ion torrent

Torrent Suite™ Software 5.6

HELP

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Revision A.0

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

ThermoFisher SCIENTIFIC

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

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

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Revision	Date	Description
A.0	Sept. 14, 2017	Updates for Torrent Suite Software 5.6 new features: • Redesigned Completed Runs and Reports pages • Updates to the RunTransfer plugin • Updates to variantCaller plugin • Updates to IonReporterUploader plugin • Addition of smallRNA plugin • New configuration method for Ion Mesh • Updated content for Data Management • Search enhanced for Plan templates and Planned Run pages • Display Chef timestamps for library and template preparation • Ability to mark "reserved" Chef plans as complete when Chef has connectivity issues

Contents

Introduction

The Torrent Suite™ Software is organized according to the three main phases of the sequencing lifecycle:

- **Plan** — The **Plan** tab contains both templates (reusable experiment designs) and planned runs (executable instructions for individual sequencing runs). Select the experimental design for a template that can be reused many times for sequencing runs. Template details include application, reference, BED files, project, plugins, and the export destinations for results files.
- **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.

Templates are organized by research application (and by product for some applications):

Research application	Description
All	
AmpliSeqDNA	For Ion AmpliSeq™ research applications (DNA and exome), including the Ion AmpliSeq™ Any Genome, and Custom Ion AmpliSeq™ panels.
AmpliSeqRNA	For Ion AmpliSeq™ research applications (RNA), including Oncomine Immune Response Research Assay.
DNA and Fusions	For Ion AmpliSeq™ research applications such as Oncomine Focus Fusions, Oncomine Focus DNA & Fusions, Colon and Lung Research Panel v2.
Generic Sequencing	For your own applications that do not fit in the other categories. Use this research application to provide all the choices for the experiment. Your choices are not restricted based on a common application workflow.
Human Identification	For templates to run Applied Biosystems™ Precision ID set of panels.

Research application	Description
Immunology	For all Immunology research applications. For example, Ion AmpliSeq™ Immune Repertoire Assay.
Immune Repertoire	For the Ion AmpliSeq™ Immune Repertoire research application.
Inherited Disease	For Ion AmpliSeq™ Inherited Disease Panel and Oncomine BRCA Research Panels.
Oncology – ImmunoOncology	For all ImmunoOncology research applications. For example, Oncomine™ Immune Response Research Assay.
Oncology – Liquid Biopsy	For your liquid biopsy oncology research panels.
Oncology – Solid Tumor	For your solid tumor oncology research panels.
Pharmacogenomics	For Ion AmpliSeq™ Pharmacogenomics Research Panels imported from Ampliseq.com.
Reproductive	Ion Reproseq Aneuploidy research applications.
RNA Seq	For RNA sequencing research applications.
TargetSeq	For TargetSeq™ research applications, with parameters optimized for hybridization-based target enrichment.
Whole-Genome	For 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	For the Ion 16S™ Metagenomics kit.
16S Target Sequencing	For the Ion 16S™ Metagenomics kit.

User versus Administrator roles

In Torrent Suite™ Software, the User role allows the creation and execution of planned runs on a sequencing instrument. The Administrator 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. For more information on Administrator functions, see “Software administration“ on page 300.

Plan a run

The following steps describe how to plan templates and planned runs that fit into your Ion S5™, Ion S5™ XL, Ion PGM™, or Ion Proton™ sequencing workflow.

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. The Torrent Suite™ Software assigns your new plan a run code.
4. Enter the run code directly on the Ion sequencing instrument to start the sequencing. The planned run automates the process from sequencing through data analysis and data handling.

   Plan templates and planned runs allow you to enter run information via 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.

   On the sequencer, information for a planned run is applied to the current Run Info screen by entering the short code of the planned run, 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

You can set up **Samples** and **Sample Sets** when you create Planned Runs, or before you begin.

1. In the **Plan** tab, click **Samples**, then click **Enter Samples Manually**.
2. Do one of the following:
   - Create sample sets manually.
   - Import samples and sample sets.
   - Manage sample attributes.

Enter new sample

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

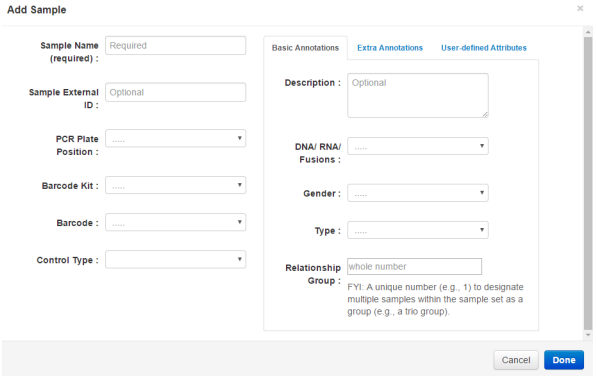
   Note: The Sample Name field is required. If you do not name the sample, you will get an error.
4. Click **Done** in the **Add Sample** window.

   The sample is not saved until you click **Save Sample Set** and select a sample set. If you log out of Torrent Suite™ Software and do not save it to a set, the sample is not saved.
5. Click **Save Sample Set** in the **Enter Samples** list.

Your new sample is now available in the **Enter Samples** list.

Create sample sets manually

1. In the **Plan** tab, click **Samples**, then click **Enter Samples Manually**.
2. Click **Enter New Sample**. The following window appears:



(Optional) If you have sample pairs, set Relationship Group numbers to reflect pairs. For example, DNA and RNA samples from the same sample would have the same Relationship Group number.

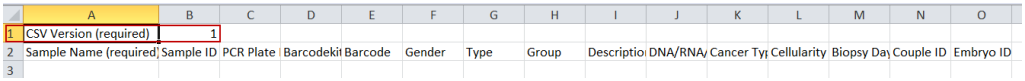
   a. Fill in the information as required.
   b. Click **Done**. The attributes appear on the **Enter Samples** list.
   c. Enter additional samples.
   d. Name **Sample Set** or add samples to an existing sample set.

3. Click **Save Sample Set**.

Import samples to create a sample set

If you have multiple samples, you can import the samples with a comma separated values (CSV) file.

Note: The latest Sample File Format CSV template has a top row that indicates the version of the template. If you are using sample CSV files that you created with versions prior to Torrent Suite Software 5.2 , you must create a new CSV file with a new template that is downloaded in version 5.2 or later. To create a new CSV file, copy and paste the contents of your existing sample CSV file into the new template under the version row.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	CSV Version (required)	1													
2	Sample Name (required)	Sample ID	PCR Plate	Barcodekit	Barcode	Gender	Type	Group	Descriptio	DNA/RNA/	Cancer Ty	Cellularity	Biopsy Da	Couple ID	Embryo ID
3															

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.
2. Click **Sample File Format** to download a CSV template.

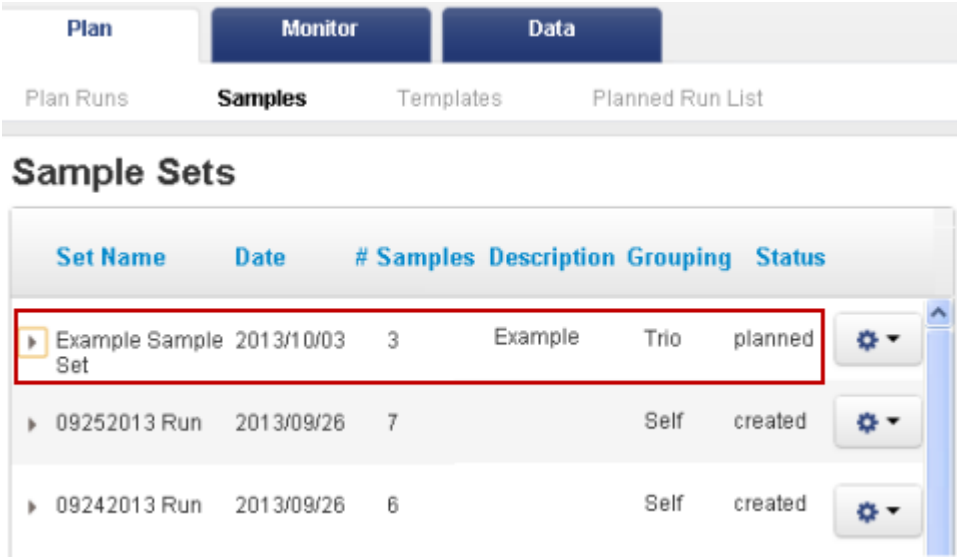
3. Open the CSV template, then enter sample information into the cells, then save it to your computer.
   See "Sample information" on page 20 for more details about fields that are used in the sample import file.
4. Click **Select File**, then browse to and upload the sample import file.
5. Click **Add Sample Set**, then enter a **Sample Set Name**, **Group Type**, and *(optional)* **Description**.
   The software automatically imports the samples into the **Sample Sets** list.
6. Click **Save & Finish**.

Example use of a sample set

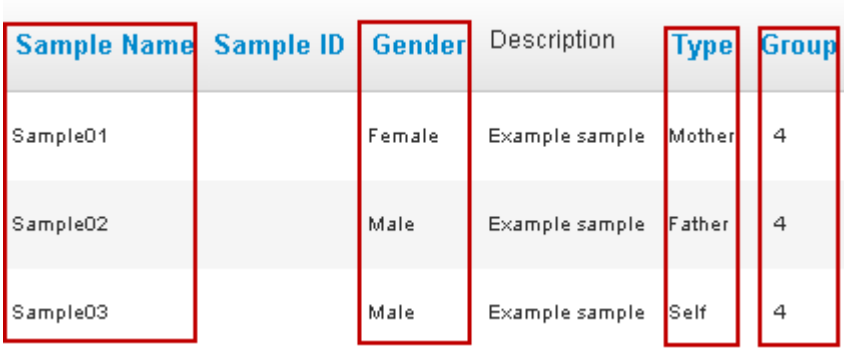
This example shows a trio sample set and how the run plan reads the sample set information for Ion Reporter™ Software users.

In the sample set

A trio sample set, named Example Sample Set, is shown in the main **Sample Sets** listing:



Expand **Example Sample Set** entry to open the details for the sample set:



The sample set contains three samples that are related and eventually analyzed as related samples in one Ion Reporter™ Software analysis.

- **Type** is the Ion Reporter™ Software relationship type information.
- 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.)

Sample attributes

You can add fields to samples for sample management. Attributes that you create appear in the sample listing, in the Add Sample dialog, and in the CSV file that is used to import sample information.

You can add to the sample attributes that are available when you enter sample information. Each attribute that you add is available in:

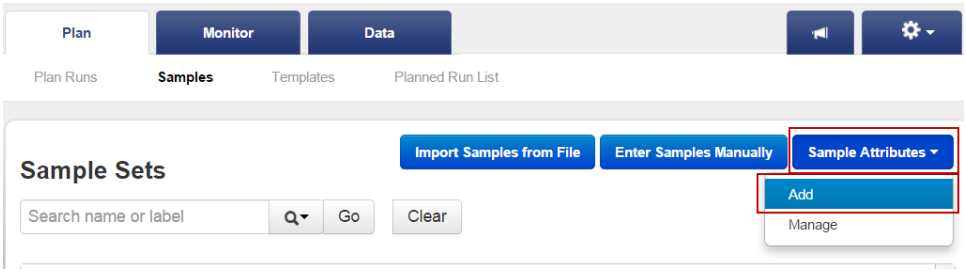
- Lists of samples and sample sets in the Torrent Browser
- The **Add Sample** window in the software
- The CSV file that is used to import sample information.

An attribute can be made mandatory, in which case it must be entered with every sample.

Note: Although you create an attribute in the Sample Sets window, the attribute is applied to individual samples, not to the sample set itself.

Add a sample attribute

1. To create a new sample attribute, in the **Plan** tab, click **Samples**, then click **Sample Attributes** ▸ **Add**.



2. In the **Add Attribute** window, enter the following:
   - **Attribute Name**
   - **Attribute type**
   - **Description**

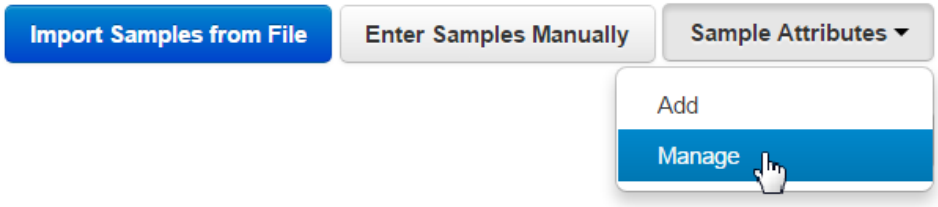
   Note: If you want the attribute to be required with every sample, select the **Is Mandatory** checkbox. If the **Attribute Type** is set to Integer, you can only enter numeric characters (whole numbers) for this attribute.

Hide a sample attribute

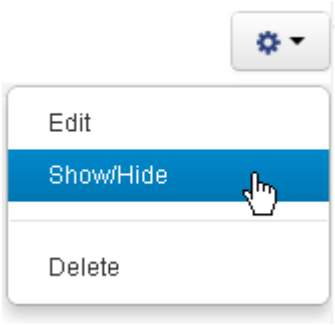
If you hide an attribute, that attribute no longer appears in sample listings or in the **Add Sample** dialog. If you hide a mandatory attribute, that attribute is no longer mandatory.

You can hide a sample attribute in the attribute manage screen.

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



2. In the **Sample Attributes** list, click **Settings (⚙)** ▸ **Show/Hide** next to the attribute:



Sample information

This table describes the fields in the **Add Sample** dialog. The same fields are used in a CSV file that is used to import samples.

Field	Description
Sample Name (Required)	Must follow Ion Reporter™ Software sample name limits. If the actual sample name already exists in Ion Reporter™ Software, a string such as _v1 or _v2, etc., is added to the sample name.
Sample External ID	A field for your own use.
PCR Plate Position	The well number of the sample in the PCR plate.
Barcode Kit	The name and/or catalog number of the barcode kit used to make a library from the sample.
Barcode	The name of the specific barcode used to generate a library from the sample.
Control Type	The name of the control used when preparing and sequencing the sample.
Basic Annotations	
Description	An open text entry field.

Field	Description
DNA/RNA/Fusions	The type of library created from the sample.
Gender	The gender of the sample. **IMPORTANT!** Do not leave this field empty. Select **Unknown** if the gender is not known. Several workflows in Ion Reporter™ Software, especially copy number variation detection and genetic disease screening, are limited when the gender is not known, and they return unexpected results when the gender is incorrectly specified for a sample.
Type	The relationship type for this sample, used by Ion Reporter™ Software.
Relationship Group	The group number of the sample set of which the sample is a member. This is identical to the Set ID in the IonReporterUploader plugin, and is used to identify related samples.
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 typeInteger, only numeric characters (whole numbers) can be entered into the field for that attribute.

Vocabulary and field restrictions

When sample sets are used to automate integration with Ion Reporter™ Software, the sample information must follow the rules for Ion Reporter™ Software samples.

The following sample relationships are supported:

- Self
- Tumor, Normal
- Control, Sample
- Father, Mother, Self

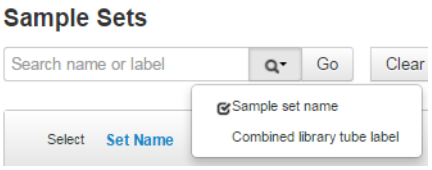
Note: Self is used both for a single sample and for the proband sample in a trio. A single sample is not related to other samples and is analyzed by itself. Always supply a value for gender. If gender is not known, select unknown.

Search samples

To search samples, use the steps that follow:

1. In the **Plan** tab, click **Samples**.
2. Enter a search name or label in the text field.

3. Click , then select **Sample set name** or **Combined library tube label**.

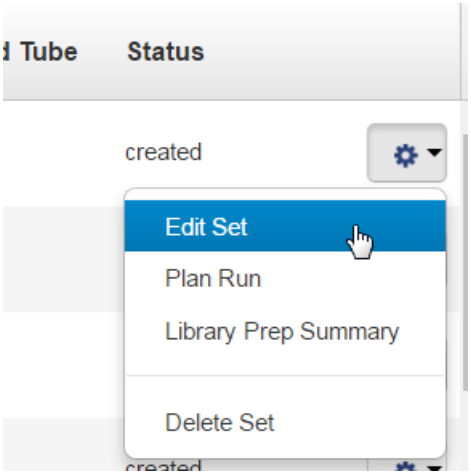


4. Click **Go**.

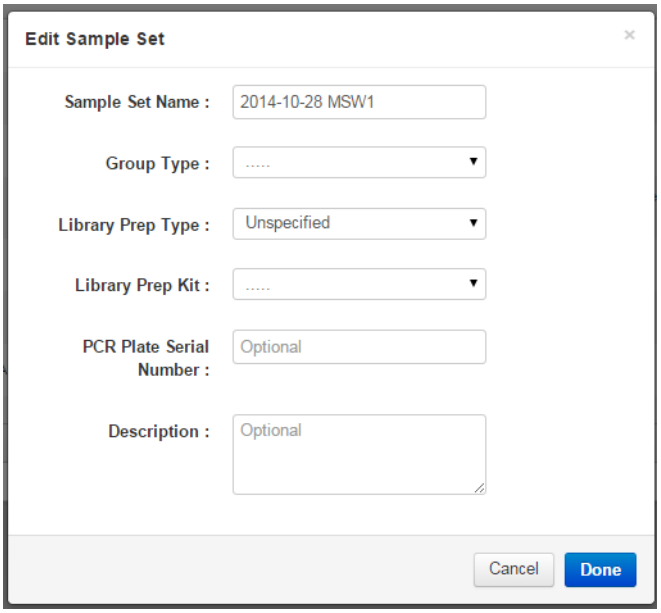
Edit samples

To edit samples:

1. In the **Plan** tab, click **Samples**.
2. Click **Settings ( ) ▸ Edit Set**.

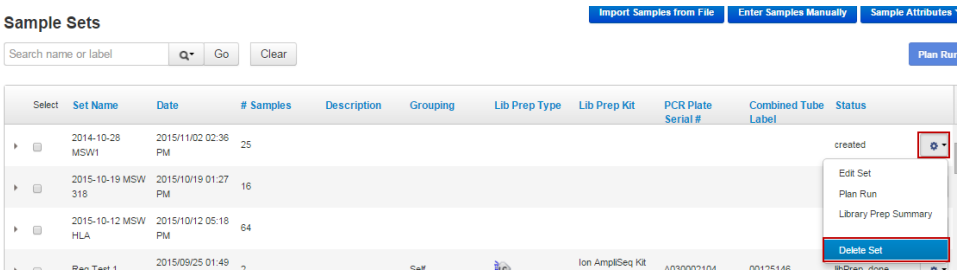


3. Make any desired changes, then click **Done**.



Delete samples

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

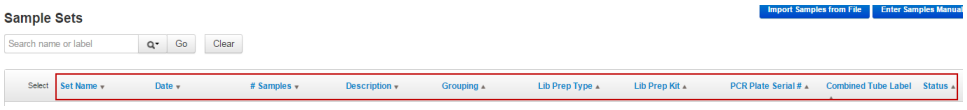


3. Click **Yes, Delete!**.

Sort samples

To sort samples:

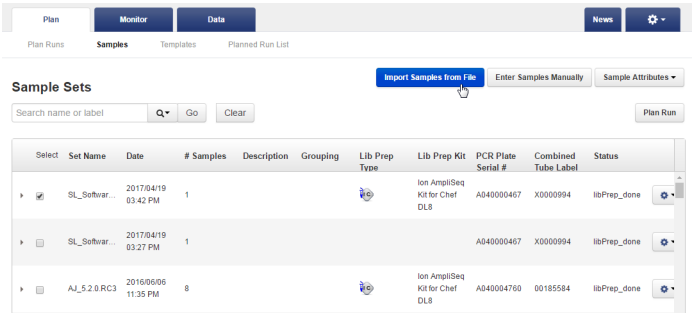
1. Click any column header to sort the sample rows alphabetically or numerically.



2. Click **Clear** to remove sorting.

Import samples

1. In the **Plan** tab, click **Samples**, then in the Favorites list, select the application group that you want to import the sample into.
2. Click **Import Sample from File**.



3. In the **Import Samples** dialog, click **Select Sample File to Import**, then select the CSV file to import.
4. Click **Select a Sample Set to Receive Samples**, then click **Save & Finish**.
   The system loads, parses, validates the file, then saves if no errors are found.

Plan an instrument run

About Planned Runs

Planned Runs are the instructions that contain settings and other details used for sequencing runs, including:

- location of the Torrent Suite Server
- location of BED and hotspot files
- library barcodes
- types of kits
- sample information
- types of chips

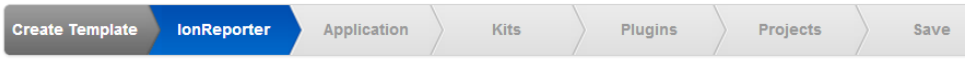
Differences between templates and planned runs

Templates and planned runs have much the same information.

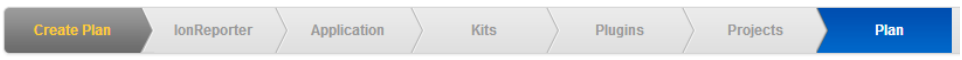
- Planned runs are created from templates.
- Templates do not have sample names and run names.
- Planned runs are executable on the sequencing instrument.
- Add a sample name and run name to a template to create a planned run.
- The planned run wizard opens in the last page, so that if you accept all the template settings, all you need do is supply are the run name and sample names and save the new planned run.
- The last page of the wizard is different for templates and planned runs. The planned run last page requires the run name and sample names. (Templates do not contain this information.)

The wizard pages for a template and a planned run are the same except for the last page. The planned run last page requires the run name and sample names.

- Template wizard:



- Planned run wizard:



- Plan by sample:

Fore more detailed information see “Templates “ on page 391 and “Plan Tab“ on page 394 in the Reference section.

Customizing and editing templates

Typically you copy a product template and customize the new template with your choices for project organization and data export handling. Then you reuse your new template to create many planned runs, as needed. Each run plan has the correct settings (from the original template). Or you can edit your template when experimental or data handling changes are required.

A planned run performs template preparation on the Ion Chef™ instrument, executes sequencing on your Ion sequencing instrument, and automates your decisions for post-sequencing data analysis and data management.

Create a planned run with AmpliSeq™ DNA template

AmpliSeq™ DNA/Exome/RNA templates (also known as panels) can be downloaded from AmpliSeq.com. The necessary BED files for those templates are automatically installed with the templates. Also, you can edit the templates that are downloaded from AmpliSeq.com or clone those templates to meet your specific needs. The AmpliSeq™ DNA templates are used to create planned runs for various AmpliSeq™ panels, such as Ion AmpliSeq™ Exome and Ion AmpliSeq™ Inherited Disease Panel. You can select your Ion Reporter™ account, kits, plugins, and parameter settings.

