ion torrent

Torrent Suite™ Software 5.10

USER GUIDE

Publication Number MAN0017598

Revision A.0

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

ThermoFisher SCIENTIFIC

Manufacturer: Life Technologies Corporation | 200 Oyster Point Blvd | South San Francisco, CA 94080 | USA

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision History: Pub. No. MAN0017598

Revision	Date	Description
A.0	5 June 2018	Updates for Torrent Suite™ Software 5.10 new features. • Support for Ion AmpliSeq™ HD Planned Run templates and dual barcode sets • Updates to Dashboard view • New reference annotation files for pre-loaded references • Updates to Reference Management section • Updates to variantCaller section • Updates to Integration with Ion Reporter™ Software section • Updates to Review Run Reports section

Contents

Get started with Torrent Suite™ Software

Torrent Suite™ Software allows you to view sequencer activity, plan and monitor sequencing runs, and review sequencing data for an Ion PGM™ Sequencer or Ion Proton™, Ion S5™, Ion S5™ XL, and Ion GeneStudio™ S5 Series sequencers.

Torrent Suite™ Software is organized in these sections, which appear as tabs:

- **Home** — View sequencer activity and learn about new software releases and product launches.
- **Plan** — Access both Planned Run templates (reusable experiment designs) and Planned Runs (executable instructions for individual sequencing runs). Select the experimental design for a Planned Run template that can be reused multiple times for sequencing runs.
- **Monitor** — View the status of your system and running jobs, including thumbnail quality graphs for current runs. The quality graphs provide near real-time information on your runs, so that you know early on about any instrument problems.
- **Data** — View summaries of completed runs, detailed run reports, and plugin results. Also download output files, download the run report, review the Planned Run settings, and group result sets into projects for data management such as archiving or pruning of result files.

Install Torrent Suite Software

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

Use these steps to install Torrent Suite™ Software:

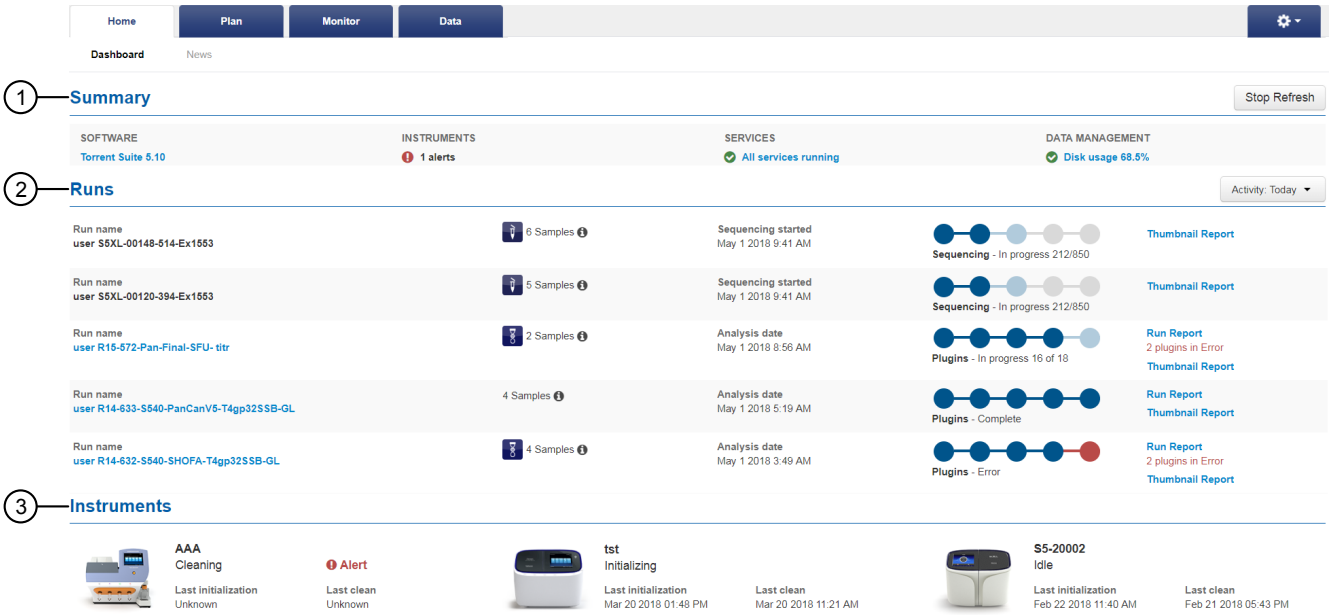
1. Log on to the Torrent Browser as an ionadmin user.
2. Click ⚙ **(Settings)**. Ensure that there are no active jobs running.
3. Click ⚙ **(Settings)** ▸ **Configure** ▸ **Admin Interface**.
4. Click **Update Server**.
5. Click **Check for updates**.
6. When the **Available** message appears, click **Update Server** to start the update process.

7. On the User Agreement (EULA) screen, scroll down to read and then accept terms to begin the update.

8. When finished, ensure that the "Upgrade completed Successfully!" message appears.

Dashboard at a glance

You can open the dashboard to see active and recent sequencing runs. The dashboard provides an overview of activity on all of your sequencers. Click **Home** tab, then click **Dashboard**.



1. Summary section
2. Run Report section
3. Instruments section

The **Summary** section includes:

- **Software** link – Opens the **Releases** screen, where you can click **Update** to update your software when new versions are released.
- **Instruments** column – Shows the number of instruments connected, offline, and those that have alerts.
- **Services** column – Reports statuses, such as services that are running, nodes down, and RAID storage statuses.
- **Data Management** column – Reports disk usage and data management activity.
- **Stop Refresh** stops the **Auto Refresh** function, which occurs every 30 seconds by default. Stop the 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. It includes:

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

  Note: 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 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.
- The **Activity** dropdown 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 **Run Report** links to completed run reports for the time interval you 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 **Instruments** section shows instrument statuses, which may include:

- **Alerts** – Instruments with an operational error.
- **Analyzing** – Instruments currently in use.
- **Cleaning** – Instruments currently undergoing a cleaning process.
- **Idle** – Instruments 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.

Note: Click ❗ **Alert information** to see details about an operational error. Then, resolve the issue on the sequencer.

News

Thermo Fisher Scientific makes announcements about new products, software releases and other news in the **News** screen. Click **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, but also allows server configuration, user configuration, base caller configuration, reference management, and data management. See “Software administration“ on page 285 for more information on Administrator functions.

Plan a run

The following steps describe how to use Planned Run templates and Planned Runs that fit into your Ion S5™, Ion S5™ XL, Ion PGM™, or Ion Proton™, and Ion GeneStudio™ S5 System sequencing workflows.

1. Decide on your sequencing application and sequencing product (such as an Ion AmpliSeq™ panel).
2. Select a pre-installed template with defaults for your application and sequencing product, or create your own template from scratch to customize your template.
3. Create new Planned Runs from your templates, adding the names of the samples to be sequenced.

   Plan templates and Planned Runs allow you to enter run information through the Torrent Suite™ Software instead of directly on the Ion sequencer. The use of templates and Planned Runs reduces the chance of error and wasted runs, reduces setup time on the sequencing instrument, and increases instrument throughput.
4. Start run on 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 menu list of Planned Runs. You can also overwrite (change) Planned Run information directly on the sequencer.

Register for a new account

Each new account requires administrator approval. It is not active until approval is granted.

Follow these steps to register for a new user account:

1. On the sign in page for Torrent Suite™ Software, click **Register**.
2. Enter the new user information, then click **Submit**.
   Upon the approval of an administrator, the new account is created.

Samples and Sample Sets

Samples in Torrent Suite™ Software are files that store information about genetic material from a single source. 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.

You can pre-select the attributes that you want to associate with each sample before you start to plan your templating and sequencing runs, and then organize samples into Sample Sets that you can select and then reuse when you create Planned Runs. This enables you to enter the information for the Sample Set just once, then reuse the Sample Set. See “Plan by Sample Set“ on page 55 for more information.

Grouping samples into Sample Sets is also helpful for using barcodes that have attributes assigned to each individual barcode. See “Barcodes and barcode sets“ on page 262 for more information. If you create Sample Sets prior to planning the run, you can enter barcodes and the barcode attributes just once when you create the Sample Set, and then select one or more Sample Sets when you create the Planned Run. This can save data entry time and reduce the likelihood of errors.

There are two ways to enter samples into Torrent Suite™ Software. You can enter information individually for each sample, or import samples 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 used an Ion AmpliSeq™ library preparation kit. If you want 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

You can use Torrent Suite™ Software to create a Sample Set by manually entering sample information into the software without the use of external CSV files. This process is useful for small Samples Sets. For Sample Sets that contain numerous samples, import samples as described in "Import samples with a CSV file" on page 19. To create a Sample Set manually, enter individual samples into the Torrent Suite™ Software, and then create a new Sample Set to add your samples to.

Alternatively, you can add your new samples to an existing Sample Set.

1. In the **Plan** tab, click **Samples**, then click **Enter Samples Manually**.
2. Click **Enter New Sample**.
    a. In the **Add Sample** dialog box, complete the fields as described in "Sample information" on page 20.

    Note: **Sample Name** is the only required field. Use the **Relationship Group** field to designate a group of related samples within the Sample Set. For example, DNA and RNA samples from the same sample would have the same **Relationship Group** number.

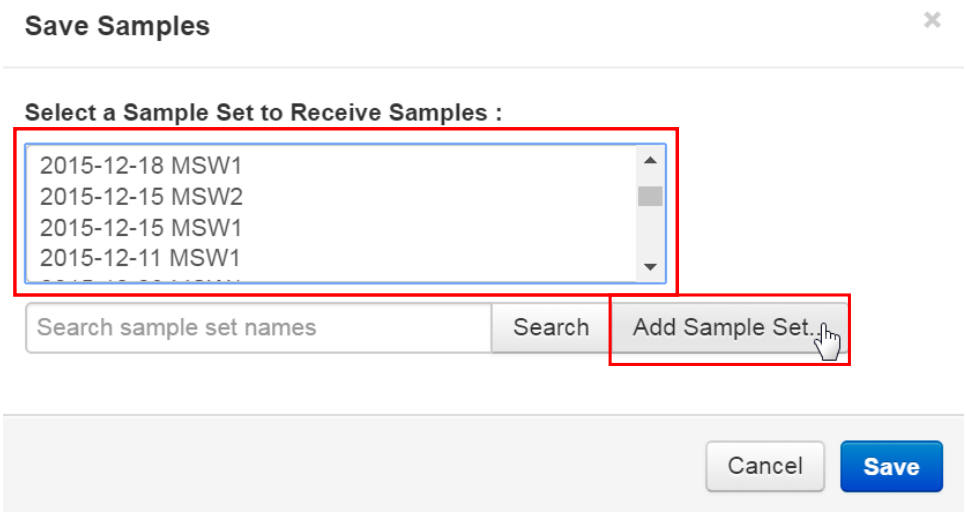
    b. Click **Done**.
    Your new samples and their attributes appear in the **Enter Samples** list.

    c. Enter additional samples as needed.

    CAUTION! The Sample Set is not saved until you click **Save Sample Set**. If you log out of the Torrent Server and do not save the Sample Set, the new sample(s) will not be saved.

3. Click **Save Sample Set**.

4. In the **Save Samples** dialog box:



- Select a Sample Set in the list to which you want to add the sample.
- Click **Add Sample Set** to create a new Sample Set, then complete the Sample Set information fields:

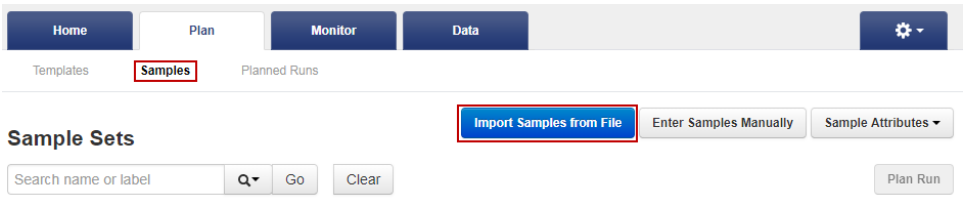
Field	Description
Sample Set Name	**(Required)** Enter a name for your Sample Set.
Group Type	*(Optional)* Select the Group Type that describes your Sample Set.
Library Prep Type	*(Optional)* Specify how your library was prepared.
Library Prep Kit	*(Optional)* Select the Library Prep Kit used to prepare your library.
PCR Plate Serial Number	*(Optional)* Enter PCR Plate Serial Number.
Description	*(Optional)* Provide a unique description for your Sample Set.

5. Click **Save**.
   Your new sample(s) are now added to the **Sample Sets** list. You can now use your Sample Set to create a Planned Run. The information from your Sample Set and individual samples within your Sample Set will be pre-populated in the fields of the Planned Run workflow bar steps and Planned Run template. See "Plan by Sample Set" on page 55 for more information.

Import samples with a CSV file

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

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



2. *(Optional)* To create a new sample file from the sample CSV file that is available in Torrent Suite™ Software:
   a. In the **Import Samples** section, click **Sample File Format** to download a sample CSV template.

      Note: The sample file format CSV contains the version of the CSV file in the top row, and sample attributes in separate columns. You must use a CSV file format that was downloaded from Torrent Suite™ Software 5.2, or later. Earlier software versions used a different format. To create a new CSV file, copy and paste the contents of your existing sample CSV file into the new file format.

   b. Open the CSV template and enter sample information into the cells, then save it to your computer. See "Sample information" on page 20 for more information.

3. Upload the sample file and optionally add a new Sample Set to receive the samples:
   a. In the **Import Samples** section, click **Select file** then navigate to and upload the sample import file.

   b. Click a Sample Set CSV file, then click **Open**.

   c. Select a Sample Set to receive the samples.

      To optionally add a new Sample Set to receive the samples, click **Add Sample Set**, and then complete the Sample Set information fields:

Field	Description
Sample Set Name	**(Required)** Enter a name for your Sample Set.
Group Type	*(Optional)* Select the Group Type that describes your Sample Set.
Library Prep Type	*(Optional)* Specify how your library was prepared.
Library Prep Kit	*(Optional)* Select the Library Prep Kit used to prepare your library.

Field	Description
PCR Plate Serial Number	*(Optional)* Enter PCR Plate Serial Number.
Description	*(Optional)* Provide a unique description for your Sample Set.

4. Click **Save & Finish**.
   The system loads, parses, and validates the file, then saves the samples and Sample Sets if no errors are found.

Sample information

This table describes the fields that you use to enter sample attribute information when you create a Sample Set manually, or import samples with a CSV file.

Note: All fields are optional except **Sample Name**. Some fields, however, are required if you transfer data to Ion Reporter™ Software, as described in the following table.

Field	Description
Sample Name	An open text entry field that allows any combination of alphanumeric characters plus spaces, periods (.), hyphens (-), and underscores (_). There is a 255-character limit. **Note:** These character limits 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, etc., is added to the sample name.
Sample External ID	*(Optional)* If you manage samples in an external system (for example, a LIMS), you can enter the identifier from that system in this field.
PCR Plate Position	*(Optional)* The well number of the sample in the PCR plate.
Barcode Kit	The name of the barcode kit used to make a library from the sample. The same barcode kit must be used for all samples in a Sample Set.
Barcode	The name of the specific barcode in the selected barcode kit. Assign a unique barcode to each sample in a Sample Set.
Control Type	The control type used when preparing the sample. Leave this field blank if no control is used.
Basic Annotations	
Description	An open text entry field.
DNA/RNA/Fusions	The type of library created from a sample.

Field	Description
Gender	The gender of the sample. IMPORTANT! If you are using Ion Reporter™ Software, do not leave this field blank. Select **Unknown** if the gender is not known. Several workflows in Ion Reporter™ Software (for example, copy number variation detection and genetic disease research) are limited when the gender is not known. The workflows can return unexpected results when the gender is incorrectly specified for a sample.
Type	The relationship type for this sample, used by Ion Reporter™ Software. Type is used in conjunction with the **Relationship Group** field, described below. For example, a relationship group can contain two samples, one with a type **Tumor** and another with a type **Normal**. The following sample relationships are supported by Ion Reporter™ Software: • Self • Tumor, Normal • Control, Sample • Father, Mother, Self **Note: Self** is used both for 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.
Relationship Group	A whole number used to define a sample as part of a relationship group. It is used in conjunction with the **Type** field, described above. 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.
Extra Annotations (used for specialized applications, such as preimplantation genetic screening (PGS) research or oncology research)	
Cancer Type	The type of cancer in the sample.
Cellularity %	The percentage of tumor cells in the sample.
Biopsy Days	The timepoint post-fertilization at which the biopsy was taken from an embryo.
Cell Number	The cell count of the biopsied material.
Couple ID	An identifier for use with the Reproductive research application.
User-defined Attributes	
<user defined>	If you create additional sample attributes, each attribute will be 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 into the field for that attribute.

Sample attributes

When you add a sample to a Sample Set in Torrent Suite™ Software, you must enter information to describe and identify each sample, with characteristics such as gender, sample origin, 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 Torrent Suite™ Software. Each attribute that you add will appear in:

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

A sample attribute can be made mandatory, in which case the user is 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 **Add Sample** dialog box.

Note: 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 workflow. If you select the Trio Sample Set grouping, Torrent

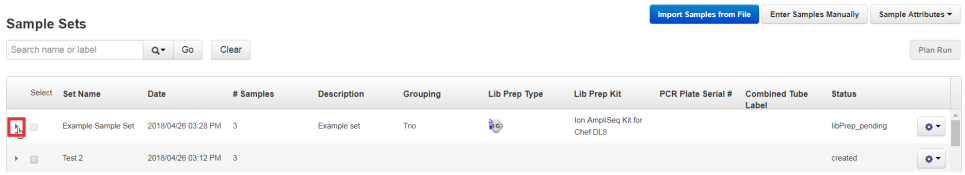
Suite™ Software will automatically select a Trio Ion Reporter™ Software workflow when you create a Planned Run.

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

- **Gender** is the gender of the sample.
- **Type** is the Ion Reporter™ Software relationship type used for **Example Sample Set**.
- The **Group** 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.)

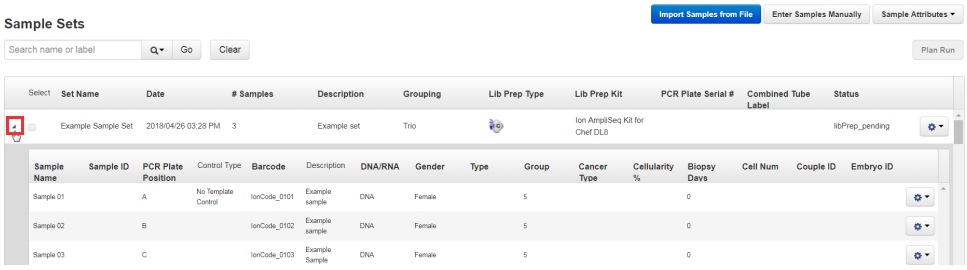
1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** table, find a sample set that you want to view. For more information on locating your sample set, see "Find a Sample Set" on page 26 and "Sort Sample Sets" on page 27.

   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.

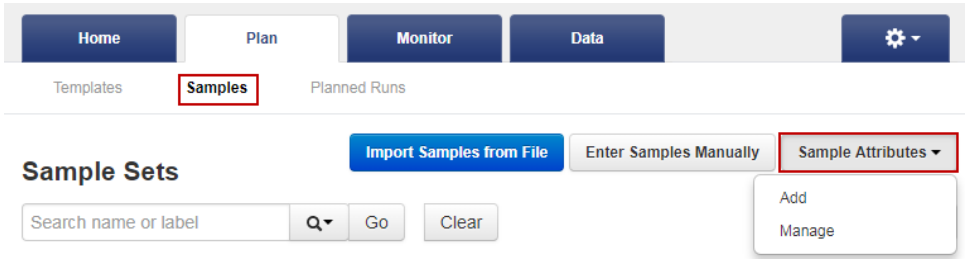
3. Click the arrow in the row of the Sample Set to expand the table and view all samples within the Sample Set.



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

Add a sample attribute

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**

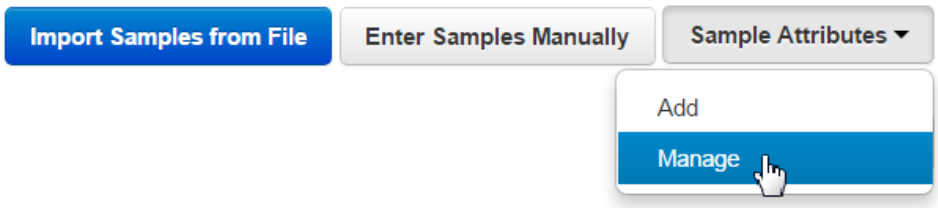
     Note: If the **Attribute Type** is set to Integer, you can only enter numeric characters (whole numbers) for this attribute.
   - *(Optional)* **Description**
3. *(Optional)* Select the **Is Mandatory** checkbox if you want the attribute to be required for every sample.
4. Click **Save**.

Manage sample attributes

If you add user-defined sample attributes to the 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, and then click one of the following:

Action	Description
Edit	• In the **Edit Attribute** dialog box, you can edit one or all of the following fields: a. **Attribute Name** b. **Attribute Type** c. **Attribute Description** • You can also change whether the attribute is required or optional by selecting or deselecting the **Is Mandatory** check box. **Note:** The attribute that is mandatory will be designated by the selected check box in the **Required** column of the **Sample Attributes** table. An absence of the selected check box designates the attribute as optional.
Show/Hide	• If you hide an attribute, that attribute no longer appears in the list of samples, Sample Sets, and **Add Sample** dialog box. **Note:** The attribute that is shown will be designated by the selected check box in the **To Show** column of the **Sample Attributes** table. An absence of the check box designates the attribute as hidden. • If you hide a mandatory attribute, that attribute is no longer mandatory.
Delete	Deleting a sample attribute permanently deletes that attribute from the Torrent Server.

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. This information can be useful for troubleshooting an Ion Chef™ Instrument run.

Note: This information is not available if a Library Prep Kit is not selected when the sample is created, of if the run did not use an Ion AmpliSeq™ library preparation kit.

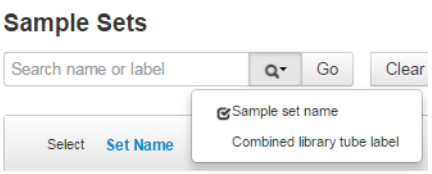
1. In the **Plan** tab, click **Samples**.
2. In the row that contains the Sample Set of interest, click **(Actions)** ▸ **Library Prep Summary**.
   The following information is listed for the Sample Set:
   - **Library Prep Type**
   - **PCR Plate Type**
   - **PCR Plate Serial Number**
   - **Combined 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**

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

Find a Sample Set

To find a Sample Set:

1. In the **Plan** tab, click **Samples**.
2. In the text field, enter a search term for the Sample Set name, or a Combined Tube Label (Sample Tube Label).
3. Click Q▾, then select **Sample set name** or **Combined library tube label**.

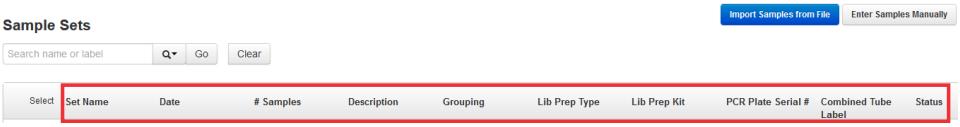


4. Click **Go**.
   The search results appear in a new table.
5. Click **Clear** to return to the complete list of Sample Sets.

Sort Sample Sets

To 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.



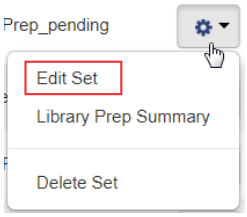
Note: Click the column header again to reverse the order of the column contents.

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

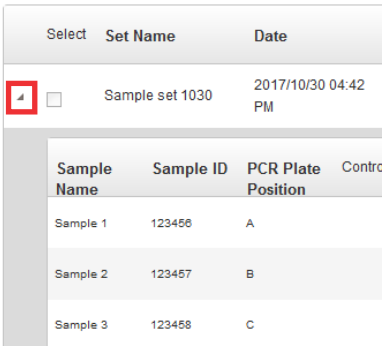
Edit a Sample Set

To edit a Sample Set:

1. In the **Plan** tab, click **Samples**.
2. Click ⚙ **(Actions)** ▸ **Edit Set** in the row of the Sample Set that you want to edit.



3. In the **Edit Sample Set** dialog box, make any desired changes in the following fields, then click **Done**.
   - Sample Set Name
   - Group Type
   - Library Prep Type
   - Library Prep Kit
   - PCR Plate Serial
   - *(Optional)* Description
4. To edit an individual sample in 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. In the **Edit Sample** dialog box, edit or enter information in the following fields if needed, then click **Done**.
      - Sample Name
      - Sample External ID
      - PCR Plate Position
      - Barcode Kit
      - Barcode

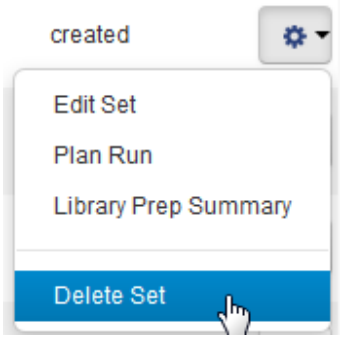
- Control Type
- Basic Annotations:
  - Description
  - DNA/RNA/Fusions
  - Gender
  - Type
  - Relationship Group

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

To delete a Sample Set:

1. In the **Plan** tab, click **Samples**.
2. Click ⚙ **(Actions)** ▸ **Delete Set** in the row of the Sample Set you want to delete.



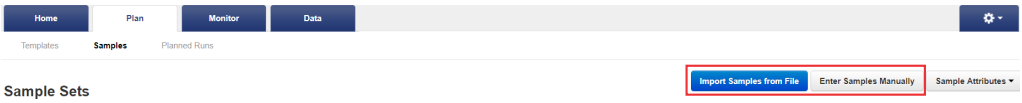
3. Click **Yes, Delete!**.

Note: To delete a sample from a Sample Set, see "Edit a Sample Set".

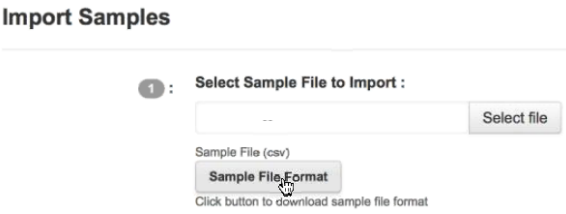
Create an Ion AmpliSeq™ on Ion Chef™ Sample Set

Ion AmpliSeq™ on Ion Chef™ samples can be automatically tracked in Torrent Suite™ Software from library preparation through sequencing and data analysis. First create a Sample Set in the Torrent Browser, 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 Sample Set, you can either import samples from a file, or enter them manually. The following is an example of importing samples from a file.



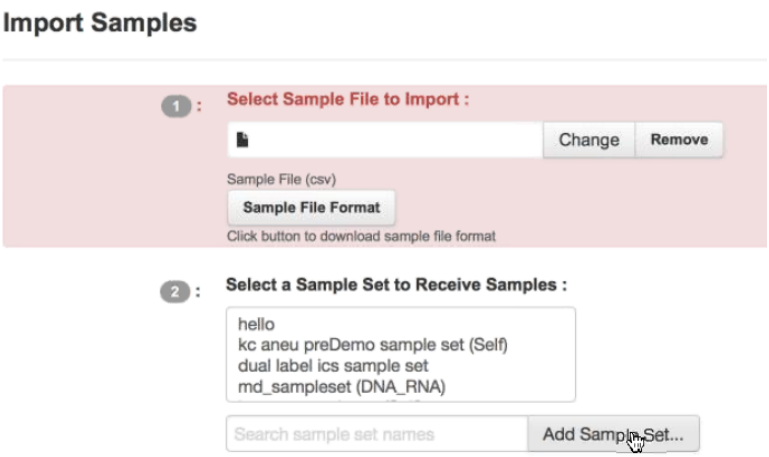
1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.
2. In the Import Samples dialog, click **Sample File Format** button.

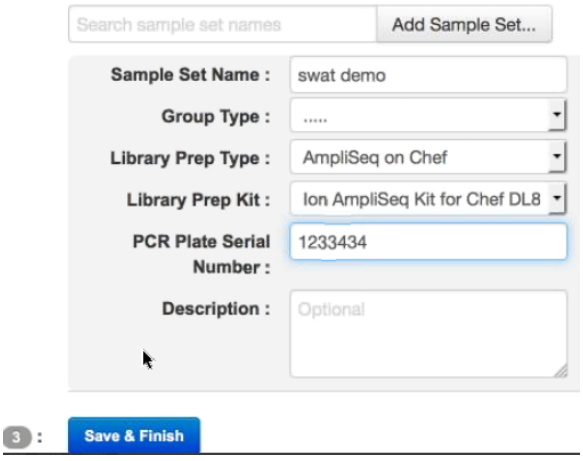
   A CSV template downloads.
3. Open the file, then enter sample names, PCR Plate positions, and Barcodes used. Save to your computer.

   Note: You can also enter sample names in the CSV file, then supply the plate position, barcode, and other information later from dropdown lists by editing the Sample Set in the **Sample Sets** screen. See "Edit a Sample Set" on page 27 for further information.
4. Click **Select File**, select your CSV file, then click **Open**.

5. Click **Add Sample Set**.



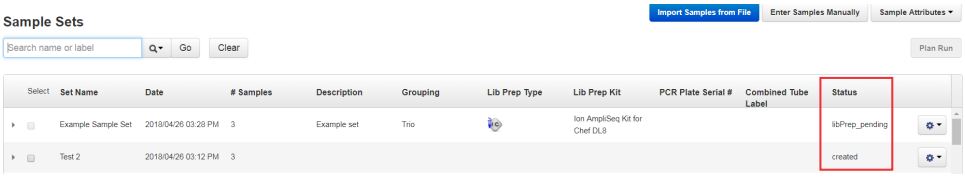
6. In the **Add Sample Set** dialog:
   a. Enter a Sample Set name.
   b. Select the appropriate **Group Type**.
   c. Set **Library Prep Type** to **AmpliSeq on Chef**.
   d. Set **Library Prep Kit** to **Ion AmpliSeq Kit on Chef DL8**.
   e. Enter or scan the PCR plate serial number.
   f. Enter any optional information in the **Description** field, then click **Save & Finish**.



A new Sample Set is created.

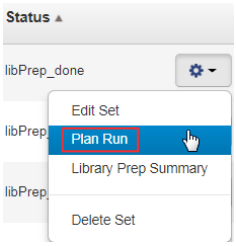
7. In the **Plan** tab, click **Samples**.

   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	New Sample Set ready for a library prep 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 prep run and the combined library is ready for a template run.

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



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

See "Plan and execute an instrument run" on page 32 for further information on how to create or edit a Planned Run template.

Plan and execute an instrument run

Planned Runs are the digital instructions for the sequencing instrument that contain specifications for sample preparation, sequencing, data export, and post-sequencing data analysis. Some key details that you can specify in a Planned Run include:

- Library barcodes
- Sample information
- Template kit
- Chip type
- Reference library
- Target and hotspot region BED files
- Plugins

Torrent Suite™ Software is pre-loaded with many Planned Run templates that contain pre-defined settings for common sequencing applications. 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. Templates also help reduce the chances of errors, because information is stored and then applied to Planned Runs, rather than entered manually for each run.

Planned Run templates

Planned Run templates are categorized into research applications that describe the sequencing techniques, or specialized products, for which each template is used. Some templates are found in more than one category.

Research application	Contains templates for . . .
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.

Research application	Contains templates for . . .
Generic Sequencing	Applications that do not fit in the other categories. Use this research application to provide all 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.
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 Panels 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

We recommend that you create a customized 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 system template then edit the settings to meet the requirements for your Planned Run.

IMPORTANT! Before you create a custom Planned Run template, we recommend that you upload the most current **Reference Library**, **Target Regions**, and **Hotspot Regions** BED files on the Torrent Server. See "Reference Management" for more information. Contact your local service representative to obtain the most current BED files.

1. In the **Plan** tab, click **Templates**, then select the desired **Research Application** from the left navigation menu.
2. In the Planned Run list, 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 each field in the **Save** step.

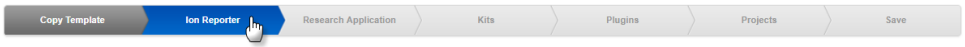
Field	Description
Template Name (required)	Enter a name for you custom Planned Run template.
Set as Favorite	Select the **Set as Favorite** checkbox to add your custom template to the **Favorites** list.
Analysis Parameters	Select the **Default** radio button to accept default analysis parameter settings (recommended). Advanced users can customize analysis parameters by selecting the **Custom** radio button and editing appropriate analysis fields. For more information, see "Create and select an analysis parameter set" on page 348.
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[1]	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.
hotspots[2]	Select the hotspots (BED or VCF) file appropriate for your sample.

[1] Check with your service representative for updates to ensure the most current files are being used. See "Upload a target regions file" on page 240 for BED file installation instructions.

[2] See "Upload a hotspots file" on page 242 for BED file installation instructions.

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

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. See "Ion Reporter™ step in the workflow bar" on page 38 for more information.



5. Click **Next**.

6. In the **Research Application** step, ensure that the correct **Research Application** and **Target Technique** are selected. See "Research Application step in the workflow bar" on page 40 more information, then click **Next**.

7. In the **Kits** step, enter or select the required information. See "Kits step in the workflow bar" on page 41 for more information.

8. Click **Next**.

9. In the **Plugins** step (see "Plugins step in the workflow bar" on page 44 for more information), select from the available plugins, and configure the selected plugins as required (see "Plugin configuration" on page 113 for more information). Click **Next**.

10. In the **Projects** step, select the project or projects to receive data from the runs that use this template, then click **Next**. See "Projects step in the workflow bar" on page 45 for more information.

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

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

Create a Planned Run from a template

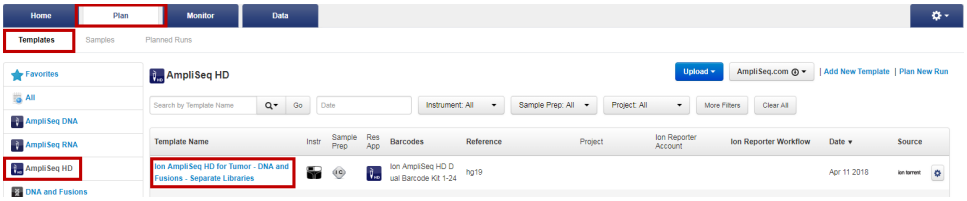
You can create Planned Runs from preinstalled or custom templates of run settings. Preinstalled Planned Run templates are included in the software for standard research applications and kits, such as Ion AmpliSeq™ or Oncomine™ workflows. Custom Planned Run templates can be created by users as described in "Create a custom Planned Run template" on page 34.

Note: When creating a Planned Run from a template, some settings and fields are already defined by the template and some remain to be selected or filled in. The fields and settings that are predefined can vary between templates. You can change any of the settings to create your Planned Run, even those that were predefined in the template.

Follow the steps below to create a Planned Run from a template.

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

2. Click on the template name in the **Template Name** column of the templates table.



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

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

4. Fill out additional fields in the **Plan** step as necessary. See "Plan step in the workflow bar" on page 45 for more information about the individual settings.

   Note: As you make your selections, your 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 on other steps in the workflow bar to enter or change settings as needed.

6. When you have completed your selections, review the settings in **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.

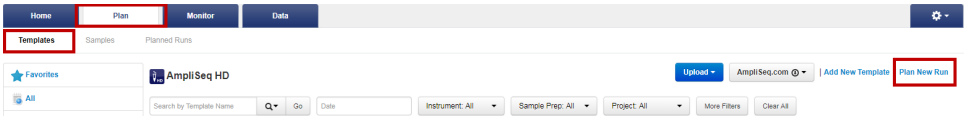
Create a Planned Run without a template

You can create a Planned Run with no predefined settings, as described in this section.

Note: We recommend creating Planned Runs from preinstalled or custom templates in Torrent Suite™ Software. 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). See "Create a custom Planned Run template" on page 34 and "Create a Planned Run from a template" on page 35 for more information.

To create a Planned Run without a template, follow the steps below.

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. See "Ion Reporter™ step in the workflow bar" on page 38 for more information.

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

3. Click **Next**.

4. In the **Research Application** step, ensure that the correct **Research Application** and **Target Technique** are selected. See "Research Application step in the workflow bar" on page 40 for more information, then click **Next**.

5. In the **Kits** step, enter or select the required information. See "Kits step in the workflow bar" on page 41 for more information.

6. Click **Next**.

7. In the **Plugins** step (see "Plugins step in the workflow bar" on page 44 for more information), select from the available plugins, then configure the selected plugins as required (see "Plugin configuration" on page 113 for more information).

8. Click **Next**.

9. In the **Projects** step, select the project or projects that will receive data from the runs that use this template. See "Projects step in the workflow bar" on page 45 for more information.

10. Click **Next**.

11. In the **Plan** step, enter a name for the plan in the **Run Plan Name** field, specify the reference and BED files, and enter or upload your sample information. See "Plan step in the workflow bar" on page 45 for more information about the individual settings.

12. 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.

Steps in the workflow bar

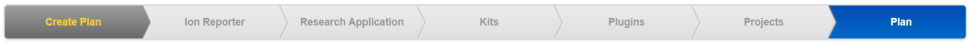
Torrent Suite™ Software guides you through steps to provide the information required to create Planned Runs and templates, and then execute a Planned Run. You can use the steps in the following ways:

- Create a Planned Run that is based on a template that is pre-populated with information specific for your instrument run.
- Create custom Planned Run templates that fit your sequencing needs, and save the templates to reuse for future Planned Run creation.
- Create a unique Planned Run that is not based on a template.

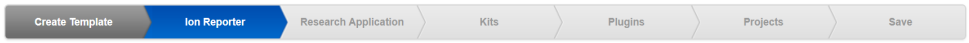
Note: Most templates have a corresponding Ion AmpliSeq™ or Oncomine panel.

When you create a Planned Run from a template, the Create Plan workflow bar opens in the last page, so that if you accept all the template settings, you only need to supply a run plan name and sample names to create a Planned Run.

- Create Plan workflow bar:



- Create Template workflow bar:



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

Note: You can also download a CSV file and customize it to create multiple Planned Runs without using the workflow bar. See "Create multiple Planned Runs" on page 61 for more information.

Ion Reporter™ step in the workflow bar

Note: Selections on this page apply only to Ion Reporter™ Software users. The Ion Reporter™ Software is not included with Torrent Suite™ Software and is available under separate license. See "Integration with Ion Reporter™ Software" on page 172 for more details.

When you create a Planned Run or a Planned Run template, you can add settings to automatically transfer run results to Ion Reporter™ for further analysis and visualization. You can directly transfer results in one of the following ways:

- If you select an Ion Reporter™ account and a workflow, then also select the option to automatically upload after run completion, an Ion Reporter™ 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™ without selecting an Ion Reporter™ Software workflow. In this case, you can then launch the analysis manually in Ion Reporter™. This approach is commonly used to annotate the VCF files by using the Annotation-only workflow in Ion Reporter™ Software . For details, see the *Ion Reporter™ Software Help* (Pub. No. MAN0017204).

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

1. In the **Ion Reporter** step in the workflow bar, select the Ion Reporter™ account that you want to transfer output files to for analysis. The selected account is the one that you will use to view and further analyze the files in Ion Reporter™ Software.

   Note: If the Ion Reporter™ account is not configured, click **Configure** to add another account. See "Set up an account for IonReporterUploader plugin" on page 173 for more information.

2. Select a **Sample Grouping** that corresponds to the sample relationship in Ion Reporter™ Software. When you select a **Sample Grouping**, the workflow menu in Ion Reporter™ Software displays only workflows that are appropriate for the sample.

3. Select an option in the **Existing Workflow** dropdown list:

Option	Description
Select **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.
Select an Ion Reporter™ Software workflow for your sample type.	The workflow is automatically launched in Ion Reporter™ 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)* If you want to create a new workflow, click **Create New Workflow** to open Ion Reporter™ Software in a new browser window. In Ion Reporter™ Software, create your new workflow, then save it. When you return to your Torrent Suite™ Software, refresh your browser. You can then select the newly created 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 if you want to review the completed run results and then manually upload the data to Ion Reporter™ Software. IMPORTANT! You must 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. If you select a workflow, an Ion Reporter™ analysis is launched immediately after the run. Successful analyses are then available to you in Ion Reporter™ when you sign into Ion Reporter™ Software with the account used in the setup.

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

Research Application step in the workflow bar

In the **Research Application** step, select your Research Application and Target Technique.

Note: Available choices in subsequent steps are restricted to those compatible with the selected Research Application and Target Technique.

1. Select the **Research Application**.

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 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 variants (SNPs, InDels and CNVs) from targeted DNA libraries, as well as gene fusions from matching targeted RNA libraries using Ion AmpliSeq™ HD technology. This 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 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 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 Ion AmpliSeq™ Pharmacogenomics Research Panels that are 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**.
    - AmpliSeq DNA
    - AmpliSeq Exome
    - AmpliSeq HD - DNA
    - AmpliSeq HD - DNA and Fusions (single library)
    - AmpliSeq HD - DNA and Fusions (separate libraries)
    - Other
    - TargetSeq
    - Whole Genome
    - AmpliSeq DNA and Fusions
    - AmpliSeq RNA
    - 16S Targeted Sequencing
    - Tag Sequencing
    - RNA Sequencing

Kits step in the workflow bar

This step is used to set up all information needed for sample preparation and sequencing, including the chip and kits used. Selections in this step will also influence how the data is analyzed post-sequencing.

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

Field	Selection
Instrument	Select the sequencing instrument system being used (for example, Ion GeneStudio™ S5 Systems).
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. 2. From the list, select the templating kit that was used.
Sequencing Kit	Select the sequencing kit used (for example, Ion S5™ Sequencing Kit).
Chip Type	Select the sequencing chip type that will 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).

Field	Selection
Mark as duplicate reads	Marks duplicate reads in the BAM file, after a run is completed. Do not use with Ion AmpliSeq™ data. See "About the Mark as Duplicates Reads option" on page 43 for details. To remove duplicates from the BAM file, select the FilterDuplicates plugin in the Plugins step in the workflow bar. See "Plugins step in the workflow bar" on page 44 for more information.
Enable Realignment	Select this option to perform realignment, an optional step that is executed immediately after TMAP. This steps adjusts the alignment, primarily in the CIGAR string. See "TMAP Modules" on page 360 for more information.

2. *(Optional)* Customize the **Advanced Setting** parameters. See "Advanced Settings – Kits step in workflow bar" on page 42 for more information.

   IMPORTANT! We recommend using the default settings. Consult your local field representative before modifying any **Advanced Setting**.

Advanced Settings – Kits step in workflow bar

IMPORTANT! We recommend using the default settings. Consult your local field representative before modifying any parameters.

1. In the **Kits** step in the workflow bar, expand the **Advanced Settings** box, then select **Customize**.

2. Edit one of the following parameters.

Setting	Description
Templating Protocol	Script the Ion Chef™ Instrument follows to perform the templating reaction. We recommend you do NOT change this setting.
Forward Library Key	Select your forward library key. If you have a custom forward library key, select it here.
Test Fragment Key	Enter your test fragment key.

Setting	Description
Base Calibration Mode	Select one of the following options: • **Default Calibration** – allows a random subset of wells to be used for base calibration. This option uses TMAP to align the training subset of wells and is recommended if a good reference for the template is available. • **Enable Calibration Standard** – allows wells belonging to the Calibration Standard to be selected as the training subset. • **Blind Calibration** – uses the same random subset of wells as Default Calibration but does not require an alignment step to generate the calibration model. This option is recommended if the template does not align well to a reference genome or if no reference is specified. • **No Calibration** See your template kit user guide for more details.
Forward 3' Adapter	Select your forward 3' adapter.
Flow Order	Select the flow order. See “” on page 371 for additional details.

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 may 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.

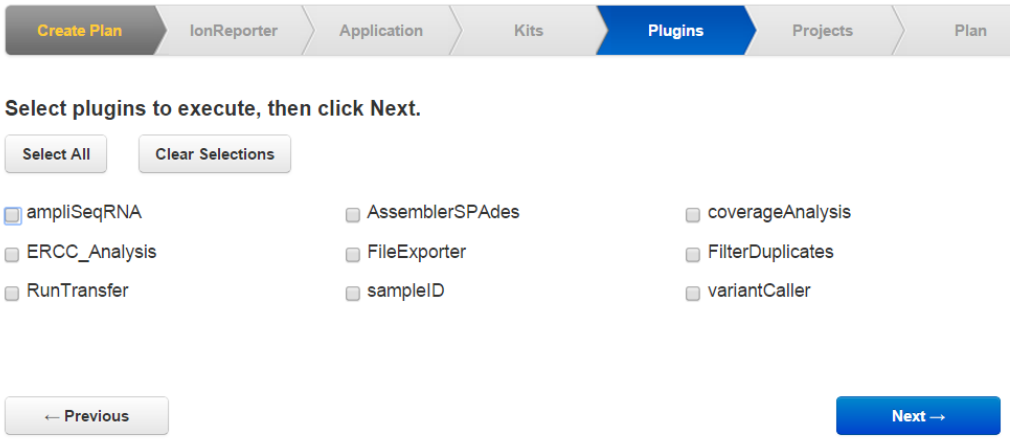
Torrent Suite™ Software uses an Ion-optimized approach that considers the read start and end positions by using both the 5' alignment start site and the flow in which the 3' adapter is detected. Duplicate reads are flagged in the BAM in a dedicated field. Use of the Torrent Suite™ Software method is 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

Plugins expand the analysis capabilities of in Torrent Suite™ Software. The plugin results are added to the run report and can be used for a variety of purposes. You can set the plugin to run automatically after every run if you add the plugin, and configure it if required, to the Planned Run or Planned Run template.

In the **Plugins** step in the workflow bar, select the plugins to include in a Planned Run, or a Planned Run template. Plugins run automatically during run analysis, and plugin analysis results appear in the run report.



Note:

- The list of available plugins depends on which plugins are active on the Torrent Server that is connected to the instrument you use. Plugins that are installed, configured, and enabled on your 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 variantCaller and coverageAnalysis plugins, a **Configure** link appears for that plugin. For information on configuring each plugin, see "Pre-installed plugins" on page 120.
- If you select the IonReporterUploader 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 IonReporterUploader plugin, see "Variant calls in Torrent Suite Software" on page 191.

Projects step in the workflow bar

In the **Projects** step in the workflow bar, 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 "Organize run results with projects" on page 82.

In the scrolling list, select the checkbox next to each project name. You can also search for or add projects as described below.

Note: The list includes all projects that are created on the Torrent Server.

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

Plan step in the workflow bar

1. In the **Plan** step in the workflow bar, enter or select the required information in each of the following fields.

Field[1]	Description
Run Plan Name (required)	Enter a name for the Planned Run.
Analysis Parameters	Select the **Default** radio button to accept default analysis parameter settings *(recommended)*. Advanced users can customize analysis parameters by selecting the **Custom** radio button and editing appropriate analysis fields. For details, see "Create and select an analysis parameter set" on page 348.
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[2]	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.
Hotspots[3]	Select the hotspots (BED or VCF) file appropriate for your sample.

[1] Depending on your sequencing application, fields can vary.

[2] Check with your service representative for updates to ensure the most current files are being used. See "Upload a target regions file" on page 240 for BED file installation instructions.

[3] See "Upload a hotspots file" on page 242 for BED file installation instructions.

2. 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.

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, fill out the following fields:

Option	Description
Number of barcodes	For barcoded samples, enter the number of barcodes to be used in this run in the **Number of barcodes** field, then click ✓ to the right of this field. Alternatively, click on the first barcode and ⬇ appears. Click ⬇ and all subsequent barcodes will be numbered from the first barcode value.
Number of chips	For non-barcoded samples, enter the number of chips to be used in this run in the **Number of chips** field, then click ✓.

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

5. In the **Sample Tube Label** field(s), scan or enter the barcode of the Ion Chef™ sample tubes to be used in the run.

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

7. *(Optional)* Select a radio button next to the sequencing application (such as "Oncology" or "Pre-implantation Genetic Screening") to populate required sample information fields into the **Samples Table**.

8. Complete the following fields in the **Samples Table**.

   - Save the samples table to a CSV file, fill out all required sample information, then upload the samples table to automatically populate the **Samples Table**.
     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** 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 Torrent Suite™ Software.

Field[1]	Description
Barcode	For barcoded samples, select a barcode from the dropdown list.
Sample (required)	Enter a unique sample name for each sample. Do not duplicate sample names.

Field[1]	Description
Control Type	*(Optional)* Click the **Control Type** column header to expand the **Control Type** column, then select the control type from the dropdown list.
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** from the dropdown list for each sample.
Reference	If using different reference and BED files for one or more samples, click the **Reference** column header to expand the **Reference** sections, then select Reference, Target Regions, and hotspots files from the dropdown list for each sample.
Annotations	Click the **Annotations** column header to expand the annotation fields specific for your application (e.g., cancer type or Embryo ID) and complete the required field information.
Ion Reporter workflow	Select the Ion Reporter™ workflow specific for your run from the dropdown list. If you do not see your workflow, select the **Show All Worklows** checkbox in the column header.
Relation	Select sample relationship group.
Gender	Select "Male", "Female", or "Unknown" from the dropdown list.
IR Set ID	The IR Set ID is set to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same Set ID are considered related samples and are launched in the same analysis (e.g, normal sample and its corresponding tumor sample). Do not give unrelated samples the same Set ID value even if the value is zero or blank.

[1] Depending on your sequencing application, fields can vary.

9. *(Optional)* Add a note about your Planned Run in the **Add a note** field.

10. *(Optional)* If using LIMS meta data, enter the text that is associated with the Torrent Suite™ Planned Run in the **Add LIMS Meta Data** field.

11. *(Optional)* In the **Monitoring Theresholds** pane, adjust Bead Loading (%), Key Signal (%), and Usable Sequence (%) minimum thresholds for your Planned Run.

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

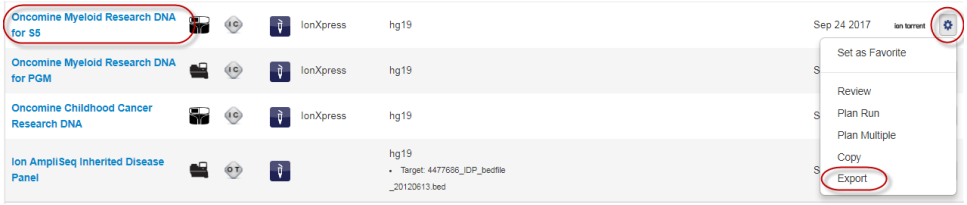
12. When you are finished with all of your selections, scroll to the bottom of the screen, then click **Plan Run**.

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

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 Torrent Server and import the template into Torrent Suite™ Software on that server (see "Import a Planned Run template" on page 48). 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.

In the **Plan** tab, in the **Templates** screen, locate the template that you want to export. 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.

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

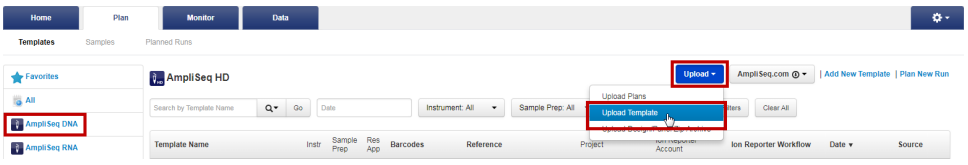
Import a Planned Run template

You can import Planned Run template settings that have been exported to a CSV file. This is useful for transferring settings between different Torrent Servers. (To export the settings, see "Export a Planned Run template" on page 48.)

Note: You can change the settings in an exported CSV file and then import, but be careful to preserve the column headings and layout.

To import a CSV file containing template settings:

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 appears listed in the application group. Exported 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**.

Review the 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.

2. In the **Summary** section, click **Report Actions** ▸ **Review Plan**.
   A Review Plan summary lists the details of the Planned Run.

Category	Subcategories
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 PCR Duplicates
	Base Calibration Mode
	Enable Realignment
Barcodes & Samples	Sample Tube Label
	Chip Barcode

Category	Subcategories
	Barcode
Analysis Parameters Used (Default)	BeadFind Args
	Analysis Args
	Pre-BaseCaller Args for Calibration
	Calibration Args
	BaseCaller Args
	Alignment Args
	IonStats Args
History	Log of system actions
Monitoring	Bead Loading (%)
	Key Signal (1-100)
	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

Import panel files and parameters from AmpliSeq.com

You can import panel files and parameters for an Ion AmpliSeq™ Ready-to-Use, On-Demand, or Made-to-Order design. The package includes primer pool results panel file, hotspot BED files, and JSON parameters files required to produce your sequencing results. For Ready-to-Use and Community Panels (but not Made-to-Order Panels), 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 194. Human, animal, and plant reference BED files are also available for import. Before importing, you need to link your Torrent Server account with your AmpliSeq.com account. For more information about Ion AmpliSeq™, see *Ion AmpliSeq™ Designer: Getting Started User Guide* (Pub. No. MAN0010907).

Link your Torrent Server account to AmpliSeq.com

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

1. In the Torrent Browser, click **(Settings)** ▸ **Accounts**.
2. If needed, update or enter any account information in the **User Profile/Account Information** section, then click **Submit**.
3. Click **Link to AmpliSeq**.

   Note: You may need to scroll down the screen to view the link button.

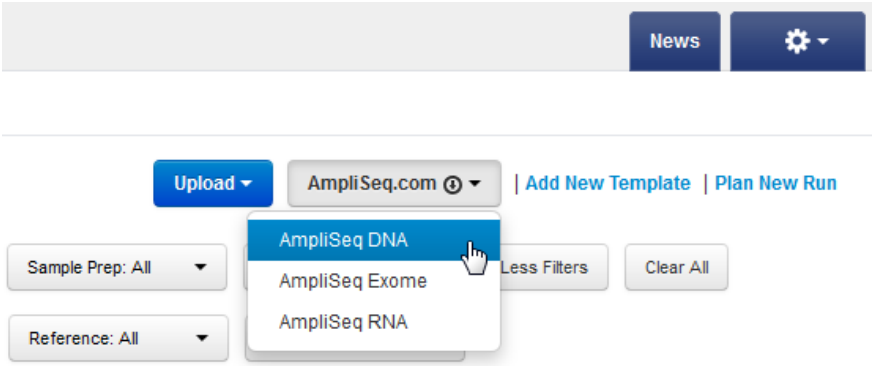
4. Enter your AmpliSeq.com sign in information, then click **Save**.

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

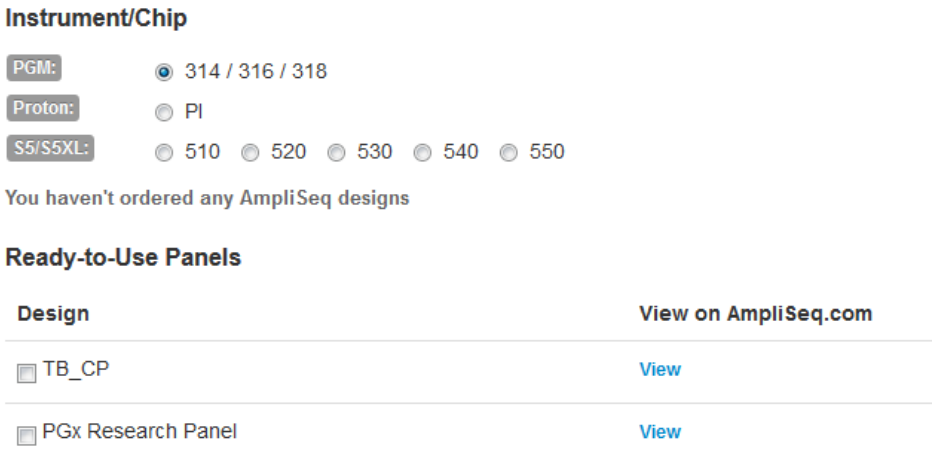
Note: To unlink your Torrent Server account, click **Unlink username@domain.com**.

Import Planned Run template parameters from AmpliSeq.com

1. In the **Templates** screen, click **AmpliSeq.com**, then select from the dropdown list the type of panel you want to import: AmpliSeq DNA, AmpliSeq Exome, or AmpliSeq RNA.



2. In the next screen, select your instrument and chip type, then select a panel or panels from the list of available panels.



Note:

- Some panels do not have optimized variantCaller plugin parameters available for all chips and sequencers. A warning identifies panels for which optimized variantCaller parameters have not been developed for the selected chip type.

- If you have ordered Ion AmpliSeq™ Made-to-Order designs, a list for selecting the panel files for import also appears on this screen.
- If you have downloaded a panel's ZIP archive 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. Click **Import Selected**.

4. The Torrent Browser opens a download and progress dialog box. Refresh your browser to track the progress. When the **Status** column shows "Complete", return to the **Templates** screen.
   The new template is listed.

Upload panel files from Ampliseq.com

You can upload panel files from **AmpliSeq.com** that are saved in a storage location to Torrent Suite™ Software. Use this procedure when only part of the panel files are available from the compressed archive, rather than the complete set of panel files.

