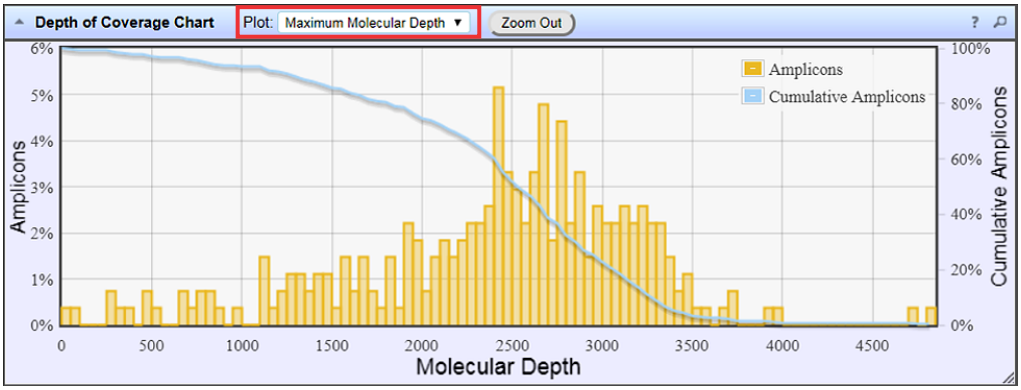



**Figure 6 Representative Depth of Coverage Chart**

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.

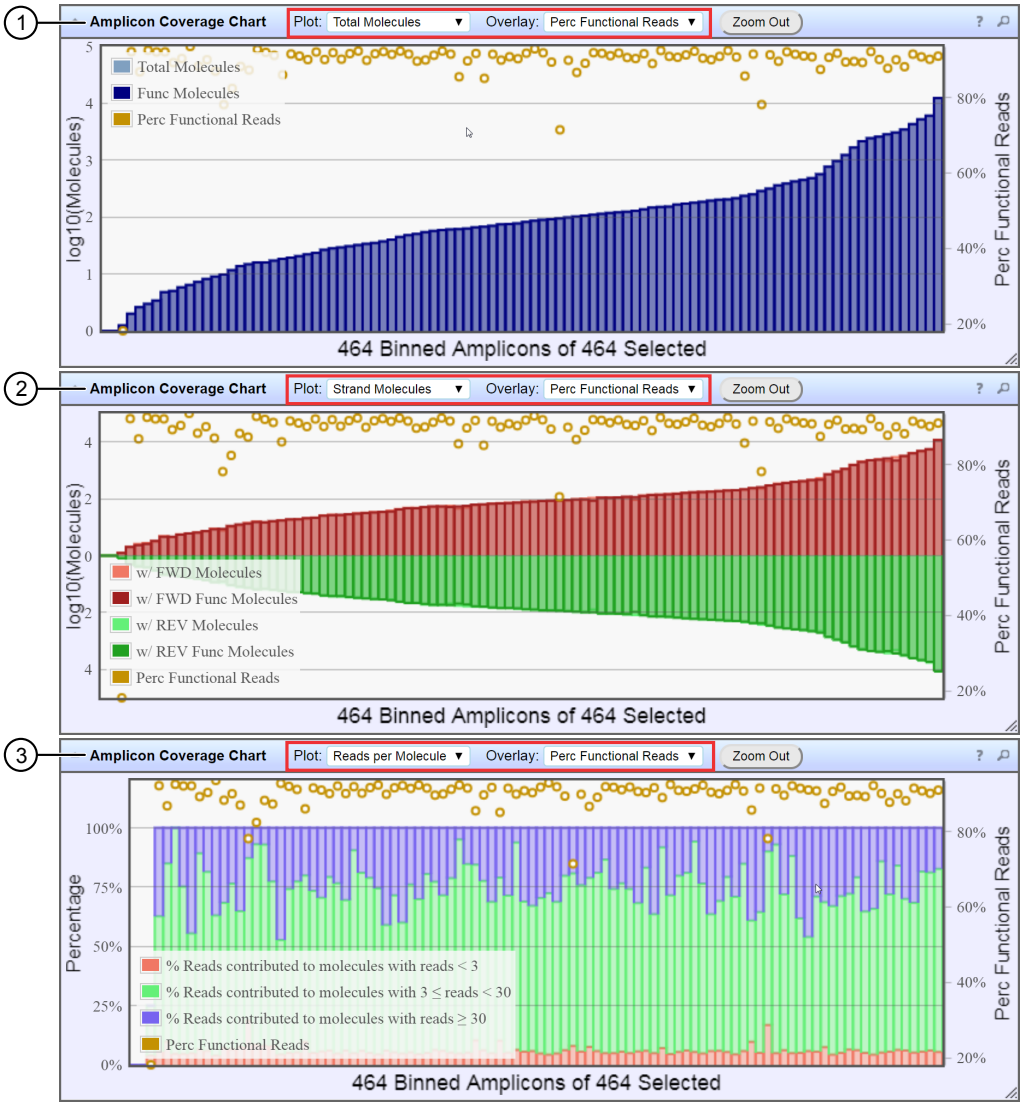



**Figure 7 Representative Amplicon Coverage charts**

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. Depending 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. You can zoom in on a subset of amplicons by using your mouse to draw a rectangle that contains the region of interest. Use **Zoom Out** to revert to the initial view.

1. Representative **Total Molecules** plot with an **Perc Functional Reads** overlay
2. Representative **Strand Molecules** plot with an **Perc Functional Reads** overlay
3. Representative **Reads per Molecule** plot with an **Perc Functional Reads** overlay

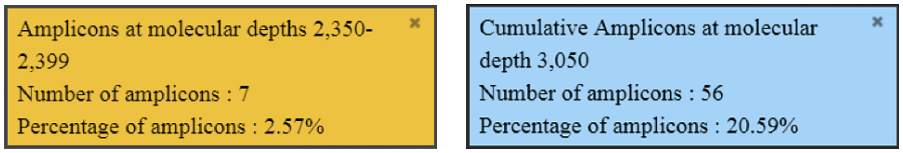



**Figure 8 Example detail panes**

In most plots, you can click a data point to open a detail pane for that data. 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. For example detail panes, see Figure 8.

## 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.

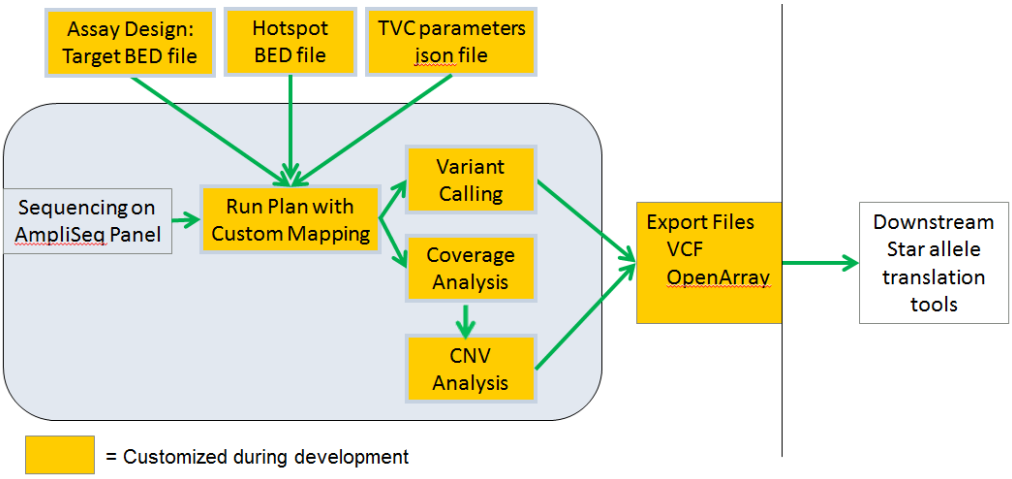
The following is an example of the content of a results file that is generated by the molecularCoverageAnalysis plugin.

| 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 Amplicon molecular coverage summary file contains the following fields.<br>• `contig_id`: the name of the chromosome or contig (from contiguous) of the reference for this amplicon.<br>• `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.<br>• `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).<br>• `region_id`: the ID for this amplicon as given as the 4th column of the targets BED file.<br>• `gene_id or attributes`: the gene symbol or attributes field as provided in the targets BED file.<br>• `func_mol_cov`: the number of molecules (functional molecules) which are available for the variantCaller plugin.<br>• `lod`: LOD (limitation of detection) calculated from the number of functional molecules.<br>• `strict_func_umt_rate`: the percentage of functional molecules used with strict molecular tags.<br>• `func_mol_cov_loss_due_to_strand`: the percentage of functional molecules loss due to strand bias.<br>• `fwd_only_mol_cov`: the number of molecules containing forward strand only.<br>• `rev_only_mol_cov`: the number of molecules containing reverse strand only.<br>• `both_strands_mol_cov`: the number of molecules containing both forward strand and reverse strand.<br>• `r2m_conv_rate_all`: the percentage of reads contributed to functional molecules.<br>• `reads_per_func_mol`: the average reads per functional molecules.<br>• `perc_to_mol_(<3_reads)`: the percentage of reads contributed to small size molecules(size<3).<br>• `perc_to_mol_(>=3&<30_reads)`: the percentage of reads contributed to median size molecules (size ≥3 && size <30).<br>• `perc_to_mol_(>=30_reads)`: the percentage of reads contributed to large size molecules (size ≥30). |

# PGxAnalysis plugin

The PGxAnalysis plugin analyzes sequencing output from the Ion AmpliSeq™ Pharmacogenomics panel, a hotspot panel that interrogates pharmacogenomically relevant variants in samples for genotyping and CYP2D6 copy number detection for research use. It requires two other Torrent Suite™ Software plugins: the variantCaller plugin for genotyping and coverageAnalysis plugin for CYP2D6 copy number detection.

The figure describes the pipeline of analyses.




For details about how to set up Torrent Suite™ Software Planned Runs that incorporate the Ion AmpliSeq™ Pharmacogenomics template and the PGxAnalysis plugin, see the following documents at the Thermo Fisher Scientific website (**thermofisher.com**):

- *Customization Guidelines for Ion AmpliSeq™ Pharmacogenomics Research Panels* (Pub. No. MAN0014300)
- *Create a Planned Run using the Ion AmpliSeq™ Pharmacogenomics Research Panel Plugin* (Pub. No. MAN0013730)

## Review PGxAnalysis 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 **PGxAnalysis** to view the plugin summary.
4. Click the **PGxAnalysis.html** link to open the Pharmacogenomics Analysis Report.

5. View the plugin analysis reports.
   - Click the **variantCaller_out** link to view the Variant Caller Report. See Chapter 8, "Variant calls in Torrent Suite™ Software" for details on variantCaller plugin results interpretation.
   - Click the **coverageAnalysis_out** link to view the Coverage Analysis Report. See "Review coverageAnalysis plugin results" on page 150 for details on coverageAnalysis plugin results interpretation.

## RunTransfer plugin

Use the RunTransfer plugin to do the following:

- Transfer the signal processing output files from a completed run to a different Ion Torrent™ Server.
- Rerun an analysis of the transferred signal processing files on the new server. The Run Summary that includes the transferred files is listed in the **Completed Runs & Reports** for the server that receives the transfer, as if it is generated on that server. The results of the analysis are contained in the ISP images of the Run Summary.

The files that are transferred are the BaseCaller input category of files, including the 1.WELLS file. This file contains observations from the instrument that are captured electronically.

For Ion Proton™ analyses, you can configure the option to transfer only thumbnail files or transfer the 96 block files in a full chip run.

### RunTransfer plugin configuration

The RunTransfer plugin requires global configuration to connect to the Ion Torrent™ Server that receives transferred files.

The following configuration settings are used by the plugin:

| Setting | Description |
|---|---|
| **IP address or fully qualified hostname** | The IP address or fully qualified host name of the receiving Ion Torrent™ Server. |
| **Remote TS Username (default ionadmin)** | The user name of the administrator-level user on the receiving Ion Torrent™ Server. The default administrator user name on a new Ion Torrent™ Server is **ionadmin**, but this can be changed. |
| **Password** | The password of the administrator-level user on the receiving Ion Torrent™ Server. |
| **Upload Path (default /results/uploads/)** | The path of the directory used to store transferred files and analyses on the receiving Ion Torrent™ Server. |

*(continued)*

| Setting | Description |
|---|---|
| **Data set type** | Select an option based on the following considerations:<br>• **Thumbnails/PGM**—This option transfers all files, including thumbnails. However, the plugin does not run if full chip sequencing runs are performed on the Ion Proton™ System, Ion S5™ System, and Ion GeneStudio™ S5 System. With this setting, a warning is issued and the plugin does not run if the instrument used a Full Chip run.<br>• **Thumbnails/PGM and Full Chip**—This option transfers only thumbnails of Ion PGM™ System, Ion Proton™ System, Ion S5™ System, and Ion GeneStudio™ S5 System data sets. Use this option if disk space is limited on the destination or network bandwidth is limited. |

## Review RunTransfer plugin results

After the sequencing run completes, you can review information about the run reports that were transferred to another Ion Torrent™ Server.

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 **RunTransfer** to view the plugin summary.
4. Click the **Report Name** link to open the summary for your report.
5. To view the plugin summary, click the **RunTransfer** link.
   In the **RunTransfer** section, review a list of the files that were transferred. If the plugin is configured to use the data type set as **Thumbnails/PGM**, these files are shown:
   1.wells
   analysis.bfmask.bin
   processParameters.txt
   avgNukeTrace_ATCG.txt
   avgNukeTrace_TCAG.txt
   bfmask.stats', 'bfmask.bin'
   analysis.bfmask.stats
   analysis_return_code.txt
   sigproc.log
   avgNukeTrace_ATCG.txt
   avgNukeTrace_TCAG.txt
   analysis.bfmask.stats

explog.txt
Bead_density_20.png
Bead_density_70.png
Bead_density_200.png
Bead_density_1000.png
Bead_density_raw.png
Bead_density_contour.png

## 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 plugin can be used with the Ion AmpliSeq™ Sample ID Panel, which is a human SNP genotyping panel, to ensure that the accuracy of samples 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. Ion AmpliSeq™ Sample ID Panel contains nine primer pairs that can be combined with any Ion AmpliSeq™ Ready-to-Use Panel or Custom Panel.

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 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”.

# Plugins available on Connect

Two plugins are supported by Thermo Fisher Scientific and are available on Connect at **https://apps.thermofisher.com/apps/publiclib/#/plugins**. These plugins are not preinstalled in the Torrent Suite™ Software. For details about plugins that are included with the software, see “Preinstalled plugins” on page 135.

| Plugin name | Description |
|---|---|
| “RNASeqAnalysis plugin” on page 183 | Analyzes cDNA reads. This plugin is an RNA transcript alignment and analysis tool for use with the reference genomes hg19 and mm10. |
| “smallRNA plugin” on page 196 | Analyzes small RNA reads with an emphasize on microRNA molecules. Use with reference genome hg19 only. |

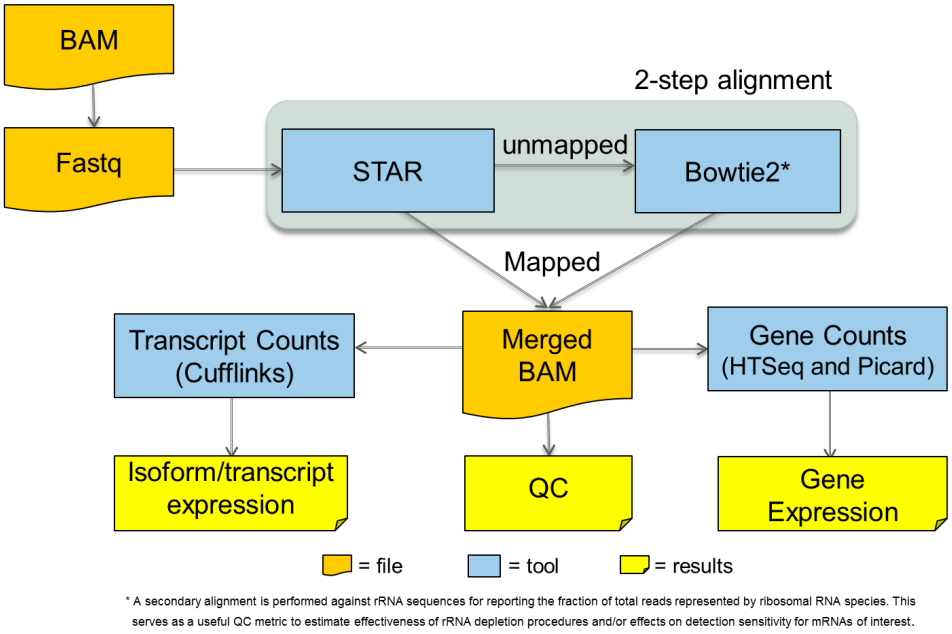
## RNASeqAnalysis plugin

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 260.

| 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:<br>• **hg19**, *or*<br>• **mm10** |
| **Regenerate indices** | Check to ensure that any pregenerated indexing and annotation files 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 (approximately 3 hours for human-sized genomes).<br>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 260.

## 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 189.

| 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 Cuflinks 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 187.

| 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 frequence 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 191. |
| Correlation Heatmap | For details, see "Correlation heatmap" on page 193. |
| Correlation Plot | For details, see "Correlation plot" on page 194. |
| Gene Heatmap | For details, see "Gene heatmap" on page 195. |
| Isoform Heatmap | For details, see "Isoform heatmap" on page 195. |

## 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.

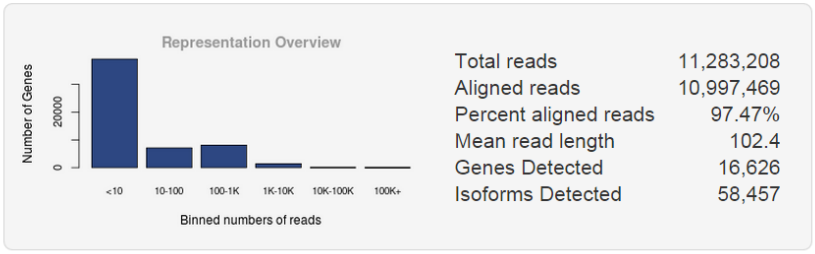
| File Size | Date | File |
|---|---|---|
| 5.5M | 2015-06-02 | IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genes.fpkm_tracking |
| 24M | 2015-06-02 | IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.isoforms.fpkm_tracking |
| 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



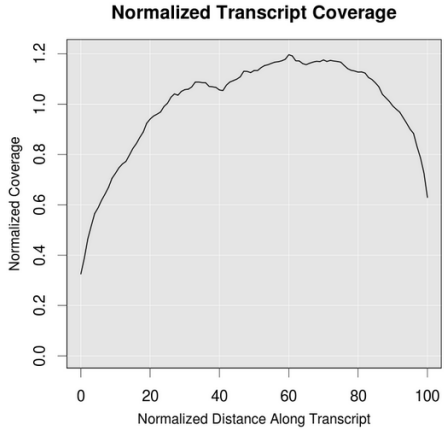

**Gene Mapping Summary**—A summary of reads mapped to genes of annotated reference.

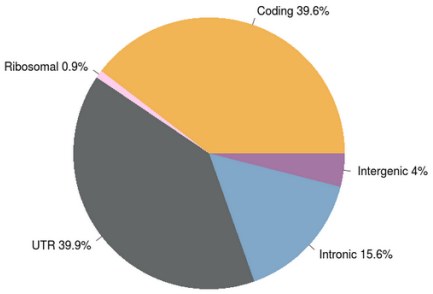
| Gene Mapping Summary | |
|---|---|
| Reference genes | 55,765 |
| Reads mapped to genes | 7,390,706 |
| Genes with 1+ reads | 26,969 |
| Genes with 10+ reads | 16,626 |
| Genes with 100+ reads | 9,531 |
| Genes with 10,00+ reads | 1,429 |
| Genes with 10,000+ reads | 35 |
| Isoforms Annotated | 230,756 |
| Isoforms Detected | 58,457 |

**Base Mapping Summary**—A summary of base reads aligned to genetic features of an annotated reference.

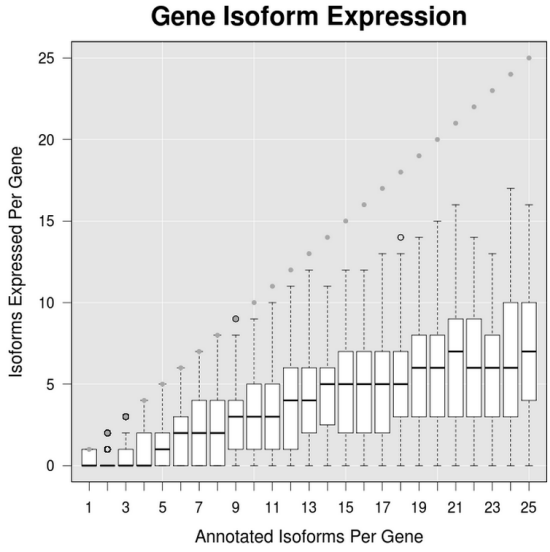
| Base Mapping Summary | |
|---|---|
| Total base reads | 1,155,834,791 |
| Total aligned bases | 914,778,477 |
| Percent aligned bases | 79.14% |
| Percent coding bases | 39.68% |
| Percent UTR bases | 39.93% |
| Percent ribosomal bases | 0.94% |
| Percent intronic bases | 15.65% |
| Percent intergenic 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' .



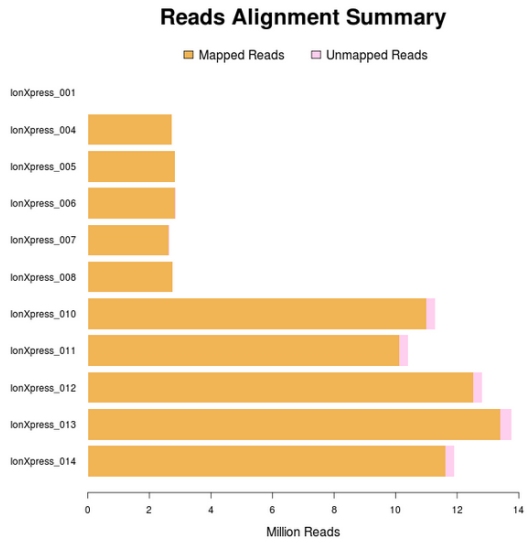




**Isoform Expression**— plots showing the variation of isoforms that are expressed at FPKM ≥ 0.3 for each set of genes that are grouped by the number of anticipated (annotated) . Whiskers points Q1-1.5xIQR to Q3+1.5xIQR. Only genes with 25 or less isoforms are represented in this plot. The data and a plot for all genes are available for download using the download reports links at the bottom of the screen.

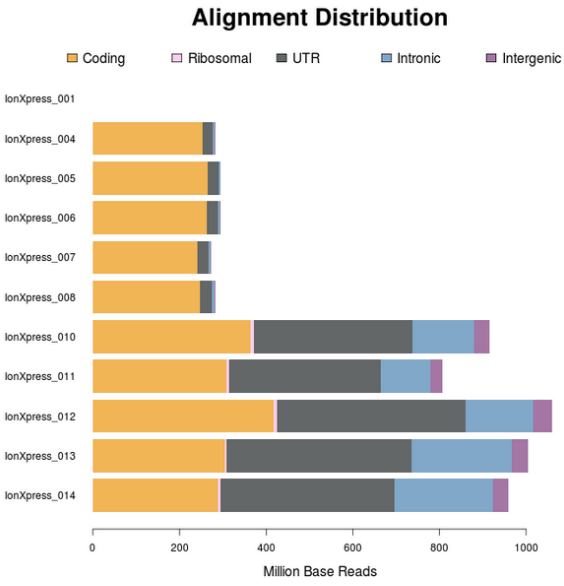


## Distribution plots

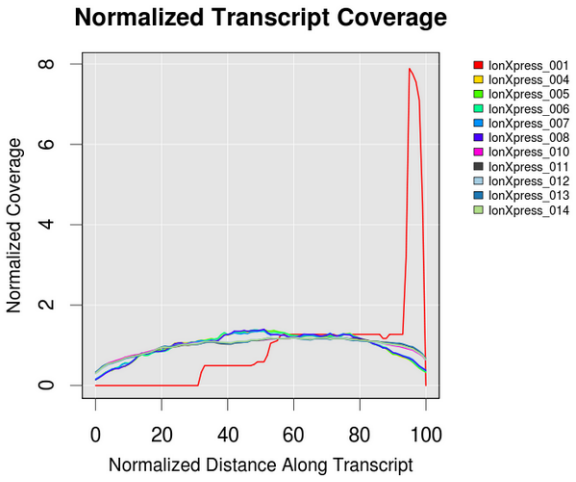
**Reads Alignment Summary**—A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the barcode summary table.



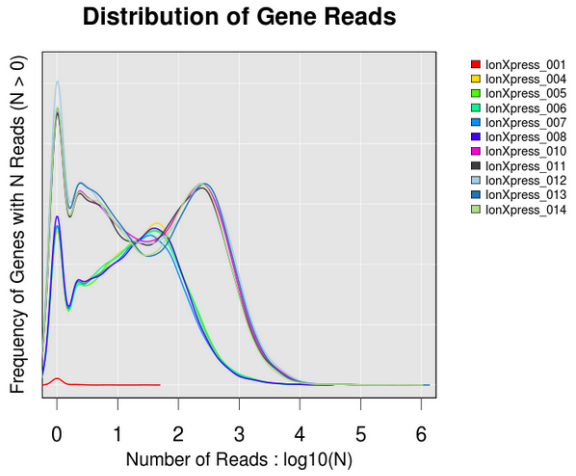

**Alignment Distribution**—A graphical summary of the distribution of reads to genomic features.



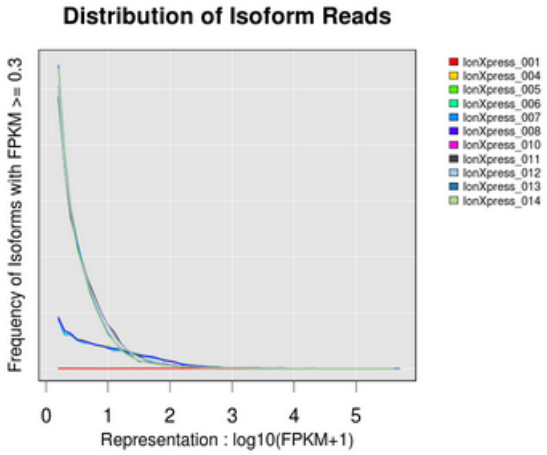

**Normalized Transcript Coverage**—An overlay of individual normalized transcript coverage plots for each barcode.




**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.

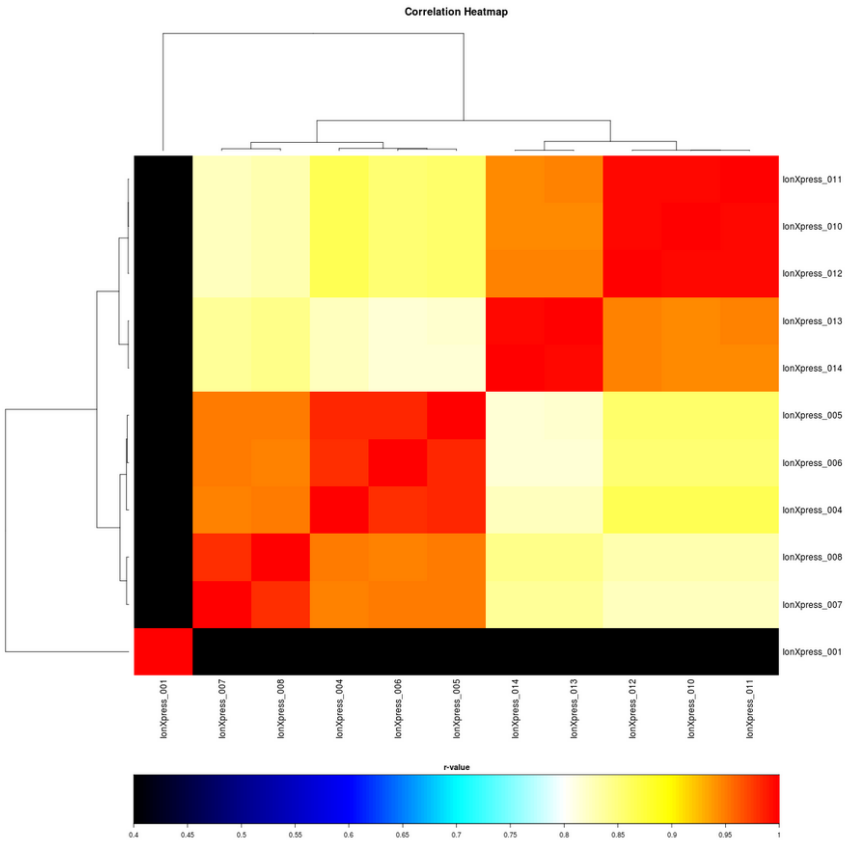



**Distribution of Isoform Reads**—Distribution of transcript isoforms across barcodes showing the counts of isoforms having similar FPKM values. All curves are plotted on the same y-axis, normalized to the highest count, and scaled for FPKM values ≥0.3.