Note: To modify default parameters, see “Configure and select a custom analysis parameter set“ on page 412.

1. In the **Plan** tab, click **Templates**, then in the Favorites list, select **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.
   The wizard launches and displays the **Plan** screen.
3. Add samples, ensure that the default settings, enter a plan name, then click **Plan Run**.
4. Run the plan on your sequencing system.

Create a Planned Run with DNA and Fusions templates

The AmpliSeq™ DNA templates are used to create planned runs for various AmpliSeq™ panels, such as Ion AmpliSeq™ RNA Lung Fusion Panel and Ion AmpliSeq™ Colon and Lung Fusion Panel. You can select your Ion Reporter™ account, kits, plugins, and parameter settings.

Note: To modify default parameters, see "Configure and select a custom analysis parameter set" on page 412.

1. In the **Plan** tab, click **Templates**, then in the Favorites list, select **DNA and Fusions**.
2. Select a template that matches your panel. For instance, if you are using an Ion AmpliSeq™ RNA Lung Fusion Panel, select a template with the same name from the DNA and Fusions category.
   The wizard launches and displays the Plan page.
3. Add samples, confirm the default settings, enter a plan name, then click **Plan Run**.
4. Run the plan on your sequencing system.

Plan a run using Human Identification templates

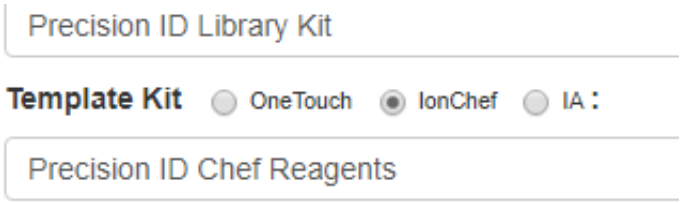
Human Identification (HID) templates are used to create Planned Runs for various Applied Biosystems™ Precision ID panels. These templates pre-populate your Planned Run with parameters for the selected panel. You can then select additional settings to plan your run.

Note: To modify the default parameters, see "Configure and select a custom analysis parameter set" on page 412.

1. In the **Plan** tab, click **Templates**, then in the **Favorites** list, select **Human Identification**.
2. Select a template that matches your panel and sequencer.
   The wizard launches and displays the **Plan** screen.
3. Select the reference and BED files, enter the samples, confirm the default settings, then enter a plan name.
4. To verify kit information, click the **Kits** tab.

   Note: All templates default to the Ion Chef™ Instrument.

5. *(Optional)* If you are using the Ion OneTouch™ 2 System, select **One Touch** in the **Template Kit** section.



IMPORTANT! Do not select IA. The IA workflow is not compatible with the Precision ID panels.

The correct template and sequencing kit should populate.

6. *(Optional)* Click in the appropriate box, then select the sample preparation kit, control sequence, and barcode set from the dropdown list.

7. When you have completed your selections, click **Plan Run** at the bottom right of the **Plan** tab to save the run. The run is listed on the Planned Runs page under the name that you specified.

Plan a run with RNA Seq templates

RNA Seq templates are used to create Planned Runs for Ion Total RNA Seq Kits. These templates will pre-populate your Planned Run with parameters for whole transcriptome and small RNA sequencing applications. You can then select additional settings to plan your run.

Note: To modify the default parameters, see "Configure and select a custom analysis parameter set" on page 412.

1. In the **Plan** tab, click **Templates**, then in the Favorites list, select **RNA Seq**.

2. Select the template that matches your application.
   The wizard launches and displays the **Plan** page.

3. Enter the samples, confirm the default settings, and enter a plan name.

4. To change kit information, click on the **Kits** step in the Workflow bar.

   Note: If you are using Ion PI™ Hi-Q™ Sequencing Kit or the Ion 540™ Kit with the Ion Chef™ Instrument, select the **Ion Chef** option next to **Template Kit**, and select the kit name. Click on the **Details** button to select the **Whole Transcriptome RNA** workflow, which was optimized in Torrent Suite™ Software 5.2.1.

5. When you have made all your selections, click **Plan Run**.

6. Run the plan on your sequencing system.

Plan a run using Generic Sequencing template

The Generic Sequencing templates are used to create planned runs for various applications, such as the System Generic Sequencing or the MuSeek Library. You can select your Ion Reporter™ account, kits, plugins, and parameter settings.

Note: To modify default parameters, see "Configure and select a custom analysis parameter set" on page 412.

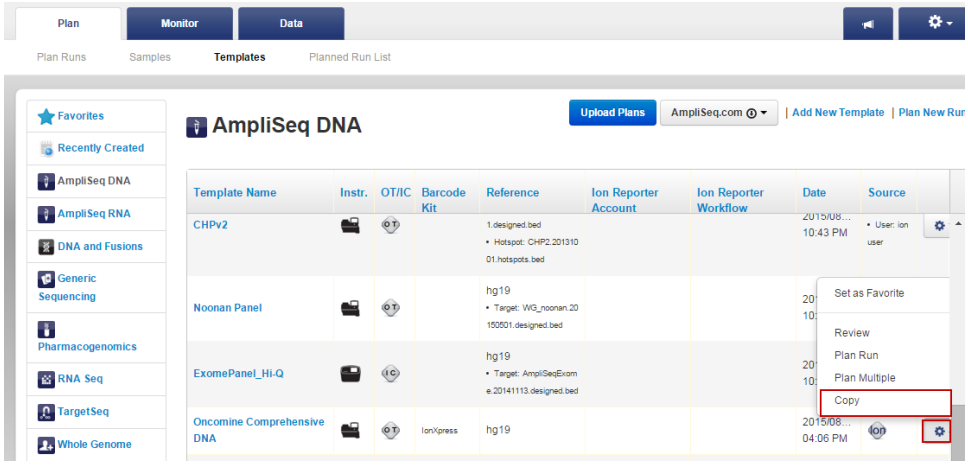
1. In the **Plan** tab, click **Templates**, then in the Favorites list, select **Generic Sequencing**.
2. Select a template that best matches your application. For instance, if you are using a MuSeek library, select the template with the same name from the Generic Sequencing category.
   The wizard launches and displays the Plan page.
3. Add samples, confirm or change the default settings, and enter a plan name, then click **Plan Run**.
4. Run the plan on your sequencing system.

Copy a template

You can copy the settings in existing template into a new custom template.

Note: To modify default parameters, see "Configure and select a custom analysis parameter set" on page 412.

1. In the **Plan** tab, click **Templates**, then in the Favorites list, click **Settings** (⚙) ▸ **Copy** in the row of the template you want to start with.



The wizard launches and displays the Save page.

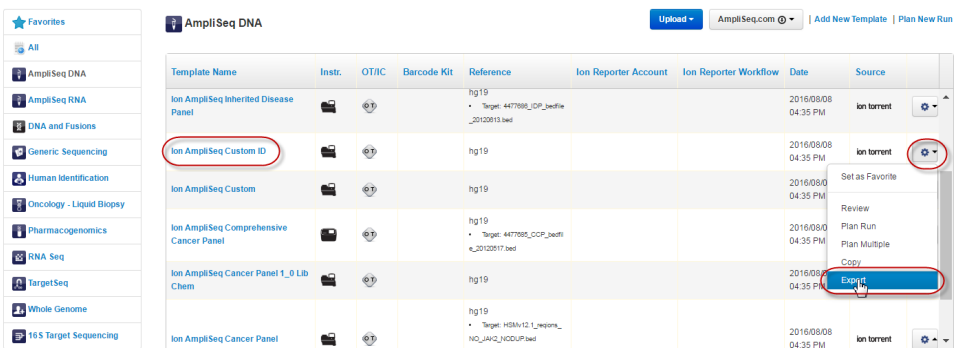
2. Enter a name for the template.

3. If desired, go back to previous steps in the workflow bar and adjust the settings.
4. In the Save page, confirm your selections, then click **Copy Template**.

Export a template

You can export the settings in an existing template into a CSV file.

1. In the **Plan**tab, click **Templates**, then in the Favorites list, click **Settings** (⚙) ▸ **Export** in the row of the template you want to start with.



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

2. Double-click on the CSV file to open it in a spreadsheet application such as Microsoft™ Excel™.

Note: Templates are exported in a format that can be imported back into Torrent Suite™ Software. You can change the parameters in the CSV file and then re-import the CSV file.

Import a template

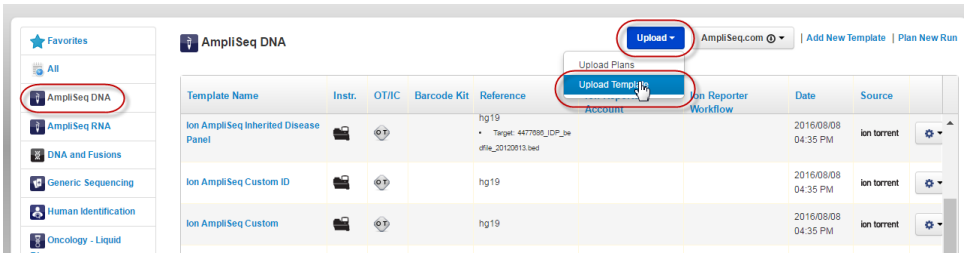
You can import all the parameters in a template in the form of a CSV file.

Note: The CSV file must be formatted correctly for import. We recommend exporting a template (see "Export a template" on page 30) and using the exported CSV file as a model. You can change the parameters in the exported CSV file and then rename and import the file.

To import a template:

1. In the **Plan** tab, click **Templates**, then in the Favorites list, select the application group that you want to import the template into.

2. Click **Upload** ▸ **Upload Template**.



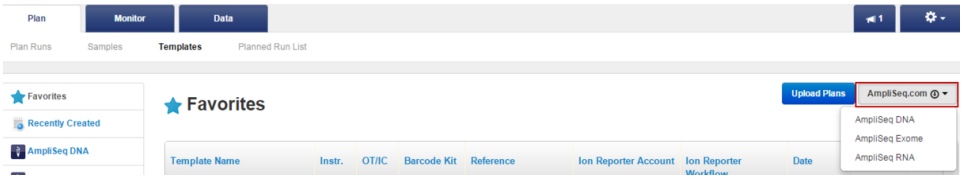
3. In the **Import Plan Template** dialog, click **Choose File**, select the CSV file to import, then click **Load**.
   The template appears listed in the application group.

Create a template with Ion AmpliSeq.com Import

To create a template for an Ion AmpliSeq™ panel or an Ion AmpliSeq™ custom design, use the **AmpliSeq.com** import button. For community and fixed panels (not for custom panels), the variantCaller plugin is pre-enabled in your new template and the variantCaller plugin is pre-configured with parameters that are optimized for the panel. Later you can further customize the variantCaller plugin parameters. There are three types of Ion AmpliSeq™ templates: DNA, RNA, and Exome. Human, animal, and plant reference genomes are also available. Start with the template group that matches your experiment type. Your choices of AmpliSeq.com panels to import are limited the group types (DNA, RNA, or Exome).

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

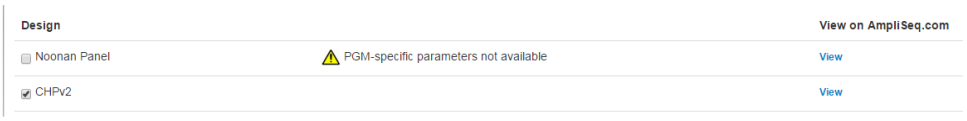
2. Click the **AmpliSeq.com** button, then select the type of panel you want to import: AmpliSeq DNA, AmpliSeq Exome, or AmpliSeq RNA.



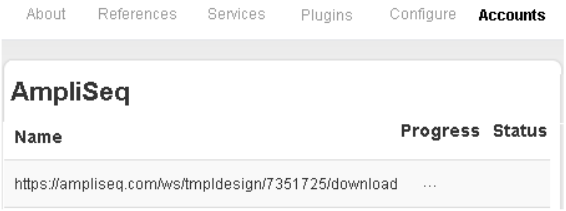
3. Enter your ampliseq.com username and password (if necessary).

4. Select your instrument and chip.

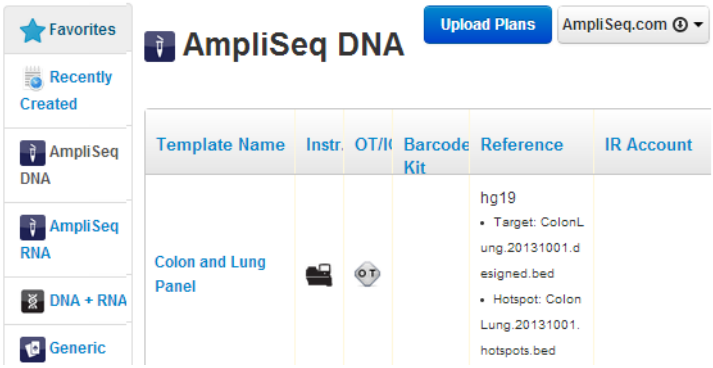
5. The Torrent Browser lists the available panels. Some panels do not have optimized variantCaller plugin parameter sets available for multiple chips and sequencers. A caution warning denotes choices for which optimized variantCaller plugin parameters have not be developed for the selected chip type, which can lead to suboptimal variant calls. The *Show solutions which were not ordered* link appears if you have unordered custom designs. Click this link if you want to import one of those designs. Enable the checkbox for the panel or panels you want to import, and click Import Selected.



6. The Torrent Browser opens a download and progress dialog. Refresh your browser to track the progress, then view the completion status.



7. When the Status column shows "Completed", go back to the Templates tab, and you see the new template.



Plan by sample set

When you base your run plan on a sample set, the run plan wizard reads the sample set information and adds it to the appropriate wizard screens. For barcoded runs, the barcode information from your sample set is added in the plan wizard. This approach both saves you time and reduces the probability of error compared to manual barcode assignments on data sets with many files.

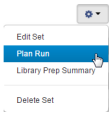
The plan-by-sample-set feature is recommended for the following:

- Ion Reporter™ Software users setting up multi-sample analyses
- Sample sets that include many samples.

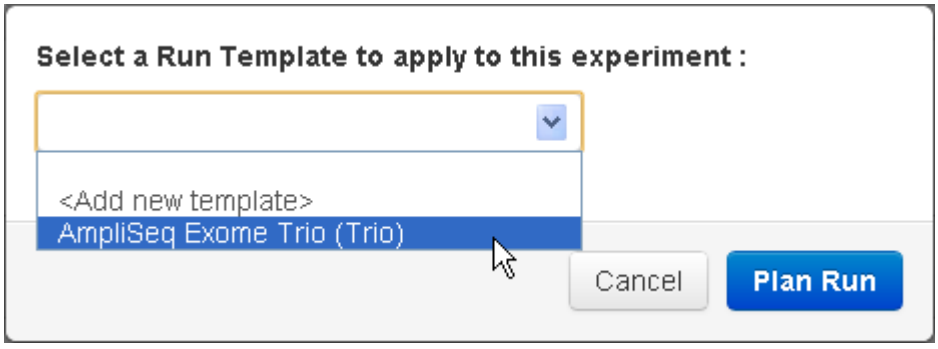
Follow these steps to start a run plan based on your sample set:

1. In the **Plan** tab, click **Samples**, then find your sample set in the **Sample Sets** screen.

2. Click **Settings (⚙) ▸ Plan Run** for your sample set to start to create a Planned Run:



3. Select a workflow that supports the sample set. The wizard opens a popup menu listing workflows that support your sample set. This example started with a trio sample set and offers trio-compatible workflows (on this server, there is only one trio workflow):



Note: If you do not see the template that you are looking for, select **Show All Templates**.

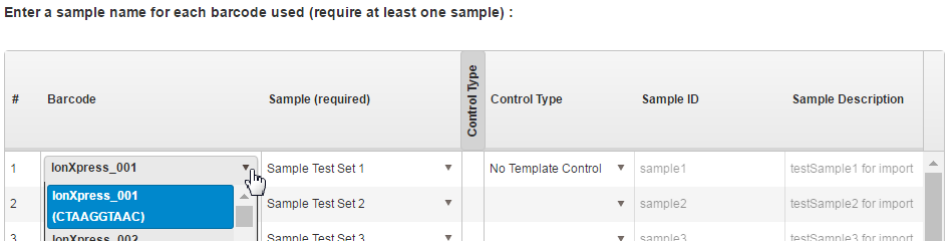
The wizard opens to the Barcoding step in the Workflow bar, with the selected sample sets displayed in a table at the bottom of the screen.

4. In the **Default Reference & BED Files** region of the screen, select the appropriate reference library and BED files for the target and hotspot regions that are covered by the selected panel.

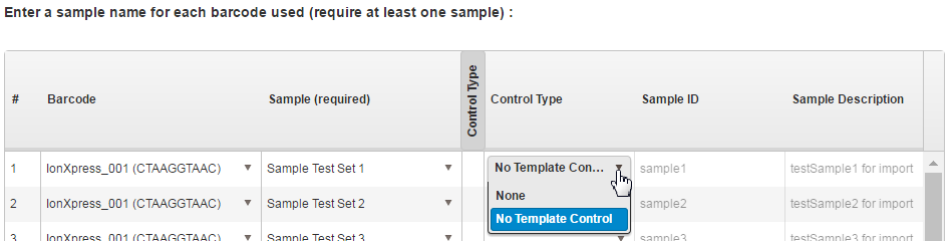
5. Select the barcoding kit that is used from the dropdown list. For tracking purposes, you can enter any text that is written on the sample tubes in the **Sample Tube Label** field.

6. The **Chip ID** field can be used to track the barcode number that is printed on the chip.

7. In the table at the bottom of the screen, select the barcode that is used to prepare each sample from the dropdown list in the **Barcode** column.



8. To identify No Template Control samples, click the **Control Type** column heading in the table, then select **No Template Control** from the dropdown list.

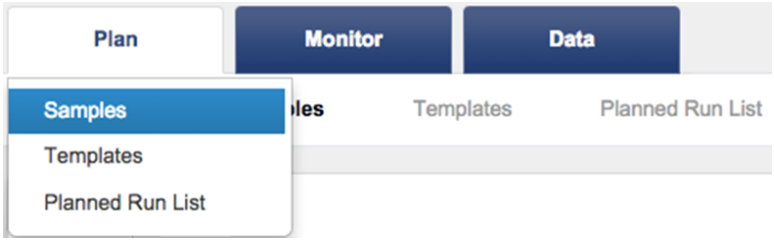


Note: The No Template Control option can be used to indicate negative control samples.

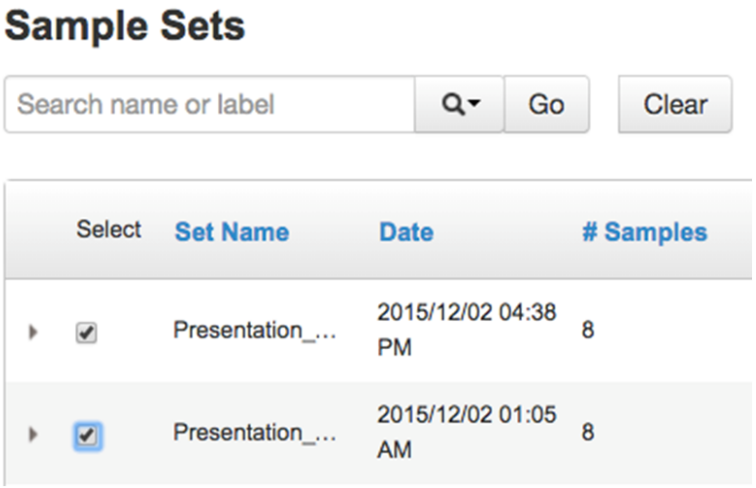
Include multiple sample sets in one planned run

Torrent Suite™ software allows multiple samples sets to be used in a single planned run. The sample sets must correspond to AmpliSeq™ library preparations and use the same barcode kit to be a part of a single planned run.

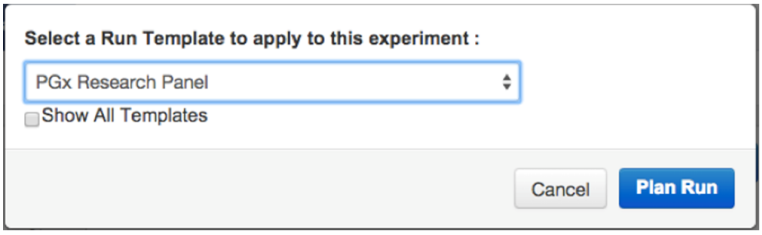
1. In the **Plan**tab, click **Samples**.



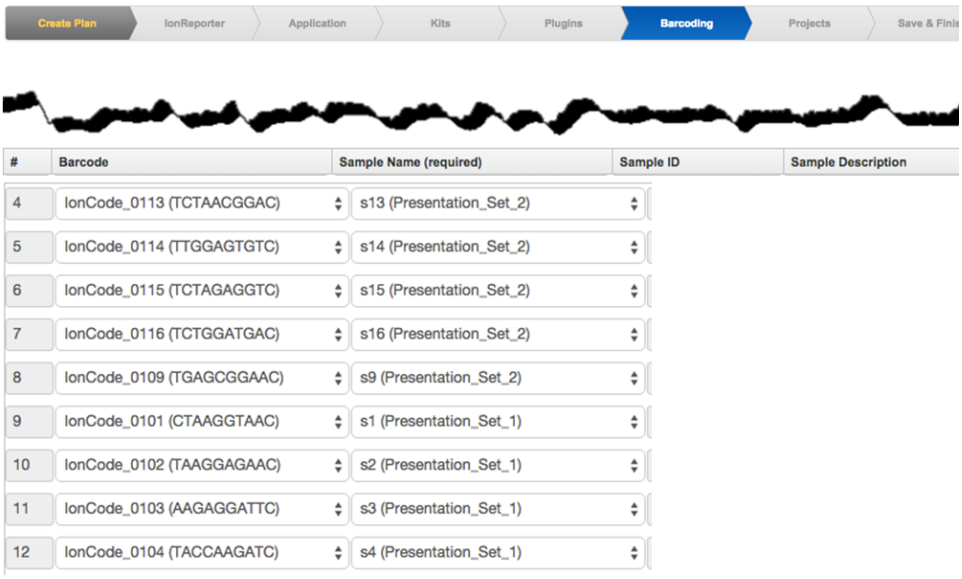
2. Select multiple samples sets, ensuring that they use the same barcode kit.



3. Select the Run Template to apply to this experiment, then click Plan Run.



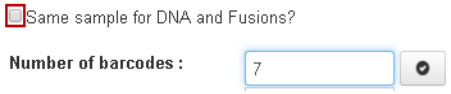
In the Barcoding step in the Workflow bar of the Planned Run wizard, you can now see the sample sets you added in the barcode table.



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. Copy the Ion AmpliSeq™ Colon Lung template.
2. Enter the number of samples.
3. Deselect "Same sample for DNA and Fusions" option.