Note: If the entire compressed directory of panel files from **AmpliSeq.com** are available, see instead "Import Planned Run template parameters from AmpliSeq.com" on page 51.

1. In the **Plan** tab, click **Templates**.
2. Click **Upload ▸ Upload Design/Panel Zip Archive** and the **Import Panel from Zip Archive** dialog box opens.
   a. Click **Select File** and browse to your compressed panel file.
   b. Select instrument type and chip type.
   c. Click **Upload Panel from Zip Archive**.
3. Close the upload dialog box.
4. To check the status of the upload, click **(Settings) ▸ Reference**, then click **Upload History**.

   Note: If you don't choose the correct chip type you will receive a validation error.

Search for a template

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

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

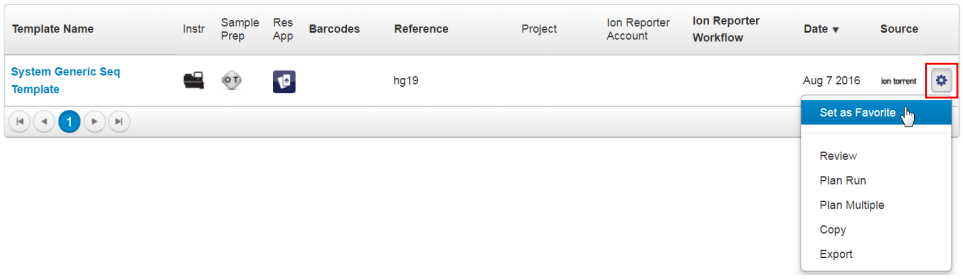
To...	Actions...
Search the list	1. Click **All** in the research applications menu on the left to search all templates. Alternatively, select a research application from the menu to search for templates within a specific research application. 2. Enter a search term in the **Search by Template Name** field, then click **Go**.
Sort the list	Click on any bolded column header in the list of templates to sort the order in which the templates are displayed. Click on the column header a second time to reverse the sort order.
Limit the list to recent runs	In the **Date** field select a preset range, or click **Date Range**, then select **Start** and **End** dates.
Filter the list	Select from one or more Filter dropdown lists to limit the list of templates. Click **More Filters** to see all available filters. Within a Filter, enter text into the **Find** field to limit the filter choices. To remove a filter, de-select the filter choice or click **Clear** in the Filter dropdown list. **Note:** 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.

Save a Planned Run template to Favorites

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

1. In the **Plan** tab, click **Templates**, then search for a template (see "Search for a template" on page 54) you want to add to **Favorites**.
2. In the row of the template, click ⚙ **(Actions)** ▸ **Set as Favorite** to add your Planned Run template to the **Favorites** list.



3. In the **Plan** tab, click **Templates**, then click **Favorites** at the top of the research applications list to display the list of favorite Planned Run templates that are saved on your Torrent Server.

Plan by Sample Set

You can define samples and pre-select the attributes that you want to associate with each sample before you start to plan your templating and sequencing runs. You can then organize samples into Sample Sets that are available to select and reuse when you create Planned Runs.

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

Create a Planned Run with Sample Sets

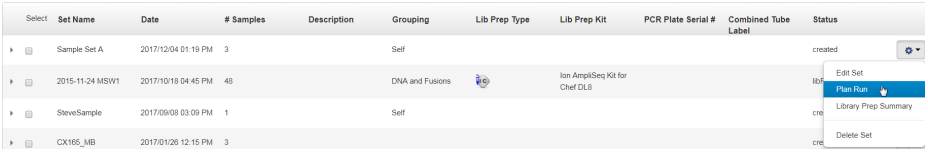
If you set up your samples before you plan an instrument run, you can add one or more Sample Sets to your Planned Run.

Sample Sets must correspond to AmpliSeq™ library preparations and use the same barcode kit to be included in a single Planned Run.

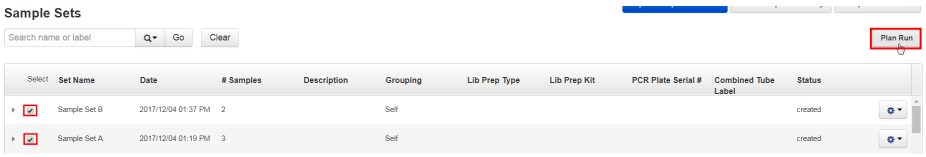
1. In the **Plan** tab, in the **Samples** screen, find the Sample Set(s) that you want to add to the Planned Run.

2. Select one or more Sample Sets to add to the Planned Run.

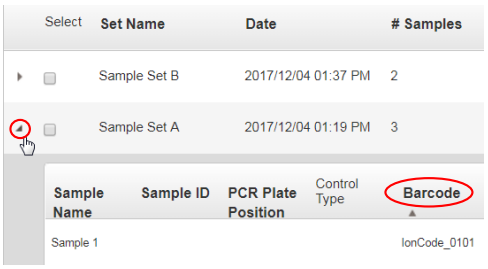
   - To plan a run using one Sample Set, click ⚙ **(Actions)** ▸ **Plan Run** in the row of the Sample Set.



   - To plan a run using multiple Sample Sets, select the checkboxes next to the Sample Sets you want to add to the Planned Run, then click **Plan Run**.

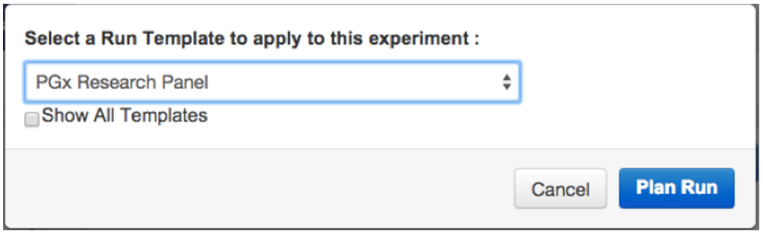


IMPORTANT! Ensure that all Sample Sets used in the Planned Run use the same barcode kit. To verify the barcode kit used, expand the Sample Set entry to view its details.



The **Select a Run Template to apply to this experiment** dialog lists Planned Run templates that support your Sample Set.

3. Select a Run Template to use for the experiment, then click **Plan Run**.



Note: 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 in each of the following fields.

Field[1]	Description
Analysis Parameters	Select the **Default** radio button to accept default analysis parameter settings *(recommended)*. Advanced users can customize analysis parameters by selecting the **Custom** radio button and editing appropriate analysis fields (see "The Command Line Args (Advanced) tab" on page 349.
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[2]	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.
hotspots[2]	Select the hotspots (BED or VCF) file appropriate for your sample.

[1] Depending on your sequencing application, fields can vary.
[2] 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 the **Sample Tube Label** field(s), scan or enter the barcode of the Ion Chef™ sample tubes that will be used in the run.

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

8. Fill out or select the following fields in 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 automatically populate the **Samples Table**.
     a. Click **Save Samples Table** button 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** in Torrent Suite™ Software with sample information supplied by the CSV file.
   - Alternatively, manually enter sample information into the **Samples Table** using the Torrent Suite™ Software.

Field[1]	Description
Barcode	For barcoded samples, select a barcode from the dropdown list.
Sample (required)	Select a sample that is a part of the selected sample set(s) from the dropdown list.
Control Type	Click on the **Control Type** column header to expand the **Control Type** 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 27.
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 27.
DNA/Fusions	For DNA and Fusions application, select **DNA** or **Fusions** from the dropdown list for each sample.
Reference	If using different reference and BED files for one or more samples, click the **Reference** column header to expand the **Reference** sections and select Reference, Target Regions, and hotspots files from the dropdown list for each sample.
Annotations	Click the **Annotations** column header to expand the annotation fields specific for your application (e.g., cancer type or Embryo ID) and complete the required field information.
Ion Reporter workflow	Select the Ion Reporter™ workflow specific for your run from the dropdown list. If you do not see your workflow, select the **Show All Worklows** checkbox in the column header.
Relation	Select sample relationship group.

Field[1]	Description
Gender	Select **Male**, **Female**, or **Unknown** from the dropdown list.
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 Set ID are considered related samples and are launched in the same analysis (e.g, normal sample and its corresponding tumor sample). Do not give unrelated samples the same Set ID value even if the value is zero or blank.

[1] Depending on your sequencing application, fields can vary.

9. Continue with the steps to create the Planned Run. See "Steps in the workflow bar" on page 37 for more information.

10. Click **Save & Finish**.

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

Create a Planned Run for mixed samples with a template

To plan a run for DNA and Fusion sample pairs and several individual Fusion or DNA samples, start with a fusions template, then alter it to accommodate single samples on the same chip. The example that follows is 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 you use, then in that row click **(Actions)** ▸ **Copy**.

3. Enter a **Template Name** and 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.

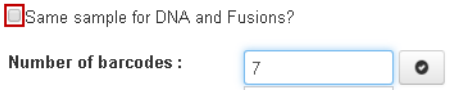
5. In the **Ion Reporter** step of the workflow bar, select an Ion Reporter account and workflow, ensure the **DNA and Fusions** Sample Grouping is selected, then click **Next**.

6. Ensure that the **DNA and Fusions** Research Application and **AmpliSeq DNA and Fusions** Target Technique is selected, then click **Next**.

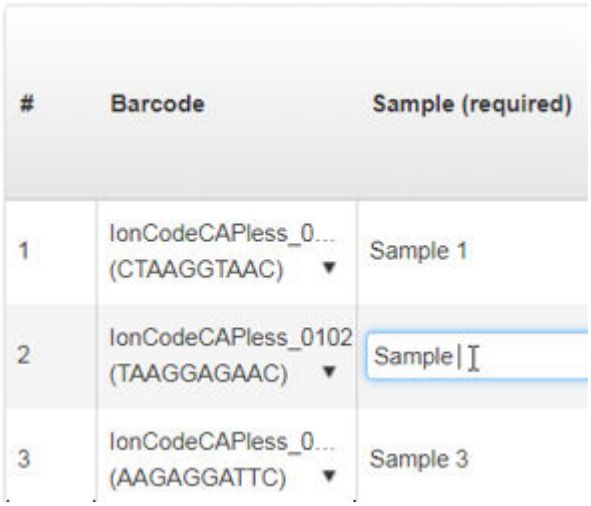
7. Click **Plan** in the workflow bar.

8. Enter the number of barcodes, then click ✔.

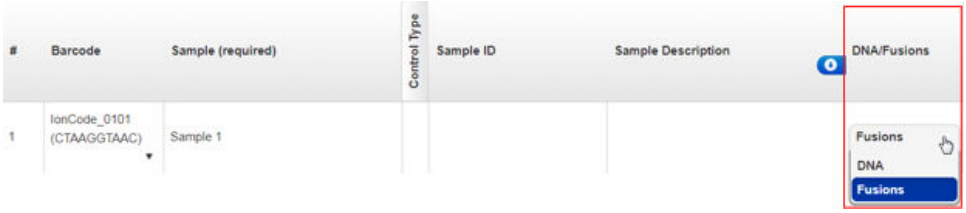
9. Deselect the **Same sample for DNA and Fusions** option.



10. Rename the samples if desired. To rename the samples, click on the sample name in the **Samples** table, then enter a new name in the text field.

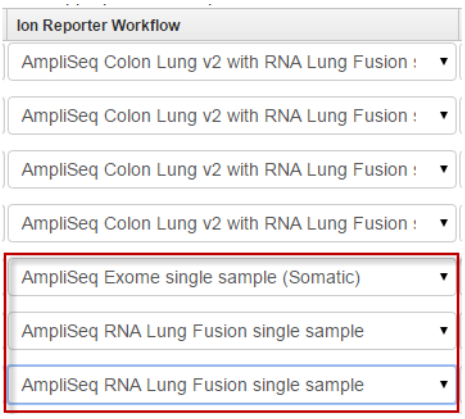


11. Change DNA/Fusions selections to match samples. To change the DNA/Fusions selection for a sample, navigate to the DNA/Fusions column in the **Samples Table** and select DNA or Fusions from the dropdown list in the row of the sample.



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

13. Select the appropriate Ion Reporter workflows. To select an Ion Reporter workflow, click on the existing selection in the **Samples Table**, then select from the dropdown list.



14. Select a **Relation** and**Gender** in the **Samples Table**, then click **Plan Run**. See "Create a Planned Run with Sample Sets" on page 56 for more information.

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. In Torrent Suite™ Software 5.4 and earlier, each row of the CSV batch planning template contained data for an individual Planned Run. In Torrent Suite™ Software 5.6 and later, each column in the CSV file represents an individual Planned Run. Each row contains the run parameters for each individual Planned Run. In Torrent Suite™ Software 5.4 and later, 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.

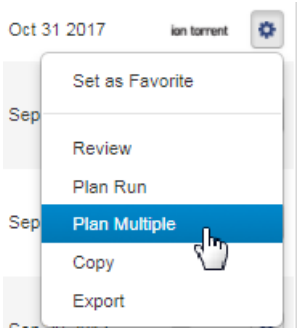
Note: The latest CSV batch planning template file indicates the version of the template in the top row. This version number is required. When you download the template from the Torrent Suite™ Software, the version is automatically included.

Create multiple Planned Runs for non-barcoded libraries

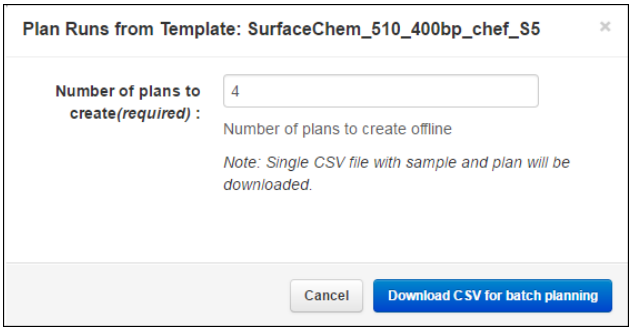
You can create multiple Planned Runs with a template CSV file. To create multiple Planned Runs for individual non-barcoded libraries, a single batch planning template CSV file is required.

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, 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.

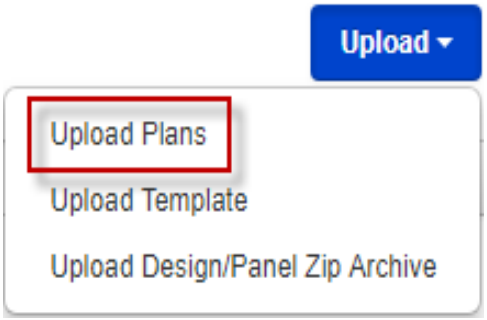
	A	B	C	D	E
1	CSV Version (required)	2			
2	Plan Parameters	Plan 1	Plan 2	Plan 3	Plan 4
3	Template name to plan from (required)	System Generic Seq Template	System Generic Seq Template	System Generic Seq Template	System Generic Seq Template
4	Plan name (required)				
5	Sample (required)				
6	Sample Description				
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

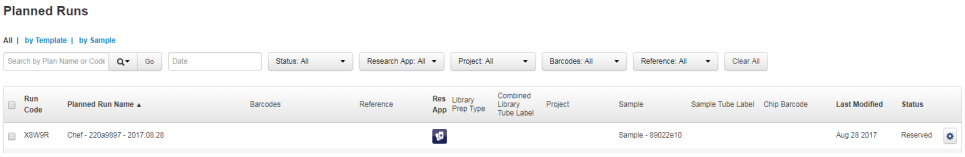
6. Name, then save the CSV file.

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



8. In the **Upload Plan Runs** dialog, 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.

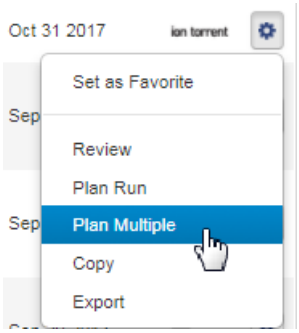
Create multiple Planned Runs for barcoded libraries

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

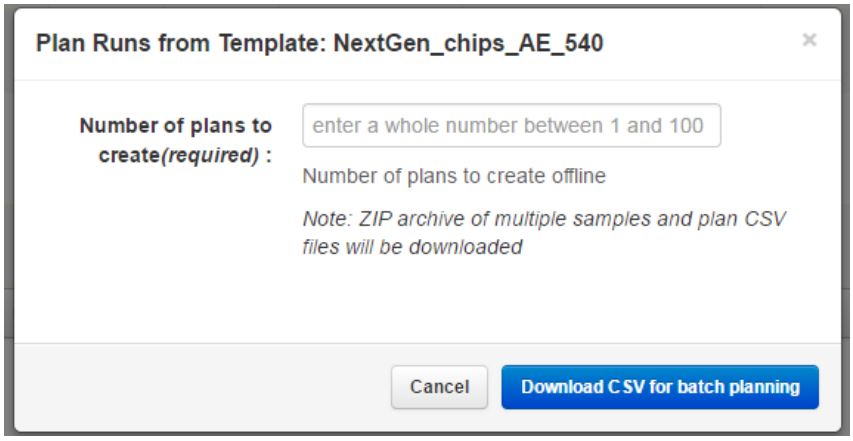
- A master CSV file that is used to specify the plan name, kits, chips, projects, and plugin selections.
- A sample CSV file for each Planned Run.

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.

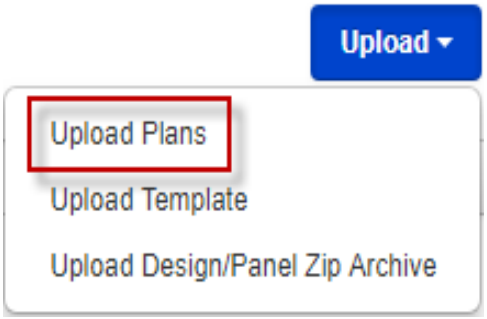
6. Save the CSV file.

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, 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.

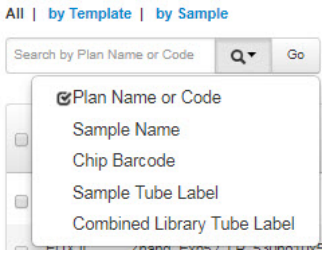
Planned Runs list

The **Planned Runs** screen under the **Plan** tab lists Planned Runs that are ready to execute on an Ion Chef™ Instrument or sequencer. Planned Runs are listed by date, and the table includes basic information about each run, including its status in the sequencing workflow (see "Planned Run status" on page 67).

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

Manage Planned Runs

Click **Planned Runs** under the **Plan** tab to access the functions listed below.

To...	Do the following...
View all Planned Runs	Click **All** above the list.
View Planned Runs that were created from a 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 field above the **Planned Runs** list, click **(Search By)** and select the search type from the options.  2. Enter your search terms in the field, then click **Go**.
Filter the list	Select your filter criteria from the dropdown lists above the list of **Planned Runs**. 
Clear all search and filter settings and display the complete list	Click **Clear All** above the list.
Sort the list	Click a column header to sort the list by the information in that column. Only column headers in bold font are clickable.
View multiple pages in the list	Click the page number and scroll buttons below the list.

To...	Do the following...
Display a list of the samples in the Planned Run	Hover over the **(Info)** icon in the **Sample** column.
Display a list of projects that the run results will be transferred to	Hover over the **(Info)** icon in the **Project** column.
Delete Planned Runs	Select the checkbox next to each Planned Run, then click **Delete Selected** below the list. Alternatively, click **(Actions)** in the Planned Run row, then select **Delete**. **Note:** 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 will open. 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 Planned Run row, then select **Copy**. The **Copy Plan** workflow will open. 2. Edit the settings in any of the steps, enter the name of the new plan in the **Run Plan Name** field, then click **Copy Plan** in the **Save** step.
Transfer a Planned Run to another Torrent Server	See "Transfer a Planned Run to another Torrent Server" on page 70.

Planned Run status

The following status types are displayed in the **Status** column of the **Planned Runs** list.

Note: When you select a Planned Run on a sequencer and begin sequencing, the Planned Run is removed from the list.

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™ run is complete. **Note:** 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™ run completes.
Planned	The Planned Run is available and ready for use by a sequencing instrument.
Voided	The Ion Chef™ run is canceled through the Ion Chef™ screen.

Note: You can change the status of an Ion Chef™ run under specific circumstances. See "Manually change an Ion Chef™ Instrument run status" on page 67.

Manually change an 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 might 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.

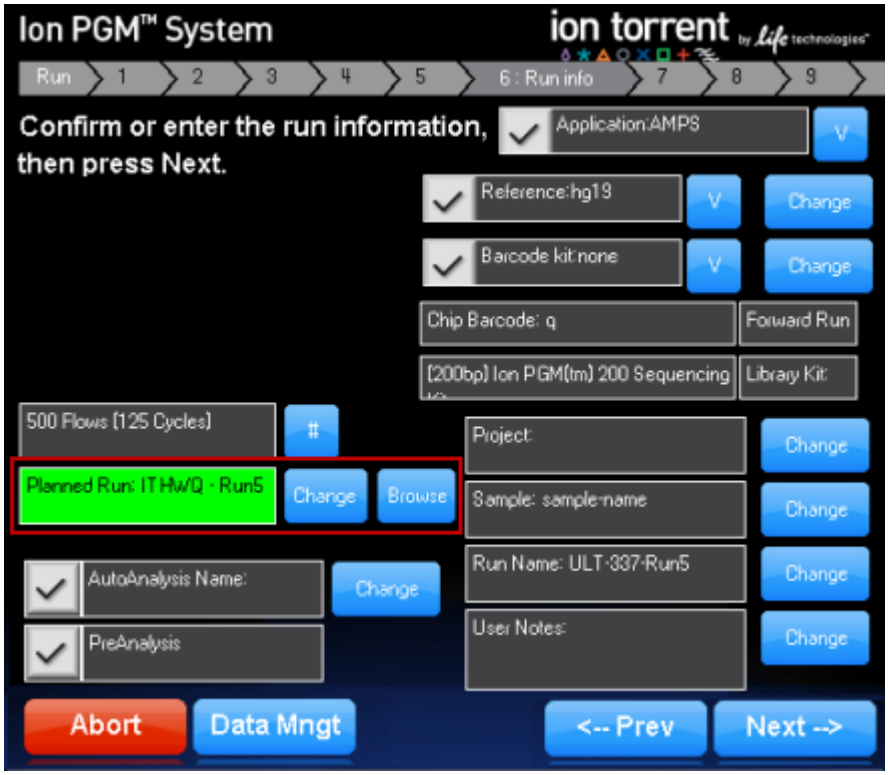
1. In the **Plan** tab, click **Planned Runs**.
2. Locate the Planned Run of interest (with a status of **Reserved**), then click ⚙ **(Actions) ▸ Completed on Chef**.
   The status for the Ion Chef™ Instrument Run on the **Planned Runs** screen changes from **Reserved** to **Planned**. The sequencer 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, you select it on the sequencer touchscreen, after which the run is removed from the **Planned Runs** list.

Depending on your sequencing system, follow these steps to select a Planned Run and start a sequencing run:

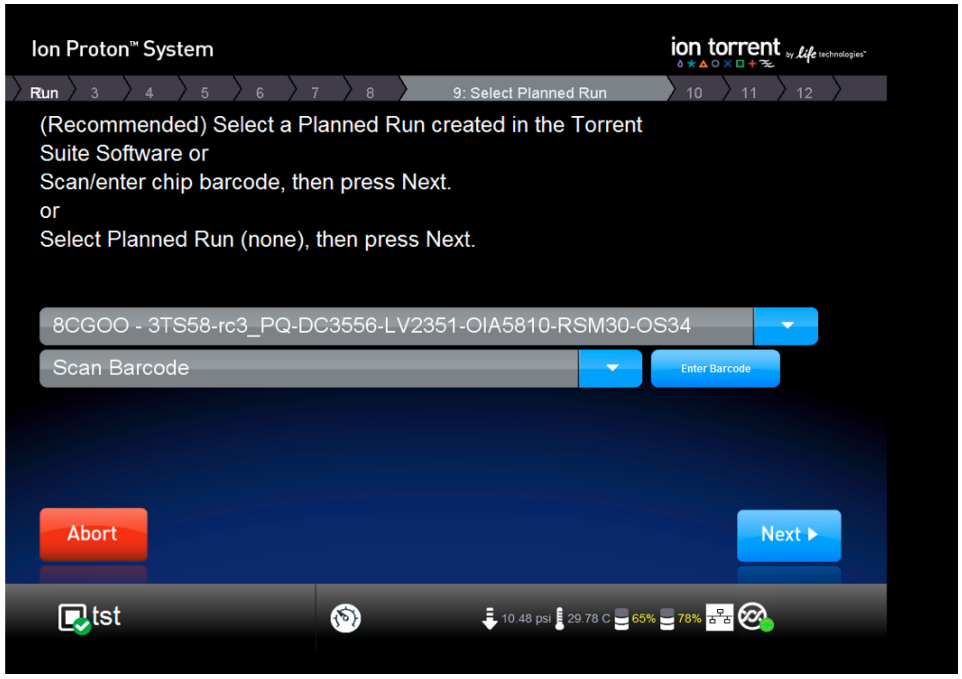
- For the Ion PGM™ System, press **Run** in the home screen, then follow the on-screen instructions. Pending run information is populated into the **Run info** screen. Press the **Browse** button to select a different Planned Run from the Planned Runs list, if needed. Press **Next -->** when you are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



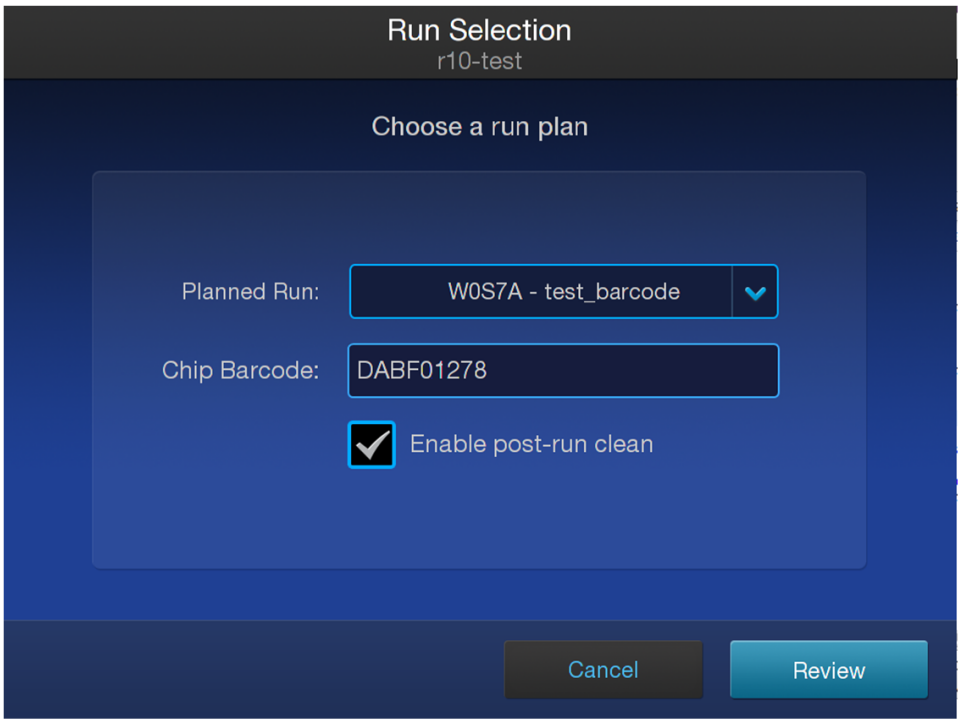
You can also type the Planned Run short code (for example, ITHWQ) into the **Planned Run:** text field on the run information dialog:

- For 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 dropdown list, scan or enter the chip barcode, then press **Next ►**. Pending run information is populated into the next screen. Follow the on-screen instructions to

confirm the run information, load and calibrate the chip, and start the sequencing run.

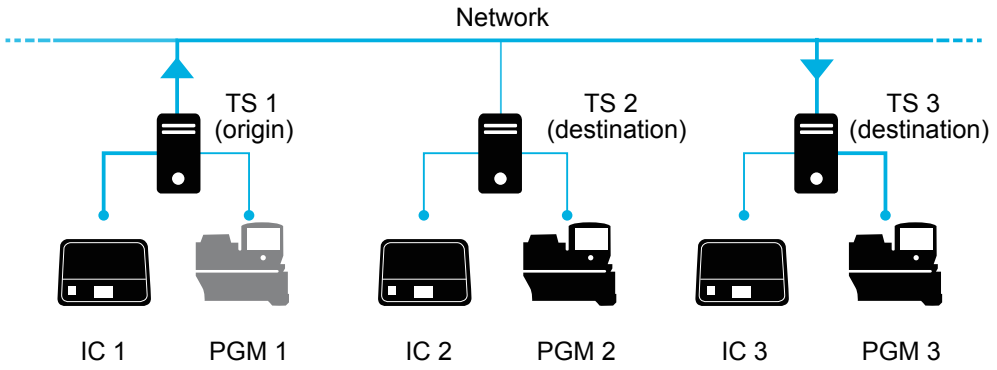


- For the Ion GeneStudio™ S5 System and Ion S5™ System, select your run from the **Run Selection** screen, then press **Review**. On the **Select Run** screen, ensure that the run selections are correct, then press **Start run**.



Transfer a Planned Run to another Torrent Server

If you have multiple Torrent Servers and sequencers on a network, you can create a Planned Run on one server, then transfer it to a different 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 field service representative must first set up this networking capability.



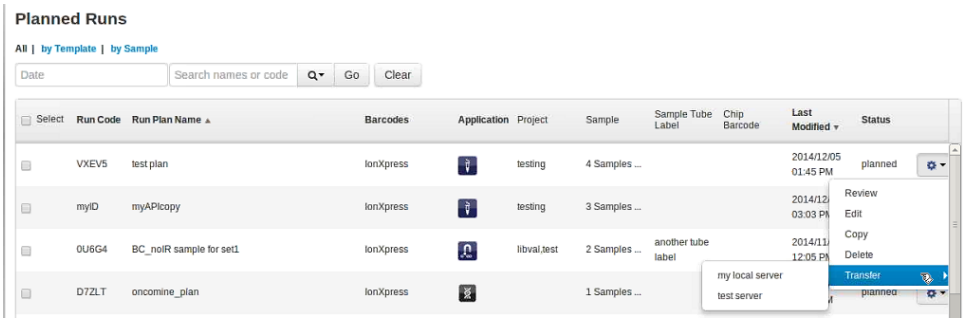
For example, as shown in the diagram, a Planned Run can be set up on the first Torrent Server (TS 1) and run on its associated Ion Chef™ Instrument (IC 1). But if the associated Ion PGM™ Sequencer (PGM 1) is busy or offline, you can transfer the Planned Run to TS 3 or TS 2 to perform the run on those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

To transfer a Planned Run:

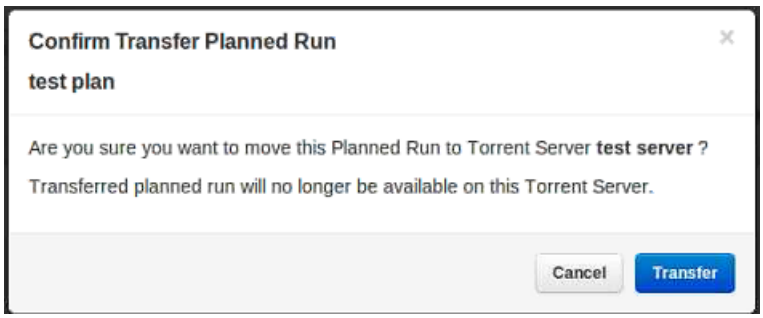
1. In the **Planned Runs** screen, find the Planned Run that you want to transfer.

   Note: 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**).

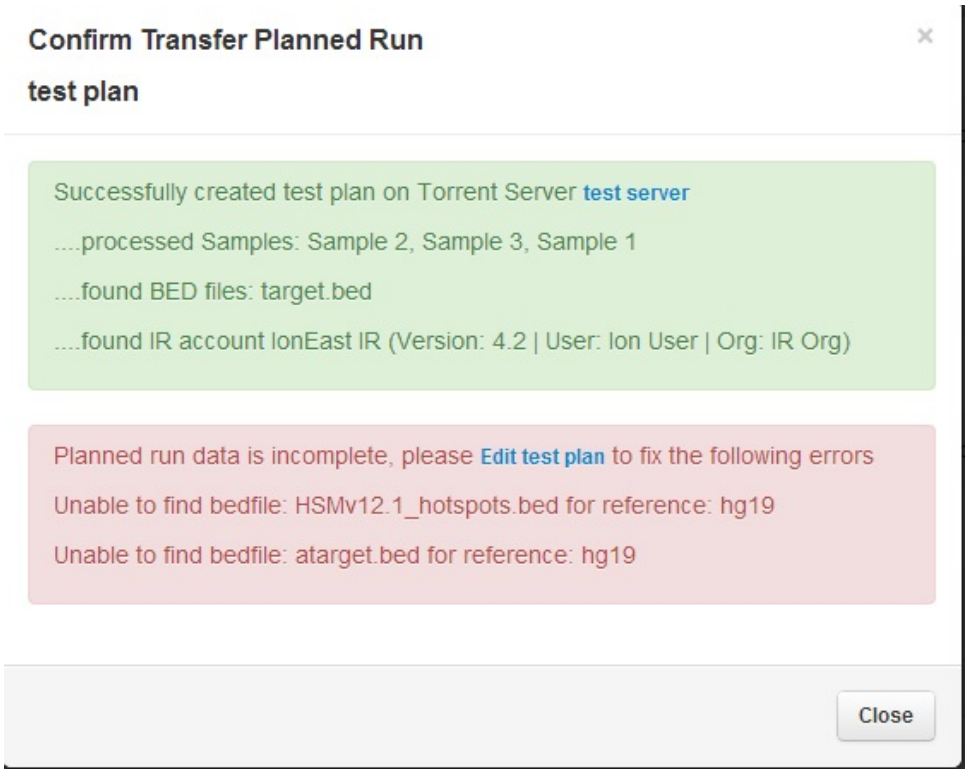
2. Click ⚙ **(Actions)** for the selected Planned Run, select **Transfer**, then select the name of the destination Torrent Server on the network.



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 displays what is missing. Edit the transferred Planned Run on the destination server to add the files or other missing settings to perform the sequencing run.



Note:

- 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 Torrent Server, use the RunTransfer Plugin. See "RunTransfer plugin" on page 152 for more information.

Monitor instrument runs

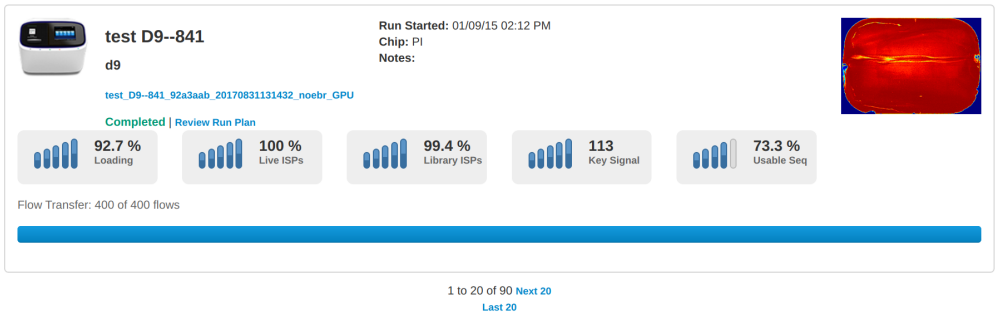
Monitor the sequencing run

In Torrent Suite™ Software you can monitor information about instrument runs through reported metrics, thumbnail graphs, and other indicators. You can also review the Planned Run settings for a sequencing run that is currently in progress on the instrument.

Note: Active sequencing runs and all runs completed within the previous 7 days are available to view.

View the thumbnail graphs and metrics of an sequencing run in progress to quickly determine 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 is generated that displays run metrics and a heatmap image of chip loading as they become available. You can see at a glance in the heatmap image, and in the thumbnail graphs if any run quality metrics are flagged ( ) as falling below the thresholds defined in your Planned Run.

Metric	Description
Loading	Addressable wells on the chip which have detectable loading.
Live ISPs	Loaded wells which 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.

See “Stop a sequencing run“ on page 285 for more information if you decide to abort the sequencing run based on the chip loading metrics observed.

Monitor an Ion Chef™ run

In the **Monitor** tab, you can view details about Ion Chef™ runs from the previous 7 days.

Note: The **Planned Runs** screen also contains information about the status of Ion Chef™ runs. See "Planned Run status" on page 67.

In the **Monitor** tab, click **Ion Chef** to view the following parameters.

Parameter	Definition
Last Updated	Date and time of the currently displayed run status.
Sample Set	See "Samples and Sample Sets" on page 16 for more information.
Plan	Ion Chef™ run plan in progress.
Chef Instrument	Identity of the Ion Chef™ Instrument in use.
Library Prep Progress	Shows the progress of an Ion AmpliSeq™ library preparation run. For an Ion AmpliSeq™ on Ion Chef™ run only.
Library Prep Status	Displays the current stage of the library preparation run. (Not started, In progress, or Complete)
Template Prep Progress	Shows the progress of a template preparation run.
Template Prep Status	Displays the current stage of the template preparation run. (Not started, In progress, or Complete)
Estimated Time Remaining	Estimated time remaining until the run is completed.
Estimated Time Until User Intervention	Estimated time and date when the run pauses for QC, or is completed.

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

View data for runs in progress

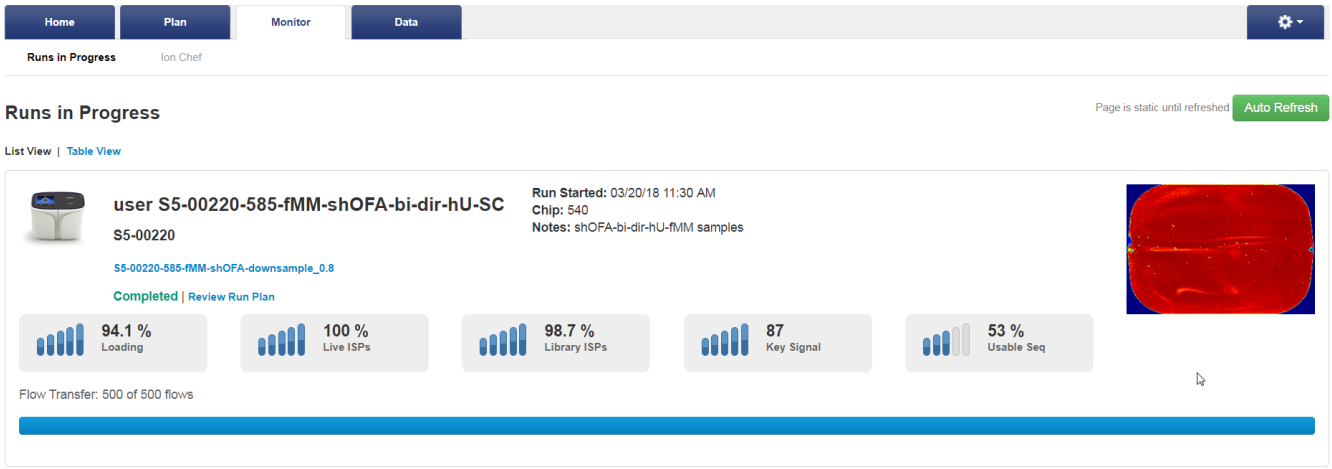
You can view data for runs in progress on the **Monitor** tab in Ion Reporter™ Software.

This section has two views:

- **List View** has 20 runs per page, with details shown for each run.
- **Table View** has 1 run per row in columns that you can sort by clicking a column head. This view displays only the parameters that are associated with each report.

Note: Both the **List View** and **Table View** show 20 runs at a time. If you have more than 20 runs, multiple pages are shown.

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



2. To monitor details about Ion Chef™ runs, click **Ion Chef** in **Table View**.

Auto Refresh the Monitor tab

Auto Refresh updates your **Runs in Progress** page every 20 seconds. Without **Auto Refresh**, the page is a static display of information at the time you opened the page.

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

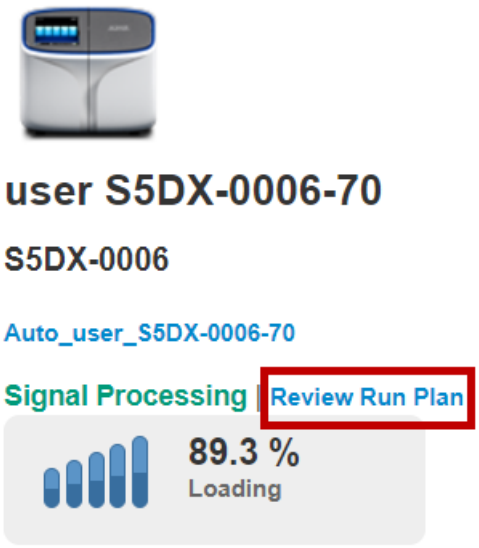
  Note: The Ion Chef page does not automatically refresh.

  a. In the **Monitor** tab, click **Ion Chef** ▸ **Auto Refresh**.
  b. Click **Stop Refresh** to turn the **Auto Refresh** feature off.

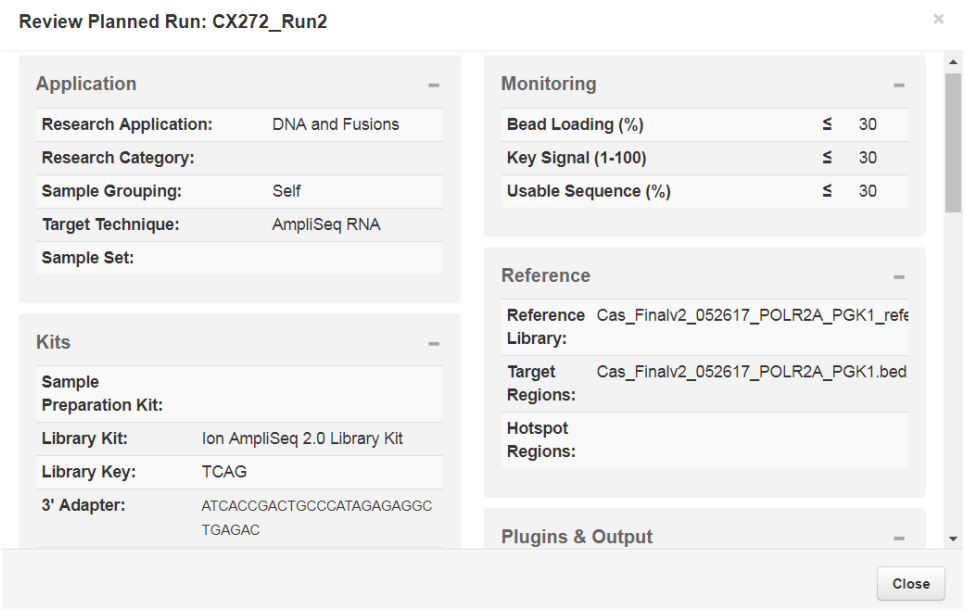
Review the Planned Run settings

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 displays the Planned Run information and settings.



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

Manage Completed Runs and Reports

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.	Enter a search term in the **Search** field, then click **Go**.
Sort the list.	Select a sort order from the **Sort** dropdown list (List View or Table View), or click on any bolded column header (List View only). Click on the column header a second time to reverse the sort order.
Limit the list to recent runs.	In the **Date** field select a preset range, or click **Date Range**, then select a **Start** and **End** date.
Filter the list.	Select from one or more Filter dropdown lists to limit the **Completed Runs & Reports** list. Click **More Filters** to see all available filters. Within a Filter enter text into the **Find** field to limit the filter choices. To remove a filter, de-select the filter choice or click **Clear** in the Filter dropdown list.
View favorites.	Click ☆ adjacent to the **Search** field to limit the list to completed Runs designated as favorites.

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

Reanalyze a run

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

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Search, filter, or sort the list to find your run report of interest.
   See "Search for a Run report" on page 76 for more information.

3. In **Table View** mode, in the row of the run of interest, click **(Actions) ▸ Reanalyze**.

   Alternatively, switch to **List View**, find your run of interest, then click **Reanalyze**.

4. Enter a **Report Name**, 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. Does not use the Use data from previous report field, but reprocesses from the DAT files. You can optionally use both the Analysis args and Basecaller args fields. • Select **Base Calling** (default) to reanalyze from 1.wells files. Uses the **Use data from previous result:** field and optionally the Basecaller args field, but reprocesses from the 1.wells file. Does not use the Analysis args field.
Use data from previous result:	Select the previous result from the dropdown list if more than one result is available. This option applies only when starting reanalysis from **Base Calling**.
Analysis Parameters:	See "Create and select an analysis parameter set" on page 348 for more information.

5. *(Optional)* In the menu, click **Analysis Options**, then edit the fields if needed.

Field	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 used.
Mark as Duplicate Reads:	Select to have PCR duplicates flagged in the BAM file.
Base Calibration Mode:	Base calibration 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)* In the menu, click **Reference & Barcoding**, then edit the fields to set the default options, if needed.

Option	Description
Default Alignment Reference:	Select the default reference file from the dropdown list.
Default Target Regions BED File:	Select the default Target Regions BED file from the dropdown list.
Default Hotspot Regions BED File:	Select the default Hotspot Regions BED file from the dropdown list.
Barcode Set:	Select the default barcode set from the dropdown list.

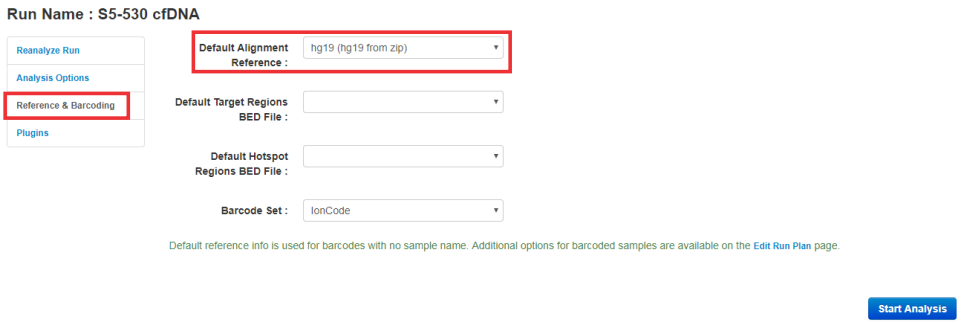
7. *(Optional)* In the menu, click **Plugins**.
   a. Select the plugin to include in the reanalysis.
   b. *(If needed)* Configure the plugin, then click **Save Plugin Settings**.
      See "Plugin configuration" on page 113, or the configuration topic specific to the selected plugin.
   c. Repeat steps a and b to include additional plugins in the reanalysis.
8. Click **Start Analysis**.

Change the default alignment reference

Use the following procedure to change the default alignment reference for an analysis.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In either **Table View** or **List View**, find the run of interest.
   See "Search for a Run report" on page 76 for help finding an individual run.
3. Click **(Actions)** ▸ **Reanalyze** in the row of the run that you want to reanalyze.
4. In the **Reanalysis** screen for the run, click **Reference & Barcoding**.

5. Select a new reference from the **Default Alignment Reference** dropdown list.



Note: If different references were selected for each barcode in the first run, use the per-barcode reference selection utility here as well.

6. Click **Start Analysis**.
   See "Create a custom configuration for the variantCaller plugin" on page 197, and "Run the variantCaller plugin manually" on page 193 for more information.

Edit a Run report

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

Note: System default Run templates cannot be edited. Create a copy of the Run template to make changes.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Search, filter, or sort the list to find your run report of interest.
   See "Search for a Run report" on page 76 for more information.
3. Open the **Edit Run** wizard.
   - In **Table View**, click ⚙ **(Actions)** ▸ **Edit** in the row of the Run report that you want to edit.
   - In **List View**, identify the Run report of interest, then click **Edit**.

   Note: The **Edit Run** wizard opens to the **Save** screen.

4. Click a step in the workflow bar to access the respective screens where edits can be entered.

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 edit fields if 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.

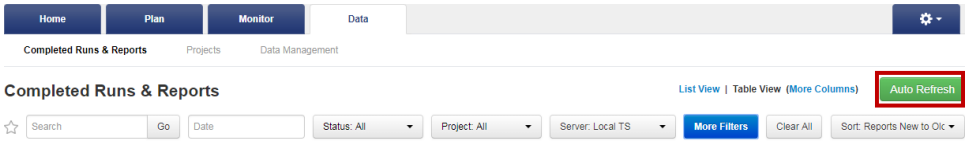
In each case, you must reanalyze the run after editing the barcode information. These steps apply only to completed runs.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In table or list view, navigate to your run of interest.
   See "Search for a Run report" on page 76 for help finding an individual run.
3. Click **(Actions)** ▸ **Edit** in the row of the run.
   The **Edit Run** screen appears.
4. 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 page to automatically refresh

Auto Refresh updates your **Completed Runs & Reports** page every 20 seconds. Without **Auto Refresh**, the page is a static display of information at the time you opened the page.

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



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

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. See "Reanalyze a run" on page 76 for more information.
		You can send the customer support archive to your Thermo Fisher Scientific support contact for review. See "Get technical support files" on page 108 for more information.

Organize run results with projects

Projects are groups of result sets that you can use to organize results into unique categories that are useful for your organization. A project might 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).
- Rename, or delete a project.
- View a history log for 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.

Search for projects by name

The following instructions apply to both, Projects and Result Sets lists.

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

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

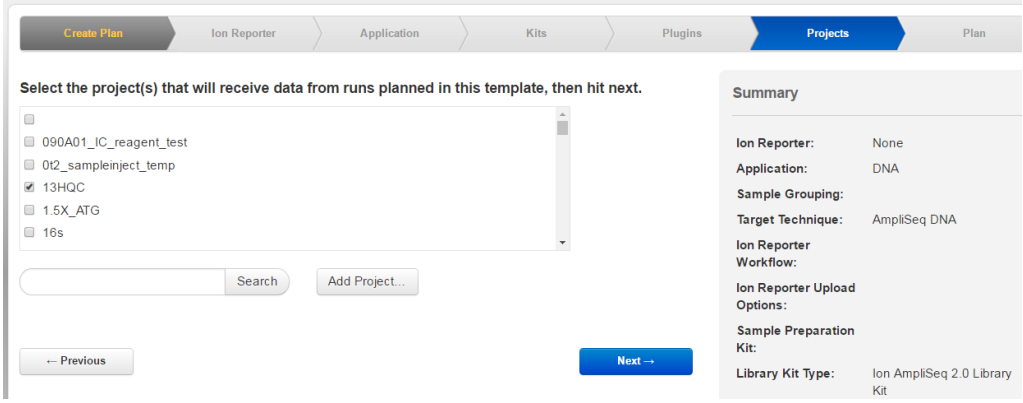
To...	Steps...
Search the list	Enter your search term into the **Search names** field, then click **Go**. The displayed information in the table is limited only to the names that match or contain the search string. **Note:** The **Search names** field takes a complete or partial name. For example, the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

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

Add a project to a Planned Run

You can designate which result sets are included in projects before runs are completed when you add a project to Planned Run or a run template.

When you create a Planned run, search for and enter project names in the **Projects** step in the Workflow bar:

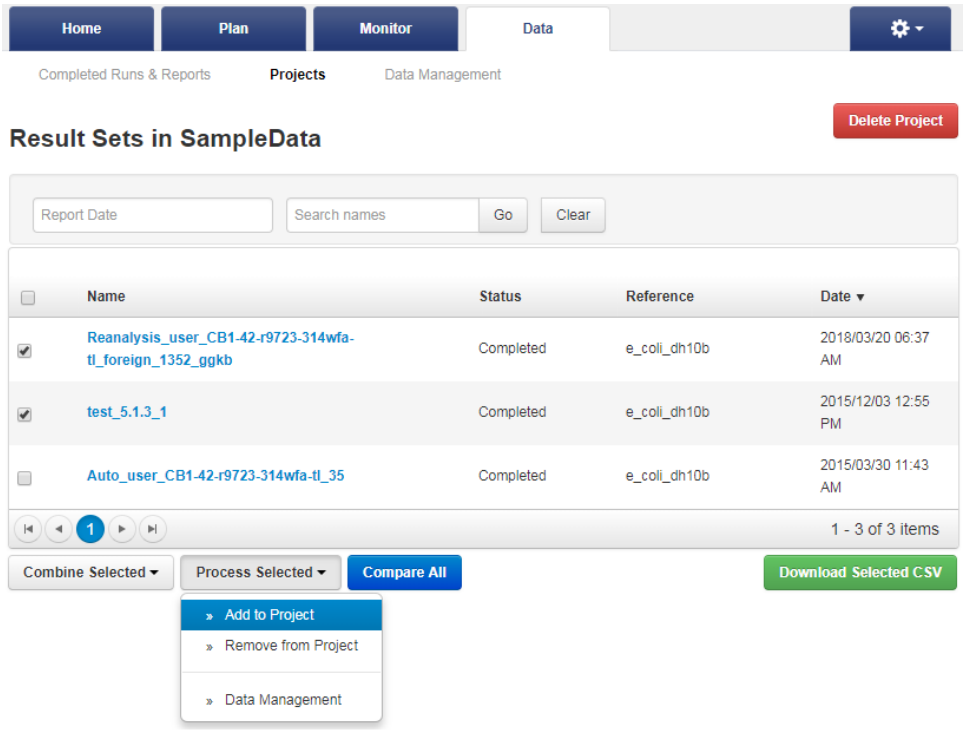


See “Plan and execute an instrument run“ on page 32 for more details.

Add selected results to another project

1. In the **Data** tab, click **Projects** to see the list of projects.
2. Click on the project name in the **Name** column of the **Projects** table 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**.



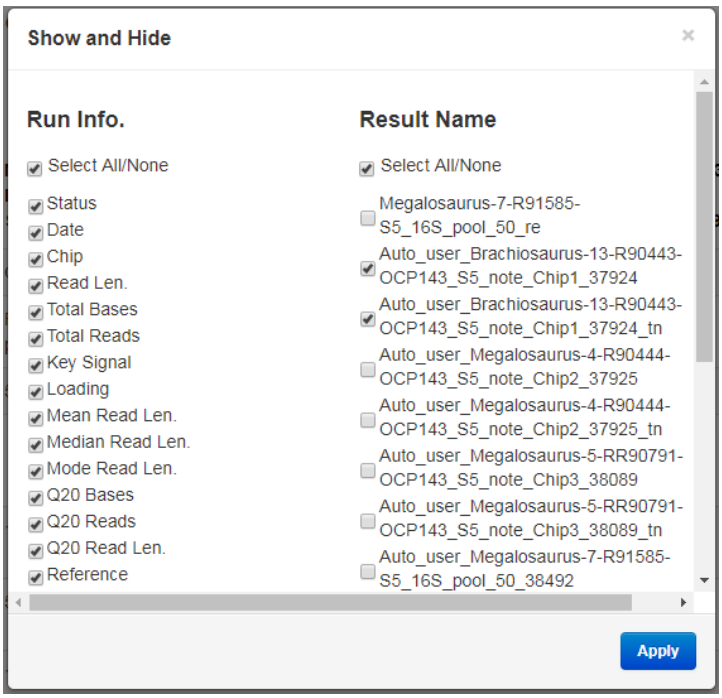
4. Select the checkbox for each project that the result sets are to be copied to, then click **Add projects**.

Compare reports of runs in a project

You can compare report metrics for multiple runs side-by-side if the reports are assigned to a Project.

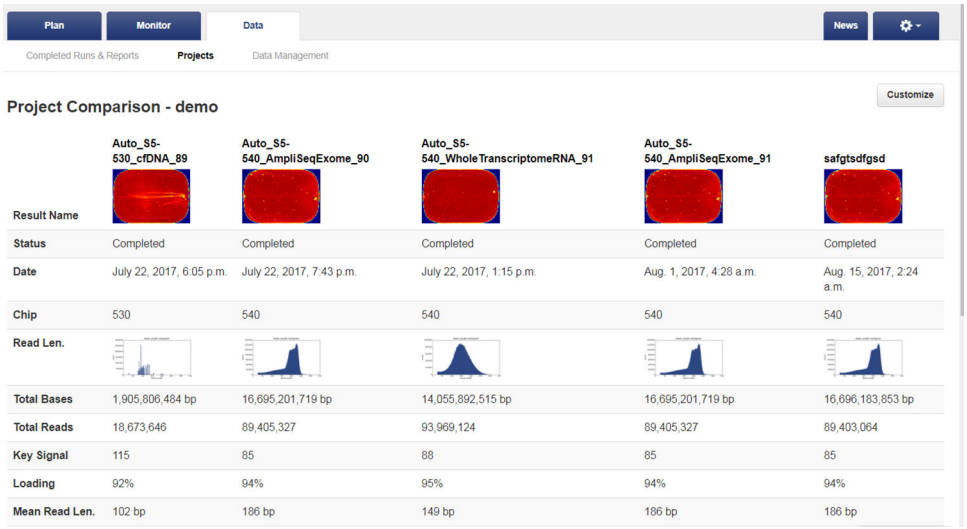
1. In the **Data** tab, click **Projects**.
2. Select a project.
3. Click **Compare All** to view all the runs in the project side-by-side.
4. *(Optional)* Click **Customize**.
   The **Show and Hide** dialog box appears.
5. *(Optional)* In the **Run Info** column, un-check metadata to remove it from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

6. *(Optional)* In the **Result Name** column, un-check 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 275, "Manually archive run data" on page 276 and "Manually delete run data" on page 277.