## Correlation heatmap

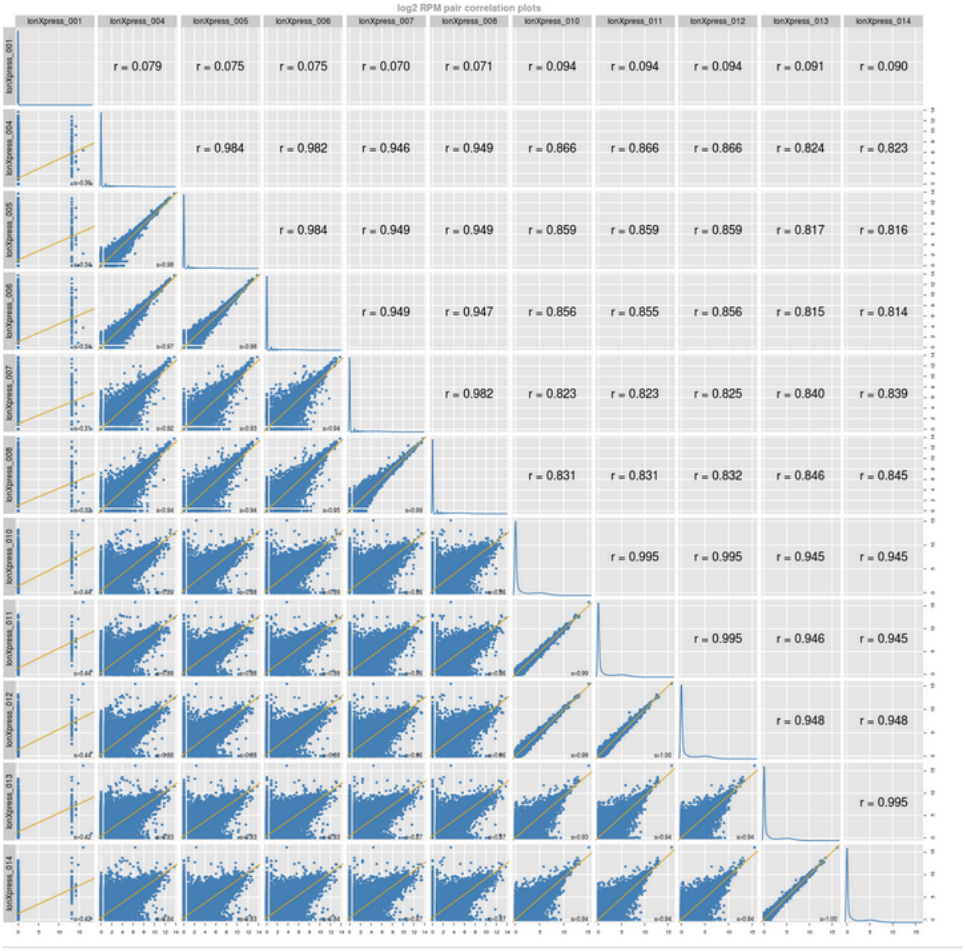
The correlation heatmap is a heatmap of Spearman correlation r-values for comparing log2 RPM reads pair correlation barcodes. The dendrogram (lines on the top and the left side of the heatmap) reflects the ordering of barcodes as being most similar by these values.



## Correlation plot

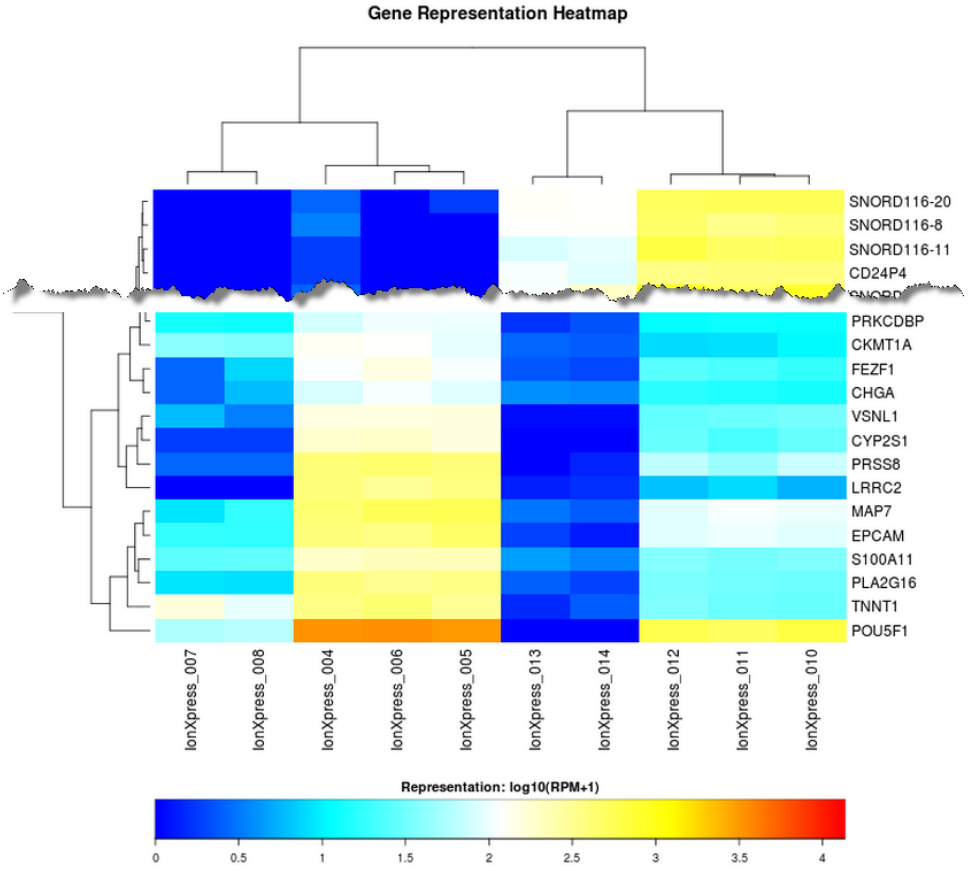
The correlation plot is a barcode read pair correlation plot. Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show 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

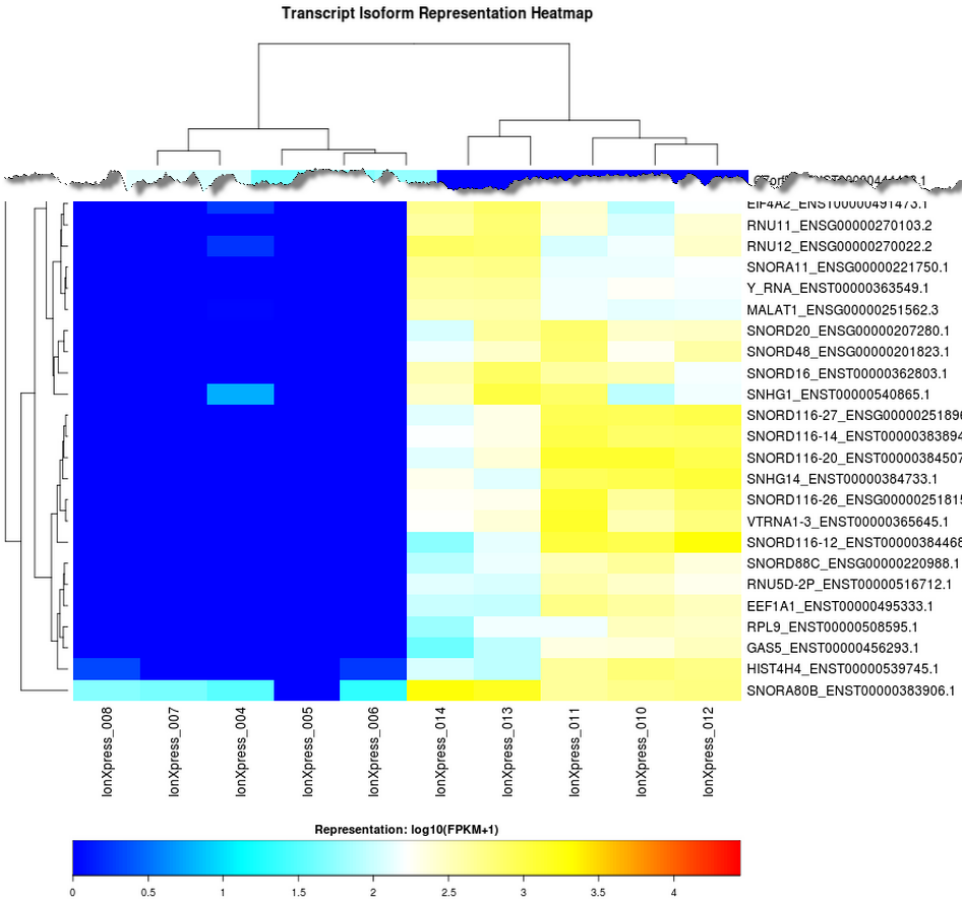
The following is a gene representation heatmap of 250 genes showing the most variation in representation across barcodes as measured by the coefficient of variant (CV) of normalized read counts for genes that have at least one barcode with at least 100 RPM reads. The heatmap is plotted using log10 of 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.




# smallRNA plugin

Use the smallRNA plugin to analyze microRNA (miRNA) reads. Reads are aligned to mature miRNAs using the TMAP or bowtie2 alignment software that is bundled with the plugin. Unmapped reads are further aligned to the whole-genome to rescue miRbase unaligned reads and count other RNA molecules (tRNAs, rRNAs, mRNAs, and so on). miRNA raw counts are generated using featureCounts software.

## smallRNA plugin

The RNASeqAnalysis plugin can be configured with the either the hg19 reference genome or the microRNA reference when you plan a run.

| Setting | Description |
|---|---|
| **microRNA reference** | **mirbase (build 20)**–Use to align against a mirBase reference, constructed from the mirBase GFF file with 10 bp padding.<br>**genome**–Use to align against the whole genome. |
| **Rescue reference** | **hg19** |

## Review smallRNA plugin results

After your sequencing run completes, review 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 **smallRNA** to view the plugin results.
4. In the **smallRNA** section, click **smallRNA.html** link to open the **smallRNA Analysis Report** for all barcodes.
   - In the barcode table, click individual barcode names to see the results for an individual barcode.

| Report | Description |
|---|---|
| **Download the Statistics Summary** | An overview of read mapping statistics and smallRNA molecules. |
| **mirBase mapping quality (page_)** | Mapping quality output from Qualimap. |
| **Download the mirRNA Mature Counts** | A table with per mature RNA read counts. |
| **Download the miRNA Per Precursor 5p-3p Counts** | A table with 5p-arm and 3p-arm read counts on the same line. |
| **Download the miRNA High Confidence Mature Counts** | A table with per mature read counts for miRNAs identified as high confidence miRNAs in mirBase build 21 |
| **Download the miRNA Per Precursor 5p-3p Counts** | A table with 5p-arm and 3p-arm read counts on the same line. Restricted to miRNAs identified as high confidence miRNAs in mirBase Build 21. |
| **Download the mirBase alignments (BAM) file (genomic coordinates)** | mirBase alignments converted to genomic coordinates when mirBase was used as reference. |
| **Download the mirBase alignments index (BAI) file** | Index file for the mirBase alignment. |
| **Download Output files (page_)** | A page that provides the ability to download all output files individually. |

   - Click **Download Barcode Summary Report** to download the data into downloadable tab-separated spreadsheet, or PDF report.
   - Click **Download absolute reads matrix** to download a table that lists absolute reads for the genes that are found on each barcode.

# 8 Variant calls in Torrent Suite™ Software


- About the variantCaller plugin . . . . . 198
- Run the variantCaller plugin . . . . . 199
- Create a custom configuration for the variantCaller plugin . . . . . 205
- Apply configuration settings to specific barcodes . . . . . 206
- Create and use a custom parameters setting . . . . . 208
- Review variantCaller plugin run results . . . . . 209
- Save adjusted parameters to a variantCaller plugin configuration . . . . . 224
- variantCaller plugin advanced parameters . . . . . 225


## 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 within a targeted subset of that reference in Torrent Suite™ Software.

You can set the plugin to run automatically after a sequencing run is completed, or you can run the plugin manually for completed runs.

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” on page 202.

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 custom 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 60.

# 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 completes, 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 completed. For more information, see “Run the variantCaller plugin manually” on page 201.

---

**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 54. 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 depending 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” on page 202.
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.

## 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 custom configuration for the variantCaller plugin” on page 205.

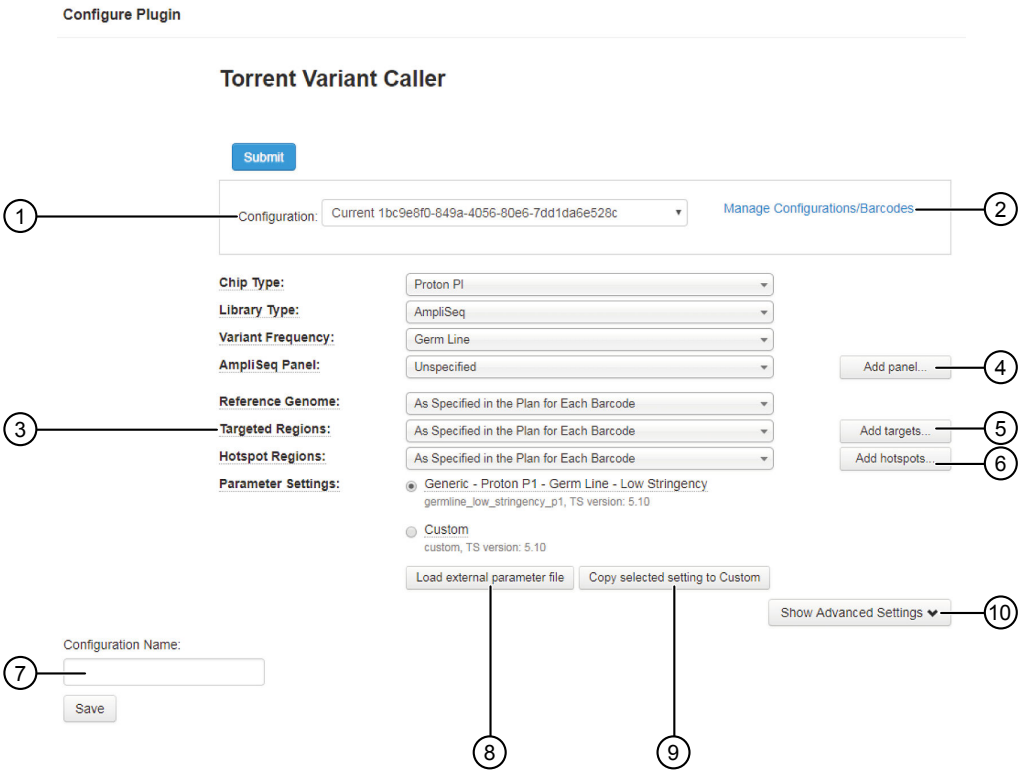
1. In the **Data** tab, click **Completed Runs & Reports** screen, then click the **Report Name** link for the completed sequencing run of interest.
2. Click **Plugins** ▸ **Select Plugins to Run**, then click the name of the plugin that you want to run.
3. In the **Select a Plugin to Run** dialog box, 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 depending 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” on page 202.
5. When your changes are complete, click **Submit**.

   The variantCaller plugin reruns, then applies the changes that you made.

# variantCaller plugin configuration

Use the **Torrent Variant Caller** screen to configure the variantCaller plugin. For information about how to get to this screen, see “Configure the variantCaller plugin to run as part of a Planned Run” on page 200




1. Configuration
2. Manage Configurations/Barcodes
3. Settings included in a predefined or custom 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 or a custom configuration for the variantCaller plugin that includes settings for a reference genome, targeted regions, hotspots, and parameter settings.<br><br>For more information, see “Create a custom configuration for the variantCaller plugin” on page 205. |

*(continued)*

| Setting | Details |
|---|---|
| **Manage Configurations/Barcodes** [1] | Manage and apply configurations for barcodes when you run the variantCaller plugin manually. For more information, see "Apply configuration settings to specific barcodes" on page 206.<br><br>(*Optional*) To run the variantCaller plugin manually for a limited number of barcodes, select **Skip this barcode** for the barcodes that you do not want to include in the plugin run.<br><br>For more information, see "Apply configuration settings to specific barcodes" on page 206. |
| **Chip Type** [1] | The chip type that is used in the sequencing run.<br><br>If you change this option, it affects only the fields that are selected by default in the **variantCaller Configuration** screen. It does not affect the sequencing run. |
| **Library Type** [1] | Library type options include:<br>• **Whole Genome**<br>• **AmpliSeq**<br>• **AmpliSeq HD**<br>• **TargetSeq**<br><br>When **Library Type** is set to **AmpliSeq**, read trimming is automatically applied to remove the primers from reads. |
| **Variant Frequency** [1] | Variant frequency options include:<br>• **Somatic**—Detects somatic variants at low allele frequencies.<br>• **Germline**—Detects germline variants that are expected to be present at low allele frequencies.<br>• **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 60.<br><br>When an Ion AmpliSeq™ panel is selected, the plugin configuration screen automatically selects 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.<br><br>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.<br><br>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.<br><br>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 270. |
| **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.<br><br>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.<br><br>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 272.<br><br>**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. |

*(continued)*

| Setting | Details |
|---|---|
| **Add Hotspots** | Upload a hotspots file. |
| **Parameter Settings** | Option to use parameter settings that are predefined in the variantCaller plugin, or to use **Custom** 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.<br>• 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** .<br>• Select **Custom** to change the advanced parameter settings. For more information, see “variantCaller plugin advanced parameters” on page 225. |
| **Load external parameter file** | Imports a file that contains parameter settings. |
| **Copy selected settings to Custom** | Creates a custom parameter setting that is based on a generic parameter setting. For more information, see “Create and use a custom parameters setting” on page 208. |
| **Configuration Name** [1] | Names and saves a custom 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 225.<br>Hover your mouse 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 custom configuration for the variantCaller plugin

You can create a custom configuration for the variantCaller plugin in Torrent Suite™ Software. Later, when you run the plugin manually, you can apply the custom 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**.
   The variantCaller plugin configuration screen opens.

4. Set plugin configuration parameters.
   For more information, see "variantCaller plugin configuration" on page 202 and "Create and use a custom parameters setting" on page 208.

5. In **Configuration Name**, enter a name for the configuration, then click **Save**.

The new configuration is included in the **Configuration** drop-down 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 custom configurations, for the variantCaller plugin and apply these custom configurations to specific barcodes when you run the variantCaller plugin manually from a completed Torrent Suite™ Software run Report. By applying specific custom configurations to individual barcodes, you can refine your analysis results.

You can use configurations for barcoded runs to:

- 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.

| To | Action |
|---|---|
| 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.

| Action | Description |
|---|---|
| Edit a configuration. | 1. Click **Edit**. The **Configure Plugin** dialog box becomes specific to the configuration selected.<br>2. Change the existing selections as desired.<br>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.<br>3. Click **Show Advanced Settings**, then edit the parameters if needed.<br>4. Click **Save**. |
| Delete a configuration. | 1. Click **Delete**.<br>2. Click **OK** to confirm. |
| Create a new configuration. | 1. Click **Add**.<br>2. Enter a configuration name.<br>3. Make selections from the available lists.<br>4. Click **Save**. |

# Create and use a custom parameters setting

You can create a custom parameters setting that you can save and reuse. The custom parameters setting that you create can be used with a manual run of the variantCaller plugin. To use a customized parameter file that is designed for your customized panel or other special requirements, you must create a custom parameters setting, then run the variantCaller plugin manually with the custom 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 225.

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**.

| To . . . | Do this . . . |
|---|---|
| Use a JSON parameters file that you have saved on your computer. | 1. Click **Load external parameter file**.<br>2. Browse to the JSON file, then click **Open**. |
| Edit a predefined parameters setting. | 1. In **Configuration**, select the predefined parameter setting that you want to edit.<br>2. Click **Copy selected to Custom**.<br>3. If you agree to save the new custom parameter setting, click **OK**.<br>4. Click **Show Advanced Settings** to enter changes for the custom 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 custom 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:

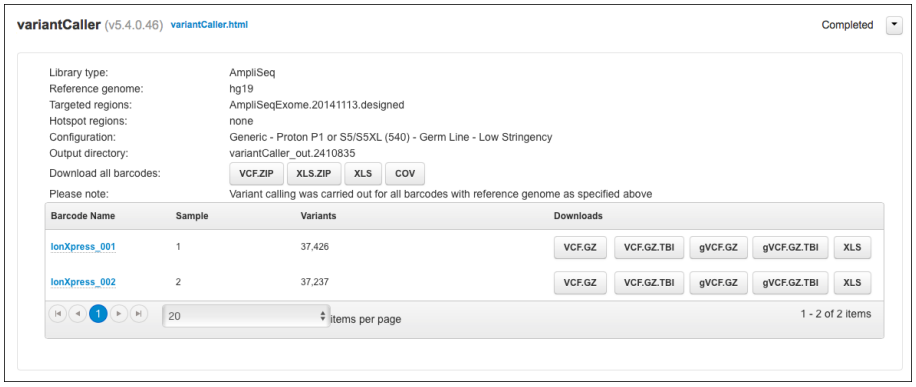
- Review the summary of the variantCaller plugin run for each barcode used.
- Review the library type, reference genome, targeted regions, hotspots file, and parameter settings that were used in the run.
- Download data files for all barcodes and each individual barcode or sample.

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 browser, you can:

- 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 TVC-processed reads.
- Download data files for variant calls and coverage for the specific barcode.
- View variant calls in IGV.

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.

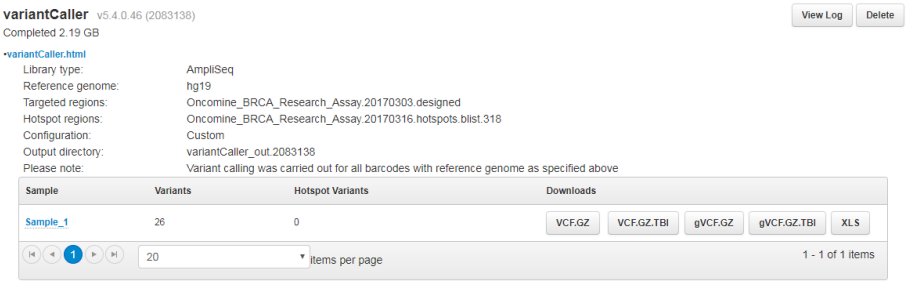
1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the **Completed Runs & Reports** list, find the run of interest, then click the report link in the **Report Name** column in the row of the run.
3. In the left navigation menu, click **variantCaller** to navigate to the variantCaller results 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.




**Downloads 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.

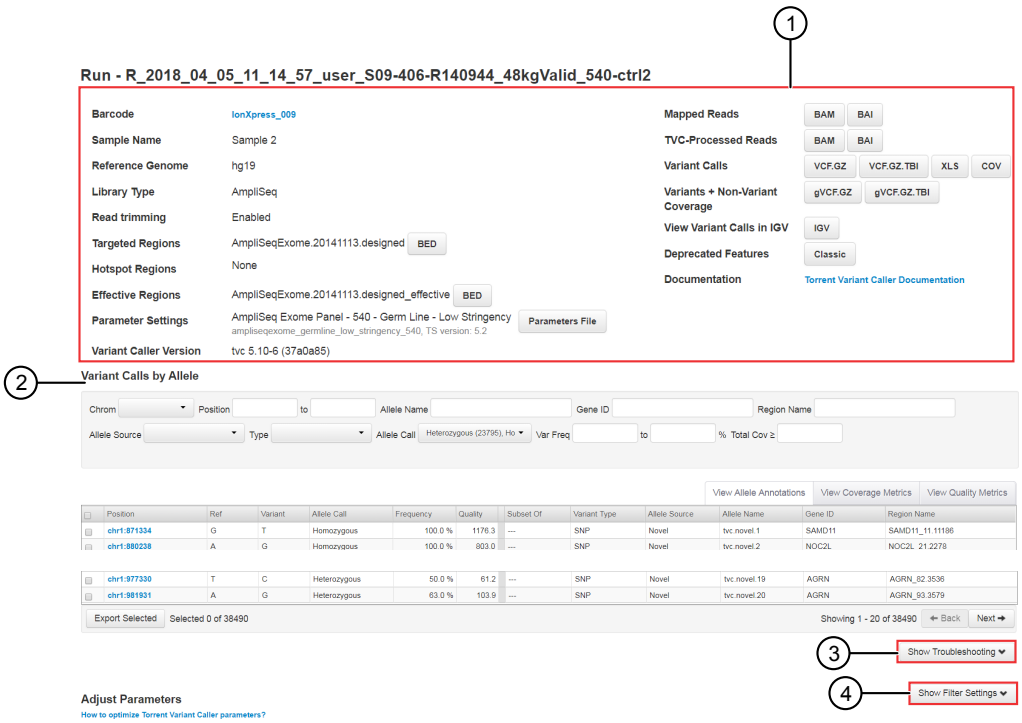



4. To open the detailed variantCaller plugin report for a specific barcode or sample, do one of the following in the variantCaller run summary table:
   - In the **Barcode name** column in a barcoded sequencing run report, click the barcode name link.
   - In the **Sample** column in a report from a 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 213.
3. Export the variant data files for troubleshooting. For more information, see “Export files for troubleshooting” on page 223.
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 224.

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. |

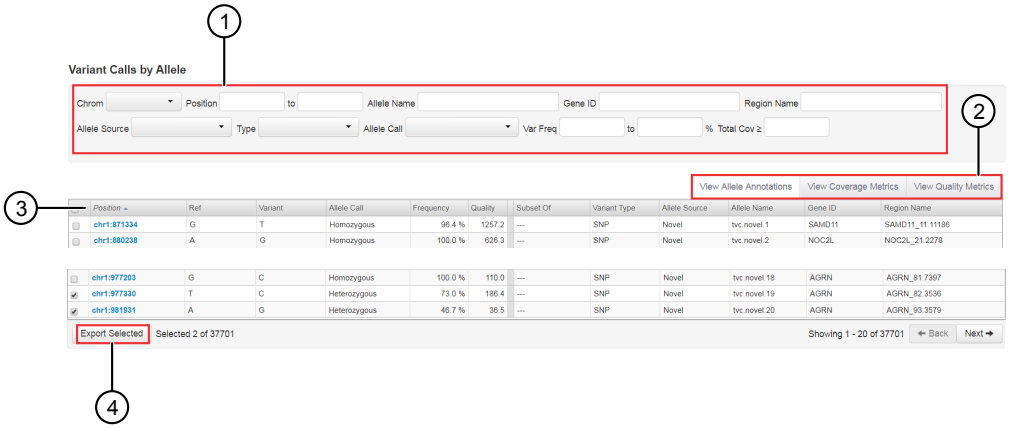
*(continued)*

| Download option | Description |
|---|---|
| **Effective Regions** | **BED**—The BED file that specifies the regions actually 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. |
| **Mapped Reads** | • **BAM**—The BAM file that is input to the Torrent Variant Caller Pipeline for discovering and evaluating variants. Note that realignment may be applied.<br>• **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. 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.<br>• **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.<br>• **VCF.GZ.TBI**—The index file for VCF.GZ.<br>• **XLS**—The file that contains a list of variant alleles in the tab-separated file format, which can be opened in Microsoft™ Excel™.<br>• **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.<br>• **gVCF.GZ.TBI**—The tabix index file for gVCF.GZ. |
| **View Variant Calls in IGV** | **IGV**—The JNLP file that can be opened using (IGV) to visualize the variant calls. |

## 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, you can select one of the following tabs.
   - **View Allele Annotation**. For more information, see “View allele annotations ” on page 215.
   - **View Coverage Metrics**. For more information, see “View coverage metrics ” on page 215.
   - **View Quality Metrics**. For more information, see “View quality metrics” on page 216.
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 217.

| 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. |

*(continued)*

| Column | Description |
|---|---|
| **Allele Call** | The zygosity (homozygouse or heterozygous) or type (absent or No Call) of the allele that is called by the zygosity (homozygouse 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.<br><br>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 47. |
| **Quality** | The Phred-scored quality.<br><br>For variants found by the Long INDEL Assembler, this value is always set to 50. Larger values mean higher confidence in the call.<br><br>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.<br><br>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** (Optional) | An indication (0 or 1) of whether the variant allele is a possible polypoidy allele (PPA). Only absent alleles can be labeled as PPA; heterozygous and homozygous alleles are not treated as PPA.<br><br>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 225. |

## 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.<br>• SNP—single nucleotide polymorphism.<br>• IND—insertion.<br>• DEL—deletion.<br>• MNP—multiple nucleotide polymorphism or the substitution of a block sequence by the block of another length.<br>• COMPLEX—Block substitution of sequence by a block of unequal length. |
| **Allele Source** | Allele source is called as:<br>• Hotspot—for alleles included in the hotspots file.<br>• Novel—for all other alleles. |
| **Allele Name** | The allele name as defined in the hotspots file.<br>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.<br>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 225. |

*(continued)*

| Column | Description |
|---|---|
| **Read Cov +** | Total read coverage on the forward strand, after downsampling. |
| **Read Cov -** | 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** [1] | The frequency of this allele across all reads. |
| **Total Mol Cov**[1] | The number of molecules covering this location. |
| **Allele Mol Cov**[1] | The number of detected molecules containing this allele. |
| **Allele Mol Freq**[1] | The frequency of molecules containing this allele. |
| **Allele Cov +** [1] | Allele coverage on the forward strand, after downsampling. |
| **Allele Cov -** [2] | Allele coverage on the reverse strand, after downsampling. |
| **Strand bias** | The discrepancy between allele frequencies on the forward and reverse strands. |

[1] This column is unavailable for sequencing runs that use Tag Sequencing or Ion AmpliSeq™ HD as the target technique.