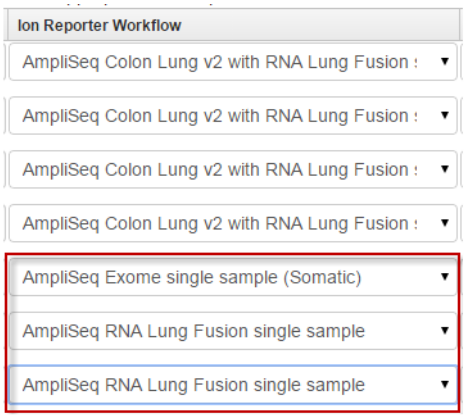
4. Renumber the samples.

#	Barcode	Sample (required)
1	IonSelect-1 (CTAAGGTAAC)	Sample 1
2	IonSelect-2 (TTACAACCTC)	Sample 1
3	IonSelect-3 (CCTGCCATTCGC)	Sample 3
4	IonSelect-4 (TGGAGGACGGAC)	Sample 4
5	IonSelect-5 (TGAGCGGAAC)	Sample 5
6	IonSelect-6 (CCTTAGAGTTC)	Sample 6
7	IonSelect-7 (TCCTCGAATC)	Sample 7

5. Change DNA/RNA selections to match samples.

DNA/Fusions
DNA
Fusions
DNA
Fusions
DNA
Fusions
Fusions

6. Select cancer types to match samples.



7. Select appropriate Ion Reporter workflows.
8. Enter Relation.
9. Enter gender.
10. Enter Analysis set IDs.
11. Click **Plan Run**.

Create multiple planned runs

You can create multiple planned runs based on a template with a CSV file. A template version of this file in Torrent Suite™ Software. Each column in the CSV template represents an individual planned run. Each row contains the plan parameters for each of the planned runs. Beginning in Torrent Suite™ Software 5.4, you can add information to the planned runs that is related to Ion Reporter™ Software for each sample within a run, including account, workflow, and workflow-related attributes such as gender, relation and SetID.

In versions earlier than Torrent Suite™ Software 5.4, data for each planned run was contained in a row in the CSV template. A column-based format is now used for each planned run.

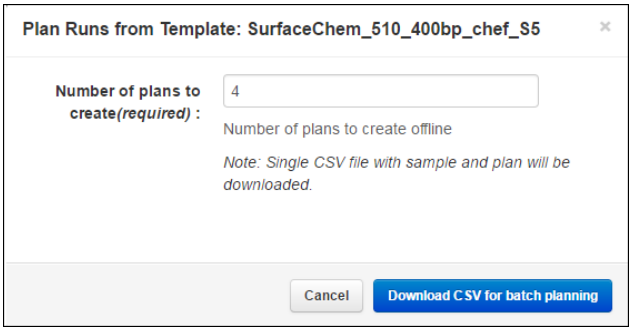
Note: The latest Plan Runs from Template CSV file indicates the version of the template in the top row. This version number is required. When you download the Plan Runs from Template CSV, the version is automatically included.

Create multiple planned non-barcoded planned runs

You can create multiple planned runs based on a template with a CSV file. To create a planned run without barcodes, use a single CSV template.

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 **Settings** ( ) ▸ **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. Download the CSV template file.

   - To create multiple planned runs from an non-barcoded template, enter Template name, Plan name and Sample. In this example, the template creates four non-barcoded planned runs.

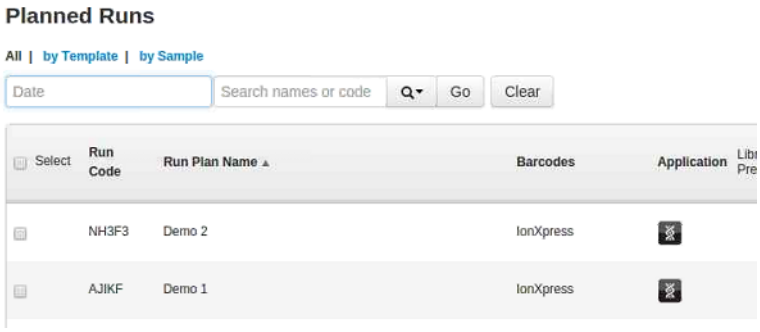
	A	B	C	D	E
1	CSV Version (required)	2			
2	Plan Parameters	Plan 1	Plan 2	Plan 3	Plan 4
3	Template name to plan from (required)	System Generic Seq Template	System Generic Seq Template	System Generic Seq Template	System Generic Seq Template
4	Plan name (required)				
5	Sample (required)				
6	Sample Description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

5. Save the CSV file.

6. In the Torrent Suite™ Software **Plan** tab, click **Templates**.

7. Click **Upload** ▸ **Upload Template**, then click **Choose File**, then select the edited CSV template.

8. Click **Load**.
   The system parses the files, then creates the planned runs.

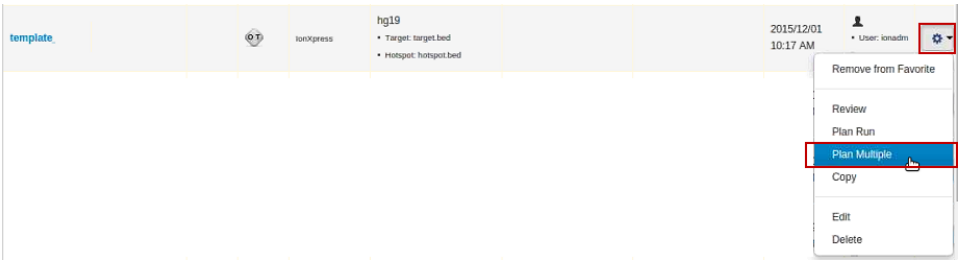


Create multiple barcoded planned runs

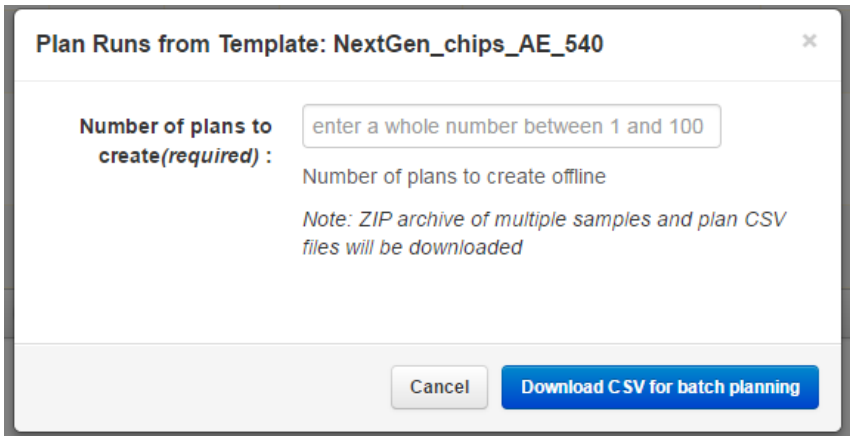
If you want to create barcoded planned runs, use multiple CSV templates as follows:

- a master CSV file that you use to specify the plan name, kits, chips, projects, and plugin selections
- one 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 **Settings** (⚙) ▸ **Plan Multiple**.

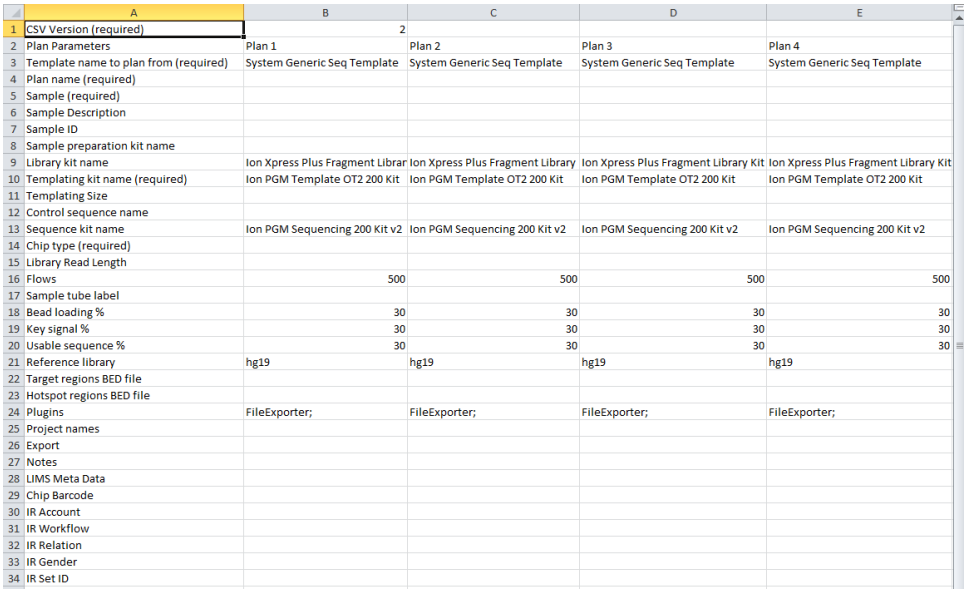


3. In the Plan Runs from Template dialog, enter the number of planned runs that you want to create, then click **Download CSV for batch planning**.



4. Download the compressed file, then decompress it.

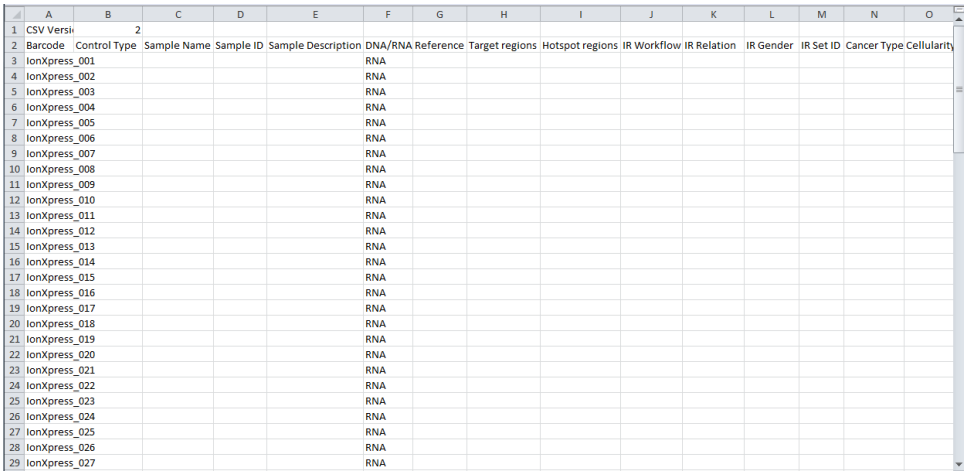
5. Open the tsPlan file appended with *master.csv* and enter the Template name, Plan name and Sample. In this example, the template creates four barcoded planned runs.



	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				

6. Save the CSV file.

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



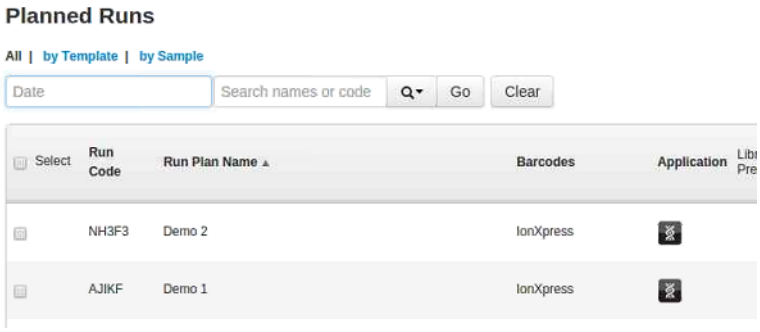
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	CSV Versi	2													
2	Barcode	Control Type	Sample Name	Sample ID	Sample Description	DNA/RNA	Reference	Target regions	Hotspot regions	IR Workflow	IR Relation	IR Gender	IR Set ID	Cancer Type	Cellularity
3	IonXpress_001					RNA									
4	IonXpress_002					RNA									
5	IonXpress_003					RNA									
6	IonXpress_004					RNA									
7	IonXpress_005					RNA									
8	IonXpress_006					RNA									
9	IonXpress_007					RNA									
10	IonXpress_008					RNA									
11	IonXpress_009					RNA									
12	IonXpress_010					RNA									
13	IonXpress_011					RNA									
14	IonXpress_012					RNA									
15	IonXpress_013					RNA									
16	IonXpress_014					RNA									
17	IonXpress_015					RNA									
18	IonXpress_016					RNA									
19	IonXpress_017					RNA									
20	IonXpress_018					RNA									
21	IonXpress_019					RNA									
22	IonXpress_020					RNA									
23	IonXpress_021					RNA									
24	IonXpress_022					RNA									
25	IonXpress_023					RNA									
26	IonXpress_024					RNA									
27	IonXpress_025					RNA									
28	IonXpress_026					RNA									
29	IonXpress_027					RNA									

8. Add the Master CSV template and all of the Sample CSV templates to a compressed directory.

9. In the Torrent Suite™ Software **Plan** tab, click **Templates**.

10. Click **Upload** ▸ **Upload Template**, then click **Choose File** and select the compressed directory of CSV templates.

11. Click **Load**.
    The system parses the files, then creates the planned runs.



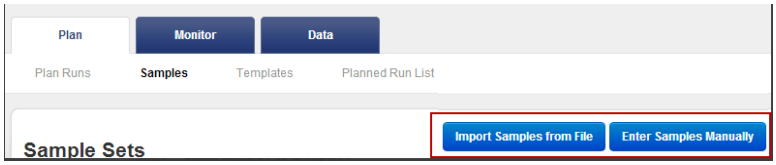
Analyze Ion AmpliSeq™ on Ion Chef™ samples

Ion AmpliSeq™ on Ion Chef™ samples can be analyzed.

The process involves creating a Torrent Suite™ Sample Set, preparing an Ion Chef™ library, creating a Torrent Suite™ planned run, preparing an Ion Chef™ or OneTouch2 template, and sequencing on an PGM™, Ion Proton™, or Ion S5™ sequencer.

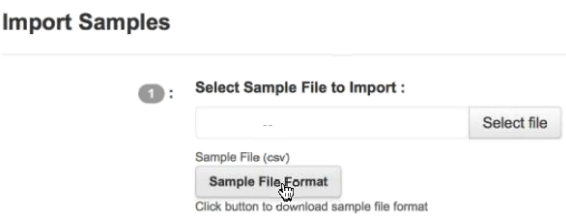
Create Sample Set:

1. Import samples from a file or enter them manually.



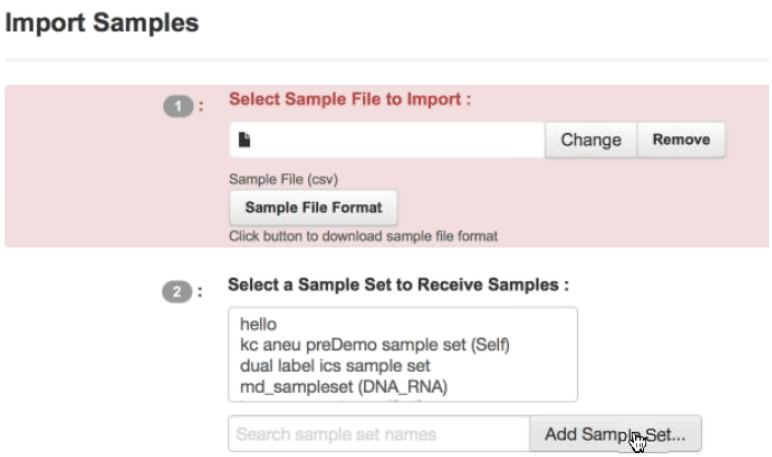
Note:

2. This example imports samples from a file.
3. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.
4. On the Import Samples window, click **Sample File Format** button. A CSV template downloads.



5. Click it, then enter sample names, PCR Plate positions, and DNA or RNA at minimum. Save to your desktop.
6. Now, click the **Select File** button, select your CSV file, then click **Open**.

7. Click **Add Sample Set**.

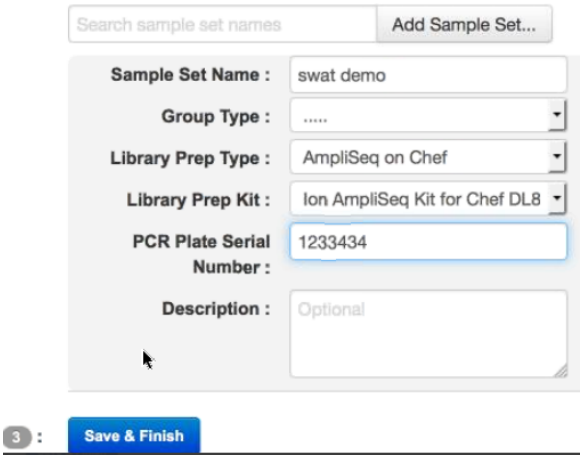


8. Name your new sample set.

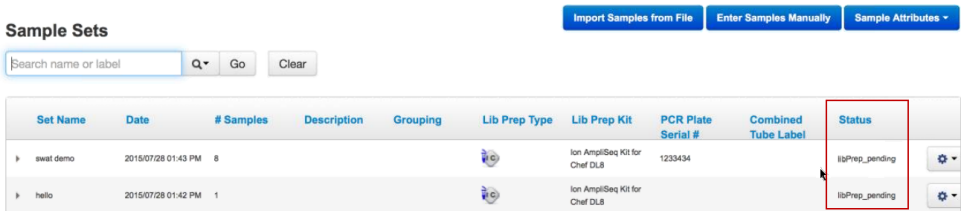
9. Set Library Prep Type to **AmpliSeq on Chef**.

10. Set Library Prep Kit to **Ion AmpliSeq Kit on Chef DL8**.

11. Enter PCR plate serial number, then click **Save & Finish** The software creates your new sample set..

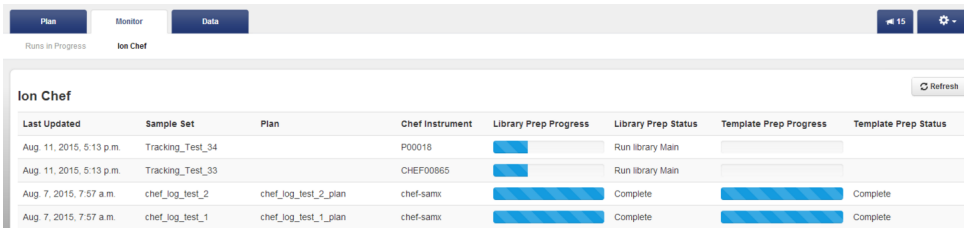


12. In the **Plan** tab, click **Samples** ▸ **Sample Set**. Check its status in the Status column. Either libPrep_pending, libPrep_reserved, libPrep_done, planned, Voided, or Run are displayed.



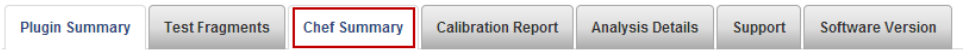
13. Notice a new icon for AmpliSeq™ on Ion Chef™ in the Lib Prep Type column.

14. Monitor the Ion Chef™ library and templating steps from **Monitor ▸ Ion Chef**. When the sequencing run is complete, view the Ion Chef™ run report.



15. Click **Data**, **Completed Runs & Reports,** then select your Ion Chef™ run.

16. Scroll to the bottom of the Run Summary screen, then select **Chef Summary**.



17. Review the Chef Library Prep Info and Chef Template Prep Info sections.

Handle a failed analysis run

If an analysis run fails, determine the cause of the failure and, possibly, restart the run.

Determine the fault cause

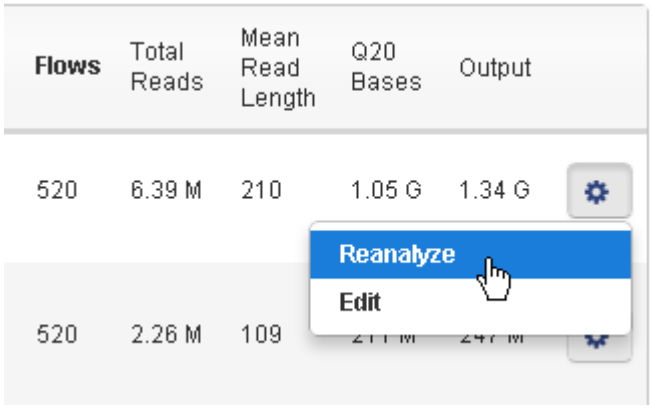
If an analysis run fails, make the following checks:

1. Has the Ion PGM™ or Ion Proton™ Sequencer completely transferred the data for the run? Go to the sequencer Data Management screen to ensure complete data transfer. If you are not sure the data was transmitted. you can retransfer it.
2. In the **Data** tab, click **Completed Runs & Reports tab** to ensure that the file transfer was complete. Also, check if there are any error messages, such as **User Aborted**. Look for a status of Error or Pending.
3. If the report was generated, check if there are any messages on the report itself.
4. Click the **Support** link towards the bottom of the run report (above the **Plugin Summary** row of buttons). Click **View the Report Log** or **Download the Customer Support Archive**. You can send the customer support archive to your Ion Torrent™ contact for review.
5. If you cannot determine the cause of the fault, try restarting the run.