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	If you select Mark as duplicate, Torrent Suite™ Software flags duplicate reads in the BAM file. For some applications, duplicate reads coming from PCR cause problems in downstream analysis. The presence of duplicate reads can create the appearance of multiple independent reads supporting a particular interpretation, when some reads are in fact duplicates of each other with no additional evidence for the interpretation. **Note:** Marking duplicate reads is not appropriate for Ion AmpliSeq™ data, because many independent reads are expected to share 5' alignment position and 3' adapter flow as each other. Marking duplicates on an Ion AmpliSeq™ run risks inappropriately flagging many reads that are in fact independent of one another.
Overwrite sample name	If your combined samples have duplicate names, you can select Overwrite Sample Names and 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, information fields are separated by a comma. Each comma-separated field is listed in a separate column. You can open the files with a spreadsheet software such as Microsoft™ Office Excel™ or OpenOffice.org.

The file has many fields per entry, as described in the following table:

Field	Description
Report	Name of the analysis run report
Status	Status of the analysis (e.g., 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
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

Field	Description
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 50bp with 99% or greater accuracy
Library_100Q20_Reads	Reads of length at least 100bp with 99% or greater accuracy
Library_200Q20_Reads	Reads of length at least 200bp 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
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 50bp

Field	Description
Library_100Q47_Reads	Number of perfect reads of length at least 100bp
Library_200Q47_Reads	Number of perfect reads of length at least 200bp
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 the sample was sequenced
Run Directory	Location of the raw DAT files on the 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

Field	Description
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

Note: Rows 4-11 (marked by *) contain test fragments metrics. The other rows contain library read metrics.

Remove result sets from project

You can remove a result set from a project.

Note: This option does not delete the selected run reports and their result sets. It only removes them from the current project.

1. In the **Data** tab, click **Projects** to see the list of projects.
2. Click on the project name in the **Name** column of the **Projects** table 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 remove from the project, then click **Process Selected ▸ Remove from Project**.

Review run reports

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

Note: 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 also complete several tasks from the run report, including:

- Upload output files from the run report to Ion Reporter™ Software. See "Run IonReporterUploader plugin manually" on page 179.
- Review, edit, or copy the Planned Run settings for the sequencing run. See "Review the Planned Run settings" on page 49 and "Create a custom Planned Run template" on page 34 for more information.
- Rerun the data analysis for the run. See "Reanalyze a run" on page 76 for more information.
- Archive, edit, or delete data for the run. See "Data management" on page 268 for more information.
- Toggle between different run reports for the same sample. See "Open a run report" on page 95 for more information.
- Run Torrent Suite™ Software plugins. See "Run a plugin manually from the sequencing run report" on page 115 for more information.
- Download the run report or plugin summary in PDF format. See "Download run report PDF" on page 103 and "Download plugin report PDF" on page 116 for more information.

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
- AQ20
- Perfect

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 Aligned 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 percent (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 percent 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.

There are many alignment algorithms 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. See “TMAP Modules“ on page 360, or contact your Thermo Fisher Scientific representative or Technical Support for more information.

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 this is the case, the Torrent Suite™ 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 Torrent Suite™ Software gives you 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.

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 and can be used to quickly determine 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

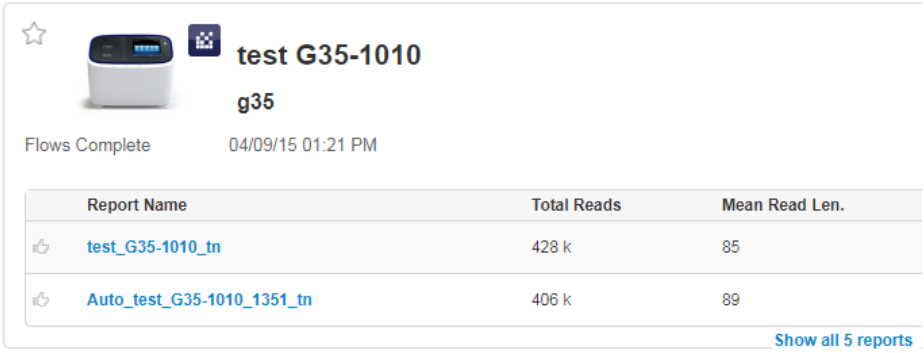
generated. The full chip reports contain all the data points and should be used for your analysis review.

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:

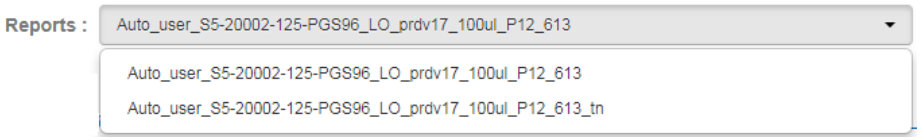
   - In the **List View**, locate the pane that contains details about your run of interest, then click the link in the **Report Name** column to view details about any of the available reports for that run.



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

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

     Note: If you want to open another report that is associated with your run of interest, click the **Reports** dropdown list, then select the report 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 generates 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.

Unaligned reads metrics

This table describes the Ion Sphere™ particle (ISP) density, summary, and read length quality metrics for the unaligned sequencing reads.

Metric	Description
Density	
Total Bases	Number of filtered and trimmed base pairs reported in the output BAM file.
Key Signal	The reported key signal is the average signal for all ISPs that identically match the library key.
ISP Loading	Percentage of chip wells that contain a live ISP. (The percentage value considers only potentially addressable wells.)
ISP Summary	
Total Reads	Total number of filtered and trimmed reads independent of length reported in the output BAM file.
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.
Loading	Percentage of chip wells that contain a live ISP. (The percentage value considers only potentially addressable wells.) This percentage is calculated by dividing the number of loaded ISPs by the number of potentially addressable wells.

Metric	Description
Empty Wells	Percentage of chip wells that do not contain an ISP. (The percentage value considers only potentially addressable wells.) The percentage is calculated by the number of potentially addressable wells, minus the number of loaded ISPs, divided by the number of potentially addressable wells.
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 Test Fragment (TF) ISPs. This number is calculated by dividing library ISPs by the number of loaded ISPs minus TF ISPs.
No Template	Percentage of chip wells that do not contain a DNA template. This percentage is calculated by the number of loaded ISPs minus TF ISPs divided by the number of loaded ISPs plus TF ISPs.
Clonal	Percentage of clonal ISPs (all library and TF ISPs that are not polyclonal). An ISP is clonal if all of its DNA fragments are cloned from a single original template. All the fragments on such a bead 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 single beads by the number of live wells.
Polyclonal	Percentage of polyclonal ISPs (ISPs carrying clones from two or more templates). A high polyclonal percentage indicates that library input is too high and should be titrated down. Enrichment does not filter out polyclonal ISPs, it only removes template-negative ISPs. This percentage is calculated by dividing polyclonal ISPs by live ISPs.
Final Library	Percentage of reads that pass all filters and are recorded in the output BAM file. This value can be different from the Total Reads due to specifications associated with read trimming beyond a minimal requirement resulting in Total Reads being slightly less than Final Library. This percentage is calculated by dividing final library ISPs by clonal ISPs.
% Test Fragments	Percentage of live ISPs with a key signal that is identical to the TF key signal. This percentage is calculated by dividing TF ISPs by clonal ISPs.
% Adapter Dimer	Percentage of ISPs with an insert length of less than 8 bp. This percentage is calculated by dividing primer-dimer ISPs by clonal ISPs.
% Low Quality	Percentage of ISPs with a low or unrecognizable signal. This percentage is calculated by dividing low quality ISPs by clonal ISPs.

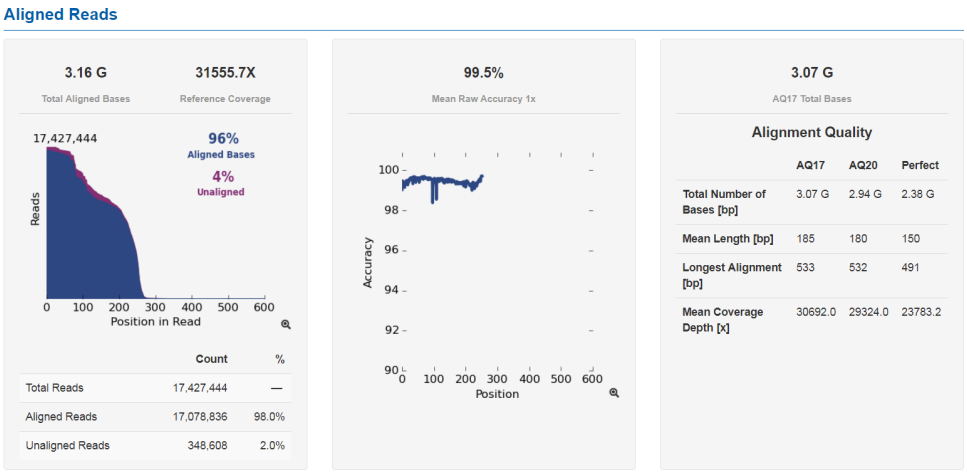
Metric	Description
Addressable Wells	Total number of wells on the chip minus excluded wells.
With ISPs	Number (and percentage) of addressable wells that were determined to be *positive* for the presence of an ISP in the well. Positive is determined by measuring the diffusion rate of a flow with a different pH. Wells containing ISPs have a delayed pH change due to the presence of an ISP slowing the detection of the pH change from the solution. This percentage is calculated by dividing the number of wells with ISPs by total addressable wells.
Live	Number (and percentage) of wells that contain an ISP with a signal of sufficient strength and composition to be associated with the library or Test Fragment key. This value is the sum of the following categories: • Test Fragment • Library This value is calculated by dividing live ISPs by wells with ISPs.
Test Fragment (TF)	Number (and percentage) of live ISPs with a key signal that was identical to the Test Fragment key signal. This number is calculated by dividing TF ISPs by live ISPs.
Library	Number (and percentage) of live ISPs with a key signal that is identical to the library key signal. This number is calculated by dividing library ISPs by live ISPs.
Library ISPs	Predicted number of live ISPs that have a key signal identical to the library key signal (the same value as Library).
Filtered: Polyclonal	ISPs carrying clones from two or more templates. This number is calculated by dividing polyclonal ISPs by library ISPs.
Filtered: Low quality	Low or unrecognizable signal. This number is calculated by dividing low quality ISPs by library ISPs.
Filtered: Adapter-dimer	Insert length of less than 8 bp. This number is calculated by dividing primer-dimer ISPs by library ISPs.
Final Library ISPs	Number (and percentage) of Library ISPs passing all filters, which are recorded in the output BAM file. This value can be different from the Total Reads due to specifications associated with read trimming beyond a minimal requirement resulting in total reads being slightly less than Final Library reads. This number is calculated by dividing final library ISPs by library ISPs.
Read length	
Mean Read Length	Average length, in base pairs, of called reads.

Metric	Description
Median Read Length	Median length, in base pairs, of called reads.
Mode Read Length	Mode length, in base pairs, of called reads.

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.

Aligned reads metrics

A completed run report in Torrent Suite™ Software includes metrics for aligned reads that include Alignment summary, Raw Accuracy, and Alignment Quality.

Metric	Description
Alignment Summary	
Total Aligned Bases	Total number of bases aligned to the reference sequence, excluding the library key, barcode, and 3' adapter sequences.
Reference Coverage	Ratio of the total aligned bases divided by the number of bases in the reference sequence. Reference coverage does not account for any enrichment that may have been done to selectively amplify a subset of the reference sequence.
Total Reads	Total number of reads after filtering.
Aligned Reads	The number of reads that align to the reference sequence expressed as a total count and percentage of the total aligned bases.

Metric	Description
Unaligned Reads	The number of reads that do not align to the reference sequence expressed as a total count and percentage of the total aligned bases.
Raw Accuracy	
Mean Raw Accuracy 1X	The mean raw accuracy across each individual base position in a read calculated as, 1– (Total errors in the sequenced reads)/Total bases sequenced.
Alignment Quality	
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)
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)
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)
Mean Coverage Depth (X)	The ratio of the depth of coverage for a given error rate at each base in the target region to the length of the target region. (AQ17 ≤2% error rate, AQ20 ≤1% error rate, Perfect = no measurable error)

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 on 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.

Note: 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. 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.

Column	Description
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 run report 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.

Details about completed runs

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

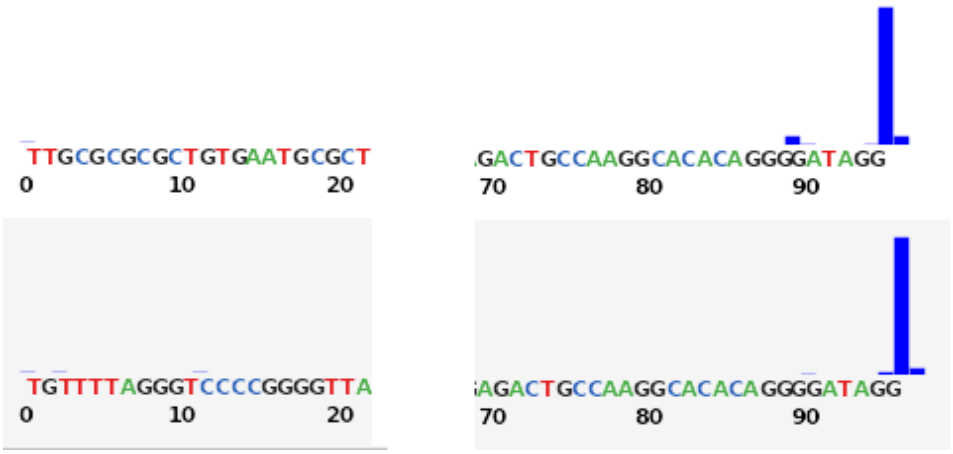
View test fragments for a completed run

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. See "Test fragments" on page 260 for more information.
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. Click on the thumbnail histogram in the **Read length histogram** column 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 (the ends only 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 to open the report of interest.
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™ run

You can view a summary for an Ion Chef™ Instrument from the 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 **Chef Summary** tab.

The following parameters are listed.

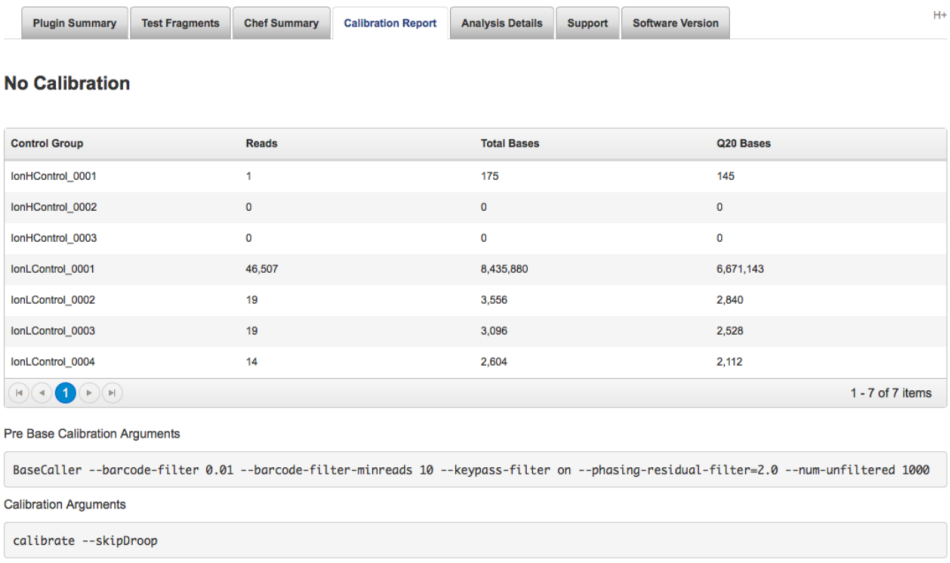
- Chef Last Updated
- Chef Instrument Name
- Chef Operation Mode
- Sample Position
- Tip Rack Barcode
- Chip Type 1
- Chip Type 2
- Chip Expiration 1
- Chip Expiration 2
- Templating Kit Type
- Chef Flexible Workflow
- Reagent Expiration
- Reagent Lot Number
- Reagent Part Number
- Reagent Cartridge Serial Number
- Solution Lot Number
- 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. See "Advanced Settings – Kits step in workflow bar" on page 42 for more information on setting calibration options.

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.



Note: Another way to access calibration details is to click **Report Actions ▸ Review Plan**, then scroll down to **Advanced Parameters**. See "Review the Planned Run settings" on page 49 for more information.

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.

   The parameters in the following list are available in a completed run report.

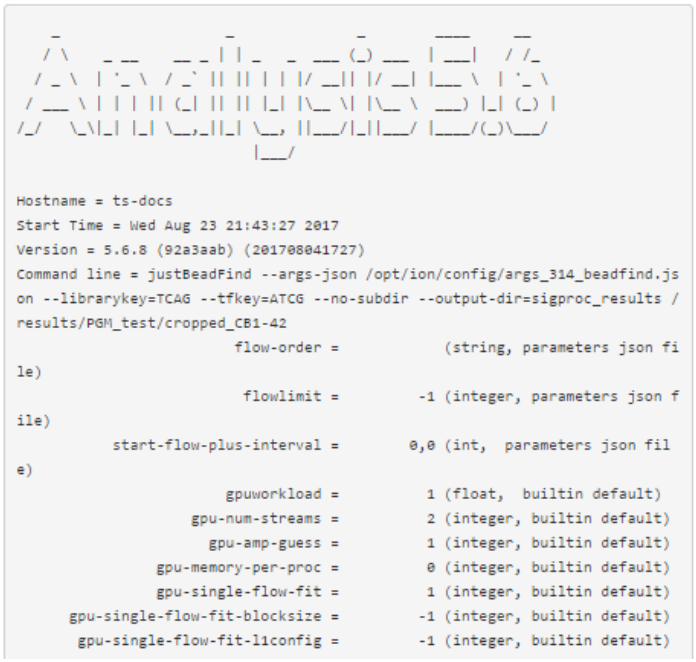
   - Run Name
   - Run Date
   - Run Cycles
   - Run Flows
   - Project
   - Sample
   - Sample Tube Label
   - Reference
   - Instrument
   - Flow Order
   - Library Key
   - TF Key
   - Chip ID or Chip Barcode
   - Chip Check
   - Chip Type
   - Chip Data
   - Chip Lot Number
   - Barcode Set
   - Analysis Name
   - Analysis Date
   - Analysis Flows
   - runID
   - Chef Package Version
   - Start Time
   - Completion Time

Get technical support files

You can view the report error log when troubleshooting a completed run. If you need further assistance, you can also generate a customer support archive that you can share with a customer support representative.

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.
   An example error report is as follows:



```
   _                 _             _       ____     __
  / \    _ __    __ _ | | _   _  ___ (_) ___  | ___|   / /_
 / _ \  | '_ \  / _` || || | | |/ __|| |/ __| |___ \  | '_ \
/ ___ \ | | | || (_| || || |_| |\__ \| |\__ \  ___) |_| (_) |
/_/   \_\|_| |_| \__,_||_||_| \__, ||___/|_||___/ |____/(_)\___/
                              |___/

Hostname = ts-docs
Start Time = Wed Aug 23 21:43:27 2017
Version = 5.6.8 (92a3aab) (201708041727)
Command line = justBeadFind --args-json /opt/ion/config/args_314_beadfind.js
on --librarykey=TCAG --tfkey=ATCG --no-subdir --output-dir=sigproc_results /
results/PGM_test/cropped_CB1-42
                        flow-order =            (string, parameters json fi
le)
                         flowlimit =         -1 (integer, parameters json f
ile)
          start-flow-plus-interval =        0,0 (int,  parameters json fil
e)
                       gpuworkload =          1 (float,  builtin default)
                   gpu-num-streams =          2 (integer, builtin default)
                     gpu-amp-guess =          1 (integer, builtin default)
               gpu-memory-per-proc =          0 (integer, builtin default)
               gpu-single-flow-fit =          1 (integer, builtin default)
     gpu-single-flow-fit-blocksize =         -1 (integer, builtin default)
      gpu-single-flow-fit-l1config =         -1 (integer, builtin default)
```

4. If you are unable to resolve an issue with the error report, 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.

5. Email the report to your support representative. If you would like to be able to upload these support files directly, ask your support representative how to enable Customer Support Archive.

View software versions used in a run

You can view version information for the Torrent Suite™ Software that was used for a completed 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 the **Software Version** tab.

IMPORTANT! The version numbers shown in the example may be different from your current version of the software depending on the date of the run. See ⚙ **(Settings) ▸ About** in Torrent Suite™ Software for a complete list of modules and version on your server. See the *Torrent Suite™ Software Release Notes* for details about the versions in a specific release.

Parameter	Description
Torrent Suite	Version of Torrent Suite™ Software used to generate the analysis.
Datacollect	Version of the Datacollect package. This collects data from the configuration file.
LiveView	Version of the LiveView package.
Script	Version of the Script package.
ion-alignment	Version of the Torrent Suite™ Software alignment module used for this analysis.
ion-analysis	Version of the analysis pipeline that was used to generate the analysis. The analysis pipeline includes signal processing, base calling, and TMAP alignment.
ion-db reports	Version of the ion-dbreports package.
ion-gpu	Version of the graphics processing unit (GPU) driver.
ion-plugins	Version of the pre-installed plugins.
ion-torrentR	Version of the TorrentR stats package.
tmap	Version of the TMAP alignment package.

Manage plugins for data analysis

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

Install or upgrade plugins

On Thermo Fisher Cloud, you can install or upgrade the following:

- Upgrades for a plugin that is pre-installed in Torrent Suite Software. For details about plugins that are included with Torrent Suite Software, see "Pre-installed plugins" on page 120.
- The RNASeqAnalysis plugin and smallRNA plugin. These plugins are supported by Thermo Fisher Scientific, but not pre-installed in the Torrent Suite Software.

1. Sign in to the **Thermo Fisher Cloud**.
2. Click on the **Apps** icon ( ).
3. In **AppConnect**, under **Resource Libraries**, click **Plugins**.
4. *(Optional)* Click a category at the top of page.
   The list of plugins is narrowed to only plugins included 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. Click **(Settings)** ▸ **Plugins** ▸ **Install or Upgrade Plugin** in Torrent Suite™ Software.
7. Click **Select File**, then 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**.

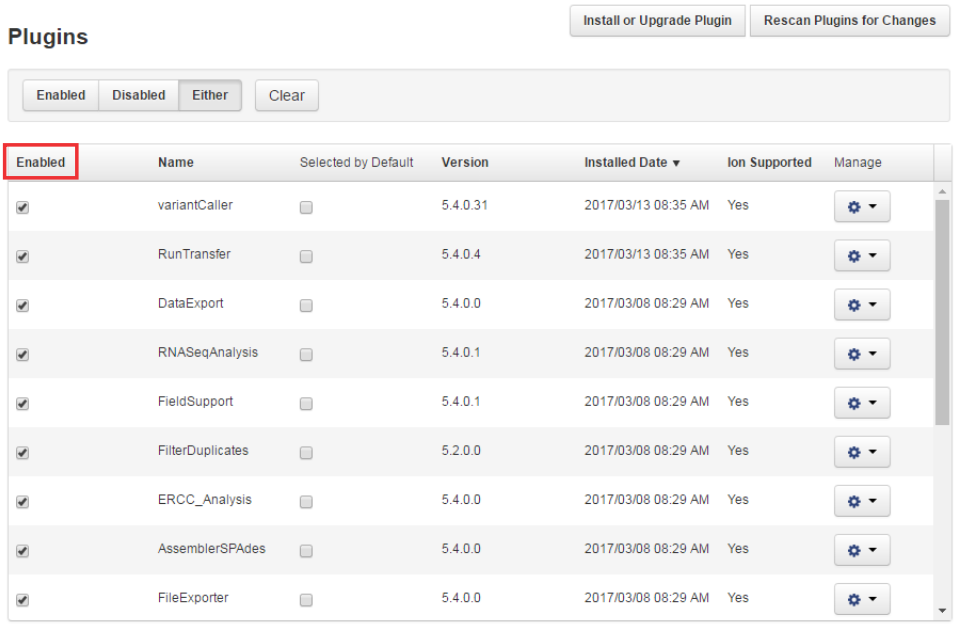
The plugin is now visible in Torrent Suite™ Software.

Enable an installed plugin

IMPORTANT! To make a plugin available to users, you must enable the plugin. The plugin must be installed before it can be enabled.

Follow these steps to enable an installed plugin:

1. Sign in to Torrent Suite™ Software.
2. Click ⚙ **(Settings)** ▸ **Plugins**.
   The installed plugins are listed.



3. Select the **Enabled** checkbox next to any installed plugin, to make it available to users.
   Changes to the settings described in this procedure take effect immediately.

Uninstall a plugin

To uninstall a plugin from your Torrent Suite™ Software:

1. Sign in to your Torrent Browser.
2. Click ⚙ **(Settings)** ▸ **Plugins**. The installed plugins are listed.
3. In the row of the plugin you want to remove, click ⚙ **(Actions)** ▸ **Uninstall**.
4. Click **Yes, Uninstall!** to confirm you want to uninstall the plugin.

Plugin configuration

Some plugins have settings that can be configured by users. For these plugins, there are typically three different ways they can be configured:

- **Global configuration:** For plugins that can be configured globally, administrator-level users can change the settings for all users of the software on a particular server (see "Configure plugins globally" on page 113). These default settings can be overridden when setting up a Planned Run or Planned Run template, or when running the plugin manually.

  Note: Some plugins require configuration, and fail unless a user first enters certain settings. For example, some plugins require that a user enter a file directory for 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

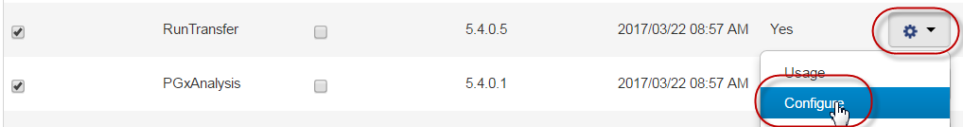
The following pre-installed plugins can be configured globally:

- Data Export
- ERCC Analysis
- File Exporter
- Ion Reporter Uploader
- Run Transfer

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

To change the global configuration of a plugin that is listed above, perform the following steps:

1. Sign in as an administrator, then click **(Settings)** ▸ **Plugins**.
2. In the **Manage** column for the plugin of interest, click **(Actions)** ▸ **Configure**.



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

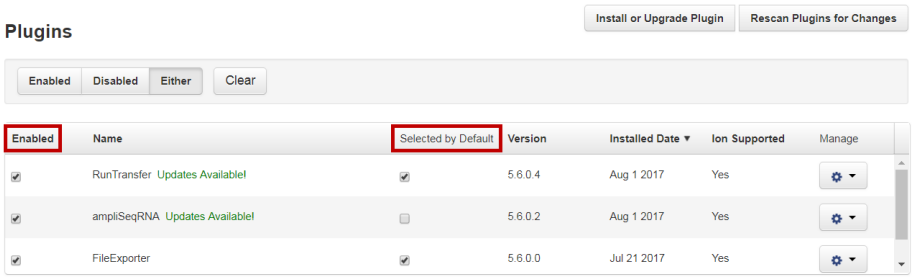
3. To save your changes, click **Submit** or **Save Configuration**.

Configure a plugin to run by default after every run

You can use the following settings for any plugin that is installed in your Torrent Suite™ Software, whether it is pre-installed or if it is downloaded from the Thermo Fisher Cloud.

Note: 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 115.

- To set the plugin to run automatically after every run:
  a. Click ⚙ **(Settings)** ▸ **Plugins**.
  b. Ensure that the **Enabled** checkbox next to the plugin name that you want to run by default is selected.



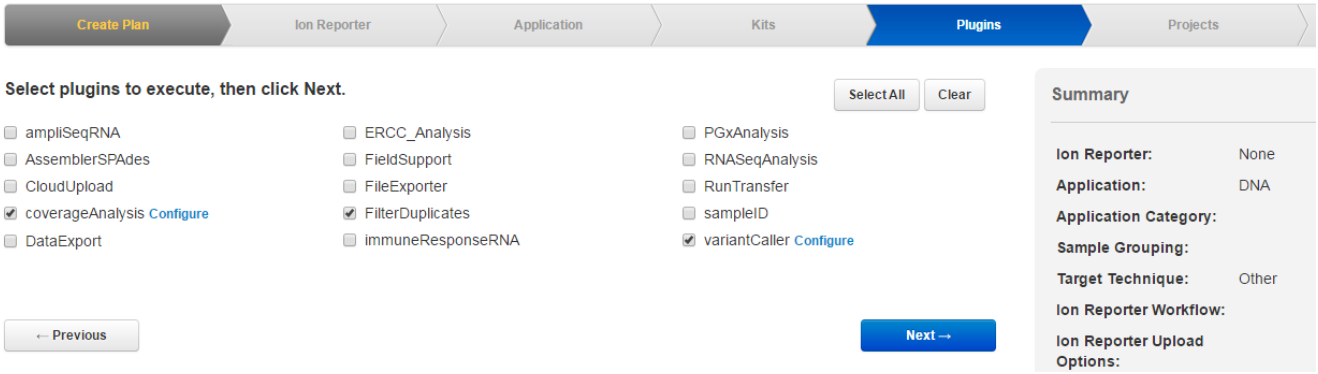
  c. Select the **Selected by Default** checkbox next to the plugin name.

  The plugin is now set to perform its function after every sequencing run.

  Note: 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 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 "Pre-installed plugins" on page 120.

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

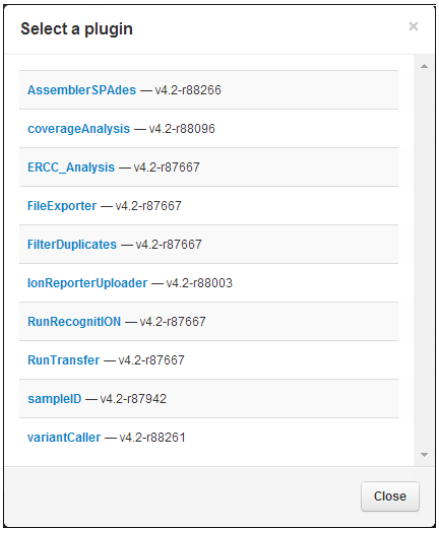
f. When all changes to the Planned Run have been made, click **Plan** in the workflow bar, then click **Plan Run**.

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

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. Configure the plugin if needed. If prompted, select the desired plugin options, then click **Submit** to start the analysis. Alternatively, click **Close** to close dialog without running a plugin. For detailed plugin configuration information for available plugins, see "Pre-installed plugins" on page 120.

   Note: If the plugin does not require configuration, analysis starts immediately without a confirmation screen. To cancel a plugin run that is in progress, click **Stop**.

Download plugin report PDF

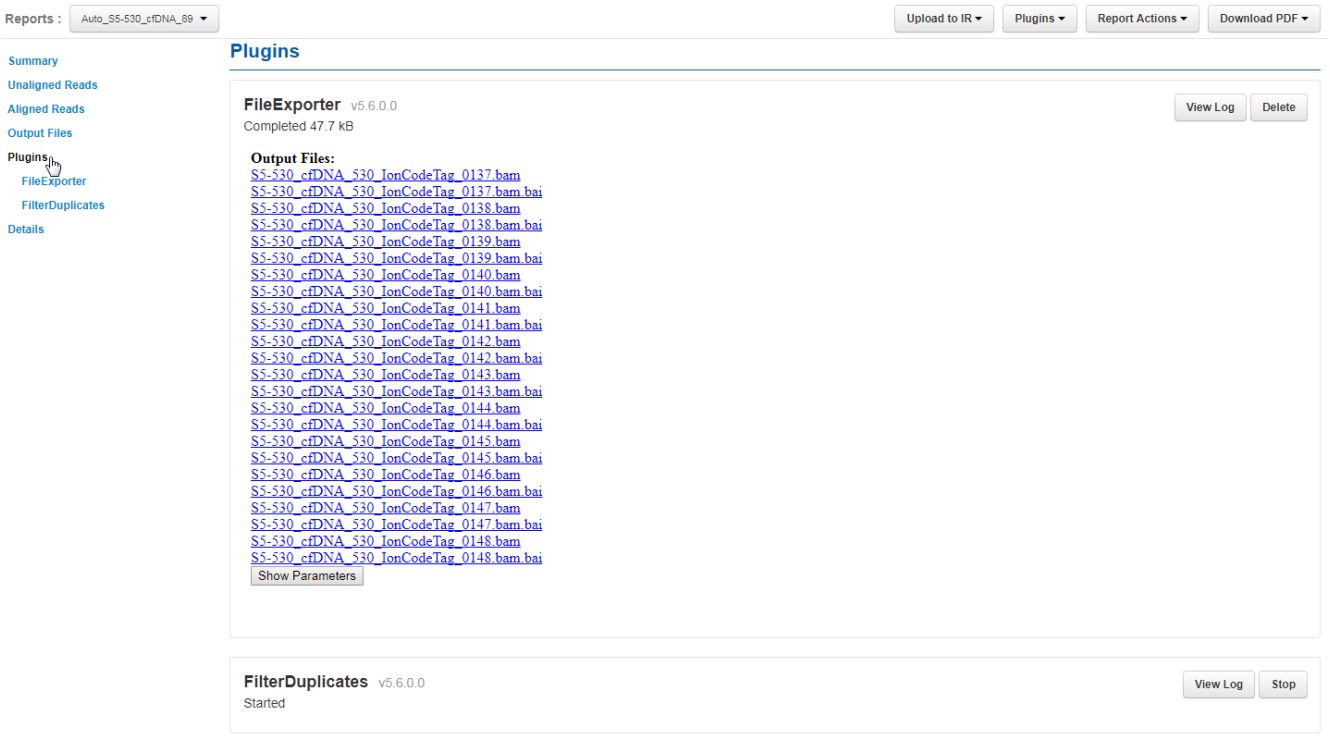
You can download a PDF of the plugin reports.

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 for of the plugin reported 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.

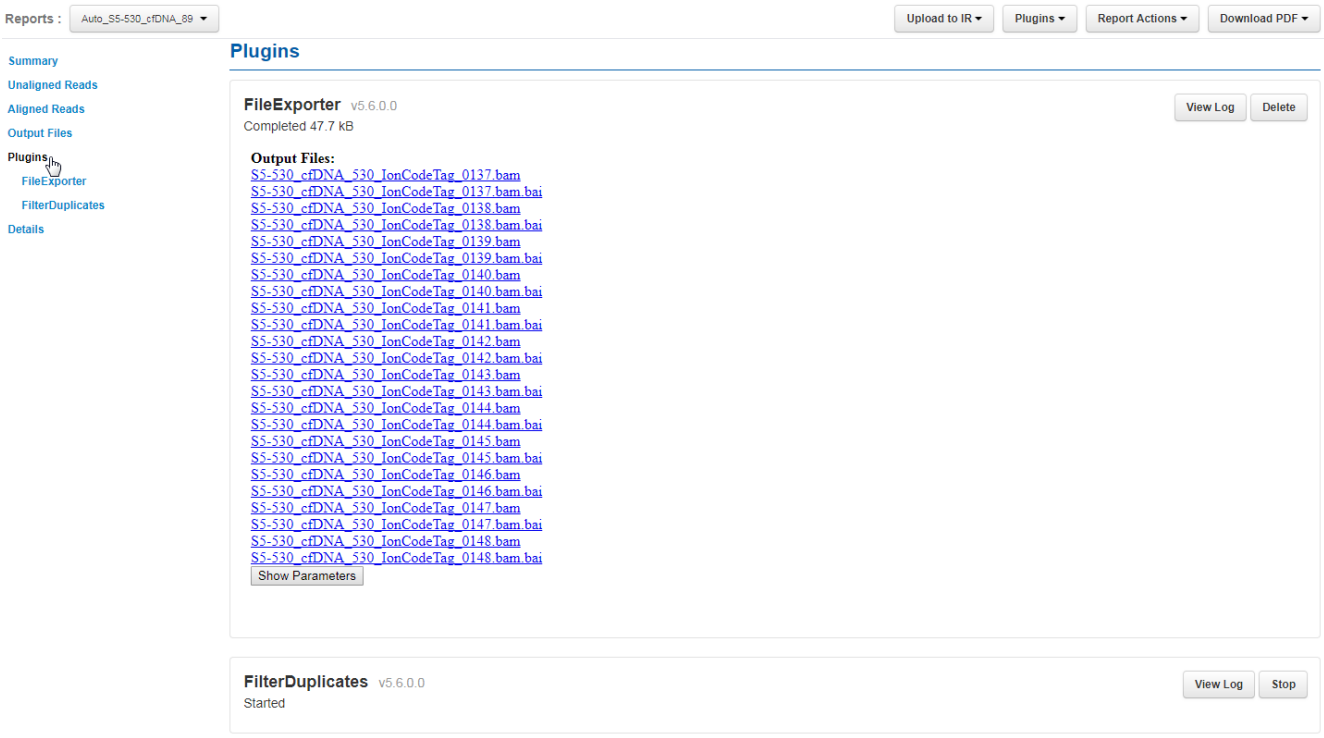
1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
2. In the left navigation menu, click **Plugins**, or the name of the plugin that has the log you want to view.
3. Click **View Log** to the right of the plugin name.
  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

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

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

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

Processing 0 barcodes...

Skipping IonXpressRNA_004:
ERROR: Analysis requires alignment to a reference

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

Delete a plugin result

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.

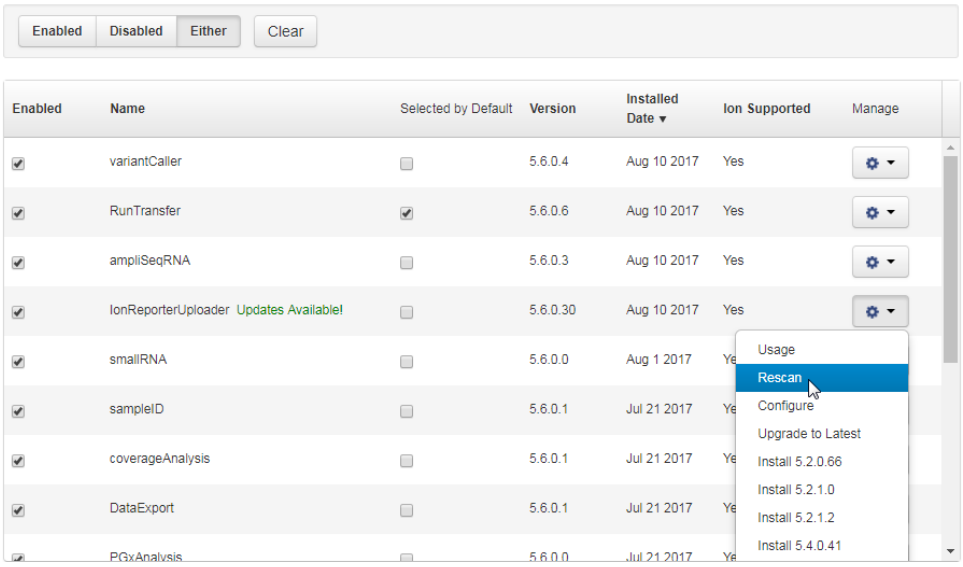


3. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
4. In the left navigation menu, click the plugin name, or scroll to the **Plugins** section of the run report.
5. Click **Delete** to the right of the plugin name.
   The plugin results are deleted from the run report.

Rescan a plugin

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

1. Sign in to Torrent Suite™ Software.
2. Click ⚙ **(Settings)** ▸ **Plugins**. The installed plugins are listed.



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

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

   Note: You can also rescan the output files from the list of reports when you view the usage for a plugin. For details see, "View IonReporterUploader plugin status details" on page 182.

Pre-installed plugins

The following table describes the plugins that are pre-installed with Torrent Suite™ Software.

Plugin name	Description
ampliSeqRNA	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. For details, see "ampliSeqRNA plugin" on page 121.
AssemblerSPAdes	Performs an initial level analysis on assembly and provides metrics. The plugin is ideal for genomes less than 50 megabases in size. For details, see "Assembler SPAdes plugin" on page 125.
coverageAnalysis	Generates statistics and graphs to describe the level of sequence coverage that is produced for targeted genomic regions. For details, see "coverageAnalysis plugin" on page 129.
DataExport	Exports data from a sequencing run to an external hard drive or a removable media, such as a USB drive. For details, see "DataExport plugin" on page 139.
ERCC_Analysis	Indicates whether a problem exists with library preparation or sequencing for runs that use the ERCC RNA Spike-In Mix. For details, see "ERCC_Analysis plugin" on page 140.
FieldSupport	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 technical support or your Field Application Scientist.
FileExporter	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 Variant Caller plugin output files (when available), and compresses output files. For details, see "FileExporter plugin" on page 144.
FilterDuplicates	Removes duplicate reads and creates BAM files that do not contain the duplicate reads. For details, see "FilterDuplicates plugin" on page 147.

Plugin name	Description
immuneResponseRNA	Use the immuneResponseRNA plugin to quantify gene expression levels for the Oncomine™ Immune Response Research Assay. For details, see "immuneResponseRNA plugin" on page 148.
IonReporterUploader	Transfers run results files to Ion Reporter™ Software. For details, see "Integration with Ion Reporter™ Software" on page 172.
PGxAnalysis	Used with the Ion AmpliSeq™ Pharmacogenomics Research Panel, which is a targeted gene panel that allows the interrogation of pharmacogenomics variants in samples for genotyping and CYP2D6 copy number detection. For details, see "PGxAnalysis plugin" on page 151.
RunTransfer	Transfers the signal processing output of a completed sequencing run from one Torrent Server to another Torrent Server, then runs an analysis of the transferred files on the receiving Torrent Server. For details, see "RunTransfer plugin" on page 152.
sampleID	Uses sample fingerprinting to identify any cross-contamination between samples or between barcodes in a run. For details, see "sampleID plugin" on page 154.
variantCaller	For details, see "variantCaller plugin" on page 155.

ampliSeqRNA plugin

The ampliSeqRNA plugin is used with the Ion AmpliSeq™ Transcriptome Human 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. See "Import a preloaded reference sequence" on page 230 for more information on the references and "View and manage target regions files" on page 238 for more information on target regions files.

Reference	Target Regions File
hg19_AmpliSeq_Transcriptome_ERCC_v1	hg19_AmpliSeq_Transcriptome_21K_v1
hg19_AmpliSeq_Transcriptome_V1.1	hg19_AmpliSeq_Transcriptome_21K_v1
hg19_AmpliSeq_Transcriptome_V1.2	hg19_AmpliSeq_Transcriptome_v1.2
AmpliSeq_Mouse_Transcriptome_V1	AmpliSeq_Mouse_Transcriptome_v1

ampliSeqRNA plugin configuration

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

Note: 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 79. Alternatively, you can use reanalyze the run. For details, see "Reanalyze a run" on page 76.

Setting	Description
The following settings can be configured when you or select the ampliSeqRNA plugin as part of a Planned Run or Planned Run template.	
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses if they have a relatively low number of reads, such as those that can result from barcode contamination. A warning appears in the barcode summary report if any barcodes were discounted from the analysis. This setting is ignored for runs not employing barcodes. Typically, the Filter Barcodes 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.

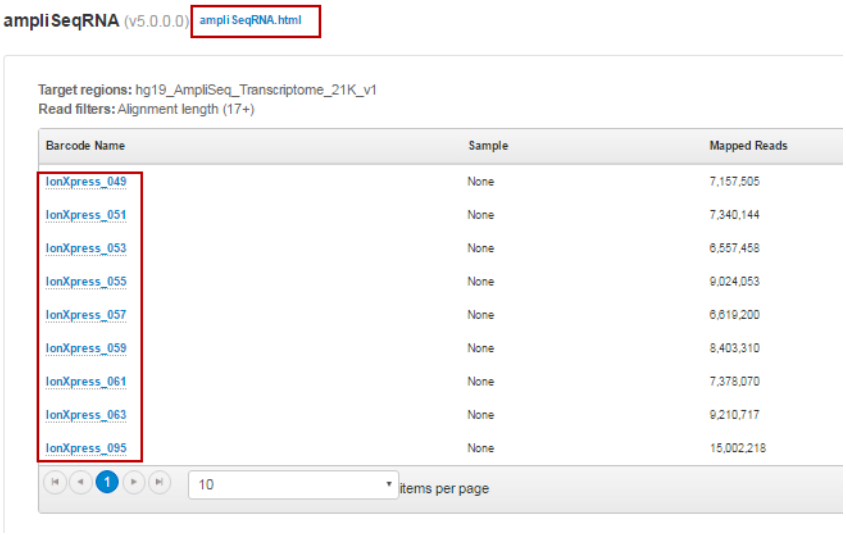
Setting	Description
The following settings can be configured when you run the ampliSeqRNA plugin manually.	
Library Type	ampliSeqRNA is selected automatically and is currently the only **Library Type** that the ampliSeqRNA plugin is designed to work with. Note: If the Planned Run specified a different application, a dialog box will warn you that the plugin may not be appropriate for the run.
Targeted Regions	This is set to the target regions file used in the Planned Run. **Note:** You can override the default Target Regions setting that each barcode uses. This might be useful to specify a subset of genes of interest, or to correct the original Planned Run.
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses. Typically, the **Filter Barcodes** 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.

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.

Graphical report	Description
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.
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, compatible with Microsoft™ Excel™ or similar applications.

Report	Description
Barcode Summary Report	A table listing each barcode's 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 Affymetrix™ software 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 will be represented as the log2 of the ratio of RPM reads of the experiment barcode to the control barcode.

Assembler SPAdes plugin

The Assembler SPAdes plugin is a De-Bruijin 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.

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

Assembler SPAdes plugin configuration

The configuration options for the Assembler SPAdes plugin are described in the following table.

The following settings can only be configured 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.

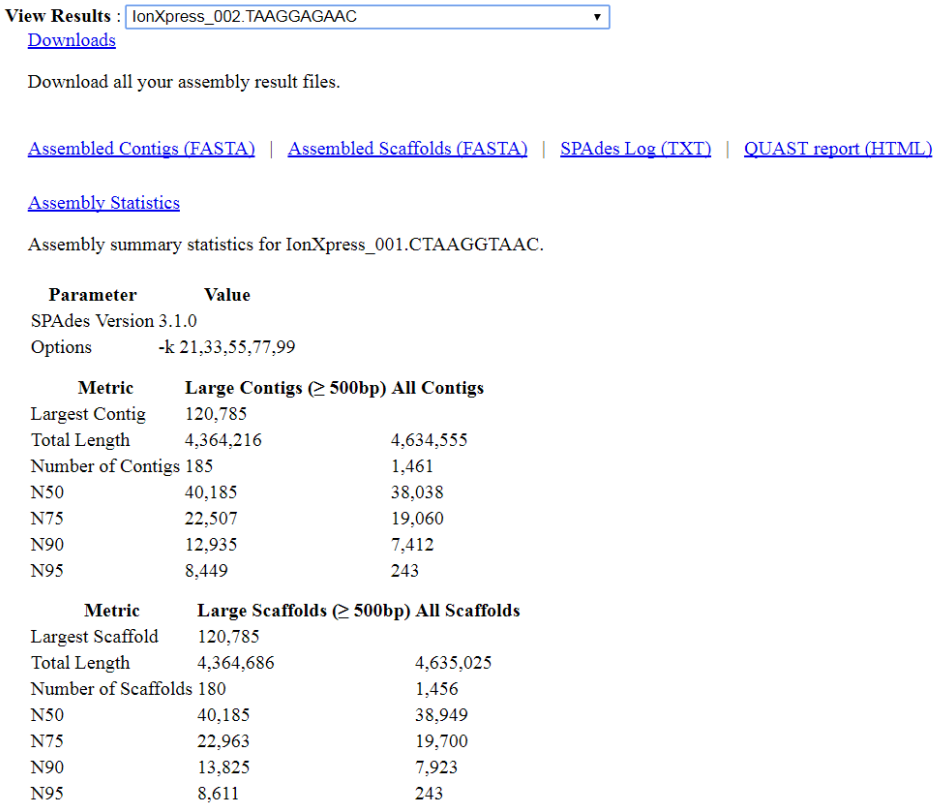
Setting	Description
Fraction of reads to use	The default setting of 100% is recommended, and handles most changes in coverage. If you enter a value of less than 100%, the reads are randomly sub-sampled.
Only process barcodes	By default, the plugin processes all barcodes in the analysis and produces a 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. **Note:** 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.

Setting	Description
Assembly settings	Set this menu as follows: • **Uniform coverage** (default setting)—This is used for data with average GC (35–68%) content. This setting uses the default kmers. • **Non-uniform coverage**—Choose this setting for data with low GC (<35%) content. This setting uses the default kmers. • **Highly non-uniform coverage**—Choose this setting for data with high GC (>68%) content. This setting uses a different set of kmers. • **Custom...**—Choose this setting to enter user-defined **K** and **Mode** settings. – **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. **Note:** Each additional kmer adds a fixed amount to the processing time (for example, using 2 kmers takes twice as long as 1 kmer). – **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 **Summary Statistics**, click **Assembly Statistics**.

Note: 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.

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 only be changed 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 only be changed after the run is complete if the plugin is run manually. Target regions can be overwritten by the specific barcode targets. Select the targeted regions file from the dropdown 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. Select the checkbox to assign specific target region files to individual barcodes. 1. Select a specific barcode from the **Barcode** dropdown list. 2. Select the specific targeted regions file from the dropdown list to associate with the selected barcode. 3. Click **Add**. 4. Repeat steps 1 through 3 to associate additional barcodes with specific target region files. **Note:** Alternatively, you can copy and paste the barcode/target file pairs manually. Barcodes without a target region specified above will assume the default target specified by the **Target Regions** option. For targeted applications, any barcode targets specifically set to None, or defaulting to the **Target Regions** set as None, will be omitted from subsequent analysis. Note: When the **Barcode-specific Targets** option is deselected, all barcodes will use the targets specified by the Target Regions, even if there are barcode-specific targets listed in the text box.

Setting	Description
Advanced options	
Minimum Aligned Length	Specify the minimum aligned length that is required to ensure that the read is included in an analysis.
Minimum Mapping Quality	Specify a minimum value that reads must exceed to be included in the analysis.
Tier 1 Coverage Depth	Specify the first-tier coverage depth at which percentage of target coverage is reported. This value must be at least 2, because the coverage depth output will always be 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 lists the samples, the number of mapped reads, the percentage of valid reads, and the percentage of targets detected. A series of log2 RPM pair-correlation plots are included for rapid correlation analysis. 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 **Summary** screen.
4. Click a link in the **Barcode Name** column of the summary table to open a detailed **Coverage Analysis Report** window for that barcoded sample. Alternatively, click the **coverageAnalysis.html** link to open the summary table for all barcodes in a new window.
5. 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

The library type determines which statistics are presented. This table shows the statistics for an Ion AmpliSeq™ DNA report. Some of these statistics are not available for other library types or can be replaced by alternative statistics. Definitions are in tooltips.

Statistic	Description
Number of mapped reads	Total number of reads mapped to the reference.
Number of reads on target	Total number of reads mapped to any targeted region of the reference. A read is 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.
Target Base Coverage	Summary statistics for targeted base reads of the reference. A base covered by multiple target regions is only counted once per sequencing read.
Bases in target regions	The total number of bases in all specified target regions of the reference.
Percent of reads on target	The percentage of reads mapped to any targeted region relative to all reads mapped to the reference.
Total aligned base reads	The total number of bases covered by reads aligned to the reference.

Statistic	Description
Total base reads on target	The total number of target bases covered by any number of aligned reads.
Percent base reads on target	The percent of all bases covered by reads aligned to the reference that covered bases in target regions.
Bases in targeted reference	The total number of bases in all target regions of the reference.
Bases covered (at least 1x)	The total number of target bases that had at least one read aligned over the proximal sequence. Only the aligned parts of each read are considered. For example, unaligned (soft-cut) bases at the 5' ends of mapped reads are not considered. Covered target reference bases can include sample DNA read base mismatches, but does not include read base deletions in the read, nor insertions between reference bases.
Average base coverage depth	The average number of reads of all targeted reference bases.
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.
Average base read depth	The average number of reads of all targeted reference bases that were read at least one time.
Genome Base Coverage	Summary statistics for base reads of the reference genome.
Genome base coverage at *N* x	The percentage of reference genome bases covered by at least *N* reads.
Target coverage at *N* x	The percentage of target bases covered by at least *N* reads.
Targets with no strand bias	The percentage of all targets that did not show a bias toward forward or reverse strand read alignments. An individual target has read bias if it has at least 10 reads and the percentage of forward or reverse reads to total reads is greater than 70%.
Amplicon Read Coverage	Summary statistics for reads assigned to specific amplicons. Each sequence read is assigned to exactly one of the amplicons specified by the targets file. Reads are assigned to particular amplicon targets based if their (5') mapping location being sufficiently close to the end of the amplicon region, taking the read direction (mapping strand) into account.
Number of amplicons	The number of amplicons specified in the target regions file.

Statistic	Description
Percent assigned amplicon reads	The total number of reads that were assigned to individual amplicons. 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 this way it is assigned to the amplicon region that has the greatest overlap of aligned sequence.
Average reads per amplicon	The average number of reads assigned to amplicons.
Uniformity of amplicon coverage	The percentage of bases in all targeted regions (or whole-genome) covered by at least 0.2x the average base read depth.
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 at least 10 reads and the percentage of forward or reverse reads to total reads is greater than 70%.
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 based composition bias	A number that represents the proportion of amplicons showing low representation (<.2x mean reads) in the lower and/or upper quartiles of amplicons ordered by increasing GC base pair content of their insert sequences. The value is relative to that in the center 50th percentile of amplicons and weighted by the standard deviation of representation over all amplicons.

Example statistics

The following is an example of the plugin statistics for an Ion AmpliSeq™ run.

Note: Almost every statistic, plot, link, and functional widget in the report provides tooltips with definitions. Hover over a heading or description in the report to view the tooltip.

Coverage Analysis Report

Sample Name: T3

IonXpress_006_R_2016_02_11_17_46_25_user_p04-574

Library type: AmpliSeq Exome
Reference: hg19 (DNA)
Target regions: AmpliSeqExome.20131001.designed

Number of mapped reads	40,285,453
Percent reads on target	94.86%
Average base coverage depth	110.0
Uniformity of base coverage	92.05%

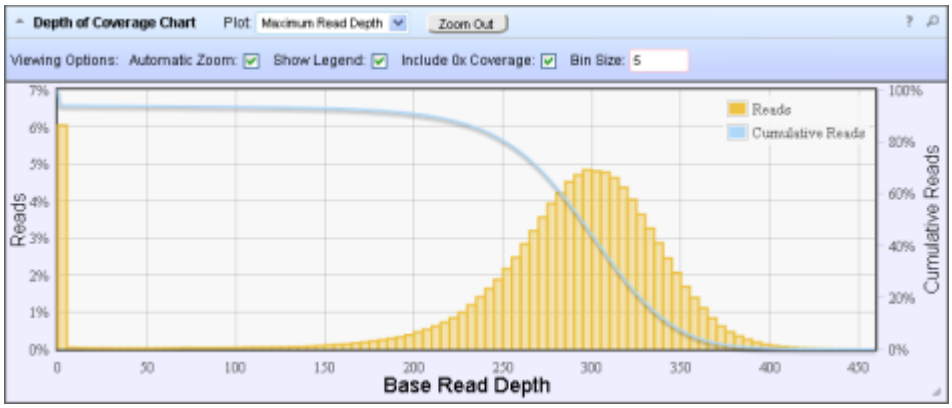
Amplicon Read Coverage		Target Base Coverage	
Number of amplicons	293,903	Bases in target regions	57,742,646
Percent assigned amplicon reads	94.86%	Percent base reads on target	94.86%
Average reads per amplicon	130.0	Average base coverage depth	110.0
Uniformity of amplicon coverage	93.33%	Uniformity of base coverage	92.05%
Amplicons with at least 1 read	99.50%	Target base coverage at 1x	99.20%
Amplicons with at least 20 reads	95.21%	Target base coverage at 20x	92.92%
Amplicons with at least 100 reads	56.84%	Target base coverage at 100x	46.00%
Amplicons with at least 500 reads	0.30%	Target base coverage at 500x	0.19%
Amplicons with no strand bias	90.41%	Target bases with no strand bias	76.69%
Amplicons reading end-to-end	19.19%	Percent end-to-end reads	51.22%

The **Reference Coverage** chart is an overlay of where target regions are defined and overlap on the reference.

Example charts

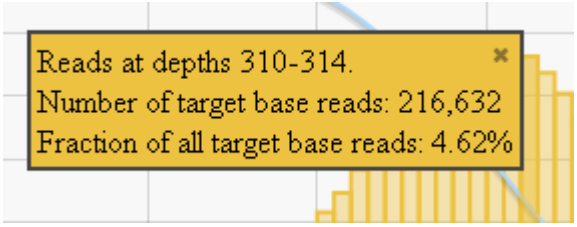
Many of the charts that are generated by the coverageAnalysis plugin include a **Plot** menu that allows you to change characteristics of the chart. For example, you can show both strands.