[2] This column is unavailable for sequencing runs that use Tag Sequencing 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). |

*(continued)*

| Column | Description |
|---|---|
| **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). |

For candidates that are filtered out, the filtering reason is highlighted in the table.

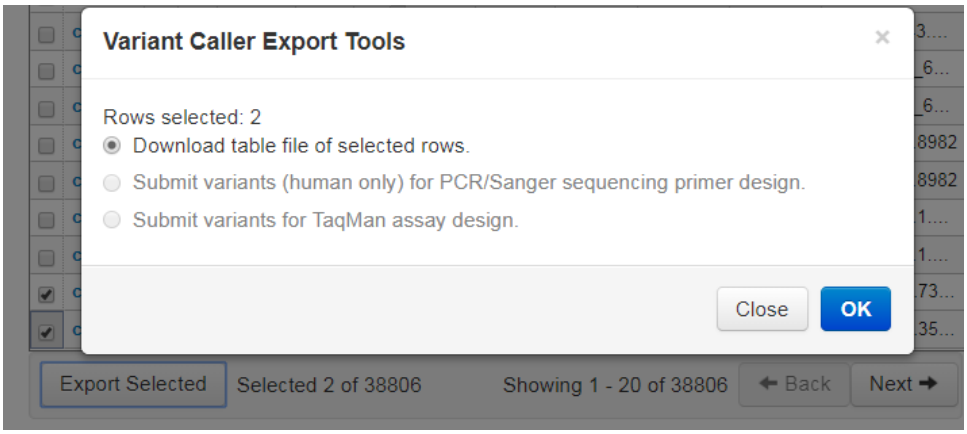
| lele coverage | allele coverage + | allele coverage - | strand bias |
|---|---|---|---|
| 29 | 21 | 8 | 0.5897 |
| 23 | 15 | 8 | 0.5522 |
| 15 | 15 | 0 | 0.5000 |
| 15 | 15 | 0 | 0.5000 |
| 288 | 133 | 155 | 0.5000 |
| 95 | 88 | 7 | 0.5028 |
| 20 | 20 | 0 | 0.5000 |
| 5 | 0 | 5 | 0.5000 |
| 259 | 102 | 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. 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.

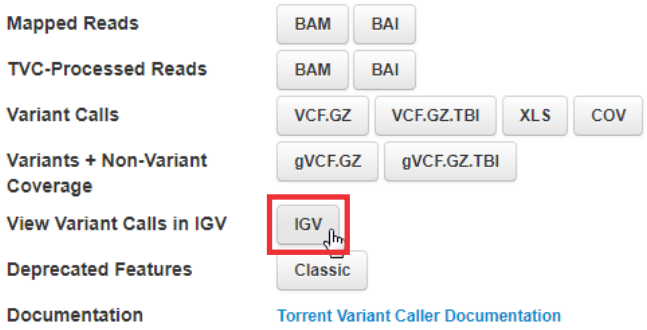
# Troubleshoot variantCaller plugin results

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).

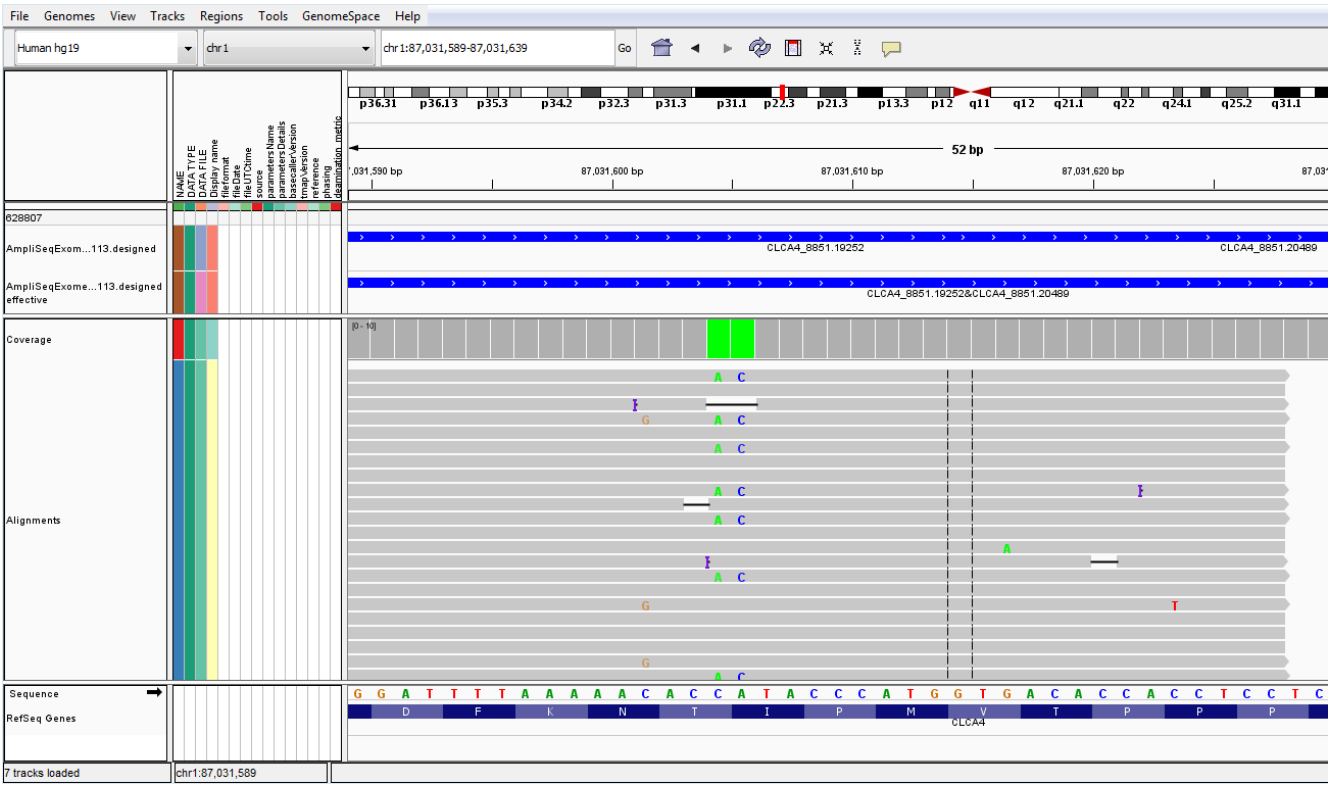
## Find false negatives with an alignment viewer

When an expected variant is not called by the variantCaller plugin, an alignment viewer, such as Integrative Genomics Viewer (IGV) or 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 the IGV or 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.

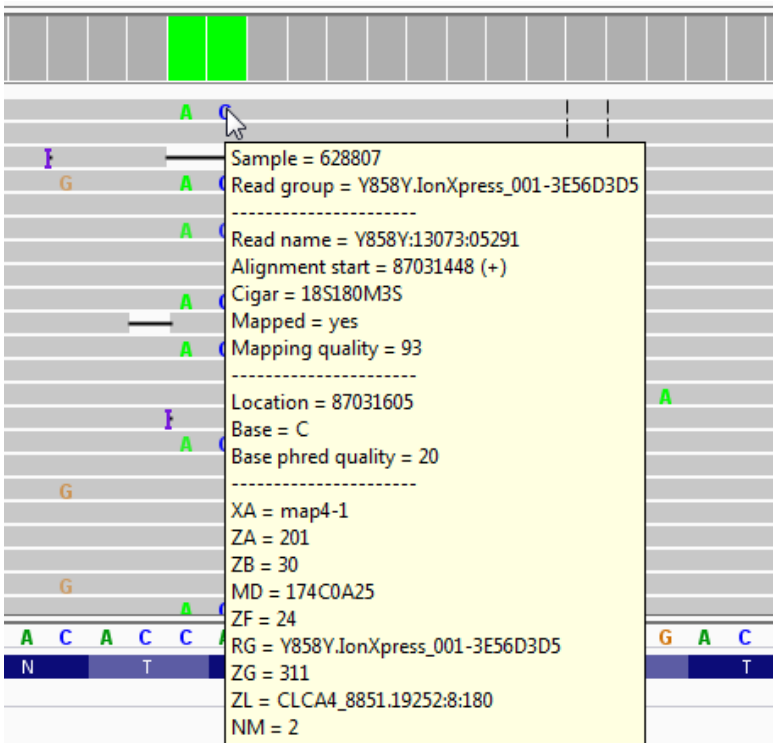
1. On the detailed variantCaller plugin summary report screen, click **IGV** to open the viewer.



2. 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.



3. Hover over a variant read to view read analytics.




4. Adjust plugin parameters.

5. 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:

| 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 225.

  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:

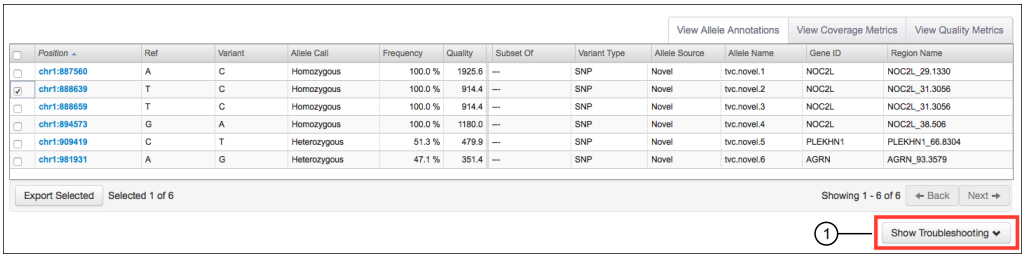
- Adjust parameters that control the homopolymer calls. This can increase the report of false negatives.
- If you are repeatedly running a panel, manually curate specific sites (positions), since the false positive tends to occur in the same positions.

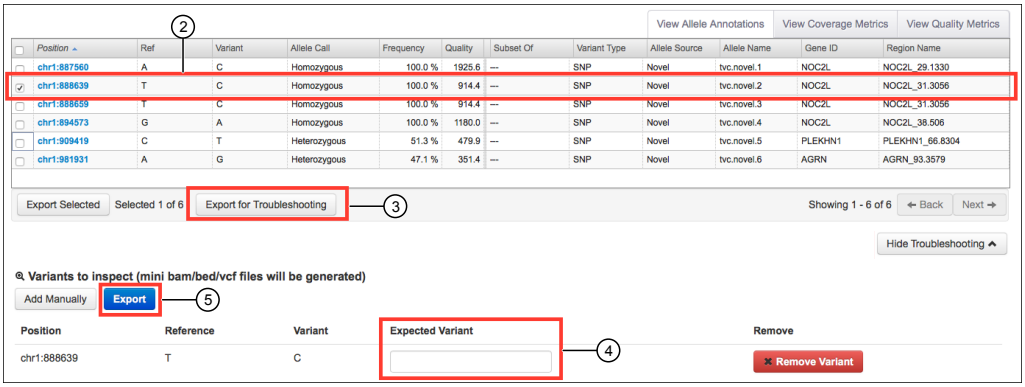
The variantCaller plugin does not support manual curation. Manual curation is available in the command-line version.

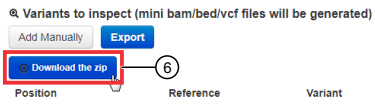
## Export files for troubleshooting

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**.

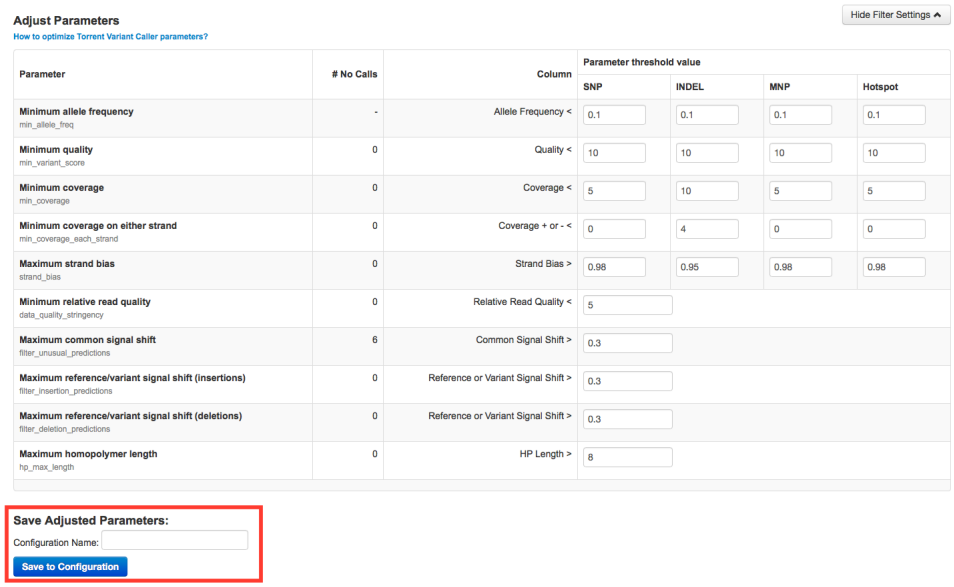
The compressed directory of miniature BAM, BED, and VCF files is downloaded to the directory, according to your browser settings.

# 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. 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 optimize 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 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` | X is one of the allele types in {indel, snp, mnp, hotspot}.<br>A variant evaluation parameter: The presence of the allele of the type is defined by which allele frequency is greater than this value.<br>**Allowed values:** Decimal between 0 and 1<br>**Suggested trial value:** between 0.01 (somatic) and 0.2 (germline) |

**Torrent Variant Caller advanced parameters and settings** *(continued)*

| Parameter | Description |
|---|---|
| `X_min_variant_score` | X is one of the allele types in {indel, snp, mnp, hotspot}.<br>A filter parameter: A called allele of the type needs to have a QUAL score greater than this Phred-scaled value.<br>**Filter reason:** quality score.<br>**Related VCF fields:** QUAL<br>**Allowed values:** Decimal values ≥ 0<br>**Suggested trial value:** > 10 |
| `X_min_coverage` | X is one of the allele types in {indel, snp, mnp, hotspot}.<br>A filter parameter: The location of a called allele of the type needs to have a coverage greater than this value.<br>**Filter reason:** MINCOV<br>**Related VCF fields:** FRO, FAO<br>**Allowed values:** Integers ≥ 0<br>**Suggested trial value:** between 5 and 20 |
| `X_min_cov_each_strand` | X is one of the allele types in {indel, snp, mnp, hotspot}.<br>A filter parameter: Minimum coverage required on each strand for a the type of allele to be called.<br>**Filter reason:** PosCov or NegCov<br>**Related VCF fields:** FSRF, FSRR, FSAF, FSAR<br>**Allowed values:** Integers ≥ 0<br>**Suggested trial value:** ≥ 3 |
| `X_strand_bias` | X is one of the allele types in {indel, snp, mnp, hotspot}.<br>A filter parameter: A candidate allele of the type will be 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.<br>**Filter reason:** STDBIAS and STDBIASPVAL<br>**Related VCF field:** STB<br>**Allowed values:** Decimal numbers between 0.5 (requires perfect balance on both strands) and 1.0 (tolerates extremely strong strand bias)<br>**Suggested trial value:** 0.95 |

**Torrent Variant Caller advanced parameters and settings** *(continued)*

| Parameter | Description |
|---|---|
| `X_strand_bias_pval` | X is one of the allele types in {indel, snp, mnp, hotspot}.<br>A filter parameter: A candidate allele of the type will be filtered out if its strand bias p-value is less than `X_strand_bias_pval`l and its strand bias is greater than `X_strand_bias`.<br>**Filter reason:** STDBIAS and STDBIASPVAL<br>**Related VCF field:** STBP<br>**Allowed values:** Decimal numbers between 0 and 1<br>**Suggested trial value:** 0.01 for strand bias filter, 1 for no strand bias filter |
| `X_min_var_coverage` | X is one of the allele types in {indel, snp, mnp, and hotspot}.<br>A filter parameter: Minimum number of variant coverage after flow-evaluation required to make the call.<br>**Filter reason:** VarCov<br>**Related VCF field:** FAO<br>**Allowed values:** Integer ≥ 0<br>**Suggested trial value:** 3 (TagSequencing or AmpliSeq HD), 0 (other) |
| `downsample_to_coverage` | Reduce coverage in over-sampled locations to this value to save computational time.<br>**Allowed values:** Integers ≥1<br>**Suggested trial value:** 400 (germline), 2000 (somatic) |
| `use_fd_param` | (experimental in Torrent Suite™ Software 5.4)<br>A filtering parameter: Use Flow Disruptiveness (FD) instead of allele types (INDEL, SNP, MNP) as the criterion to select the parameter set.<br>If turned on, the (non-FD, weak FD, strong FD) allele applies the (INDEL, SNP, MNP) parameters, respectively.<br>If powered on, the (non-FD, weak FD, strong FD) allele applies the (INDEL, SNP, MNP) parameters, respectively.<br>**Allowed values:** 0: do not use FD parameters, 1: use FD parameters. |

**Torrent Variant Caller advanced parameters and settings** *(continued)*

| Parameter | Description |
|---|---|
| `data_quality_stringency` | A filter parameter: A called variant needs to have a mean log-likelihood difference per read greater than this Phred-scaled value.<br>**Filter reason:** STRINGENCY<br>**Related VCF field:** MLLD<br>**Allowed values:** Decimal numbers ≥ 0<br>**Suggested trial value:** ≥ 6.5 |
| `filter_unusual_predictions` | A filter parameter: 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.<br>**Filter reason:** PREDICTIONSHIFTx<br>**Related VCF fields:** RBI = sqrt(FWDB ^ 2 + REVB ^ 2)<br>**Allowed values:** Decimal numbers ≥ 0<br>**Suggested trial value:** 0.3 |
| `filter_deletion_predictions` | A filter parameter: Filter out a deletion if the observed clusters deviate from predictions more than this amount.<br>**Filter reason:** PREDICTIONVarSHIFTx or PREDICTIONRefSHIFTx<br>**Related VCF fields:** VARB, REFB<br>**Allowed values:** Decimal numbers ≥ 0<br>**Suggested trial value: 0.2** |
| `filter_insertion_predictions` | A filter parameter: Filter out an insertion if the observed clusters deviate from predictions more than this amount.<br>**Filter reason:** PREDICTIONVarSHIFTx or PREDICTIONRefSHIFTx<br>**Related VCF fields:** VARB, REFB<br>**Allowed values:** Decimal numbers ≥ 0<br>**Suggested trial value:** 0.2 |
| `min_callable_prob` | A reporting parameter (TagSeq/AmpliSeq HD only): The minimum callable probability for the calculation of Limit Of Detection (LOD).<br>• 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.<br>• **Allowed values:**Decimal numbers between 0 and 1<br>• **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.<br>**Allowed values:** Integers ≥ 0<br>**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.<br>**Allowed values:** Integer ≥ 1<br>**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.<br>**Allowed values:** strict-trim (requires match), sloppy-trim (does not require match)<br>**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.<br>**Allowed values:** Integers ≥ 0<br>**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.<br>**Allowed values:** Integers ≥ 1<br>**Suggested trial value:** 3 |
| `outlier_probability` | A variant evaluation parameter: probability that a read comes from none of the models under consideration.<br>The variantCaller plugin will make NOCALL with filter reason REJECTION if FXX is too high.<br>**Related VCF field:** FXX<br>**Allowed values:** Decimal numbers between 0 and 1.0<br>**Suggested trial value:** between 0.005 and 0.01 |

**Torrent Variant Caller advanced parameters and settings** *(continued)*

| Parameter | Description |
|---|---|
| `prediction_precision` | A variant evaluation parameter: The number of pseudo data points suggesting our predictions match the measurements without bias.<br>**Allowed values:** Decimal numbers ≥ 0.1<br>**Suggested trial value:** 1.0 |
| `max_flows_to_test` | A variant candidate evaluating parameter: The maximum number of scoring flows being used.<br>**Allowed values:** Integers > 0<br>**Suggested trial value:** 10 |
| `suppress_recalibration` | A variant evaluation parameter: Homopolymer recalibration values should not be used when set.<br>**Allowed values:** 0 = enable recalibration, 1 = disable recalibration<br>**Suggested trial value:** 0 |
| `do_snp_realignment` | A variant candidate evaluating parameter: Realign reads in the vicinity of SNP candidates when set.<br>**Related VCF content:** REALIGNEDx<br>**Allowed values:** 0 = do not realign, 1 = realign<br>**Suggested trial value:** 0 |
| `do_mnp_realignment` | A variant candidate evaluating parameter: Realign reads in the vicinity of MNP candidates when set.<br>**Related VCF content:** REALIGNEDx<br>**Allowed values:** 0 = do not realign, 1 = realign<br>**Suggested trial value:** 0 |
| `realignment_threshold` | A variant candidate evaluating parameter: Maximum allowed fraction of reads where realignment causes an alignment change.<br>**Related VCF content:** SKIPREALIGNx<br>**Allowed values:** Decimals between 0 and 1<br>**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.<br>**Allowed values:** Decimal numbers between 0 and 1<br>**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.<br>**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 will be filtered out.<br>**Filter reason:** HPLEN<br>**Related VCF field:** HRUN<br>**Allowed values:** Integers ≥ 1<br>**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`.<br>**Filter reason:** HPINSLEN, HPDELLEN<br>**Related VCF field:** HRUN<br>**Allowed values:** Vector of positive integers (for example, [1,2,3]) with size matches `hp_del_len` and `hp_ins_len`<br>**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`.<br>**Filter reason:** HPINSLEN<br>**Related VCF field:** HRUN<br>**Allowed values:** Vector of non-negative integers (for example, [1,2,3]) with size matches `hp_del_len` and `hp_indel_hrun`.<br>**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'.<br>**Filter reason:** HPDELLEN<br>**Related VCF field:** HRUN<br>**Allowed values:** Vector of nonnegative integers (for example, [1,2,3]) with size matches `hp_ins_len` and `hp_indel_hrun`.<br>**Suggested trial value:** [] |
| `use_position_bias` | A filter parameter: Enable the position bias filter when set.<br>**Filter reason:** POSBIAS, POSBIASPVAL<br>**Allowed values:** 0 = disable, 1= enable<br>**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`.<br>**Filter reason:** POSBIAS, POSBIASPVAL Related VCF field: POSBIAS<br>**Allowed values:** Ddecimal numbers between 0 and 1<br>**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`.<br>**Filter reason:** POSBIAS, POSBIASPVAL Related VCF field: POSBIASPVAL<br>**Allowed values:** Decimal numbers between 0 and 1<br>**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.<br>**Filter reason:** POSBIAS, POSBIAS-PVAL<br>**Allowed values:** Decimal numbers between 0 and 1<br>**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.<br>**Filter reason:** NOCALLxPredictedSSE, NOCALLxPositiveSSE, NOCALLxNegativeSSE<br>**Related VCF fields:** SSEP, SSEN<br>**Allowed values:** Decimal numbers between 0 and 1<br>**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.<br>**Related VCF field:** PPA<br>**Allowed values:** 1 = report PPA, 0 = do not report<br>**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.<br>**Allowed values:** 0 = does not generate indel candidates, 1 = generates INDEL candidates<br>**Suggested trial value:** 1 |
| `allow_snps` | Candidate generation parameter: Allow SNP candidates to be generated when set.<br>**Allowed values:** 0 = does not generate SNP candidates, 1 = generates SNP candidates<br>**Suggested trial value:** 1 |
| `allow_mnps` | Candidate generation parameter: Allow MNP candidates to be generated when set.<br>**Allowed values:** 0 = does not generate MNP candidates , 1 = generates MNP candidates<br>**Suggested trial value:** 1 |
| `allow_complex` | Candidate generation parameter: Allow complex variant candidates to be generated when set.<br>**Allowed values:** 0 = does not generate complex candidates, 1 = generates complex candidates<br>**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.<br>**Allowed values:**Decimal numbers between 0 and 1<br>**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.<br>**Allowed values:** Decimal numbers between 0 and 1<br>**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.<br>**Allowed values:** Integers ≥ 0<br>**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).<br>**Allowed values:** ≥ 0<br>**Suggested trial value:** 4 |
| `read_snp_limit` | A read filtering parameter: Do not use reads with number of SNPs above this.<br>**Allowed values:** Integers ≥ 0<br>**Suggested trial value:** 10 |
| `read_max_mismatch_fraction` | A read filtering parameter: Ignore reads with fraction of mismatch greater than this value.<br>**Allowed values:** Decimal numbers between 0 and 1<br>**Suggested trial value:** 1.0 |
| `min_cov_fraction` | A read filtering parameter: Do not use reads with fraction of covering the best assigned unmerged target region below this.<br>**Allowed values:** Decimal numbers between 0 and 1<br>**Suggested trial value:** 0.9 (TagSequending and Ion AmpliSeq™ HD), 0 (otherwise) |
| `read_mismatch_limit` | A read filtering parameter: Do not use reads with number of mismatches (where 1 gap open counts 1) above this value.<br>**Allowed values:** Integers ≥ 0 (0 disables the filter)<br>**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.<br>Impact: Increasing values make INDEL calls less sensitive but more specific.<br>**Allowed values:** Integers ≥ 5<br>**Suggested trial value:** 11 and 30 |
| `min_var_freq` | Minimum frequency of the variant to be reported.<br>Impact: Increasing values make INDEL calls less sensitive but more specific.<br>**Allowed values:** Decimal numbers between 0 and 1<br>**Suggested trial value:** 0.1 and 0.4 |
| `min_var_count` | Minimum support for a variant to be evaluated.<br>Impact: Increasing values make INDEL calls less sensitive but more specific.<br>**Allowed values:** Integers > 1<br>**Suggested trial value:** 3 and 30 |
| `short_suffix_match` | Minimum assembled sequence match on both sides of the variant.<br>Impact: Increasing values make INDEL calls less sensitive but more specific.<br>**Allowed values:** Integers > 2<br>**Suggested trial value:** 4 and kmer_len |
| `min_indel_size` | Minimum size indel reported by assembly.<br>Impact: Increasing values make INDEL calls less sensitive but more specific.<br>**Allowed values:** Integers > 0<br>**Suggested trial value:** 2 and 30 |

*(continued)*

| Parameter | Description |
|---|---|
| `max_hp_length` | Variants containing HP larger than this are not reported.<br>Impact: Increasing values make INDEL calls more sensitive but less specific.<br>**Allowed values:** Integers > 1<br>**Suggested trial value:** 2 and 11 |
| `relative_strand_bias` | Variants with strand bias above this are not reported.<br>Impact: Increasing values make INDEL calls more sensitive but less specific.<br>**Allowed values:** Decimal numbers between 0 and 1<br>**Suggested trial value:** 0.6 and 1.0 |
| `output_mnv` | Enables reporting of complex variants.<br>**Allowed values:** 1 = report complex variants, 0 = don't report<br>**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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## 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 245.