Restart a run

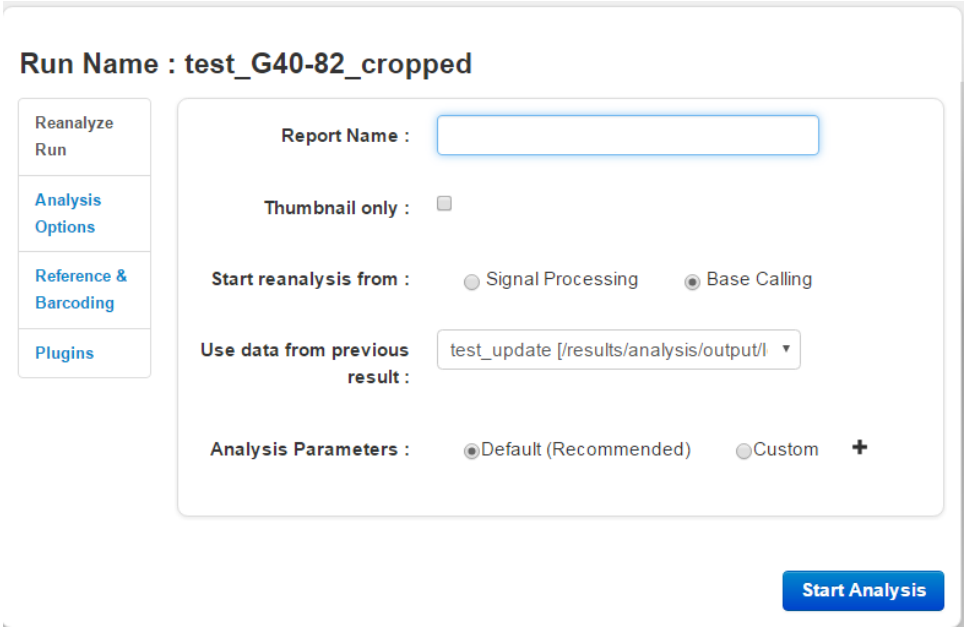
Follow these steps to restart an analysis run:

1. In the **Data** tab, click **Completed Runs & Reports**, then find the name for the report that you want to reanalyze.
   - In the **Table View**, click **Settings (⚙) ▸ Reanalyze** in the row of the run that you want to reanalyze:

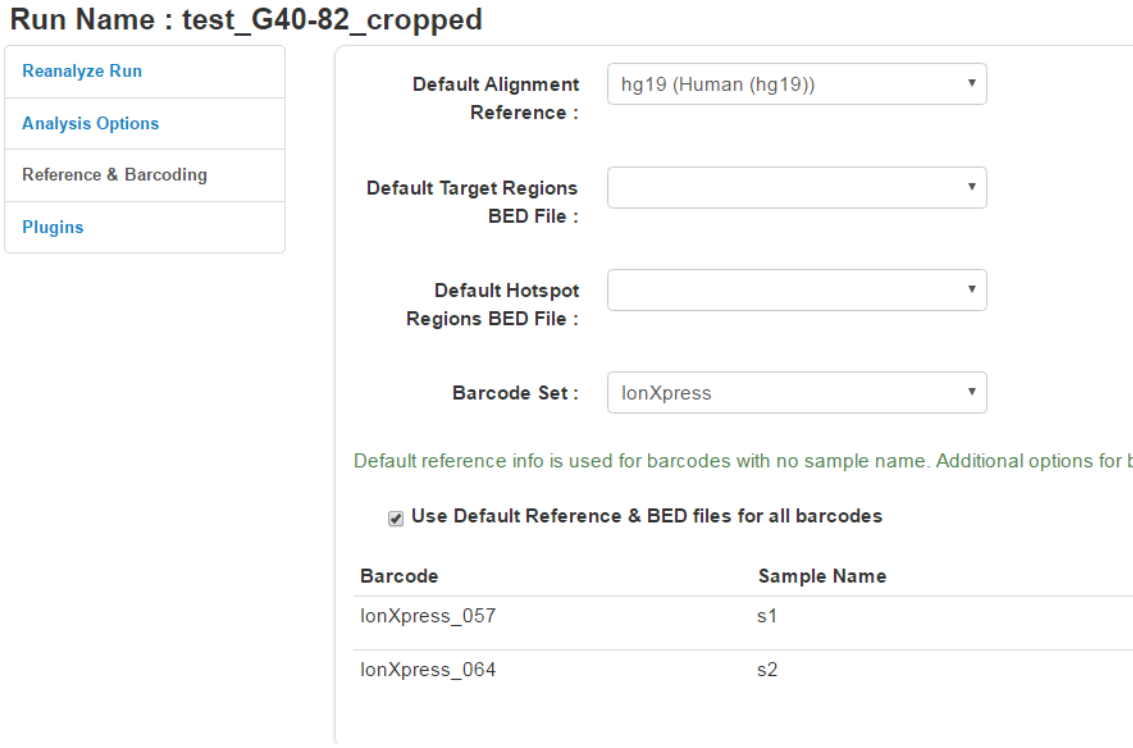


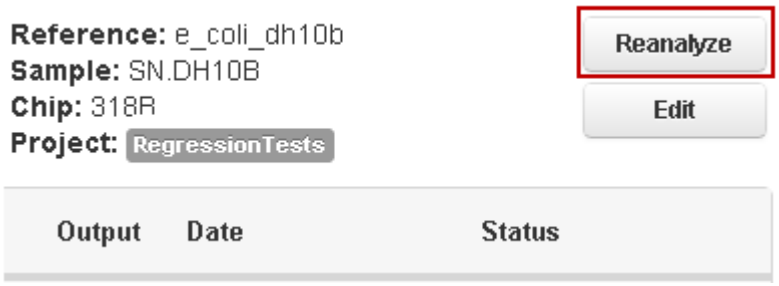
   - In the list view, click **Reanalyze** in the row of the run that you want to reanalyze:

The main run analysis dialog opens:

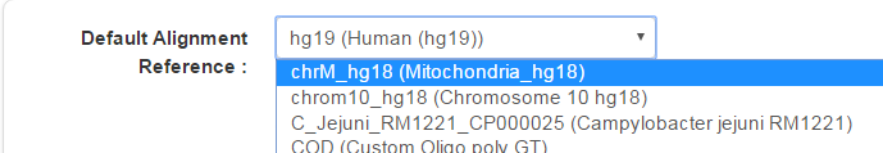


2. *(Optional)* Click **Reference & Barcoding** to display the additional options for references. Here you can select a different reference for the entire run or a specific reference for each barcode.

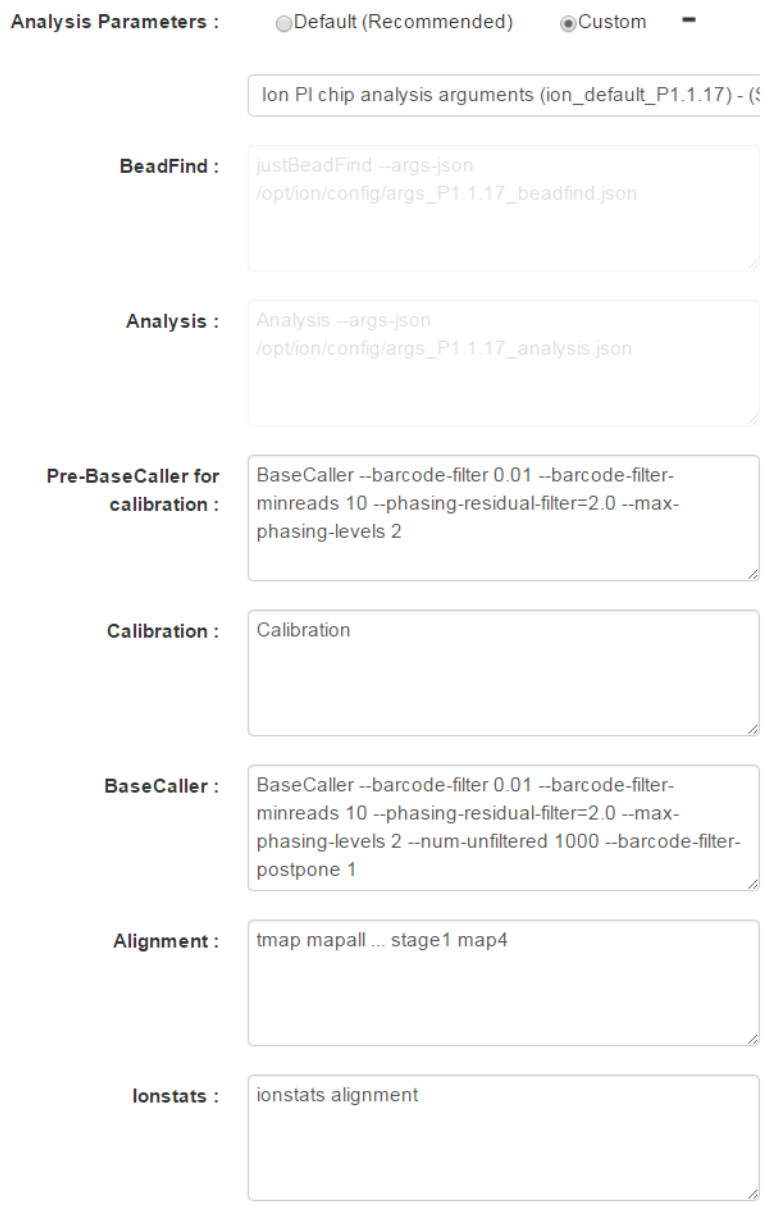




3. *(Optional)* In the **Default Alignment Reference** section, select a different reference for this run from the list of available references.



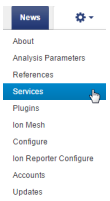
4. (*Optional*) Click **Analysis Options**, then click **Custom** to modify other options as appropriate.



5. (*Optional*) Click **Plugins** to select one or more plugins to run.

6. Click **Start Analysis**.

7. Click **Settings** (⚙) ▸ **Services** to ensure that the job has started and is listed in **Active Jobs**:

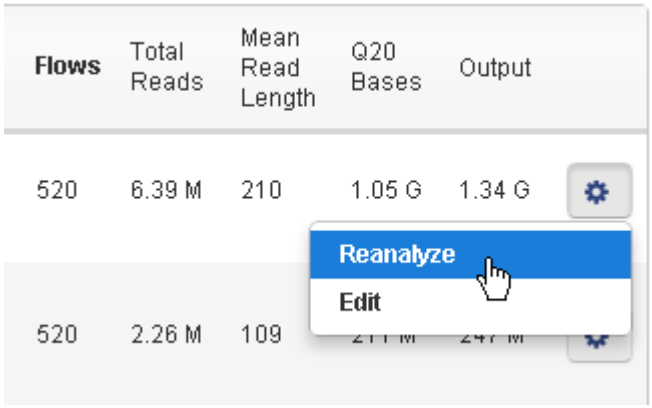


Realign a run to a different reference genome

This section describes how to rerun an analysis with alignment to a different reference genome.

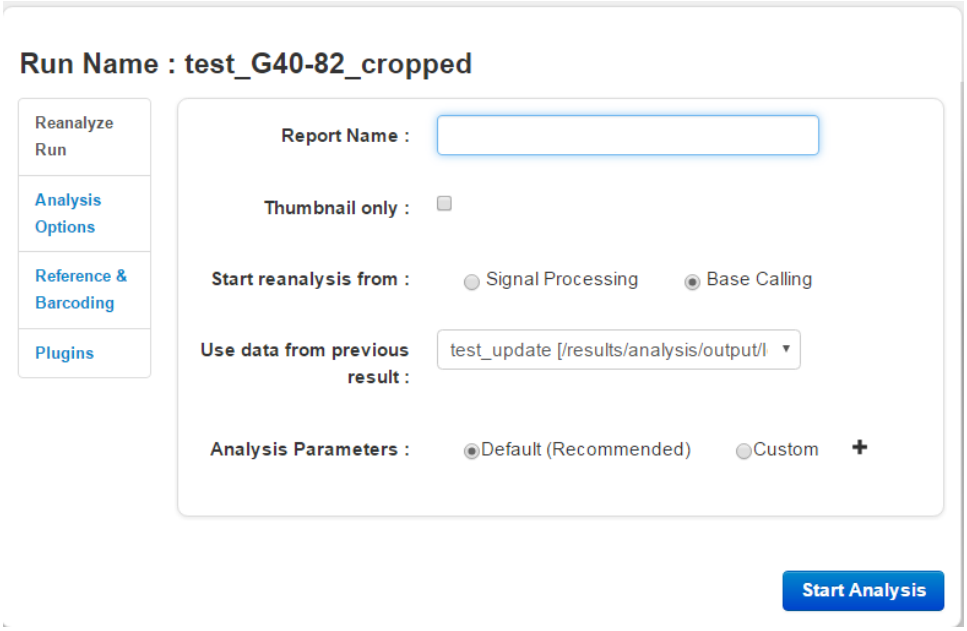
These steps create a new run report.

1. In the **Data** tab, click **Completed Runs & Reports**, then find the name for the report that you want to reanalyze.
   - In the **Table View**, click **Settings** (⚙) ▸ **Reanalyze** in the row of the run that you want to reanalyze:

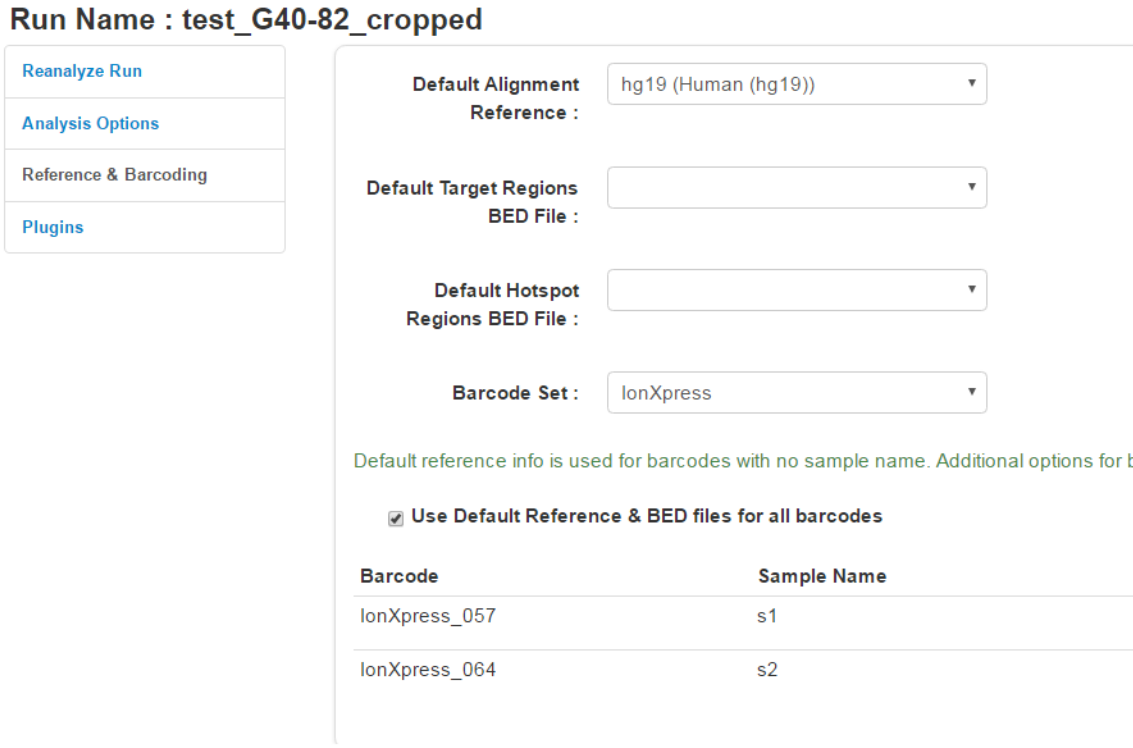


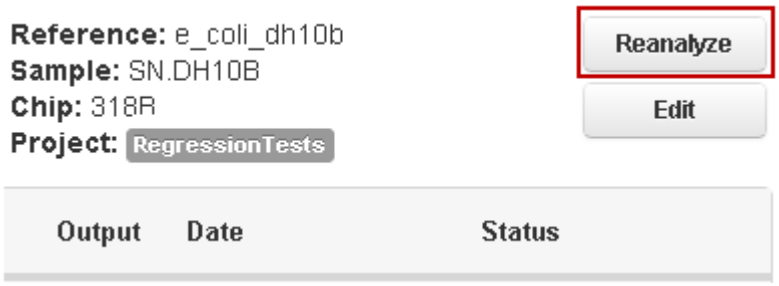
   - In the list view, click **Reanalyze** in the row of the run that you want to reanalyze:

The main run analysis dialog opens:

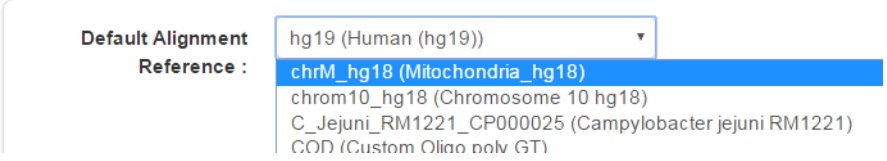


2. *(Optional)* Click the **Reference & Barcoding** tab to display the additional options for references. Here you can select a different reference for the entire run or a specific reference for each barcode.





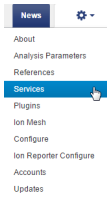
3. *(Optional)* In the **Default Alignment Reference** section, select the reference for this run from the list of available references.



4. (*Optional*) Click **Analysis Options**, then modify other options as appropriate.

5. Click **Start Analysis**.

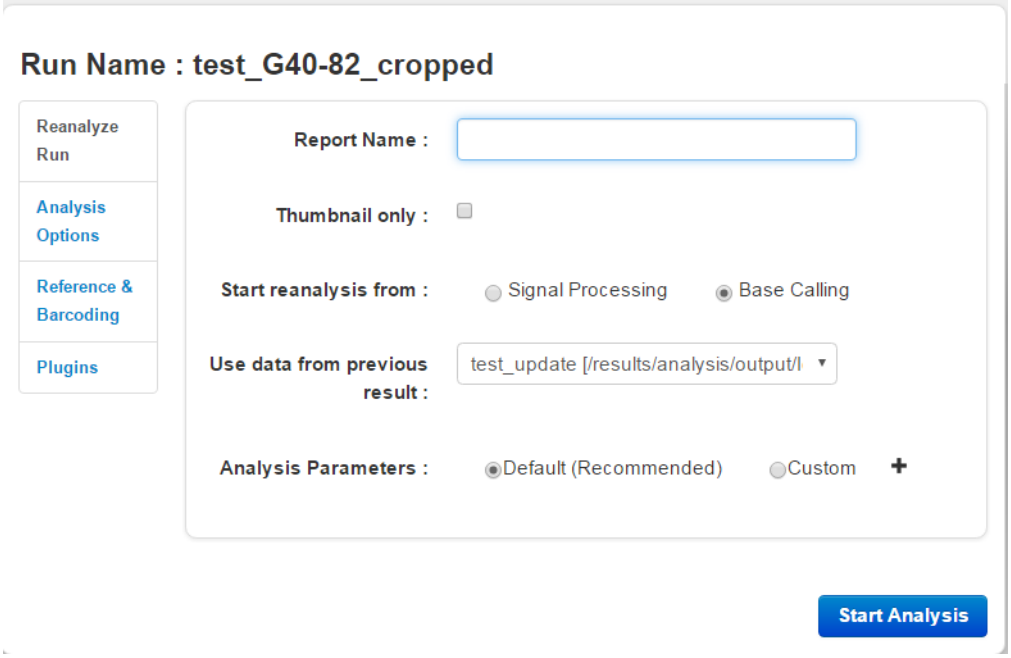
6. Click **Settings (⚙) ▸ Services** to ensure that the job has started and is listed in **Active Jobs**:



Reanalyze a run

Click **Reanalyze** to enter a name for the new run report and the reanalysis starting point:

Click **Reanalyze** to enter a name for the new run report and the reanalysis starting point:



Setting	Description
Report Name	The name of the new run report (the result of the reanalysis).
Thumbnail only	Displays thumbnail view of report.
Start reanalysis from	The Analysis Pipeline proceeds through three stages: Signal Processing, Base Calling, and Alignment. Normally report generation proceeds through all three steps. If you have already generated a report, it is possible to reanalyze the experiment and skip the earlier stages of the pipeline. For example, you can change the genome that is used for Alignment. After changing the genome for the experiment on the Runs screen using the Edit field, you need to reanalyze data to produce a new report using the new genome. Because there is no need to repeat the time consuming Signal Processing and Basecalling steps, you can use the output from an existing report as a starting point for Alignment. The report is completed much more quickly. You can restart the analysis from these points:

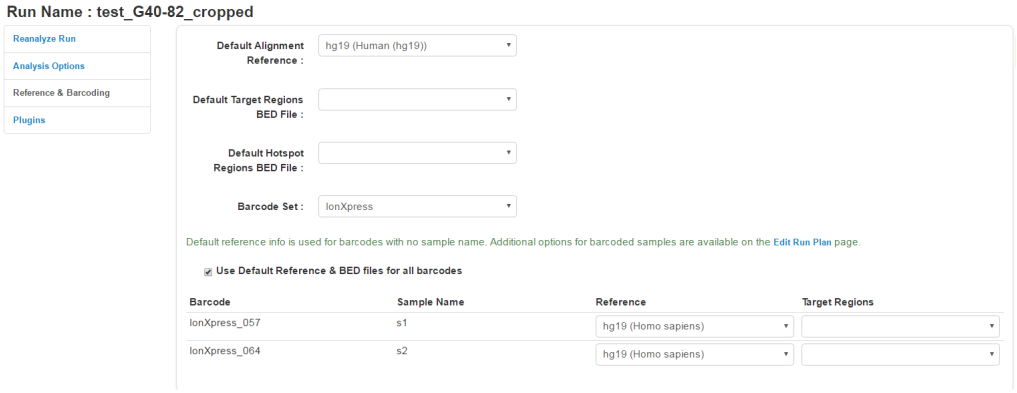
Setting	Description
	• **Signal Processing** (Default) Does not use the **Use data from previous report** field. Reprocesses from the DAT files. You can optionally use both the **Analysis args** and **Basecaller args** fields. • **Base Calling** Uses the **Use data from previous report** field and optionally the **Basecaller args** field. Reprocesses from the .wells file. Does not use the **Analysis args** field **.**
Use data from previous result	This option applies only when starting reanalysis from Base Calling. In this case, the results from a previous report are used as input for reanalysis.
Analysis Parameters	**Default (Recommended)** are the parameters determined to best fit the factory template. **Custom** interface allows you to change many aspects of the analysis parameters. For more information, see"Configure and select a custom analysis parameter set" on page 412

Reference & Barcoding settings

The References tab contains these settings:

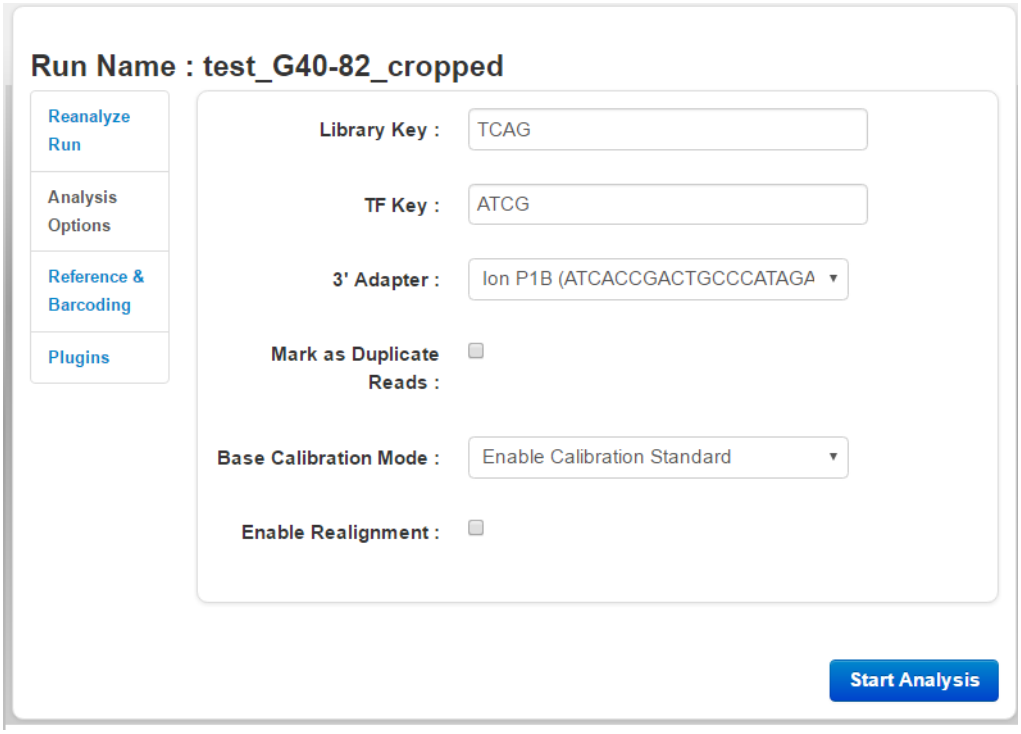
Setting	Description
Default Alignment Reference	The genomic reference to align to. Use this menu to change the reference used for alignment in the new analysis.
Default Target Regions BED File	Targeted regions of interest file. Analysis is restricted only to regions listed in this file.
Default Hotspot Regions BED File	Hotspots file. The variant caller includes each hotspot position in its output VCF file. Variant caller filter scores are provided for each hotspot position that does not have a variant called.
Barcode Set	The DNA barcode set.