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

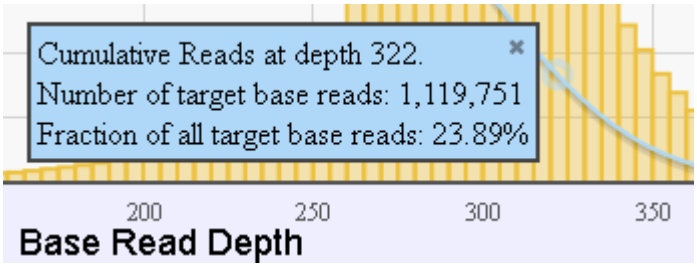


In the **Depth of Coverage** chart above, the left Y-axis (% reads) is the number of reads at a particular read depth (or bin of read depths) as a percentage of the total number of base reads. The right Y-axis (% cumulative reads) is the cumulative count of the number of reads at a given read depth count is at least read depth, as a percentage of the total number of reads. If your analysis includes a regions of interest file, this chart reflects only target regions (reads that fall within a region of interest).

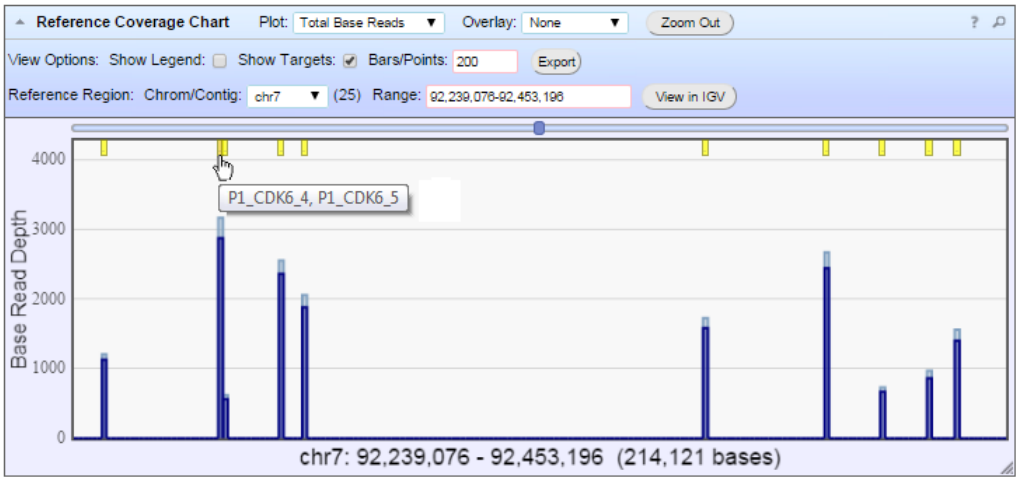
In most charts you click on a data point to open a detail panel for that data:



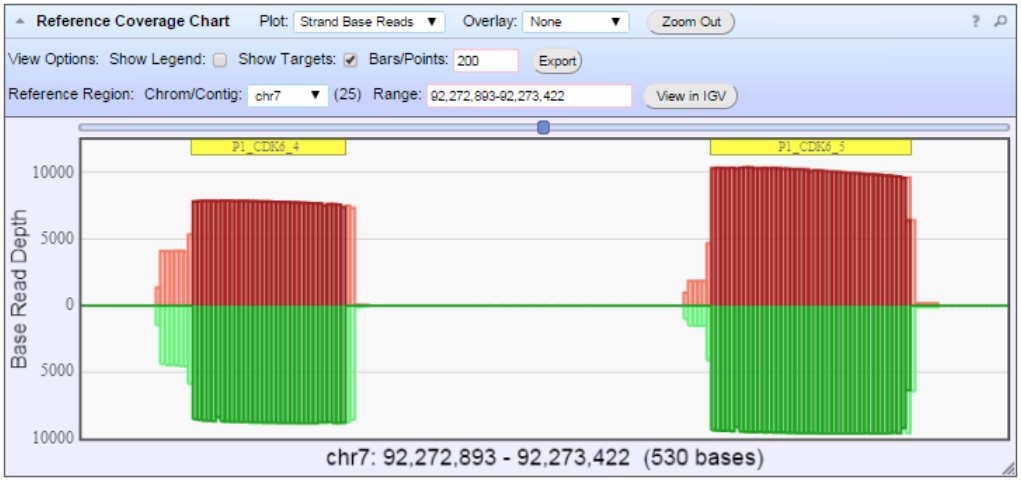
In this chart, the blue curve measures the cumulative reads at that read depth or greater. Click a point on the blue curve to open the blue detail panel for that read depth:



The following **Reference Coverage Chart** is shown with the **Strand Base Reads** option:



You can also zoom in on a region of interest.

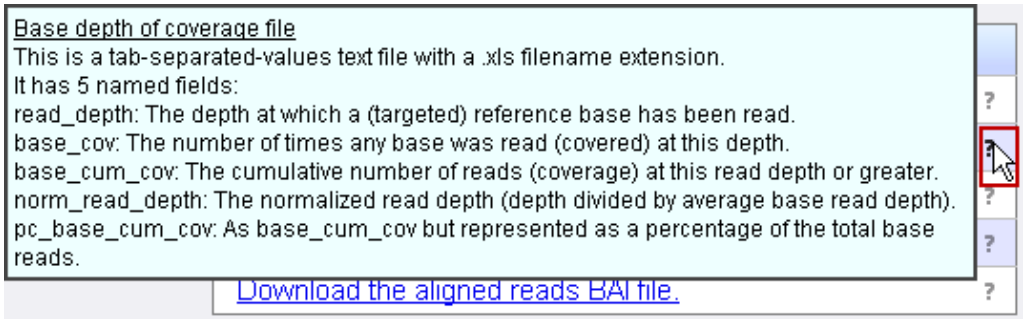


Output files

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

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

Click a question mark next to the file ? to open a description of the file:



The list of files depends on the application type selected. The following list is for an Ion AmpliSeq™ DNA run.

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 these fields: • **read_depth** The depth at which a (targeted) reference base has been read. • **base_cov** The number of times any base was read (covered) at this depth. • **base_cum_cov** The cumulative number of reads (coverage) at this read depth or greater. • **norm_read_depth** The normalized read depth (depth divided by average base read depth). • **pc_base_cum_cov** As base_cum_cov but represented as a percentage of the total base reads.

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

File	Description
Chromosome base coverage summary	Base reads per chromosome summary data used to create the default view of the Reference Coverage Chart. This file contains these fields: • **chrom** The name of the chromosome or contig of the reference. • **start** Coordinate of the first base in this chromosome. This is always 1. • **end** Coordinate of the last base of this chromosome. Also its length in bases. • **fwd_reads** Total number of forward strand base reads for the chromosome. • **rev_reads** Total number reverse strand base reads for the chromosome. • **fwd_ontrg** (if present) Total number of forward strand base reads that were in at least one target region. • **seq_reads** Total sequencing (whole) reads that are mapped to individual contigs.
Aligned reads BAM file	Contains all aligned reads used to generate this report page, 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 transfer files to another system quickly. When you configure the plugin, you select which file categories from the run are included in the export.

Note: 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, as well as 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 export is complete, the report appears 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 the Torrent Suite™ Software is busy performing additional processing functions, plugin run times are longer.

Note: You can configure the ERCC_Analysis plugin to run automatically. However, automatic execution is not recommended, unless most of the analyses on the 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). The value you enter on the ERCC Plugin Configuration screen is used when the plugin is auto-run and when a user manually launches the plugin without entering a value. Users can override this value on a per-run basis when they manually launch the plugin.
Minimum transcript counts	The minimum number of reads that an ERCC transcript must have to be included in the analysis.
ERCC pool used	Select the ERCC transcript pool used when preparing the library.
Barcodes of interest	**IMPORTANT!** If you configure a Planned Run or Planned Run template to execute the ERCC_Analysis plugin, and your experiment uses the Ion Total RNA-Seq Kit v2, you must select a barcode option: • Select **IonXpressRNA** if your experiment uses this kit. • Select **RNA_Barcode_None** if your experiment does not use a barcode kit

Review 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 **Completed Runs & Reports** screen, click the report name to open.
3. In the **Summary**, click **ERCC_Analysis** to see the **ERCC_Analysis** pane. The following settings are shown.

4. After the analysis is complete, click the **ERCC_Analysis.html** link or individual **Barcode Name** link to open the ERCC Report and view the analysis results. The following table describes the settings that are shown.

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). The value you enter on the ERCC Plugin Configuration screen is used when the plugin is auto-run and when a user manually launches the plugin without entering a value. Users can override this value on a per-run basis when they manually launch the plugin.
Minimum transcript counts	The minimum number of reads that a given 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	Select a barcode from the dropdown list for **Add a specific barocde**. 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.

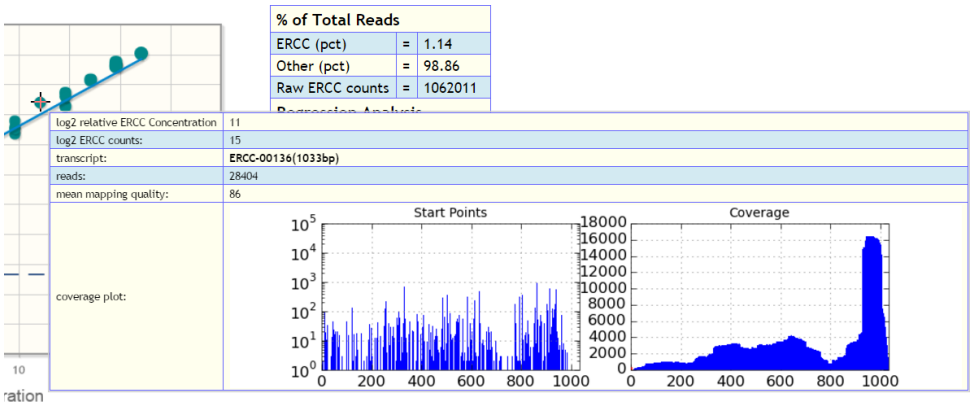
Interpret the ERCC Dose Response plot

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—and 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 (e.g., ≥0.9) value. Although there are 92 transcripts 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

There are two ways to look at the details of a particular ERCC transcript. To view all the details regarding a particular ERCC transcript, you should view both.

- Hover your mouse-cursor over a point in the ERCC Dose Response plot to display a popup window that shows details regarding that transcript. Overlapping points on the plot can be resolved by zooming in on the plot to more easily distinguish 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 the **Reset Zoom** button 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.
transript	The ERCC transcript identifier including length in base pairs (bp).
reads	The number of reads that map to the particular transcript.
mean mapping quality	Points in the display are color coded based on the mapping quality.

- 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 will be > 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.

Parameter	Description
Unique Start Sites	The number of base positions that have only one read starting at the position.
Coverage CV	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 will be >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**—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. Enable and run this plugin only under the guidance of Thermo Fisher Scientific Technical Support.

IMPORTANT! Enable and run this plugin only when directed by Thermo Fisher Scientific Technical Support or your Field Applications Scientist.

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

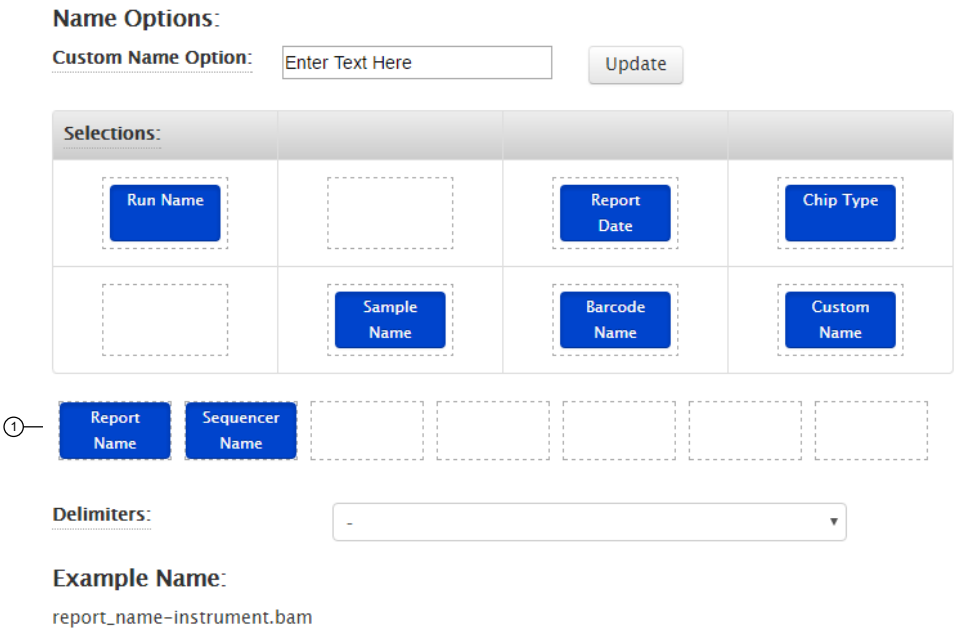
1. Select from the following options to choose the file types that you export:

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 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.

   Note: The BAM files load quickly so may appear first in the list of links. The other file formats take longer to download.

4. Ensure that the status of the plugin run is **Completed**. You can click **Plugins** ▸ **Refresh plugins** at the top of the **Summary** 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.

5. When the list contains all 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

The FilterDuplicates plugin allows you 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.

Note: The **Mark as Duplicate Reads** feature in the main analysis pipeline, enabled in the **Kits** step of Planned Run creation, marks reads as duplicates but does not remove them from the BAM files.

Review FilterDuplicates plugin results

After the sequencing run completes, review the FilterDuplicates plugin results, and download the BAM files with duplicate reads removed.

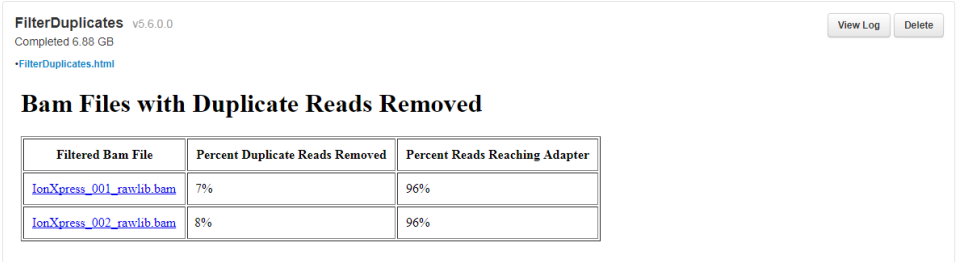
1. In the **Data** tab, click **Completed Runs & Reports**.

2. In the list of runs, locate 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.

   Note: BAM files load quickly, so 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 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.

immuneResponse RNA 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 Affymetrix™ Transcriptome Analysis Console (TAC) 3.1 software.

The immuneResponseRNA plugin requires a **Target Regions** BED file and an associated **Reference Library** FASTA file. See "Reference Management" on page 227 for more information on installing these files.

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
(Optional) Add new gene list	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, locate 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.

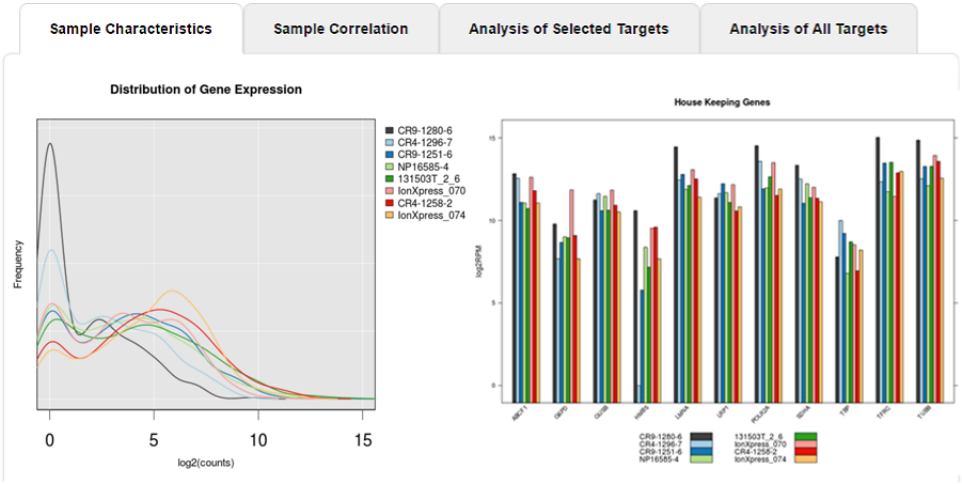
   Note: The BAM files load quickly so may appear first in the list of links. The other file formats (VCF, XLS and FASTQ) take longer to download.

5. In the **Analysis Summary**, review your **Mapped Reads**, **Valid Reads**, and **Targets** detected by barcode.

Column	Description
Barcode Name	The barcode used for the sample.
Sample	Sample name as it was entered in the sequencing Run Plan.
Mapped Reads	Number of reads that map to the reference sequences.
Valid Reads	Percentage of mapped reads ≥50% amplicon length.
Targets ≥1 reads	Number of targets/genes with at least 1 read.
Targets ≥2 reads	Number of targets/genes with at least 2 reads.
Targets ≥10 reads	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** (only present if a **Genes of interest** subset .bed file was selected), or **Analysis of All Targets** tabs to review the data graphically.



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 each barcode's sample name, 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.

Report hyperlink	Description
Download RPM data (normalized by total read counts)	A table listing RPM (Read count Per Million mapped reads) for each barcoded sample along with gene annotations. RPM is calculated as: (read count) × 10^6 / total number of mapped reads
Download mean housekeeping scaled log2 RPM data	A table listing housekeeping-gene normalized, log2-transformed read counts for each barcoded 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: $log2(\text{count}+1) - \frac{\sum(log2(hk\ \text{counts}+1))}{\text{number of } hk \text{ gene}} + log2(10^6)$ These values are useful for differential analysis when a large proportion of the target genes (non-housekeeping genes) are expected to be differentially expressed or when the expression levels of the housekeeping genes in the 2 groups differ significantly.
Download CHP files normalized by RPM	The RPM data is converted to CHP file format for use with Affymetrix™ Transcriptome Analysis Console (TAC) software. The downloaded .ZIP file contains all the CHP files from the sequencing run. Each barcoded sample has 1 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 4 experiments using genomic DNA and H_2O as negative control samples.

IonReporterUploader plugin

Analysis files that are generated in the Torrent Suite™ Software can be directly transferred to an Ion Reporter account in Ion Reporter™ Software with the IonReporterUploader plugin.

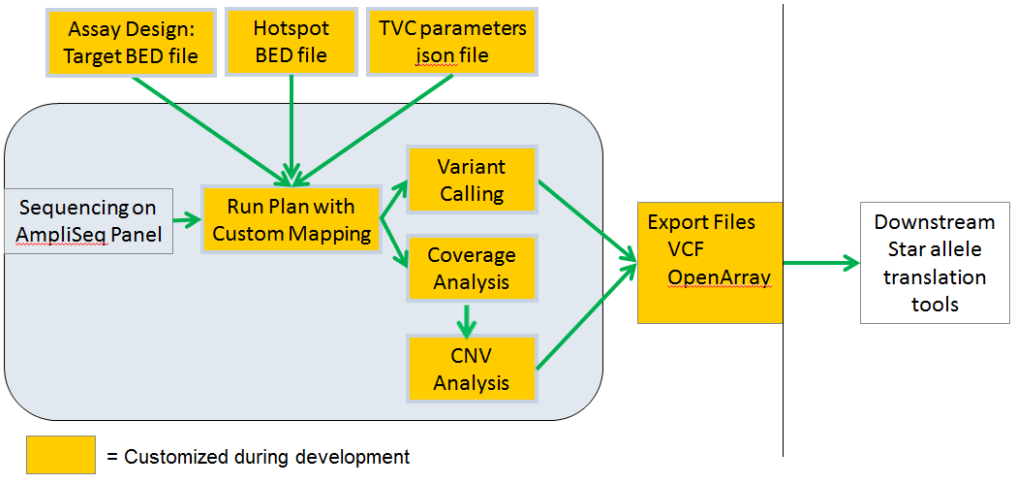
Ion Reporter™ Software uses the Torrent Suite™ Software output BAM file for analysis. The Ion Reporter™ Software annotation-only workflow also accepts the VCF output file of the variantCaller plugin. Use the IonReporterUploader plugin to transfer these BAM and VCF output files to Ion Reporter™ Software.

For details about the IonReporterUploader plugin, see "Integration with Ion Reporter™ Software" on page 172

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 plugins: the variantCaller plugin for genotyping and coverageAnalysis plugin for CYP2D6 copy number detection.

The figure below 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™ Pharmocogenomics Research Panels* (Pub. No. MAN0014300)
- *Create a Planned Run using the Ion AmpliSeq™ Pharmocogenomics 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, locate the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click the **PGxAnalysis** link 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 "Variant calls in Torrent Suite Software" on page 191 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 130 for details on coverageAnalysis plugin results interpretation.

RunTransfer plugin

Use the RunTransfer plugin to complete the following:

- Transfer the signal processing output files from a completed run to a different 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.

Note: 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 thumbnail files only or transfer the 96 block files in a full chip run.

RunTransfer plugin configuration

The RunTransfer plugin requires global configuration to connect to the 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 Torrent Server.
Remote TS Username (default ionadmin)	The username of the administrator-level user on the receiving Torrent Server. The default administrator username on a new Torrent Server is **ionadmin**, but this can be changed.
Password	The password of the administrator-level user on the receiving Torrent Server.
Upload Path (default /results/uploads/)	The path of the directory used to store transferred files and analyses on the receiving Torrent Server.

Setting	Description
Data set type	Select an option based on the following considerations: • **Thumbnails/PGM:** This option will transfer all files, including thumbnails. However, the plugin will not run if full chip sequencing runs were performed on the Ion Proton™ System, Ion S5™ System and Ion GeneStudio™ S5 System. With this setting, a warning will be issued and the plugin will not run, if the instrument used a Full Chip run. • **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 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 Torrent Server.

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 **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, you can see a list of the files that were transferred. If the plugin is configured to use the data type set as **Thumbnails/PGM**, you will see these files:
   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 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 non-coding SNPs. The Sample ID panel contains nine primer pairs that can be combined with any Ion AmpliSeq™ Ready-to-Use or Custom Panel.

For the samples to work with this plugin, the Ion AmpliSeq™ library must have be prepared with Ion AmpliSeq™ sample tracking amplicons.

Note: The sampleID plugin is pre-configured and does not require input.

Review sampleID 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 **sampleID** to view the plugin summary.
4. Click **sampleID.html** to open the sampleID report in the browser tab. Then, you can open a detail report and other data files:
5. *(Optional)* Scroll to the File Links table, and click on 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), multi-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 pre-set 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 “Variant calls in Torrent Suite Software“ on page 191.

Plugins available only on Thermo Fisher Cloud

There are two plugins that are supported by Thermo Fisher Scientific and available on the Thermo Fisher Cloud at **https://apps.thermofisher.com/apps/publiclib/#/plugins**. These plugins are not pre-installed in the Torrent Suite Software. For details about plugins that are included with Torrent Suite Software, see "Pre-installed plugins" on page 120.

Plugin name	Description
RNASeqAnalysis	Analyzes cDNA reads. This plugin is an RNA transcript alignment and analysis tool for use with the reference genomes hg19 and mm10. For details, see "RNASeqAnalysis plugin" on page 157.
smallRNA Analysis	Analyzes small RNA reads with an emphasize on micro RNA molecules. Use with reference genome hg19 only.

smallRNA plugin

Use this plugin to analyze micro RNA reads. Reads are aligned to mature micro RNAs using the tmap or bowtie2 alignment software. 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. **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.

RNASeqAnalysis plugin

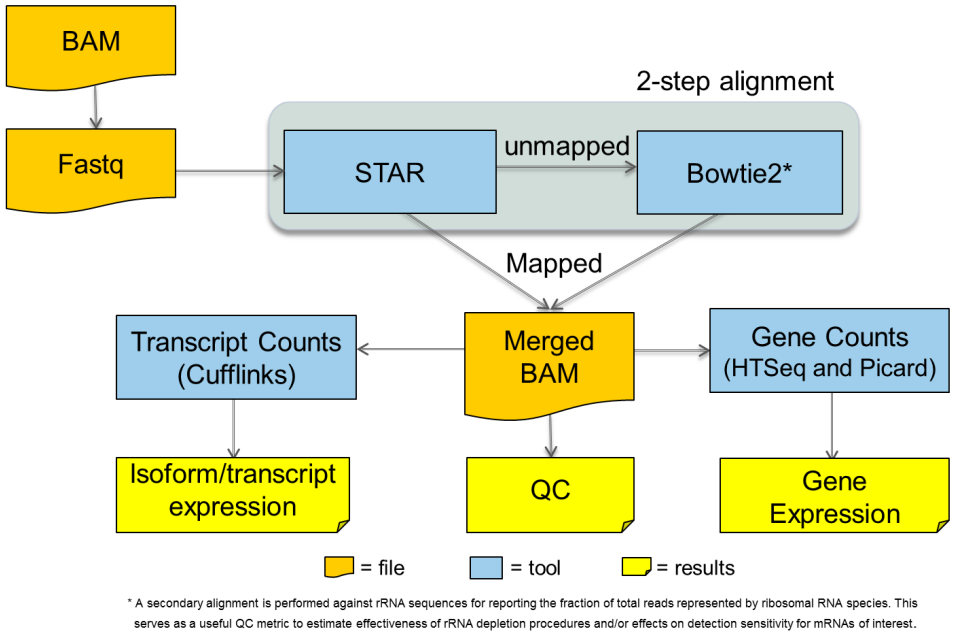
The RNASeqAnalysis plugin is an RNA Transcript Alignment and Analysis tool for use with reference genomes hg19 and mm10.

Note: In order to use the hg19 or mm10 genomes with this plugin, the reference genomes must first be imported from the preloaded references page. Also, annotation files for the human and mouse references are available for import. See “Import a

preloaded reference sequence“ on page 230 for more information on downloading references and annotation files.

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.

Note: 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 from the dropdown list: • **hg19**, *or* • **mm10**
Regenerate indices	Check to ensure any pre-generated indexing and annotation files for the specified reference are deleted before the analysis starts. New files will be generated as needed, which may add several hours to the plugin run time (~3 hours for human-sized genomes). Using this option is necessary if the plugin previously failed during (STAR) reference index generation of the reference sequence was updated.

Note: To use the mouse mm10 Reference Genome with this plugin, first import the preloaded Ion reference genome to Torrent Suite™ Software. See "Import a preloaded reference sequence" on page 230 for more information.

Review RNASeqAnalysis plugin run results

After your sequencing run completes, 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, you can 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 163.

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 161.

Link name	Download description
Reference table	Plot that shows the number of genes that have reads in log10 counting bins.
Gene Mapping Summary	Summary of reads mapped to genes of the annotated reference.
Base Mapping Summary	Summary of base reads aligned to genetic features of an annotated reference.

Link name	Download description
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 165.
Correlation Heatmap	For details, see "Correlation heatmap" on page 168.
Correlation Plot	For details, see "Correlation plot" on page 168.
Gene Heatmap	For details, see "Gene heatmap" on page 169.
Isoform Heatmap	For details, see "Gene heatmap" on page 169.

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
- Download the Gene Read Counts
- Download Output Files (page)
- Download Cufflinks Output Files (page)