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

# Install the IonReporterUploader plugin on a Ion Torrent™ Server

The IonReporterUploader plugin is installed automatically on a 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 348 and “Update off-cycle release plugins” on page 351.

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.

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**IMPORTANT!** When you upgrade to a new version of Ion Reporter™ Software, you must reconfigure the IonReporterUploader plugin with a 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 a 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.

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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 into 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** dropdown menu, then click **Save and Generate**.

Note:

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 as needed.

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 | Description |
|---|---|
| Ion Reporter™ Software on Connect | Select **Ion Reporter Cloud** |
| Ion Reporter™ Software on 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 | Description |
|---|---|
| **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 ConnectEnter the access code.<br>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 a 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, you must 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 64.

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 239.
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 your browser. You can select the newly created analysis workflow in the **Existing Workflow** menu.

9. Select an **Ion Reporter Upload 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.<br><br>**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 44.

11. If appropriate, enter the gender of a sample.

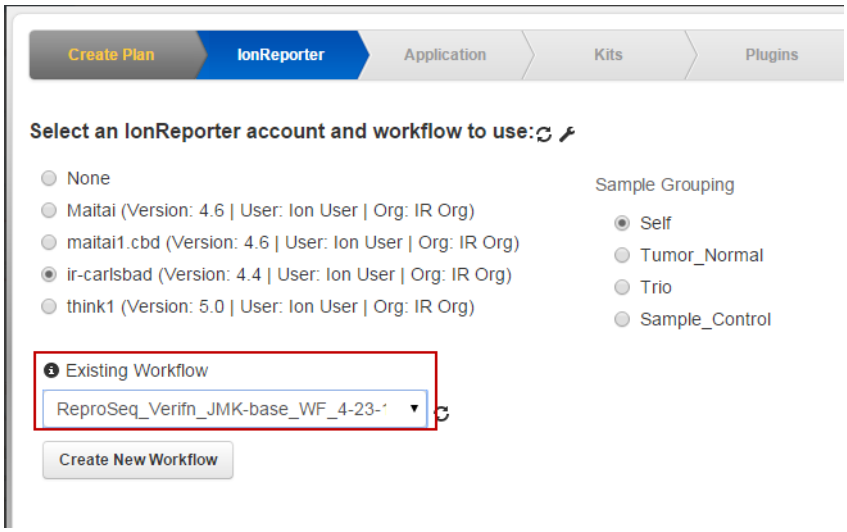
    For more information, see “Sample gender” on page 245.

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.

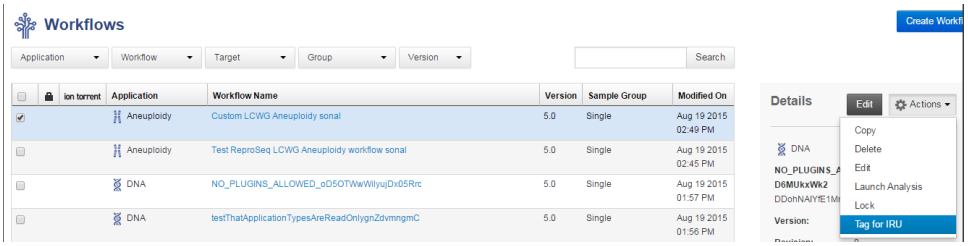
13. The Planned Run is ready to run on your sequencing system.

# Manage the Ion Reporter™ Software analysis workflow list

You can reduce the number of Ion Reporter™ Software analysis workflows that are listed when you create a Planned Run or Planned Run template in Torrent Suite™ Software. 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 when you plan instrument runs in Torrent Suite™ Software.

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.
- If the gender of the sample is not specified or is specified as "Unknown", the Integrative Genomics Viewer uses female as the gender.

# 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.

   **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.

   a. 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.

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 depending 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 239.

| Setting | Description |
|---|---|
| **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. |

*(continued)*

| Setting | Description |
|---|---|
| **Username** | Enter your Ion Reporter™ Software user name (your email address). |
| **Password** | For Ion Reporter™ Software on ConnectEnter the access code for use with the IonReporterUploader plugin setup.<br>For Ion Reporter™ Server, enter the password. |
| **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 245.

| 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 250. | |
| **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

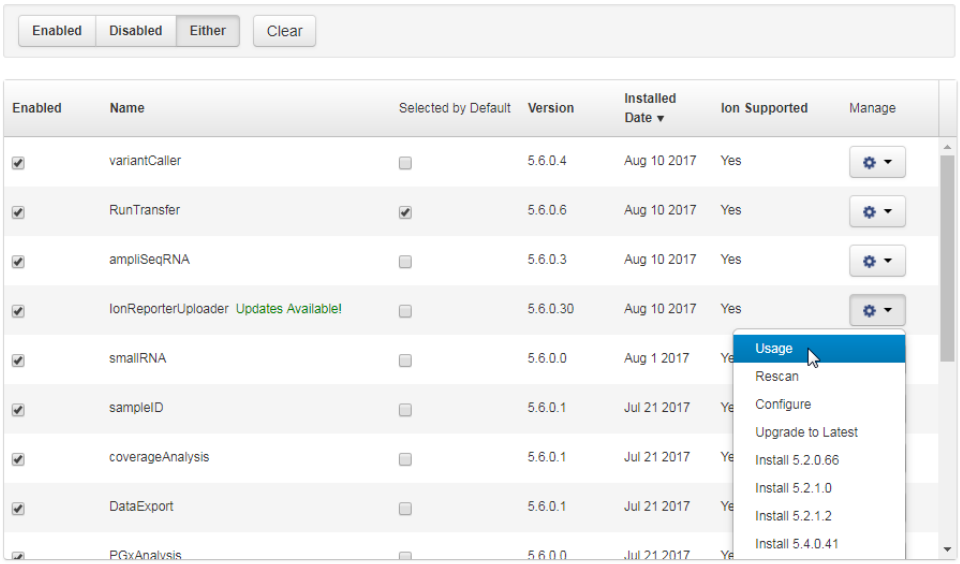
You can monitor the progress of the transfer of analysis results files from Torrent Suite™ Software to Ion Reporter™ Software.

| To monitor IonReporterUploader plugin progress through… | See details in… |
|---|---|
| Email | Email notifications sent for each plugin run:<br>• When the plugin starts to transfer your files.<br>• When the upload to Ion Reporter™ Software is complete.<br>Email notifications are sent to the email address of the Ion Reporter™ Software user whose authentication token was used to configure the plugin. |
| Torrent Suite™ Software | “View plugin run status” on page 133. |
| Log files | “Open a plugin log” on page 133. |

## 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 sizes of the plugin output.

1. Click ⚙ **(Settings)** ▸ **Plugins**.
   The installed plugins are listed.
2. Click ⚙ **(Actions)** ▸ **Usage** in the row of the IonReporterUploader plugin.

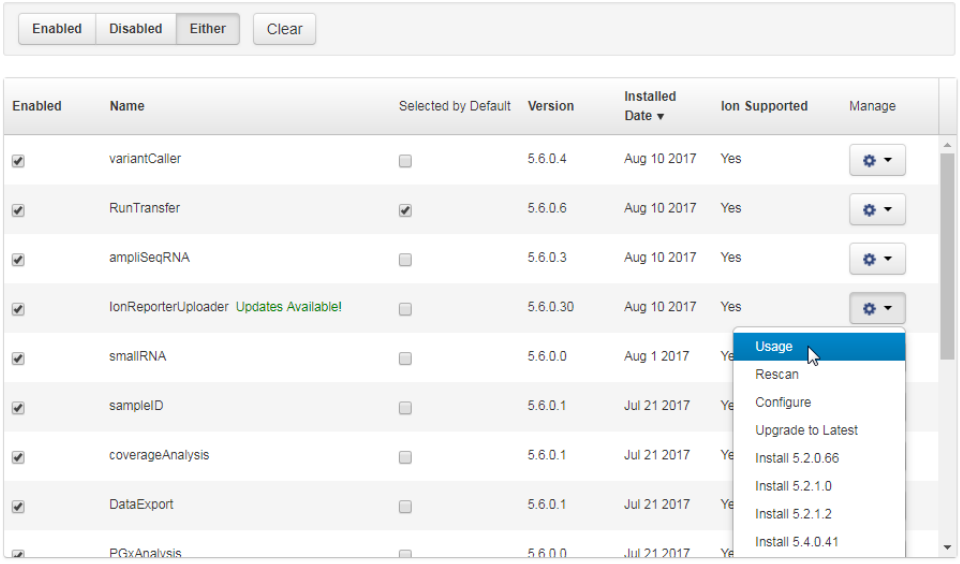


3. View the following information from the list of run reports:
   - Time that a plugin run started and ended.
   - Status of the plugin run.
   - Size of the plugin run result output files.

## Delete IonReporterUploader plugin report files

**IMPORTANT!** This action permanently deletes the IonReporterUploader plugin report for a run and cannot be undone.

1. Sign in to Torrent Suite™ Software.
2. Click **Settings (⚙) ▸ Plugins**. The installed plugins are listed.
3. Click **Settings (⚙)Usage** for IonReporterUploader plugin:




4. Click **Delete** on the row for the plugin report that contains the files that you want to delete.

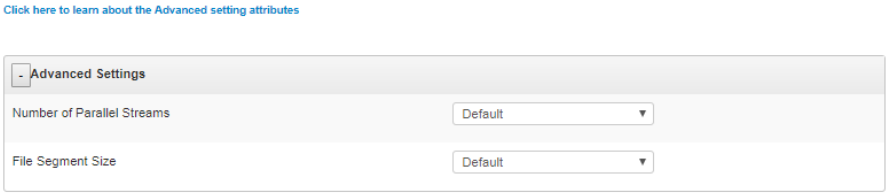
The output files for the plugin report are deleted.

# 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.



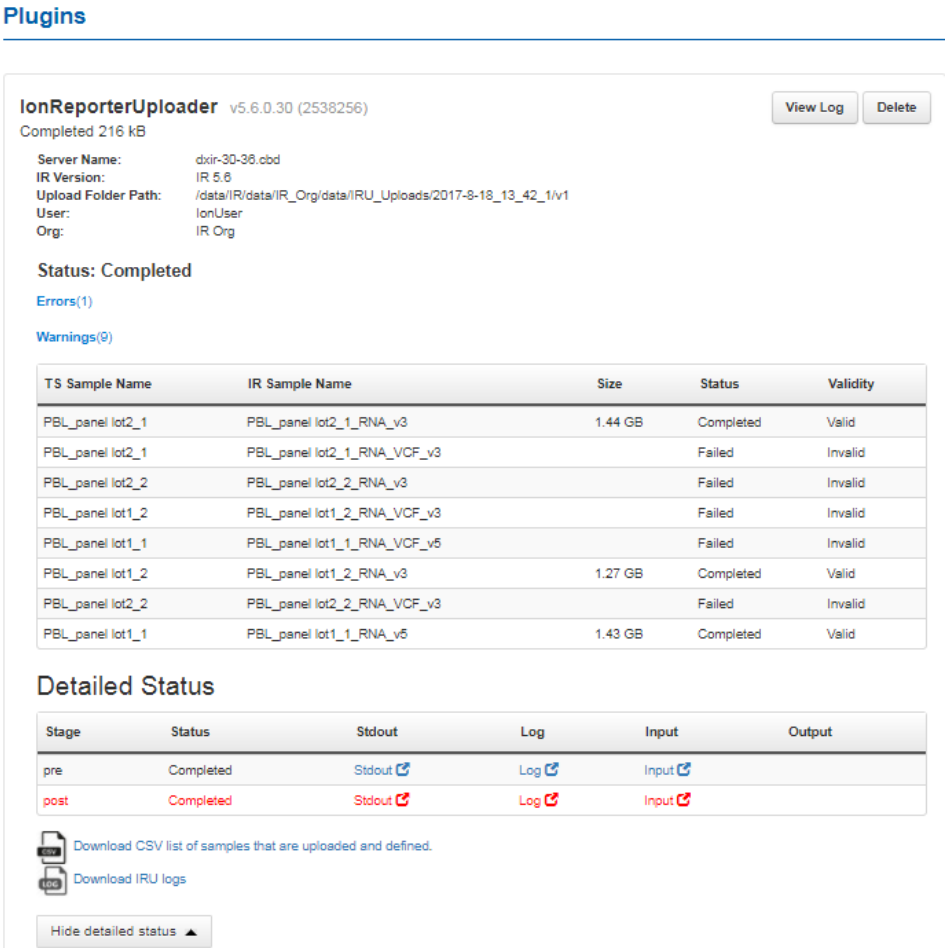

   b. Set File Segment Size to **Default** (recommended), or **16MB**, **32MB**, **64MB**, or **128MB**.

# 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. From the IonReporterUploader plugin, you can view information that is related to the data transfer, including 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 failed to upload.

| 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 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

Typically the BAM file output of your Torrent Suite™ Software analysis is 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 | TS analysis pipeline | Any except annotation-only |
| VCF file | TS Variant Caller (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:

- A 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 directory `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.

---

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` directory to a convenient location on the target computer.

## Run IonReporterUploader command-line utility

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

Instructions to use the IonReporterUploader command-line utility are downloaded with the utility. For more information, see the *IonReporterUploader Command-Line Utility User Guide* (Pub. No MAN0017648.

# 10 References management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## 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 by clicking ⚙ **(Settings)** ▸ **References**, and using options 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. |
| **Hotspots** | Upload hotspots files to add them to your Planned Runs. The variantCaller plugin generates output files that include these positions whether or not 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. |

*(continued)*

| Reference type | Description |
|---|---|
| **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 containing 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 using the TMAP aligner that comes preinstalled on the Ion Torrent™ Server. For more information, see “TMAP modules” on page 325.

## 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'`, while 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, while 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 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's 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 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 your 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 custom reference sequence files

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 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 containing 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 and save them to your local storage. For more information, see “Target regions and hotspots files” on page 266 and “Import reference sequence files” on page 259.

## Import a preloaded reference sequence file

Validated reference sequence genome files suitable for use with Torrent Suite™ Software are preloaded on the Ion Torrent™ Server. These reference files can be easily imported into the software using this procedure. After you import the reference genome file, the references can enabled for use in Planned Runs and data analysis.

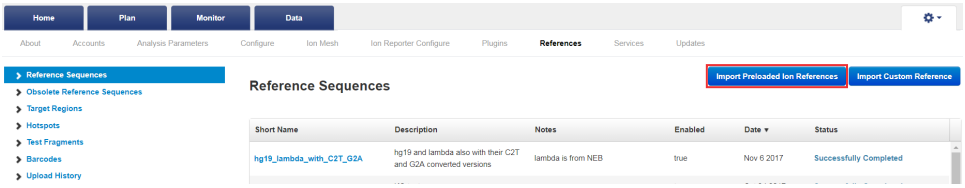
You can also edit the reference genome information, or permanently delete the reference file from the software. For more information, see "View a reference sequence file" on page 262.

---

**IMPORTANT!** If you edit or delete a reference sequence file, the change affects all users.

---

1. Click ⚙ **(Settings)** ▸ **References**.
2. Click **Import Preloaded Ion References**.




   The **Ion References** and **Downloads** lists are displayed.

3. In the **Ion References** list, find the file of interest.

| Option | Description |
|---|---|
| Reference | To import a reference file, click **Import** in the row of the reference file to import. |
| Annotation Files | To download annotation files that pertain to the reference file that you are importing:<br>1. Click **Import Annotation Files**.<br>2. In the **Import Annotation Files** dialog box, select the annotations that you want to import, then click **Import Selected**. |

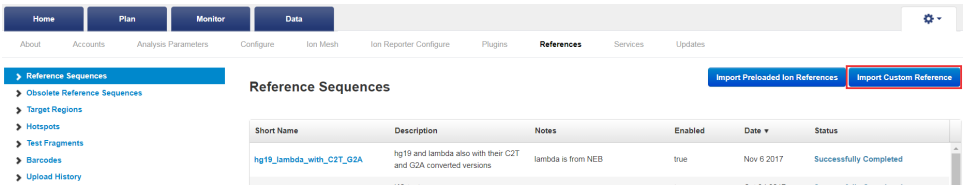
   The file import status appears in the row of the selected file in the **Ion References** list. When the status is **complete**, a compressed folder in ZIP file format is added to the **Downloads** list and the reference genome is added to the **Reference Sequences** table. You can now use the reference genome in a Planned Run.

## Import a custom reference sequence file

If you want to use a custom reference genome sequence in a Planned Run and data analysis, you can import a custom reference file into the Torrent Suite™ Software from your local storage or a remote server location.

**IMPORTANT!** First-time users should review custom reference file rules and restrictions to avoid uploading errors. For more information, see "Guidelines for importing custom reference sequence files" on page 259.

1. Click ⚙ **(Settings)** ▸ **References**.
2. Click **Import Custom Reference**.




3. In the **Add New Reference Genome** dialog box, do one of the following.
   - 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** (Required) | 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** (Required) | Enter a longer, more descriptive reference genome name. The description usually includes the genus, species, version, and other identifying reference genome information. |

*(continued)*

| Item | Description |
|---|---|
| **Version**<br>(Required) | Enter the genome version number and the accession number, if there is one (for example, "hg19", "gi\|39933080\|NC_005296.1"). |
| **Notes**<br>(Optional) | Use to record any 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 262. If you encounter any errors during file upload, see “Troubleshooting file import/upload errors” on page 408.

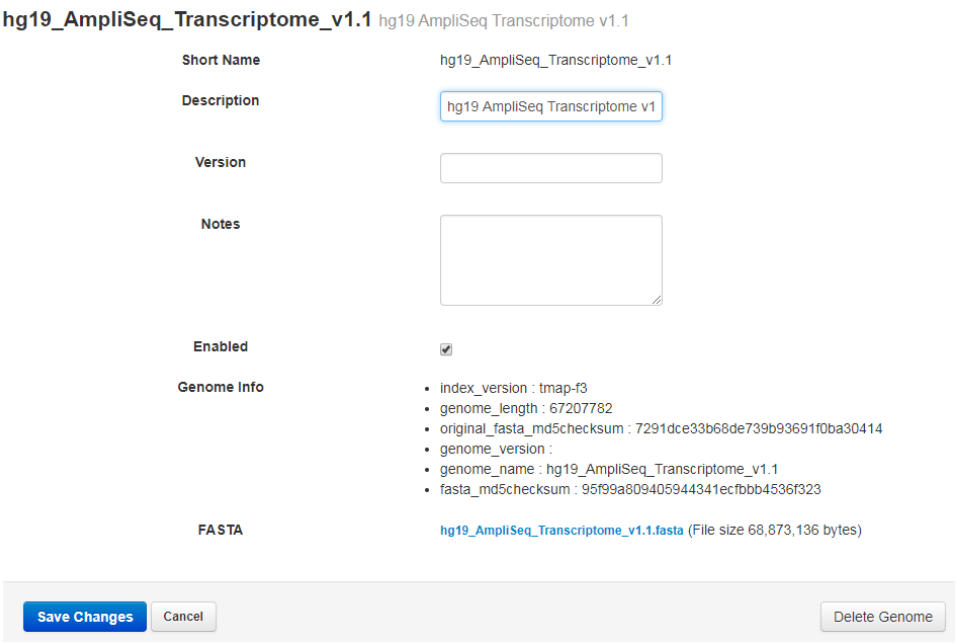
## View a reference sequence file

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 on how to import the file into Torrent Suite™ Software , see “Import reference sequence files” on page 259.

1. Click **(Settings)** ▸ **References**.
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 Ion References**, then click **complete** in the row of the imported Ion reference file.

   The file properties screen opens.



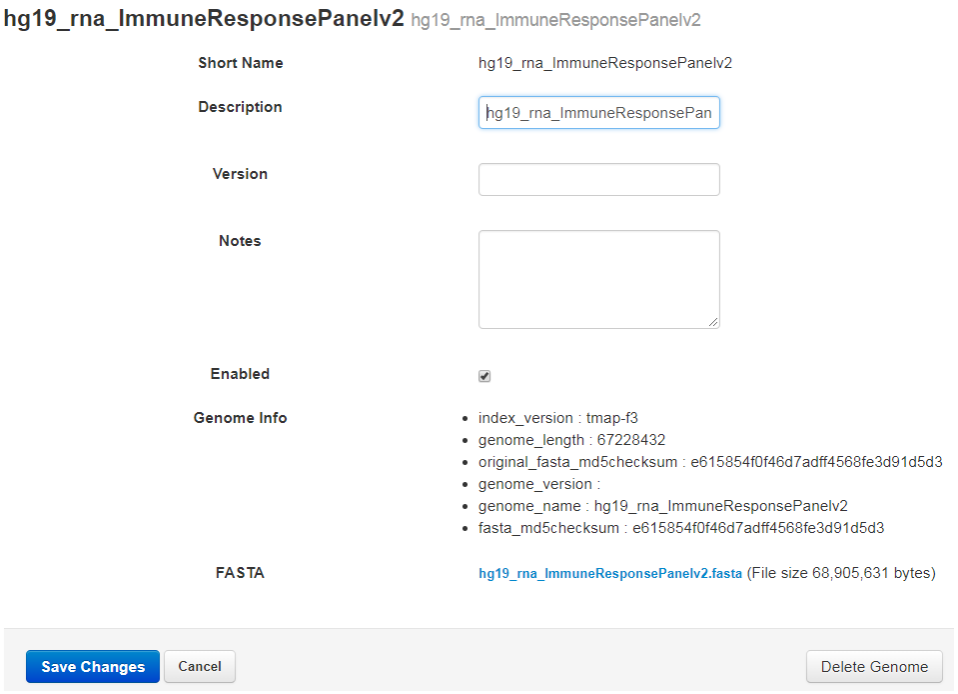

3. Click the FASTA file link to view the FASTA format reference sequence in the browser window.

## 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 259.

1. Click **(Settings)** ▸ **References**.
2. In the **Reference Sequences** table, in the **Short Name** column, click the reference sequence file name.
   The file properties screen opens.




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)** ▸ **References**.
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

A reference sequence becomes obsolete and is listed in the **Obsolete References Sequences** table after Torrent Suite™ Software is updated with a release that includes a new TMAP index.

Torrent Suite™ Software automatically records the libraries that were installed before the upgrade, then automatically creates the list of obsolete reference sequences.

Each list is different, depending 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 270 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.

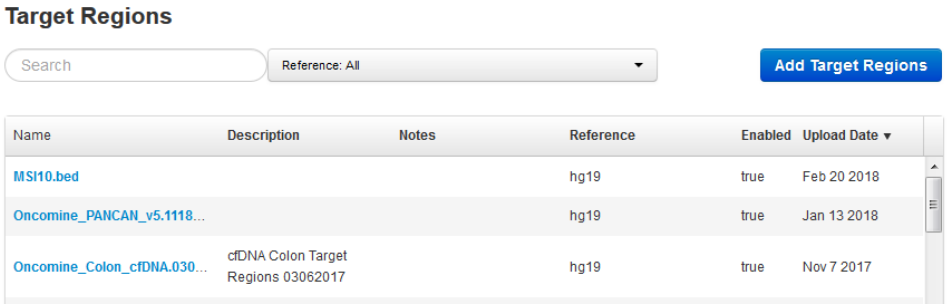
- The variantCaller plugin calls variant candidates at hotspot positions with higher sensitivity than candidates at other positions. You can customize specific variantCaller parameters separately for hotspot candidates.
- Torrent Suite™ Software accepts VCF files as hotspots files, but the VCF file must 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)** ▸ **References**.

2. In the left navigation pane, click **Target Regions**.
   Available target regions files 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 that displays information such as the number of targets, genes, covered bases, description, and notes. 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 | *(Optional)* Additional information about the target regions file. |
| Enabled | Select the checkbox to enable use of the file in a Planned Run.<br>Deselect the checkbox to prevent use of the file in a Planned Run. |

5. Enter any additional information if needed, then click **Save Changes**.

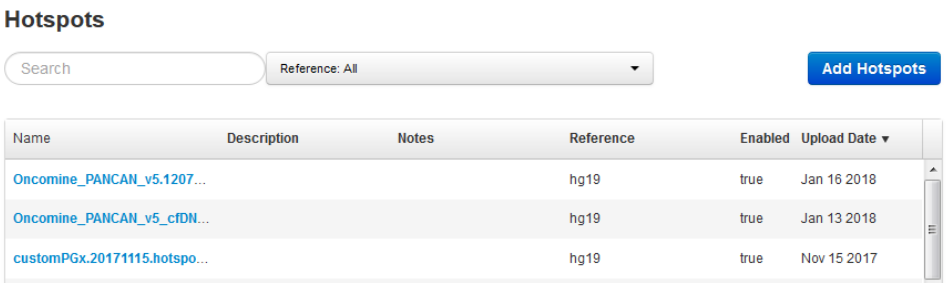
6. To delete a file from the Ion Torrent™ Server, click **Delete**.

## View and manage hotspots files

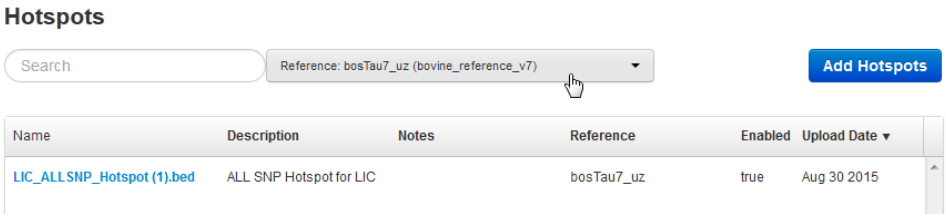
You can upload hotspots files for use with a specific reference sequence. After hotspot files are uploaded, the files are available when that reference sequence is selected for use in a Planned Run or variantCaller plugin configuration. When you select a hotspots file in run planning, the file instructs 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)** ▸ **References**.

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 that displays file information such as the number of loci, description, and notes. Scroll to view the 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 | *(Optional)* Additional information about the hotspot file. |
| Enabled | Select the checkbox to enable use of the file in a Planned Run.<br>Deselect the checkbox to prevent use of the file in a Planned Run. |

5. Enter any additional information if needed, then click **Save Changes**.

6. To delete a 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 ensures 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. The new file appears in the **Target Regions** list in the **Plan** step of the Planned Run workflow bar, and also appears in the **Target Regions** table in the **References** tab.

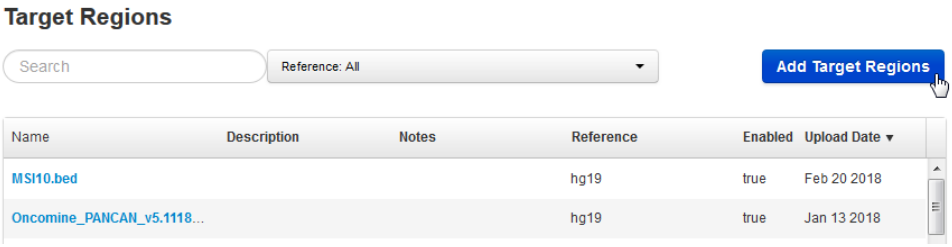
---

**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.

---

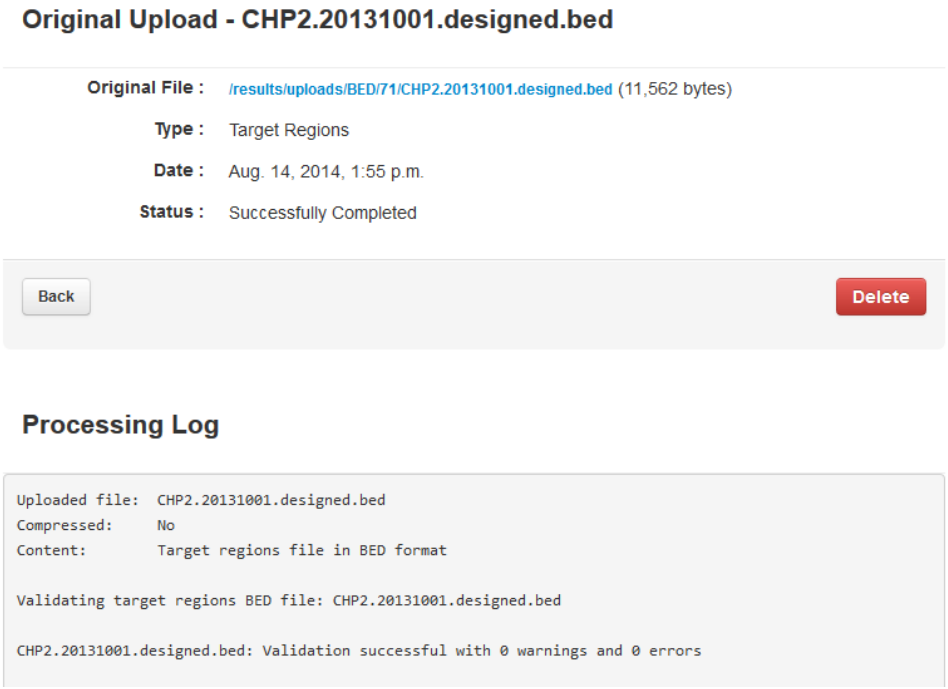
1. Click **(Settings)** ▸ **References**, then click **Target Regions** in the left navigation menu.
2. In the **Target Regions** screen, click **Add Target Regions**.