Select specific references for specific sample barcodes.



Analysis Options

An example Analysis Options dialog is shown here:

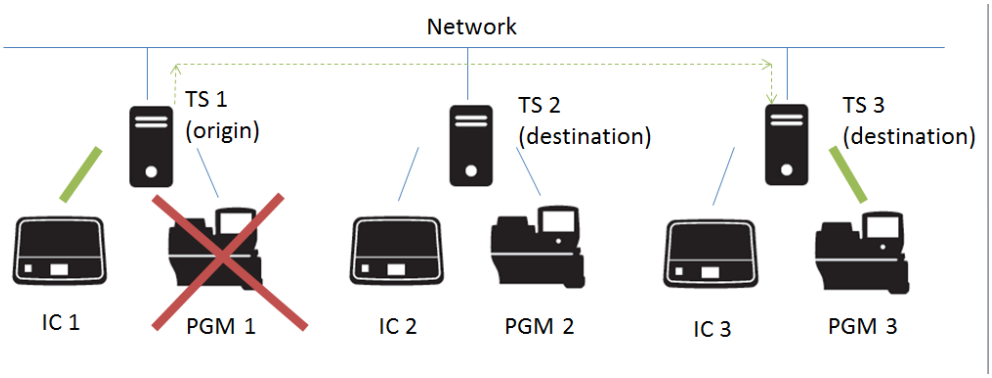


The Analysis Options tab contains these settings:

Setting	Description
Library Key	Enter the sequence used to identify library reads. Example: "TCAG".
TF key	Enter the sequence used to identify test fragment reads. Example: "ATCG".
3' Adapter	Enter the name and sequence of the 3' adapter.
Mark as Duplicate Reads	Enable Filter out PCR duplicates. Useful when reanalyzing combined BAM files. Do not use with Ion AmpliSeq™ data.
Base Calibration Mode	Select one of the four options that are available: Default Calibration, Enable Calibration Standard, Blind Calibration, and No Calibration.
Enable Realignment	*(Optional)* Perform realignment, an optional analysis step that is executed right after TMAP. This step adjusts the alignment, primarily in the CIGAR string.

Share a Planned Run among multiple Torrent Servers

If you have multiple Torrent Servers and multiple sequencers, you can create a Planned Run on one Torrent Server. If the dedicated sequencer is offline, you can now transfer your Planned Run to another Torrent Server, then run it on a different sequencer. However, an administrator or an Ion Torrent™ field service representative must first set up this networking capability.



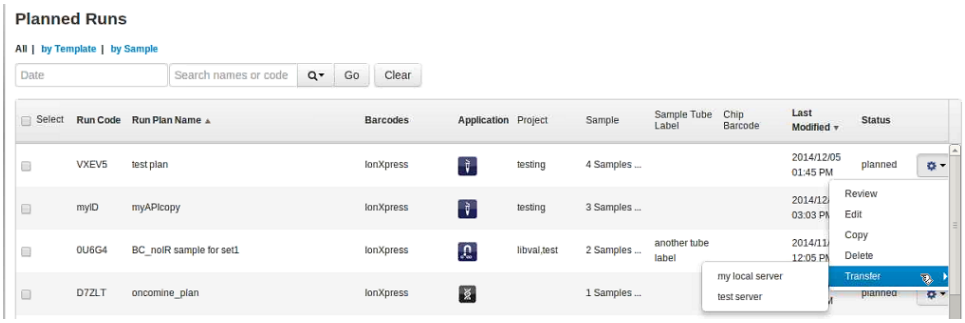
Using this diagram as an example, a Planned Run and associated chip can be set up on Torrent Server 1 (TS) 1 and Ion Chef™ 1 (IC 1). If Ion PGM™ 1 (PGM 1) is offline, you can transfer the planned run to TS 3, then run it on PGM 3 (or transfer to TS 2 and run it on PGM 2, if also networked).

To transfer a Planned Run:

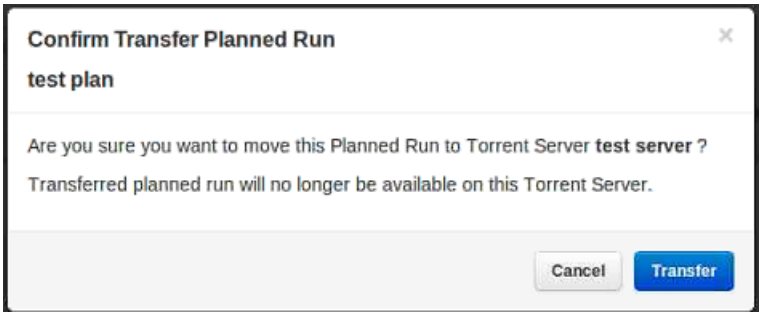
1. On the *origin* Torrent Server, create a plan for Ion Chef™, execute Ion Chef™ plan, then monitor the Ion Chef™ run.

2. After the Ion Chef™ run is complete, browse to the Planned Run list in Torrent Suite™ Software on the *origin* Torrent Server.

3. Click Settings (⚙) for the selected Planned Run, select **Transfer**, then click the *destination* Torrent Server.

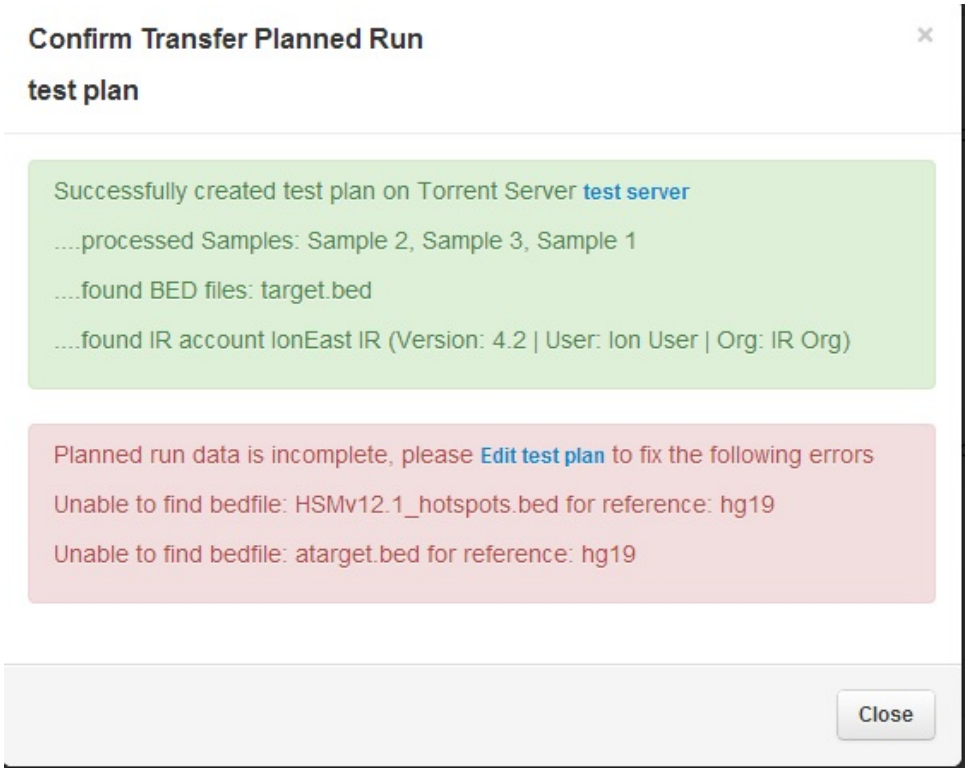


4. A confirmation window appears. Check the information, then click **Transfer**.



Note: You can no longer access this planned run on the origin server after it has transferred. A status window appears. If the Planned Run copied successfully, a green box states what copied correctly. If any BED files are missing on the destination server, a red box states what is missing. Your Planned Run is transferred if the copy is successful. However, you need to edit the transferred Planned Run on the destination server and add the BED files or other missing

data to have a successful sequencing run. Click the Edit [plan name] link in the confirmation dialog to correct the Planned Run on the destination server.

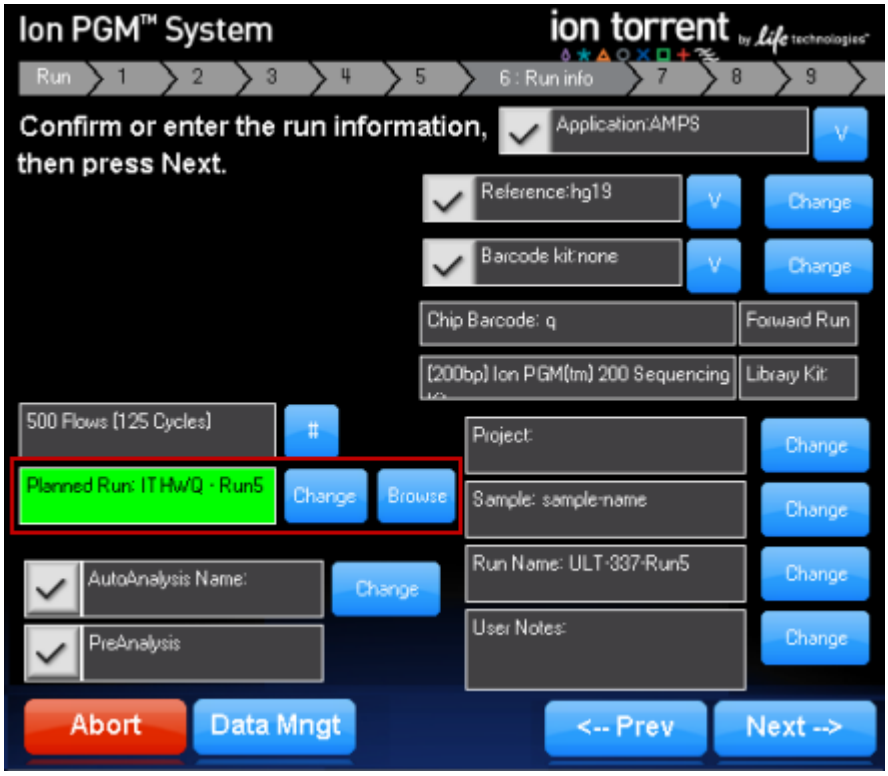


Note: If you need to move the results of a run back to the origin Torrent Server, you can use the Run Transfer Plugin to move results from the destination Torrent Server to the origin Torrent Server (or any other networked Torrent Server).

Execute a Planned Run on your sequencer

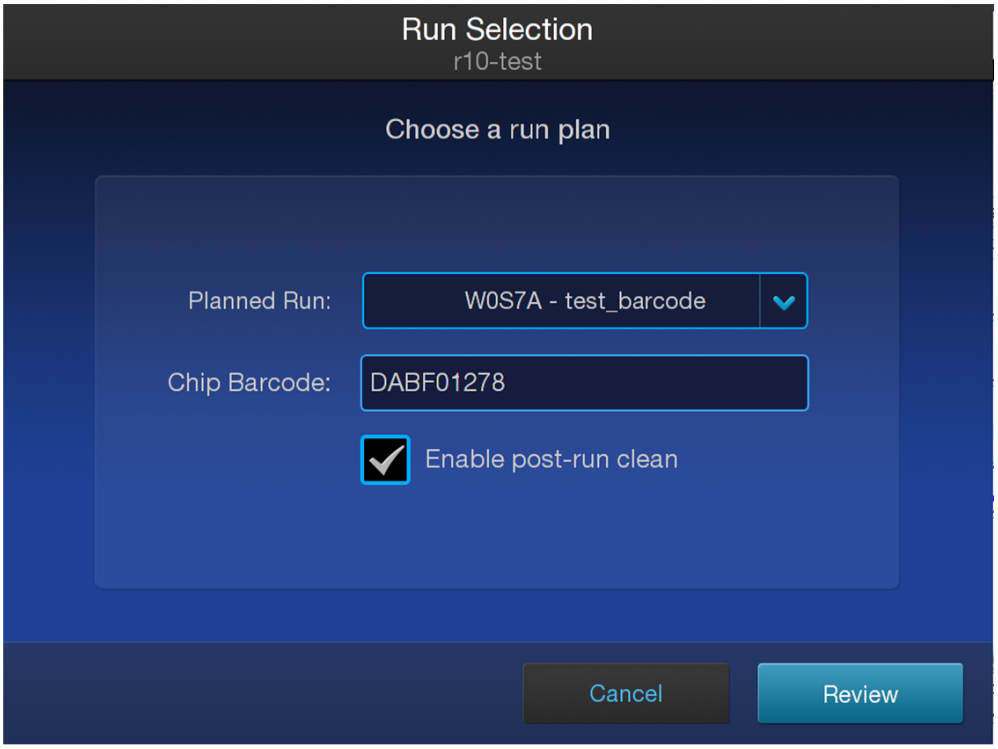
A Planned Run that you create in the Torrent Suite™ Software is executed on the Ion Torrent™ sequencer by selecting it from the run information dialog. With the **Browse** button, you can select a planned run from a list of runs previously created on the Torrent Suite™ Software. The **Change** button allows you to select a planned run via its run code.

The pending run information is populated into the run information dialog. You can optionally change run information on the **Run Info** screen. When ready, click **Next -->** to start your Ion Torrent™ sequencing run. Your Planned Run is removed from the **Plan ▸ Planned Runs** table when you approve the run confirmation.



The Planned Run short code can be entered by entering it manually from the touch screen. You can also type the Planned Run short code (for example, ITHWQ) into the **Pending Run:** text field on the run information dialog:

On the Ion S5™ Sequencer, select your run from the **Run Selection** screen.



Monitor instrument runs

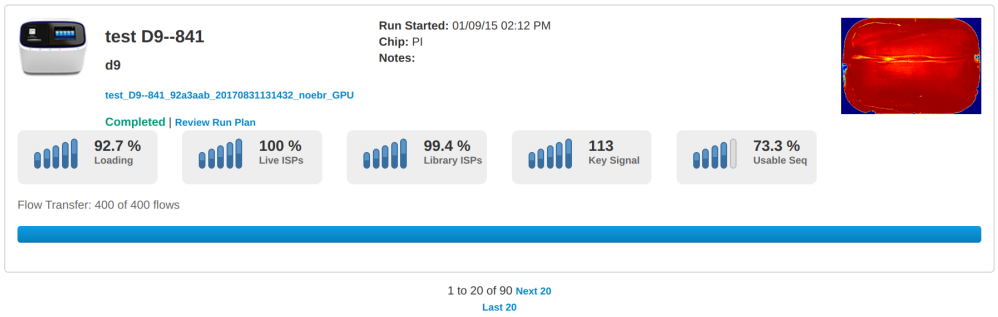
Monitor the sequencing run

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 avaialble 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 301 for more information if you decide to abort the sequencing run based on the chip loading metrics observed.

Monitor an Ion Chef™ run

1. In the **Monitor** tab, click **Ion Chef**.
   Runs in the previous 7 days are listed. The listed parameters are shown in the following table.

Parameter	Definition
Last Updated	Date and time of the currently displayed run status.
Sample Set	See "Samples and Sample Sets" on page 16 for details.
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.

2. 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 need to remove ISP samples at the QC pause, or remove loaded chips for sequencing.

Manually change Ion Chef™ run status

An Ion Chef™ Instrument run must have a status of **Planned** before an Ion PGM™, Ion Proton™, or Ion S5™ Sequencer can start a sequencing run. If connectivity is temporarily lost or interrupted between an Ion Chef™ Instrument and Torrent Suite™ Software, the status of the Ion Chef™ run might be marked as **Reserved**, even if you observe in the laboratory that 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**.

   There are four status types, which are displayed in the **Status** column, that can be assigned to a Planned Run:

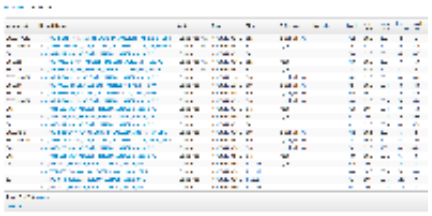
Status	Description
Pending	The Planned Run is available and ready for use by an Ion Chef™ Instrument, and is unavailable for use by a sequencing instrument.
Reserved	The Planned Run is in use by an Ion Chef™ Instrument, and is unavailable for use until the current Ion Chef™ run completes.
Planned	The Planned Run is available and ready for use by a sequencing instrument.
Voided	The Ion Chef™ run is cancelled through the Ion Chef™ screen.

2. For the Planned Run of interest, click ⚙ ▸ **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.

Data views for runs in progress

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

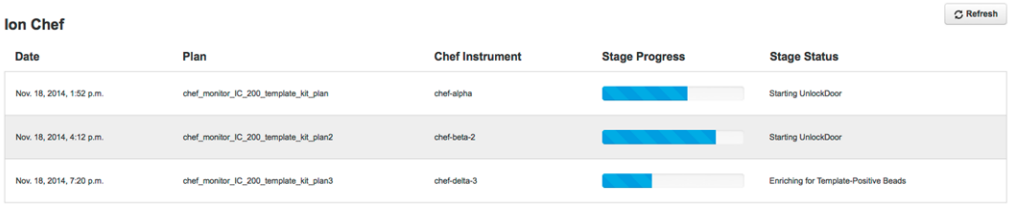


This section has two views:

- **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, and you can paginate.

Also in the **Monitor** tab, you can click Ion Chef™ to access views of current runs 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:

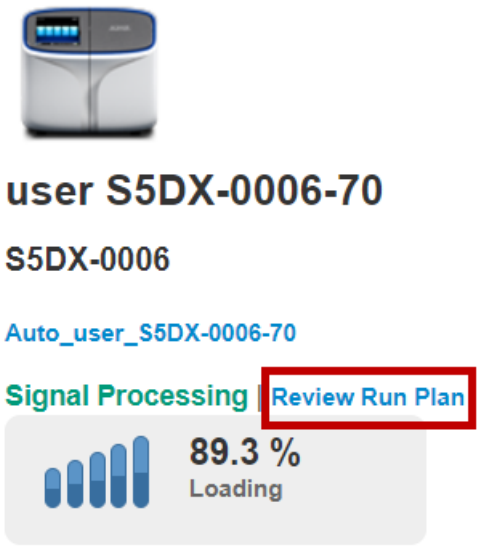
  Note: The Ion Chef page does not automatically refresh.

  a. In the **Monitor** tab, click **Ion Chef** ▸ **Refresh**.

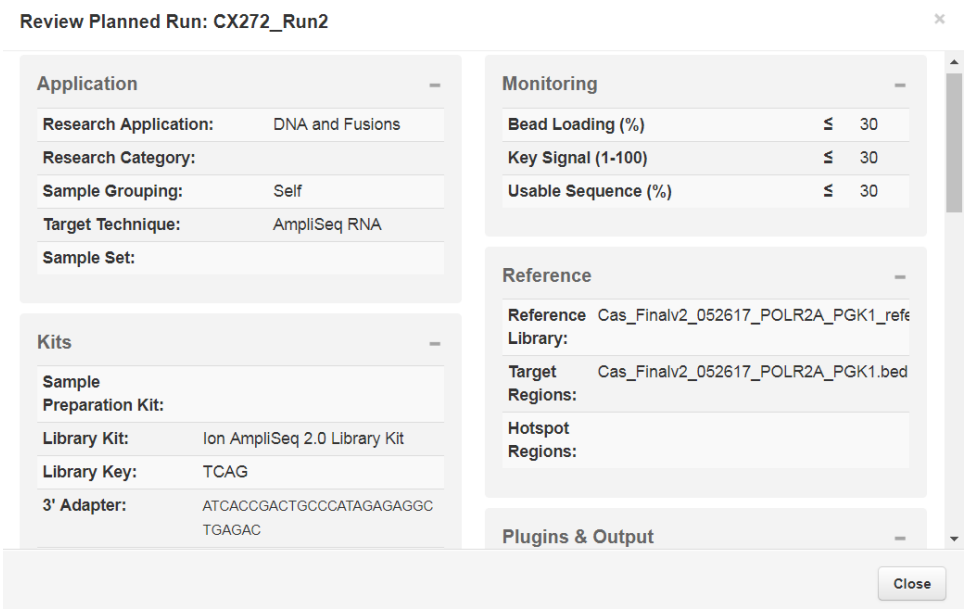
Review the Planned Run settings

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 report

You can reanalyze a run report 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 65 for more information.

3. In **Table View** mode, in the row of the Run report of interest, click **Settings** (⚙) ▸ **Reanalyze**.

   Alternatively, switch to **List View**, find your Run report 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 and Ion S5™ System data.
Start reanalysis from:	Select Signal Processing to reanalyze from DAT files. Select Base Calling (default) to reanalyze from 1.wells files.
Use data from previous result:	Select the previous result from the dropdown list if more than one result is available.
Analysis Parameters:	See "Configure and select a custom analysis parameter set" on page 412 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 if needed to set the default options.

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 116, or the configuration topic specific to the selected plugin.

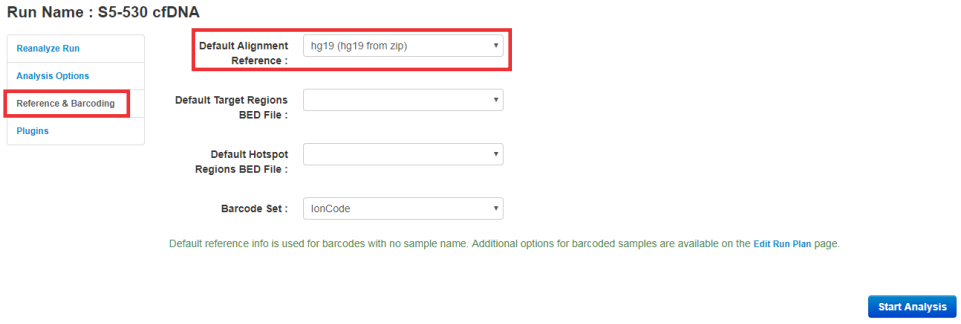
c. Repeat steps a and b to include additional plugins in the reanalysis.