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
```

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

Output Files - 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

Cufflinks Output Files - Provides a list of links to Cufflinks output files.

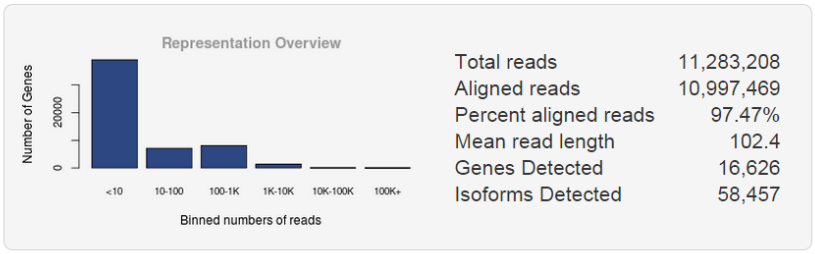
File Size	Date	File
5.5M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genes.fpkm_tracking
24M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.isoforms.fpkm_tracking
0	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_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 on any barcode of interest in the **RNASeqAnalysis Report** to see graphs for the selected barcode.

Reference table - Plot showing the number of genes with reads in log10 counting bins.

Reference: hg19



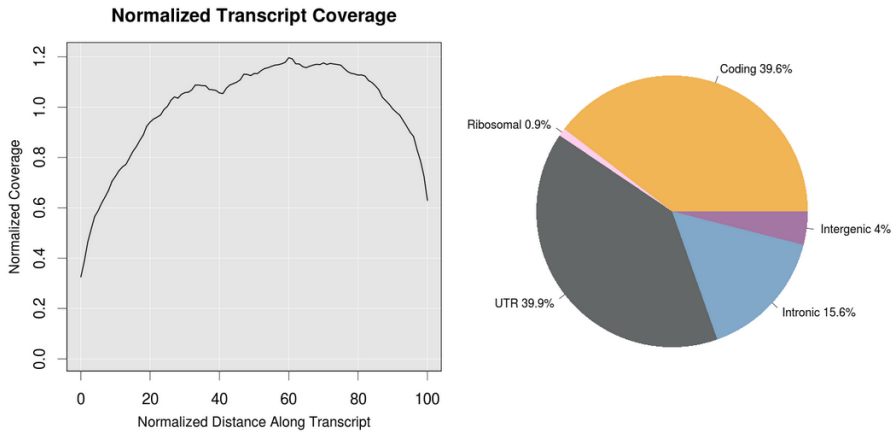
Gene Mapping Summary - Summary of reads mapped to genes of annotated reference.

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 - Summary of base reads aligned to genetic features of an annotated reference.

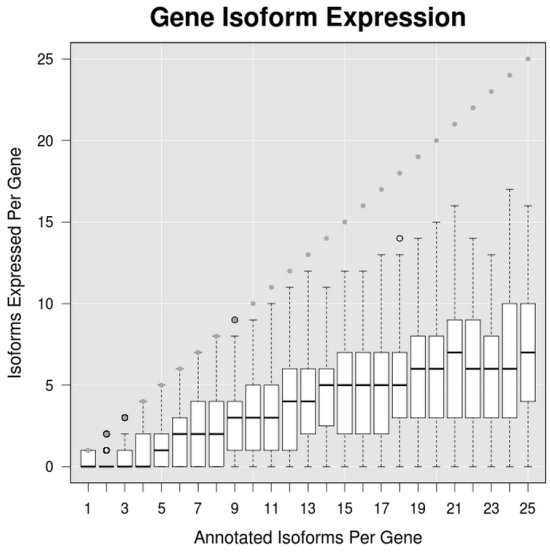
Base Mapping Summary	
Total base reads	1,155,834,791
Total aligned bases	914,778,477
Percent aligned bases	79.14%
Percent coding bases	39.68%
Percent UTR bases	39.93%
Percent ribosomal bases	0.94%
Percent intronic bases	15.65%
Percent intergenic bases	3.98%
Strand balance	0.4980

Normalized Transcript Coverage - A plot of normalized transcript coverage; the frequency of base reads with respect to the length of individual transcripts they are aligned to in the 3' to 5' orientation.



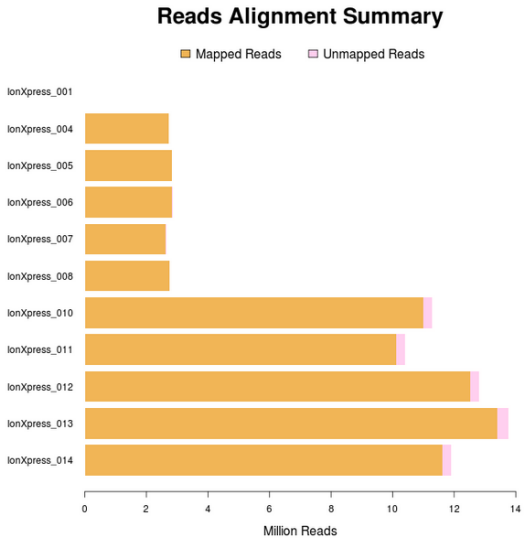
Gene Isoform Expression - Box plots showing variation of isoforms expressed at FPKM ≥ 0.3 for each set of genes grouped by the number of anticipated (annotated) isoforms. Whiskers are defined by points within Q1-1.5xIQR to Q3+1.5xIQR. Only

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.

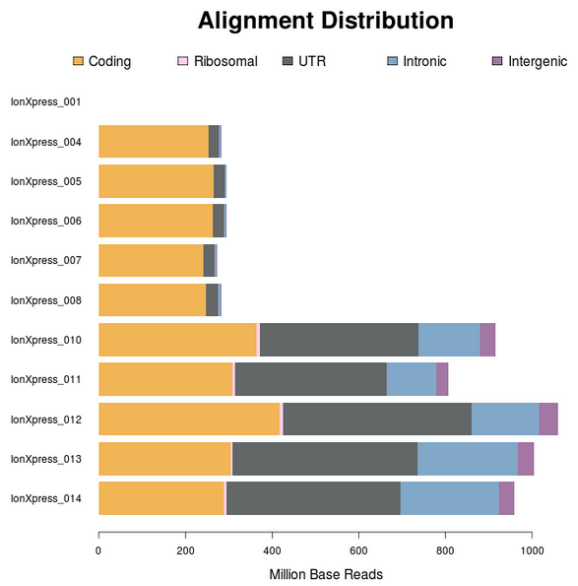


Distribution Plots

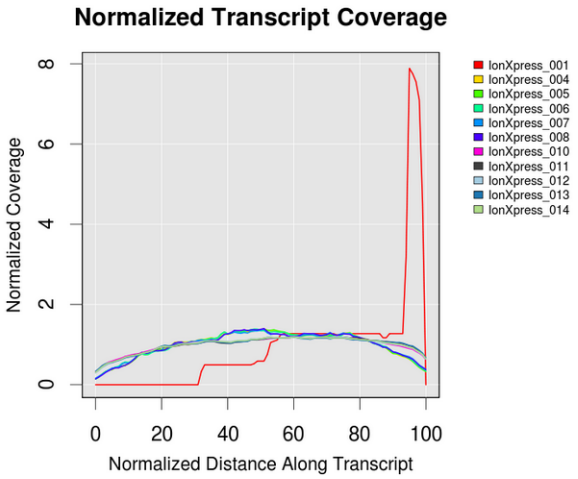
Reads Alignment Summary- A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the barcode summary table.



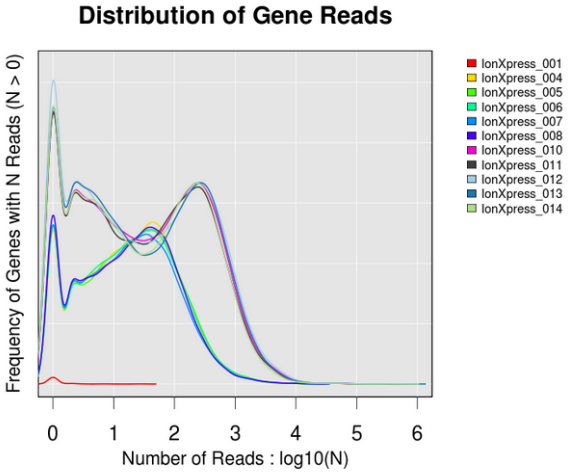
Alignment Distribution - A graphical summary of the distribution of reads to genomic features.



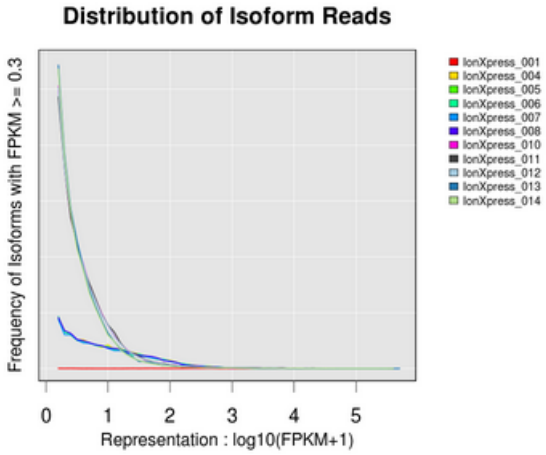
Normalized Transcript Coverage - An overlay of individual normalized transcript coverage plots for each barcode.



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 is fitted to a Gaussian kernel using the default R 'density' function.

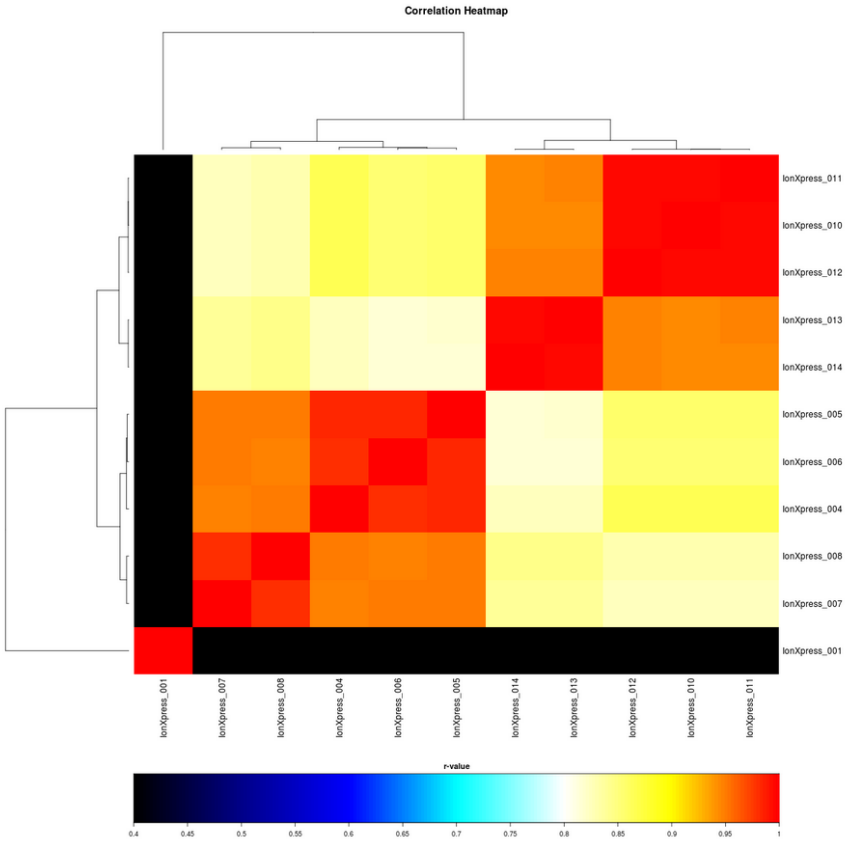


Distribution of Isoform Reads - Distribution of transcript isoforms across barcodes showing the counts of isoforms having similar FPKM values. All curves are plotted on the same y-axis, normalized to the highest count and scaled for FPKM values ≥0.3.



Correlation heatmap

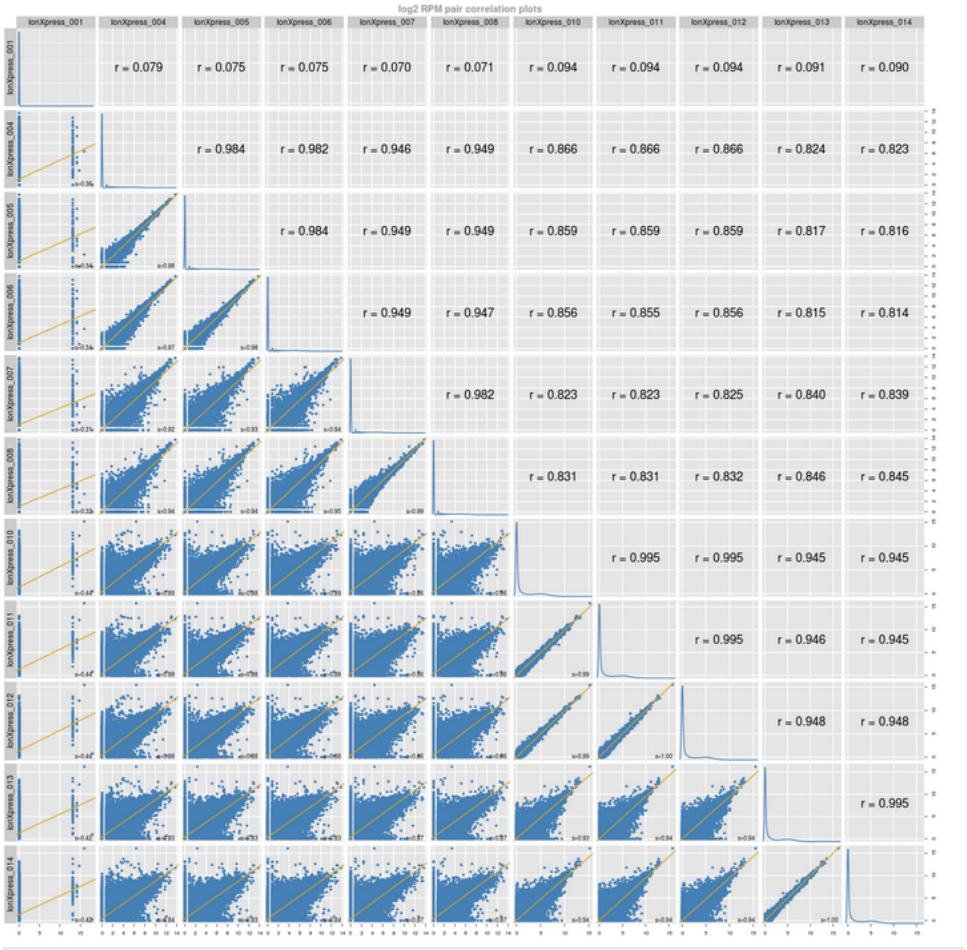
A heatmap of Spearman correlation r-values for comparing log2 RPM reads pair correlation barcodes, with dendrogram reflecting ordering of barcodes as being most similar by these values.



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)

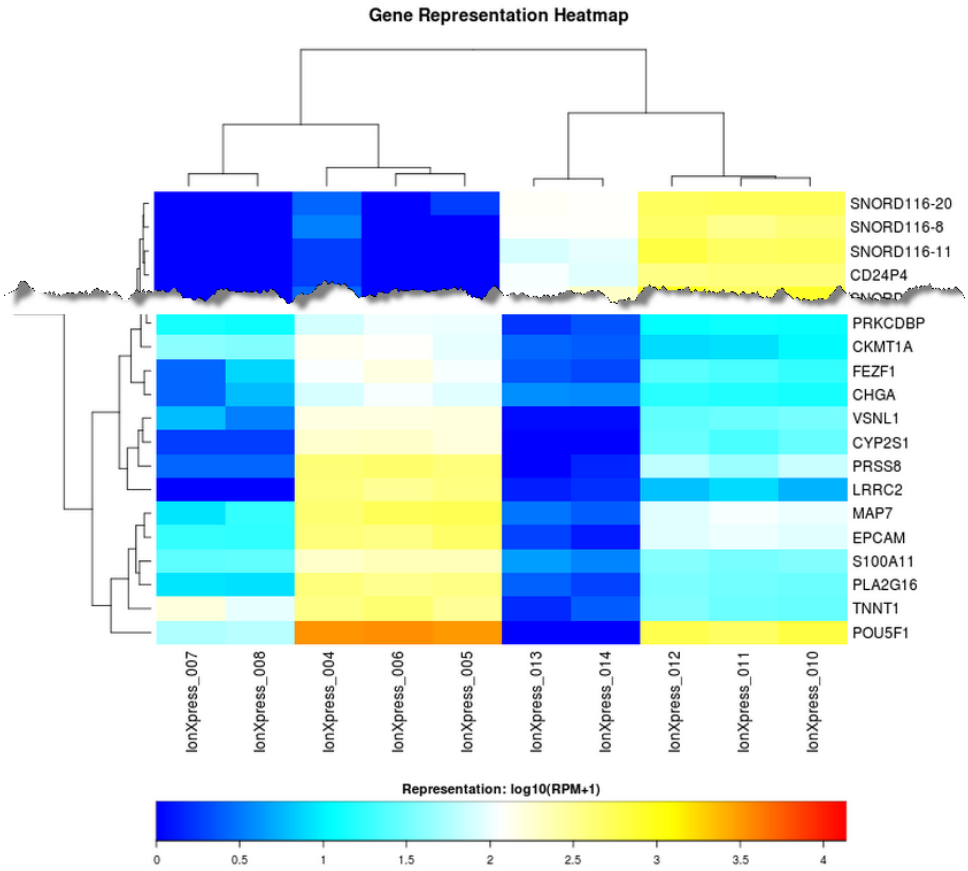
values for each barcode. (If only one barcode has reads, a density plot is displayed.) Click the plot to open an expanded view in a new window.



Gene heatmap

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

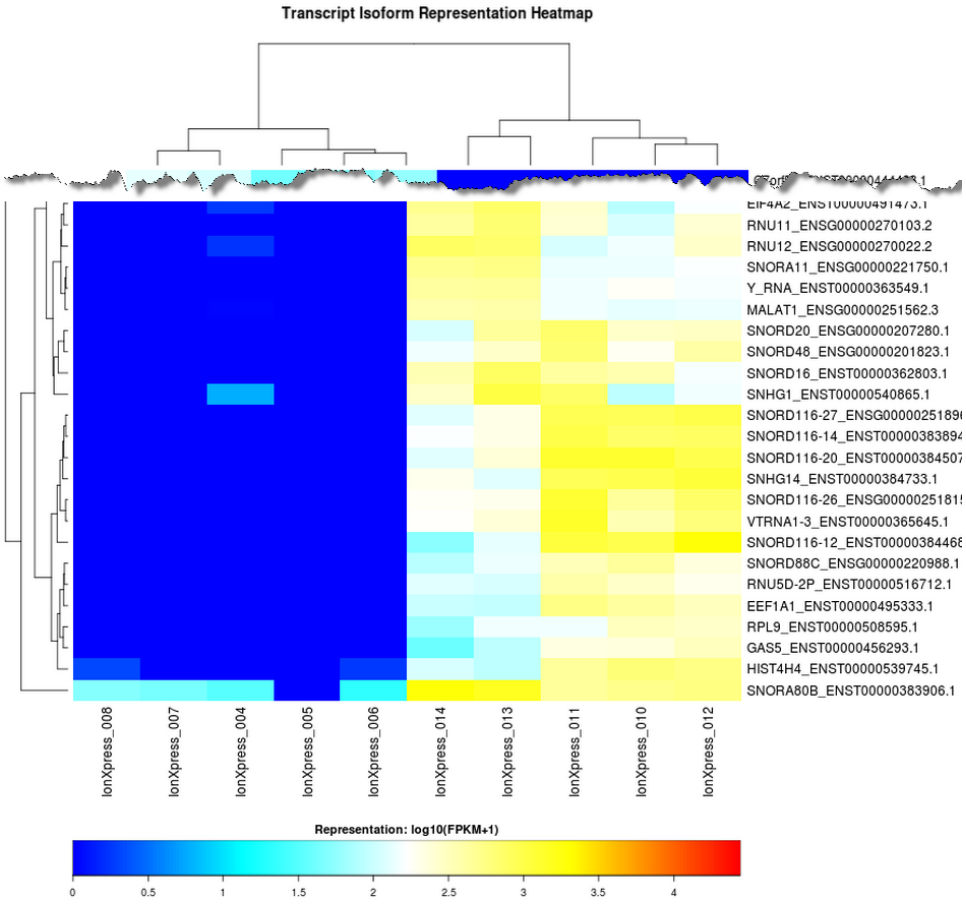
reads, plotted using log10 of those counts. For this plot, barcodes will be omitted if they have less than 100,000 total reads.



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.



Integration with 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, provided that an annotation-only workflow is used to process the files in Ion Reporter™ Software.

Note: Ion Reporter™ Software is available under separate license and is not included with Torrent Suite™ 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 file from a completed run report to Ion Reporter™ Software and either:
  - Available as analyses in Ion Reporter™ Software. In this case, the output files are transferred to Ion Reporter™ Software and the workflow of your choice Ion Reporter™ Software is automatically launched on your newly transferred samples.
  - Available 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 manually upload of data to Ion Reporter™ Software Use this option if, for example, you want to upload data to Ion Reporter™ Software multiple servers. To manually upload data from Torrent Suite™ Software to Ion Reporter™ Software, do one of the following:
  - Run the 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.

Note: 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 179.

Install the IonReporterUploader plugin on a Torrent Server

The IonReporterUploader plugin is automatically installed on a 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 289 and "Update off-cycle release plugins" on page 290.

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/**.

Note: An administrative account is not required for this procedure.

1. Click ⚙ **(Settings)** ▸ **Plugins**.
   The list of plugins opens.
2. Click **Install or Upgrade Plugin**.
3. Click **Upload a Plugin file**, then browse to and select the **IonReporterUploader.zip** file that you downloaded. Click **Open**, 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 the plugin is run, or when you manually upload output files to Ion Reporter™ Software. You can upload the Torrent Suite™ Software output files to any of the Ion Reporter™ Software accounts that are available in Torrent Suite™ Software.

IMPORTANT! When you upgrade to a new version of Ion Reporter™ Software you must reconfigure your 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.

---

1. Sign in to Torrent Suite™ Software as either an administrative user or a standard user.
2. Click ⚙ **(Settings)** ▸ **Ion Reporter Configure**:
   The **Ion Reporter Uploader Account Configuration** screen opens.

3. Click **Add Account**, then select an account type:

Option	Description
Ion Reporter™ Software on Thermo Fisher Cloud	Select **Ion Reporter Cloud**
Ion Reporter™ Software on Ion Reporter™ Server	Select **Ion Reporter**
Ion Reporter™ Software on Thermo Fisher Cloud—China version	Select **Ion Reporter Cloud - China**

4. Enter the appropriate information into the **Add Ion Reporter account** screen.

   Note: Ask your system administrator for values for a local Ion Reporter™ Server.

Setting	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 run plan template or run the Ion Reporter Uploader 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™ Software server.
Port	443 is the port number that is automatically populated.
Username	Enter your Ion Reporter™ Software username (your email address)
Password	Enter the password you use to Sign in to Ion Reporter™ Software

5. Select one of the following options:

   - **Default** The account that is configured by default in the run templates and Planned Runs. If the main account is for file transfers, enable the **Default** checkbox. You can change the default account later when 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.

   Note: This option is available if multiple versions of Ion Reporter™ Software are available and multiple accounts are configured.

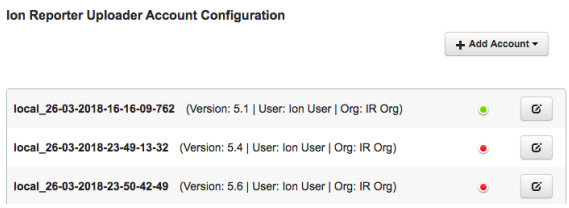
6. Click **Add**.

7. (*Optional*) The IonReporterUploader plugin can manage multiple configurations. To add another configuration, repeat the procedure.

   Two email notifications are sent each time that a IonReporterUploader plugin finishes a run. The first is sent when the plugin run starts. Another is sent when the upload to Ion Reporter™ Software is finished. The notifications are sent to the email address of the Ion Reporter™ Software user whose is signed in when the IonReporterUploader plugin is launched.

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 manually upload output files to Ion Reporter™ Software. The accounts are also listed when you configure a Ion Reporter™ Software Planned Run or Planned Run template.

Observation	Possible cause	Recommended action
Ion Reporter account is not configured Red status on Ion Reporter Software account configuration screen. 	If you change your account password, you will see a red dot in the status column of the IonReporterUploader account.	When you change your password, click **Edit** and enter your new password.
	If you upgrade the account, you will see a red dot in the status column of the IonReporterUploader 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.

Automatically transfer Torrent Suite™ Software output to Ion Reporter™ Software

To transfer output files from a Torrent Suite Software analysis to Torrent Suite™ 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 a 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 this up, you must include select the IonReporterUploader plugin *and* select the Ion Reporter™ Software workflow in the Planned Run.

- Made available in Ion Reporter™ Software as output files (BAM and VCF files) that can be later 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 if you want to annotate the VCF files, using the Annotation-only workflow in Ion Reporter™ Software.

  Note: VCF files are available as output files if you configure the Ion Reporter™ Software plugin. For details, see "Variant calls in Torrent Suite Software" on page 191.

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.

We recommend that you use the plan by Sample Set feature when you configure the Ion Reporter™ Software in your Planned Run or template. For details, see "Plan by Sample Set" on page 55.

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 instance, if you are using an Ion AmpliSeq™ Exome Panel, select the AmpliSeq™ DNA template with the same name.

3. Add samples, ensure the default settings, then enter a plan name, then select **Ion Reporter** in the workflow bar.

4. Select the Ion Reporter™ 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 the Torrent Suite™ Software manually to Ion Reporter™ Software.

   If the Ion Reporter™ account is not configured, click **Configure** to add an account. See "Set up an account for IonReporterUploader plugin" on page 173 for more information.

5. Select a **Sample Grouping** that corresponds to the sample relationship in Ion Reporter™ Software. When you select a **Sample Grouping**, the workflow menu in Ion Reporter™ Software displays only workflows that match the type of workflows selected.

6. Select an option in the **Existing Workflow** menu:

Option	Description
Select a workflow Ion Reporter™ Software for your sample type.	The workflow will be automatically launched Ion Reporter™ Software with the sample data from the run. Successful analyses will be available in Ion Reporter™ Software with the account and organization that you selected.
Select **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 will also be available, if you ran the variantCaller plugin.

7. *(Optional)* Click **Create New Workflow** to open Ion Reporter™ Software in a new browser window. In Ion Reporter™ Software, create your new workflow, then save it.

   When you return to your Torrent Suite™ Software, refresh your browser. You can then select the newly created workflow in the **Existing Workflow** menu.

8. Select an **Ion Reporter Upload Option**:

Option	Description
Review results after run completion, then upload to Ion Reporter	Use this option if you want to review the completed run report and then manually transfer the data files to Ion Reporter™ Software. IMPORTANT! After you review the results in **Completed Runs & Results**, you must click **Upload to IR > Upload as Planned** to upload the data to Ion Reporter™ Software.
Automatically upload to Ion Reporter after run completion	Run results are automatically uploaded to Ion Reporter™ Software. If you select a workflow, an Ion Reporter™ analysis is launched immediately after the run. Successful analyses are then available in Ion Reporter™ when you log into with the account that is included in the setup.

9. Continue with the steps to create the Planned Run. See “Steps in the workflow bar“ on page 37 for more information.

10. If appropriate, enter the gender of a sample. For details, see “Sample gender“ on page 179.

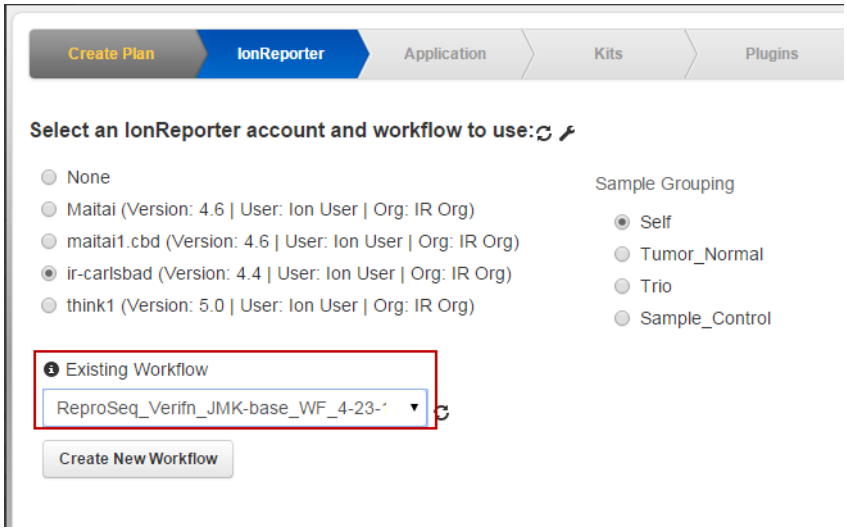
    Note: If the gender of a sample is not specified or if the sample gender is specified as "Unknown", IGV assumes that the gender of a sample is female.

11. To save the Planned Run or Planned Run template, do one of the following 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.

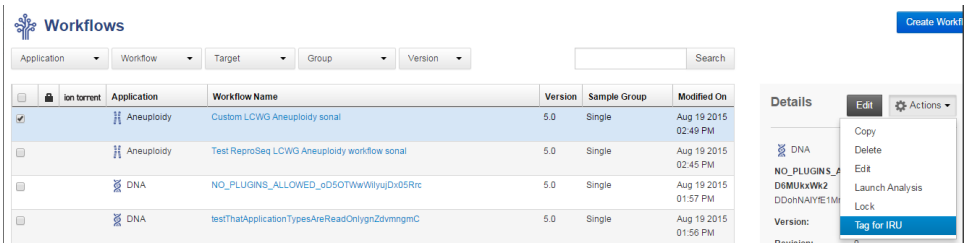
12. The Planned Run is ready to run on your sequencing system.

Manage the Ion Reporter™ Software workflow list

You can reduce the number of Ion Reporter™ Software workflows that are listed when you create a Planned Run or 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 into Ion Reporter™ Software.
2. In the **Workflows** tab, click **Overview**.
3. Select a workflow, then click **Actions** ▸ **Tag for IRU**.



The **Tag for IRU** in the **Details** section for the workflow is changed to **Yes**. Only 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 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 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 was transferred from Torrent Suite™ Software to Ion Reporter™ Software, go to the **Sample ▸ Sample Management** screen 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 raw data files, and add the correct gender metadata to the new samples.
- If the gender of the sample is not specified or specified as "Unknown", the Integrative Genomics Viewer (IGV) uses female as the gender.

Run IonReporterUploader plugin manually

You can run 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.

Note: For barcoded runs, you can select which barcodes that you want to include in your plugin results. You can also select the barcode kit on the instrument prior to the run, then run the plugin manually when the run is complete. The barcode kit that you enter on the sequencer will be used in the run; the barcode kit that you select on the instrument overwrites the barcode kit selected in the Planned Run.

When you run the plugin manually, you can select whether to upload only VCF files, BAM files, or both VCF and BAM files. You might want to run IonReporterUploader plugin manually if after a sequencing run is completed, for example, you want to annotate variants only 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.

You can also see the barcoded samples that were used the sequencing run. You have the option to 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, IonReporterUploader will 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 Ion Reporter™ Uploader 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 begins. Upload times vary depending on the speed of your internet connection and the size of the dataset being transferred. You will receive an email notification 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:

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 Browser users. Use only the alphanumeric, dash, underscore, and space characters.
Server	Enter: 40.dataloader.ionreporter.iontorrent.com
Port	Enter: 443
Username	Enter your Ion Reporter™ Software username (your email address)
Password	Enter the password you use to sign in to Ion Reporter™ Software

Setting	Description
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:

Note: You can select barcodes for the samples or samples that were used in the sequencing run. By selecting these barcodes, you can select which samples that you want to upload to Ion Reporter™ Software. For details, see "Run IonReporterUploader plugin manually" on page 179.

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 that you will use to upload files to Ion Reporter™ Software
Upload Options	
BAM	Select this option to upload BAM files only
VCF	Select this option to upload VCF files only
BAM and VCF	Select this option to upload both BAM and VCF files
Advanced Settings For details on these settings, see "Tune Ion Reporter™ Software speed parameters" on page 183.	
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 **16MB**, **32MB**, **64MB**, or **128MB**

IonReporterUploader plugin file transfer progress

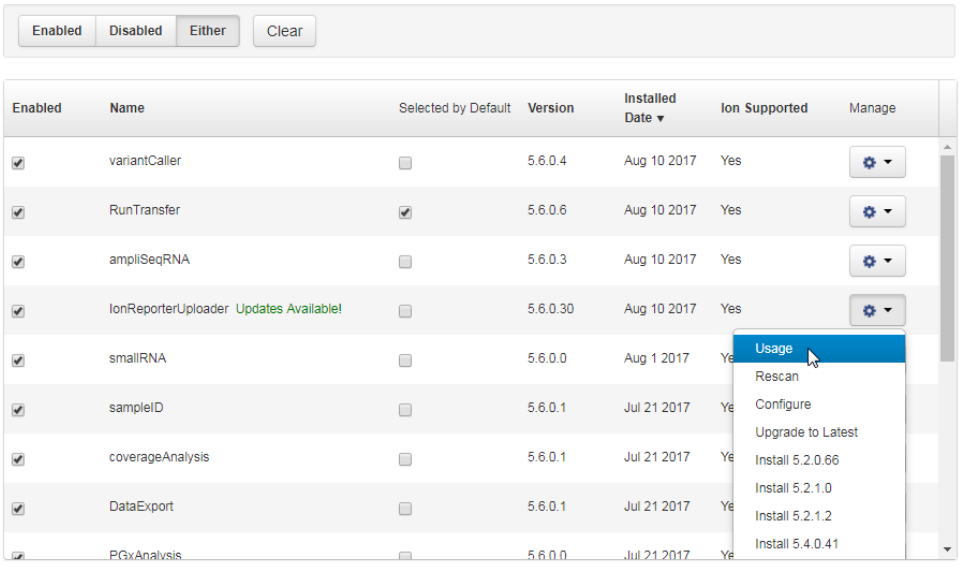
You can monitor the progress of the transfer of analysis results files from Torrent Suite™ Software to Ion Reporter™ Software.

To monitor IonReporterUploader plugin progress through the following	See details in
Email	The two email notifications for sent for each plugin run: • When the plugin starts to transfer your files • When the upload to Ion Reporter™ Software is finished The 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 116.
Log files	"Open a plugin log" on page 117.

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



You can view the following information from the list of run reports:

- Time that the plugin runs 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.

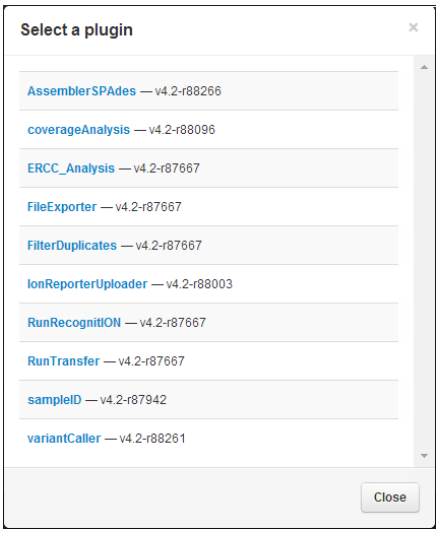
For details, see "Delete a plugin result" on page 118.

Tune Ion Reporter™ Software speed parameters

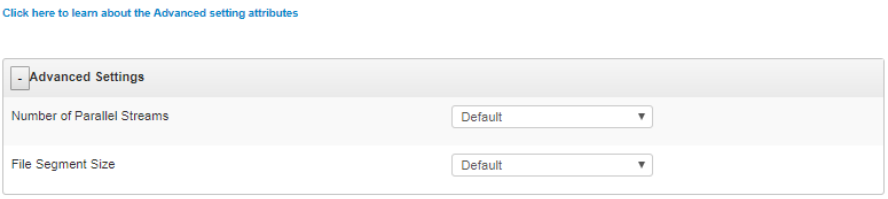
You can adjust speed parameters for the Ion Reporter™ Software plugin to change the rate at which files are uploaded.

Note: Update these settings only if file transfers from Ion Reporter™ Software plugin are difficult or slow with the default settings.

1. In Torrent Suite™ Software, 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**.

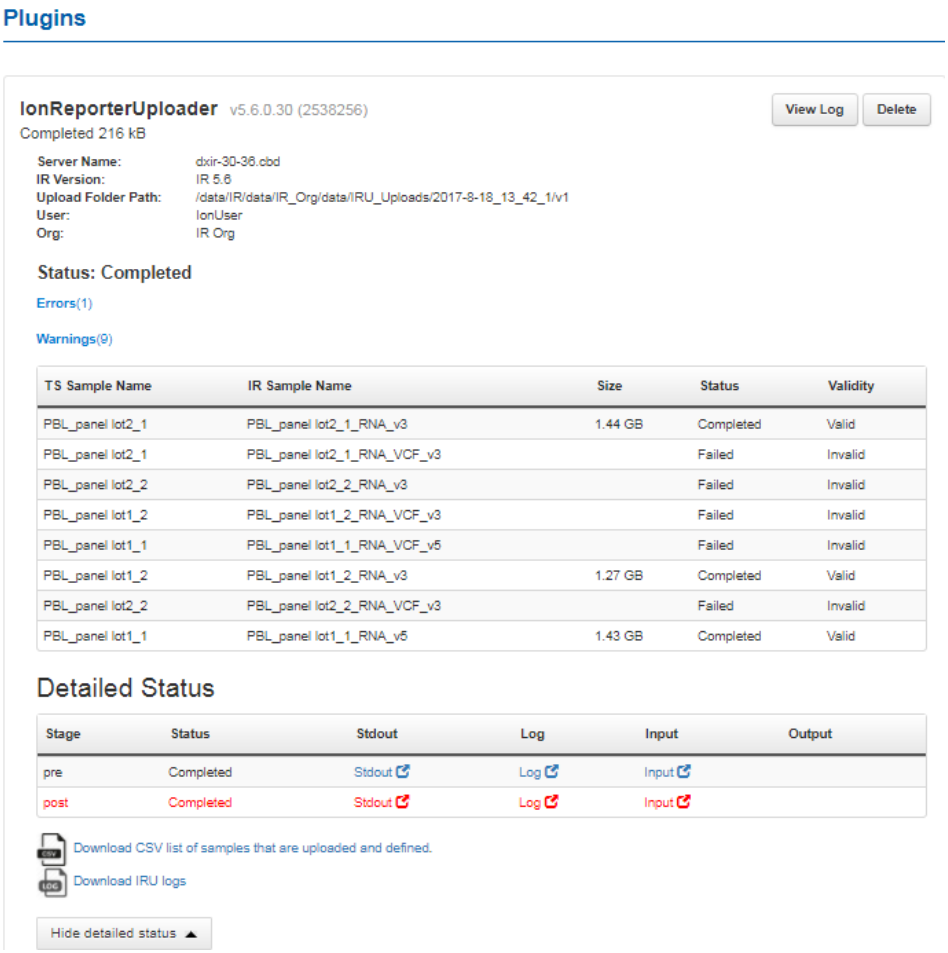
Review IonReporterUploader plugin results

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, you can view information 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 IonReporterUploader, 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 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**

To do this	Click
Download IonReporterUploader of the 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 Ion Reporter™ Software runs through the following major analysis phases:

1. Mapping
2. Variant calling
3. Annotation

This table shows 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 Ion Reporter™ Software workflow
BAM file	TS analysis pipeline	Any except annotation-only
VCF file	TS Variant Caller (variantCaller) plugin	Annotation-only

The Ion Reporter™ Uploader plugin by default uploads both the BAM file and the VCF file from your 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

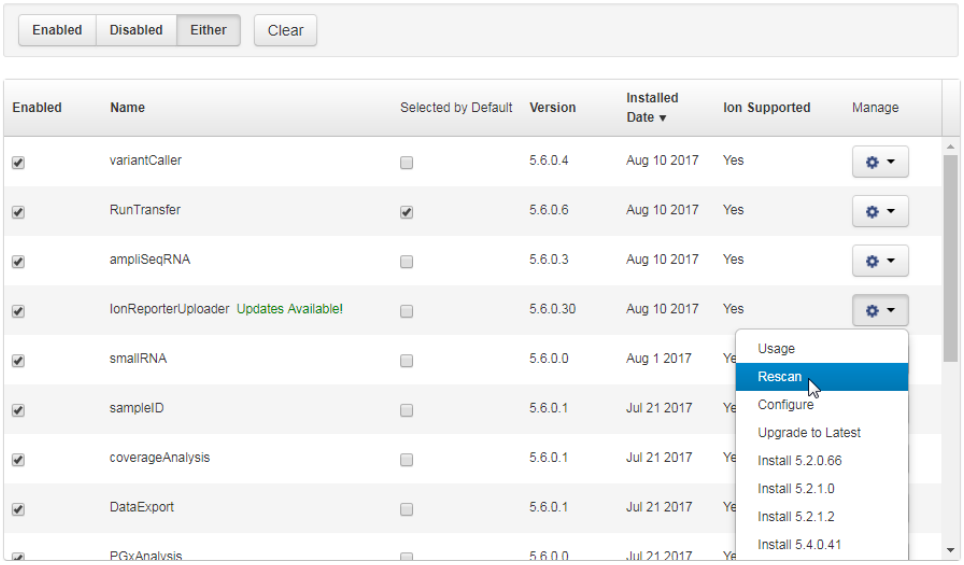
Each output file type is required as input to the next analysis phase. In almost all cases, the Ion Reporter™ Software analysis phases are performed in order.

The exception is the annotation phase. The annotation-only workflow runs this phase by itself. (All other workflows include the annotation phase as their last analysis phase.) The annotation-only workflow requires as input a VCF file, which can be generated from either an Ion Reporter™ Software analysis, an Ion Reporter™ Software analysis variantCaller plugin analysis, or a different source.

Rescan a plugin

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

1. Sign in to Torrent Suite™ Software.
2. Click ⚙ **(Settings)** ▸ **Plugins**. The installed plugins are listed.



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

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

   Note: You can also rescan the output files from the list of reports when you view the usage for a plugin. For details see, "View IonReporterUploader plugin status details" on page 182.

IonReporterUploader command-line utility

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 login ID to transfer run data from Torrent Suite™ Software to Torrent Suite™ 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. The IonReporterUploader command-line utility can be run on your Torrent Server, or can be run on any standard (and current) computer that runs Linux™ Operating System, Mac OS™, or Windows™ Operating System. You can enter IonReporterUploader command-line utility options through command line arguments, or through a properties file.

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

The Ion Reporter™ Software can be run on any of these systems:

- Your Torrent Server
- A standard Linux™ machine
- A standard Windows™ (XP or later) machine
- A standard Macintosh™ machine

Note: 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.

The IonReporterUploader command-line utility is an alternative to the IonReporterUploader plugin that is included with Torrent Suite™ Software. For information on IonReporterUploader plugin, see the Torrent Suite™ Software Help, or the *Torrent Suite™ Software 5.8 User Guide*.

Download IonReporterUploader command-line utility

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

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

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

---

1. Sign in to Ion Reporter™ Software, then click **Settings (⚙) ▸ Download Ion Reporter Uploader**.
2. Click the filename `IonReporterUploader-cli.zip`, then download the file to the target computer.
3. Extract the downloaded `IonReporterUploader-cli.zip` file, then copy the `IonReporterUploader-cli` directory to a convenient location on the target computer.

Run Ion Reporter Uploader 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.

Variant calls in Torrent Suite Software

The variantCaller plugin calls single-nucleotide polymorphisms (SNPs), multi-nucleotide 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). See "variantCaller plugin configuration" on page 194 for more information.

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 choose a predefined configuration that is included with Torrent Suite™ Software.

Note: Pre-configured plugin settings are available as predefined or custom configurations that are available when you configure the variantCaller plugin. Use these configurations to save significant time setup time. Predefined configurations for use with the AmpliSeq and AmpliSeq HD research applications can also be downloaded from ampliseq.com. See "Import panel files and parameters from AmpliSeq.com" on page 50.

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.

- If you want 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, if you want to use a reference genome, target regions file, or hotspots file, for one or more barcodes, that is specified in the Planned Run.

Note: 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.

Note: If the variantCaller plugin runs automatically, you can still re-run the plugin manually after a sequencing run is completed. See "Run the variantCaller plugin manually" on page 193 for more information.

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.

   Note: You must run the variantCaller plugin manually after the run, if you want to use a reference genome, target regions file, or hotspots file, for one or more barcodes, that is specified in the Planned 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. See "Plan step in the workflow bar" on page 45 for more information. 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 will use 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.

Note: If the variantCaller plugin runs automatically, you can still rerun the plugin manually after a sequencing run is completed.

IMPORTANT! The variantCaller plugin parameter settings are saved in 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**.

   Note: The variantCaller plugin parameter settings change according to the selections you make. Default settings might different depending on whether the sequencing data is from an Ion PGM™, Ion Proton™, Ion S5™, and Ion GeneStudio™ S5 Systems.

5. Select settings that are appropriate for the sequencing instrument used, the experiment, and the frequency of the variants of interest. See "variantCaller plugin configuration" on page 194 for more information.

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, or you can apply a different configuration for individual barcodes. See "Create a custom configuration for the variantCaller plugin" on page 197 for more information.

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 appears.

   Note: The variantCaller plugin parameter settings change according to the selections you make. Default settings might different depending on whether the sequencing data is from an Ion PGM™, Ion Proton™, Ion S5™, and Ion GeneStudio™ S5 Systems.

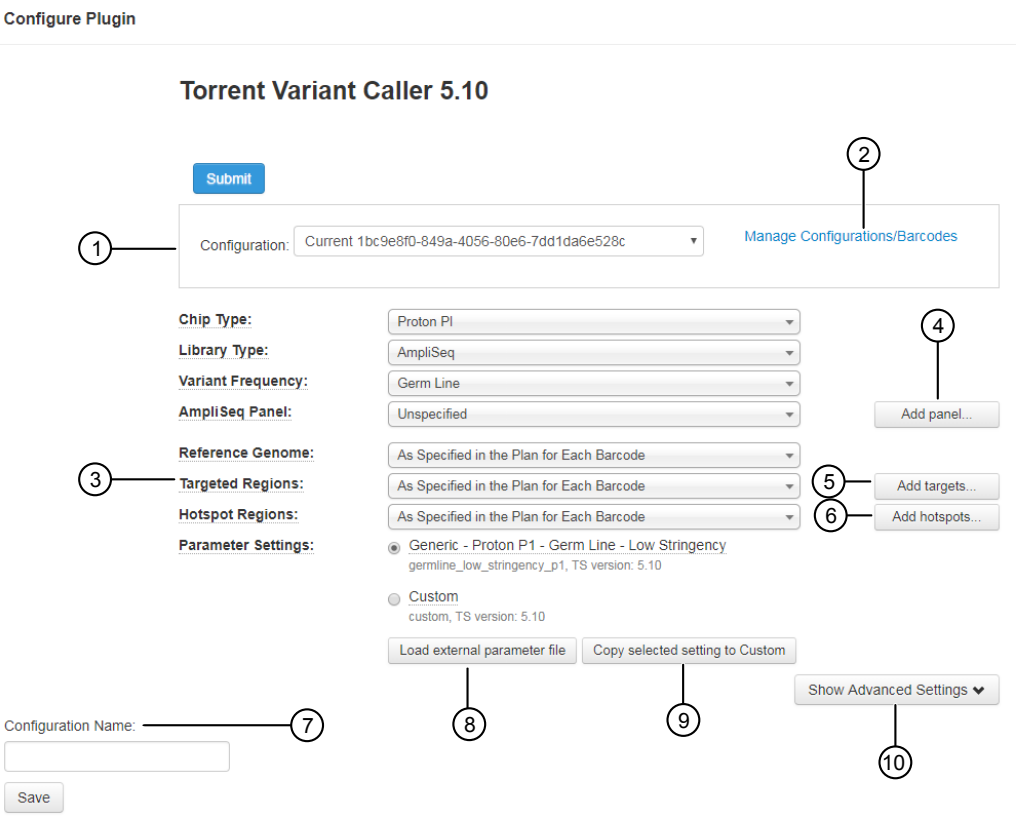
4. Select settings that are appropriate for the sequencing instrument used, the experiment, and the frequency of the variants of interest. See "variantCaller plugin configuration" on page 194 for more infomation.

5. When your changes are complete, click **Submit**.

   Note: To cancel a plugin run that is in progress, click **Stop**.

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

variantCaller plugin configuration



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]	Select a reusable predefined or a custom configuration for the variantCaller plugin that includes settings for a **Reference Genome**, **Targeted Regions**, **Hotspots**, and **Parameter Settings**. See "Create a custom configuration for the variantCaller plugin" on page 197 for more information.

Setting	Details
Manage Configurations/Barcodes [1]	Manage and apply configurations for barcodes when you run the variantCaller plugin manually. See "Apply configuration settings to specific barcodes" on page 198 for more information. (*Optional*) If you want 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. "Apply configuration settings to specific barcodes" on page 198 for more information.
Chip Type [1]	The chip type that is used in the sequencing run. 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: Whole Genome, AmpliSeq, AmpliSeq HD, or TargetSeq. 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: • **Somatic** – This option detects somatic variants at low allele frequencies. • **Germline** – This option detects germline variants that are expected to be present at low allele frequencies. • **Rare Somatic**This option detects rare somatic variants in FFPE or cfDNA samples. The option is available if and 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 50 for more information. **Note:** 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.
Reference Genome [1]	Reference genome that is for use with variant calling. **Note:** If you want 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.

Setting	Details
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. If you want 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. **Note:** Before a targeted regions file can be selected, it must be uploaded from inTorrent Suite™ Software and associated with a specific reference genome. See "Upload a target regions file" on page 240 for more information.
Add targets	Upload a target regions file.
Hotspots	Hotspots Regions files are BED or VCF files that define variant alleles of interest. Hotspots Regions 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 Regions file affects only the variantCaller plugin, not other parts of the analysis pipeline. If you don't specify a Hotspots Regions file, the software will only report the de novo variants that show as present. In contrast, if a Hotspots Region file is used, the variant calls and the filtering metrics for each hotspot allele is reported in the output VCF file, including data for absent or NOCALL variants. If you want to configure Hotspots Regions files to use the same Hotspots Regions file that is included in the current Planned Run, select the **As Specified in the Plan for Each Barcode** option. Before a hotspots file can be selected, it must be uploaded from inTorrent Suite™ Software and associated with a specific reference genome. See "Upload a hotspots file" on page 242 for more information. IMPORTANT! A carefully designed Hotspot Region file is highly recommended to optimize the overall performance of variant calling. Please contact your field bioinformatics specialist (FBS) for more information.
Add hotspots	Upload a hotspots file.

Setting	Details
Parameter Settings	Option to use parameter settings that predefined in the variantCaller plugin, or to use **Custom** parameter settings. For Ion AmpliSeq™ experiments, panel templates from AmpliSeq.com might contain parameter settings that are optimized for the variantCaller plugin and are available for use during variantCaller plugin configuration. • The variantCaller plugin provides generic parameter settings that are optimized for the selected **Chip Type**, **Library Type**, and **Variant Frequency** and the parameters settings that are downloaded from AmpliSeq.com. • Select **Custom** to change the advanced parameter settings. See "variantCaller plugin advanced parameters" on page 216 for more information.
Load external parameter file	Option to import a file that contains parameter settings.
Copy selected settings to Custom	Option to create a **Custom** parameter setting that is based on a generic parameter setting. See "Create and use a custom parameters setting" on page 200for more information.
Configuration Name [1]	Option to name and save a custom configuration when changes are made to the predefined settings.
Show Advanced Settings	Configure advanced parameter settings. See "variantCaller plugin advanced parameters" on page 216 for more information. Hover your mouse of 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. Select the variantCaller plugin, then click **Configure**.
   The variantCaller plugin configuration screen opens.
4. Set plugin configuration parameters. See "variantCaller plugin configuration" on page 194 and "Create and use a custom parameters setting" on page 200 for more information.
5. Enter a name for the configuration in the **Configuration Name** field, then click **Save**.

The new configuration is included to 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.

  Note: 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, click **variantCaller**.
3. In the **Configure Plugin** dialog, click **Manage Configurations/Barcodes**, then click **OK**.
4. In the **Setup** tab, you can 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 the **Set All** dropdown list, select the configuration to apply to all barcodes.
Apply a specific configuration to an individual barcode.	In the **Configuration** column, select the configuration from the dropdown list of available configurations for each individual barcode.

5. *(Optional)* Create a new configuration or edit an existing one.

Action	Description
Edit a configuration	1. Click **Edit**. The Configure Plugin dialog becomes specific to the configuration selected. 2. Change the existing selections as desired. **Note:** To add a new Panel, Target Regions, or hotspots file, you will be redirected to the appropriate **Settings** (⚙) page. You must close the **Configure Plugin** dialog, then reopen the dialog to access the new file. 3. Click **Show Advanced Settings**, edit the Parameters as necessary, then click **Hide Advanced Settings** when complete. 4. Click **Save**.
Delete a configuration	1. Click **Delete**. 2. Click **OK** to confirm.
Create a new configuration	1. Click **Add**. 2. Enter a **Configuration Name**. 3. Make selections from the available dropdown lists. 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. If you want to use a customized parameter file that is designed for your customized panel or other special requirements, you must create a custom parameter setting, then run variantCaller plugin manually with the custom parameter setting.

The parameter file in the variantCaller plugin is a JSON text file that contains the variant calling parameters listed in "variantCaller plugin advanced parameters" on page 216.

1. In the **Data** tab, in the **Completed Runs & Reports** screen, click on the report name of the run 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 an JSON parameters file that you have saved on your computer	1. Click **Load external parameter file** 2. Browse to the JSON file, then click **Open**.
Edit a predefined parameters setting	1. In the **Configuration** dropdown list, select the predefined parameter setting that you want to edit. 2. Click **Copy selected to Custom**. 3. If you agree to save the new custom parameter setting, click **OK**. 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 your variantCaller plugin run completes, you can access variantCaller run results from the run report page in Torrent Suite™ Software. On the run report page, in the variantCaller pane you can:

- Review the summary of the variantCaller 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 as well as each individual barcode or sample.

You can also access the detailed variantCaller plugin summary report for each barcode or sample from the variantCaller pane on the run report page. In the detailed variantCaller plugin summary report browser, you can:

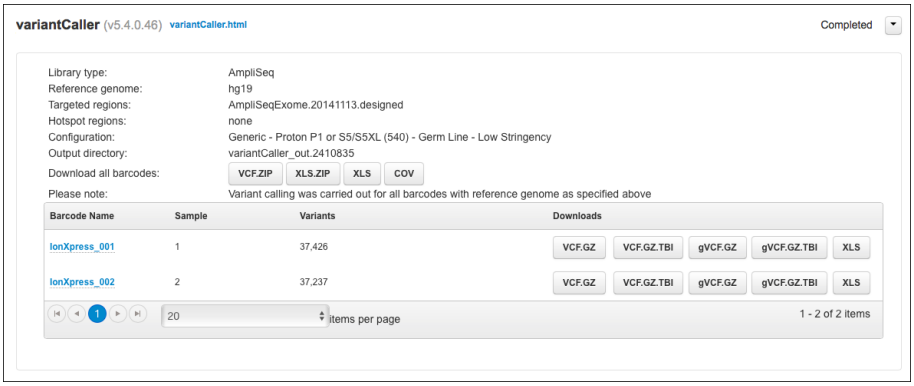
- View variant call information by allele for the specific barcode, such as allele location on the chromosome, allele annotations, coverage metrics, and quality metrics.
- Review the library type, reference genome, targeted regions, hotspots, and parameter settings that were used in the run.
- Download BED files and Parameters File used for the specific barcode.
- Download BAM and BAI files for the mapped and TVC-processed reads.
- Download data files for variant calls and covereage for the specific barcode.
- View variant calls in IGV.

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, locate the run of interest, then click the report link in the **Report Name** column in the row of the run.

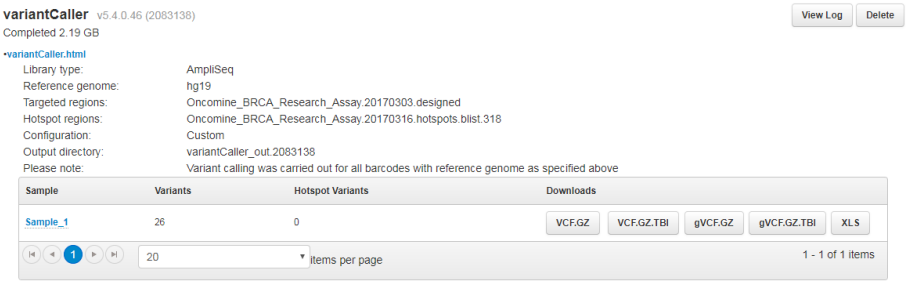
3. In the left navigation menu, click **variantCaller** to navigate to the variantCaller results pane.
    - If the sequencing run contains barcodes, the plugin report includes a list of the barcodes that were used and file download options for all barcodes and each individual barcode.



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™.

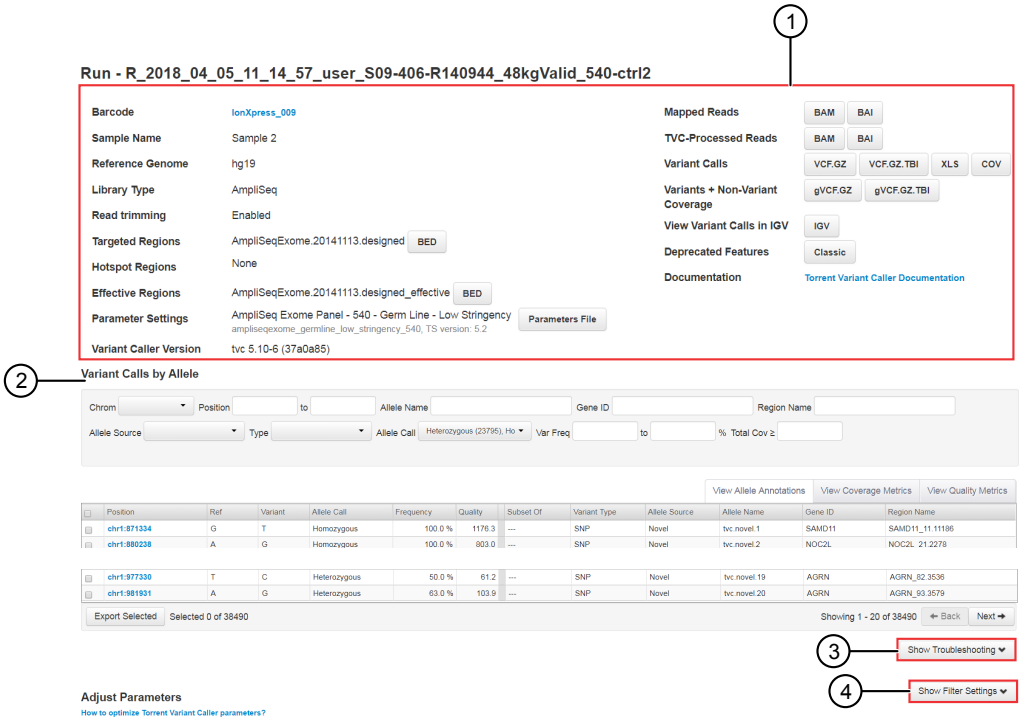
- If the sequencing run does not contain barcodes, the plugin report contains information for the sample used in the run and the file download options for the sample.



4. To open the detailed variantCaller plugin summary report for a specific barcode or sample, click one of the following links in the variantCaller run summary table:
   - Barcode name link in the **Barcode name** column in a barcoded sequencing run report.
   - Sample name link in the **Sample** column in a report from a sequencing run that does not contain barcodes.

Detailed variantCaller plugin summary report

The detailed variantCaller plugin summary report contains variantCaller run information, results, and the associated files for download that are specific for an individual barcode or sample.



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 205.
3. Export the variant data files for troubleshooting. For more information, see "Export files for troubleshooting" on page 214.
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 215.

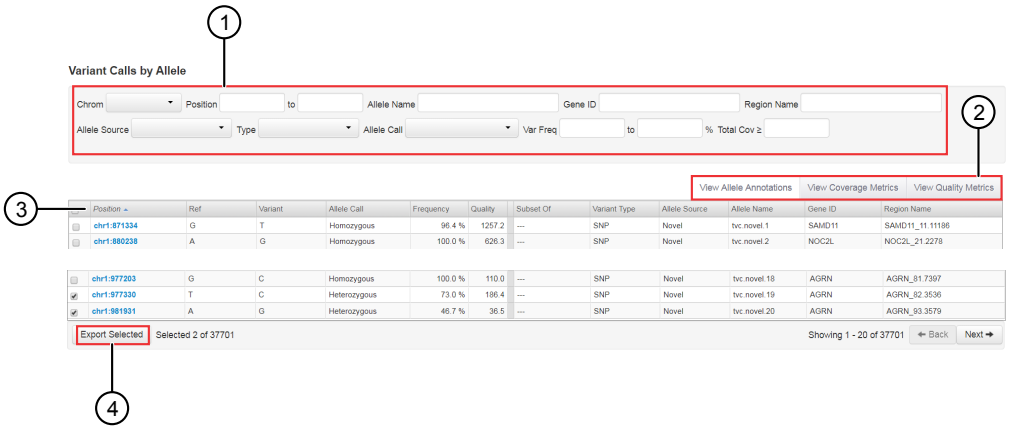
Download options for an individual barcode or the sample used in the run

Download option	Description
Target Regions	**BED** – The BED file that specifies the genomic positions of interest
Hotspot Regions	**BED** – The BED file that specifies the variant alleles of interest
Effective Regions	**BED** – The BED file that specifies the regions 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

Download option	Description
Mapped Reads	• **BAM** – The BAM file that is input to Variant Caller Pipeline for discovering and evaluating variants. Note that realignment may be applied. • **BAI** – The BAI file that contains the index information for the corresponding BAM file
Torrent Variant Caller-Processed Reads	• **BAM** – The BAM file that is processed by Torrent Variant Caller. Note that read trimming and read filtering may be applied. In Tag Sequencing and AmpliSeq HD runs, the BAM file may contain consensus reads that are obtained by compressing the reads in the Mapped BAM that originate from the same DNA molecule. • **BAI** – The BAI file that contains the index information for the corresponding BAM file
Variants Calls	• **VCF.GZ -** The compressed VCF file that contains the variant calls • **VCF.GZ.TBI -** The index file for VCF.GZ • **XLS -** The file that contains a list of variant alleles in the tab-separated file format, which can be opened in Microsoft™ Excel™. • **COV -** The file that contains coverage analysis for each variant call, which can be opened in Microsoft™ Excel™.
Variants + Non-Variant Coverage	• **gVCF.GZ -** The bgzip-compressed genome-VCF file that contains the variant calls • **gVCF.GZ.TBI -** The tabix index file for gVCF.GZ
View Variant Calls in IGV	**IGV -** The JNLP file that can be opened using IGV to visualize the variant calls.

Variant Calls by Allele table

The **Variant Calls by Allele** table is located in the detailed variantCaller plugin summary report. The table displays the details about each variant 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 allele. You can also export the information to be saved to your local storage.



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 choose one of the following tabs.
   - **View Allele Annotation**; see "View allele annotations " on page 206 for more information.
   - **View Coverage Metrics**; see "View coverage metrics " on page 207 for more information.
   - **View Quality Metrics**; see "View quality metrics" on page 208 for more information.
3. • Click the column header to sort variant alleles by the values in the column.
4. Export the information associated with the selected variant alleles to an XLS file. The exported XLS file holds all the information about the selected variants, including the information listed in the **View Allele Annotation**, **View Coverage Metrics**, and **View Quality Metrics** tabs. For more information, see "Export variant calls to a file" on page 208.

Key variant information

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(s).
Variant	Variant allele base(s).
Allele Call	The zygosity (Homozygouse or Heterozygous) or type (Absent or No Call) of the allele that is called by the The zygosity (Homozygouse or Heterozygous) or type (Absent or No Call) of the allele all by the variant caller.
Var Freq	Frequency of the variant allele.

Column	Description
Quality	Phred-scored quality field. [1] Larger values mean higher confidence in the call. Quality is calculated by posterior probability that the variant allele frequency is greater than the cutoff (min_allele_freq in the parameter file, if a variant call is made), or posterior probability that the variant allele frequency is below this cutoff (if a reference call). The posterior probability that is computed as conditional on the reads observed includes sampling variability. **Note:** 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 in this field can mean either the reads do not distinguish the variant well, there is insufficient depth to resolve, or the observed allele frequency is near the cutoff.
LOD[2]	The Limit Of Detection (LOD) at the genome location, estimated based on the number of detected molecules.
(Optional) **PPA**	An indication (0 or 1) of the variant allele is a Possible Polypoidy Allele (PPA) or not. The column is available if the parameter "report_ppa" is set to be 1. For Torrent Suite™ Software 5.4 and 5.6, heterozygous or homozygous alleles are labeled as PPA. For Torrent Suite™ Software 5.8 and later, heterozygous and homozygous alleles are no longer treated as PPA. Only absent alleles can be labeled as PPA.

[1] For variants found by Long Indel Assembler, this value is always set to 50.

[2] This column is available only for sequencing runs that use the Tag Sequencing or AmpliSeq HD as the Target Technique in the Planned Run. See "Research Application step in the workflow bar" on page 40 for more information.

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 hotspot calls only.
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.

Column	Description
Variant Type	The type of the variant called. • SNP– single nucleotide polymorphism • IND–insertion • DEL–deletion • MNP– multiple nucleotide polymorphism or the substitution of a block sequence by the block of another length • COMPLEX– Block substitution of sequence by a block of unequal length
Allele Source	Allele source is called as: • Hotspot–for alleles included in the hotspots file. • Novel–for all other alleles.
Allele Name	The allele name as defined in the hotspots file. For novel alleles, in Torrent Suite™ Software 5.4 and later, 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.

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

[1] 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". See downsample_to_coverage in **variantCaller plugin advanced parameters**.

[2] Hidden in 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.

Note: This tab does not appear in Tag Sequencing runs.

Column	Description
Common Signal Shift	Distance between predicted and observed signal at the allele locus. [RBI]
Reference Signal Shift	Distance between predicted and observed signal in the reference allele. [REFB]
Variant Signal Shift	Distance between predicted and observed signal in the variant allele. [VARB]
Relative Read Quality	Phred-scaled mean log-likelihood difference between the prediction under reference and variant hypothesis. [MLLD]
HP Length	Homopolymer length.
Context Error +	Probability of sequence-specific error on the forward strand (reported only for deletion variants).
Context Error -	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).

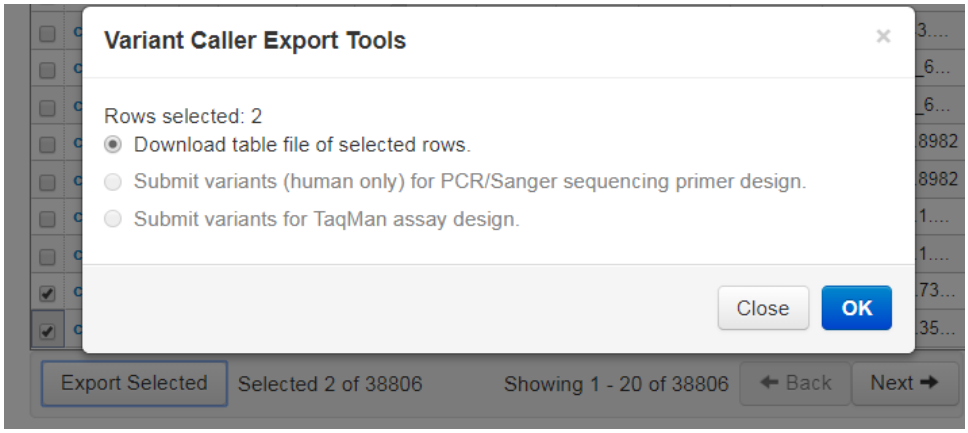
For candidates that are filtered out, the filtering reason is highlighted in the table.

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 your 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 (including 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 on 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.
   - Click the barcode name link in the **Barcode name** column in a barcoded sequencing run report.
   - Click the sample name link in the **Sample** column in a report from a sequencing run that does not contain barcodes.
3. In the **Variant Calls by Allele** table, select the checkbox in the row of each variant you want to export, then click the **Export Selected**.



4. Select **Download table file of selected rows**, then click **OK**.
   The `subtable.xls` file is created and dowloaded to your computer.
5. *(Optional)* View the file and save to your local storage using a descriptive file name.

Troubleshoot variantCaller plugin results

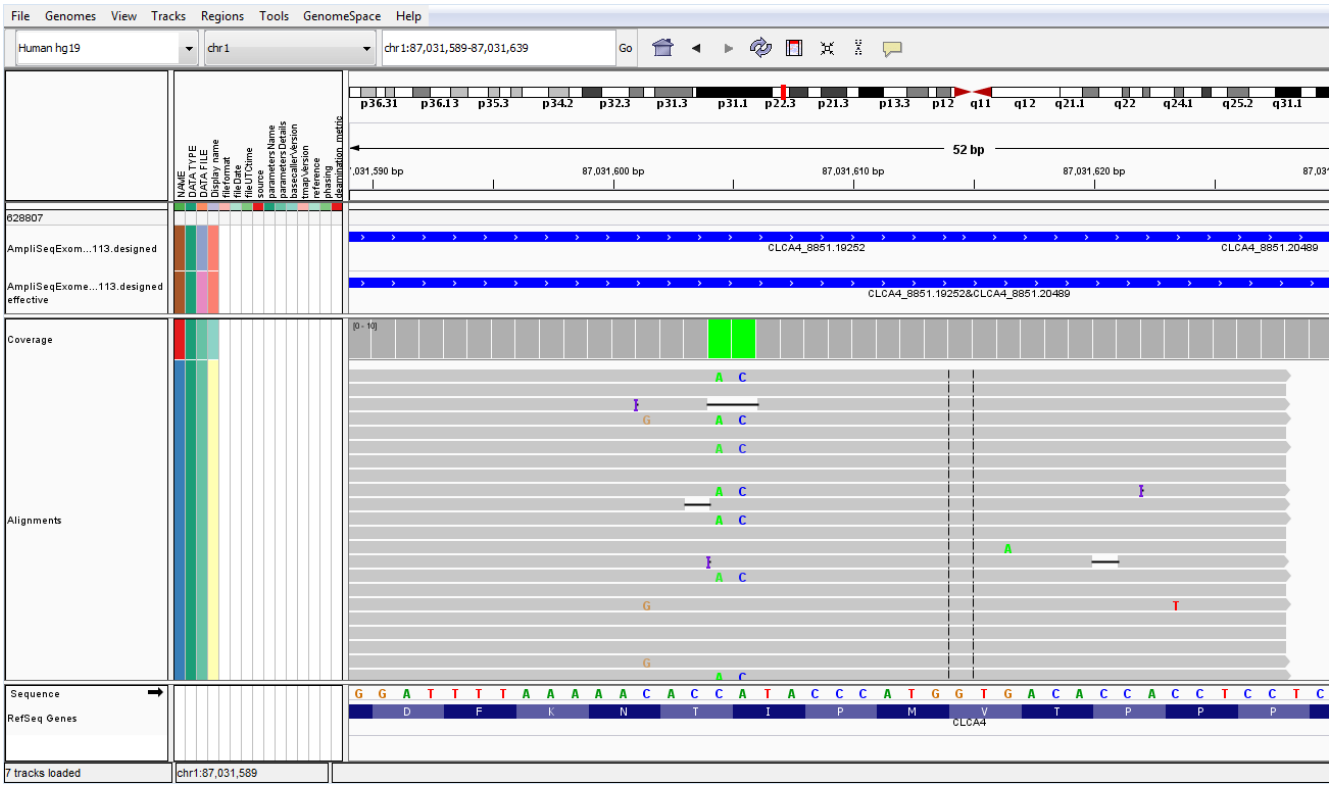
You can troubleshoot results in the variantCaller plugin detailed report to analyze why expected variants were missed (false negatives) or to examine false positives. Plugin parameters can be adjusted and the plugin can be rerun. You can also export troubleshooting results to share them with a field bioinformatics specialist (FBS).

Find false negatives with an alignment viewer

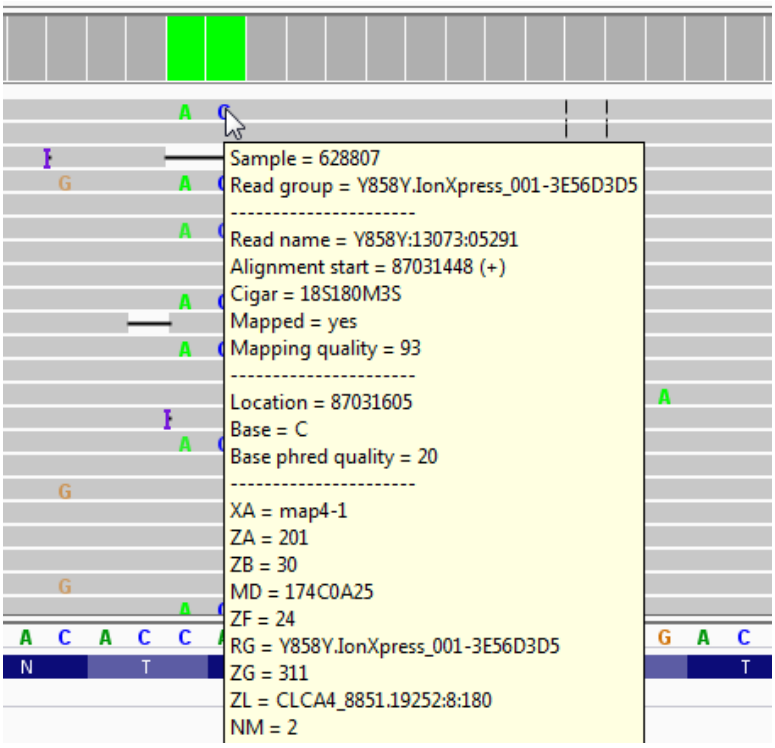
When an expected variant is not called by the variantCaller plugin, an alignment viewer, such as Integrative Genomics Viewer (IGV) or Ion Reporter™ Genomic Viewer (IRGV) can help you confirm the absence of the variant in the sample, or help you understand how to adjust the plugin parameters to enable 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 using the variantCaller plugin report

You can also 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 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 field (filtered reason).
  c. Relate the reason to plugin run parameters using the parameter definitions in “variantCaller plugin advanced parameters“ on page 216.
  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:

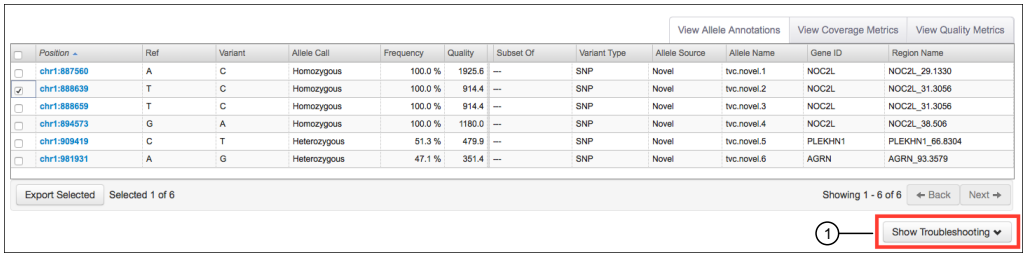
- Adjust parameters that control the homopolymer calls. This can increase the report of false negatives.
- If you are repeatedly running a panel, manually curate specific sites (positions), since the false positive tends to occur in the same positions.

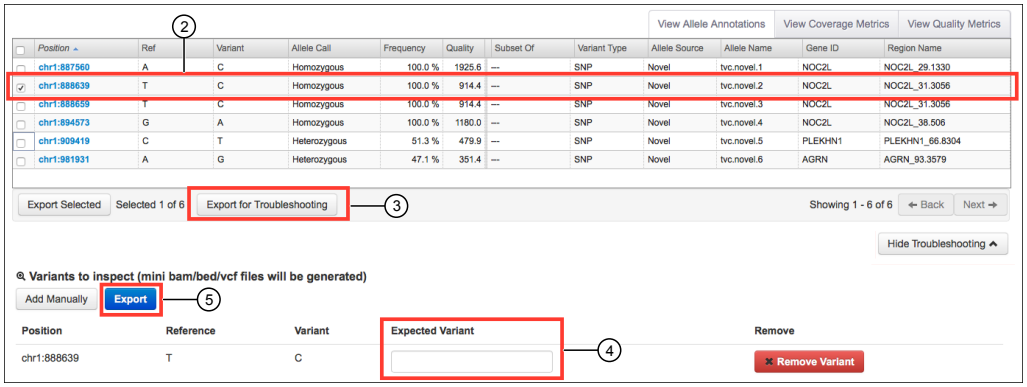
Note: Currently, the variantCaller plugin does not support manual curation. Manual curation is available in the command-line version.

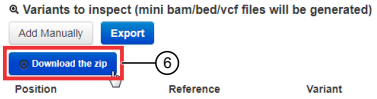
Export files for troubleshooting

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**.

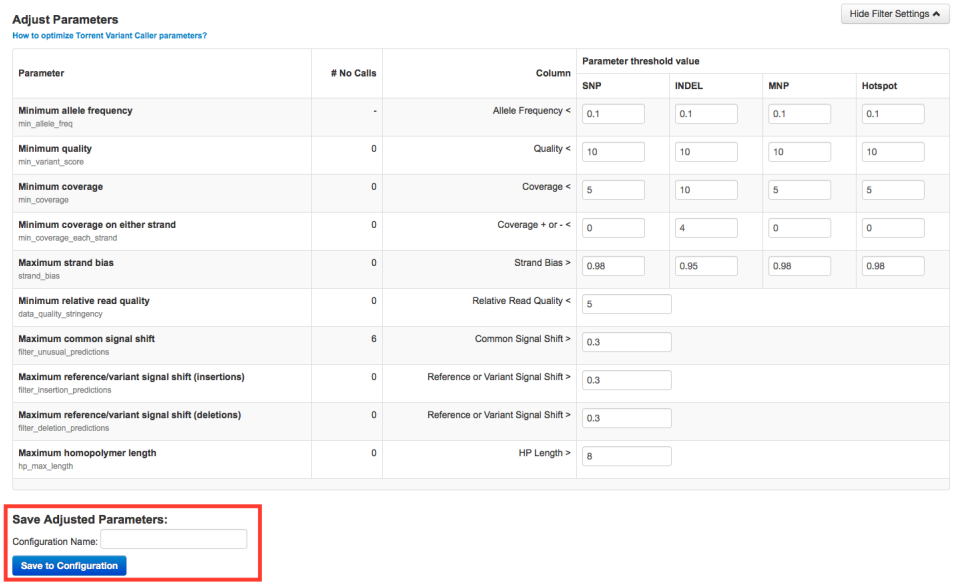
The compressed directory of miniature BAM, BED, and VCF files is downloaded to the directory, according to your browser settings.

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.

Note: 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 page.
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 advanced users only. Contact your field bioinformatics specialist (FBS) if you need assistance.

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, we highly recommend that you consider the trade-off between sensitivity and specificity for INDEL calls when you optimize customized parameters.

The variant calling pipeline in variantCaller plugin consists of three modules: FreeBayes, Torrent Variant Caller, and Long Indel Assembler. FreeBayes, as a candidate generator, generates lists of variant candidates. The lists of candidates are then evaluated by Torrent Variant Caller to examine the evidence of the variants in flow space. Yet, some long INDELs may not be properly aligned to the reference genome and, hence, cannot be discovered and evaluated by FreeBayes and Torrent Variant Caller, respectively. The Long Indel Assembler aims to call long INDELs of this type. There is a unification step in the pipeline that merges the variants called by Torrent Variant Caller and the Long Indel Assembler into one VCF file that is available to the user.

IMPORTANT! These parameters are recommended for advanced users only.

Advanced parameters for the variantCaller plugin

Parameter	Description
X_min_allele_freq	X is one of the allele types in {indel, snp, mnp, hotspot} A variant evaluation parameter: The presence of the allele of the type is defined by which allele frequency is greater than this value **Allowed values:** Decimal between 0 and 1 **Suggested trial value:** between 0.01 (somatic) and 0.2 (germline)
X_min_variant_score	X is one of the allele type in {indel, snp, mnp, hotspot} A filter parameter: A called allele of the type needs to have a QUAL score greater than this Phred-scaled value. **Filter reason:** quality score. **Related VCF fields:** QUAL **Allowed values:** decimal values >=0 **Suggested trial value:** >10
X_min_coverage	X is one of the allele type in {indel, snp, mnp, hotspot} A filter parameter: The location of a called allele of the type needs to have a coverage greater than this value **Filter reason:** MINCOV **Related VCF fields:** FRO, FAO **Allowed values:** Integers >= 0 **Suggested trial value:** between 5 and 20

Parameter	Description
X_min_cov_each_strand	X is one of the allele type in {indel, snp, mnp, hotspot} A filter parameter: Minimum coverage required on each strand for a the type of allele to be called. **Filter reason:** PosCov or NegCov **Related VCF fields:** FSRF, FSRR, FSAF, FSAR **Allowed values:** Integers >= 0 **Suggested trial value:** >= 3
X_strand_bias	X is one of the allele type in {indel, snp, mnp, hotspot} 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. **Filter reason:** STDBIAS and STDBIASPVAL **Related VCF field:** STB **Allowed values:** Decimal numbers between 0.5 (requires perfect balance on both strands) and 1.0 (tolerates extremely strong strand bias) **Suggested trial value:** 0.95
X_strand_bias_pval	X is one of the allele type in {indel, snp, mnp, hotspot} 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_snp_strand_bias **Filter reason:** STDBIAS and STDBIASPVAL **Related VCF field:** STBP **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.01 for strand bias filter, 1 for no strand bias filter
X_min_var_coverage	X is one of the allele types in {indel, snp, mnp, and hotspot}. A filter parameter: Minimum number of variant coverage after flow-evaluation required to make the call. **Filter reason:** VarCov **Related VCF field:** FAO **Allowed values:** Integer >=0 **Suggested trial value:** 3 (TagSequencing or AmpliSeq HD), 0 (other)
downsample_to_coverage	Reduce coverage in over-sampled locations to this value to save computational time **Allowed values:** Integers >= 1 **Suggested trial value:** 400 (germline), 2000 (somatic)

Parameter	Description
use_fd_param	(experimental in Torrent Suite Software 5.4) A filtering parameter: Use Flow Disruptiveness (FD) instead of allele types (INDEL, SNP, MNP) as the criterion to select the parameter set. If turned on, the (non-FD, weak FD, strong FD) allele applies the (INDEL, SNP, MNP) parameters, respectively. If powered on, the (non-FD, weak FD, strong FD) allele applies the (INDEL, SNP, MNP) parameters, respectively. **Allowed values:** 0: do not use FD parameters, 1: use FD parameters.
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 **Filter reason:** STRINGENCY **Related VCF field:** MLLD **Allowed values:** Decimal numbers >= 0 **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. **Filter reason:** PREDICTIONSHIFTx **Related VCF fields:** RBI = sqrt(FWDB ^ 2 + REVB ^ 2) **Allowed values:** Decimal numbers >= 0 **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 **Filter reason:** PREDICTIONVarSHIFTx or PREDICTIONRefSHIFTx **Related VCF fields:** VARB, REFB **Allowed values:** Decimal numbers >= 0 **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. **Filter reason:** PREDICTIONVarSHIFTx or PREDICTIONRefSHIFTx **Related VCF fields:** VARB, REFB **Allowed values:** Decimal numbers >= 0 **Suggested trial value:** 0.2

Parameter	Description
min_tag_fam_size	An evaluation parameter for Tag Sequencing and AmpliSeq HD runs only: Minimum number of reads with same molecular tag required to form a functional molecular family. **Allowed values:** Integer >=1 **Suggested trial value:** 3
min_fam_per_strand_cov	An evaluation parameter for Tag Sequencing and AmpliSeq HD runs only: Minimum required coverage of reads on each strand in a bi-directional functional molecular tag family. **Allowed values:** Integers >=0 **Suggested trial value:** 1
tag_trim_method	An evaluation parameter for Tag Sequencing and AmpliSeq HD only: Requirement of the molecular tag of the read must match the format specified in the Planned Run. **Allowed values:** strict-trim (requires match), sloppy-trim (does not require match) **Suggested trial value:** sloppy-trim
indel_func_size_offset	An evaluation parameter for Tag Sequencing and AmpliSeq HD runs only: requires a family size >= (min_tag_fam_size + this value) to be functional for calling HP-INDEL. **Allowed values:** Integers >=0 **Suggested trial value:** 0
heavy_tailed	A variant evaluation parameter: (2*heavy_tailed-1) is the degree of freedom of the t-distribution for modeling the heavy tail in signal residual distribution **Allowed values:** Integers >= 1 **Suggested trial value:** 3
outlier_probability	A variant evaluation parameter: probability that a read comes from none of the models under consideration. The variantCaller plugin will make NOCALL with filter reason REJECTION if FXX is too high. **Related VCF field:** FXX **Allowed values:** Decimal numbers between 0 and 1.0 **Suggested trial value:** between 0.005 and 0.01
prediction_precision	A variant evaluation parameter: The number of pseudo data points suggesting our predictions match the measurements without bias **Allowed values:** Decimal numbers >= 0.1 **Suggested trial value:** 1.0

Parameter	Description
max_flows_to_test	A variant candidate evaluating parameter: The maximum number of scoring flows being used. **Allowed values:** Integers > 0 **Suggested trial value:** 10
suppress_recalibration	A variant evaluation parameter: Homopolymer recalibration values should not be used when set **Allowed values:** 0 = enable recalibration, 1 = disable recalibration **Suggested trial value:** 0
do_snp_realignment	A variant candidate evaluating parameter: Realign reads in the vicinity of SNP candidates when set **Related VCF content:** REALIGNEDx **Allowed values:** 0 = do not realign, 1 = realign **Suggested trial value:** 0
do_mnp_realignment	A variant candidate evaluating parameter: Realign reads in the vicinity of MNP candidates when set **Related VCF content:** REALIGNEDx **Allowed values:** 0 = do not realign, 1 = realign **Suggested trial value:** 0
realignment_threshold	A variant candidate evaluating parameter: Maximum allowed fraction of reads where realignment causes an alignment change **Related VCF content:** SKIPREALIGNx **Allowed values:** Decimals between 0 and 1 **Suggested trial value:** 1
min_ratio_for_fd	A filter parameter: Claim flow-disruption if the portion of reads that are flow-disrupted >= this value **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.1
indel_as_hpindel	A filter parameter: A flag indicating whether INDEL filters or SNP filters should be applied to non-HP indels **Allowed values:** 0 (false), 1 (true)
hp_max_length	A filter parameter: HP indels of more than this length will be filtered out **Filter reason:** HPLEN **Related VCF field:** HRUN **Allowed values:** Integers >= 1 **Suggested trial value:** 8

Parameter	Description
hp_indel_hrun	A filter parameter: Define the HRUN for filtering HP-INDEL variants with lengths specified by 'hp_del_len' and 'hp_ins_len'. **Filter reason:** HPINSLEN, HPDELLEN **Related VCF field:** HRUN **Allowed values:** vector of positive integers (e.g. [1,2,3]) with size matches 'hp_del_len' and 'hp_ins_len' **Suggested trial value:** []
hp_ins_len	A filter parameter: Filter out HP-INS variants whose INS length <= the corresponding entry of this vector if the HRUN is defined in 'hp_indel_hrun'. **Filter reason:** HPINSLEN **Related VCF field:** HRUN **Allowed values:** vector of non-negative integers (e.g. [1,2,3]) with size matches 'hp_del_len' and 'hp_indel_hrun' **Suggested trial value:** []
hp_del_len	A filter parameter: Filter out HP-DEL variants whose DEL length <= the corresponding entry of this vector if the HRUN is defined in 'hp_indel_hrun'. **Filter reason:** HPDELLEN **Related VCF field:** HRUN **Allowed values:** vector of non-negative integers (e.g. [1,2,3]) with size matches 'hp_ins_len' and 'hp_indel_hrun' **Suggested trial value:** []
use_position_bias	A filter parameter: Enable the position bias filter when set **Filter reason:** POSBIAS, POSBIASPVAL **Allowed values:** 0 = disable, 1= enable **Suggested trial value:** (AmpliSeq) 1, (other) 0
position_bias	A filter parameter: Filter out a variant if the position bias is greater than position_bias and the position bias p-value is less than position_bias_pval **Filter reason:** POSBIAS, POSBIASPVAL Related VCF field: POSBIAS **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.75

Parameter	Description
position_bias_pval	A filter parameter: Filter out a variant if the position bias is greater than position_bias and the position bias p-value is less than position_bias_pval **Filter reason:** POSBIAS, POSBIASPVAL Related VCF field: POSBIASPVAL **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.05
position_bias_ref_fraction	A filter parameter: Skip the position bias filter if (reference read count) / (reference and alt read count) <= this value **Filter reason:** POSBIAS, POSBIAS-PVAL **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.05
error_motifs	The file name of the error motif file
sse_prob_threshold	A filter parameter: Filter threshold for motif-predicted error probability **Filter reason:** NOCALLxPredictedSSE, NOCALLxPositiveSSE, NOCALLxNegativeSSE **Related VCF fields:** SSEP, SSEN **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.02
report_ppa	A reporting parameter: Report Possible Polyploidy Alleles (PPA) in the VCF file and the variant calls in the XLS files. **Related VCF field:** PPA **Allowed values:** 1 = report PPA, 0 = do not report Note: This feature is experimental and by default is set to report_ppa = 0 (do not report).

Long Indel Assembler advanced settings

The Long Indel Assembler advanced settings parameters control the behavior of the Long Indel Assembler, a module within the variantCaller plugin.

IMPORTANT! These parameters are recommended for advanced users only.

Parameter	Description
kmer_len	Size of the smallest k-mer used in assembly Impact: Increasing values make indel calls less sensitive but more specific **Allowed values:** Integers >= 5 **Suggested trial value:** 11 and 30
min_var_freq	Minimum frequency of the variant to be reported Impact: Increasing values make indel calls less sensitive but more specific **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.1 and 0.4
min_var_count	Minimum support for a variant to be evaluated Impact: Increasing values make indel calls less sensitive but more specific **Allowed values:** Integers > 1 **Suggested trial value:** 3 and 30
short_suffix_match	Minimum assembled sequence match on both sides of the variant Impact: Increasing values make indel calls less sensitive but more specific **Allowed values:** Integers > 2 **Suggested trial value:** 4 and kmer_len
min_indel_size	Minimum size indel reported by assembly Impact: Increasing values make indel calls less sensitive but more specific **Allowed values:** Integers > 0 **Suggested trial value:** 2 and 30
max_hp_length	Variants containing HP larger than this are not reported Impact: Increasing values make indel calls more sensitive but less specific **Allowed values:** Integers > 1 **Suggested trial value:** 2 and 11

Parameter	Description
relative_strand_bias	Variants with strand bias above this are not reported Impact: Increasing values make indel calls more sensitive but less specific **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.6 and 1.0
output_mnv	Enables reporting of complex variants **Allowed values:** 1 = report complex variants, 0 = don't report **Suggested trial value:** 0

FreeBayes advanced settings

These parameters control the behavior of the FreeBayes module, which performs read filtering and generates lists of variant candidates.

IMPORTANT! These parameters are recommended for advanced users only.

Parameter	Description
allow_indels	Candidate generation parameter: Allow INDEL candidates to be generated when set **Allowed values:** 0 = does not generate indel candidates, 1 = generates INDEL candidates **Suggested trial value:** 1
allow_snps	Candidate generation parameter: Allow SNP candidates to be generated when set **Allowed values:** 0 = does not generate SNP candidates, 1 = generates SNP candidates **Suggested trial value:** 1
allow_mnps	Candidate generation parameter: Allow MNP candidates to be generated when set **Allowed values:** 0 = does not generate MNP candidates , 1 = generates MNP candidates **Suggested trial value:** 1
allow_complex	Candidate generation parameter: Allow complex variant candidates to be generated when set **Allowed values:** 0 = does not generate complex candidates, 1 = generates complex candidates **Suggested trial value:** 1

Parameter	Description
gen_min_alt_allele_freq	A candidate generation parameter: A non-HP-INDEL candidate needs to have an allele frequency greater than this value in the pileup **Allowed values:**Decimal numbers between 0 and 1 **Suggested trial value:** 0.02 to 0.15
gen_min_indel_alt_allele_freq	A candidate generation parameter: An HP-INDEL candidate needs to have an allele frequency greater than this value in the pileup **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.02 to 0.15
gen_min_coverage	A candidate generation parameter: A variant candidate location needs to have coverage depth greater than this value **Allowed values:** Integers >= 0 **Suggested trial value:** 6
min_mapping_qv	A read filtering parameter: Minimum mapping quality value required for a read to be counted (for both candidate generation and variant evaluation) **Allowed values:** >= 0 **Suggested trial value:** 4
read_snp_limit	A read filtering parameter: Do not use reads with number of SNPs above this **Allowed values:** Integers >= 0 **Suggested trial value:** 10
read_max_mismatch_fraction	A read filtering parameter: Ignore reads with fraction of mismatch greater than this value **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 1.0
min_cov_fraction	A read filtering parameter: Do not use reads with fraction of covering the best assigned unmerged target region below this. **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.9 (TagSequending and 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. **Allowed values:** Integers >= 0 (0 disables the filter) **Suggested trial value:** 5 (Tag Sequencing and AmpliSeq HD), 0 (other)

Parameter	Comments	Recommended value
tvcargs	This field is for internal use	"tvc"
tmapargs	The desirable arguments for aligning the BAM file	"tmap mapall . . . -J 25 --end-repair 15 --do -repeat-clip --context stage1 map4" (ampliseq), "tmap" (others)|
unifyargs	This field is for internal use	"tvcutils unify_vcf"

Reference Management

References in Torrent Suite™ Software include the 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 Torrent Server that is connected to the sequencing instrument.

- **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**
  You must upload these files to add them to your Planned Runs.
- **Hotspots**
  You must upload these 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**
  You can use these known sequences to monitor system characteristics.
- **Barcodes**
  You must 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 ampliseq.com zip files.

Reference sequences

In order to identify genetic variations within a nucleic acid sample, sequencing reads are aligned to a reference genome sequence. The 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.

Note: 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 Torrent Server. For more information, see "TMAP Modules" on page 360.

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. See the Genome Reference Consortium's website: **ncbi.nlm.nih.gov/grc/human/data** for more information. There are several notable differences between the hg19 reference in the 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 the 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 Genome Browser FTP server at **hgdownload.soe.ucsc.edu/goldenPath/hg19** or the Downloads page at **hgdownload.soe.ucsc.edu/downloads.html#human**.

Hard masked PAR regions in chromosome Y

The mammalian Y chromosome contains regions that are virtually 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 duplication must be taken into account when performing sequence analysis 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 will align to both the X and the Y PAR sequences. This affects the mapping quality of the reads in these regions as well as creates issues 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 the 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 page at **hgdownload.soe.ucsc.edu/downloads.html#human**.

Import reference sequences

Torrent Suite™ Software includes validated reference sequence files that you can download onto your 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 Torrent Server, the reference sequences are available for use when you set up Planned Runs and when Torrent Suite™ Software performs data analysis.

Import a preloaded reference sequence

Torrent Suite™ Software provides a list of validated reference genome files that you can download onto your Torrent Server. These reference files can be easily imported into the Torrent Suite™ Software using this procedure. After you complete the import, the references can be used in Planned Runs and data analysis in the Torrent Suite™ Software.

You must be signed in to your Torrent Server as an administrator to have permission to import reference files.

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

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

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

Option	Description
Reference	Click **Import** in the row of the file to download a reference.
Annotation Files	Then, to download annotation files. 1. Click **Import Annotation Files**. 2. In the **Import Annotation Files** dialog box, select the annotations you want to import, then click **Import Selected**.

The file import status is displayed in the row of the file in the **Ion References** list. When **complete**, a compressed directory 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.

After the reference genome file is imported, you can edit the reference genome information, enable the reference for use in Planned Runs, or permanently delete the reference file from your Torrent Server. For more information, see "View a reference sequence file" on page 233.

Import a custom reference sequence

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 "Custom reference sequence guidelines" on page 232.

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

3. In the **Add New Reference Genome** dialog box.
   - Upload a FASTA file from your local storage.
     a. Select the **Upload File** tab.
     b. Click **Select File**, then navigate to and select the file from your local storage.
     c. Click **Open**.
   - Upload a FASTA file from a remote server.
     a. Select the **Install via URL** tab.
     b. In the **Reference URL *(required)*** field, enter the file path to the reference file (for example, http://updates/iontorrent.com/reference_downloads_mm10.zip).

4. Complete the information required in the following fields. This informaton is displayed in various report outputs and the **Reference Sequences** table.

Field	Description
Short name ***(required)***	Enter a recognizable short form of the genome name. Make sure 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. The field accepts 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.
Version ***(optional)***	Enter the genome version number and the accession number, if there is one (for example, "hg19", "gi|39933080|NC_005296.1").
Notes ***(optional)***	Use this field 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 233. If you encounter any errors during file upload, see "Error handling" on page 233.

Custom reference sequence guidelines

The following are guidelines 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. The 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 Torrent Suite™ Software uploads a genome containing other IUPAC characters, each such character is replaced with N.
- Prepare any related Target Regions, Hotspot files, and Reference Annotation files to upload with the reference genome file and save them to your local storage. See "Target Regions and Hotspots" on page 237 and "Import reference sequences" on page 230 for more information.

Error handling

Observation	Possible cause	Recommended action
Reference genome file cannot be opened	• An invalid FASTA file was uploaded. • No FASTA header found at line 1. • Genome information text file may have been deleted.	1. Delete the existing reference sequence entry. 2. Identify and correct formatting errors in the FASTA file. 3. Upload the corrected file.

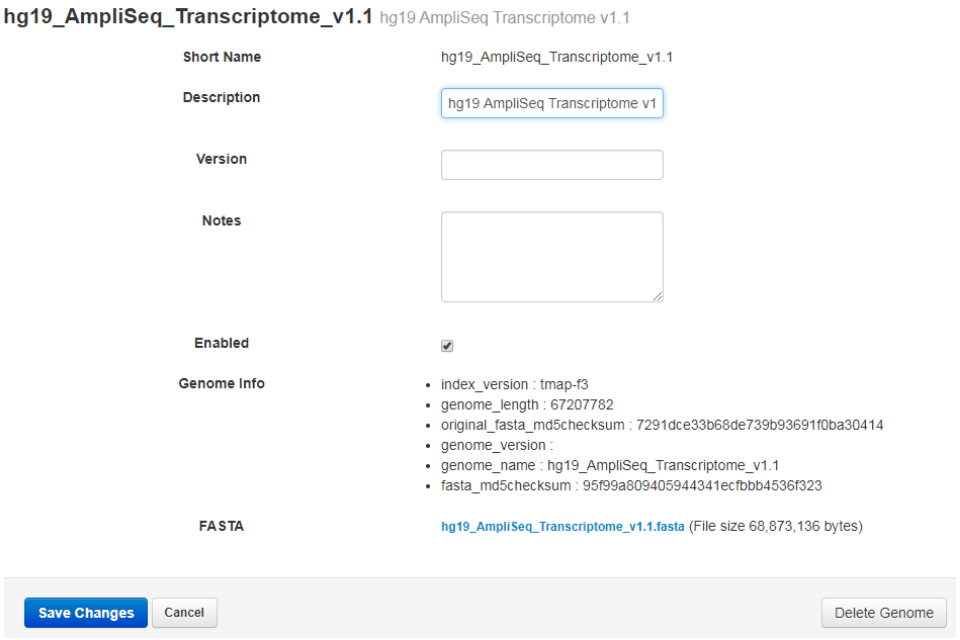
View a reference sequence file

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.

To view the reference sequence file, you must first import the file into Torrent Suite™ Software. For more information, see "Import reference sequences" on page 230.

1. Click **(Settings)** ▸ **References**.
   The **Reference Sequences** screen opens.
2. Perform one of the following actions to access the reference sequence file properties screen.
   - In the **Reference Sequences** table, in the **Short Name** column, click on 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 on the FASTA file link to view the FASTA format reference sequence in the browser window.

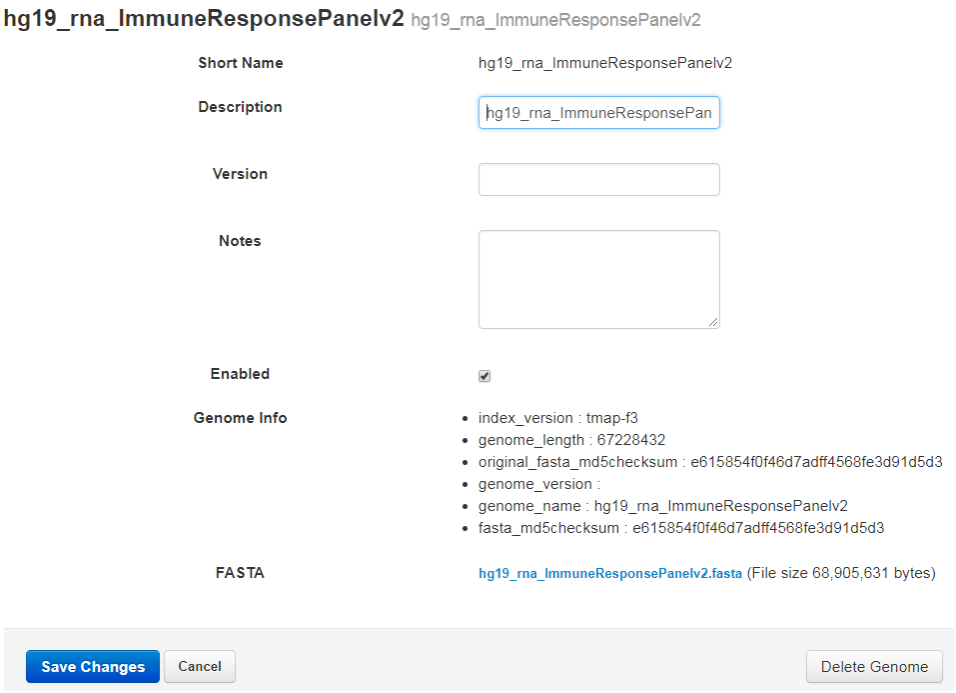
Edit reference sequence file properties

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 sequences" on page 230.

1. Click ⚙ **(Settings)** ▸ **References**.
   The **Reference Sequences** screen opens.

2. In the **Reference Sequences** table, in the **Short Name** column, click on the reference sequence file name.
   The file properties screen opens.



3. These file properties can be edited:

Property	Description
Description	Description of the reference.
Version	Version number or letter.
Notes	Any details.
Enabled	References are enabled by default. To disable a reference that is not being used anymore, deselect Enabled.

Note: You cannot edit the **Short Name** field. To change or reuse the reference sequence file short name, you must delete the existing reference sequence file from your Torrent Server and then upload a new file.

4. Click **Save Changes**.

Permanently delete a reference sequence file

After you import a reference sequence file into Torrent Suite™ Software, you can permanently delete the file from your 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 you no longer require the reference for your Planned Runs.

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

   IMPORTANT! Recovery of a deleted reference sequence is not supported.

   The reference is removed from the **Reference Sequences** table and is permanently deleted from your Torrent Server.

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.

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

Note: 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

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 BED file format only.
- **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 the variantCaller plugin only; 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 pages 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

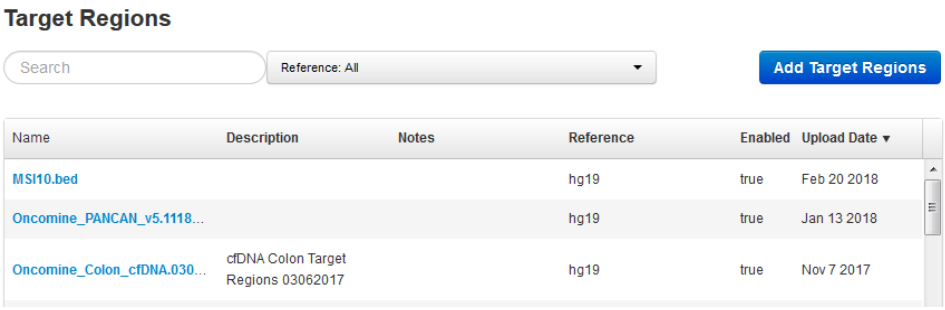
- Target regions BED files provide an option to restrict analysis to regions of interest. Do not specify a target regions BED file in the **Plan** step of the Planned Run workflow if you want variant analysis to span an entire genome. Use the **Whole Genome** application with a reference sequence to support whole genome analysis.
- All regions that are included in the target regions BED file you select are analyzed. Follow the instructions in “Modify a BED file“ on page 245 (before uploading your target regions BED file) to delete lines representing regions containing variants that you do not want to call.
- The BED file coordinates (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 the internal segment only, 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 Torrent Suite™ Software 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 at **AmpliSeq.com**. For Oncomine™ panel target regions and hotspots files, contact your field service representative.

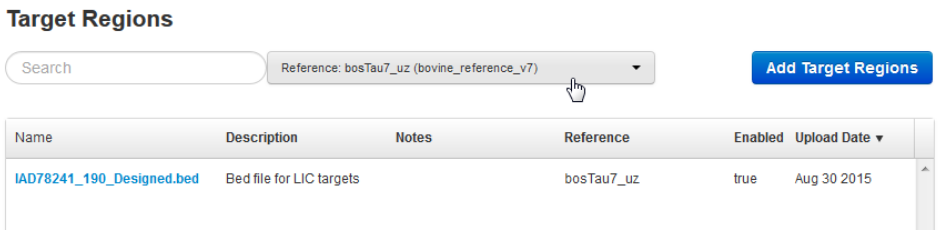
View and manage target regions files

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 available on your Torrent Server, click **(Settings)** ▸ **References**.
2. In the left navigation menu, click **Target Regions**.
   Available target regions files are listed in the **Target Regions** table.



3. Enter a term in the **Search** field, or select a reference from the **Reference** dropdown list to filter the list.



4. Click a target regions file name to open a **Target Regions Details** screen that displays file information, including the number of targets, genes, and covered bases, description, and notes. Scroll to view original upload information. Enter any additional information if needed, then click **Save Changes**.

File information field	Target regions detail
Processed File:	File name; click to download the BED file to view on your computer
Reference:	Reference genome species
Number of Targets:	Number of target regions included in the file
Number of Genes:	Number of genes included in the target regions
Covered Bases:	Number of bases covered by the target regions
Description:	Short description of the file
Notes:	*(Optional)* Additional information
Enabled:	Select the checkbox to enable use of the file in Planned Runs; deselect to prevent use in a Planned Run

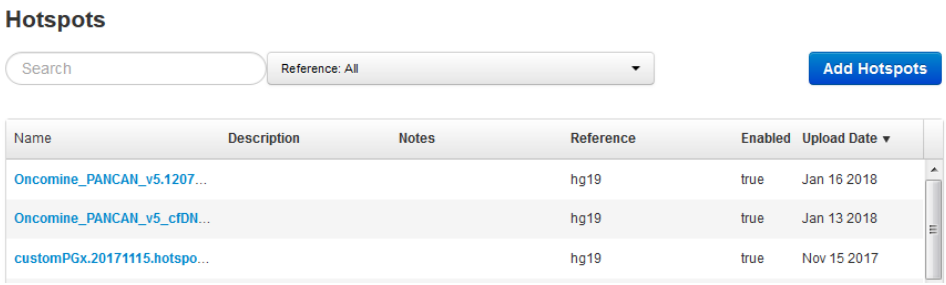
5. To delete a file from the Torrent Server, click **Delete**.

View and manage hotspots files

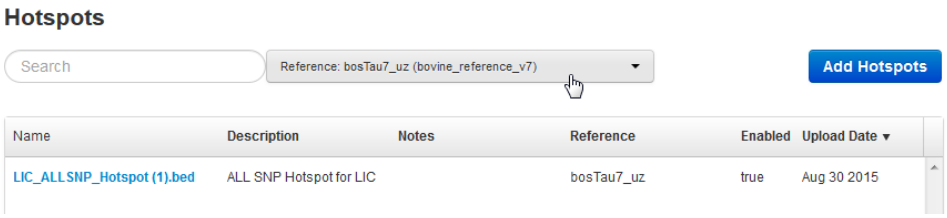
You can upload hotspots files for use with a specific reference. After hotspot files are uploaded, the files are available when that reference is selected for use in a Planned Run or variantCaller plugin configuration. When you select a hotspots file in run planning, the file instructs the variantCaller plugin to report on loci, known to be frequently altered, in its output files.

1. To view the hotspots files available on your Torrent Server, click **(Settings)** ▸ **References**.

2. In the left navigation menu, click **Hotspots**. Available hotspots files are listed in the table.



3. Enter a term in the **Search** field, or select a reference sequence from the **Reference** dropdown list to filter the list.



4. Click the name of a hotspots file to open a **Hotspots Details** screen that displays file information, including the number of loci, description, and notes. Scroll to view original upload information. Enter any additional information if needed, then click **Save Changes**.

File information field	Hotspots detail
Processed File:	File name; click to download the BED file to view on your computer
Reference:	Reference genome species
Number of Loci:	Number of hotspot alleles included in the file
Description:	Short description of the file
Notes:	*(Optional)* Additional information
Enabled:	Select the checkbox to enable use of the file in Planned Runs; deselect to prevent use in a Planned Run

5. To delete a file from the Torrent Suite™ Software, click **Delete**.

Upload a target regions file

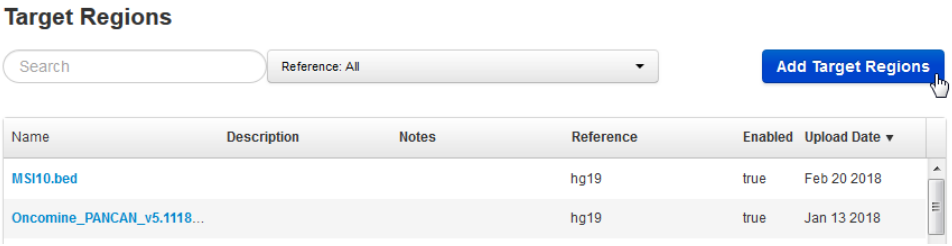
You can upload a target regions file from your computer to Torrent Suite™ Software. Target regions files are BED files only. Supported file extensions are .bed, .zip, and bed.gz. During file upload, the software validates the BED file, then ensures that the coordinate regions of the BED file are valid for the selected reference genome. The new BED file is then available as an option when you create a Planned Run. Your new file appears in the **Target Regions** dropdown 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 region 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.

To upload a target regions BED file for use with a reference sequence.

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

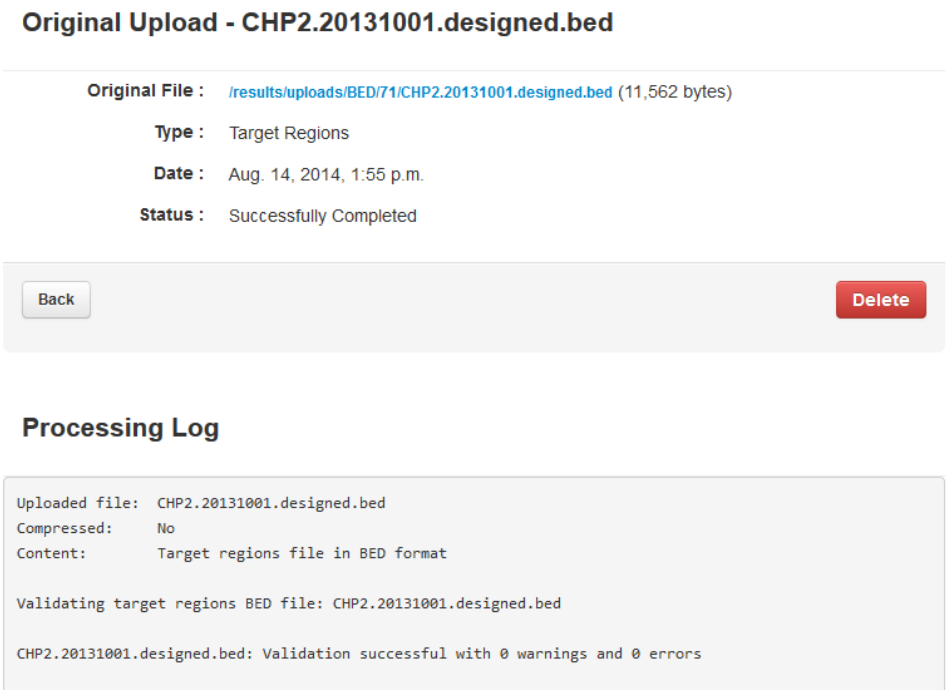


3. In the **New Target Regions** screen, click **Select File**, then navigate to the file to be uploaded.
4. Select the reference sequence from the **Reference** dropdown list.

   IMPORTANT!
   - Be careful to select the correct reference sequence because the new target regions file can only be used 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 is updated to **Successfully Completed** after the upload finishes. Errors are reported in the **Processing Log** pane.



Note: For large files, validation can take several minutes. Refresh your browser to check that validation is complete.

Upload a hotspots file

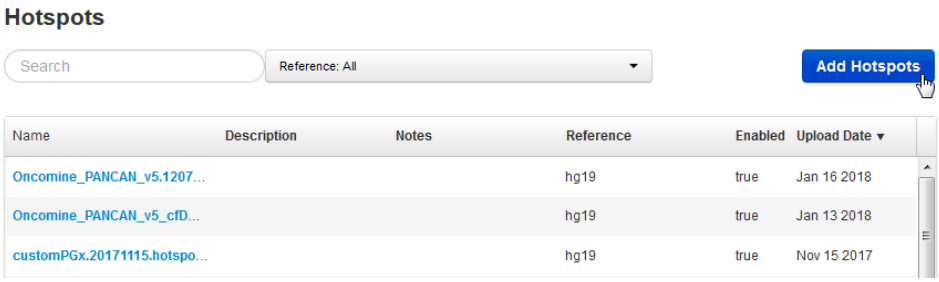
You can upload a hotspots file from your computer to Torrent Suite™ Software. Hotspots files can be BED or VCF files. Supported file extensions are .bed, .vcf.gz, .zip, bed.gz, and .vcf.gz. During file upload, the software validates the BED or VCF file, then ensures that the coordinate regions of the 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! You must 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.

To upload a hotspots BED or VCF file for use with a reference sequence.

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

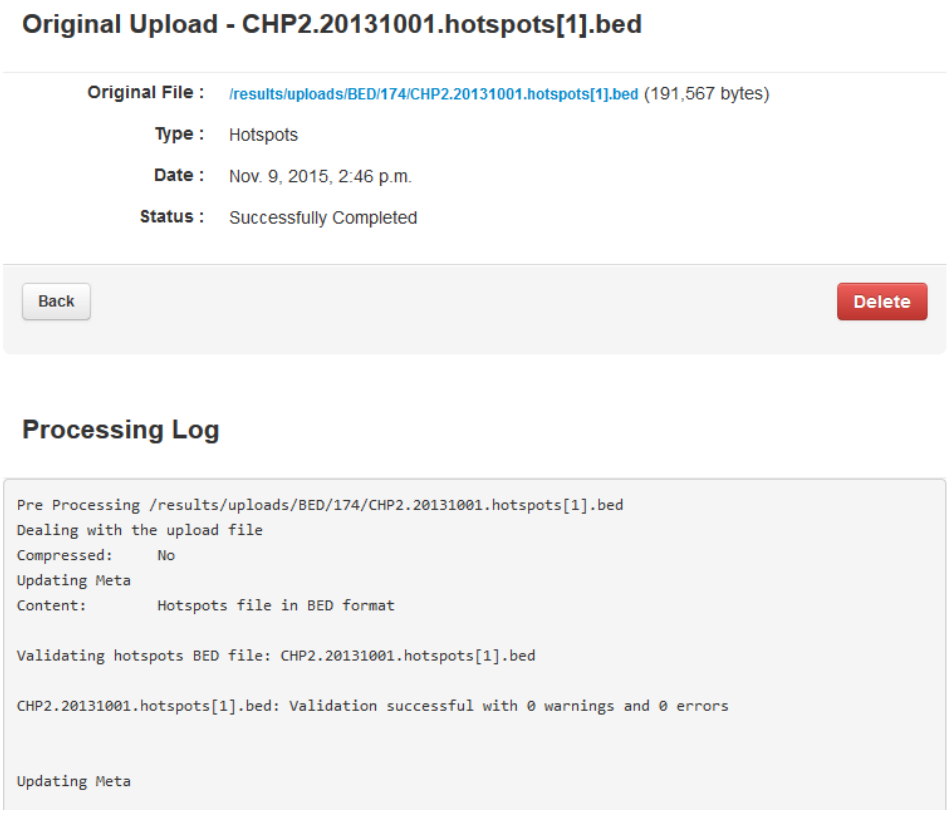


3. In the **New Hotspots** screen, click **Select File**, then navigate to the file to be uploaded.
4. Select the reference sequence from the **Reference** dropdown list.

   IMPORTANT!
   - Ensure that you select the correct reference sequence because the new hotspots file can only be used 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 is updated 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** dropdown list in the **Plan** step of the Planned Run workflow bar, and also appears in the **Hotspots** table in the References tab.

Uploading errors

Some types of errors do not appear in the **Processing Log** section. Major problems with files can prevent file uploading.

Observation	Possible cause	Recommended action
File validation cannot be attempted.	• Incorrect file format • Incorrect file extension • The .zip file contains no files or multiple files • A corrupted .zip or .gz file	1. Check for possible errors in the source BED or VCF file. 2. Fix the errors, or replace the file, then re-attempt the upload.

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.

BED File Formats and Examples

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

BED files are text files with tab-separated fields.

Target regions files format

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 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	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)	Name of the chromosome. This name must be an exact match with a chromosome in the reference.
chromStart	unsigned int64	Starting position of the feature (zero-based).
chromEnd	unsigned int64	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 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	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)	Name of the chromosome. This name must be an exact match with a chromosome in the reference.
chromStart	unsigned int64	Starting position of the feature (zero-based).
chromEnd	unsigned int64	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"

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 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 following is an example track line:

```
track name="ASD270245" description="AmpliSeq Pool ASD270245" ?
type=bedDetail
```

The track line includes these tab-separated fields:

Field	Type	Description
Name	string	A unique design identifier. Optional.
Description	string	Description of the design. Optional.
Type	string	Must be "bedDetail" (without quotes). Required.
ionVersion	string	Introduced in the Torrent Suite™ Software 4.0 release.

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)	Name of the chromosome. This name must be an exact match with a chromosome in the reference.
chromStart	unsigned int64	Starting position of the feature (zero-based).
chromEnd	unsigned int64	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	Customer-specified ID. If missing, set to '.'. This field is not used currently.
GeneSymbol	string	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 BED file format is for Fusion panels.

The additional columns are:

Field	Type	Description
Score	Unsigned int64	Score. If missing, set to "."
Strand	string (+ or -)	Strand. If unknown, set to "+".

BED files generated by AmpliSeq.com custom designs

The track line for BED files generated by AmpliSeq.com custom designs follows 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	Description of the design.
Type	string	"bedDetail" (without quotes).
ionVersion	string	Introduced in the Torrent Suite™ Software 4.0 release. When set to "4.0" or higher, indicates that the BED file supports the Extended BED Detail format.
db	string	The UCSC Assembly ID.
reference	string	The Torrent Server reference ID. Present for AmpliSeq.com 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 files format

The track line is required in a HotSpots BED file. The following is an example track line:

The track line includes these tab-separated fields:

Field	Type	Description
Name	string	A unique design identifier. Optional.
Description	string	Description of the design. Optional.
Type	string	Must be "bedDetail" (without quotes). Required.
db	string	The UCSC Assembly ID. Optional.
reference	string	The Torrent Server reference ID. Optional for hg19. Required for GRCh38.

The following is an example track line:

```
track name="ASD270245" description="HotSpots locations for
AmpliSeq ASD270245" type=bedDetail db=hg38 reference=GRCh38.p2
```

In HotSpots BED files, the coordinates lines require the following tab-separated fields:

Field	Type	Description
chrom	string (chars >= 0x20, other than \tab)	Name of the chromosome. This name must be an exact match with a chromosome in the reference.
chromStart	unsigned int64	Starting position of the feature (zero-based).
chromEnd	unsigned int64	Ending position of the feature (not inclusive). Must be greater than chromStart.
HotSpotName	string	This ID is either the COSMIC ID, dbSNP ID, or user-defined. If missing, the following string is used "chrom" + ":" + "chromStart" + "-" + "chromEnd"

Field	Type	Description
HotSpotAlleles	string	This field describes the variant, using this format (see examples below): `REF=` *reference_allele* ; `OBS=` *observed_allele* ; `ANCHOR=` *base_before_allele*
AmpliconID	string	Amplicon ID. If missing, the following string is used "chrom" + ":" + "chromStart" + "-" + "chromEnd"

The HotSpotAlleles field

This field specifies the alleles 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 completely optional. In fact, it is recommended that the ANCHOR key it is NOT provided for TS >= 4.2.
  - 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 hotspot file refers to the orientation of the Ion AmpliSeq™ 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 to what is reported on the HotSpotAlleles column. 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

AmpliSeq.com 3.0 and later uses this format for the following fixed panels:

- CCP
- CFTR
- CHP v2
- Ion AmpliSeq™ Exome

New fixed panels introduced for AmpliSeq.com 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, if supplied by the user, or '.'	User-supplied name or id for the region.
Description	key-value pairs separated by semicolon, or '.' if empty	Contains a '.' or one or more of the following: • GENE_ID= • SUBMITTED_REGION= • Pool= These key-value pairs are described in the next table.

This table describes the key-value pairs that are supported in the Description column:

Key	Description
GENE_ID	A gene symbol or comma-separated list of gene symbols. If no gene symbol is available, this key is absent. Example: GENE_ID = brca1 Example: GENE_ID = brca1, ret
Pool	The Ampliseq.com pool or pools containing this amplicon. Example: Pool=2 If an amplicon is present in multiple pools, the pools are delimited with "," a comma, with the primary pool listed first. For example, if an amplicon is present in pools 1 and 3, and 1 is the primary pool, the entry is: Pool=1,3. Single-pool designs do not include the Pool= key-value pair.
SUBMITTED_REGION	The region name provided by the user during theAmpliSeq.com design process. If a region name is not provided, this key is absent. Example: SUBMITTED_REGION=Q1
CNV_ID	A gene symbol used to specify a copy number region for the cnv pca algorithm. This will take precedence over the GENE_ID and once CNV_ID can span multiple GENE_IDs.
CNV_HS	A CNV region hotspot. This can be a value of either 0 or 1. A 1 will report as a hotspot (HS) in the output VCF file from the CNV PCA algorithm. A 0 will not be 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 these fields (Id and Descriptor) 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 GENE_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

The score and strand fields in uploaded 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

This page describes the target regions Browser Extensible Display (BED) format used with Ion AmpliSeq™ RNA fusion designs. BED files are text files with tab-separated fields.

Track line

The track line is required in the target regions BED file. The following is an example track line:

```
track name=
"Fusions 2.6"
description=
"AmpliSeq RNA"
type=bedDetail ionversion="4.0"
```

The track line includes these tab-separated fields:

Field	Type	Description
Name	string	A unique design identifier. Optional.
Description	string	Description of the design. Optional.
Type	string	Must be "bedDetail" (without quotes). Required.
ionVersion	string	Introduced in the Torrent Suite™ Software 4.0 release (AmpliSeq.com 3.0 and higher fixed panels). When set to "4.0", indicates that the BED file supports the **Extended BED Detail format**. Optional. This field relates to BED File format version only, not the version of panel designs.

Columns

This format includes 8 required columns separated by a tab (`\t`) character:

Field	Type	Description
chrom	string (chars >= 0x20, other than \tab)	Name of the chromosome. This name must be an exact match with a chromosome in the reference.
chromStart	unsigned int64	Starting position of the feature (Insert Start not the Amplicon Start). Must be zero-based.
chromEnd	unsigned int64	Ending position of the feature (not inclusive) (Insert End not the Amplicon End). Must be greater than chromStart.
AmpliconID	string	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 currently.
Strand	string (+ or -)	Strand. If unknown, set to '+'.
ID	string	Customer-specified ID. If missing, set to '.'. This field is not used currently.
Key-value pairs	string	Multiple attributes specified as semi-colon 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.

Note: 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	Type of the event. Allowed values: • Fusion • CONTROL or ExpressionControl • Driver_Gene or 5p3pAssay • GeneExpression • RNA_Hotspot	TYPE=Fusion
FP_TRANSCRIPT_ID	Transcript ID for the Five Prime Gene partner. This key value pair is only for Fusion type Targets.	FP_TRANSCRIPT_ID=ENSG00000156735
TP_TRANSCRIPT_ID	Transcript ID for the Three Prime Gene Partner.(This field is absent for CONTROL type amplicons).This key value pair is only for Fusion type Targets.	TP_TRANSCRIPT_ID=ENSG00000077782
BREAKPOINT	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	Name of the Five Prime Gene partner in the Fusion.This key value pair is only for Fusion type Targets.	FP_GENE_ID=BAG4
FP_GENE_STRAND	Strand for the Five Prime Gene partner. Allowed values are '+' and '-'.This key value pair is only for Fusion type Targets.	FP_GENE_STRAND=+
FP_EXON_NUMBER	Exon number in the Five Prime Gene. Use comma separated values if there the Amplicon spans multiple Exons.This key value pair is only for Fusion type Targets.	FP_EXON_NUMBER=2

Key	Value	Example
TP_GENE_ID	Name of the Three Prime Gene Partner in the Fusion. (This field is absent for CONTROL type amplicons.) This key value pair is only for Fusion type Targets.	TP_GENE_ID=FGFR1
TP_GENE_STRAND	Strand for the Three Prime Gene partner. Allowed values are '+' and '-'. (This field is absent for CONTROL type amplicons.). This key value pair is only for Fusion type Targets.	TP_GENE_STRAND=-
TP_EXON_NUMBER	Exon number in the Three Prime Gene.Use comma separated values if there the Amplicon spans multiple Exons. This key value pair is only for Fusion type Targets.	TP_EXON_NUMBER=6
FP_CHROM	Chromosome of the Five Prime Gene. This key value pair is only for Fusion type Targets.	FP_CHROM=chr8
FP_START	Start position for the Five Prime Segments. Use comma separated values if there are multiple segment Starts. This key value pair is only for Fusion type Targets.	FP_START=38050257
FP_END	End position for the Five Prime Segments. Use comma separated values if there are multiple segment Ends. This key value pair is only for Fusion type Targets.	FP_END=38050313
TP_CHROM	Chromosome of the Three Prime Gene. (This field is absent for CONTROL type amplicons.) This key value pair is only for Fusion type Targets.	TP_CHROM=chr8
TP_START	Start position for the Three Prime Segments, Use comma separated values if there are multiple segment Starts. This key value pair is only for Fusion type Targets.	TP_START=38283673

Key	Value	Example
TP_END	End position for the Three Prime Segments. Use comma-separated values if there are multiple segment Ends.	TP_END=38283763
HOTSPOT_POSITION	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	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	Name of the Gene for non-fusion type targets. For Fusion targets, we have FP_GENE_ID and TP_GENE_ID.	GENE_ID=LMNA
TRANSCRIPT_ID	Transcript Id for non-fusion type targets. For fusion targets, we have FP_TRANSCRIPT_ID and TP_TRANSCRIPT_ID.	TRANSCRIPT_ID=ENST00000389048
GENE_STRAND	Strand of the Gene. This key is for all non-fusion type targets. For fusion targets, we have FP_GENE_STRAND and TP_GENE_STRAND.	GENE_STRAND=+
EXON_NUM	Exon number(s) in the Gene. For fusion targets, we have FP_EXON_NUM and TP_EXON_NUM.Use comma separated values if there the Amplicon spans multiple Exons.	EXON_NUM=3,4
START	Start position of the Target segment. Use comma separated values if there are multiple segment starts in genomic space. This key is for all non-fusion type targets. For fusion targets, we have FP_START and TP_START.	START=53586113,53585786

Key	Value	Example
END	End position of the Target segment. Use comma separated values if there are multiple segments in genomic space. This key is for all non-fusion type targets. For fusion targets, we have FP_END and TP_END.	END=53586228,53585803
MIN_READ_COUNT	Minimum number of reads needed to call the particular target as present/absent. This value is optional and if present, it will override the universal minimum read count threshold (eg: 20). 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=382837
63;FP_TRANSCRIPT_ID=ENSG00000156735;TP_TRANSCRIPT_ID=ENSG000000
77782 ? ITGB7.ENCTRL.E14E15 ? 1 ? 132 ? ? AMP99 ? . ? ?
+ ? . ? TYPE=CONTROL;FP_GENE_
ID=ITGB7;FP_CHROM=chr12;FP_EXON_NUM=14,15;FP_START=53586113,535
85786;FP_END=53586228,53585803;FP_GENE_STRA
ND=-;FP_TRANSCRIPT_ID=ENSG00000139626
```

Test fragments

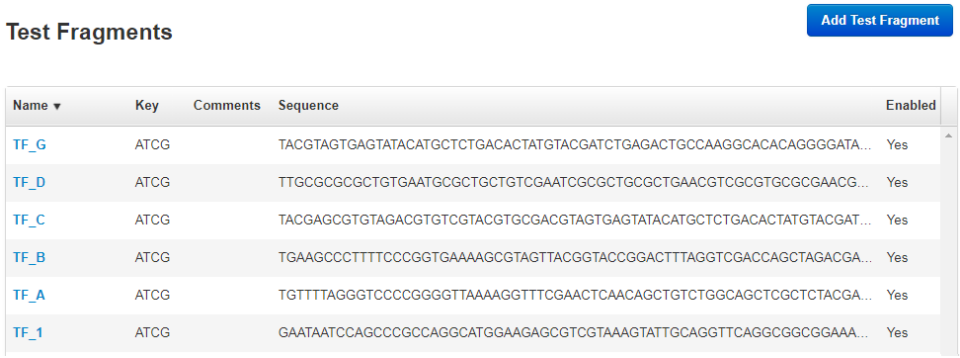
Test fragments are known genetic sequences used to measure the quality of your chip loading and sequencing run. You can include test fragments in your sequencing run and, after the run completes, review the **Details** section of the run report to evaluate the quality of your loading and sequencing run. 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 will only go through the loading and sequencing portions of the workflow. Test fragments are displayed when there are at least 1,000 high-quality reads, with an 85 percent 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 on a test fragment name to view the test fragment details, including the complete test fragment sequence.

3. *(Optional)* Add comments about the test fragment in the **Edit Test Fragment** dialog box.

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.
   The **Test Fragments** dialog box opens.

2. Click **Add Test Fragment**.

3. In the **Add New Test Fragment** dialog box, fill out the information in the following fields.

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.

   Note: If you enter an invalid character or duplicate test fragment, you will not be able to 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 in the Sequence field.
- Change whether or not the test fragment is enabled.

1. Click **(Settings)** ▸ **References**, then click **Test Fragments** in the left navigation menu.
   The **Test Fragments** table appears.
2. Click a link in the **Name** column.
   The **Edit Test Fragment** dialog box appears.
3. To manage the test fragments, perform the following actions in the **Edit Test Fragment** dialog box:
   - Edit the **Name**, **Key**, **Sequence**, or **Comments** fields, or select/deselect the **Enabled** checkbox, then click **Save**.
   - Click **Delete** to permanently remove the test fragment from Torrent Suite™ Software.
   - Click **Cancel** to exit the **Edit Test Fragment** dialog box without saving.

Any changes made appear in the **Test Fragments** table. 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 multi-plexed. 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. See “Plan step in the workflow bar“ on page 45 and “Run a plugin manually from the sequencing run report“ on page 115.

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. See "Output files" on page 101 for more information.

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 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 Torrent Server.

You can also transfer DNA barcode sets between two different Torrent Servers.

IMPORTANT! The creation of custom barcode sets is recommended for advanced users only. Thermo Fisher Scientific recommends that you contact a field bioinformatics specialist (FBS) for assistance with custom barcode sets.

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 **Barcodes** in the left navigation menu.
2. In the **DNA Barcodes** table, click on the barcode name in the **Name** column.
   A table listing all the individual barcodes within the barcode set and their associated information appears.
3. Click any column header in bold to sort the display of that column.

If you want to edit the existing barcode set, you can 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 263.

Download a DNA barcode set CSV file

You can download a DNA barcode set CSV file and save it on your local storage. This feature can be used to transfer DNA barcode sets between two different 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 Torrent Server.

1. Click ⚙ **(Settings)** ▸ **References**, then click **Barcodes** in the left navigation menu.
2. In the **DNA Barcodes** 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 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 264.

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 **Barcodes** in the left navigation menu. The **DNA Barcodes** 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, click **(Actions)** ▸ **Download** in the **Action** column.
      A CSV file is automatically downloaded to your computer.

   b. Edit the CSV file as described in "DNA barcode set CSV file set-up" on page 265, then save it to your local storage.

3. In the **Barcodes** screen, click **Add new DNA Barcodes**.

4. In the **Add New DNA Barcodes** dialog box, in the **Barcode Set Name** field, 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 set-up" on page 265.

   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 & Save**.
   The custom DNA barcode set is added to the **DNA Barcodes** table in the **Barcodes** screen and is now available to be used in a Planned Run.

DNA barcode set CSV file set-up

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 (e.g., 1, 2, 3,...) **Note:** Only numerical characters are accepted in this field.
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	String	**(Required)** The 5′– barcode sequence. **Note:** 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". **Note:** Upper-case G, C, A, and T are allowed.
annotation	--	Use this field for any barcode-specific notes.
end_sequence	String	The 3′– barcode sequence. **IMPORTANT!** This sequence is required for libraries that were prepared using dual barcode technology. **Note:** 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". **Note:** • This sequence is available only for libraries that were prepared using dual barcode technology. • Upper-case G, C, A, and T are allowed.

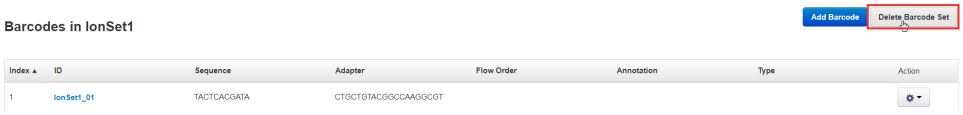
Delete a custom DNA barcode set

After you import a custom DNA barcode set into Torrent Suite™ Software, you can delete it from your Torrent Server if you no longer want to use the set in your Planned Runs.

IMPORTANT! Do not delete the any system barcode set.

1. Click ⚙ **(Settings)** ▸ **References**, then click **Barcodes** in the left navigation menu.
2. Select the barcode set to be deleted.
   - In the **DNA Barcodes** table, in the row of the barcode set to be deleted, click ⚙ **(Actions)** ▸ **Delete**.
   - In the **DNA Barcodes** table, in the row of the barcode set, click ⚙ **(Actions)** ▸ **Edit**.
   - In the **DNA Barcodes** table, in the **Name** column, click on the name of the custom DNA barcode set.

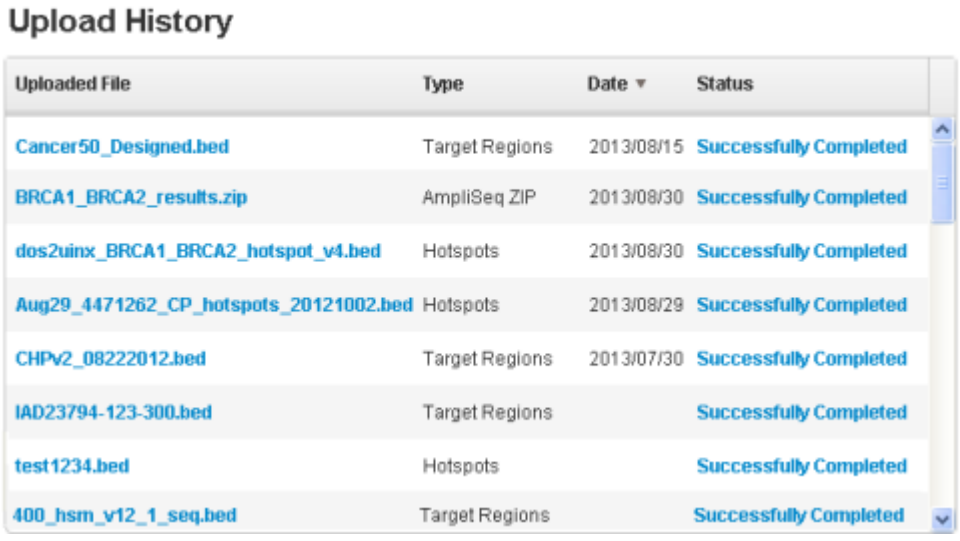
   The **Confirm Delete Barcode Set** dialog opens.
3. Click **Delete Barcode Set**.



4. Click **Yes, Delete!** to confirm.
   The DNA barcode set is permanently deleted from your 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	File name of the uploaded file.
Type	Indicates if the file is a Target Regions, Hotspots, or ZIP file.
Date	The date that the file was uploaded.
Status	A report of whether the files was successfully uploaded. Any errors that occur during upload will be shown in this column.

Data management

To avoid data loss and ensure that sufficient disk space is available on the server, you can configure Torrent Suite™ Software to automatically archive and delete sequencing data that are no longer needed.

You 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.

Ion instrument data types

Data that are generated from Ion sequencers consists of the following types of files:

- Signal Processing Input (.dat)
- Basecalling Input (1.wells)
- Output files (.bam, plugin output, etc.)
- Intermediate files

For more details about these file types, see "Analysis pipeline overview" on page 351.

Recommendations on when to archive each file type are listed in the following table.

File type	Details
Signal Processing Input	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. Keep the Signal Processing Input data if you want to reanalyze the run starting from raw signal processing data. However, on the Ion Proton™ or Ion S5™ Systems, Signal Processing Input data are used on the instrument, then deleted. These files are only available on the Torrent Suite™ Software as thumbnails for the Ion Proton™,Ion S5™ Systems or Ion GeneStudio™ S5 Systems.

File type	Details
Basecalling Input	Signal Processing Input files are converted to a single condensed Basecalling Input file representing the processed signal. 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. On the Ion Proton™, Ion S5™ Systems, and and Ion GeneStudio™ S5 Systems, Basecalling Input data are transferred to the Torrent Server and are available for reanalyses.
Output files	Output files consist of all BAM files, run reports, and plugin results. It is important to keep and archive these files. Delete output files *only* if you are sure that you no longer need the files.
Intermediate files	Intermediate files contain information used for debugging runs. You can delete these files immediately after instrument runs, without affecting data.

Archive or delete data automatically

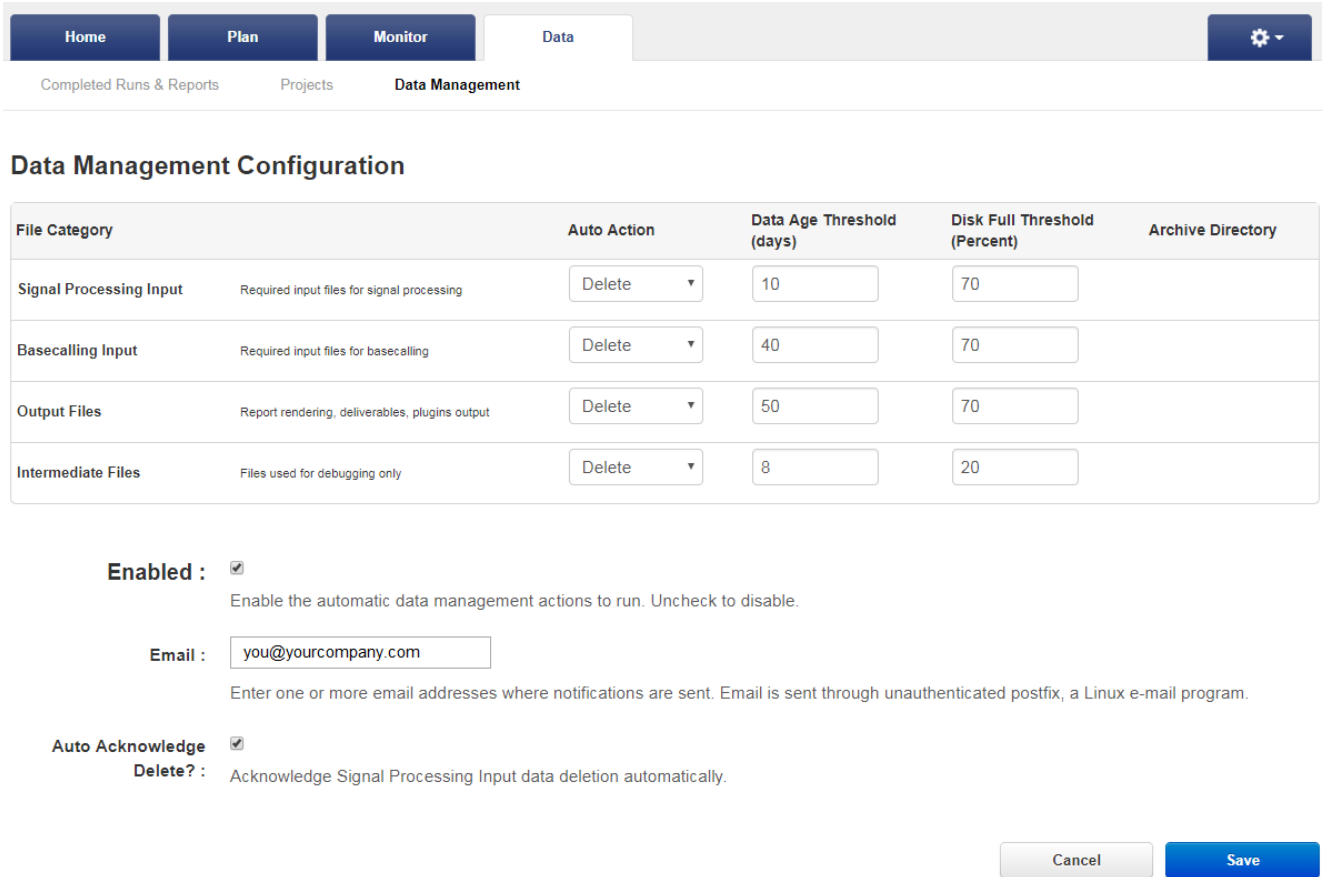
To avoid data loss, it is critical that sufficient disk space is available on the server. Therefore, it is important to have a strategy to monitor disk space and archive or delete data as needed.

- You can configure your Torrent Server to archive data to a mounted drive automatically after a data age threshold is met. Data that you assign to be automatically archived are copied to the designated location, then deleted from the Torrent Server. Automatic archiving helps to maintain available disk space, and simplifies management of data that you want to save to another volume.
- You can also configure your Torrent Server to delete data automatically when thresholds of filled disk space and data age are met. Automatic deletion of files is important to maintain available disk space, and simplifies removal of data that are no longer necessary to keep.
- You can assign automated archive or delete actions to each data file category independently of the others.
- An administrator-level role is required for configuring data management.

IMPORTANT! When you configure your 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	1. **Data Age Threshold (days)**: Set the number of days that you want data stored on the server before data are archived. 2. **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 stored.
Delete	1. **Data Age Threshold (days)**: Set the number of days that data are stored on the server before data are deleted. 2. **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. Enter an email address in the **Email:** field to receive notifications for automatic data management actions.

   Note: If you use a Linux™ mail server, you may 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.

   Note: 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**.

Import data for data transfers or restoration

You can import data from a mounted storage drive such as an external server or USB drive. The import function can be used to transfer data between servers or restore data that has been archived.