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.

## 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 ensures 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.

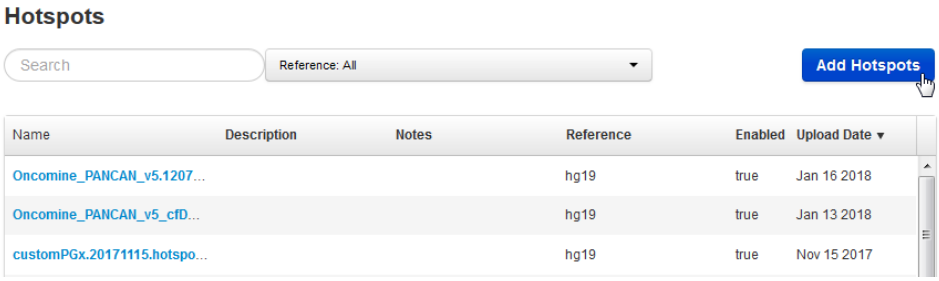
---

**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)** ▸ **References**, then click **Hotspots** in the left navigation menu.
2. In the **Hotspots** screen, click **Add Hotspots**.

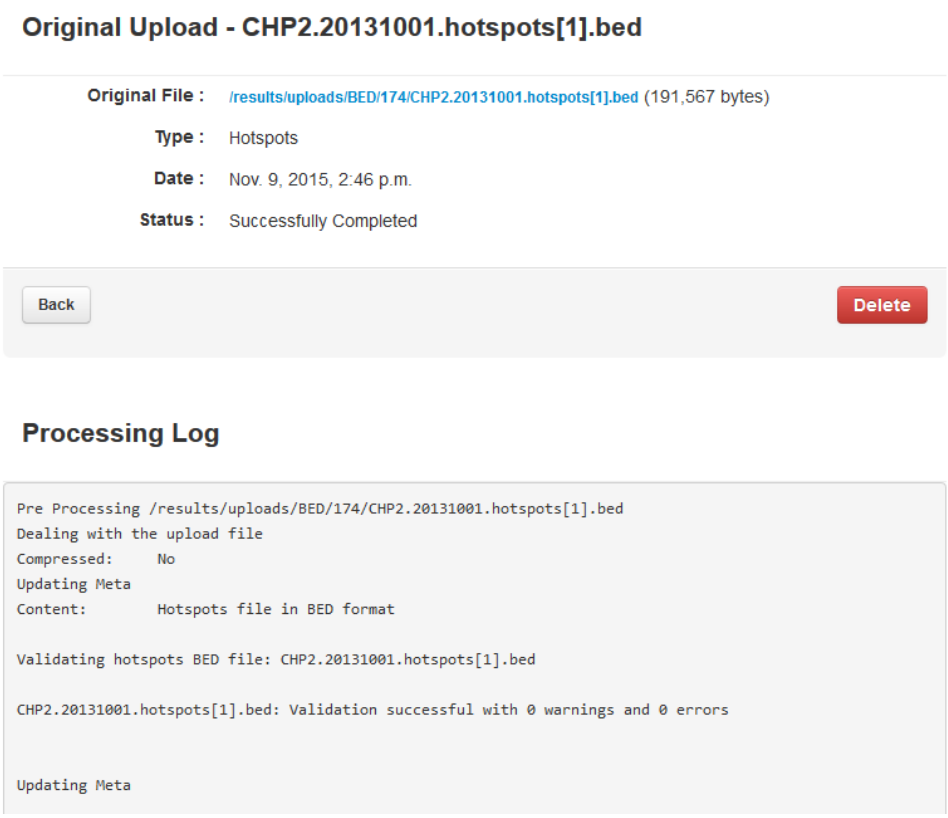



3. In the **New Hotspots** screen, click **Select File**, then navigate to the file to be uploaded.
4. Select the reference sequence from the **Reference** list.

   **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 also appears in the **Hotspots** table in the **References** tab.

## BED file formats and examples

The Browser Extensible Display (BED) format is used for both target regions files and hotspot files. Torrent Suite™ Software also accepts the Variant Call Format (VCF) for hotspot files.

BED files are text files with tab-separated fields.

### 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 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 ≥ 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`. |

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 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 >= 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`. |
| `AmpliconID` | String | The amplicon ID. If missing, the following string is used `"chrom"` + `":"` + `"chromStart"` + `"-"` + `"chromEnd"` |

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 of the 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 these tab-separated fields:

| Field | Type | Description |
|---|---|---|
| `name`<br>(Optional) | String | A unique design identifier. |
| `description`<br>(Optional) | String | A description of the design. |
| `type`<br>(Required) | String | Must be `bedDetail` (without quotes). |
| `ionVersion` | String | Introduced in the Torrent Suite™ Software 4.0 release. |

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 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. |
| `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 ≥ 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`. |
| `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). Required. |
| `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. |
| `color` | String | Code for color track in UCSC Genome Browser (when uploaded from **AmpliSeq.com**. |
| `priority` | String | Sets the order for color track in UCSC Genome Browser (when 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`<br>(Optional) | String | A unique design identifier. |
| `description`<br>(Optional) | String | A description of the design. |
| `type`<br>(Required) | String | Must be `bedDetail` (without quotes). |
| `db`<br>(Optional) | String | The UCSC Assembly identifier. |
| `reference`<br>(This field is optional for hg19, and is required for GRCh38.) | String | The Ion Torrent™ Server reference identifier. |

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. In fact, it is recommended that the ANCHOR key is NOT 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:<br>• `GENE_ID=`<br>• `Pool=`<br>• `SUBMITTED_REGION=`<br>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.<br>Example: `GENE_ID=brca1`<br>Example: `GENE_ID=brca1, ret` |
| `Pool` | The Ion AmpliSeq™ Designer pool or pools containing this amplicon.<br>Example: `Pool=2`<br>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`.<br>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.<br>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. |

*(continued)*

| Key | Description |
|---|---|
| CNV_HS | A CNV region hotspot. This can be a value of either 0 or 1.<br>• 1 reports as a hotspot (HS) in the output VCF file from the CNV PCA algorithm.<br>• 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`<br>(Optional) | String | A unique design identifier. |
| `description`<br>(Optional) | String | The description of the design. |
| `type`<br>(Required) | 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).<br>When set to "4.0", indicates that the BED file supports the Extended BED Detail format..<br>Optional. This 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 ≥ 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 (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 '+'. |
| `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:<br>• `Fusion`<br>• `CONTROL` or `ExpressionControl`<br>• `Driver_Gene` or `5p3pAssay`<br>• `GeneExpression`<br>• `RNA_Hotspot` | `TYPE=Fusion` |
| `FP_TRANSCRIPT_ID` | The transcript ID for the 5′ gene partner.<br>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.<br>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.<br>This key value pair is only for fusion type targets. | `FP_GENE_ID=BAG4` |
| `FP_GENE_STRAND` | The strand for the 5′ gene partner. Allowed values are '+' and '-'.<br>This key value pair is only for fusion type targets. | `FP_GENE_STRAND=+` |

*(continued)*

| Key | Value | Example |
|---|---|---|
| FP_EXON_NUMBER | The exon number in the 5′ gene. Use comma-separated values if the amplicon spans multiple exons.<br>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.<br>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.<br>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.<br>This key value pair is only for fusion type targets. | TP_EXON_NUMBER=6 |
| FP_CHROM | The chromosome of the 5′ gene.<br>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.<br>This key value pair is only for fusion type targets. | FP_START=38050257 |

*(continued)*

| Key | Value | Example |
|---|---|---|
| FP_END | The end position for the 5′ segments. Use comma-separated values if there are multiple segment Ends.<br>This key value pair is only for fusion type targets. | FP_END=38050313 |
| TP_CHROM | The chromosome of the 3′ gene. This field is absent for CONTROL type amplicons.<br>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.<br>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 |

*(continued)*

| Key | Value | Example |
|---|---|---|
| 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=+ |
| EXON_NUM | The exon number(s) 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 |

*(continued)*

| Key | Value | Example |
|---|---|---|
| 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).<br>Example usage—For EGFR deletion assay, we would use a higher read count threshold (greater than 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.

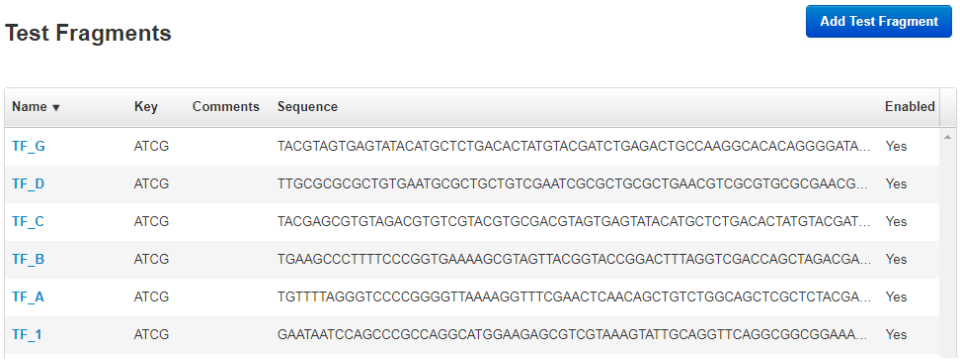
Test fragments are displayed when there are at least 500 high-quality reads, with an 85% match against the appropriate template in the **Test Fragment** list.

## View test fragment details

You can view existing test fragment nucleotide sequences used for quality metrics.

Torrent Suite™ Software provides six test fragments by default: TF_A, TF_B, TF_C, TF_D, TF_G, and TF_1.

1. Click **(Settings)** ▸ **References**, then click **Test Fragments** in the left navigation menu.
   The **Test Fragments** table appears.




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 custom test fragment

You can add custom test fragments to Torrent Suite™ Software.

1. Click **(Settings)** ▸ **References**, then click **Test Fragments** in the left navigation menu.

2. In the **Test Fragments** screen, click **Add Test Fragment**.

3. In the **Add New Test Fragment** dialog box, complete the following information.

| Option | Description |
|---|---|
| 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 | (*Optional*) Enter any comments about the test fragment. |

   If you enter an invalid character or duplicate test fragment, you can not save your changes.

4. Click **Save**.

Your new test fragment and its details are displayed in the test fragment table.

## Edit or delete a custom test fragment

You can edit or delete custom test fragments in Torrent Suite™ Software.

**WARNING!** Do not modify the test fragment sequences for the test fragments that are supplied by Torrent Suite™ Software: TF_A, TF_B, TF_C, TF_D, TF_G, and TF_1. For example, TF_1 is used to assess the quality of bead loading. TF_C is used to assess the quality of a templating and library matching.

On your own test fragment (not test fragments supplied by Torrent Suite™ Software), you can make the following edits:

- Change the test fragment name, key, or comments.
- Change the test fragment nucleotide sequence.
- Change whether or not the test fragment is enabled.

1. Click **(Settings)** ▸ **References**, 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 54 and "Run a plugin manually from the sequencing run report" on page 132.

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 into one of the barcodes 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 106.

A sequencing run on the Ion PGM™, Ion Proton™, Ion S5™, and Ion GeneStudio™ S5 Systems 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 custom 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 custom 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, and import the custom 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 custom barcode sets is for advanced users only. For help with creating custom 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)** ▸ **References**, 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 within the barcode set and their associated information.
3. Click any column header to sort the display of that 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 297.

## Download a DNA barcode set CSV file

You can download a DNA barcode set CSV file and save it on 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 custom DNA barcode set onto your Ion Torrent™ Server.

1. Click ⚙ **(Settings)** ▸ **References**, 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 containing 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 custom DNA barcode set into Torrent Suite™ Software. For more information, see "Add a custom DNA barcode set" on page 298.

## Add a custom DNA barcode set

You can create custom DNA barcode sets and add them to Torrent Suite™ Software for use in Planned Runs. To add a custom barcode set, you must generate a Comma-Separated Value (CSV), save the file to your local storage, then import the file into Torrent Suite™ Software.

All custom DNA barcode set files must have a .csv extension.

1. Click ⚙ **(Settings)** ▸ **References**, then click **Barcode Sets** in the left navigation menu.
   The **Barcode Sets** table lists all the available custom and system DNA barcode sets.

2. *(Optional)* Create a custom 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 automatically downloaded to your computer.

   b. Edit the CSV file as described in “DNA barcode set CSV file setup” on page 299, 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 custom DNA barcode set.

5. *(Optional)* Create a custom DNA barcode set CSV file using the example CSV file.
   a. Click **Download the example file**.
      A CSV file is automatically 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 299.

   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 custom 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.

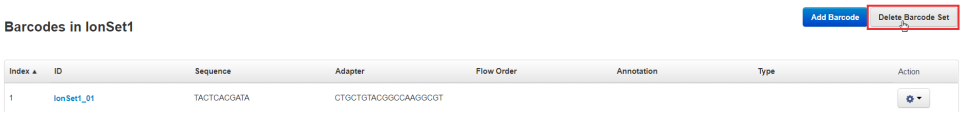
| Column name | Type | Description |
|---|---|---|
| `index` | Integer | A unique index for an individual barcode (for example, 1, 2, 3,...)<br>Only numerical characters are accepted. |
| `id_str` | String | A unique name for an individual barcode entry. Typically the name of the barcode kit followed by a 3′– to 4′– digit unique identifier, such as IonXpress_001 or IonCode_0101. |
| `sequence`<br>(Required) | String | The 5′– barcode sequence.<br>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".<br>Upper-case G, C, A, and T are allowed. |
| `annotation` | -- | Use for any barcode-specific notes. |
| `end_sequence` | String | The 3′– barcode sequence.<br>**IMPORTANT!** This sequence is required for libraries that were prepared using dual barcode technology.<br>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".<br>This sequence is available only for libraries that were prepared using dual barcode technology.<br>Upper-case G, C, A, and T are allowed. |

## Delete a custom DNA barcode set

After you import a custom 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 your Ion Torrent™ Server.

**IMPORTANT!** Do not delete the any system barcode set.

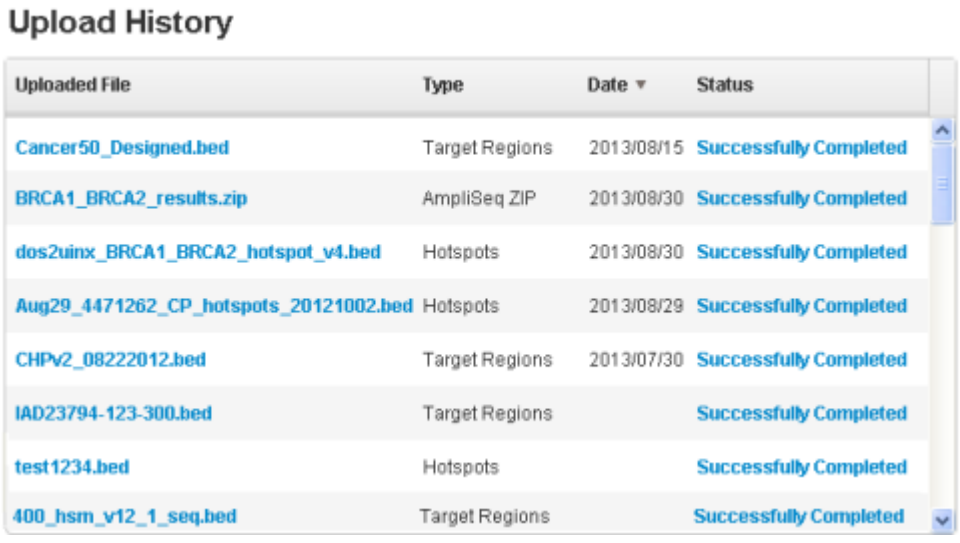
1. Click ⚙ **(Settings)** ▸ **References**, then click **Barcode Sets** in the left navigation menu.
2. In the **Barcodes** table, in the row of the barcode set to be deleted, click ⚙ **(Actions)** ▸ **Delete**.
3. In the **Confirm Delete Barcode Set** dialog box, click **Delete Barcode Set**.




4. Click **Yes, Delete!** to confirm.
   The DNA barcode set is permanently deleted from your Ion Torrent™ Server.

# Upload history

**Upload History** is 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.<br>Any errors that occur during upload are shown in this column. |

# 11 Data management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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

In the **Data** tab, click **Data Management**, then scroll to the **Disk Usage** section.

**Parameters in the Disk Usage section.**

| Parameter | Definition |
|---|---|
| Keep | File space devoted to files that are to be kept. |
| Used | File space being 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 B | Threshold above which basecaller 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. |

For details regarding automatic deletion and archive creation, see “Archive or delete data automatically” on page 353.

# View category statistics

In the **Data** tab, click **Data Management**, then scroll to the **Category Statistics** section.

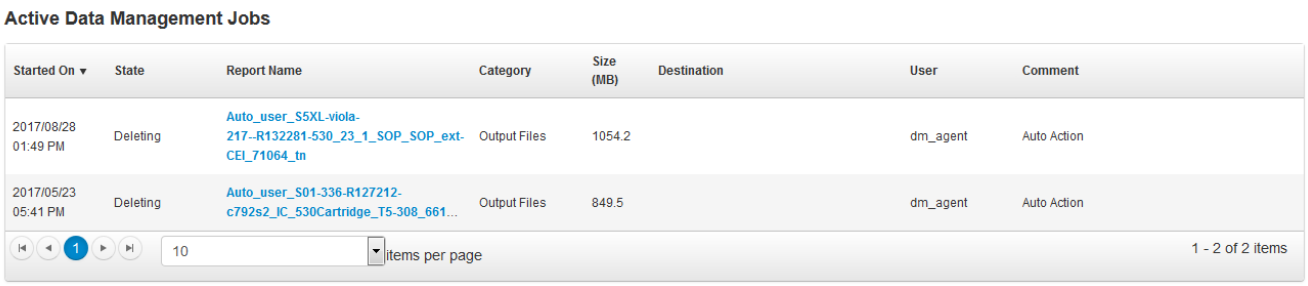
**Category statistics in the Category Statistics section.**

| Parameter | Definition |
|---|---|
| File Category Group | File type. For details, see “Ion instrument data types” on page 352. |
| 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.<br>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 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.



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 Mame | The identifier of job. |
| Category | Identifies the file as one of the following file types.<br>• Signal processing input—required input files for signal processing.<br>• Basecalling input—required input files for base calling.<br>• Output files—files for data processing.<br>• 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 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 regarding 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 a 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 can not 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 a 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 359.

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 is 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 305.

4. Click ⚙ **(Actions)** ▸ **View Log**.
   A new screen containing chronological list of actions that are taken for this run report opens. 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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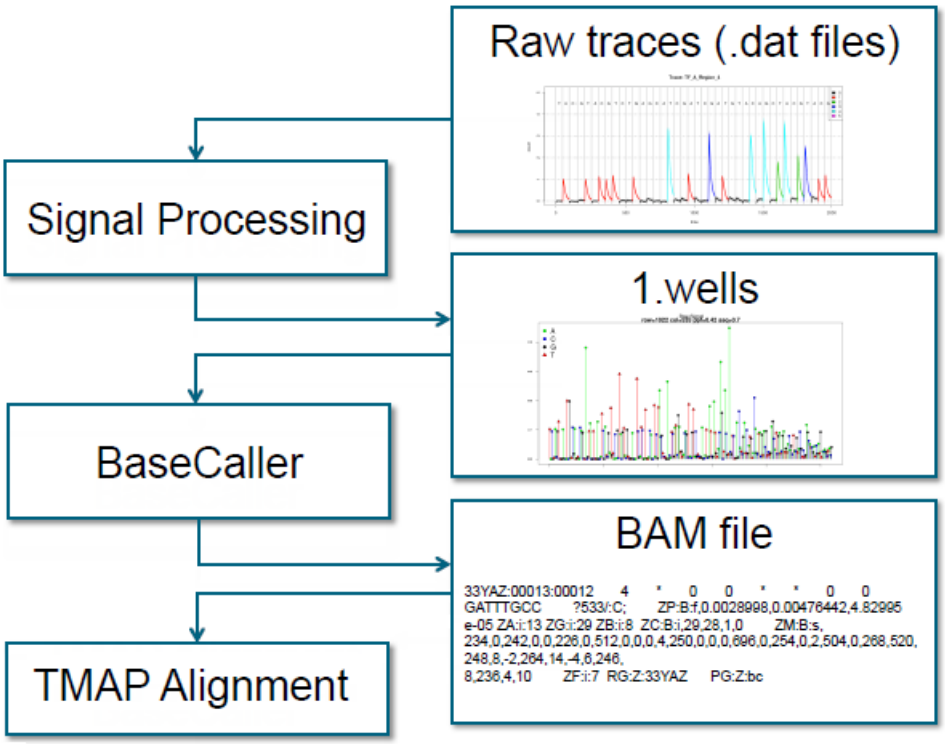
- BaseCaller module and barcode classification overview . . . . . 309
- TMAP modules . . . . . 325


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 custom tags to store sequencing and alignment information useful for downstream processing. In general, custom 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. |

*(continued)*

| BAM tag | Type | Description |
|---|---|---|
| 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.) |
| ZC | B:i | A vector of the following four values (present only if a 3' adapter was found):<br>• Field 1: The zero-based flow during which the first base of the adapter was incorporated (same as ZG).<br>• Field 2: The zero-based flow corresponding to the last insert base.<br>• Field 3: Length of the last insert homopolymer.<br>• 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. |

*(continued)*

| BAM tag | Type | Description |
|---|---|---|
| ZK | Z | The trimmed 3' 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 custom 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 custom 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 relating 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<br>(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.<br>Reads that have a distance greater than this value are counted as barcode no-matches. |
| `--barcode-mode` | 2<br>(integer) | The barcode mode.<br>• 0—Classification based on exact barcode base match.<br>• 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. (The default in 4.0, known as hard decision classification.)<br>• 2—The barcode classification is based on signal information, specifically on the squared distance between the measured signal and the predicted barcode signal. (The default in 4.4, known as soft decision classification.) |
| `--barcode-separation` | 2.5<br>(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.<br>`--barcode-separation` has no effect when `--barcode-mode` is set to 1. |

*(continued)*

| Parameter | Default | Description |
|---|---|---|
| `--barcode-filter-postpone` | 1<br>(integer) | • 0—Keeps the 4.0 behavior: barcode filtering is done independently on each block. This is the default for all Ion PGM™ analyses and for Ion Proton™ thumbnail (which consist of only a single block) processing and base calibration training stage processing.<br>• 1—The BaseCaller module does barcode pre-filtering 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. This is the default for Ion Proton™ full-chip (not thumbnail) analyses.<br>• 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.<br>Do not change this parameter. Instead accept the pipeline defaults, which are different for Ion PGM™ and Ion Proton™ analyses. |
| `--barcode-filter` | 0.01<br>(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.<br>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<br>(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<br>(boolean) | For dual barcoding runs, specifying "off" disables end barcode classification and uses the start barcodes for read classification instead of both barcodes.<br>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.<br>Using this option turned "on":<br>• End barcodes are still searched for and trimmed off the read if found.<br>• No sk tag is written in the BAM read group header.<br>• End barcodes, end barcode adapters, or PCR handles are trimmed if they are found and all stored in the YK tag.<br>• 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.<br>• 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`.<br>• 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.<br>• End barcode classification will not be 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. |

*(continued)*

| Parameter | Default | Description |
|---|---|---|
| `--trim-barcodes` | on<br>(boolean) | Trim the barcode and barcode adapter. If off, disables all other 5' trimming. |
| `--barcode-adapter-check` | 0.15 | 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.<br>0—Off. |

## Cutoff setting

Notes about 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 custom barcode sets).
- Do not set `--barcode-cutoff` greater than 2 with the Ion Xpress™ Barcode Adapters barcode set. Values greater than 2 relax the classification rules and allow incorrect barcode assignments.

A rule of thumb 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 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 close to 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 rule of thumb 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<br>`--disable-all-filters` | off | When on, disables all filtering and trimming and overrides other filtering and trimming settings. |
| `-k`, or<br>`--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<br>(integer) | Filters out reads less than this minimum read length.<br>This filter screens out poor reads early on to avoid wasting processing time on them.<br>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.<br>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.<br>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<br>(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. |

*(continued)*

| Parameter | Default | Description |
|---|---|---|
| `--trim-adapter-cutoff` | 16<br>(float) | A score cutoff value.<br>Smaller values correspond to more stringent adapter search and larger values to less stringent adapter search.<br>Set to 0 to turn off. |
| `--trim-adapter-min-match` | 6<br>(integer) | The minimum number of P1 adapter bases required in order to trim the P1 adapter. |
| `--trim-qual-window-size` | 30<br>(integer) | Window size for quality trimming. |
| `--trim-qual-cutoff` | 16<br>(float) | Cutoff for quality trimming.<br>Set to 100 to turn off. When set to 100, no reads are filtered out due to this parameter. |
| `--trim-min-read-len` | 25<br>(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`. |

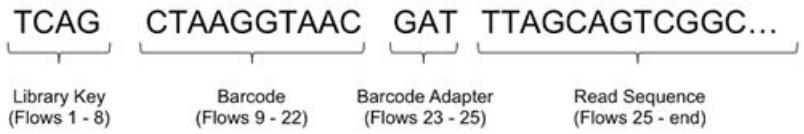
## About barcodes

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

The `barcode_bias` values show the mean signal deviation by flow: how much 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

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 are in-between two barcodes, and are not close enough to either barcode to be assigned.

```
"barcode_match_filtered": 162,
```

### Filtered

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

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 328 | BWA-short reads mapping.<br>• Very fast at finding perfect matches.<br>• Very slow at finding a set of matches with up to two mismatches. |
| "TMAP map2 options" on page 329 | BWA-long / BWASW reads mapping. |
| "TMAP map3 options" on page 330 | Simplified SSAHA, based on a k-mer lookup table. |
| "TMAP map4 options" on page 331 | Based on the BWA fastmap routine.<br>Searches for the maximum exact matches between the reads and reference. |
| "TMAP mapvsw options" on page 333 | A vectorized implementation of Smith-Waterman.<br>• A single mapping strategy that is twice as fast as the other modules.<br>• Modified to improve specificity. |
| mapall | A command to quickly map short sequences to a reference genome. |

## 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 326. 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.<br>• 0 = Do not perform pairing.<br>• 1 = Mate pairs (-S 0 -P 1).<br>• 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.<br>-1 to disable. | |
| `--seed-max-diff` | Integer | 2 | The maximum number of edits in the seed. | |
| `--seed2-length` | Integer | 48 | The secondary seed length.<br>-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 | 2000000 | The maximum number of alignment nodes. | |

*(continued)*

| Option | Type | Default | Description | |
|---|---|---|---|---|
| `--min-seq-length` | Integer | -1 | The minimum sequence length to examine.<br>-1 to disable | |
| `--max-seq-length` | Integer | -1 | The maximum sequence length to examine.<br>-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. |

*(continued)*

| Option | Type | Default | Description |
|---|---|---|---|
| `--min-seq-length` | Integer | -1 | The minimum sequence length to examine.<br>-1 to disable. |
| `--max-seq-length` | Integer | -1 | The maximum sequence length to examine.<br>-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.<br>-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.<br>-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.<br>-1 to disable |
| `--max-seq-length` | Integer | -1 | The maximum sequence length to examine.<br>-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.<br>-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.<br>-1 to disable |
| `--max-seed-length` | Integer | 48 | The maximum seed length to accept hits. |

*(continued)*

| Option | Type | Default | Description |
|---|---|---|---|
| `--max-seed-length-adj-coef` | Float | 2.0 | maximum seed length adjustment coefficient.<br>-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.<br>-1 to disable. |
| --max-seq-length | Integer | -1 | The maximum sequence length to examine.<br>-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.<br>-1 to disable |
| `--max-seq-length` | Integer | -1 | The maximum sequence length to examine.<br>-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. |

*(continued)*

| Stage | Description |
|---|---|
| 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. |
| 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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Administrative privileges allow you to configure Torrent Suite™ Software, administer Ion Torrent™ Server databases, manage user accounts, back up and restore data, and enable remote monitoring.