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. Navigate to the run of interest.
   See "Search for a Run report" on page 65 for help finding an individual run.
3. For that run, click **Settings** ( ) ▸ **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**.

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 65 for more information.

3. Open the **Edit Run** wizard.
   - In **Table View**, click **Settings (⚙) ▸ 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 ("Reanalyze a run" on page 51), but you can also use the **Edit** option for a completed Run to change barcoding for all future reanalyses. You can:

- Add barcoding to a Run.
- Change the barcode set for a Run.
- Remove barcoding from a Run.

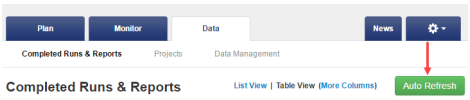
In each case, you must reanalyze the Run after editing the barcode information. These steps apply only to completed Runs.

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 65 for help finding an individual Run.
3. Click **Settings (⚙) ▸ Edit** for that 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.

Organize run results with projects

Projects are groups of results 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:

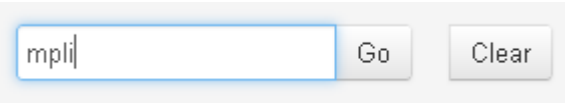
- Quickly find and view details for a group of run results
- Search the list by project name or partial name, or by date (date range, current month, current week, current day, or specific date).
- Rename, or delete a project.
- View a history log for a project.

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

Search for projects by name

You can search for project by name in the list of projects.

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



2. Enter your search criteria **Search names** field, then click **Go**. The displayed information is limited to only names that match or contain the search string.
   - 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. Wildcards are not supported in the search string.
3. Click **Clear** to cancel the search, then display unfiltered results.

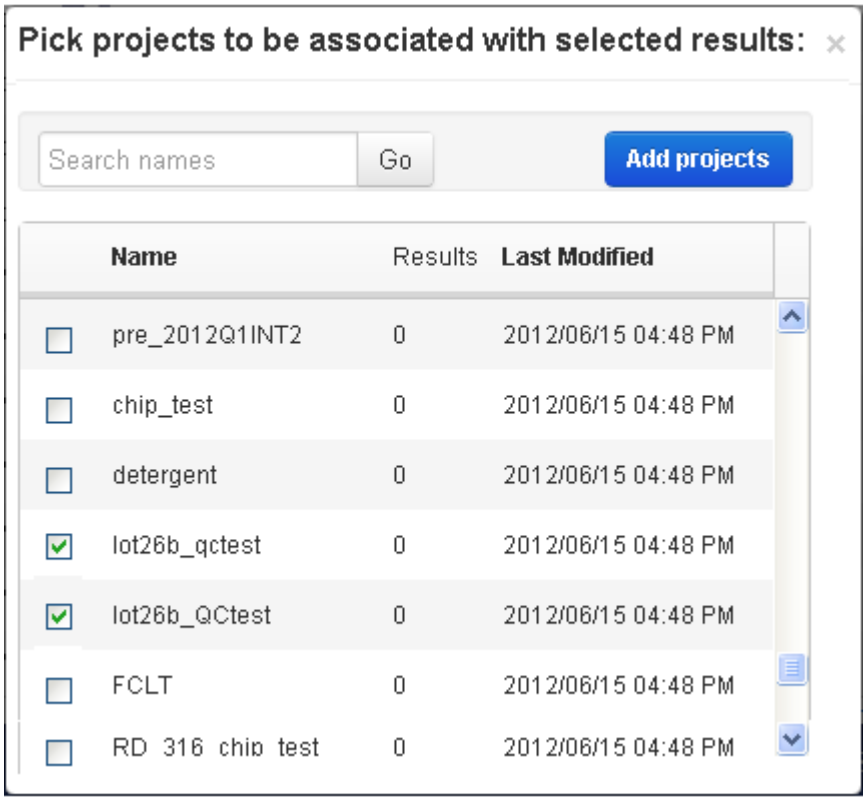
Sort projects or result set lists

Click any column heading that is in bold type to sort the list of projects or the result sets for projects. Click the heading a second time to reverse the sort.

Add a Run report to a project

You can add a completed run report to a project.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Find the Run report that you want to add to a project. For that report, click **Settings (⚙) ▸ Add Report To Project**
3. Select the project (or projects) to which you want the Run report added:



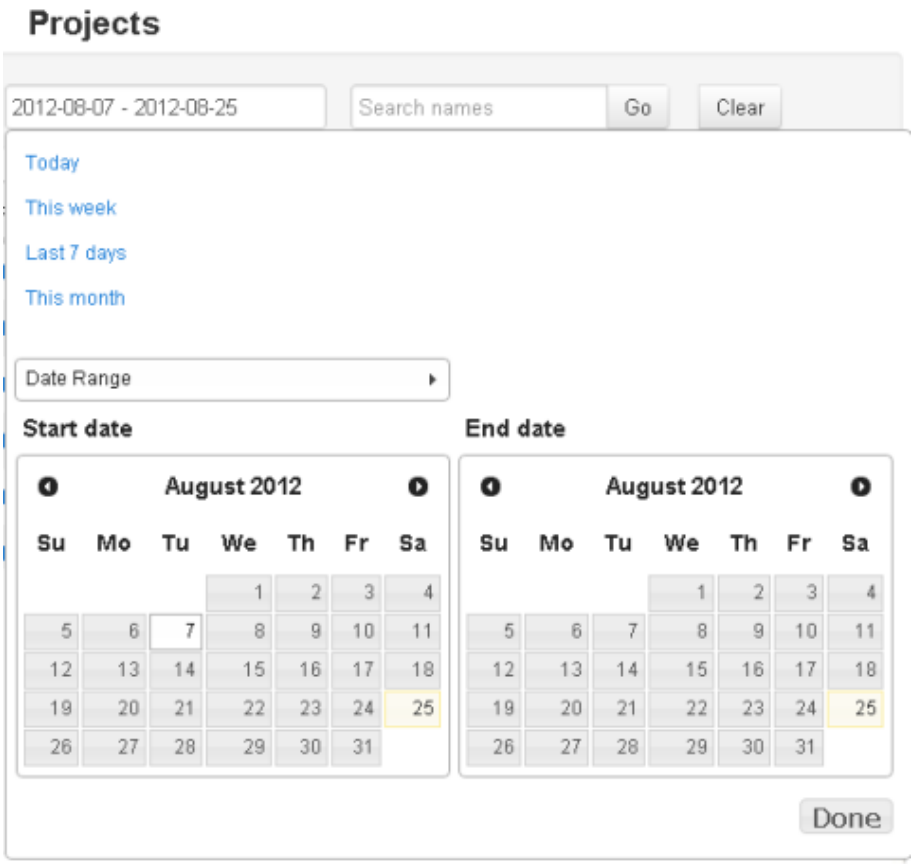
4. Click **Add projects**. The Run report is added to the selected project or projects.

Filter projects or result sets by date

You can find use dates or date ranges to search for projects, or for results sets that are contained in a project.

1. Do one of the following:
   - To search for a project, in the **Data** tab, click **Projects**.
   - To search for results sets, click a project name.

2. Click the **Report Date** field for a search that takes effect immediately (depending on server load):
   Select one of the following to find use a range of dates in the search:
   - **Today** selection enters the current date in the **Start date** field and limits the run table display only to jobs with a run date from the current date.
   - **This week** to search a date range from Monday to the current day.
   - **Last 7 days** to search the seven days before today.
   - **This month** to search the entire current month.
   - **Date Range** to search with calendars for the range start date and one for the range end date. The current date is shown in pale yellow. If a date range is currently selected, those dates are shown in white.

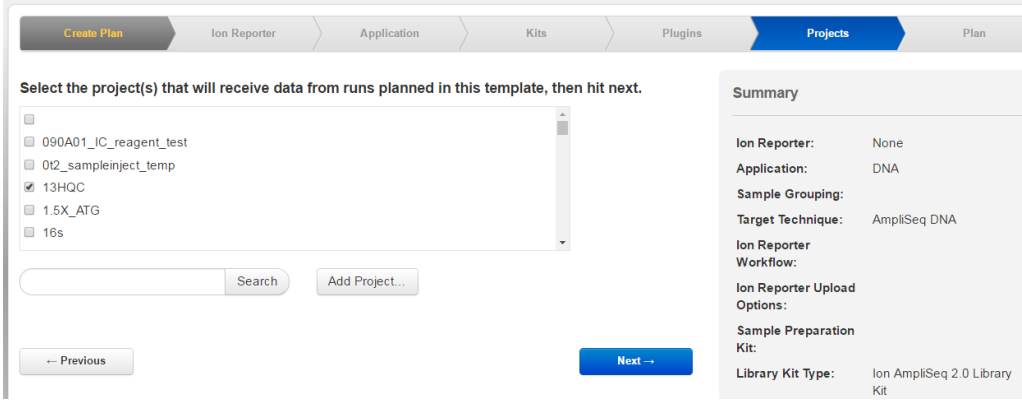


   - (*Optional*) Enter a **Date Range**, or edit the date range in the field.
   - (Optional) Click **Clear** to cancel filtering and return to the full results listing.
   - Click **Done** to perform the search.

Add a project to a Planned Run

You can designate which results 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:

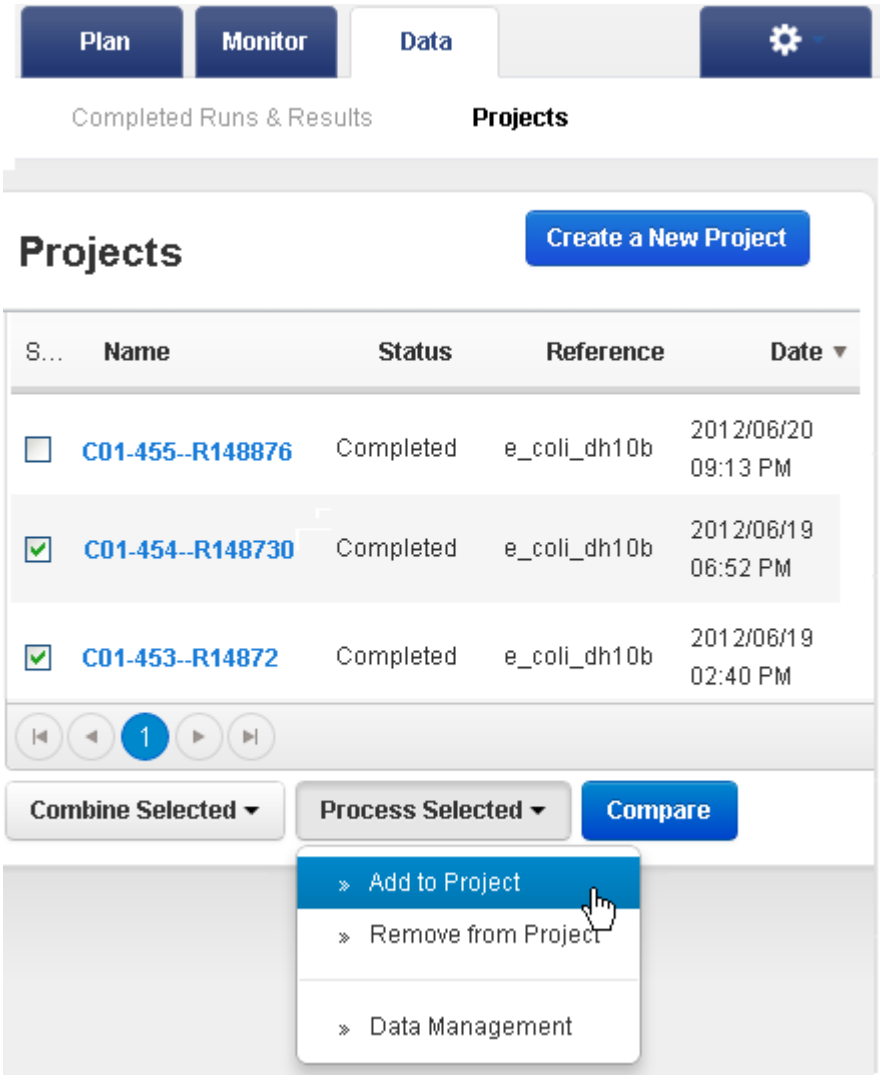


See “Plan an instrument run“ on page 25 for more details.

Add selected results to another project

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

3. Select the checkboxes for 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**.

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 results 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 290, "Manually archive run data" on page 291 and "Manually delete run data" on page 292.

Combine aligned reads from multiple run reports

You can use projects to combine aligned reads from multiple run reports. The resulting data set can be treated the same results from a single analysis run, for example to export or to use as input for a plugin. Use this option when multiple runs analyze the same tissue sample, for example when a tissue sample is run on more than one chip. All reports must be aligned to the same reference.

1. In the **Data** tab, click **Projects** to see the list of projects.
2. Select a project in the list to view the results sets in the project.
3. Select the result set or sets that you want to combine into a single Run result set.

Option	Description
Mark as duplicate	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. 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 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	

4. *(Optional)* Click **Report** to open the summary of the report, or **Log** to open the log for the report.
5. Click **Combine Selected ▸ Combine Alignments**.

The result sets are combined into a single Run report that is added to the list of projects.

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 results 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.

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. Select a project in the list to view the results sets in the project.
3. Select the checkboxes of the result sets that you want to remove from the project, then click **Process Selected ▸ Remove from Project**.

Run Reports

Introduction

A Torrent Suite™ Software run report contains statistics and quality metrics for your run. From a run report you can do the following:

- Review pre-alignment metrics such as bead loading, Ion Sphere™ Particle (ISP) density, total number of reads, filtering numbers, and mean read length
- Review alignment metrics such as total aligned bases, average coverage, and mean raw accuracy
- Download the result set
- Manually run a plugin on the run results
- Review the planned run settings
- Review the test fragments used with this run and test fragment quality metrics
- Review Chef Summary
- Review Calibration Report
- Review analysis information and Torrent Suite™ Software versions
- Review the analysis log
- Generate a zip file for technical support

A run report is divided into the following main areas:

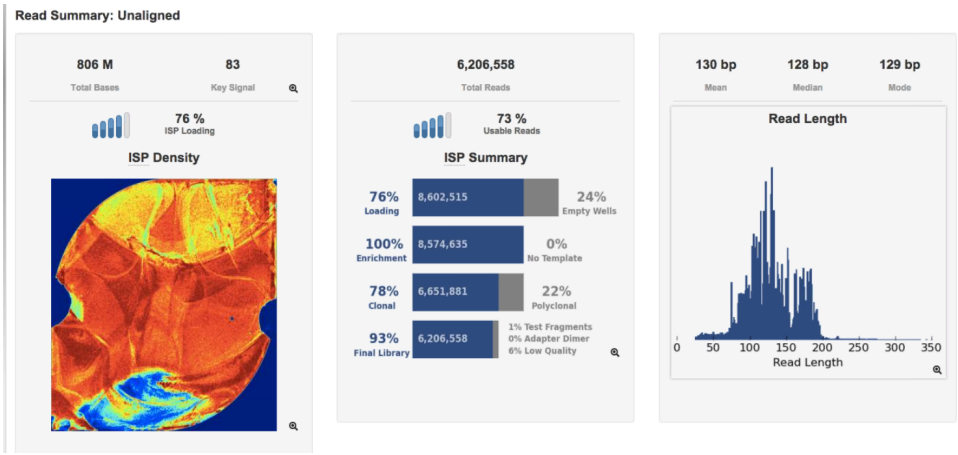
- **Report header** - Use this section to download the run report or summary in PDF format, to review the planned run settings for the run, to reanalyze the run, and to upload the run report output files to Ion Reporter™ Software. Also, change to a different result set for the same sample and use links to move to the Output Files or Plugin Summary sections of the run report.
- **Barcode Summary** - For barcoded runs, a barcode summary table appears above the Plugin Summary area.
- **Unaligned** - Metrics taken before alignment, including bead loading, ISP density and other metrics, read and filtering metrics, and read length.
- **Aligned** - Metrics on the aligned reads.
- **Plugin Previews** - Summary output of completed plugins (only if supported by the plugins that executed on this analysis).
- **Output Files** - Download read files for both before alignment and after alignment. Full-chip Ion Proton™ analyses only offer the download of aligned reads.
- **Plugin Summary** - Links to plugin reports and allows you to run plugins manually on a completed sequencing run.
- **Test Fragments** - Displays information about the performance of each test fragment included in the experiment.
- **Chef Summary** - Displays Ion Chef™ templating results.

- **Calibration Report** - Displays pre-base calibration and calibration arguments.
- **Analysis Details** - Displays a set of information about the sequencing run environment. For example, run date, sample name, chip type, instrument name, barcode set, and so on.
- **Support** - Displays a link to the report log and a link to generate information for technical support.
- **Software Version** - Displays the version of Torrent Suite™ Software and its modules.

Review pre-alignment metrics

When determining the quality of a run, first look at the unaligned metrics including: total bases, total reads, and mean and median read length. This information comes from the primary pipeline, base calling, and signal processing.

1. In the **Data** tab, click **Completed Runs & Reports** , then select a report of interest.
2. In the Unaligned section, review Total Bases, Total Reads and Mean, and Median Read Length to determine the quality of the run.

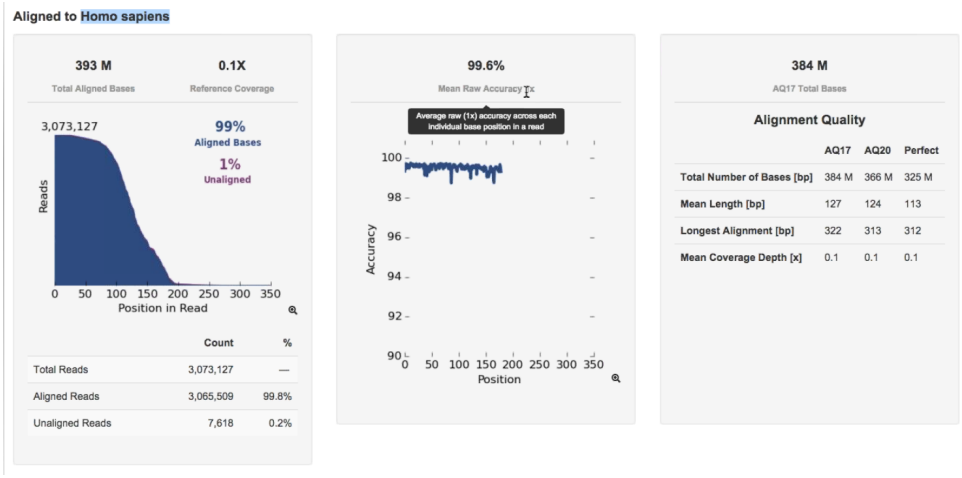


For more information on these metrics, see “Run report metrics before alignment“ on page 86.

Review alignment metrics

The secondary pipeline aligns the run to the reference. Here you can see how many bases align to the reference.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a run of interest.
2. Scroll down to the Aligned to *reference name* section, then review **Total Aligned Bases**, **Reference Coverage**, **Mean Raw Accuracy**, and **Total Bases Alignment Quality**.

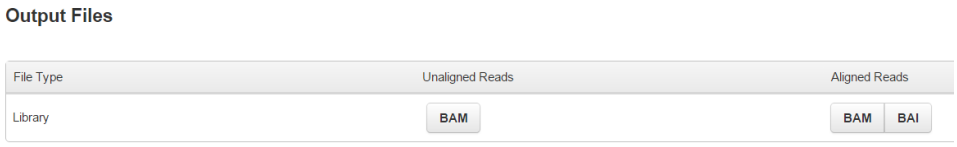


For more information on these metrics, see "Run report metrics on aligned reads" on page 95

Download results set

You can download the run results in several formats.

1. In the **Data** tab, click**Completed Runs & Reports**, then select a run of interest.
2. Scroll down to the **Output Files** selection, then choose your output type: **Unaligned reads BAM**, or **Aligned Reads BAM or BAI**.



For more information, see the "Output files" on page 101 section.

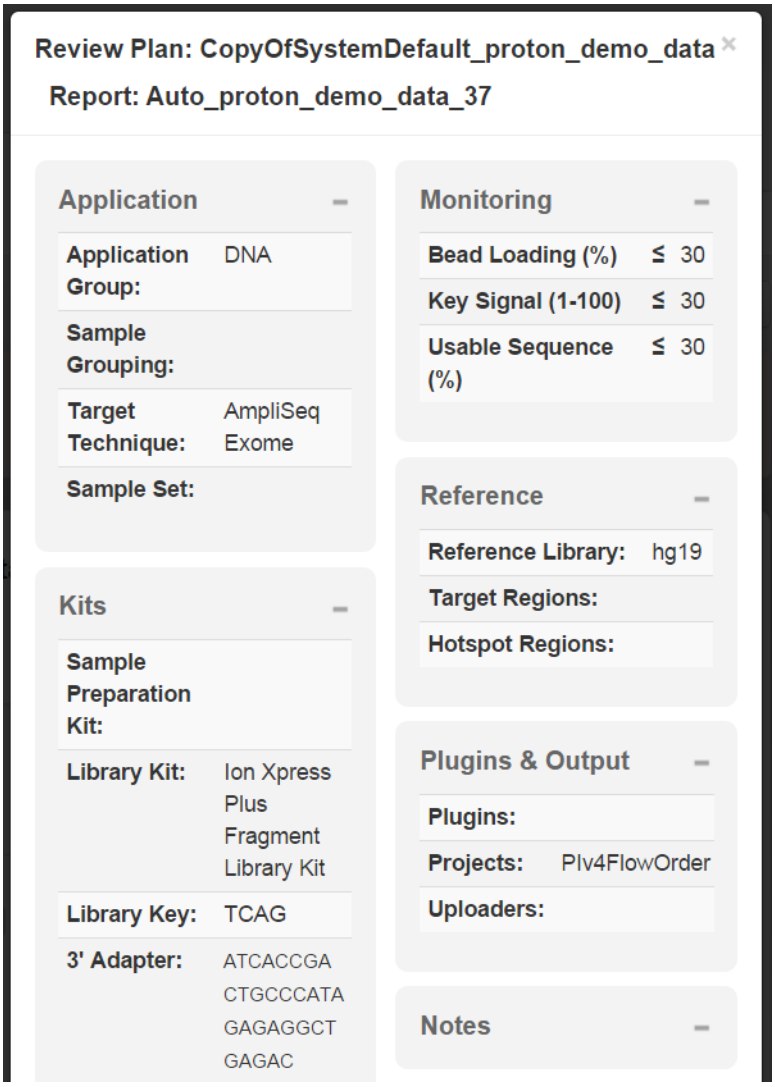
Manually run a plugin on the run results

After your run is complete, you can further your analysis by running various analysis plugins. For details, see "Manage plugins for data analysis" on page 114.