Data can only be imported from storage drives that have been mounted on your Torrent Server. For information about mounting a storage drive, see "Increase file storage and available disk space" on page 278.

Note:

- Exported and archived files on a mounted drive can be viewed and analyzed directly in Torrent Suite™ Software under **Completed Runs & Reports**, but if you unmount the storage device, the data will no longer be available. Import files before unmounting a drive to continue using them.
- Imported files appear as standard data files under **Completed Runs & Reports**.
- The **Import** function can only retrieve 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.

To import files:

1. Under the **Data** tab, click **Data Management**.
2. In the **Data Import** section of the screen, click **Import**.
3. Select a mounted Archive Directory from the dropdown list, or click **Browse** to navigate to a particular subdirectory, then click **Select**.
4. Select the file categories that you want to import with the checkboxes, then click **Import**.

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 Base Caller 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.

Note: For details regarding automatic deletion and archive creation, see "Archive or delete data automatically" on page 269.

View category statistics

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

Parameters of the **Category Statistics** section

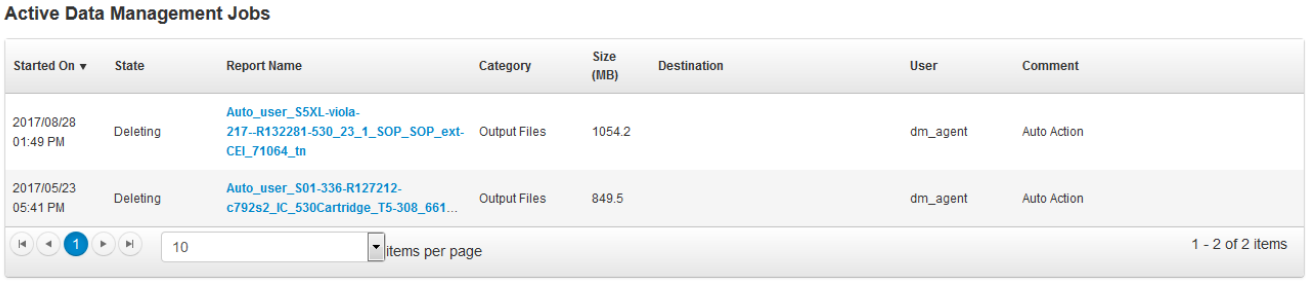
Parameter	Definition
File Category Group	File type (see "Ion instrument data types" on page 268 for details.)
Total	Number of data sets in each file category.
Keep	Number of data sets in each file category that are exempt from data management actions.
Archived	Number of data sets in each file category that have been removed from your Torrent Server by data management archival.

Parameter	Definition
Deleted	Number of data sets in each file category that have been removed from your Torrent Server by data management deletion.
In Process	Data sets that are currently archiving/deleting/importing.
Error	Error column displays the count of file categories that are currently in an error state. **Note:** 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.



Details regarding active data management jobs

Parameter	Definition
Started On	Start date and time of job.
State	Status of job/file.
Report name	Identifier of job.
Category	Identifies the file as one of the following file types. • Signal Processing Input: Required input files for signal processing. • Base calling input: Required input files for base calling. • Output files: Files for data processing. • Intermediate Files: Files used for debugging.
File size	File size of report.
Destination	Destination is the folder for archive or export action on a report.

Parameter	Definition
User	User that started the data management action. For auto-actions, "dm-agent" is the user.
Comment	Free space for notes.

2. *(Optional)* click a report to see the status of that report.

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 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.

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 next to each file category in that row.

If **Keep** is enabled, the data file will not be deleted. Instead, an error occurs if a user tries to confirm a deletion of run report data.

Manually export run data

You can manually export run data to a storage device that is mounted on the Torrent Server. When you export the data, it is copied from the Torrent Server to the archive location. The data remains on the Torrent Server, and the run results listed in the **Completed Runs & Results** screen continue to link to the data on the Torrent Server (see "Increase file storage and available disk space" on page 278 for more information).

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, you can click **Settings (⚙) ▸ Actions** to the right of the report name.
3. In the dialog, 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.

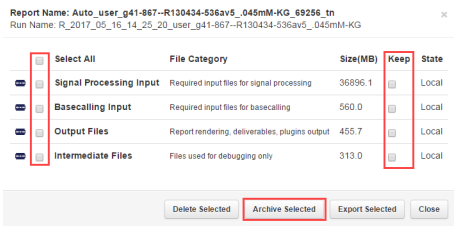
Manually archive run data

You can manually archive run data to storage device that is mounted on the Torrent Server. When you archive the data, it is moved from the 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 Torrent Server. For details, see "Increase file storage and available disk space" on page 278.

To manually archive run data:

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, 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 run data

You can manually delete run data from the Torrent Server.

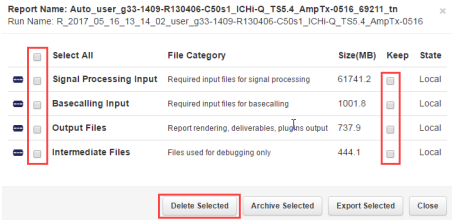
Note: For details about automatic deletion of run data, see "Archive or delete data automatically" on page 269.

IMPORTANT! Use this procedure only if you are sure that you no longer require access to the run data. After you delete data, it cannot be restored.

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 delete, then click **Process Selected**.

   If you want to delete data from only one run report, you can click **Settings** (⚙) ▸ **Actions** to the right of the report name.

   Note: If **Keep** is enabled, the data will be kept on the local hard drive. If you try to delete data, you will receive an error.
3. In the pop-up window, click 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 Torrent Server.

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.

   See "Search for run reports with disk usage status" on page 274 for details about how to search for a run in the **Disk Space Management** section.

4. Click ⚙ **(Actions)** ▸ **View Log**.
   A new screen containing chronological list of actions 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** page.

Increase file storage and available disk space

You can increase file storage space for data and results files with a Torrent Storage™ NAS 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 device	A field service engineer typically installs this device. It attaches directly to a 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 device" on page 281.
Your own NAS device	Similar to the Torrent Storage™ NAS device, but typically installed by your own system administrator.
USB Drive	Attaches directly to a 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 278.

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 and a basic understanding of disk drives and partitions are necessary.

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.

Note: These instructions only provide an overview of the required steps, and can be a helpful reminder if you are new to the Linux™ operating system. We recommend that a system administrator perform the Linux™ mount and unmount procedures. For

more detailed instructions and background information, see the Ubuntu™ documentation: **https://help.ubuntu.com/community/Mount/USB**.

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, as follows.

   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 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 the Torrent Suite™ Software.

Unmount a USB drive

Before disconnecting a USB drive, we recommend that you 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 Torrent Server: `sudo umount /dev/sdb1 /media/external`.

Connect to a Torrent Storage™ NAS device

Initial setup of your Torrent Storage™ NAS device is provided by your field service engineer. If the device is moved or disconnected for any reason (e.g., a power outage), this section provides instructions for an administrator-level user to reconnect the device to a Torrent Server.

- If the Torrent Storage™ NAS device connects directly to the Torrent Server, see “Connect directly to a Torrent Storage™ NAS device“ on page 282.
- If the device connects over a network to the Torrent Server, see “Connect over a network to a Torrent Storage™ NAS device“ on page 283.

Connect directly to a Torrent Storage™ NAS device

If your Torrent Server, Ion S5™ Instrument, or Ion GeneStudio™ S5 System is connected directly to your Torrent Storage™ NAS device, use the following steps to mount the device.

Note: You must be signed in as an administrator-level user to perform these steps.

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

   Note: To update your software, see "Update Torrent Suite™ Software" on page 286.

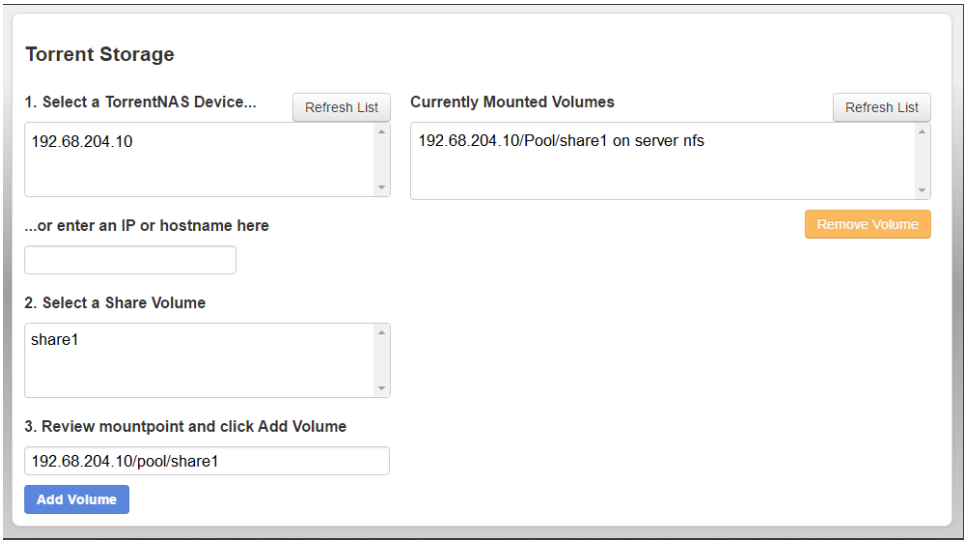
2. Click **Settings (⚙) ▸ Configure**, then scroll to **Torrent Storage**.

3. Locate the IP address of the Torrent Storage™ NAS device in the **Select a TorrentNAS Device** list. It may take several seconds for the list to populate.

   Note: If the Torrent Storage™ NAS device is not automatically detected in ≤1 minute, confirm that the correct network ports are connected, then click **Refresh List**.

4. Select the IP address of the device in the **Select a TorrentNAS Device** list.

5. Select a volume on the device under **Select a Share Volume**, 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 device

If your Torrent Server, Ion S5™ Instrument, or Ion GeneStudio™ S5 System is installed on the same network as your Torrent Storage™ NAS device, use the following steps to mount the device.

Note: You must be signed in as an administrator-level user to perform these steps.

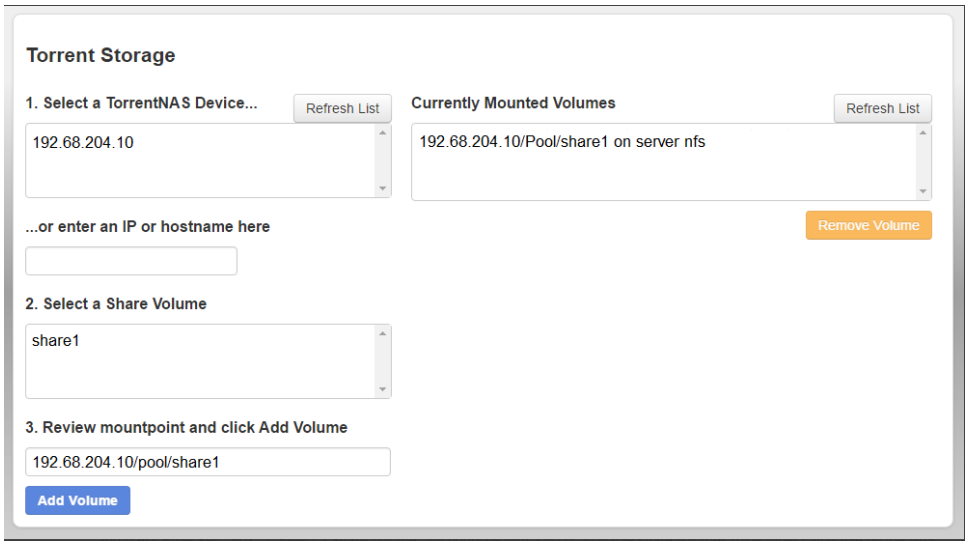
1. In Torrent Suite™ Software, click **Settings** (⚙) ▸ **About**, then confirm that the Torrent Suite™ Software version is 5.2 or later.

   Note: To update your software, see "Update Torrent Suite™ Software" on page 286.

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

3. Enter the IP address of the Torrent Storage™ NAS device in the **...or enter an IP or hostname here** field, then press the **Enter** key.

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

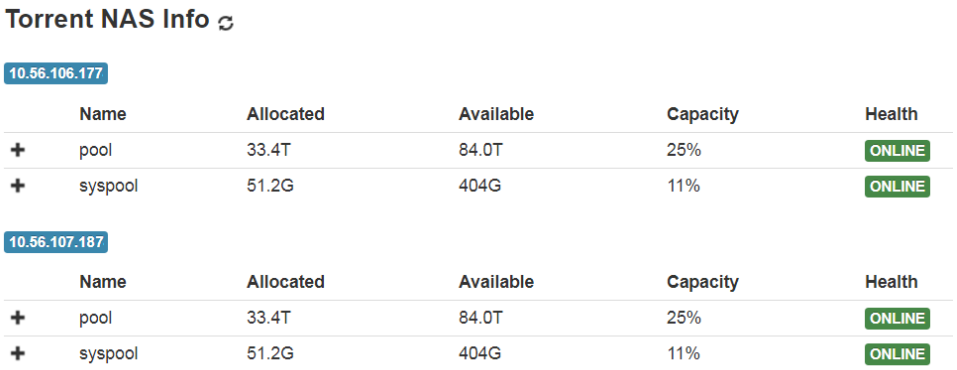


The storage volume is connected to the server and is listed in **Currently Mounted Volumes**.

Monitor the Torrent Storage™ NAS device

You can check the status of a Torrent Storage™ NAS device in Torrent Suite™ Software.

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



Software administration

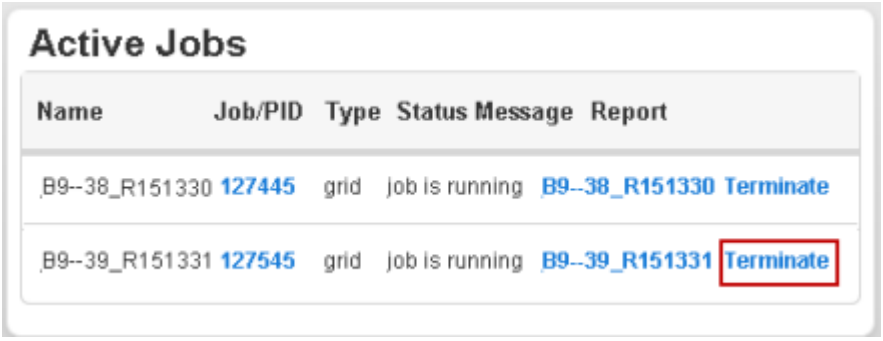
Administrative privileges allow you to configure Torrent Suite™ Software, administer Torrent Server databases, manage user accounts, backup and restore data, and enable remote monitoring. An `ionadmin` account is required for the procedures in this section.

Note: An `ionuser` account does not include sufficient privileges for these procedures.

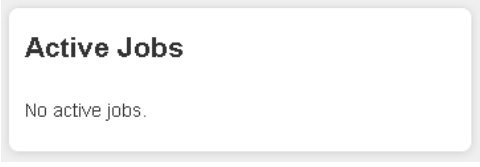
Stop a sequencing run

Use the following procedure to stop an analysis job for a run that has started but 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**.



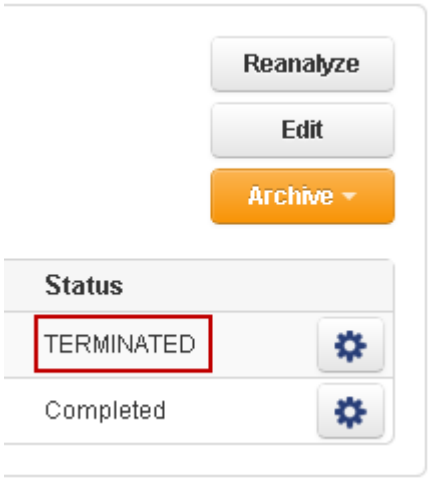
3. Click **Terminate** for the sequencing Run that you want to stop.
4. In the confirmation dialog, click **Yes** to end the run, or click **No** for the analysis job continue.
5. Refresh your browser to update the information in the **Active Jobs** section. The run is removed from the **Active Jobs** list, which displays **No active jobs** if no other runs are active.



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

The status is **TERMINATED** next to the name of the sequencing Run that you stopped.

Note: You can always start a new analysis run.



Torrent Suite™ Software updates

The instructions in this section describe how to update your 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 Thermo Fisher Scientific product.

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

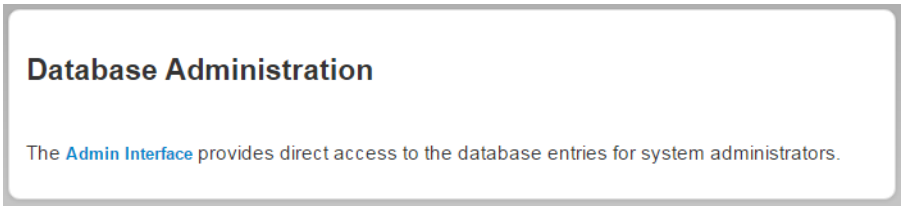
Update Torrent Suite™ Software

Updates to Torrent Suite™ Software cause the software web services to restart. Ensure that no sequencing runs are active on the server or are queued to start.

IMPORTANT! These procedures require an administrative (`ionadmin`) account. A user account such as `ionuser` does not include sufficient privileges for these procedures.

1. Sign in to Torrent Suite™ Software with your Administrator account.
2. Click ⚙ **(Settings)** ▸ **Configure**.

3. Scroll to the **Database Administration** section, then click **Admin Interface**.



   If you are prompted to Sign in, use your `ionadmin` account.
   The Site administration page opens.

4. Click **Update Server** in the **Management Actions** section:

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

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

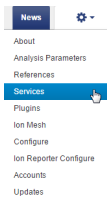
6. Click **Check** to check for updates.

7. If software updates are available, click **Update Server** to update Torrent Suite™ Software on the server.

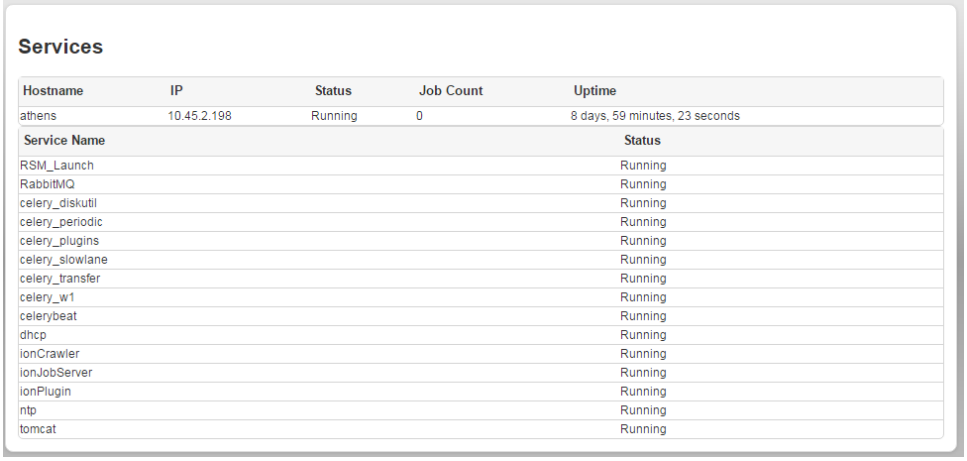
8. When the software update is complete, click **Back to Main Site**, then click ⚙ **(Settings)** ▸ **About**.

9. Review the Torrent Suite™ Software version number in the Releases list to ensure that it reflects the update that you completed.

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



11. Under **Status** in the **Services**, 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

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

Check for off-cycle updates

To check if there are any new products, plugins, or instrument updates, open the Updates Page.

Note: To update Torrent Suite™ Software, see "Update Torrent Suite™ Software" on page 286.

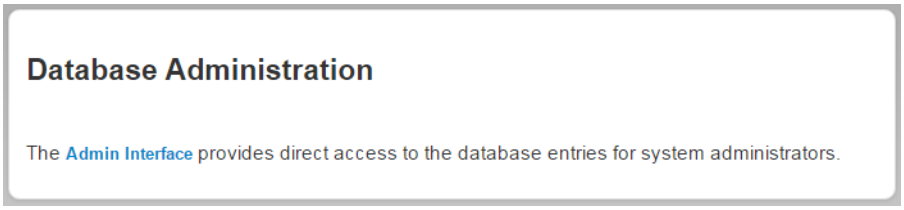
1. Click ⚙ **(Settings)** ▸ **Updates** and the Updates Page appears.
2. Determine if any updates listed are relevant to your work.
   - To update products, see "Enable off-cycle product updates" on page 289.
   - To update plugins, see "Update off-cycle release plugins" on page 290.
   - To manually install update files, see "Install off-cycle bundles without Internet Access" on page 290.

Lock current Torrent Suite™ Software version

You can prevent users from installing updates to Torrent Suite™ Software. Use this procedure to lock the current version of Torrent Suite™ Software.

1. Sign in with your Administrator account.
2. Click ⚙ **(Settings)** ▸ **Configure**.

3. Scroll to the **Database Administration** section, then click the **Admin Interface** link.

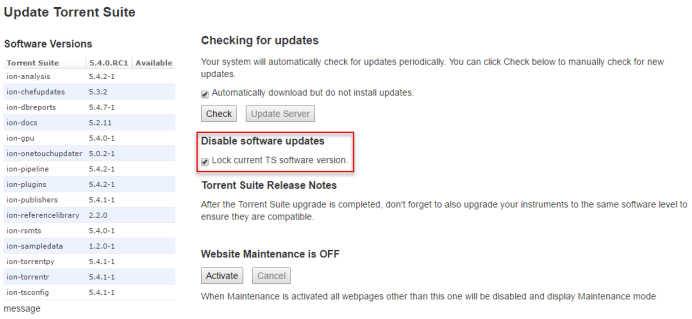


   If you are prompted to Sign in, use your Administrator account.

4. Click the **Update Server** link in the **Management Actions** section.

   Software versions that are currently available are listed and the area below the list indicates whether updates are available. For example, **No updates** indicates that updates are not available.

5. In the **Software Versions** list, click the **Lock current TS software version** checkbox to prevent accidental updates to your software:



Enable off-cycle product updates

You 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. Sign in to Torrent Suite™ Software as administrator.
2. Click **(Settings)** ▸ **Updates**.
3. Scroll down to the **Update Products** section at the bottom of the screen.
4. 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.

Update off-cycle release plugins

You 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. Sign in to Torrent Suite™ Software as administrator.
2. Click ⚙ **(Settings)** ▸ **Updates**.
3. Scroll down to the **Update Plugins** section at the bottom of the screen.
4. Select the new Torrent Suite™ Software plugin that you want to install and click **Update**.
   Your installed version of Torrent Suite™ Software is updated to include the new plugin that you selected.

Install off-cycle bundles without Internet Access

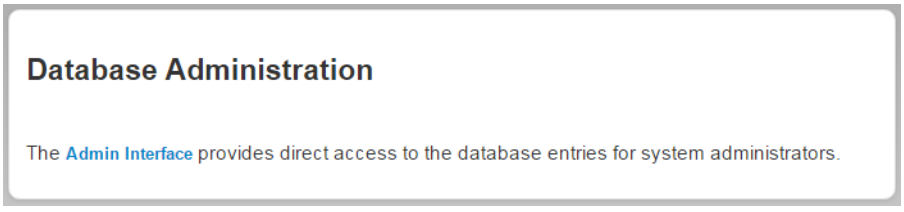
If your Torrent Server is not connected to the Internet, follow the steps below to manually install updates.

1. Request USB device from your Thermo Fisher Scientific representative or Technical Support.
2. Insert the USB device into your Torrent Server.
3. Click ⚙ **(Settings)** ▸ **Updates** and the Updates page appears.
4. Under Manual Upload, click **Click to Upload and Install**.
5. Follow prompts to upload the compressed folder.

Manage Torrent Suite™ Software user accounts

The section that follows explains how to manage user accounts from the Torrent Suite™ Software 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.

Option	Description
Add	Click **Add** to add new users.
Change	Check **Change** to modify user accounts.

Open the Site Administration screen

Administrator-level users can modify default settings for sequencing runs, using tools in the **Site Administration** screen.

IMPORTANT! Use extreme caution when modifying any of the settings in this screen. Fields that are set to incorrect values may corrupt the database or produce unpredictable results. Check with your Field Application Scientist or Field Bioinformatics Specialist if you need 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 **Site administration** screen in the **Ion Web** portal opens. After you have made changes, click **Back to Main Site** at the top of the screen to return to the software.

Approve or reject a new user account

New users can request accounts on the Torrent Suite™ Software sign on screen. An administrator must approve each request before the new account is active. An administrative account is required to approve or reject each user account request.

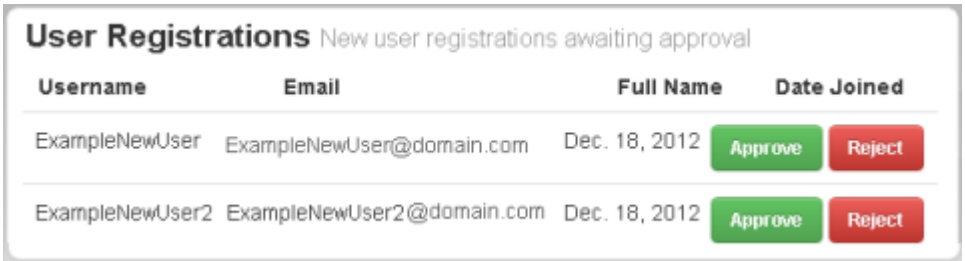
A message about new user registrations that are pending opens when you sign in as an administrator.

IMPORTANT! Use extreme caution when modifying any of the settings in this screen. Fields that are set to incorrect values may corrupt the database or produce unpredictable results. Check with your Field Application Scientist or Field Bioinformatics Specialist if you need 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 registration, click **Account Management**.

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

   Note: 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 and choose from the following.

Option	Description
Approve the registration.	Click **Approve** to approve the account for the new user, then click **Yes, Approve**.
Reject the registration.	Click **Reject** to approve the account for the new user, then click **Yes, Reject**.

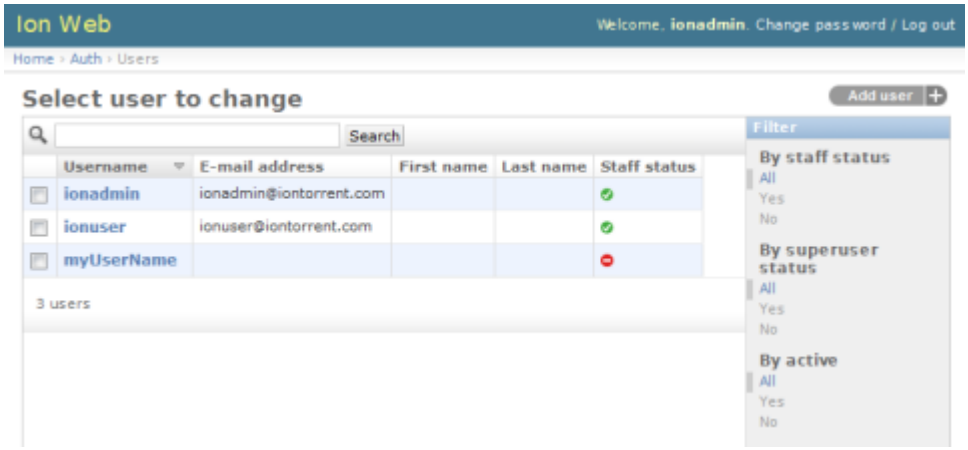
The user account is added to the list of user accounts in the Torrent Suite™ Software Site Administration screen. For details, see "Manage Torrent Suite™ Software user accounts" on page 290.

After you have made changes, click **Back to Main Site** at the top of the screen to return to the software.

Modify a user

Administrator-level (Superuser) users can modify the access level for an existing user (Staff), using tools in the **Site administration** screen.

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 **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 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 to modify user information, such as user name, password, first and last name, email address, or Active/unactive status. Confirm 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.

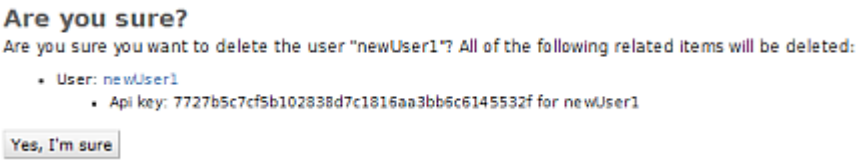
The user account is modified.

After you have made changes, click **Back to Main Site** at the top of the screen to return to the software.

Delete a single user account

Administrator-level users can delete a user account, using tools in the **Site administration** screen.

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 **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** page, click the **Username** of the user to be deleted.
6. At the bottom-left of the **Change user** page, click **Delete**.
7. Ensure that you want to delete the user by clicking **Yes, I'm sure**.



The user is deleted.

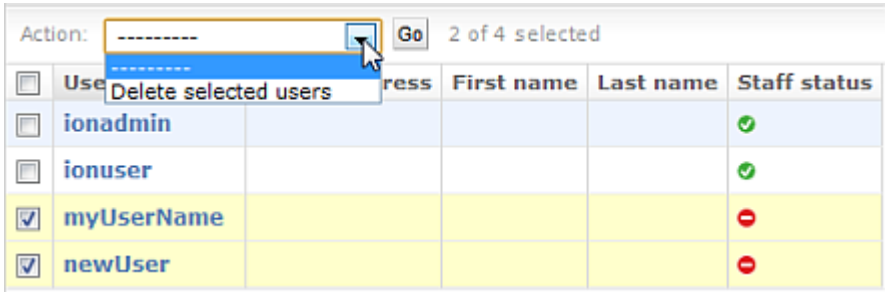
After you have made changes, click **Back to Main Site** at the top of the screen to return to the software.

Delete multiple user accounts

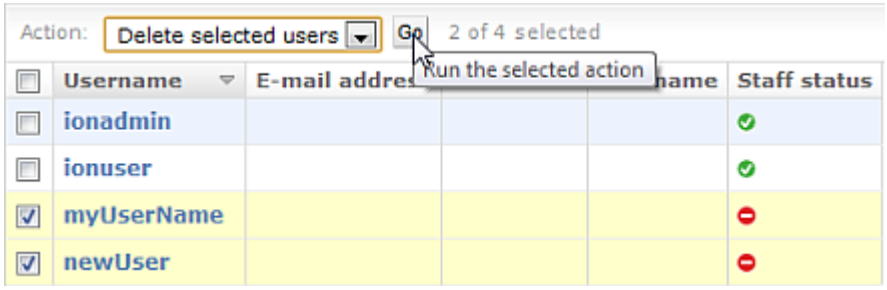
Administrator-level users can delete multiple users, using tools in the **Site administration** screen.

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 **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** page, check the checkbox for each users you want to delete.

6. Click the dropdown list, then select **Delete selected users**:



7. Click **Go**:



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

On the **Select user to change** page, the list of users confirms your deletions.

After you have made changes, click **Back to Main Site** at the top of the screen to return to the software.

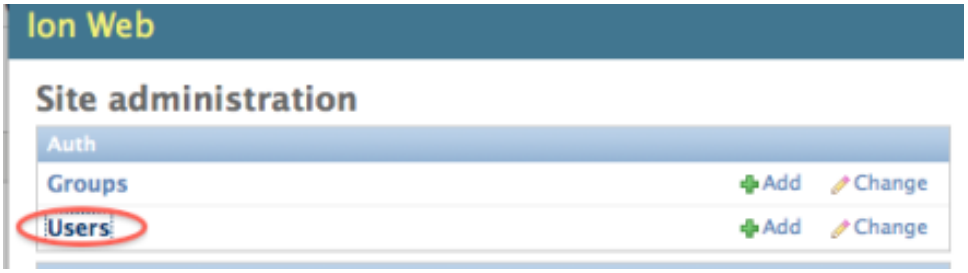
Create a new administrator account to change a password

You can change your password by creating an new superuser account, then changing the user account password.

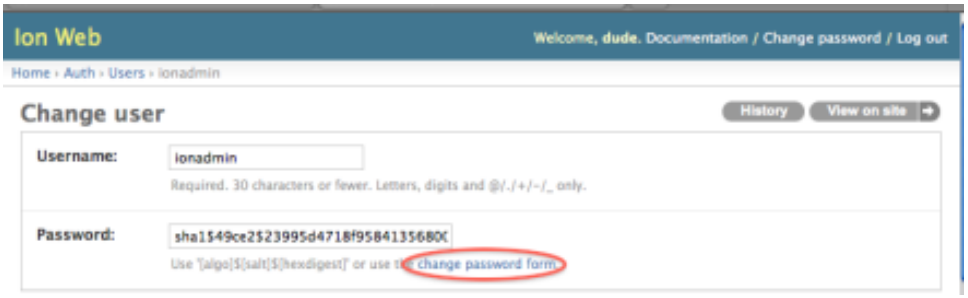
IMPORTANT!

Use extreme caution when modifying any of the settings in this screen. Fields that are set to incorrect values may corrupt the database or produce unpredictable results. Check with your Field Application Scientist or Field Bioinformatics Specialist if you need to change any of the settings or complete any of the procedures that are available through this administrative tool.

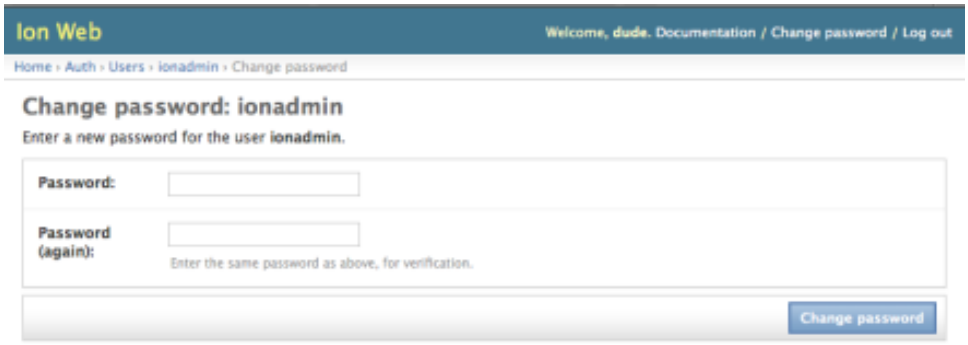
1. Run the following commands: `cd /opt/ion/iondb ./manage.py createsuperuser` in the command prompt.
2. After the new superuser account has been created, click ⚙ **(Settings)** ▸ **Configure**.
3. In the **Configure** screen, scroll down to the **Database Administration** section. Click the **Admin 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:



   Note: If you sign in with an `ionuser` account, the Auth section does not appear .
6. Select the account that you want to change the password for.
7. Click **Change password form**:



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



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

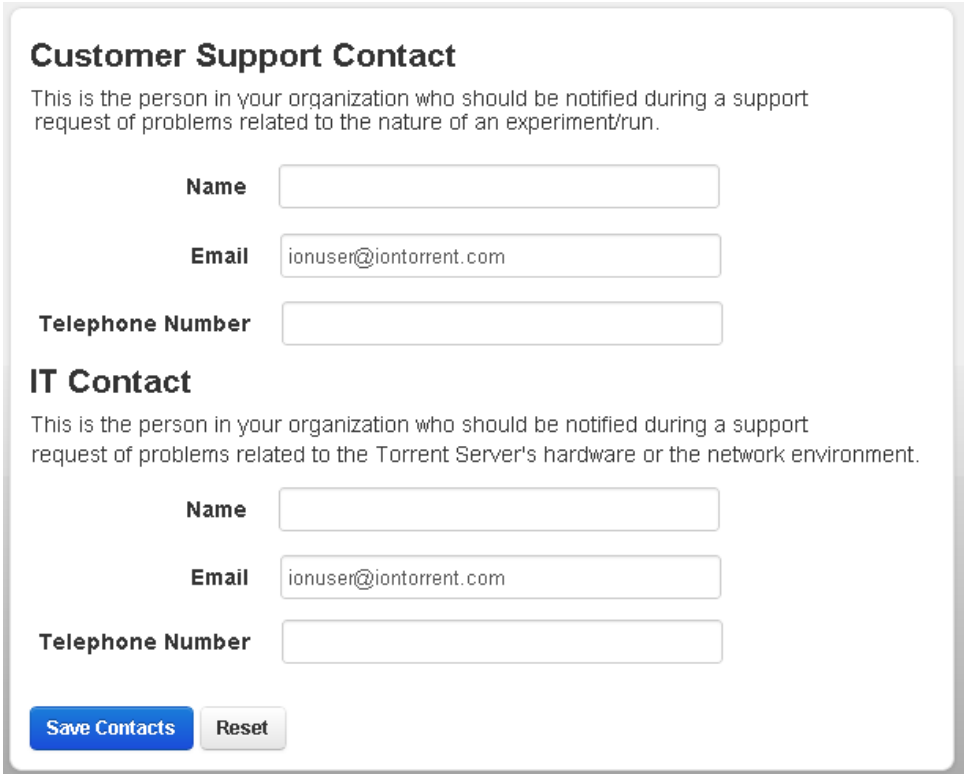
After you have made changes, click **Back to Main Site** at the top of the screen to return to the software.

Disk usage

It is critical that sufficient disk space is available on the server to avoid data loss. If needed, it is important to have a strategy that periodically monitors disk space and archives or deletes data. For details on how to check disk space on the server, see "View disk usage parameters" on page 272.

Add customer support contacts

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 displayed server name

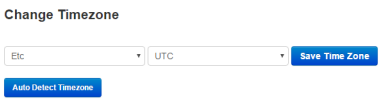
You can change the server name that appears in the Torrent Suite™ Software. By default, this name is `Torrent Server`. This change affects only the server name that is shown in the Torrent Suite™ Software, and the default bookmark name that appears in the browser when a bookmark is created.

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 for the Torrent Browser is changed.

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.

RAID Info

The RAID Info section shows the status of physical drives on an attached Torrent Storage device (Dell PowerVault MD1200):

The Show Details link opens a popup with details of the RAID drives (only one shown here):

Slot 0	
Media Error Count	0
Other Error Count	0
Predictive Failure Count	0
Firmware state	Online, Spun Up
Inquiry Data	SEAGATE ST32000444SS KS679WM0L47T
Needs EKM Attention	No
Foreign State	None
Port-0	
Port status	Active
Port-1	
Port status	Active
Drive has flagged a S.M.A.R.T alert	No
Drive Temperature	30C (86.00 F)

Refresh your browser to see changes in status. This information is not updated automatically.

Set up Ion Mesh

Ion Mesh is a feature in Torrent Suite™ Software that allows you to form a network between Torrent Servers.

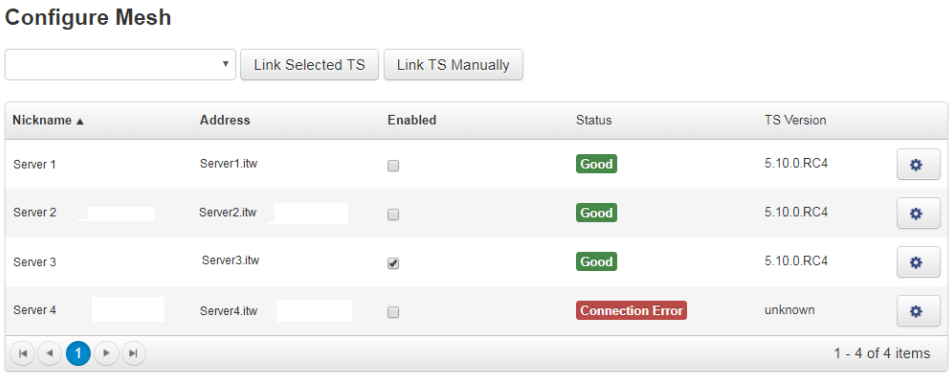
Note: Torrent Servers must be running the same software version.

When using Ion Mesh, you can:

- view all runs of interest across multiple Torrent Servers on the same data page.
- transfer Planned Runs between sequencers connected to different Torrent Servers.
- track reagent and cartridge usage across Ion Chef™ flexible workflows from different Ion Chef™ instruments connected to different Torrent Servers

Follow these steps to connect (link) or disconnect (unlink) your Torrent Server to another Torrent Server.

1. In the any tab, click ⚙ **(Settings)**, then click **Ion Mesh**.



2. Select one of the following options.

Option	Action
Link a selected Torrent Server	Select a Torrent Server from the dropdown menu, then click **Link Selected TS**.
Manually link a Torrent Server	Click **Link TS Manually**.
Edit the nickname of a linked TS	Click ⚙ **(Actions)** in the row of the Torrent Server you want to edit, then click **Edit**.
Enable/Disable a Torrent Server	• To enable a Torrent Server: select the checkbox in the **Enable** column of the **Configure Mesh** table in the row of the Torrent Server. • To disable a Torrent Server: deselect the checkbox in the **Enable** column of the **Configure Mesh** table in the row of the Torrent Server. **Note:** Disabling a Torrent Server hides that Torrent Server from Planned Runs actions and Data menus so the unlinked Torrent Server cannot be utilized by the Torrent Suite™ Software.
Unlink a Torrent Server from Ion Mesh	Click ⚙ **(Actions)** in the row of the Torrent Server you want to unlink, then click **Unlink**. **Note:** Unlinking a Torrent Server permanently removes that Torrent Server from Ion Mesh.

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

Parameter	Definition
Hostname/Address	Host name or address of the server. **Note:** If linking a selected Torrent Server, this field is automatically populated and cannot be edited.
Nickname	A common name that is assigned to the Torrent Server.
Username	Your username.
Password	Your password.

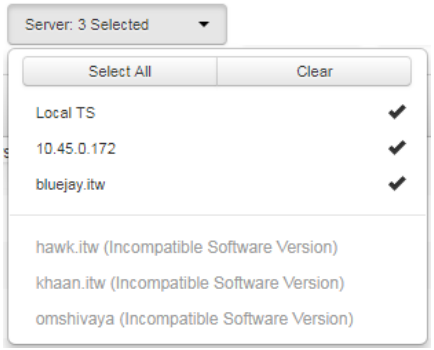
4. Click **Setup**.
The linked and enabled Torrent Servers are available to be used in the Planned Runs and the data from the linked and enabled Torrent Servers can be viewed on the same **Data** page.

View sequencing runs on multiple sequencers with Ion Mesh

Prior to viewing runs on multiple servers, you must first connect the servers using Ion Mesh. See "Set up Ion Mesh" on page 301 for more information.

1. In the **Data** tab, click **Completed Runs and Reports**.
2. Select one or more Torrent Servers that you want to view data from in the **Server** dropdown list.

   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 Torrent Server with Ion Mesh

Prior to transferring Planned Runs, first connect the Torrent Servers using Ion Mesh. See "Set up Ion Mesh" on page 301 for more information.

1. In the **Plan** tab, click **Planned Runs**.
2. Locate the row of the Planned Run you want to transfer, then click ⚙ **(Actions)** ▸ **Transfer**.
3. Select the Torrent Server you want to receive the Planned Run.

Track Ion Chef™ 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 Ion Mesh" on page 301. 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 connected in an Ion Mesh configuration, and the connection to one 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 cartridge use status is not trackable. Ion Mesh communication must be restored to verify cartridge use status before the run can start.

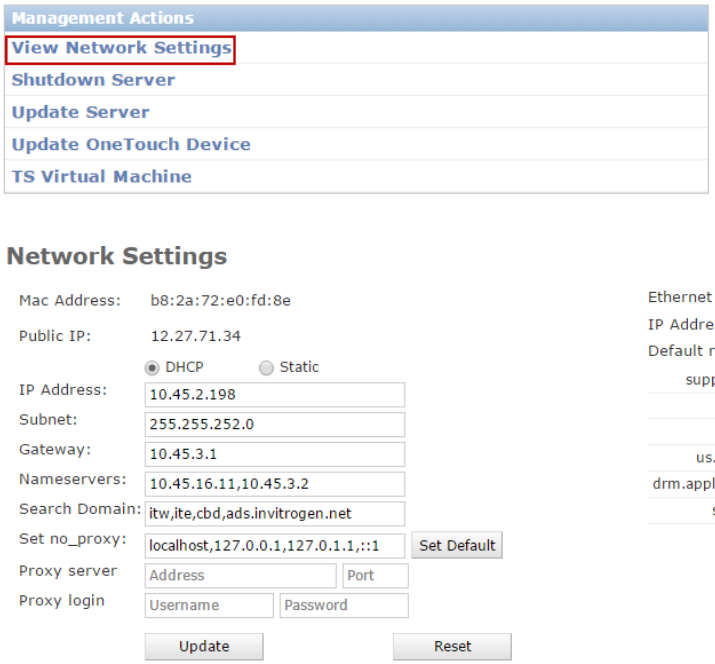
---

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Click a completed report.
3. In the run report, scroll down to **Details** section and select **Chef Summary** and **S5 Consumable Summary** tabs to track the cartridge use.

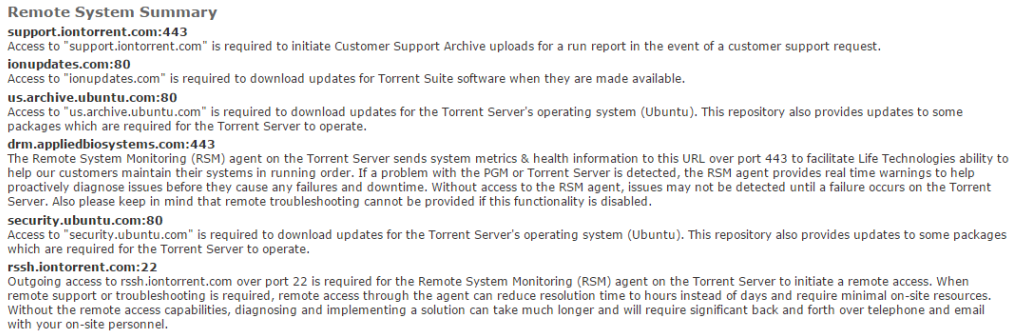
View network settings

The **Network Settings** page also describes the following ports and remote sites in its **Remote System Summary** section:

Click the **View Network Settings** link to see information about the Ion Torrent™ Server:



The Network Settings page also describes the following ports and remote sites in its **Remote System Summary** section:



Data backup and restore locations

The 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

The following instructions delete the current database.

- To restore the database, you need a complete working Torrent Server installation.
  The two scenarios for restoring a database are:
  a. Installing a new Torrent Server from the Torrent Server installation disk due to migrating the database to a new server or needing to reinstall the server.
  b. Replacing the database on an existing 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 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, there is a django error after completing the import data step. If an error is displayed on the browser UI, repeat the following steps:

a. Drop database.

b. Create database.

c. Import data.

Axeda™ Remote System Monitoring (RSM)

Overview

The Axeda™ RSM (Remote System Monitoring) agent is a software component that is installed automatically on the Torrent Server and Ion S5™, Ion PGM™, and Ion Proton™, and Ion GeneStudio™ S5 Systems through the software update process.

Approximately every 60 seconds, this 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
- Some technical details about the instrument such as temperature and hard drive status.

The agent also allows Thermo Fisher Scientific personnel to log in remotely to 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 Ion Torrent™ sequencers, remote monitoring must be provided using Axeda® Remote System Monitoring software enabled, and able to reverse ssh into the boxes. This requirement means that the Ion sequencers and Torrent Servers be connected to the Internet with outbound connections that are permitted on the following ports:

Port	Required	Use
22	Yes	Start reverse SSH tunnel for remote troubleshooting
80	Yes	Download updates from **http://updates.iontorrent.com** and **http://us.archive.ubuntu.com**
123	Yes	(UDP) NTP access to the Internet, incoming and outgoing.
443	Yes	Enable sending of basic status information to the remote monitoring server. The IonReporterUploader plugin also requires port 443 to transfer data to Ion Reporter™ Software.
5432	No	Remote access to PostgreSQL database.

Data automatically collected by the RSM agents

Field names, data types, and examples of the data being collected are described in the following tables. This information is sent automatically from the Torrent Server and Ion S5™, Ion PGM™, Ion Proton™, and Ion GeneStudio™ S5 Systems back to Thermo Fisher.

Torrent Server

Event Name	Type	Sample Value
TS.Config.biosversion	String	6.00
TS.Config.configuration	String	standalone
TS.Config.hostname	String	Ion-torrent-server
TS.Config.ipaddress	String	10.45.3.246
TS.Config.mode	String	Master
TS.Config.serialnumber	String	1SMJFP1 (Dell™ service tag)
TS.Contact.IT Contact	String	email, phone
TS.Contact.Lab Contact	String	email, phone

Event Name	Type	Sample Value
TS.Experiment	String	chip type, flow count, run type, bedfile, barcode count, seq s/n
TS.GPU	String	No problems
TS.host	String	Ion-torrent-server
TS.HW.HD./results	Analog	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

Event Name	Type	Sample Value
TS.Server.ntp	String	ok/offline/error
TS.Server.RabbitMQ	String	ok/offline/error
TS.Server.RSM_Launch	String	ok/offline/error
TS.Server.tomcat	String	ok/offline/error
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

Event Name	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 Valve Board Down Stream Errors 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)
Instrument.InstrumentName	String	Stork
Instrument.Pressure	Analog	10.2 (chart)
Instrument.Temperature	Analog	27.06 (chart)
Instrument.TYPE	String	PGM1

Event Name	Type	Sample Value
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 number and names of these entries are subject to change across software releases.

Data Item Name	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 dffffe cntArry 9 0 0 9
RunData.AutoPhFinal	Analog	7.660635

Data Item Name	Type	Sample value
RunData.AutoPhInitial	Analog	6.321023
RunData.AutoPhIterations	Analog	4
RunData.AutoPhResult	String	Pass
RunData.AutoPhTotalW1Volume	Analog	1.0
RunData.ChipGain	Analog	1.066389
RunData.ChipPixelAverage	Analog	8241
RunData.ChipPixelsInRange	Analog	164698460
RunData.ChipPixelsPinnedHigh	Analog	0
RunData.ChipPixelsPinnedLow	Analog	676
RunData.ChipTemp	Analog	81.826172
RunData.CpuTemp0	Analog	53
RunData.CpuTemp1	Analog	74
RunData.efuse	String	**********L:Q6C841,W:4,J:WC2012C00086-C00272,P:16,C:PT4,F:F6,Y:4,X:0,B:3,SB:31,B:1P,N:343**********
RunData.FpgaMasterTemp	Analog	113
RunData.FpgaSlaveTemp	Analog	118.4
RunData.GpuTempC	Analog	82
RunData.LastAutoPhRealPh	Analog	766
RunData.LastAutoPhRef	Analog	745
RunData.LastAutoPhTarget	Analog	770
RunData.R1pH	Analog	7.00
RunData.R2pH	Analog	7.00
RunData.R3pH	Analog	7.00
RunData.R4pH	Analog	7.00
RunData.W1pH	Analog	8762
RunData.W2pH	Analog	7619
RunData.W3RefpH	Analog	7.45
Status.HDPctFull	Analog	0.823612

Data Item Name	Type	Sample value
Status.SsdPctFull	Analog	6.220454
System.CpuUsagePct	Analog	7
System.Date	String	2013-01-0
System.FreeMemoryKB	Analog	129951948
System.Hostname	String	d1.ite
System.IpAddress	String	10.25.3.150
System.PhysMemTotalGB	Analog	128
System.Time	String	03:42:58 PM GMT
TYPE	String	Proton1
Version.Datacollect	String	3371
Version.DiskImage	String	2015_06_04
Version.Graphics	String	80
Version.KernelRelease	String	3.13.9-ionrt1
Version.LiveView	String	2166
Version.OIA	String	5203
Version.OS	String	17
Version.Reader FPGA	String	3d400109
Version.Reader FPGA1	Analog	33400109
Version.Reader Woddr FPGA	String	3400043
Version.Reader Woddr FPGA1	String	340004b
Version.RSM	String	24
Version.Scripts	String	2.0.63
Version.S5 Release	Analog	5.2
Version.S5 Script	String	0.1.13
Version.TSLink	String	1.0.2r5
Version.Valve FPGA	String	c010

Remote access for troubleshooting

When there is a problem with the Ion sequencer or Torrent Server, the Axeda™ RMS agent allows Thermo Fisher Scientific support personnel to remotely:

- Collect log files from the system for review.
- Restart the device.
- Upgrade software.
- Provide a remote login connection to the device for further diagnostic work.

When a problem with an Ion S5™, Ion PGM™, Ion Proton™, 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 service and support requires authorization from the technical contact to initialize a remote connection. Only after getting authorization does Thermo Fisher Scientific personnel proceed with remote troubleshooting. After the problem is resolved, you are notified. Additional authorization is required before starting any further remote help.

Troubleshoot Torrent Server

These troubleshooting recommendations apply to system level issues such as networking, disk space, and system load.

Check crawler and job server status

Access the Crawler and Jobs Server page:

Click **(Settings)** ▸ **Services**.

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 only runs if archiving has been configured.

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

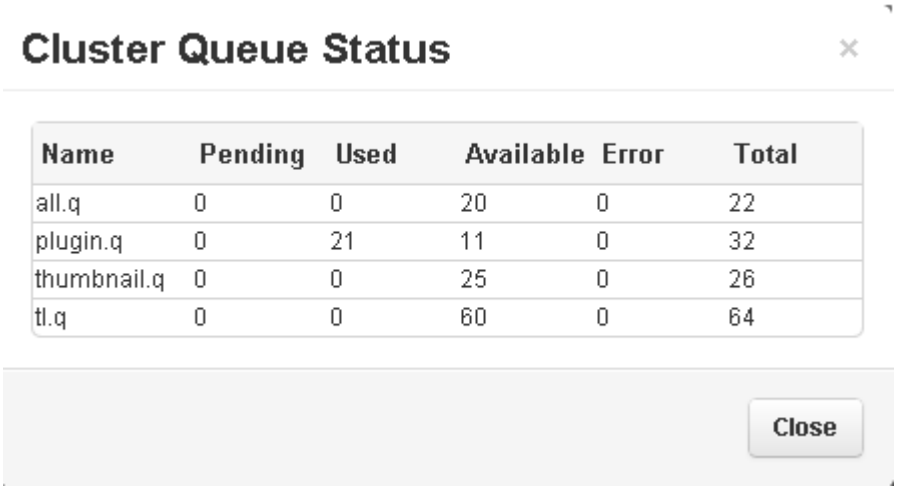
Click **(Settings)** ▸ **Services**.

The following table lists the background processes that run on Torrent Suite™ Software:

Process	Program	Startup Script	Description
Crawler	crawler.py	ionCrawler	Searches for new runs from the Ion PGM™, Ion Proton™, Ion S5™, or Ion GeneStudio™ S5 Sequencers and puts run information into the database so that they appear in the Torrent Browser **Data > Completed Runs & Reports** page.
Job Server	serve.py	ionJobServer	Sends analysis jobs to the Sun Grid Engine (SGE).
Plugin Server	ionPlugin.py	ionPlugin	Sends plugin jobs to the Sun Grid Engine (SGE).
Celeryd	manage.py	celeryd	A background job processor for Django.

Queue status

Click the **Queue Status** link in the Active Jobs section to open a table of SGE queue activity:



Restart services

Currently, there is no method to restart a process using the Torrent Browser. The easiest approach is to is to shutdown and restart the server. Before restarting the server, make sure that no Ion PGM™ or Ion Proton™ Sequencers are uploading data to the server, otherwise the file transfer is interrupted.

After restarting a process, it continues from the point where it was interrupted, and no more user interaction is needed.

1. Before restarting the server, ensure that no Ion PGM™ or Ion Proton™ Sequencers are uploading data to the server, otherwise the file transfer is interrupted.
2. Shutdown and restart the server
3. (*Optional*) Restart the processes using the scripts located in the `/etc/init.d` directory. For example, use the following command to restart the Crawler:

   ```
   user@svr:/etc/init.d$ sudo /etc/init.d/ionCrawler restart
   Stopping crawler Starting crawler pid = 26025
   ```

4. Verify that the processes are running using the `ps ax | grep py` command or the Torrent Browser UI.

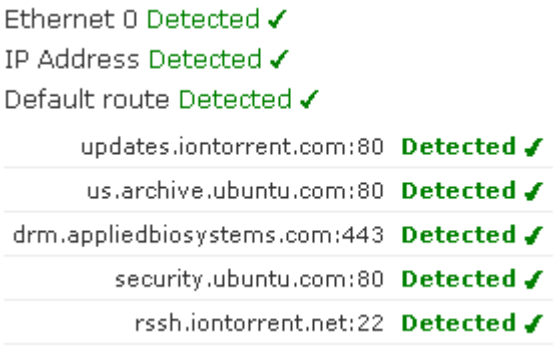
   Note: If the processes do not continue to run after being restarted, contact your Ion Torrent™ representative for assistance.

Verify network connectivity and name resolution

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

If you cannot reach the Torrent Server 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. 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 Torrent Browser performs several network checks:



5. Verify that the Torrent Server is configured correctly by reviewing the Torrent Server deployment instructions.
6. Find the IP address of the Torrent Server as described in "Verify Torrent Server IP address" on page 316.

After you are finished, click **Back to Main Site** at the top of the screen to return to the software.

Verify Torrent Server IP address

The 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 will not know what the current IP address is.