An ionadmin account is required to perform the procedures in this section. A user account, such as ionuser, does not include sufficient privileges to perform these procedures.

## Open the Site Administration screen

An administrator can use tools in the **Site Administration** screen in the **Ion Web** portal to modify default settings for sequencing runs.

---

**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. 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 functions.

4. If you are prompted to sign in, enter your administrator user name and password, then click **Sign in**.

   The **Site administration** screen opens.

5. After you have made changes, click **Back to Main Site** at the top of the screen to return to the Torrent Suite™ Software.

# Install Torrent Suite™ Software

An administrator can use these steps to install Torrent Suite™ Software.

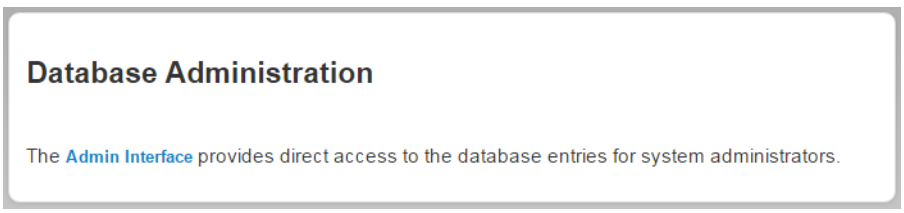
**IMPORTANT!** During an upgrade, you must use the same user account for both Ion Torrent™ Server and Ion systems (Ion PGM™ Instrument, Ion Chef™ System, Ion S5™ System, Ion S5™ XL System, and Ion GeneStudio™ S5 Systems.

1. Click ⚙ **(Settings)**. Ensure that there are no active jobs running.
2. Click ⚙ **(Settings)** ▸ **Configure** ▸ **Admin Interface**.
3. Click **Update Server**.
4. Click **Check for updates**.
5. When the **Available** message displays, click **Update Server** to start the update process.
6. On the **User Agreement (EULA)** screen, scroll down to read and then accept terms to begin the update.
7. When finished, ensure that the "Upgrade completed Successfully!" message displays.

## Manage Torrent Suite™ Software user accounts

An administrator can manage Torrent Suite™ Software user accounts from the **Site Administration** screen.

1. Click ⚙ **(Settings)** ▸ **Configure**.
2. Scroll to the **Database Administration** section, then click the **Admin Interface** link.



If you are prompted to sign in, use your administrator account.

3. In the **Site administration** screen, in the **Users** row, do one of the following:

| Option | Description |
|---|---|
| Add | Click **Add** to add a new user. |
| Change | Check **Change** to modify a user account. |

## Approve or reject 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 an administrator signs in to Torrent Suite™ Software, a message alerts the administrator that requests are pending for new user accounts.

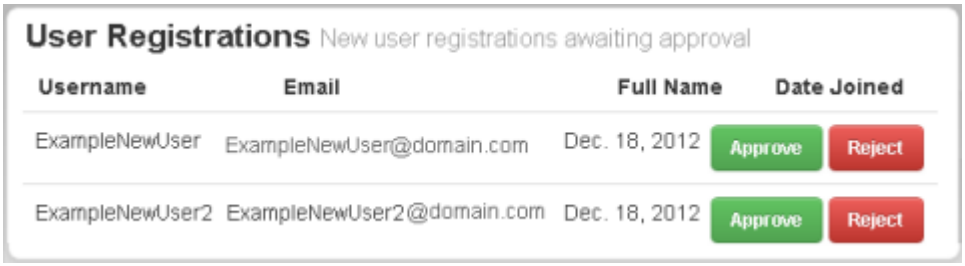
---

**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**.

   The **User Registration** section shows the pending requests for new user accounts:




   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.

2. Review the new user registration request, then choose one of the following.
   - Click **Approve** to approve the account for the new user, then click **Yes, Approve**.
   - Click **Reject** to reject the account for the new user, then click **Yes, Reject**.
3. After you have made changes, click **Back to Main Site** at the top of the screen to return to the Torrent Suite™ Software.

The user account is added to the list of user accounts in the Torrent Suite™ Software **Site administration** screen. For details, see "Manage Torrent Suite™ Software user accounts" on page 336.

## Modify a user account

An administrator can modify the access level for an existing user using tools in the **Site administration** screen.

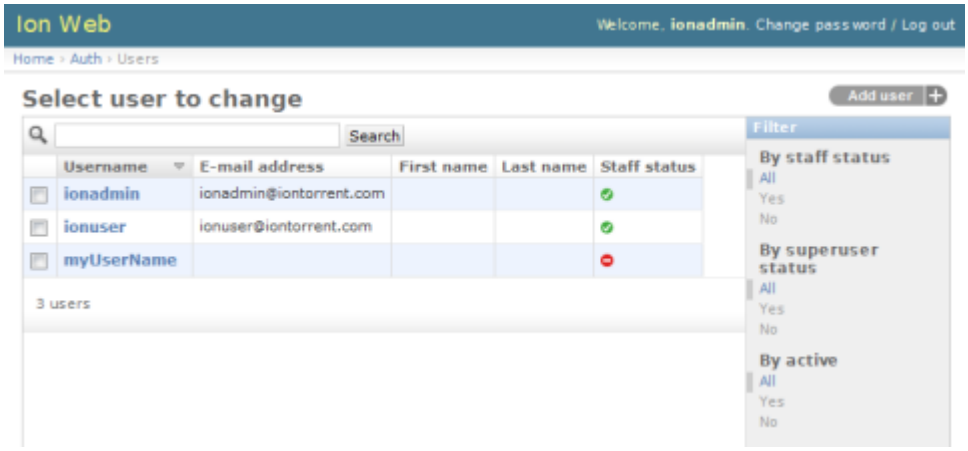
---

**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. Click ⚙ **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll down to the **Database Administration** section. Click the **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**.
   The screen in the **Ion Web** portal opens.
4. In the **Site administration** screen, in the **Users** row of the main screen, click **Change**.

5. On the **Select user to change** screen, click the **Username** 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.




6. Use the **Change user** dialog box to modify user information, such as user name, password, first and last name, email address, or active/inactive status. Confirm that permissions are selected as follows for Staff and Superusers.

| Option | Description |
|---|---|
| **Staff** (User) | **Active** |
| **Superuser** (Administrator) | **Active**, **Staff** and **Superuser** |

**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.

7. Select one of the **Save** options at the bottom of the screen to save your changes.

   The user account is modified.

8. After you have made changes, click **Back to Main Site** at the top of the screen to return to the Torrent Suite™ Software.

## Delete a single user account

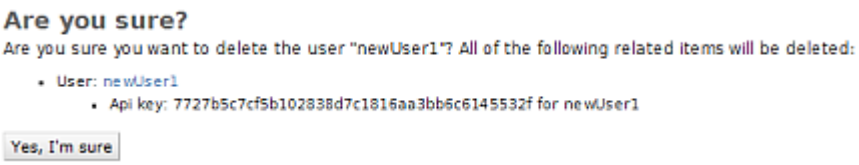
An administrator can delete a user account, using tools in the **Site administration** screen.

---

**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. Click ⚙ **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll to the **Database Administration** section. Click the **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**.
   The **Site administration** screen in the **Ion Web** portal opens.
4. In the **Users** line of the main **Site administration** menu, click **Change**.
5. On the **Select user to change** screen, click the **Username** of the user to be deleted.
6. At the bottom-left of the **Change user** screen, click **Delete**.
7. Ensure that you want to delete the user by clicking **Yes, I'm sure**.




   The user is deleted.
8. After you have made changes, click **Back to Main Site** at the top of the screen to return to the Torrent Suite™ Software.

## Delete multiple user accounts

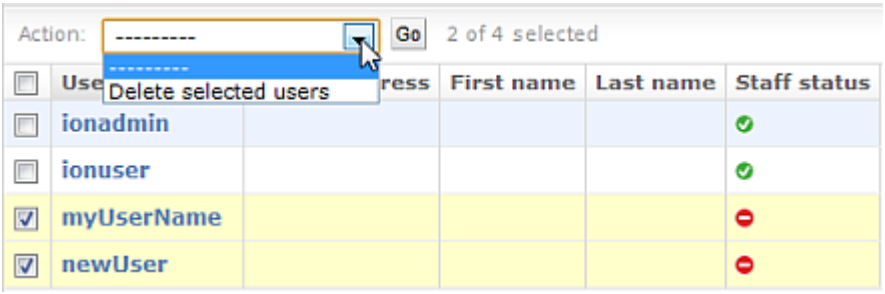
An administrator can delete multiple users using tools in the **Site administration** screen.

---

**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. Click ⚙ **(Settings)** ▸ **Configure**.
2. In the **Configure** screen, scroll to the **Database Administration** section. Click the **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**.
   The **Site administration** screen in the **Ion Web** portal opens.
4. In the **Users** line of the main **Site administration** menu, click **Change**.
5. On the **Select user to change** screen, check the checkbox for each user that you want to delete.
6. In **Action**, select **Delete selected users**, then click **Go**.




7. Ensure that the list of users that you want to delete is correct by clicking **Yes, I'm sure**.
   If you do not want to delete the users, click the back arrow on your browser.

8. Review the **Select user to change** screen to confirm that the selected users no longer appear on the list.

   The accounts are deleted.

9. After you have made changes, click **Back to Main Site** at the top of the screen to return to the Torrent Suite™ Software.

## Create a new administrator account to change a password

An administrator can change their password by creating an new administrator (superuser) account, then change the user password for that account.

---

**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.

---

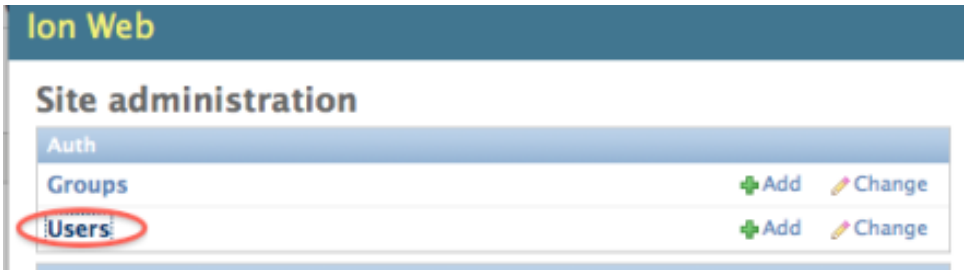
1. At the command line on the Ion Torrent™ Server, run the following command in the command prompt: `cd /opt/ion/iondb ./manage.py createsuperuser`.

2. After the new superuser account has been created, in Torrent Suite™ Software click ⚙ **(Settings)** ▸ **Configure**.

3. In the **Configure** screen, scroll to the **Database Administration** section, then click the **Admin Interface** link to access the database administration functions.

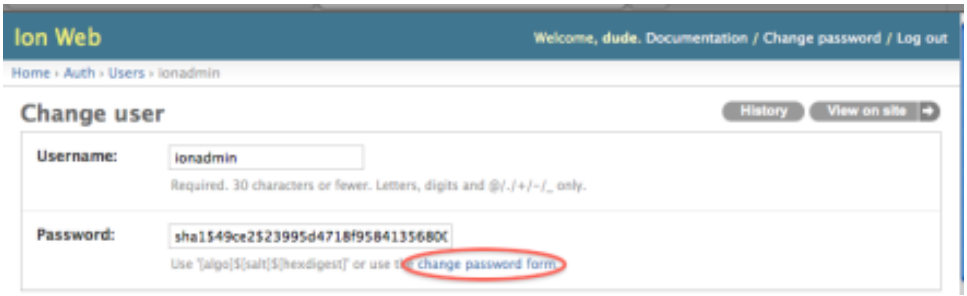
4. If you are prompted to sign in, enter your newly created user name and password, then click **Sign in**.
   The **Site administration** screen in the **Ion Web** portal opens.

5. Select the **Users** section under **Auth**:

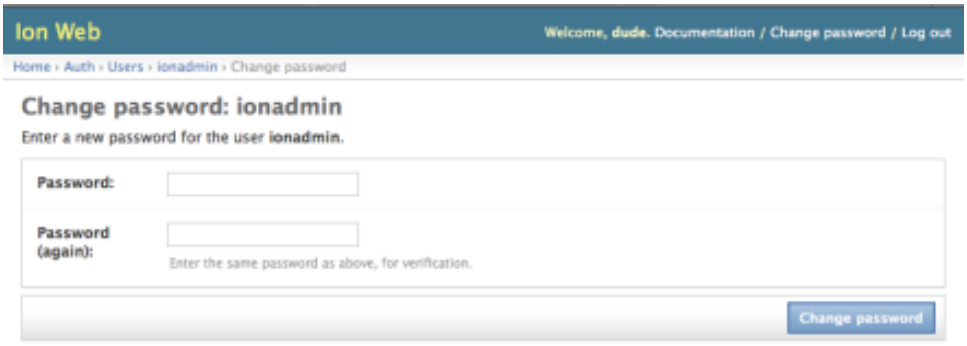



6. Select the account that you just created and that you want to change the password for.

7. Click **Change password form**:




8. Enter the new password, then click **Change Password**:



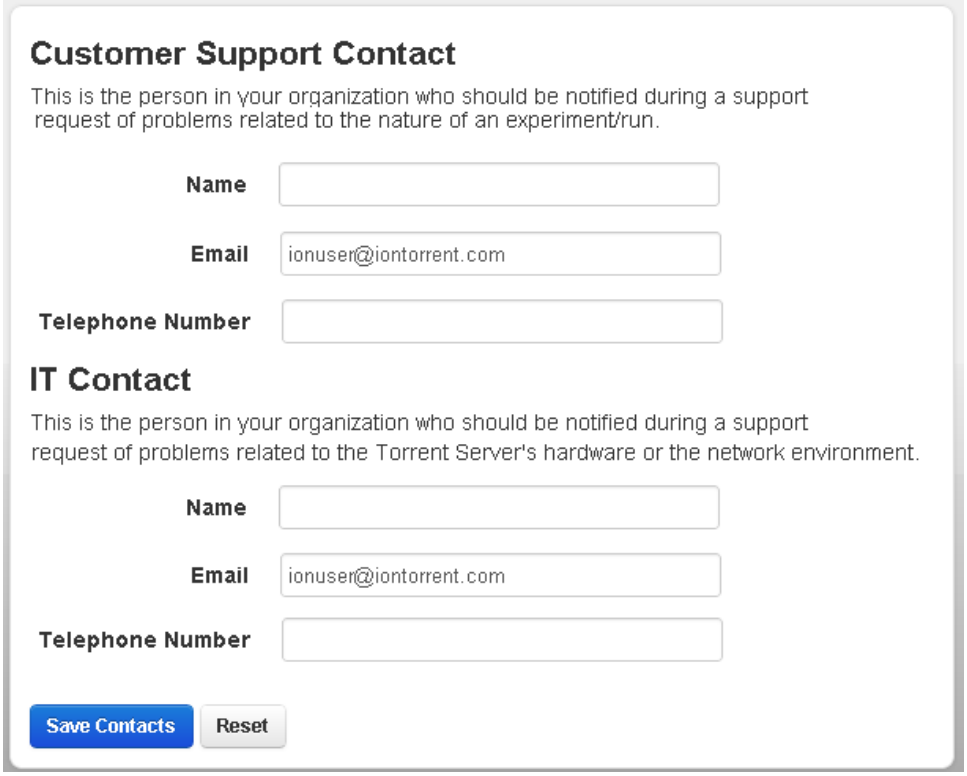

9. After you have made changes, click **Back to Main Site** at the top of the screen to return to the Torrent Suite™ Software.

You can now use your new password with your user account.

## Add customer 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.

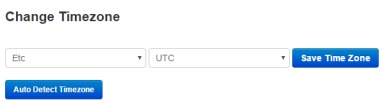
1. Click ⚙ **(Settings)** ▸ **Configure**.




2. Add the information for a customer support contact and an IT contact in your organization, then click **Save Contacts**.

## Change the time zone for the Ion Torrent™ Server

1. Click ⚙ **(Settings)** ▸ **Configure**.
2. Scroll to **Change Timezone**, select a region and a time zone, then click **Save Time Zone**.




3. () 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

You 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. Click ⚙ **(Settings)** ▸ **Configure**, then scroll to the **Customize Site Name** section.

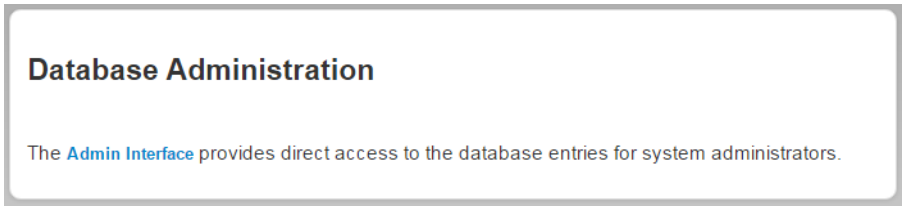
2. Enter the name of your choice, then click **Save Name**.
   The server name that is displayed in the browser is changed.

## Lock 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.

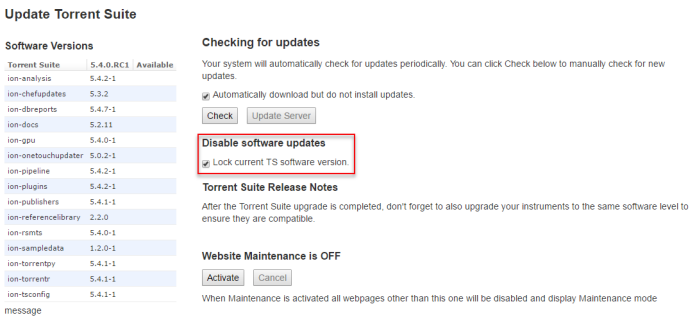
You must be an administrator to perform this procedure.

1. Click ⚙ **(Settings)** ▸ **Configure**.
2. Scroll to the **Database Administration** section, then click **Admin Interface**.




3. 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.




4. 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, depending on the type of Torrent Suite™ Software upgrade. For complete instructions, see the latest Release Notes on the software product.

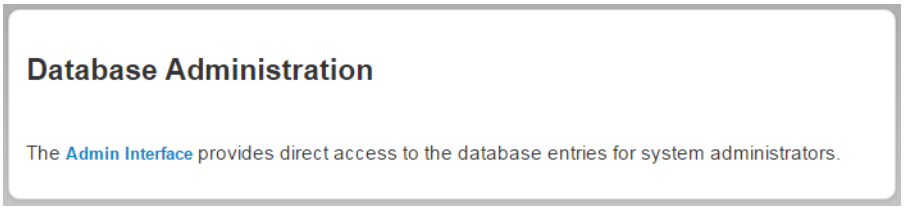
**IMPORTANT!** To ensure compatibility between the software and your instruments, you must also upgrade your instruments after the upgrade is complete.

## Update Torrent Suite™ Software

An administrator can use this procedure to update the Torrent Suite™ Software on the Ion Torrent™ Server. Updates cause the software web services to restart.

**IMPORTANT!** To ensure compatibility between the software and your instruments, you must also upgrade sequencing instruments after the Torrent Suite™ Software upgrade is complete.

1. Click ⚙ **(Settings)** ▸ **Configure**.
2. Scroll to the **Database Administration** section, then click **Admin Interface**.



If you are prompted to sign in, use your `ionadmin` account.

3. On the **Site administration** screen in the **Management Actions** section, click **Update Server**.

The **Update Torrent Suite** screen opens with information on available software versions, including whether updates are available.

4. Click **Activate** to power on website maintenance.

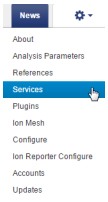
5. Click **Check** to check for updates.

6. If software updates are available, click **Update Server** to update software on the server.

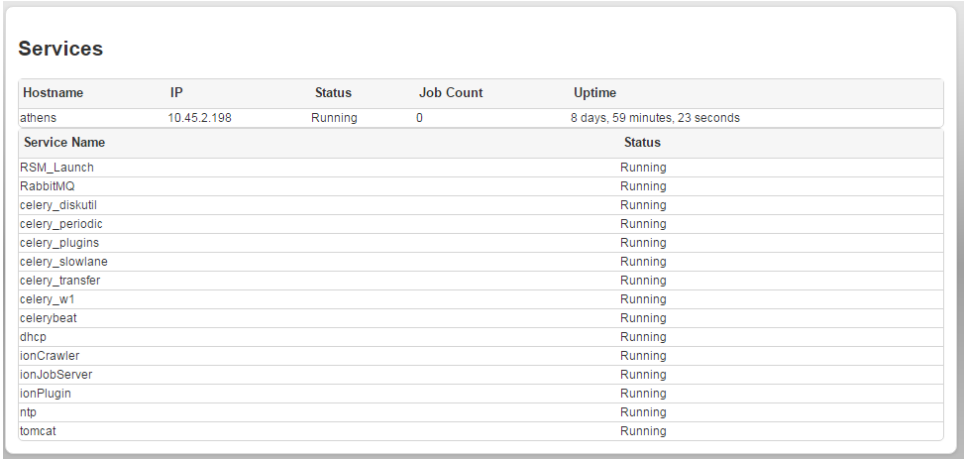
7. When the software update is complete, click **Back to Main Site**, then click ⚙ **(Settings) ▸ About**.

8. Review the software version number in the releases list to ensure that it reflects the update that you completed.

9. To ensure that the software upgrade is complete, and that the software is ready to run analysis programs, click ⚙ **(Settings) ▸ Services**.




10. In **Services** 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 |

11. To ensure compatibility between the software and your instruments, upgrade your sequencing instruments after the software upgrade is complete.

## 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 346.

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 348.
   - To update plugins, see "Update off-cycle release plugins" on page 351.
   - To manually install update files, see "Install off-cycle bundles without Internet access" on page 348.

## 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. Scroll down to the **Update Products** section at the bottom of the screen.
3. Select the desired new product and 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 Connect, an administrator can install or upgrade the following:

- 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 135.
- The RNASeqAnalysis plugin and smallRNA plugin. These plugins are supported by Thermo Fisher Scientific, but are not preinstalled in the Torrent Suite™ Software.

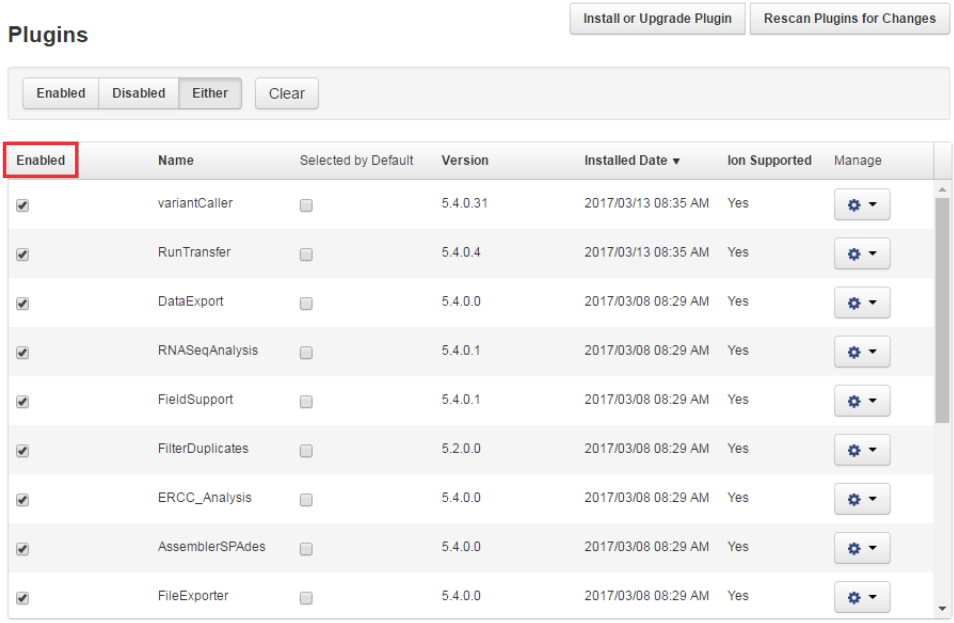
1. Sign in to the **Thermo Fisher Connect**.
2. Click the **Apps** icon ( ).
3. In **AppConnect**, under **Resource Libraries**, click **Plugins**.
4. *(Optional)* Click a category at the top of screen.
   The list of plugins is narrowed to only the plugins included that are in the selected category.
5. 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.
6. In Torrent Suite™ Software, click **(Settings)** ▸ **Plugins** ▸ **Install or Upgrade Plugin**.
7. Click **Select File**, browse to the location where you downloaded the plugin file, select the file, then click **Open**.
8. 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 installing a plugin, you must enable the plugin to make the plugin available in Torrent Suite™ Software.

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




2. In the Plugins list, select the **Enabled** checkbox next to any installed plugin, to make it available.
   The plugin is immediately available.

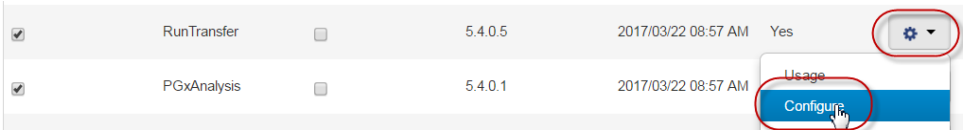
## Configure plugins globally

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
- RunTransfer 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 in the configuration dialog box vary depending on the plugin. See the plugin-specific configuration topic for more information.

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

## 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 a new plugin that you would like to use, check to see if an update is available.

1. Click ⚙ **(Settings)** ▸ **Updates**.
2. Scroll to the **Update Plugins** section at the bottom of the screen.
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

You can uninstall a plugin from your 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. If needed, 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 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

In the **Data** tab, click **Data Management**, then scroll to the **Disk Usage** section.

**Parameters in the Disk Usage section.**

| Parameter | Definition |
|---|---|
| Keep | File space devoted to files that are to be kept. |
| Used | File space being 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 B | Threshold above which basecaller 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. |

For details regarding automatic deletion and archive creation, see “Archive or delete data automatically” on page 353.

## 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 details about these file types, see “Analysis pipeline overview” on page 308.

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. On the Ion PGM™ System, you can reanalyze a run with the signal processing input file, which is available on the instrument.<br><br>Keep the signal processing input data if you want to reanalyze the run starting from raw signal processing data.<br><br>However, on the Ion Proton™ or Ion S5™ Systems, signal processing input data are used on the instrument, then deleted. These files are available on Torrent Suite™ Software only as thumbnails for the Ion Proton™, Ion S5™ Systems, or Ion GeneStudio™ S5 Systems. |
| Basecalling input files | Signal processing input files are converted to a single condensed basecalling input file representing the processed signal.<br><br>Keep or archive basecalling input data if you want to reanalyze the run. This can save time and resources because reanalyses use the basecalling input data, rather than the raw signal processing input data.<br><br>On the Ion Proton™, Ion S5™ Systems and Ion GeneStudio™ S5 Systems, basecalling input data are transferred to the Ion Torrent™ Server and are available for reanalyses. |
| Output files | Output files consist of all BAM files, run reports, and plugin results.<br><br>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.<br><br>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 if needed.

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.