Review the planned run settings

You can review the planned run settings of a completed run.

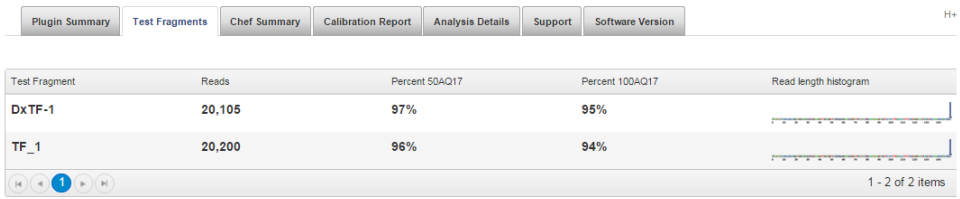
1. In the **Data** tab, click **Completed Runs & Reports**, then select a run of interest.
2. Click **Report Actions ▸ Review Plan**.
   A Review Plan window appears.



Review the test fragments and their quality metrics

If you included key signal test fragments in your run, you can review the test fragments, then evaluate their quality.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a run report of interest.
2. Scroll down to the bottom of the report, then click **Test Fragments**.

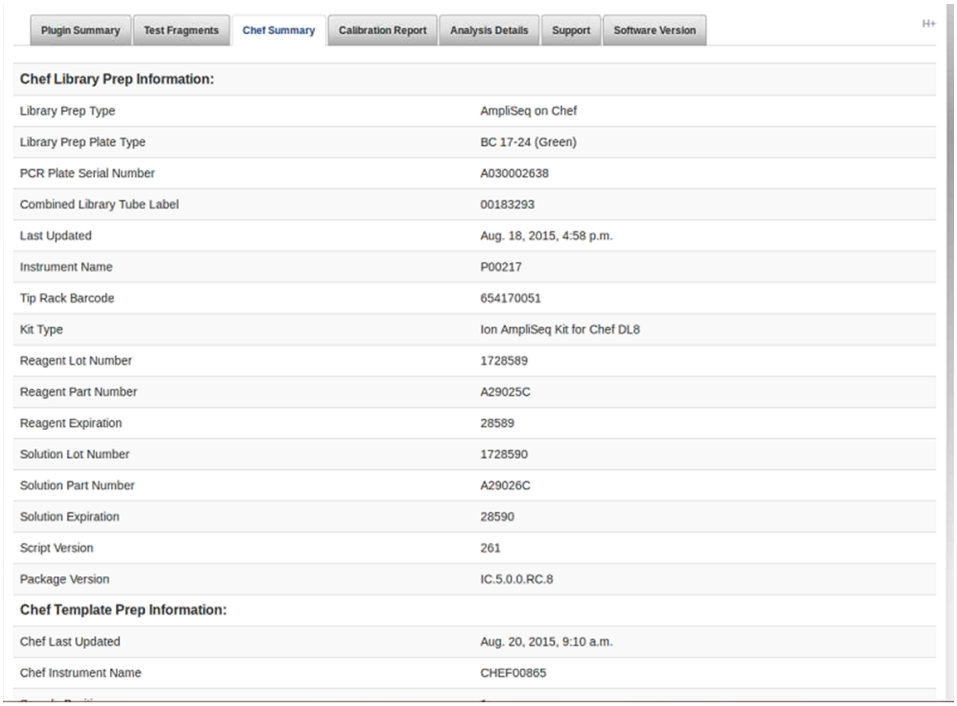


For more information, see "Test fragment report" on page 102.

Review Chef Summary

If you used an Ion Chef™ instrument, you can review library and template information.

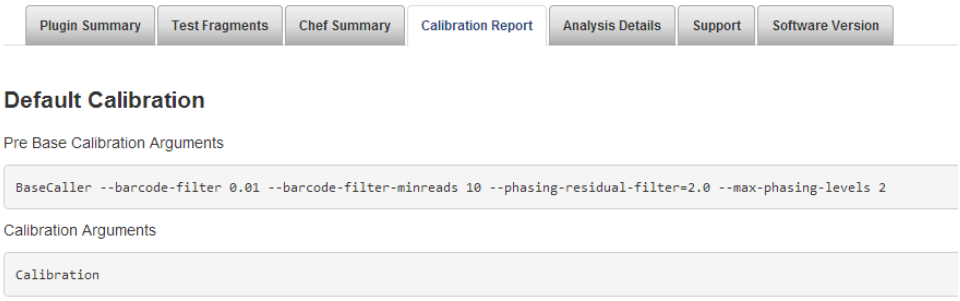
1. In the **Data** tab, click **Completed Runs & Reports**, then select a run which incorporated an Ion Chef™ instrument.
2. Scroll to the bottom of the run report, then click **Chef Summary**.
   Here you can review the library preparation and templating information.



Review calibration report

You can review calibration settings that are applied to a run in the Calibration Report.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a run report of interest.
2. Scroll down to the bottom, then click **Calibration Report**.
3. View your Pre Base Calibration Arguments and Calibration Arguments.



For more information, see "Base Calibration mode options" on page 389.

Review analysis information

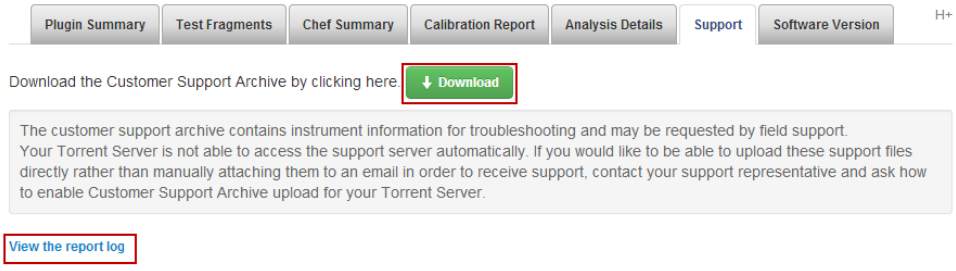
You can review the analysis details of a completed run.

1. In the **Data** tab, click **Completed Runs & Reports**, then select a run report of interest.
2. Scroll down to the bottom of the report and click **Analysis Details**.
   For more information, see "Analysis details" on page 104.

Review report error log

You can view the report error log when troubleshooting a run. If you need further help, you can generate a customer support archive to share with customer support.

1. Go to **Data ▸ Completed Runs & Reports** and select the run report of interest.
2. Scroll down to the bottom and click **Support ▸ View the report log** to see a list of errors.
3. If the error report does not help you resolve an issue with the run, click **Download** to generate a customer support archive that you can then send to your customer support representative for assistance.

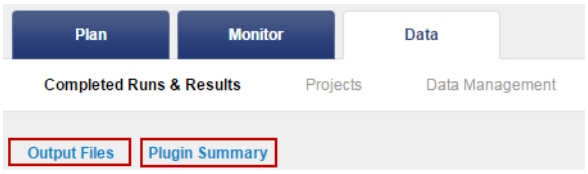


For more information, see "Support" on page 107.

Report header

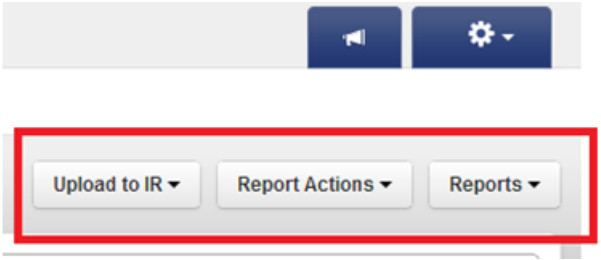
The left side of a run report header contains the following navigation links:

- **Output Files** Jumps to the Output Files area
- **Plugin Summary** Jumps to the Plugin Summary area (which also has the Test Fragment, Analysis Details, Customer Support, and Software Version buttons)

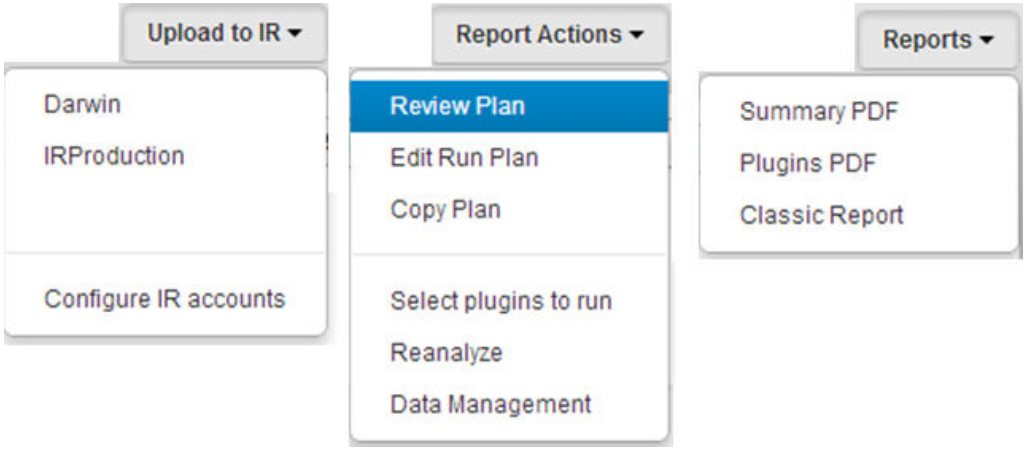


The right side of run report header contains buttons for the following:

- **Upload to IR** Copies the run report's output files to Ion Reporter™ Software.
- **Report Actions**
  - **Review Plan** Opens a summary page of the planned run information for this run
  - **Edit Run Plan** Opens an Edit Run page
  - **Copy Plan** Opens the run plan wizard with a copy of the run plan information for this run
  - **Select plugins to run** Opens the Select a plugin window
  - **Reanalyze** Starts a reanalysis of the run (you have the opportunity to changes settings first)
  - **Data Management** Opens the Data Management app, which you use to delete, archive, export, or mark as do-not-delete the files for this run report
- **Reports** Opens the run report of a different result set for the same sample
-
  - **Summary PDF** Downloads the run report summary in PDF format
  - **Plugins PDF** Downloads a summary of the plugin results in PDF format
  - **Classic Report** Opens the run report in Torrent Suite™ Software 2.x format



Drop-down options shown below:



Compare run reports

From a project listing page, you can compare report metrics for multiple runs side-by-side.

Run report metrics

This section provides background information on run metrics and detailed descriptions of a run report.

For analyses that are members of a project, you can download a CSV file of run metrics.

Run metrics overview

This section provides background information on quality metrics, read lengths, and alignment. These concepts are required to understand your run report.

The Torrent Browser Analysis Report gives performance metrics for reads whose first bases match the library key.

IMPORTANT! These reads are generated from the input library, not from the positive control **Test Fragments**.

Performance is measured based on either predicted quality or quality as measured following alignment. Q20 and AQ20 are explained as examples of predicted quality and quality following alignment.

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.

Note: Refer to **http://en.wikipedia.org/wiki/Phred_quality_score** for a more complete description of Phred values.

Quality following alignment (AQ20)

You can use Read Alignment to evaluate the quality of the sequencing reaction and the quality of the underlying library where an accurate reference is available. Reads are aligned to a reference genome. Any discrepancy in alignment to a reference (whether biological or technical, meaning a real variant or a sequencing error) is listed as a mismatch. Alignment performance metrics are reported depending on how many misaligned bases are allowed. Torrent Suite™ Software reports alignment performance at two quality levels:

- 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. As a result, 20 refers to an error rate of 1%, 17 refers to an error rate of 2%, and so on.

For example, 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 "perfect" length is the longest perfectly aligned segment.

For all 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 interpreting AQ20 values in situations where the sample sequenced has substantial differences relative to the reference used, such as working with alignments to a rough draft genome or with samples that are expected to have high mutation rates relative to the reference used. In these situations, the AQ20 lengths might be short even when sequencing quality is excellent.

Specifically, the AQ20 length is calculated as follows:

- 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 followed by 2 errors 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 two percent (2/100). The greatest length at which the error rate is one percent or less is 80 and the greatest length at which the error rate is two percent or less is 100, so the AQ20 and AQ17 lengths are 80 and 100 bases, respectively.

Alignment

In Torrent Suite™ Software, the goal is to provide you with a view on alignment that helps determine run and library quality.

There are many alignment algorithms available in the marketplace and you are encouraged to consult with a bioinformatician for the most appropriate alignment algorithm for your downstream analysis needs. Alignment algorithms are also embedded in many commercial software tools available in the Ion Torrent™ Web store. You are also encouraged to experiment with these tools.

Alignment in Torrent Suite™ Software is performed using TMAP. TMAP is currently an unpublished alignment algorithm, created by the authors of the BFAST algorithm. Contact your Ion Torrent™ representative or Technical Support for more information on TMAP.

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.BFAST: An alignment tool for large-scale genome resequencing. PMID: 19907642PLoS ONE. 2009 4(11): e7767. **http://dx.doi.org/10.1371/journal.pone.0007767**

Homer N, Merriman B, Nelson SF. Local alignment of two-base encoded DNA sequence. BMC Bioinformatics. 2009 Jun 9;10(1):175.PMID: 19508732. **http://dx.doi.org/10.1186/1471-2105-10-175**

Which reads are used in the alignment process

The alignment stage involves aligning reads produced by the pipeline to a reference genome and extracting metrics from those alignments. By default, Torrent Suite™ Software aligns all reads to the genome, however there may be situations, particularly with large genomes, where the alignment takes longer than you are willing to wait. So for such circumstances the Torrent Suite™ Software also 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 genome-specific maximum, a random sample of reads is taken and results are extrapolated to the full run. By sampling a quickly-aligned subset of reads and extrapolating the values to the full run, the software gives you sufficient information to be able to judge the quality of the sample, library, and sequencing run for quality assessment purposes.

The output of the alignment process is a BAM file. The BAM file includes an alignment of all reads, including the unmapped, 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 given a mapping quality of zero.

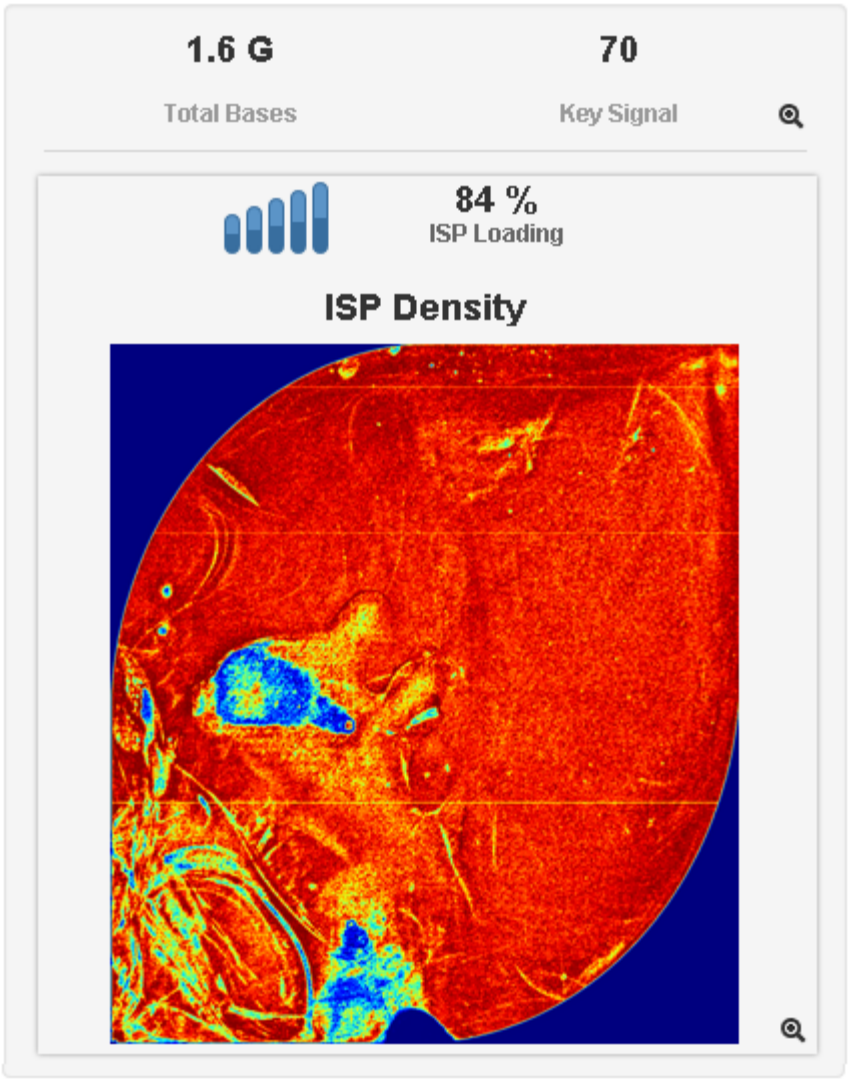
Run report metrics before alignment

The Unaligned area in the Run Summary section provides before-alignment metrics. There are three sections in the Unaligned area:

- ISP Density
- ISP Summary
- Read Length

Note: Click the magnifying glass icon in the run report to open a larger image.

ISP density



This table describes the Ion Sphere™ Particle (ISP) density metrics:

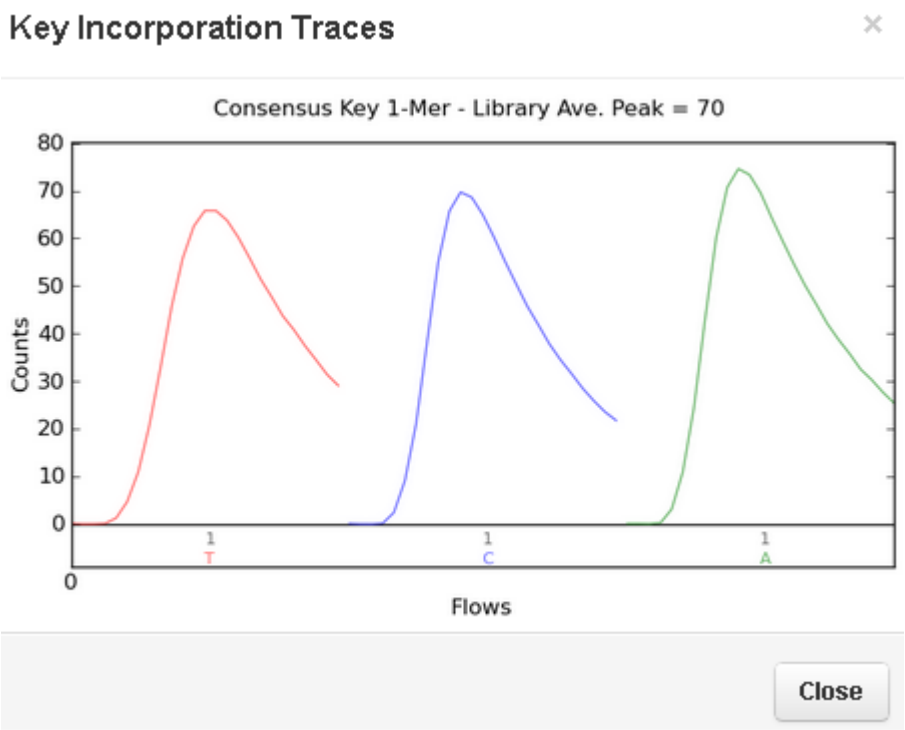
Metric	Description
Total Bases	Number of filtered and trimmed base pairs reported in the output BAM file.
Key Signal	Percentage of Live ISPs with a key signal that is identical to the library key signal.
Bead Loading	Percentage of chip wells that contain a live ISP. (The percentage value considers only potentially addressable wells.)

The ISP Density image is a pseudo-color image of the Ion Chip Plate showing percent loading across the physical surface.

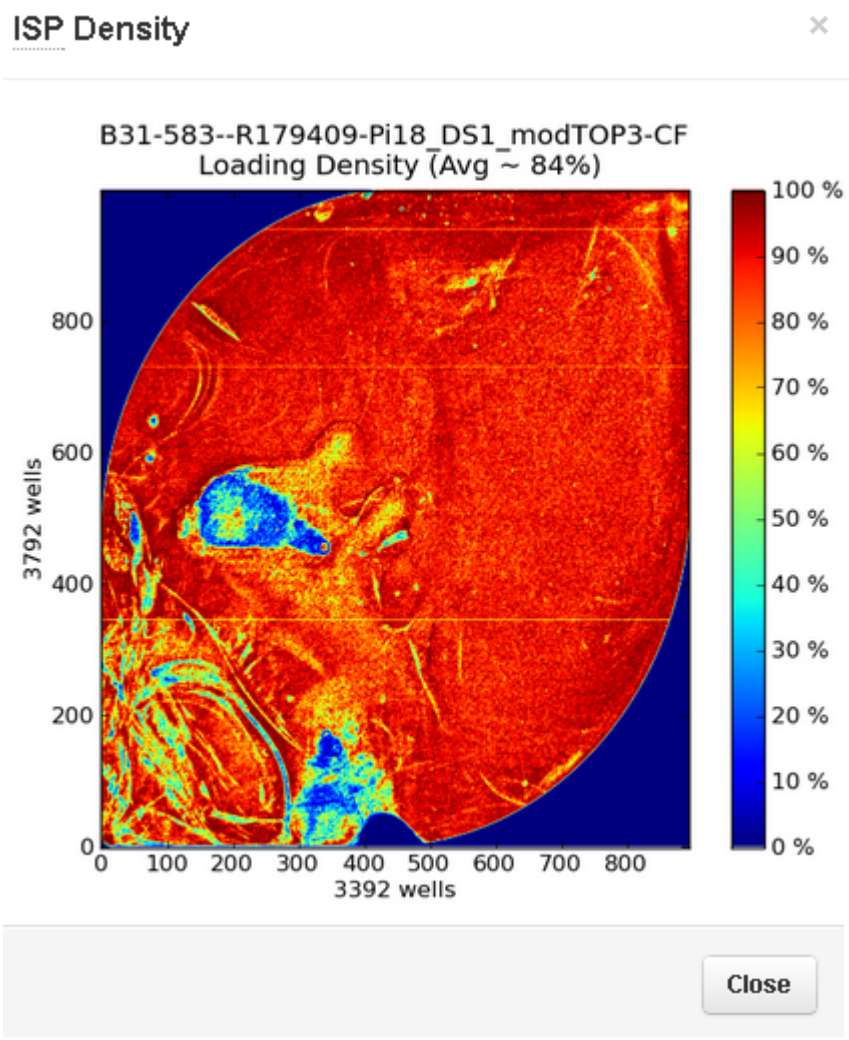
Click on the image (or the magnify icon) to open a larger version.