The Torrent Server has several Ethernet ports on the back. Make sure your site network is connected to the port labeled **LAN**, called **eth0** in Linux™ terminology. The Ethernet port are identified as **eth0**, **eth1**, ..., for as many ports as are available. On Torrent Server, **eth0** is the only port connected to your network and is configured by DHCP.

To determine the IP address assigned to **eth0**, login 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
```

Another useful check is the line beginning with **UP**, which indicated the interface is active and working:

```
UP BROADCAST RUNNING MULTICAST MTU:1500 Metric:1
```

If the **eth0** port is not available, it is possible the Ethernet cable is connected to a network, so you will 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.

If you are still concerned about network connectivity, you can test that 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 Browser running on the server, contact your site IT administrator.

Troubleshoot and configure the time service

The Torrent Server uses the Linux™ Network Time Protocol (NTP) program to synchronize its time with another time server. By default, the Torrent Server is configured to synchronize its time service to a trusted time service on the Internet. This requires that the network configuration permits the NTP network protocol to connect to that time service on the Internet.

The Torrent Server can also act as a time server for Ion PGM™ and Ion Proton™ Systems. However, if the server is not able to synchronize with the trusted time service, it does not act as a time server for the systems (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 Torrent Server and theIon PGM™ and Ion Proton™ Systems are not be able to synchronize time.

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™ orIon Proton™ Sequencer until you are confident that the data is present on the 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 Torrent Server.
2. (Optional) Manually transfer files by going to the **Data Management** ▸ **Re-transfer**, then select the an option for any of the runs in question. You can then safely re-transfer data.

Further investigation and problem resolution

After the root cause of a major problem is identified, the following more intrusive action may be needed:

- Replace failed hard disk drive
- Downgrade software packages
- Reinstall software
- Modify config files
- Add, modify, or delete database information

Please contact your Ion Torrent™ representative for assistance before you attempt any of these steps.

Customer support archive

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

Note: Under some circumstances, you can use the FieldSupport plugin to generate an archive for use by Customer Support. For details, see "FieldSupport plugin" on page 144.

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**. Click the **Support** tab.
3. Click **Download**.

A compressed archive is downloaded to the directory that you use to download files from the browser. This location will depend on your browser settings. You can attached this archive to an email for Customer Support.

Note: Torrent Server is not able to access the customer support server automatically. If you would like to upload files directly, contact your support representative and ask how to enable Customer Support Archive upload for your Torrent Server.

Customer Support Archive contents

The tables in this section describe the files included in a Customer Support Archive. Files for optional modules (such as recalibration) only appear if the optional module is run.

In the **top level** directory:

File	Description
alignment.log	Log of the final TMAP alignment process
< *RunName*>_< *AnalysisReportName*> .alignment.summary	Text format summary of sample alignment final results
alignment.summary	Text format summary of sample alignment final results (same as the file < *RunName*>_ < *AnalysisReportName*> .alignment.summary, but with a predictable file name)
backupPDF.pdf	PDF file of the analysis report and plugin results (similar to the output of the **Download as PDF** button on a run report)
Controller	Live View log of user activity on the sequencing instrument
debug	Log from data collect, the background data acquisition module

File	Description
DefaultTFs.conf	List of known Test Fragment sequences and their bases
drmaa_stderr_block.txt	Analysis pipelin eerror log for the block being executed by Sun Grid Engine
drmaa_stdout.txt	Log of events after primary analysis
drmaa_stdout_block.txt	Analysis pipeline output log for the block being executed by Sun Grid Engine
explog.txt	Initial run s settings needed for Torrent Browser analysis when being exported from instrument
explog_final.txt	Final run s settings needed for Torrent Browser analysis when being exported from instrument
InitLog.txt	Instrument auto pH log
InitValsW2.txt	pH log of the W2 solution
InitValsW3.txt	pH log of the W3 solution
RawInit.txt	Contains initialization data output
sysinfo.txt	Torrent Browser system software settings
TF.alignment.summary	Summary of test fragment alignment results in text file
uploadStatus	Log of metrics being uploaded to the Torrent Browser
version.txt	Torrent Suite™ software versions used for the analysis report

In the **`basecaller_results`** directory:

File	Description
basecaller.log	Log file for the basecaller analysis module
datasets_basecaller.json	A JSON-format file of the settings needed for basecaller to analyze the sample data
datasets_pipeline.json	A JSON-format file of the settings needed by the pipeline to run the basecallermodule
datasets_tf.json	A JSON-format file of the settings needed for basecaller to analyze the Test Fragments
< *RunName*>_< *AnalysisReportName*> .quality.summary	A quality summary of basecaller unaligned reads/bases after filtering and trimming

File	Description
quality.summary	Same as above, but with a predictable file name
TFStats.json	A JSON-format file ofTest Fragments results statistics

In the **basecaller_results/recalibration** directory:

File	Description
alignment.log	Log of the TMAP alignment process during base recalibration
alignmentQr_out.txt	Log file from the TMAP analysis module

In the **basecaller_results/unfiltered.trimmed** directory:

File	Description
alignment.log	Log of the TMAP alignment process based on unfiltered and trimmed reads
< *RunName*>_< *AnalysisReportName*>. alignment.summary	Text format summary of sample alignment results for unfiltered and trimmed reads
alignment.summary	Text format summary of sample alignment results for unfiltered and trimmed reads(same as above, but with a predictable file name)
datasets_basecaller.json	A JSON-format file of the settings needed for basecaller to analyze the sample data, when generating the raw BAM file
< *RunName*>_< *AnalysisReportName*> .quality.summary	The basecaller unfiltered and trimmed reads/bases quality summary
quality.summary	The basecaller unfiltered and trimmed reads/bases quality summary (same as above, but with a predictable file name)

In the **basecaller_results/unfiltered.untrimmed** directory:

File	Description
alignment.log	Log of the TMAP alignment process based on unfiltered and trimmed reads
< *RunName*>_< *AnalysisReportName*>. alignment.summary	Text format summary of sample alignment results for unfiltered and untrimmed reads
alignment.summary	Text format summary of sample alignment results for unfiltered and untrimmed reads(same as above, but with a predictable file name)

File	Description
datasets_basecaller.json	A JSON-format file of the settings needed for basecaller to analyze the sample data, when generating the raw BAM file
< *RunName*>_< *AnalysisReportName*> .quality.summary	The basecaller unfiltered and untrimmed reads/bases quality summary
quality.summary	The basecaller unfiltered and untrimmed reads/bases quality summary (same as above, but with a predictable file name)

In the **`sigpror_results`** directory:

File	Description
analysis.bfmask.stats	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	ATCG key signal measurements
avgNukeTrace_TCAG.txt	TCAG key signal measurements
bfmask.stats	Summary statistics of wells in the bead find stage
processParameters.txt	Parameter settings for analysis signal processing
separator.bftraces.txt	Matrix data to separate between live wells and empty wells during bead find phase
separator.trace.txt	Matrix data to separate between live wells and empty wells
sigproc.log	Log file for the analysis module

In the **`sigpror_results/dcOffset`** directory:

File	Description
dcOffset.txt	background model parameter values of dcOffset

In the **`sigpror_results/NucStep`** directory:

The files in this folder contain background model parameter values based on the location of the well in the chip.

File
NucStep_frametime.txt
NucStep_inlet_head.txt
NucStep_inlet_empty.txt
NucStep_inlet_empty_sd.txt
NucStep_inlet_step.txt
NucStep_middle_head.txt
NucStep_ middle_empty.txt
NucStep_ middle_empty_sd.txt
NucStep_ middle_step.txt
NucStep_outlet_head.txt
NucStep_ outlet_empty.txt
NucStep_outlet_empty_sd.txt
NucStep_outlet_step.txt

View system support diagnostics

System diagnostics information can help in troubleshooting network, disk space, and system status problems.

To access system diagnostics information, click **(Settings)** ▸ **About**:

Scroll down to the **More Information and Assistance** section and click **System Support Diagnostics**:

The diagnostics page has Network, System, and Data sections. A small section of each is shown here:

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

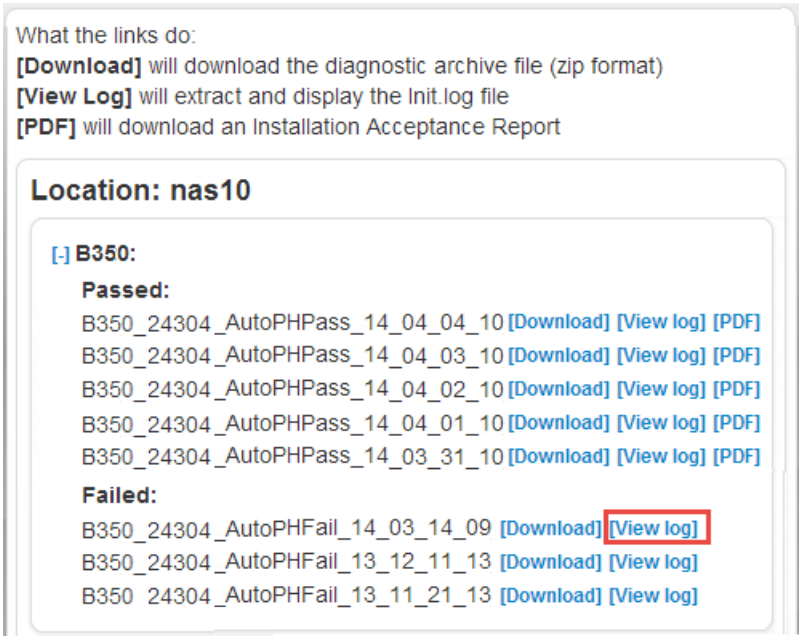
Use **Instrument Diagnostics** to investigate chip and sequencing instrument problems, such as pH levels.

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:

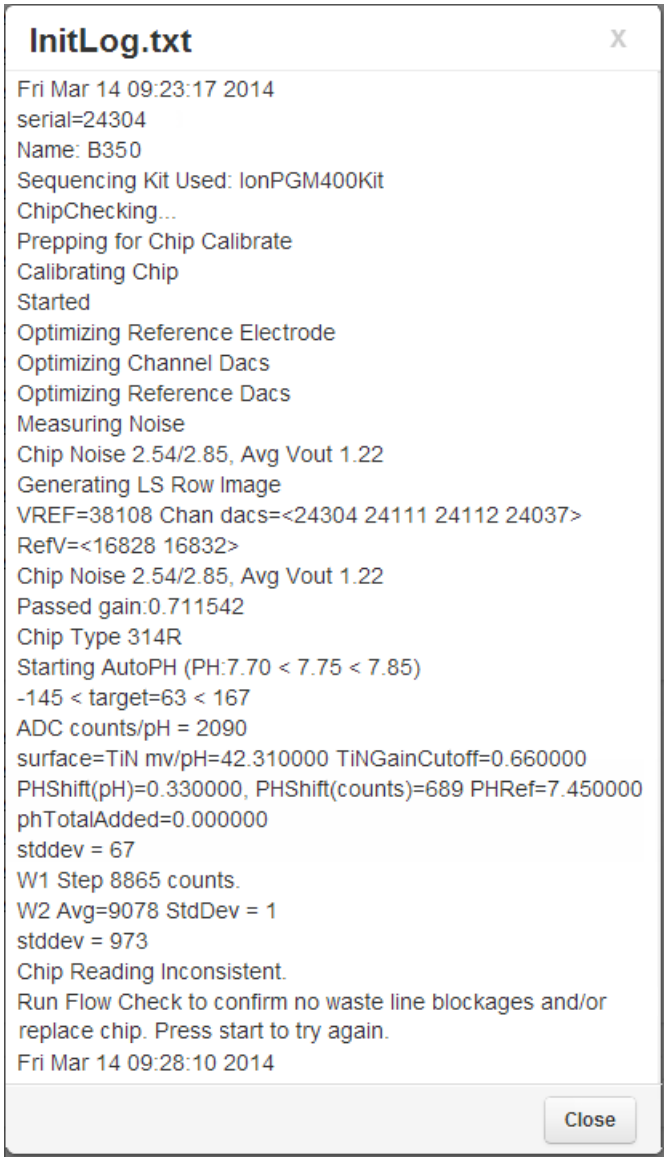
   The **Instrument Diagnostics** page lists the sequencing instruments that are associated with each of your results partitions. Passed and failed analysis runs are shown for each instrument.

   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:



Administration with command-line utilities

Monitor disk space

Use the following procedure to monitor disk space if the Torrent Browser is not available, or you want to use a command-line utility:

1. Log into the 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 Torrent™ data are stored.

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

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

You can also monitor disk space through the Torrent Browser. For details, see "View disk usage parameters" on page 272.

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 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.

Screen descriptions

User and account settings screen

How to...	Learn more about...
"Approve or reject a new user account" on page 292 "Link your Torrent Server account to AmpliSeq.com" on page 51	

Analysis Parameters screen

How to...	Learn more about...
"Create and select an analysis parameter set" on page 348	"The Command Line Args (Advanced) tab" on page 349

Configure screen

How to...	Learn more about...
"Add customer support contacts" on page 298 "Change the displayed server name" on page 299 "Update Torrent Suite™ Software" on page 286 "Manage Torrent Suite™ Software user accounts" on page 290 "Modify a user" on page 293 "Delete multiple user accounts" on page 294 "Delete a single user account" on page 294 "Archive or delete data automatically" on page 269 "Lock current Torrent Suite™ Software version" on page 288 "Change the time zone for the Ion Torrent™ Server" on page 299 "Create a new administrator account to change a password" on page 296	"Torrent Suite™ Software updates" on page 286

Ion Reporter configuration screen

How to...	Learn more about...
"Set up an account for IonReporterUploader plugin" on page 173	"IonReporterUploader plugin configuration" on page 180
"Automatically transfer Torrent Suite™ Software output to Ion Reporter™ Software" on page 175	"IonReporterUploader plugin file transfer progress" on page 182
"Manage the Ion Reporter™ Software workflow list" on page 178	Ion Reporter™ account setup troubleshooting in "Ion Reporter account is not configured" on page 175
"View IonReporterUploader plugin status details" on page 182	"Torrent Suite™ Software output and Ion Reporter™ Software analysis phases" on page 186
"Delete IonReporterUploader plugin report files" on page 183	
"Tune Ion Reporter™ Software speed parameters" on page 183	

Plugins screen

How to...	Learn more about...
"Install or upgrade plugins" on page 111	"Manage plugins for data analysis" on page 111
"Enable an installed plugin" on page 112	"Pre-installed plugins" on page 120
"Configure a plugin to run by default after every run" on page 114	"Plugin configuration" on page 113
"Run a plugin manually from the sequencing run report" on page 115	
"Uninstall a plugin" on page 112	
"Rescan a plugin" on page 119	
"Configure plugins globally" on page 113	

Product and plugin updates

How to...	Learn more about...
"Check for off-cycle updates" on page 288	"Torrent Suite™ Software updates" on page 286
"Enable off-cycle product updates" on page 289	
"Update off-cycle release plugins" on page 290	
"Install off-cycle bundles without Internet Access" on page 290	

References Sequences screen

How to...	Learn more about...
"Import a preloaded reference sequence" on page 230	"Reference sequences" on page 227
"Import a custom reference sequence" on page 231	"hg19 reference" on page 228
"View a reference sequence file" on page 233	"GRCh38 reference" on page 229
"Edit reference sequence file properties" on page 235	"Obsolete Reference Sequences" on page 236
"Permanently delete a reference sequence file" on page 236	"Target Regions and Hotspots" on page 237
"View and manage target regions files" on page 238	"Guidelines for using target regions and hotspots files" on page 237
"View and manage hotspots files" on page 239	"Hotspots files format" on page 250
"Upload a target regions file" on page 240	"Test fragments" on page 260
"Upload a hotspots file" on page 242	"Barcodes and barcode sets" on page 262
"View test fragment details" on page 261	"Upload history" on page 266
"Add a custom test fragment" on page 261	
"Edit or delete a custom test fragment" on page 262	
"View a DNA barcode sequence" on page 263	
"Download a DNA barcode set CSV file" on page 263	
"Add a custom DNA barcode set" on page 264	
"Delete a custom DNA barcode set" on page 266	

Services screen

How to...	Learn more about...
"Check crawler and job server status" on page 314	"Alternate checks" on page 330
"Restart services" on page 315	

Planned Runs screen

How to...	Learn more about...
"Manage Planned Runs" on page 65	"Plan and execute an instrument run" on page 32
"Manually change an Ion Chef™ Instrument run status" on page 67	"Planned Run templates" on page 32
"Transfer a Planned Run to another Torrent Server" on page 70	"Planned Run status" on page 67
"Execute a Planned Run on a sequencer" on page 68	

Samples screen

How to...	Learn more about...
"Create a Sample Set manually" on page 17	"Sample information" on page 20
"Import samples with a CSV file" on page 19	"Sample attributes" on page 22
"Create an Ion AmpliSeq™ on Ion Chef™ Sample Set" on page 29	"Plan by Sample Set" on page 55
"View Ion AmpliSeq™ library preparation on an Ion Chef™ System run" on page 26	
"View Sample Set attributes" on page 22	
"Add a sample attribute" on page 23	
"Manage sample attributes" on page 24	
"Find a Sample Set" on page 26	
"Edit a Sample Set" on page 27	
"Delete a Sample Set" on page 28	
"Sort Sample Sets" on page 27	
"Create a Planned Run with Sample Sets" on page 56	

Templates screen

How to...	Learn more about...
"Create a Planned Run from a template" on page 35	"Plan and execute an instrument run" on page 32
"Create a custom Planned Run template" on page 34	"Steps in the workflow bar" on page 37
"Export a Planned Run template" on page 48	
"Import a Planned Run template" on page 48	
"Import panel files and parameters from AmpliSeq.com" on page 50	
"Search for a template" on page 54	
"Save a Planned Run template to Favorites" on page 55	
"Create a Planned Run for mixed samples with a template" on page 59	
"Create multiple Planned Runs" on page 61	
"Transfer a Planned Run to another Torrent Server" on page 70	

Runs in Progress screen

How to...	Learn more about...
"Monitor the sequencing run" on page 72	Sequencing run metrics in "Monitor the sequencing run" on page 72
"Review the Planned Run settings" on page 75	"View data for runs in progress" on page 73
	"Auto Refresh the Monitor tab" on page 74

Ion Chef screen

How to...	Learn more about...
"Monitor an Ion Chef™ run" on page 73	
"Auto Refresh the Monitor tab" on page 74	

Completed Runs & Reports screen

How to...	Learn more about...
"Manage Completed Runs and Reports" on page 76	"Test fragments" on page 260
"Search for a Run report" on page 76	"Create an Ion AmpliSeq™ on Ion Chef™ Sample Set" on page 29
"Change the default alignment reference" on page 78	
"Reanalyze a run" on page 76	
"Add or change barcoding for a completed run report" on page 80	
"Reanalyze a run" on page 76	
"Edit a Run report" on page 79	
"View plugin run status" on page 116	
"Set the Completed Runs & Reports page to automatically refresh" on page 81	
"Failed status for a run" on page 81	

Run Report

How to...	Learn more about...
"Open a run report" on page 95	"Quality following alignment" on page 93
"Review unaligned reads" on page 97	"Aligned read length calculation" on page 94
"Review aligned reads" on page 100	"Alignment" on page 94
"Download output files" on page 102	"Reads used for alignment" on page 95
"View and download barcode reports" on page 102	"Predicted quality (Q20)" on page 95
"View software versions used in a run" on page 110	"Unaligned reads metrics" on page 97
"Download run report PDF" on page 103	"Aligned reads metrics" on page 100
"View consumables used in a completed run" on page 105	"Output files" on page 101
"View the summary for an Ion Chef™ run" on page 105	"Details about completed runs" on page 104
"View analysis details for a completed run" on page 108	
"Get technical support files" on page 108	

Projects screen

How to...	Learn more about...
"Add a project to a Planned Run" on page 84 "Add selected results to another project" on page 84 "Download a CSV file of metrics" on page 88 "Remove result sets from project" on page 92 "Search for projects by name" on page 83 "Organize run results with projects" on page 82 "Combine aligned reads from multiple run reports" on page 87 "Compare reports of runs in a project" on page 85 "Manage data for result sets in projects" on page 87	

Data Management screen

How to...	Learn more about...
"Archive or delete data automatically" on page 269	"Ion instrument data types" on page 268
"Import data for data transfers or restoration" on page 271	"Data management" on page 268
"View disk usage parameters" on page 272	"Dataflow file sizes" on page 345
"View category statistics" on page 272	
"View active data management jobs" on page 273	
"Search for run reports with disk usage status" on page 274	
"Keep run report data" on page 275	
"Manually export run data" on page 275	
"Manually archive run data" on page 276	
"Manually delete run data" on page 277	
"View the data management actions log" on page 277	
"Increase file storage and available disk space" on page 278	
"Connect to a Torrent Storage™ NAS device" on page 281	
"Monitor the Torrent Storage™ NAS device" on page 284	

Reference

Per-Base Quality Score System

The Ion Torrent™ per-base quality score system uses a Phred-like method to predict the probability of correct base call. The prediction is based on the quality of the base incorporation signal that was used for generating the base calls. The sequencers' quality score system uses a set of 6 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 customer configuration. The lookup table is re-trained 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 software uses the following six 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.
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 located 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 Torrent Server. The Torrent Server supports separate phred tables for each type of chip (Ion 314™ Chip, Ion 316™ Chip, Ion 318™ Chip, and Ion PI™ Chip), named phredTable.314, phredTable.316, phredTable.318 , and phredTable.p1.1.17 respectively.

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.

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.

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 purpose of this section is to highlight specific Ion Torrent™ conventions and the meaning of custom tags.

Ion Torrent™ conventions:

- Run ID: Every TS analysis gets a run ID, a 5-character string consisting of upper case letters and numbers, assigned. A reanalysis of a specific run will get a different run ID assigned. 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, 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, 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 SAM Recorder Tags

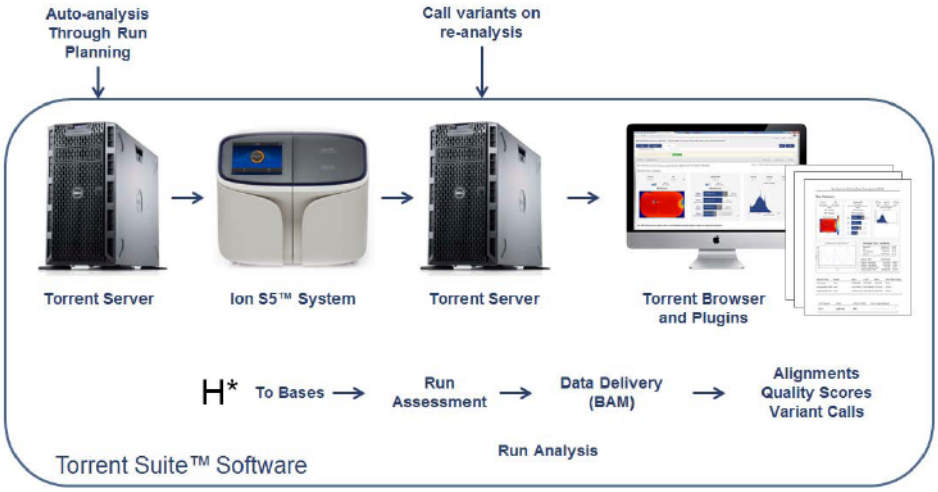
Ion Torrent uses a collection of custom tags to store sequencing and alignment information useful for downstream processing. In general, custom BAM tags starting with Z or Y are written by the BaseCaller and BAM tags starting with X stem from TMAP. As a consequence, tags starting with Z or Y are present both in aligned and unaligned BAM files whereas tags staring with X appear only in aligned BAM files.

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	Target Length, i.e., number of reference bases spanned by the alignment.
XS	i	The alignment score of next-best sub-optimal mapping.
ZA	i	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). Only present if a 3' adapter was found.
ZB	i	Number of overlapping adapter bases. (Only present if a 3' adapter was found.)
ZC	B:i	A vector of the following four values (only present if a 3' adapter was found): Field 1: The zero-based flow during which the first base of the adapter was incorporated (same as ZG) Field 2: The zero-based flow corresponding to the last insert base Field 3: Length of the last insert homopolymer Field 4: Zero-based index of adapter type found.
ZF	i	The zero-indexed flow position corresponding to the first template base after 5' trimmed region.
ZG	i	The zero-based flow during which the first base of the adapter was incorporated. (Only present if a 3' adapter was found.)
ZM	B:s	Normalized signals, which include phasing effects. Stored as floor(256*value)

Tag	Type	Description
ZP	B:f	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. Only written if a tag was trimmed.
YT	Z	The trimmed 3' unique molecular tag sequence. Only written if a tag was trimmed.
ZE	Z	The 5' trimmed sequence removed by the `extra-trim-left` command. Only written if a sequence was trimmed.
YE	Z	The 3' trimmed sequence removed by the `extra-trim-right` command. Only written if a sequence was trimmed.
ZK	Z	The trimmed 3' portion of read group specific identifiers that can vary within a read group. Only written if a tag was trimmed.
YK	Z	The trimmed 3' portion of read group specific identifiers that can vary within a read group. Only written if a sequence was trimmed.

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 Torrent Server for analysis and reporting.



The following tables show a high-level view of the dataflow. File sizes for Torrent data depend on the Torrent Suite™ Software version, chip type, and kit type.

Torrent Suite™ Software 5.10 and 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. File sizes for 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	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

* Data compression enabled.

Torrent Suite™ Software 5.10 and 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. File sizes for 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	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

Torrent Suite™ Software4.x and 400 bp kit on the Ion PGM™ Sequencer

The following table shows a high-level view of the dataflow. File sizes for 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 signal, and the number of library reads available. Your file sizes may be different. An unmapped BAM file format is used in pipeline steps before alignment.

Torrent Suite™ Software4.x and 200 bp kit on the Ion Proton™ Sequencer

The following table shows a high-level view of the dataflow. File sizes for Torrent data depend on the Torrent Suite™ Software version, chip type, and kit type. The following table shows a high-level view of the dataflow. File sizes for 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 signal, and the number of library reads available. Your file sizes may be different. An unmapped BAM file format is used in pipeline steps before alignment.

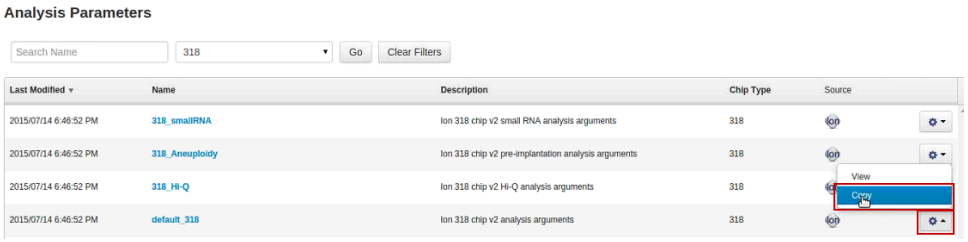
Create and select an analysis parameter set

You can copy an existing analysis parameter set, and then customize the settings and save it as a new parameter set.

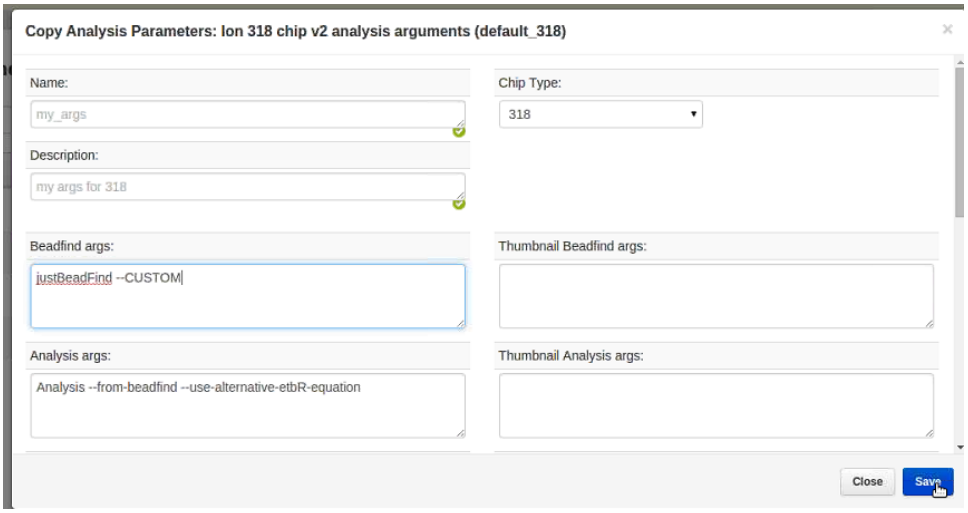
1. Click ⚙ **(Settings)** ▸ **Analysis Parameters**:
   In the **Analysis Parameters** screen, factory parameters are denoted by "Ion Torrent" in the **Source** column.

2. To filter the parameter sets by chip type, select your chip type from the **All Chips** menu.

3. Identify the parameter set that you want to copy, then click ⚙ **(Actions)** ▸ **Copy** in the table row for that set.



4. In the **Copy Analysis Parameters** dialog, enter a parameter name and description, and make any changes. Click **Save**.



Your new analysis parameter set is available in the **Analysis Parameters** table. The **Source** column lists the name of the user that created it.

Note: Click ⚙ **(Actions)** ▸ **View** in the row of the analysis parameter set to view the details for that parameter set in the list.

5. 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 of the screen, click **Details+**, and then select **Custom** and select the parameter set from the dropdown menu.  **Note:** 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 76.

Find the TMAP command for a specific analysis

See "TMAP examples" on page 361 for steps to open the run report log and search for the TMAP command. (The analysis must be completed before you can find the command.)

The Command Line Args (Advanced) tab

An example Advanced Options page is shown here:

Setting	Description
Beadfind args	Beadfind module command line arguments. Should not be modified unless instructed by Ion Torrent™ Technical Support.
Analysis args	Analysis command line arguments. Should not be modified unless instructed by Ion Torrent™ Technical Support.
Pre Basecaller args for calibration	BaseCaller command line arguments. See Basecaller arguments for information on `--barcode-mode`, `--barcode-cutoff`, and `--barcode-filter`. Other Basecaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support. This field is used only if a Base Calibration Mode other than 'No Calibration' is used.
Recalibration Args	Recalibration command line arguments.
Basecaller args	BaseCaller command line arguments. See Basecaller arguments for information on `--barcode-mode`, `--barcode-cutoff`, and `--barcode-filter`. Other Basecaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support.
Alignment Args	Arguments for the TMAP aligner. (Replaces the TMAP Args field that appears in previous releases.)

Overview of BaseCaller and Barcode Classification

This page discusses BaseCaller operations in general and issues around BaseCaller parameters, barcode classification, and filtering and trimming.

The settings of BaseCaller parameters control barcode classification as well as filtering and trimming.

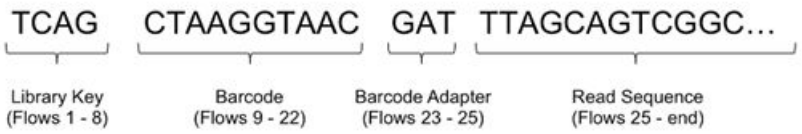
About barcodes

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 samplesduring data analysis.

The use of barcodes allows multiple samples to be sequenced together on one chip during a sequencing run, and still have the run's read data be analyzed separately afterward as distinct samples.

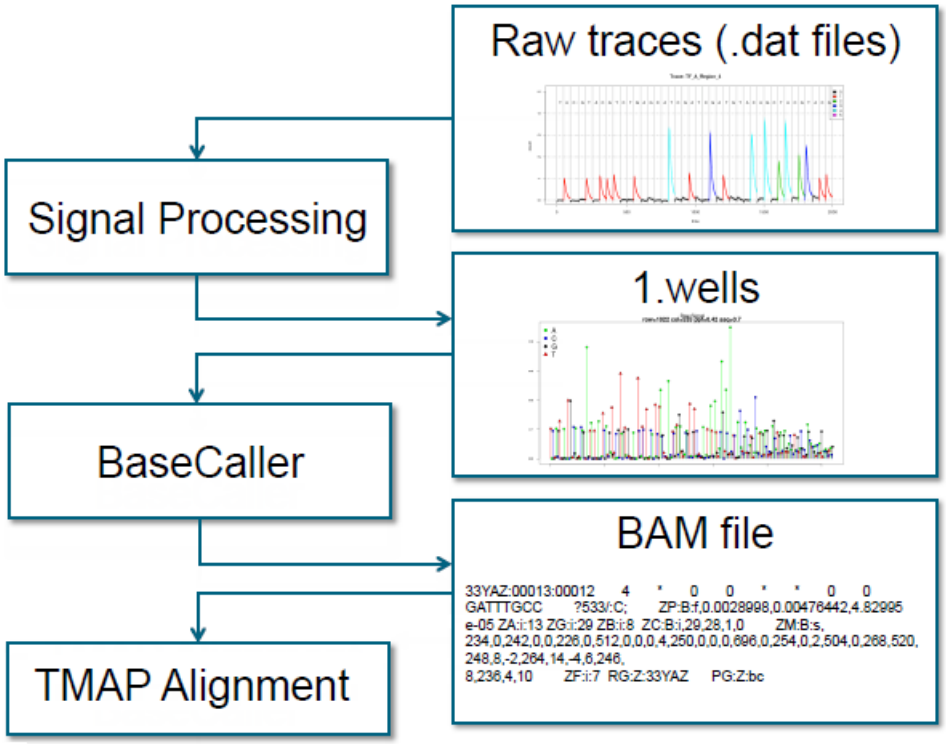
This diagram shows the placement of the barcode sequence, as well as the library key and adapters, with the read sequence (which is labeled "Template Bases"). The key is on the 5' end.

This example shows the location of the barcode sequence in both base space and flow space, using barcode IonPress_001 as an example:



Analysis pipeline overview

The beginning steps of the Torrent Suite™ Software analysis pipeline are shown below:



Steps:

1. The sequencing instrument generates DAT files of electrical signals' raw traces.
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 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 TMAP 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

Thesignal processing step marks these problematic reads but does not remove them.

Overview of BaseCaller 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.
- Filtered out reads *do not*appear in the BAM file. The BaseCaller keeps counts of these reads but there is no record of specific reads that are filtered out.

Notes on read trimming:

- Removes certain bases from the read for quality reasons.
- The read appears in the BAM file.
- The removed bases do not appear in theBAM file.

These are the steps performed in the BaseCaller:

1. Remove low-quality reads that were marked during the signal processing step.
2. Do base calling:
    1. From the signal values, create the sequence of bases.
    2. Estimate the base quality value for each base.
3. Do barcode classification:
    1. Assign each read to a barcode.
    2. Trim the barcode sequence away if `--trim-barcodes=on` is specified. (The default is 'on').
4. Trim 5' unique molecular tag (only done if `--trim-barcodes=on`).
5. Trim extra bases at the 5' end. Controlled by `--extra-trim-left` (default is 0, meaning no extra trimming).
6. Filter out reads that are too short. Controlled by `--min-read-length` and `--trim-min-read-len`.
7. Filter out reads that do not have the correct library key. Can be turned off by `--keypass-filter`.
    1. Trim 3′ unique molecular tag (only done if P1 adapter was found).
    2. Trim extra bases on the 3′ end. Controlled by --extra-trim-right (default is 0, meaning no extra trimming. Only done if P1 adapter was found).
8. Trim the P1 adapter (at the 3' end).
9. Perform quality trimming. Affect ed by `--trim-qual-window-size` and `--trim-qual-cutoff`.

Notes about quality trimming:

- The purpose of quality trimming is to identify where quality issues begin at the end of a read. We try to identify when bases fall below a quality threshold and trim both 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 the by parameter `--trim-qual-cutoff`, default 16), then we trim 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

Troubleshooting Barcode Classification Issues

Barcode classification metrics are available in the file `basecaller_results/datasets_basecaller.json` in the Torrent Suite™ Software analysis directory.

This file contains information about all barcodes, no matter whether they appear in the run report or are filtered out. This information describes the numbers of barcodes that would be included or discarded if you reanalyze with certain changed BaseCaller settings.

A sample of this file is shown here. Later examples in this page use this file:

```
"IEXL3.IonXpress_033": { "Q20_bases": 98859279,
"barcode_adapter": "GAT", "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_hist":
[ 907546, 50122, 10793, 4498, 5342 ], "barcode_errors_hist":
[ 949782, 24584, 3935 ], "barcode_match_filtered": 162,
"barcode_name": "IonXpress_033", "barcode_sequence":
"TTCTCATTGAAC", "description": "1T 058a0112 Lib6457 0bp lr2
lr226b04", "filtered": false, "index": 33, "library": "hg19/
IonXpress_033", "platform_unit": "PGM/318/IonXpress_033",
"read_count": 978301, "recalibrate": true, "sample": "None",
"total_bases": 109292583 },
```

Explanation of fields in the BaseCaller JSON file

Read count

The read_count field shows how many reads were assigned to this barcode.

```
"read_count": 978301,
```

Filtered

The `filtered` field is `true` if this barcode is filtered out and `false` if the barcode appears on the run report.

```
? "filtered": false,
```

Barcode errors histogram

The barcode errors histogram shows the number of reads with difference levels of basecalling errors in this barcode:

- **First field**: 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).
- **Second field**: The number ofreads that have one basecalling error(24584 in this example).
- **Third field**: The number of reads that have two basecalling errors(3935in 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 will be 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 distance histogram

The barcode distance histogram shows, *in signal space*, the number of reads at various squared residual distances between the predictedsignal 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 asquared 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, etc.

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 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,
```

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],
```

BaseCaller arguments

This section describes select arguments used with the BaseCaller module.

BaseCaller Parameters

This page describes BaseCaller parameters that are available when you reanalyze a completed run.

Note: The default BaseCaller parameters are tuned for Ion Torrent™ data. In most cases, you do not need to modify these settings. Modifying these parameters is recommended for advanced users only.

However, if you use a custom barcode set, please see the cautions and requirements in Design Custom Barcodes. Correct parameter settings require knowledge of your barcode's distances in signal space. The BaseCaller defaults are optimized for the IonXpress 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 please 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 beinganalyzed 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

This table lists the more common BaseCaller parameters relating to barcode classification. (All parameters listed in this table are barcode classification parameters.)

Parameter	Default	Description
--barcode-cutoff	1.0 (Float)	Maximum distance allowed in barcode matches. A threshold that sets the stringency for barcode matches. Lower values require more exact matches when assigning reads to barcodes. Higher values allow less exact matches. Reads that have a distance greater than this value are counted as barcode no-matches.
--barcode-mode	2 (Integer)	Allowed values: 1, 2 • **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.) **2**: 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.) **Note:** `--barcode-mode 0` is no longer supported.
--barcode-separation	2.5 (Float)	This setting controls how much ambiguity in barcode assignment you want to tolerate, by investigating the distances to the 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. **Note:** `--barcode-separation` has no effect when `--barcode-mode` is set to 1.

Parameter	Default	Description
--barcode-filter-postpone	1	Allowed values: 0, 1, 2 • **0**: Keeps the 4.0 behavior: b arcode filtering is done independently on each block. This is the default for all Ion PGM™ analyses and also for Ion Proton™ thumbnail (which only consist of a single block) processing and base calibration training stage processing. • **1**: BaseCaller does barcode pre-filtering at a 10x lower frequency threshold (10 times more lenient). B arcode filtering is done on the chip's full information as a whole, after the 96 blocks are merged into one. This is the default for Ion Proton™ full-chip (not thumbnail) analyses. • **2**: The BaseCaller does not do any barcode pre-filtering. All barcode classification happens after the 96 blocks are merged into one. (The setting "2" is slower than the setting "1". "2" creates more files and involves more processing than "1".) **Note:** We do not recommend that you change this parameter. Instead accept the pipeline defaults (which are different for Ion PGM™ and Ion Proton™ analyses).
--barcode-filter	0.01 (Float)	Barcode frequency threshold to be reported in the UI. The relative frequency of a barcode is the number of assigned reads divided by number of reads assigned to the most frequent barcode. Set to 0.0 to turn this filter off. The setting 0.0 causes all barcodes in the barcode set to be reported in the UI, including barcodes with no or very few reads, provided that the barcode group has at least `--barcode-filter-minreads` number of reads. (Typically barcodes with no or very few reads are not relevant to your analysis and should be filtered out.)
--barcode-filter-minreads	20 (INT)	Threshold for the minimum number of reads in a barcode group, for that group to be reported in the UI.
--trim-barcodes	on	Trim barcode and barcode adapter. If off, disables all other 5' trimming.
--barcode-adapter-check	0.15	Validate barcode adapter sequence. The parameter given is the maximum allowed squared residual per flow. This feature reduces barcode set cross contamination, e.g., between the IonXpress and IonCode barcode sets. (0=off)

The cutoff setting

Notes about the `--barcode-cutoff` parameter with `--barcode-mode` 1:

- 0 is the most restrictive setting. `--barcode-cutoff` 0 allows only reads that perfectly match a barcode in base space.
- The setting 0 works with any barcode set (both Ion Torrent™ sets and custom barcode sets).
- Do not set `--barcode-cutoff` greater than 2 with the IonXpress 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:

$$\text{barcode-cutoff} \leq (d_{min} - 1) / 2$$

T he minimum distance for the IonXpress barcode set is 5. Then the maximum recommended value for `--barcode-cutoff` is 2 for analyses that use the IonXpress barcode set.

The 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 right 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 barcodeassignment (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

This table lists the public BaseCaller parameters that are available for you to modify. However, please note that the defaults for these parameters are optimized for most scenarios and in most cases the default settings are recommended.

Parameter	Default	Description
-d, or --disable-all-filters	off	When on, disables all filtering and trimming and overrides other filtering and trimming settings.
-k, or --keypass-filter	on	When on, filters out reads that do not both produce a signal and match the library key (or the test fragment key).
--min-read-length	25 (Int)	F ilters out reads less than this minimum read length. This filter screens out poor reads early on to avoid wasting processing time on them. See also `--trim-min-read-len`, which sets the minimum length threshold that is applied after trimming.
--prefix-mol-tag	Empty	Base structure of 5' unique molecular tag (ACGTN bases) to be trimmed after the barcode adapter.
--suffix-mol-tag	Empty	Base structure of 5' unique molecular tag (ACGTN bases) to be trimmed before P1 adapter.
--extra-trim-left	0 (Int)	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 P1 adapter was found.
--trim-adapter-cutoff	16 (Float)	A score cutoff value. Smaller values correspond to more stringent adapter search and larger values to less stringent adapter search. Set to 0 to turn off.
--trim-adapter-min-match	6 (Int)	The minimum number of P1 adapter bases required in order to trim the P1 adapter.

Parameter	Default	Description
--trim-qual-window-size	30 (Int)	Window size for quality trimming.
--trim-qual-cutoff	16 (Float)	Cutoff for quality trimming. Set to 100 to turn off. When set to 100, no reads are filtered out due to this parameter.
--trim-min-read-len	25 (Int)	Filters out any readsthat fall below this minimum read length after anytrimming step. By default it is initialized with the value of 'min-read-length'.

BaseCaller filters

The BaseCaller module and its parameter settings control these types of filtering:

- Keypass
- Quality trimming
- Adapter trimming

Examples of BaseCaller parameters usage

With these examples:

- Do not remove the string "BaseCaller" from the Basecaller Args field.
- Do not change BaseCaller parameters other than those listed in the basic table or the public table (unless specifically directed to do so by Ion).

TMAP Modules

The Torrent Mapping Alignment Program (TMAP) is a sequence alignment software program optimized specifically for Ion Torrent™ data. TMAP generally operates in two phases: initial mapping, when the read sequences are roughly located in the reference genome, and alignment refinement, when each particular position of the read is aligned to the corresponding position in the reference. TMAP is capable of running 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. TMPA can use several mapping algorithms, each with its own best application. TMAP's current default is map4.

The alignment refinement phase includes initial alignment using Smith-Waterman or Needleman-Wunch algorithms, and a number of optional alignment refinement stages that are designed to compensate for specific systematic biases of the sequencing process. Thus, the reads can be re-aligned for better homopolymer alignment (`--context` option); the portions with likely phasing errors can be realigned with low indel scores (`--do realign` option); the long indels at the edges of amplicons can be salvaged with `--end-repair` option); the tandem repeats can be clipped from the read 3' tail (`--do-repeat-clip` option); the alignment can be clipped from the read signals rather than called bases (`--final-flowspace` option).

When you reanalyze a run, you can optionally change both the TMAP module (`map1`, `map2`, `map3`, `map4`, or `mapvsw`) and also change the module's parameters.

Note: The default TMAP parameters are tuned for Ion data. In most cases, you do not need to modify these settings. Modifying these parameters is recommended for advanced users.

Mapping modules

This table lists the mapping alternatives supported by TMAP. The `map4` module is the default. (Other modules are not run unless specifically called, for instance on the Reanalyze page.)

Click the module name link to see the options supported for that module.

Module	Description
map1	BWA-short reads mapping • Very fast at finding perfect matches • Very slow at finding a set of matches with up to two mismatches
map2	BWA-long / BWASW reads mapping
map3	Simplified SSAHA, based on a k-mer lookup table
map4	Based on the BWA fastmap routine Searches for the maximum exact matches between the reads and reference
Mapvsw	A vectorized implementation of Smith-Waterman • A single mapping strategy that is twice as fast as the other modules • Modified to improve specificity
Mapall	A command to quickly map short sequences to a reference genome.

Find the TMAP command for a specific analysis

See “TMAP examples“ on page 361 for steps to open the run report log and search for the TMAP command. (The analysis must be completed before you can find the command.)

TMAP examples

This example is the current default setting. Only the `map4` module is used.

```
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
```

This example is the previous TMAP default. This example uses the modules `map1`, `map2`, and `map3`, 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]	FASTAreference 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	INT	0	Sets the starting virtual file offsets that limit the range of BAM reads to process
-A	--score-match	INT	1	Score for a match
-M	--pen-mismatch	INT	3	Mismatch penalty
-O	--pen-gap-open	INT	5	Indel start penalty
-E	--pen-gap-extension	INT	2	Indel extension penalty
-G	--pen-gap-long	INT	-1	Long indelpenalty

Global pairing options

Option	alternate option	Type	Default	Description
-Q	--pairing	INT	0	The insert pairing: • 0 Do not perform pairing • 1 Mate pairs (-S 0 -P 1) • 2 Paired end (-S 1 -P 0)
		INT	-1	
		INT	-1	
		FLOAT	-1.0	
		FLOAT	-1.0	

TMAP map1 Options

This page describes the parameters for the TMAP `map1` module. The `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

Note: The `map1` module is not the current default for TMAP.

Options supported with the TMAP `map1` module (all are optional):

--seed-length	INT	32	The k-mer length to seed CALs (-1 to disable)
--seed-max-diff	INT	2	The maximum number of edits in the seed
--seed2-length	INT	48	The secondary seed length (-1 to disable)
--max-diff	NUM	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

--seed-length	**INT**	**32**	**The k-mer length to seed CALs (-1 to disable)**
--max-mismatches	NUM	3	The maximum number of or (read length) fraction of mismatches
--max-gap-opens	NUM	1	The maximum number of or (read length) fraction of indel starts
--max-gap-extensions	NUM	6	The maximum number of or (read length) fraction of indel extensions
--max-cals-deletion	INT	10	The maximum number of CALs to extend a deletion
--indel-ends-bound	INT	5	The number of bps from the end of the read
--max-best-cals	INT	32	Optimal CALs have been found
--max-nodes	INT	2000000	The maximum number of alignment nodes
--min-seq-length	INT	-1	The minimum sequence length to examine (-1 to disable)
--max-seq-length	INT	-1	The maximum sequence length to examine (-1 to disable)
Option	Type	Default	Description

TMAP map2 Options

This page describes the parameters for the TMAP `map2` module. The `map2` module implements BWA-long / BWASW reads mapping.

Note: The `map2` module is not the current default for TMAP.

Options supported with the TMAP `map2` module (all are optional):

Option	Type	Default	Description
--max-seed-hits	INT	1024	The maximum number of hits returned by a seed
--length-coef	FLOAT	5.5	The coefficient of length-threshold adjustment
--max-seed-intv	INT	6	The maximum seeding interval size
--z-best	INT	1	The maximum number of top-scoring nodes to keep on each iteration
--seeds-rev	INT	5	The number of seeds to trigger reverse alignment
--narrow-rmdup	INT	false	Remove duplicates for narrow SA hits
--max-chain-gap	INT	10000	The maximum gap size during chaining
--min-seq-length	INT	-1	The minimum sequence length to examine (-1 to disable)
--max-seq-length	INT	-1	The maximum sequence length to examine (-1 to disable)

TMAP map3 Options

This page describes the parameters for the TMAP `map3` module. The `map3` module implements a simplified SSAHA, based on a k-mer lookup table.

Note: The `map3` module is not the current default for TMAP.

Options supported with the TMAP `map3` module (all are optional):

Option	Type	Default	Description
--seed-length	INT	-1	The k-mer length to seed CALs (-1 to disable)
--max-seed-hits	INT	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	INT	8	The number of bases to increase the seed for each seed increase iteration (-1 to disable)
--hp-diff	INT	0	The single homopolymer error difference for enumeration
--fwd-search	Boolean	false	Use forward search instead of a reverse search
--skip-seed-frac	FLOAT	0.2	The fraction of a seed to skip when a lookup succeeds
--min-seq-length	INT	-1	The minimum sequence length to examine (-1 to disable)
--max-seq-length	INT	-1	The maximum sequence length to examine (-1 to disable)

TMAP map4 Options

This page describes the parameters for the TMAP `map4` module. The `map4` module is based on the BWA fastmap routine and searches for the maximum exact matches between the reads and reference.

Note: The `map4` module is the current default for TMAP.

Options supported with the TMAP `map4` module (all are optional):

Option	Type	Default	Description
--seed-step	INT	8	The number of bases to increase the seed for each seed increase iteration (-1 to disable)
--hit-frac	FLOAT	0.2	The fraction of seed positions that are under the maximum
--min-seed-length	INT	-1	The minimum seed length to accept hits (-1 to disable)
--max-seed-length	INT	48	The maximum seed length to accept hits
--max-seed-length-adj-coef (-1 to disable)	FLOAT	2.0	maximum seed length adjustment coefficient (-1 to disable)
--max-iwidth	INT	20	The maximum interval size to accept a hit
--max-repr	INT	3	The maximum representative hits for repetitive hits
--rand-repr	INT	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.

Option	Type	Default	Description
--min-seq-length	INT	-1	The minimum sequence length to examine (-1 to disable)
--max-seq-length	INT	-1	The maximum sequence length to examine (-1 to disable)

TMAP mapvsw Options

This page describes the parameters for the TMAP `mapvsw` module. The `mapvsw` module implements a vectorized implementation of Smith-Waterman.

Note: The `mapvsw` module is not the current default for TMAP.

Options supported with the TMAP `mapvsw` module (all are optional):

Option	Type	Default	Description
--min-seq-length	INT	-1	The minimum sequence length to examine (-1 to disable)
--max-seq-length	INT	-1	The maximum sequence length to examine (-1 to disable)

TMAP alignment refinement

The TMAP alignment refining 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):

Option	Description
Flow space realignment	Performs alignment of the sequence of read flow signals to simulated reference flow signal. Turned on with `--final flowspace` flag.
Context-based realignment	Realigns read with reduced cost of indels within homopolymers. Turned on with `--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 `--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 (`-G` option).

Option	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 --end-repair <MM> option on TMAP command line where <MM> is the maximum allowed mismatch percentage at the alignment edge. End repair is typically used together with `--J` option (max adapter bases for soft clipping), recommended usage is `--end-repair 25-J 15`).
Bed file	The bed file specification, given with --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 calculate: 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; on by default. Turned off with `--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 `--do-repeat-clip` flag.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Glossary

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 simply the longest perfectly aligned segment. For all of these calculations the alignment is constrained to start from position 1 in the read - in other words, no 5′ clipping is permitted.
Aligned reads	The number of bases covered by reads aligned to the reference sequence.
API	Torrent Server API (Application Programming Interface) can be used to access database records on a Torrent Server. 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 / Alignment Quality Score	An 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	Binary Aligned/Mapped file - BAM file - contains aligned reads sorted by reference location. BAM file is the binary form of the SAM (Sequence Alignment/Map) file.

Barcode	There are several applications for barcodes in Torrent Suite™ Software. Libraries can be barcoded with unique nucleic acid sequence identifiers. Library barcodes are used by the Torrent Suite™ Software 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 workflow by the Torrent Suite™ Software.
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 No. of Loaded ISPs ÷ No. of potentially addressable wells.
BED file	Browser Extensible Data file - BED file - defines chromosome positions or regions.
bp	Abbreviation for "base pair(s)".
Cellularity (%)	The percentage of tumor cells in a given sample.
Clonal (ISPs)	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 they respond in unison as each nucleotide is flowed in turn across the chip.
CNV	Copy Number Variation (CNV) is the variation in copy number of any given gene between two samples.
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 text file in which each line represents a data record with information fields separated by a comma. CSV files are easily opened using spreadsheet software, such as Microsoft™ Excel™ or OpenOffice.org 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 System template and modified to fit the user's specific needs.
DeNovo Assembly	Nucleic acid sequence data that is assembled from sequencing reads without the aid of a reference genome library sequence.
Empty wells	Wells on the 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 text file that holds nucleotide sequence information.
FASTQ	A text file that holds a list of nucleotide sequence reads generated with quality scores for each read.
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 Order	The order in which the chip is exposed to each particular dNTP. Flow order selected on Ion PGM™ or Ion Proton™ Sequencer: Samba = TACGTACGTCTGAGCATCGATCGATGTACAGC; [Default]Regular = TACG. The "regular" flow order adds bases most rapidly to sequenced molecules but is vulnerable to phase errors. The 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 will show 250/500 flows in the status column of the **Runs in Progress** table when the sequencing run is half way done.
Flows	A "flow" is the event of exposing the chip to one particular dNTP, followed by a washing step.
Fusions	A target technique used for detection and annotation of gene fusions (or translocation of genetic material) in samples.
Hotspot Regions file	A BED or a VCF file that defines regions in the gene that typically contain variants and enables Torrent Variant Caller to identify if a specific variant is present or absent. Hotspot Regions 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 Regions file affects only the variantCaller plugin, not other parts of the analysis pipeline. If you don't specify a Hotspot Regions file, the software will only tell you the difference between your sequence and the reference genome.
IA (Isothermal Amplification)	A template preparation technique that uses non-emulsion isothermal reaction to clonally amplify DNA onto an ISP surface.

INDEL	Insertion or deletion of bases in the genome of an organism.
Intermediate files	Intermediate files contain information used for debugging runs.
Ion Mesh	A network of Torrent Servers that allows the users to: • view all runs of interest across multiple Torrent Servers on the same data page. • transfer Planned Runs between different connected Torrent Servers. • perform Ion Chef™ flexible workflows for 550 chip across different Ion Chef™ instruments connected to different Torrent Servers since Torrent Suite™ Software can track reagent/cartridge usage across multiple Torrent Servers that are a part of the same Ion Mesh .
ISPs	Ion Sphere Particles (ISPs) are particles that contain multiple bound copies of the same DNA fragment.
Key Signal	Average 1-mer signal in the library key.
Library ISPs	Number of Live ISPs that have a key signal identical to the library key signal.
Library Key	A short known sequence of bases used to distinguish the library fragment from the test fragment (for example: "TCAG").
LIMS Meta Data	The Laboratory Information Management System (LIMS) is used for recording sequencing metadata. Entered text is associated with the Torrent Suite™ Planned Run and can be extracted using APIs for LIMS consumption.
Live ISPs	Number of wells that contain an ISP with a signal of sufficient strength and composition to be associated with the library or Test Fragment key.
Low Quality ISPs	ISPs with low or unrecognizable signal.
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.
Planned Run	The digital instructions for the sequencing instrument that contain specifications for sample preparation, sequencing, data export, and post-sequencing data analysis.

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. Planned Run templates can be used to create many Planned Runs.
Polyclonal (ISPs)	ISPs that carry clones from two or more templates.
Primer dimer (ISPs)	ISPs that carry 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. 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(s)	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. In Torrent Suite™ Software, related samples are designated by the same relationship group number.
SAM file	Sequence Alignment Map (SAM) is a text-based output file that stores biological sequences aligned to a reference sequence.
Sample	Genetic material from one source (for example: DNA from one patient).
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 re-analyze 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.
System template	A Planned Run template that comes pre-loaded on the Torrent Suite™ Software. System templates are designed to facilitate the user in creating a Planned Run or a customized Planned Run template for a specific research application. System templates cannot be deleted from the Torrent Server.
Target base coverage	Summary statistics for targeted base reads of the reference. A base covered by multiple target regions is only counted once per sequencing read.
Target Regions file	A BED file that specifies all the regions that a panel represents such as the amplified regions that are used with target sequencing. The complete Torrent Suite™ Software analysis pipeline, including plugins, is restricted to only these specified regions instead of analyzing the entire reference library.
Test Fragment (TF)	Known nucleotide sequence that is used to monitor system characteristics.
Test Fragment ISPs	Number (and percentage) of Live ISPs with a key signal that is identical to the Test Fragment key signal.
TF Key / Test Fragment Key	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.
uBAM file	A binary file that contains unaligned or unmapped reads.
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	Variant Call Format (VCF) file specifies the 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.

Index