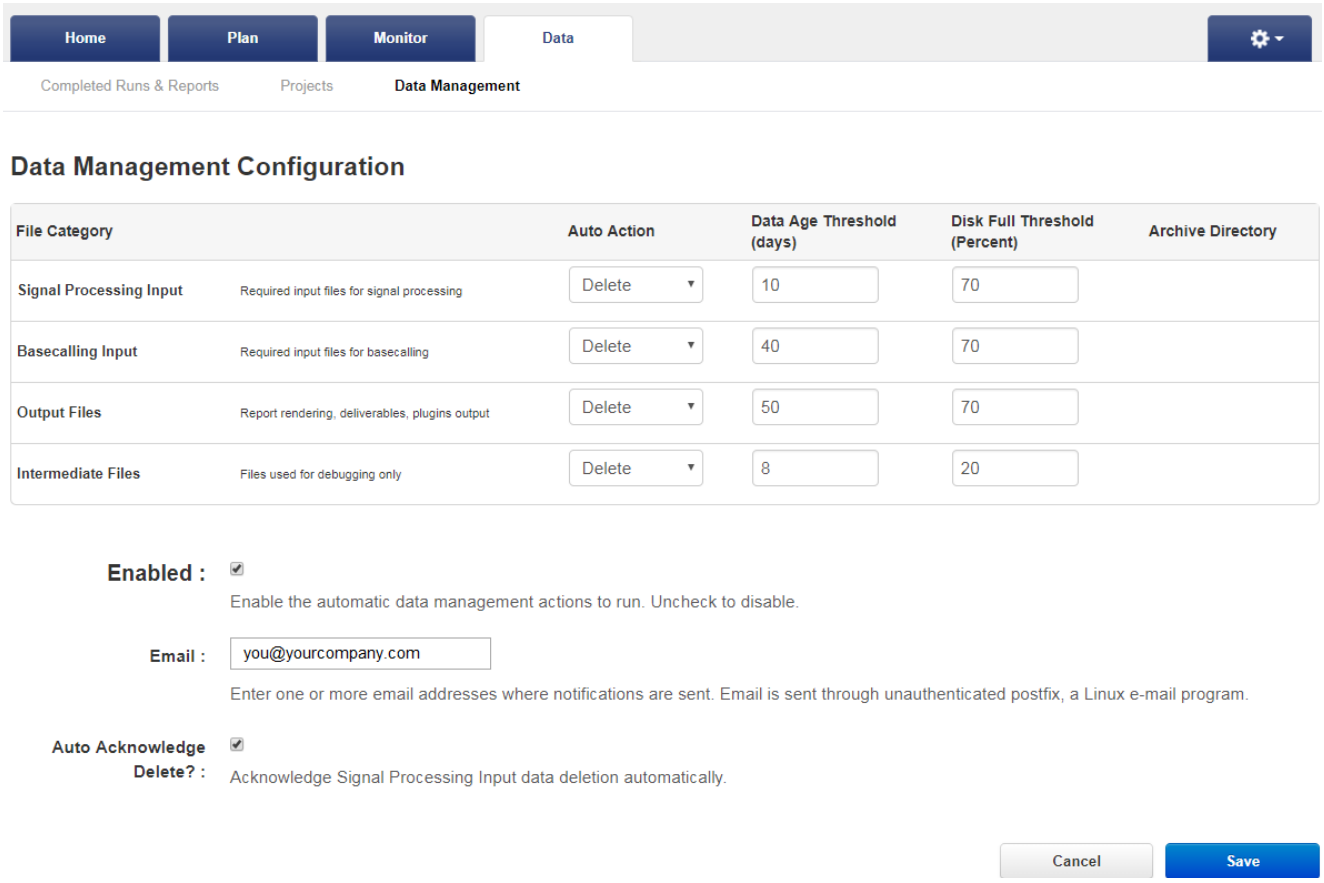
An administrator can also configure the Ion Torrent™ Server to delete data automatically when thresholds are met for filled disk space and data age. Automatic deletion of files is important to maintain available disk space, and simplifies removal of data that are no longer necessary to keep.

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, then configure:

| 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.<br>• **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.<br>• **Disk Full Threshold (Percent)**: Set the percentage of disk space that is filled on the server before data are deleted. |




3. Select the **Enabled** checkbox to enable the automatic data management you have configured. Deselect the checkbox to suspend automatic action.

4. In **Email**, enter an email address of where to 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**.

5. *(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.

6. After you have completed the configuration, click **Save**.

7. *(Optional)* On the **Data Management** screen, click **Configuration Log** in the **Configuration** section to view a record of configuration changes.

8. *(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 359.

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**.

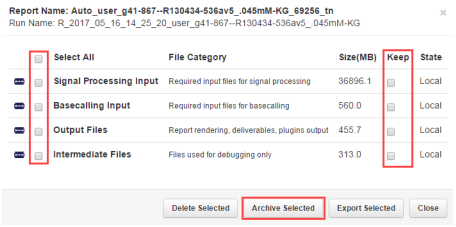
   The data for the file categories of the selected run reports are copied to the external hard drive. The data are also available in the local hard drive run results directory.

## 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 359.

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 select 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:

- Signal processing input files
- Basecalling input files
- Output files
- Intermediate files

For details about automatic deletion of select run data, see “Archive or delete data automatically” on page 353.

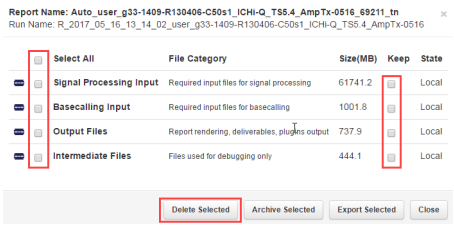
---

**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 will remove the archived copy when the archived drive is mounted. If a run was previously imported or restored, deleting will remove 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 select 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 pop-up window, 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

You can increase file storage space for data and results files with a Torrent Storage™ NAS (Network Attached Server) device, your own network access storage (NAS) device, or a USB drive.

After one or more of these storage drives has been installed and configured, you can use the drive to save data locally in the lab, transfer data between servers, store data with disk failure tolerance, and expand storage space.

| Storage method | Description |
|---|---|
| Torrent Storage™ NAS (Network Attached Server) device | A Field Service Engineer typically installs this device. It attaches directly to a Ion Torrent™ Server, Ion S5™ Instrument, or Ion GeneStudio™ S5 System, or can connect over a local network. After installation, it must be mounted on the local server as described in “Connect to a Torrent Storage™ NAS (Network Attached Server) device” on page 359. |
| Your own NAS device | Similar to the Torrent Storage™ NAS (Network Attached Server) device, but typically installed by your own system administrator. |
| USB Drive | Attaches directly to a Ion Torrent™ Server or Ion S5™ Instrument. After installation, it must be mounted on the local server as described in “Mount a USB drive” on page 362. |

## Connect to a Torrent Storage™ NAS (Network Attached Server) device

Initial setup of the Torrent Storage™ NAS (Network Attached Server) device is provided by your local Field Service Engineer. If the device is moved or disconnected for any reason (for example, a power outage), an administrator can reconnect the device to a Ion Torrent™ Server.

- If the Torrent Storage™ NAS (Network Attached Server) device connects directly to the Ion Torrent™ Server, see “Connect directly to a Torrent Storage™ NAS (Network Attached Server) device” on page 360.
- If the Torrent Storage™ NAS (Network Attached Server) device connects over a network to the server, see “Connect over a network to a Torrent Storage™ NAS (Network Attached Server) device” on page 361.

## Connect directly to a Torrent Storage™ NAS (Network Attached Server) device

An administrator can connect directly to a Torrent Storage™ NAS (Network Attached Server) device. If your Ion Torrent™ Server, Ion S5™ Instrument, or Ion GeneStudio™ S5 System is connected directly to your Torrent Storage™ NAS (Network Attached Server) device, 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 346.

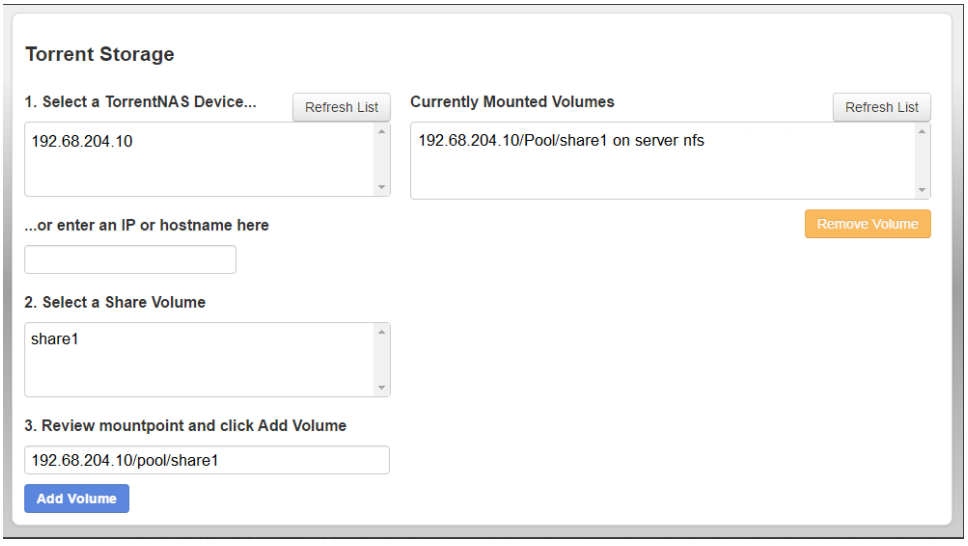
2. Click **Settings (⚙) ▸ Configure**, then scroll to **Torrent Storage**.

3. Find the IP address of the Torrent Storage™ NAS (Network Attached Server) device in the **Select a TorrentNAS Device** list. It can take several seconds for the list to populate.

   If the Torrent Storage™ NAS (Network Attached Server) device is not automatically detected in less than 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 Torrent Storage™ NAS (Network Attached Server) device

If the Ion Torrent™ Server, Ion S5™ Instrument, or Ion GeneStudio™ S5 System is installed on the same network as the Torrent Storage™ NAS (Network Attached Server) device, an administration can use the following steps to mount the device.

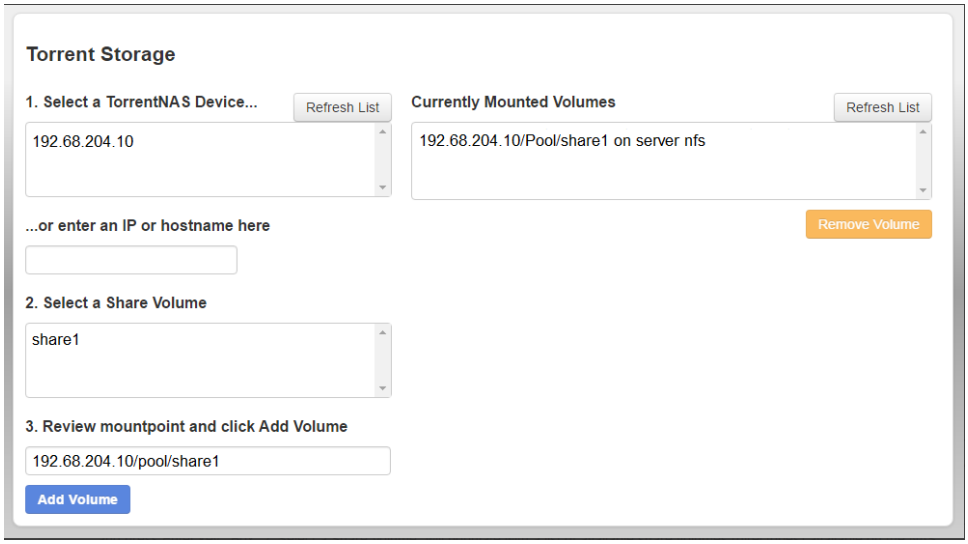
1. Click **Settings (⚙)** ▸ **About**, then confirm that the Torrent Suite™ Software version is 5.2 or later.

   To update your software, see "Update Torrent Suite™ Software" on page 346.

2. Click **Settings (⚙)** ▸ **Configure**, then scroll to **Torrent Storage**.

3. In **or enter an IP or hostname here**, enter the IP address of the Torrent Storage™ NAS (Network Attached Server) device, then press the **Enter** key.

4. In **Select a Share Volume**, select a volume on the device, then click **Add Volume**.

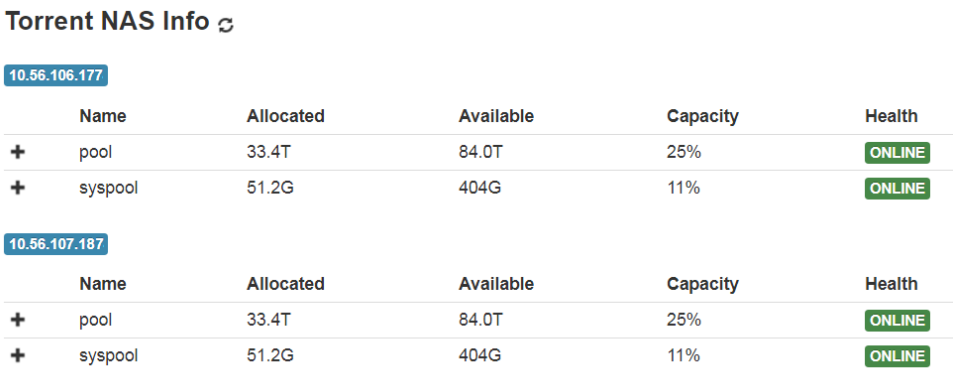



The storage volume is connected to the server and is listed in **Currently Mounted Volumes**.

### Monitor the Torrent Storage™ NAS (Network Attached Server) device

You can check the status of a Torrent Storage™ NAS (Network Attached Server) device in Torrent Suite™ Software.

1. Click **Settings (⚙) ▸ Services**.
2. Scroll to the **Torrent NAS Info** section to view information on Torrent Storage™ NAS (Network Attached Server) devices that are attached to your server through a network, including available storage capacity, usage, and health of the device.




## USB drives

### 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:

```
ionadmin@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`, depending 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).

```
ionadmin@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

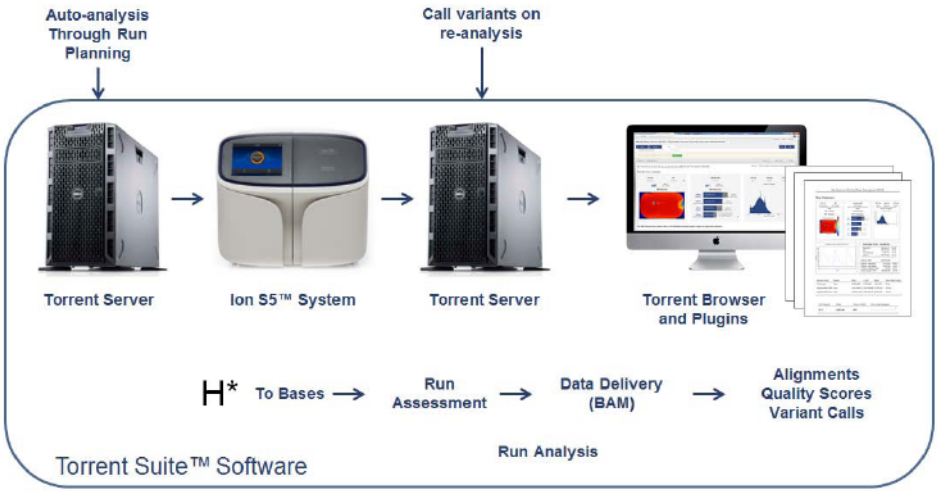
### Unmount a USB drive

**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 GeneStudio™ S5 Prime, Ion S5™ XL, Ion PGM™, or Ion Proton™ 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 the Ion GeneStudio™ S5 Plus System, Ion GeneStudio™ S5 Prime System, and Ion S5™ XL System

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 S5™ XL 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 |
|---|---|---|---|---|---|---|
| Read capacity | — | 2.5 M | 5 M | 15–20 M | 60–80 M | 100–130 M |
| Signal processing input[1] | DAT | 65 GB | 125 GB | 310 GB | 2 TB | 1 TB* |
| Signal processing output | WELLS | 9 GB | 18 GB | 45 GB | 180 GB | 330 GB |
| Base calling output | Unaligned BAM | 3 GB | 8 GB | 23 GB | 85 GB | 100 GB |
| Aligned output | Aligned BAM | 3 GB | 7 GB | 20 GB | 55 GB | 90 GB |

[1] Signal Processing input files for S5 systems are not transferred from the instrument and are not part of Ion Torrent™ Server data management.

* Data compression enabled.

## Dataflow using the 400-bp kit on the Ion GeneStudio™ S5 Plus System, Ion GeneStudio™ S5 Prime System, and Ion S5™ XL System

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 S5™ XL 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 S5 systems are not transferred from the instrument and are not part of Ion Torrent™ Server data management.

## Dataflow using the 400-bp kit on the Ion PGM™ Sequencer

The following table shows a high-level view of the dataflow using the 400-bp kit on the Ion PGM™ Sequencer. File sizes for Ion Torrent™ data depend on the Torrent Suite™ Software version, chip type, and kit type.

| Step | Resulting file type | Ion 318™ Chip | Ion 316™ Chip | Ion 314™ Chip |
|---|---|---|---|---|
| Flows | — | 900 | 900 | 900 |
| Raw image acquisition | DAT | 396 GB | 246 GB | 52 GB |
| Image processing | WELLS | 31.4 GB | 18.4 GB | 3.5 GB |
| Signal processing and base calling | BAM | 6.8 GB | 4.5 GB | 0.65 GB |

**CAUTION!** File sizes vary depending on the number of flows, the number of wells generating signals, and the number of library reads available. Your file sizes may be different. An unmapped BAM file format is used in the analysis pipeline steps before alignment.

### Dataflow using the 200-bp kit on the Ion PGM™ Sequencer and Ion Proton™ Sequencer

The following table shows a high-level view of the dataflow from the 200-bp kit on the Ion PGM™ Sequencer and Ion Proton™ Sequencer. File sizes for Ion Torrent™ data depend on the Torrent Suite™ Software version, chip type, and kit type.

| Step | Resulting file type | Ion Proton™ Chip | Ion 318™ Chip | Ion 316™ Chip | Ion 314™ Chip |
|---|---|---|---|---|---|
| Flows | -- | 500 | 500 | 520 | 520 |
| Raw image acquisition | DAT | 2.7 TB | 225 GB | 135 GB | 30 GB |
| Image processing | WELLS | 219 GB | 16.4 GB | 9.0 GB | 2.0 GB |
| Signal processing and base calling | BAM | 44 GB | 4.2 GB | 3.1 GB | 0.5 GB |

**CAUTION!** File sizes vary depending on the number of flows, the number of wells generating signals, and the number of library reads available. Your file sizes may be different. An unmapped BAM file format is used in the analysis pipeline steps before alignment.

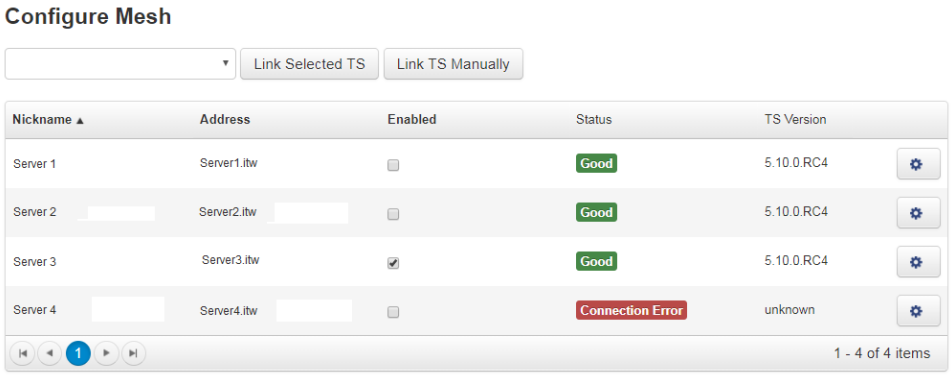
## Set up flexible workflows

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 a selected Torrent Server. | Select a Ion Torrent™ Server, then click **Link Selected TS**. |
| Manually link a Torrent Server. | Click **Link TS Manually**. |

3. Enter the following information in the **Setup Mesh Computer** dialog box.

| Option | Definition |
|---|---|
| Hostname/Address | Host name or address of the server.<br>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 must first connect the servers using Ion Mesh. For more information, see “Set up flexible workflows” on page 368.

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.
   Notice that the server name is now reflected in the **Run Name** column. Your results across multiple sequencers are ready for your review.

# Transfer a Planned Run to a 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 origin 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 368.

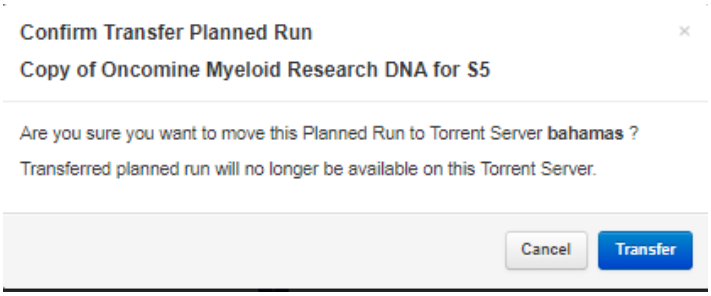
---

**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.

---

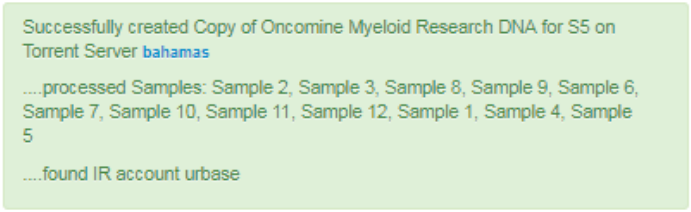
1. Sign in to Torrent Suite™ Software on the original Ion Torrent™ Server.
2. In the **Plan** tab, click **Planned Runs**.
3. Find the row of the Planned Run that you want to transfer, then click ⚙ **(Actions)** ▸ **Transfer**.
4. Select the Ion Torrent™ Server that you want to receive the Planned Run.
5. In the confirmation dialog box, confirm the information, then click **Transfer**.

   You can no longer access this Planned Run on the origin server after it has transferred.

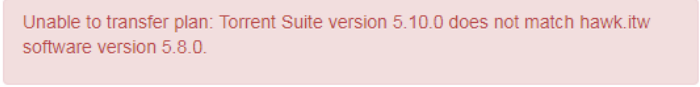



A status appears in a message box with the results of the transfer.

- A green box lists the Planned Runs that are successfully transferred.




- A red box lists any failed Planned Run transfers.



## Track Ion Chef™ Instrument flexible workflows for Ion 550™ chips with Ion Mesh

To use the flexible workflow feature and enable cartridge use tracking between Torrent Servers across multiple Ion Chef™ instruments, Torrent Servers must be linked in an Ion Mesh configuration. For further information on how to link Torrent Servers in an Ion Mesh setup, see "Set up flexible workflows" on page 368. For more information on configuring a flexible workflow on the Ion Chef™ Instrument, see the *Ion 550™ Kit – Chef User Guide* (Pub No. MAN0017275).

**IMPORTANT!** If you have Torrent Servers that are connected in an Ion Mesh configuration, and the connection to one Ion Torrent™ Server is disrupted or lost, cartridge 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 that 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 PGM™ and Ion S5™ Sequencer, and Ion GeneStudio™ S5 Systems data | `/results/< instrument_ name>` directory, by default. |
| Ion Proton™ Sequencer data | `/rawdata/<Proton_Name>` directory, by default. |
| Report data | `/results/analysis/output/Home` directory, 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 database, you need a complete working Ion Torrent™ Server installation.

The two scenarios for restoring a database are:

- Installing 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.
- Replacing 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 Connect 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 S5™, Ion PGM™, Ion Proton™, or Ion GeneStudio™ S5 System, or Torrent Suite™ Software is reported, Thermo Fisher Scientific service and 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 personnel 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 automatically on the Ion Torrent™ Server and Ion S5™, Ion PGM™, and Ion Proton™, and Ion GeneStudio™ S5 Systems 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 is collected in the Axeda™ monitoring database, where Thermo Fisher Scientific Technical Support personnel can review the information that agents collect. Because the heartbeat message is sent many times an hour, Technical Support can quickly see the following:

- If a machine is online
- The software versions 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 Ion S5™, Ion PGM™, Ion Proton™, and Ion GeneStudio™ S5 Systems and the Torrent Suite™ Software, which is required for system support. Without remote access, Thermo Fisher Scientific Field Application Scientists cannot access, view, and troubleshoot problems regarding 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.<br>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, 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 Connect 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 integration and may make remote support more difficult. You can disable telemetry services on the **System 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 from the Ion Torrent™ Server and Ion S5™, Ion PGM™, Ion Proton™, and Ion GeneStudio™ S5 Systems back to Thermo Fisher Scientific.

### Ion Torrent™ Server data

The following table lists and describes the Ion Torrent™ Server events that are collected by Axeda™ RSM agent and the Deep Laser telemetry service, then 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 ( DO NOT USE 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 |

*(continued)*

| Event name | Data type | Sample value |
|---|---|---|
| TS.host | String | Ion-torrent-server |
| TS.HW.HD./results | String | 58.99 |
| 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 |

*(continued)*

| Event name | Data type | Sample value |
|---|---|---|
| TS.Server.RabbitMQ | String | ok/offline/error |
| TS.Server.RSM_Launch | String | ok/offline/error |
| TS.Server.tomcat | String | ok/offline/error |
| 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 PGM™ data

The following table lists and describes the Ion PGM™ events that are collected by Axeda™ RSM agent and the Deep Laser telemetry service, then automatically sent to Thermo Fisher Scientific.

| Event name | Data type | Sample value |
|---|---|---|
| Instrument.Event.LastExperiment | String | R_2011_04_22_15_34_58_usr_S-1 |
| Instrument.Event.Pressure | Analog | 0 (chart) |
| Instrument.Event.ValveBoard | String | Valve Board not accessible<br>Valve Board Down Stream Errors<br>Valve Board Up Stream Errors |
| Instrument.Event.RunAborted | String | Run aborted |
| Instrument.Event.LostChipCon | String | Lost chip connection, run aborted |
| Instrument.Event.UBoot | String | U-boots don’t match |
| Instrument.Event.Kernel | String | Kernels don’t match |
| Instrument.Event.ResultsDrive | String | Results drive not accessible |
| Instrument.Event.BootDrive | String | Bad boot drive detected |
| Instrument.Event.DataDrive | String | Bad data drive detected |
| Instrument.HW.HD1 | Analog | 34.001 (chart) |

*(continued)*

| Event name | Data type | Sample value |
|---|---|---|
| `Instrument.InstrumentName` | String | Stork |
| `Instrument.Pressure` | Analog | 10.2 (chart) |
| `Instrument.Temperature` | Analog | 27.06 (chart) |
| `Instrument.TYPE` | String | PGM1 |
| `Instrument.Version.Board` | String | 4 A.1 |
| `Instrument.Version.Datacollect` | String | 180 |
| `Instrument.Version.driver` | String | 31 |
| `Instrument.Version.fpga` | String | 70 |
| `Instrument.Version.Graphics` | String | 15 |
| `Instrument.Version.LiveView` | String | 268 |
| `Instrument.Version.OS` | String | 12 |
| `Instrument.Version.Scripts` | String | 16.3.58 |

### Ion S5™, Ion Proton™, and Ion GeneStudio™ S5 System data

Ion S5™, Ion Proton™, and Ion GeneStudio™ S5 System data are divided into these 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 Ion S5™, Ion Proton™, and Ion GeneStudio™ S5 System events that are collected by Axeda™ RSM agent and the Deep Laser telemetry service are 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 |
| InstrumentState | String | Idle |
| RunData.a1a2 | String | R_2016_02_17_13_01_08_user_F4--145 W1.dat dfffe 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 |

*(continued)*

| Data item name | Data type | Sample value |
|---|---|---|
| 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 |
| 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 |

*(continued)*

| Data item name | Data type | Sample value |
|---|---|---|
| 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 |
| Version.TSLink | String | 1.0.2r5 |
| Version.Valve FPGA | String | c010 |

# Administration with command-line utilities

## Monitor disk space

You can monitor disk space if the Torrent Suite™ Software is not available, or if you want to use a command-line utility.

---

**IMPORTANT!** If there is insufficient space on the Ion Torrent™ Server, data files are retained on the Ion PGM™ Sequencer and the Ion Proton™ Sequencer until space becomes available.

---

1. Sign in to the Ion Torrent™ Server using an `ssh` client:

```
$ ssh ionadmin@ion-torrent-server
$ password: ionadmin
```

2. Enter the `df` command to display partitions and disk utilization:

```
$ df -h

ionadmin@itw-test01:~$ df -h
Filesystem         Size  Used Avail
Use% Mounted on
/dev/sda3          5.3T  372G  4.6T
8% /
none                24G  200K   24G
1% /dev
none                24G     0   24G
0% /dev/shm
none                24G   88K   24G
1% /var/run
none                24G     0   24G
0% /var/lock
none                24G     0   24G
0% /lib/init/rw
/dev/sda5           61G  524M   57G
1% /tmp
/dev/sda1          276M   29M  233M
12% /boot
/dev/sda4          3.8G  2.4G  1.3G
65% /var
nas3:/c/results2   19T    17T  1.7T
91% /results2
nas2:/c/archive/tahiti
                   19T    13T  5.3T
71% /media/archive
nas1:/c/results    19T    17T  2.1T
89% /results4
nas1:/c/results1   19T    16T  2.1T
89% /results3
```

Most growth is seen in the `/results` directories, which is where Ion Reporter™ Software data are stored.

The `Use%` column indicates how much space is being used.

You can also monitor disk space through the Ion Reporter™ Software. For details, see "View disk usage parameters" on page 302.

## Change the hostname

Use the following command to change the hostname:

```
sudo TSconfig --change-hostname
```

You must restart the server after the hostname is changed. This command automatically restarts the server.

## Change the time zone

Use the following command to change the time zone:

```
sudo TSconfig --configure-timezone
```

## 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
```

## Alternate checks

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.

# 14 Screen descriptions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## User and account settings screen

| How to... | Learn more about... |
|---|---|
| "Approve or reject a new user account" on page 337 | |
| "Link your Ion Torrent™ Server account to AmpliSeq.com" on page 60 | |

# Analysis Parameters screen

| How to… | Learn more about… |
|---|---|
| “Copy an analysis parameter set” on page 62 | “Command Line Args (Advanced) screen” on page 68 |

# Configure screen

| How to… | Learn more about… |
|---|---|
| “Add customer support contacts” on page 344<br>“Change the displayed server name” on page 345<br>“Update Torrent Suite™ Software” on page 346<br>“Manage Torrent Suite™ Software user accounts” on page 336<br>“Modify a user account” on page 338<br>“Delete multiple user accounts” on page 341<br>“Delete a single user account” on page 340<br>“Archive or delete data automatically” on page 353<br>“Lock current Torrent Suite™ Software version” on page 345<br>“Change the time zone for the Ion Torrent™ Server” on page 344<br>“Create a new administrator account to change a password” on page 342 | “Update Torrent Suite™ Software” on page 346 |

# Ion Reporter™ configuration screen

| How to… | Learn more about… |
|---|---|
| "Set up an account for IonReporterUploader plugin" on page 239 | "IonReporterUploader plugin configuration" on page 246 |
| "Automatically transfer Torrent Suite™ Software output to Ion Reporter™ Software" on page 241 | "IonReporterUploader plugin file transfer progress" on page 248 |
| "Manage the Ion Reporter™ Software analysis workflow list" on page 244 | Ion Reporter™ account setup troubleshooting in "IonReporterUploader plugin account is not configured." on page 408 |
| "View IonReporterUploader plugin status details" on page 248 | "Torrent Suite™ Software output and Ion Reporter™ Software analysis phases" on page 252 |
| "Tune IonReporterUploader plugin speed parameters" on page 250 | |

# Plugins screen

| How to… | Learn more about… |
|---|---|
| "Install or upgrade plugins" on page 349 | Chapter 7, "Manage plugins for data analysis" |
| "Enable an installed plugin" on page 350 | "Preinstalled plugins" on page 135 |
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| "Run a plugin manually from the sequencing run report" on page 132 | |
| "Uninstall a plugin" on page 351 | |
| "Rescan a plugin" on page 134 | |
| "Configure plugins globally" on page 130 | |

# Updates screen

Use the **Updates** screen to check for product and plugin updates.

| How to… | Learn more about… |
|---|---|
| “Check for off-cycle updates” on page 348 | “Update Torrent Suite™ Software” on page 346 |
| “Enable off-cycle product updates” on page 348 | |
| “Update off-cycle release plugins” on page 351 | |
| “Install off-cycle bundles without Internet access” on page 348 | |

# References Sequences screen

| How to… | Learn more about… |
|---|---|
| “Import a preloaded reference sequence file” on page 260 | “Reference sequences” on page 256 |
| “Import a custom reference sequence file” on page 261 | “hg19 reference” on page 256 |
| “View a reference sequence file” on page 262 | “GRCh38 reference” on page 258 |
| “Edit reference sequence file properties” on page 263 | “Obsolete reference sequences” on page 265 |
| “Permanently delete a reference sequence file” on page 265 | “Target regions and hotspots files” on page 266 |
| “View and manage target regions files” on page 267 | “Guidelines for using target regions and hotspots files” on page 266 |
| “View and manage hotspots files” on page 268 | “Hotspots file format” on page 280 |
| “Upload a target regions file” on page 270 | “Test fragments” on page 294 |
| “Upload a hotspots file” on page 272 | “Barcodes and barcode sets” on page 296 |
| “View test fragment details” on page 294 | “Upload history” on page 300 |
| “Add a custom test fragment” on page 294 | |
| “Edit or delete a custom test fragment” on page 295 | |
| “View a DNA barcode sequence” on page 297 | |
| “Download a DNA barcode set CSV file” on page 297 | |
| “Add a custom DNA barcode set” on page 298 | |
| “Delete a custom DNA barcode set” on page 300 | |

# Services screen

| How to… | Learn more about… |
|---|---|
| “Check crawler and job server status” on page 410<br>“Restart services” on page 419 | “Alternate checks” on page 384 |

# Planned Runs screen

| How to… | Learn more about… |
|---|---|
| “Manage Planned Runs” on page 76 | Chapter 4, “Plan and execute an instrument run” |
| “Manually change Ion Chef™ Instrument run status” on page 78 | “Planned Run templates” on page 34 |
| “Transfer a Planned Run to another Ion Torrent™ Server” on page 81<br>“Execute a Planned Run on a sequencer” on page 79 | “Planned Run status” on page 78 |

# Samples screen

| How to… | Learn more about… |
|---|---|
| “Create a Sample Set manually” on page 19 | “Sample attributes” on page 24 |
| “Create a Sample Set by importing samples from a CSV file” on page 20 | “Sample information” on page 21 |
| “Create an Ion AmpliSeq™ on Ion Chef™ Sample Set” on page 31 | “Plan by Sample Set” on page 64 |
| “View Ion AmpliSeq™ library preparation on an Ion Chef™ System run” on page 29 | |
| “View Sample Set attributes” on page 22 | |
| “Add a sample attribute to a sample” on page 23 | |
| “Manage sample attributes” on page 23 | |
| “Find a Sample Set” on page 29 | |
| “Edit a Sample Set” on page 30 | |
| “Delete a Sample Set” on page 31 | |
| “Sort Sample Sets” on page 30 | |
| “Create a Planned Run with Sample Sets” on page 65 | |

# Templates screen

| How to… | Learn more about… |
|---|---|
| “Create a Planned Run from a Planned Run template” on page 38 | Chapter 4, “Plan and execute an instrument run” |
| “Create a custom Planned Run template” on page 36 | “Steps in the workflow bar” on page 44 |
| “Export a Planned Run template” on page 42 | |
| “Import a Planned Run template” on page 42 | |
| “Import panel files and parameters from AmpliSeq.com” on page 60 | |
| “Search for a Planned Run template” on page 41 | |
| “Save a Planned Run template to Favorites” on page 43 | |
| “Create a Planned Run for mixed samples with a Planned Run template” on page 70 | |
| “Create multiple Planned Runs” on page 71 | |
| “Transfer a Planned Run to another Ion Torrent™ Server” on page 81 | |

# Runs in Progress screen

| How to… | Learn more about… |
|---|---|
| “Monitor a sequencing run” on page 83 | Sequencing run metrics in “Monitor a sequencing run” on page 83 |
| “Review the Planned Run settings” on page 86 | “View data for runs in progress” on page 85 |
| | “Auto Refresh the Monitor tab” on page 86 |

# Ion Chef screen

| How to… | Learn more about… |
|---|---|
| “Monitor an Ion Chef™ run” on page 84 | |
| “Auto Refresh the Monitor tab” on page 86 | |

# Completed Runs & Reports screen

| How to… | Learn more about… |
|---|---|
| “Search for a run report” on page 90 | “Create an Ion AmpliSeq™ on Ion Chef™ Sample Set” on page 31 |
| “Change the default alignment reference” on page 126 | “Test fragments” on page 294 |
| “Reanalyze a run” on page 124 | |
| “Add or change barcoding for a completed run report” on page 127 | |
| “Reanalyze a run” on page 124 | |
| “Edit a run report” on page 126 | |
| “View plugin run status” on page 133 | |
| “Set the Completed Runs & Reports screen to automatically refresh” on page 128 | |
| “Failed status for a run” on page 399 | |

# Run Report screen

| How to… | Learn more about… |
|---|---|
| “Open a run report” on page 91 | “Quality following alignment” on page 95 |
| “Review unaligned reads” on page 92 | “Aligned read length calculation” on page 96 |
| “Review aligned reads” on page 95 | “Alignment” on page 97 |
| “Download output files” on page 107 | “Reads used for alignment” on page 97 |
| “View and download barcode reports” on page 107 | “Predicted quality (Q20)” on page 98 |
| “View software versions used in a run” on page 113 | “Run report metrics before alignment” on page 93 |
| “Download a run report summary PDF” on page 108 | “Run report metrics after alignment” on page 100 |
| “View consumables used in a completed run” on page 110 | “Output files” on page 106 |
| “View the summary for an Ion Chef™ Instrument run” on page 111 | “View details about completed runs” on page 109 |
| “View analysis details for a completed run” on page 112 | |
| “Get technical support files for a completed run” on page 399 | |

# Projects screen

| How to… | Learn more about… |
|---|---|
| “Add a project to a Planned Run” on page 115<br>“Add selected results to another project” on page 115<br>“Download a CSV file of metrics” on page 119<br>“Remove a result set from a project” on page 124<br>“Search for projects by name” on page 114<br>“View result sets in a project” on page 116<br>“Combine aligned reads from multiple run reports” on page 118<br>“Compare reports of runs in a project” on page 116<br>“Manage data for result sets in projects” on page 118 | |

# Data Management screen

| How to… | Learn more about… |
|---|---|
| “Archive or delete data automatically” on page 353 | “Ion instrument data types” on page 352 |
| “Import data for data transfers or restoration” on page 306 | Chapter 11, “Data management” |
| “View disk usage parameters” on page 302 | “Dataflow file sizes” on page 365 |
| “View category statistics” on page 303 | |
| “View active data management jobs” on page 303 | |
| “Search for run reports with disk usage status” on page 305 | |
| “Keep run report data” on page 305 | |
| “Manually export run data” on page 356 | |
| “Manually archive run data” on page 357 | |
| “Manually delete selected data from a run report” on page 357 | |
| “View the data management actions log” on page 306 | |
| “Increase file storage and available disk space” on page 359 | |
| “Connect to a Torrent Storage™ NAS (Network Attached Server) device” on page 359 | |
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# Troubleshooting


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This appendix includes information on troubleshooting tips, resources, and where to get additional information or Technical Support assistance for Ion GeneStudio™ S5 and Ion S5™ Systems sequencing runs. 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 Ion PGM™ Sequencer, Ion Proton™, Ion S5™, Ion Chef™ Instrument, or Ion GeneStudio™ S5 Series product pages. 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. The sequencing CSA is used by Technical Support to diagnose problems. Due to the large size of the full analysis CSA, it is necessary to use the thumbnail version for troubleshooting purposes. To download this CSA:
  a. In the **Data** tab, click **Completed Runs & Reports**, then click the Report Name link with "_tn" appended.
  b. In the left navigation menu, click **Details**, or scroll to the **Details** section of the run report, then click the **Support** tab.
  c. Click **Download** to download a compressed directory that contains a PDF and HTML version of the run report, and logs that can be used for troubleshooting.
  d. Email the report to Technical Support. If you would like to be able to upload these support files directly, ask your local Field Service Engineer how to enable the Customer Support Archive function.
- Instrument log files—Instrument log files from the Ion OneTouch™ 2 or 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 resulting 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 your 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

## 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 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 **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, click **Download** to download a customer support archive. This is a compressed directory that contains a PDF and HTML version of the run report, and logs that can be used for troubleshooting.
5. (Optional) Email the customer support archive 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 function.

## 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 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 124. |
| | | 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 399. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| 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.<br>• If % templated ISPs was <10%, increase library input to target 15–25% templated ISPs.<br>• Requantify the library. If the library yield was below recommended, rebuild the library. |
| | Too much library input into template preparation. | Review Qubit™ QC assay results to determine if % templated ISPs was too high after template preparation.<br>• 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.<br>• 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 loading density from Ion OneTouch™ 2 System 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.<br>• If % templated ISPs was <10%, increase library input to target 15–25% templated ISPs.<br>• Requantify the library. If the library yield was below recommended, rebuild the library. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Low loading density from Ion OneTouch™ 2 System runs *(continued)* | Too much library input into template preparation. | Review Qubit™ QC assay results to determine if % templated ISPs was too high.<br>• 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.<br>• 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. |
| | Low pre-enriched ISP recovery. | Review Qubit™ QC assay results to determine if ISP recovery was low after template preparation. |
| | | Ensure that ISPs are properly vortexed (1 minute at maximum speed as suggested in the user guide) before the addition of amplication mix. |
| | | Ensure proper OT2 instrument setup and performance. |
| | Low enriched ISP recovery. | Ensure proper reagent setup for ES enrichment. |
| | | Ensure that enrichment beads (Dynabeads™ MyOne™ beads) are washed and resuspended in the correct washing solution. |
| | | Properly prepare the fresh melt-off solution. |
| | Improper addition of sequencing primer and polymerase. | Ensure that sequencing primer and polymerase were added. |
| | | During the primer annealing step, it is important to thoroughly resuspend the enriched ISP pellet before and after adding the sequencing primer. This helps to break up ISP clumps and ensure even sequencing primer distribution to the templates on the ISPs. |
| | | Properly mix ISPs during the primer annealing step. Improper mixing can cause lower than expected key signal and possible misidentification of template ISPs. |
| | Wrong loading protocol was used. | Use the recommended protocol for the chip type and sequencing kit. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Low percentage of live ISPs. 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. |
| | | Check Ion OneTouch™ 2 System performance by downloading the log files. |
| | 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. |
| | | Check Ion OneTouch™ 2 System performance by downloading the log files. |
| | 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<br>There are two test fragment controls: TF-C is a sequencing control and TF-1 is an amplification control. Starting in Torrent Suite™ Software 5.4, only TF-1 is present. | 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 sequencing primer was annealed and sample was incubated with polymerase. |
| | | Ensure that all reagents were from the same sequencing kit type. Do not swap reagents between kit types. |
| | | Ensure that reagents set up were correctly. |
| | | Send Customer Support Archive (CSA) to Technical Support. |
| Lower than expected 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. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Lower than expected number of live library ISPs with a key sequencing matching the library key (TCAG). *(continued)* | 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. |
| High polyclonal count | Too much library input into template preparation. | Verify library quantification and template preparation with QC (Qubit assay).<br>If the library key signal is lower than expected, % polyclonal estimate might be inaccurate. |
| High number of low quality reads.<br>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. |

## Customer Support Archive (CSA)

You can download an archive that a Technical Support representative can use to diagnose Torrent Suite™ Software issues. The Customer Support Archive (CSA) contains log files and other technical data about your Torrent Suite™ Software and analysis runs.

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 164.

### Generate a customer support archive

1. Under the **Data** tab, in the **Completed Runs & Reports** screen, click the link for your completed analysis run.
2. In the Run Summary, click the **Plugin Summary** link, or scroll down to the **Plugin Summary** section.
3. Click the **Support** tab, then click **Download**.

A compressed archive is downloaded to the directory location that you specified to download files from the browser. This location depends on your browser settings. You can attach this archive to an email to send to Customer Support.

Ion Torrent™ Server cannot access the customer support server automatically. If you would like to upload files directly, contact your local Field Service Engineer and ask how to enable the customer support archive upload for your Ion Torrent™ Server.

### 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. |
| `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. |

**Files in the top level folder** *(continued)*

| File | Description |
|---|---|
| <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. |
| <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` |
| `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.

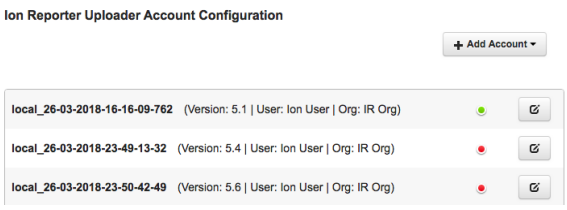
**Files in the `sigpror_results/NucStepFromBeadfind` folder**

| File |
|---|
| `dcOffset.txt` |
| `NucStep_frametime.txt` |
| `NucStep_inlet_bead.txt` |
| `NucStep_inlet_empty.txt` |
| `NucStep_inlet_empty_sd.txt` |
| `NucStep_inlet_step.txt` |

**Files in the sigpror_results/NucStepFromBeadfind folder** *(continued)*

| File |
|---|
| 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.<br>Red status appears on the Ion Reporter™ Software account configuration screen.<br>  | If you change your account password, you see a red dot in the status column of the IonReporterUploader plugin account. | When you change your password, click **Edit** and enter the access code for the account. For more information, see<br>"Set up an account for IonReporterUploader plugin" on page 239. |
| | If you upgrade the 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.<br>File validation cannot be attempted. | • Incorrect file format<br>• Incorrect file extension<br>• The ZIP file contains no files or multiple files<br>• A corrupted ZIP or GZ file | Check for possible errors in the source BED or VCF file.<br>Fix the errors, or replace the file, then re-attempt the upload. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Reference genome file cannot be opened | • An invalid FASTA file was uploaded.<br>• No FASTA header found at line 1.<br>• Genome information text file may have been deleted. | 1. Delete the existing reference sequence entry.<br>2. Identify and correct formatting errors in the FASTA file.<br>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.

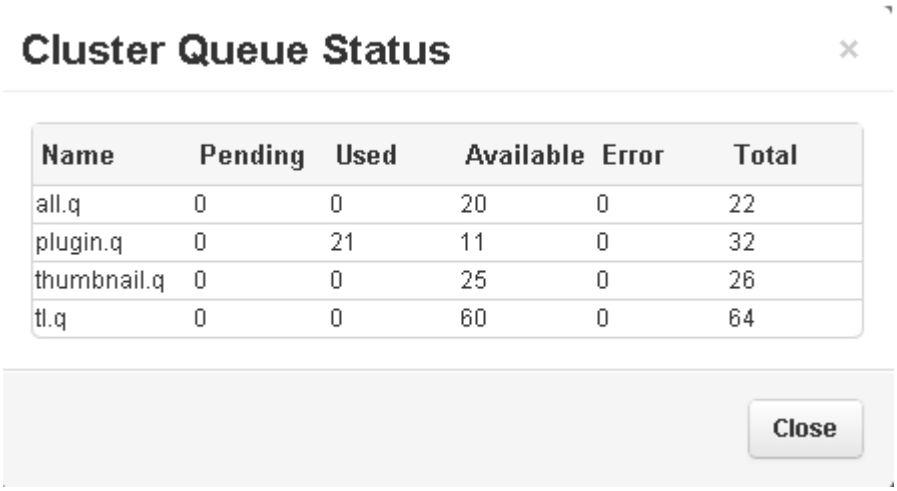
If a process is not running, a Down or Offline reason displays in the **Services** screen. An example message is The crawler is offline.

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:




## Verify network connectivity and name resolution

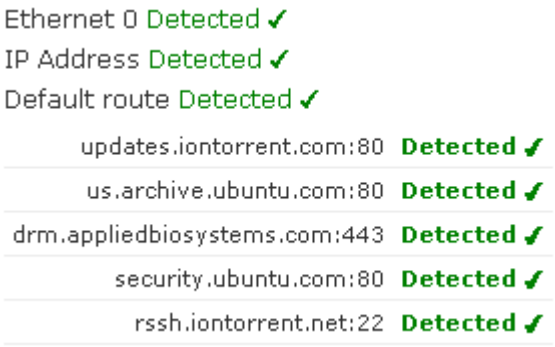
There can be many reasons for network connectivity or name resolution to fail. Use this procedure to try to resolve connectivity and name resolution problems.

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**.
   The **Site administration** screen in the Ion Web portal opens.

4. Scroll down to **Management Actions**, then select the **Network Settings** line.

   The software performs several network checks:

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 412.

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:

```
ionadmin@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** ▸ **,** , then scroll the **Database Administration**section and click the **Admin Interface** link.
   b. In the **Admin Interface** screen, scroll to the **Management Actions** section, then click **Network Settings**.
   c. Select **Static**, then enter the IP address into the **IP address** field and 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 Ion PGM™ Sequencer, Ion Proton™, Ion S5™, Ion Chef™ Instrument, or Ion GeneStudio™ S5 Series sequencers.

To 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 potentially incorrect information to other machines.

If the network configuration is blocking the NTP protocol from reaching the Internet, the Ion PGM™ Sequencer, Ion Proton™, Ion S5™, Ion Chef™ Instrument, or Ion GeneStudio™ S5 Series sequencers cannot synchronize time. If the server is restricted from reaching to the external time server, edit the NTP settings to point to your local NTP server.

Your site network administrator is probably aware of this connectivity restriction, and it is likely that IT has a time server in the network.

## Verify file transfer

Do not delete the data from the Ion PGM™ or Ion Proton™ Sequencer until you are confident that the data are present on the Ion Torrent™ Server, the analysis is successful, and the **Analysis Report** has been generated successfully.

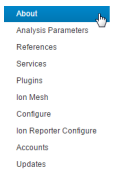
1. Verify that all files successfully transferred from the Ion PGM™ and Ion Proton™ Sequencers to the Ion Torrent™ Server.

2. (Optional) Manually transfer files by going to **Data Management** ▸ **Re-transfer**, then select an option for any of the runs in question. You can then safely transfer data again.

## 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 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.

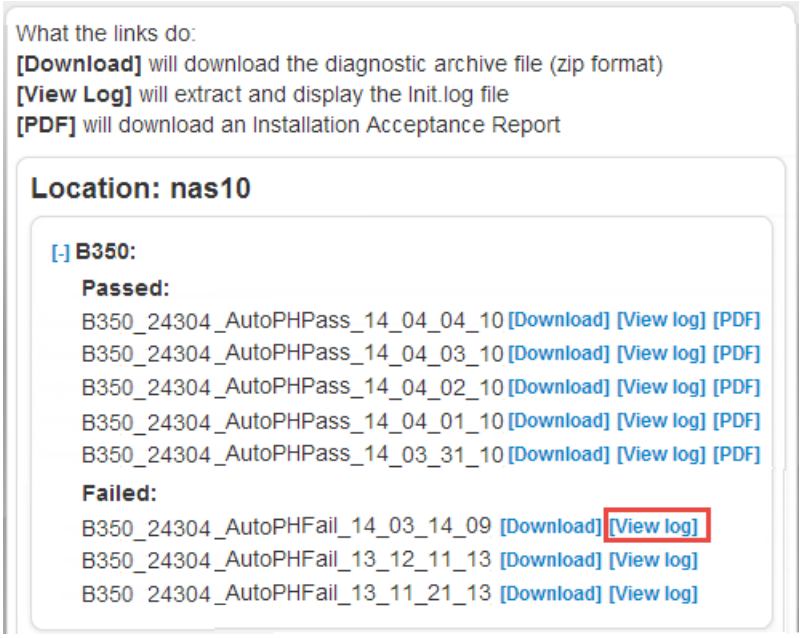
1. To access the **Instrument Diagnostics** information, click ⚙ **(Settings)** ▸ **About**:

2. Scroll down to the **More Information and Assistance** section, then click the **Instrument Diagnostics** link:

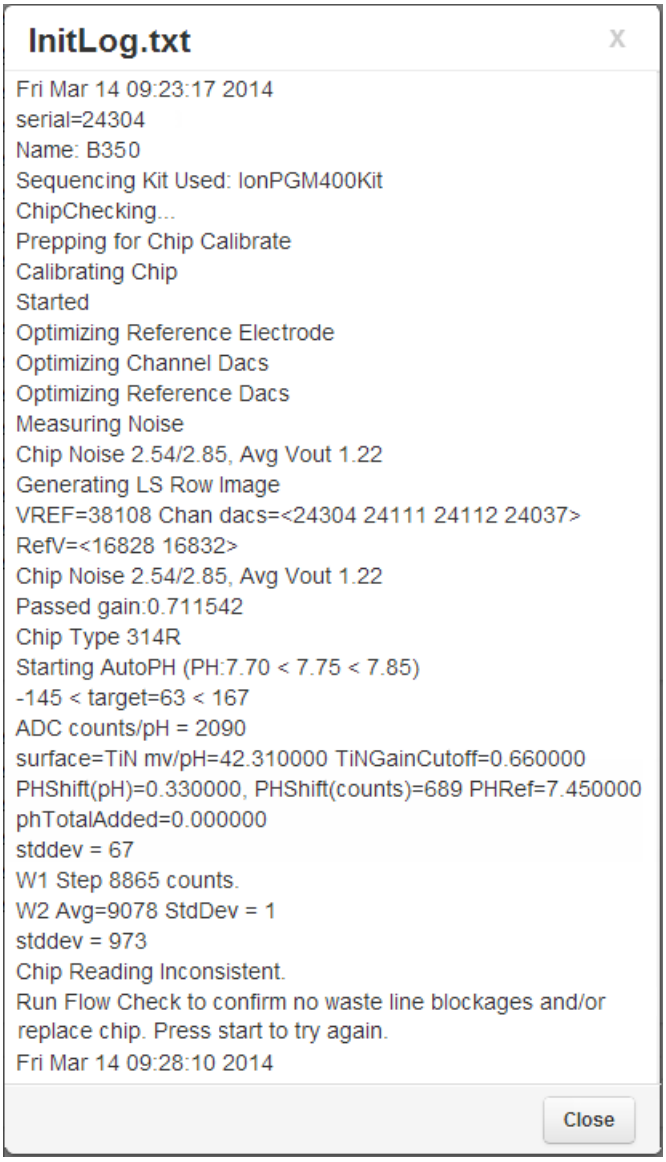
   The **Instrument Diagnostics** screen lists the sequencing instruments that are associated with each of your results partitions. Passed and failed analysis runs are shown for each instrument.

   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
X
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 can not restart the service using the Torrent Suite™ Software. The easiest approach is to shut down and restart the Ion Torrent™ Server.

After shutting down and restarting the server, all services that were running before shutting down continue from the point where they were interrupted, and no more user interaction is needed.

---

**IMPORTANT!** Before restarting the Ion Torrent™ Server, make sure that no Ion PGM™ Sequencer or Ion Proton™ Sequencer is uploading data to the server, otherwise the file transfer is interrupted.

---

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 being restarted, 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.

# Documentation and support

## Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
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  - 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.

## Limited product warranty

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# 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) | ISPs that carry DNA insert length of less than 8 bp. |
| 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 a 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. A SAM file (.sam) is a tab-delimited text file that contains sequence alignment data. A BAM file contains aligned reads sorted by reference location. |
| 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.<br>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.<br>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. |
| custom template | A user-created Planned Run template. Custom templates can be created based on a preinstalled Planned Run template and 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 target 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 regions in the gene that typically contain variants. Specifying a hotspots file to use in a run enables Torrent Variant Caller to identify if a specific variant is present or absent. A hotspot file instructs the Torrent Variant Caller to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspot file affects only the variantCaller plugin, not other parts of the analysis pipeline. If you don't specify a hotspots file, the software tells only the difference between your sequence and the reference genome. |
| 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 S5™/Ion S5™ XL or Ion GeneStudio™ S5 Series system. |
| 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 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 all of the regions that a panel represents such as the amplified regions that are used with target sequencing. The complete software analysis pipeline, including plugins, is restricted to only these specified regions instead of analyzing the entire reference library. |
| test fragment | 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 | A 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 filter. |
| VCF file | A variant call format (VCF) file specifies a variant of interest and its location. This file stores only the differences between the BAM file and the reference file. |
| wells with ISPs | Number of wells that were determined to be positive for the presence of an ISP within the well. 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. |
| XLS file | Microsoft™ Excel™ format of a VCF file. |