Key signal

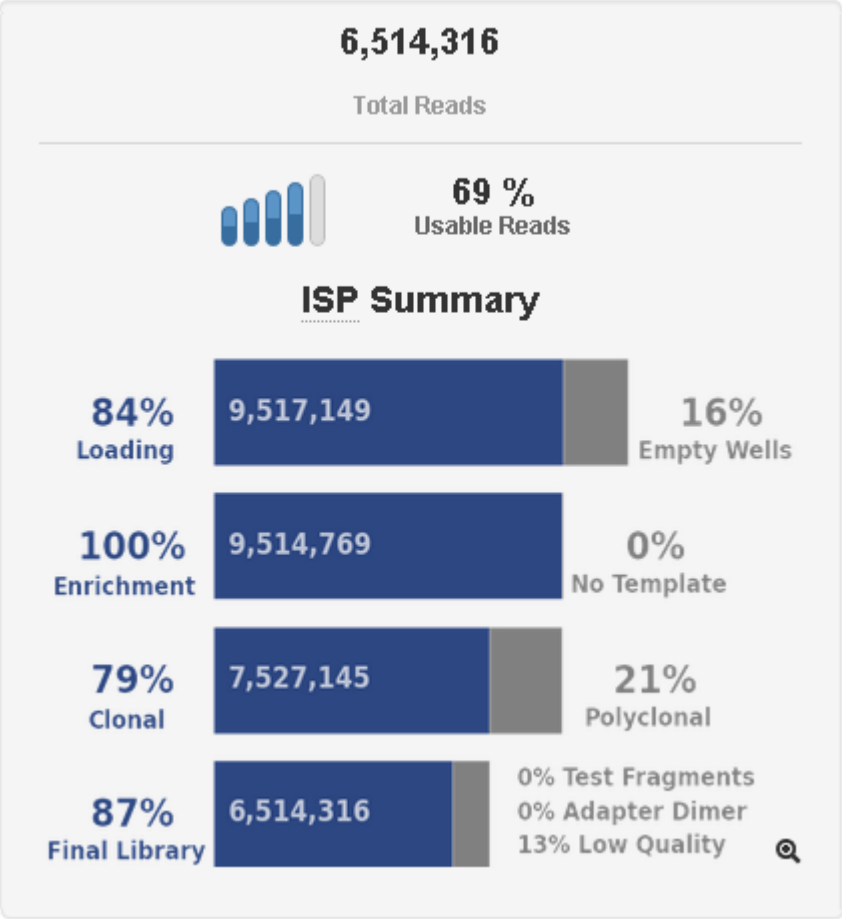
Click the magnify icon in the Key Signal area  to open the key incorporation graphs:



The key incorporation graph show the average signal readings for flows of the bases T, C, and A in the library key.



ISP summary



In the lower rows, the percentages are relative to the total in the next higher row. The first row gives percentages of loaded wells and empty wells, relative to the number of potentially addressable wells on the chip.

This table describes the ISP summary metrics:

Metric	Description	Calculation
Total Reads	Total number of filtered and trimmed reads independent of length reported in the output BAM file.	(Not calculated)
Usable Sequence	The percentage of library ISPs that pass the polyclonal, low quality, and primer-dimer filters.	Final Library ISPs/ Library ISPs
Loading	Percentage of chip wells that contain a live ISP. (The percentage value considersonly potentially addressable wells.)	No. of Loaded ISPs / No. of potentially addressable wells

Metric	Description	Calculation
Empty Wells	Percentage of chip wells that do not contain an ISP. (The percentage value considers only potentially addressable wells.)	(No. of potentially addressable wells minus No. of Loaded ISPs) / No. of potentially addressable wells
Enrichment	Predicted number of Live ISPs thathave 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 ISPs.	Library ISPs / (No. of Loaded ISPsminus TF ISPs)
No Template	Percentage of chip wells that do not contain a DNA template.	(No. of Loaded ISPs minus TF ISPs) minus (Library ISPs) / (No. of Loaded ISPs minus TF ISPs)
Clonal	Percentage of clonal ISPs (all library and Test Fragment 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).	No. of ISPs with single beads / No. of Live Wells
Polyclonal	Percentage of polyclonal ISPs (ISPs carrying clones from two or more templates).	Polyclonal ISPs / Live ISPs
Final Library	Percentage of reads which pass all filters and which are recorded in the output BAM file.This value may be different from the Total Reads due to technicalities associated with read trimming beyond a minimal requirement resulting in Total Reads being slightly less than Final Library.	Final Library / Clonal ISPs

Metric	Description	Calculation
% Test Fragments	Percentage of Live ISPs with a key signal that is identical to the test fragment key signal.	Test Fragment ISPs / Clonal ISPs
% Adapter Dimer	Percentage of ISPs with an insert length of less than 8 bp.	Primer-dimer ISPs / Clonal ISPs
% Low Quality	Percentage of ISPs with a low or unrecognizable signal.	Low quality ISPs / Clonal ISPs

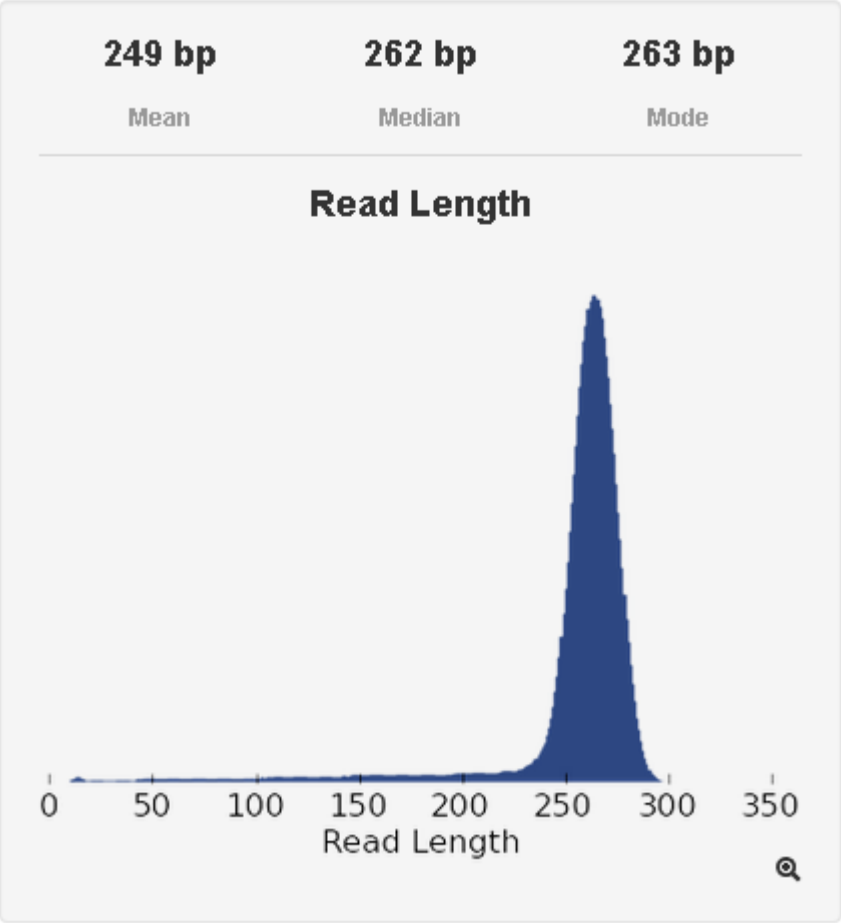
Click the ISP Summary magnify icon to open a larger version with also a table of metrics:

These metrics are described in this table:

Metric	Description	Calculation
Addressable Wells	Total number of addressable wells.	(Not calculated)
With ISPs	Number (and percentage of addressable wells) of 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.	Wells with ISPs / Total Addressable Wells
Live	Number (and percentage of wells with ISPs) of wells that contained 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	Live ISPs / Wells with ISPs

Metric	Description	Calculation
Test Fragment	Number (and percentage of Live ISPs) of Live ISPs with a key signal that was identical to the Test Fragment key signal.	Test Fragment ISPs / Live ISPs
Library	Number (and percentage of Live ISPs) of Live ISPs with a key signal that was identical to the library key signal.	Library ISPs / Live ISPs
Library ISPs	Predicted number of Live ISPs that have a key signal identical to the library key signal (the same value as shown in the well information table on the right).	Library ISPs
Filtered: Polyclonal	ISPs carrying clones from two or more templates.	Polyclonal ISPs / Library ISPs
Filtered: Low quality	Low or unrecognizable signal.	Low quality ISPs / Library ISPs
Filtered: Primer-dimer	Insert length of less than 8 bp.	Primer-dimer ISPs / Library ISPs
Final Library ISPs	Number (and percentage of Library ISPs) of reads passing all filters, which are recorded in the output BAM file. This value may be different from the **Total number of reads** in the Library Summary Section due to technicalities associated with read trimming beyond a minimal requirement resulting in **Total number of reads** being slightly less than **Final Library Reads**.	Final Library / Library ISPs

Read length

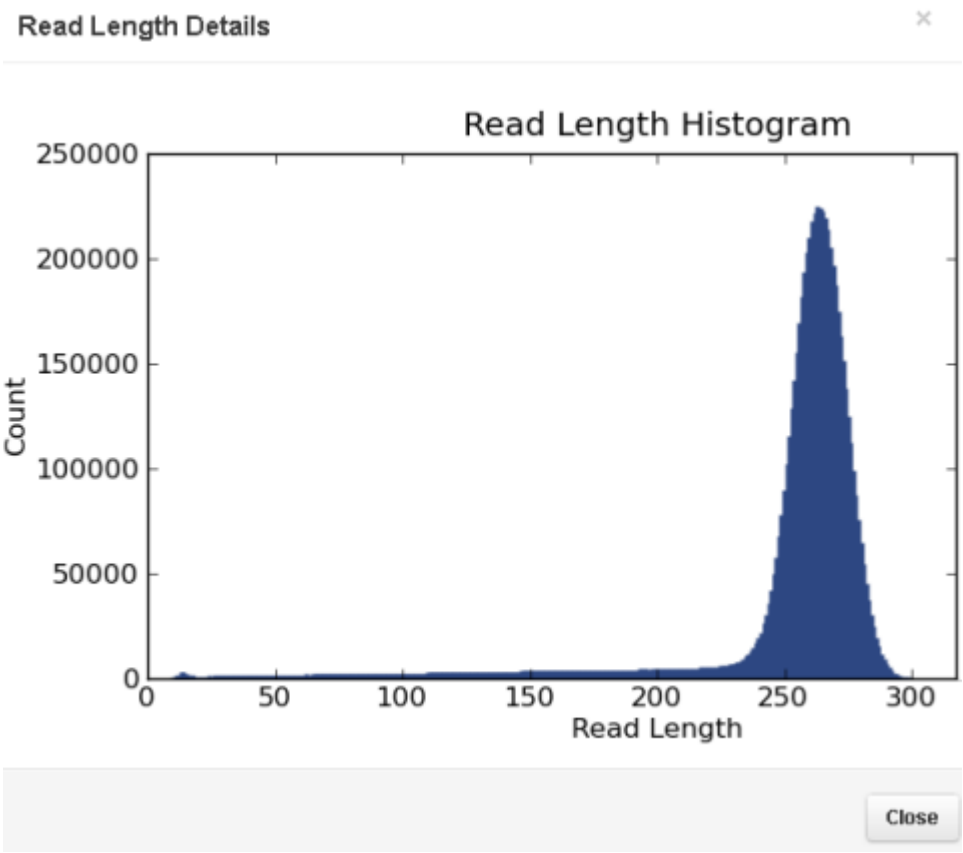


This table describes the read length metrics:

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

The read length histogram is a histogram of the trimmed lengths of all reads present in the output files.

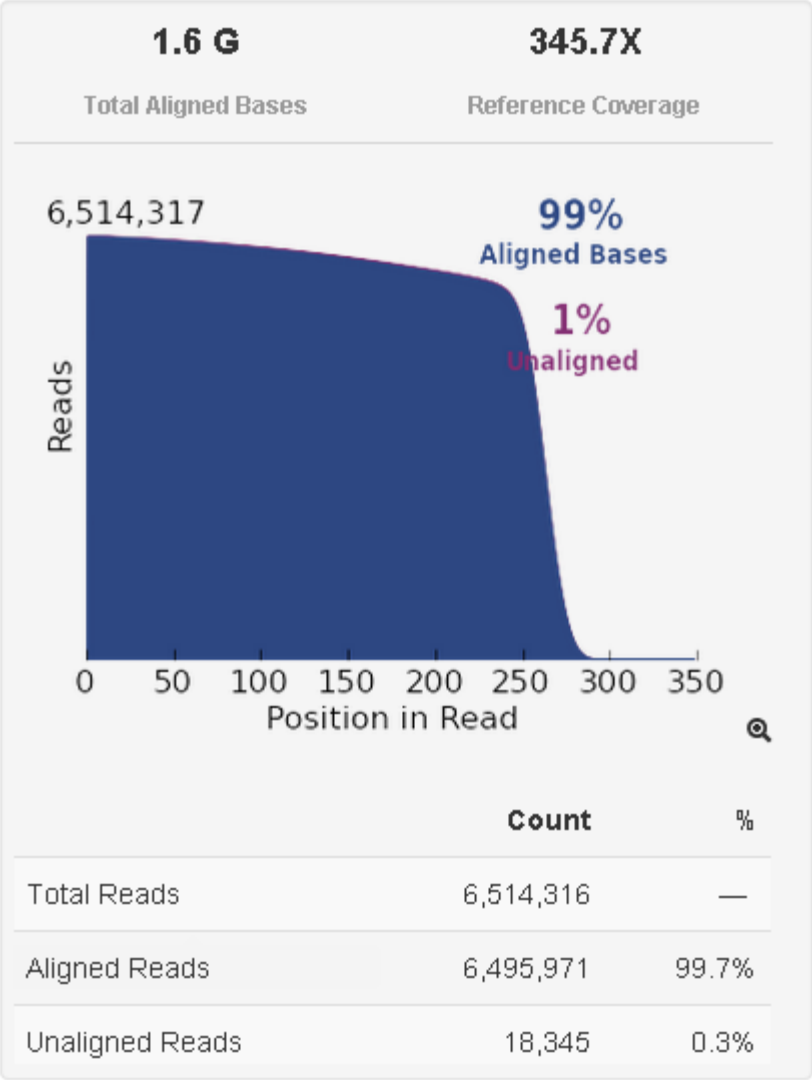
Click on the histogram to open a larger version:



Run report metrics on aligned reads

The run report provides metrics on aligned reads.

Total aligned bases



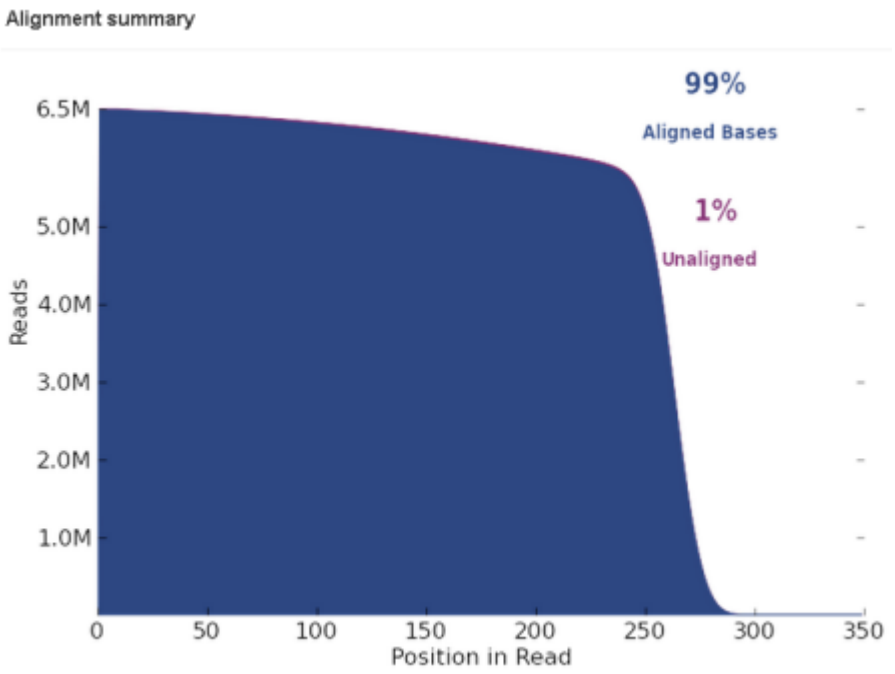
	Count	%
Total Reads	6,514,316	—
Aligned Reads	6,495,971	99.7%
Unaligned Reads	18,345	0.3%

The following table describes metrics in the Total Aligned bases area.

Metric	Description
Total Aligned Bases	Number of filtered and trimmed aligned base pairs reported in the output BAM file. Total number of bases aligned to the reference sequence. Excludes the library key, barcodes, and 3' adapter sequences.
Reference Coverage	The average of the number of reads that cover each reference position: total aligned bases divided by the number of bases in the reference sequence. Does not consider enrichment.

Metric	Description
% **Aligned Bases**	Percentage of Total Aligned Bases out of all reads.
% Unaligned	Percentage of bases not aligned to references.
Total Reads	Number of reads generated during basecalling.
Aligned Reads	Number of reads that aligned to the reference genome.
Unaligned Reads	Number of reads that did not align to the reference genome.

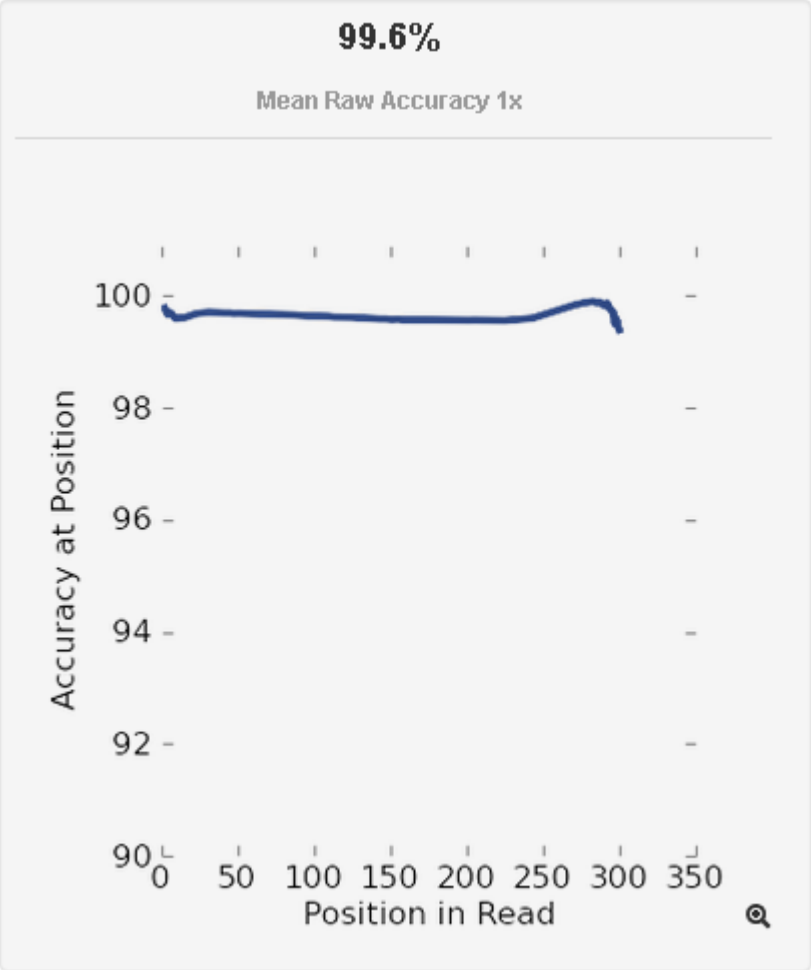
The graph in the Total Aligned reads column plots number of aligned (in blue) and unaligned (in purple) bases byposition in an aligned sequence. (The purple area cannot be seen easily when it is under 3 or 4 percent.)



For each position in an aligned sequence, the height of the blue area shows the number of aligned bases at that position. The purple area shows the number of unaligned bases at that position. Unaligned bases are not shown by the absolute height on the number of bases axis, but by the difference between the purple height and the blue height.

Raw accuracy

The graph in the Raw Accuracy column plots percent accuracy for each position in an aligned sequence:



Metric	Description
Mean Raw Accuracy 1x	Average raw accuracy of 1-mers plotted by their position in the read.

Alignment quality

Alignment quality calculations include the following:

1.5 G

AQ17 Total Bases

Alignment Quality

	AQ17	AQ20	Perfect
Total Number of Bases [bp]	1.5 G	1.5 G	1.2 G
Mean Length [bp]	248	242	202
Longest Alignment [bp]	336	327	321
Mean Coverage Depth [x]	340.3	329.2	266.5

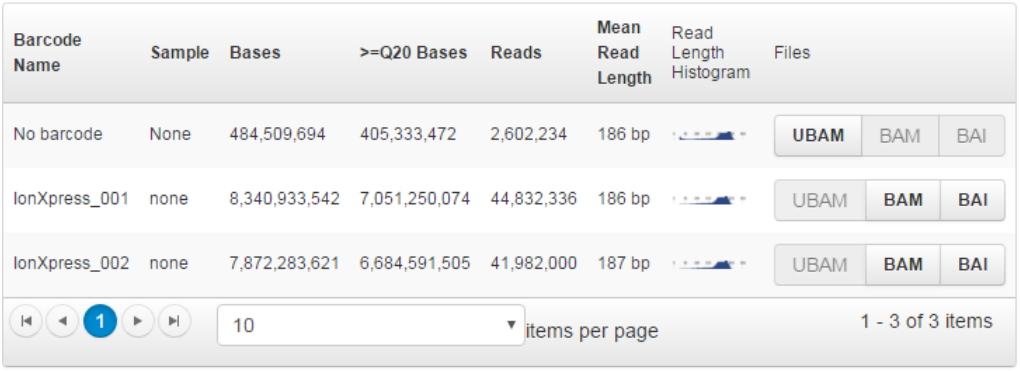
Metric	Description
AQ17	An error rate of 2% or less.
AQ20	An error rate of 1% or less.
Perfect	The longest perfectly aligned segment.
Total Number of Bases	Total number of bases at the quality level.
Mean Length	Average segment length at the quality level.
Mean Coverage Depth	Average coverage at the quality level.

Barcode reports

Barcode reports are included in the **Run Summary** runs that use barcodes. It shows key performance metrics for each barcode included in the run.

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

The barcode section of a run report displays the following information per barcode:



Column	Description	
Barcode Name	The individual barcode in the barcode set. The row labeled as **No barcode** reports on unclassified barcodes, which are reads that could not be classified as matching one of the expected barcodes in the barcode set.	
Sample	Name of the sample that was sequenced on instrument.	
Bases	Post filtering base output per barcode.	
% ≥ Q20	The percentage of reads that have a predicted quality score of Q20 or better. A Q20 score is the predicted quality of a Phred-like score of 20 or better, or one error in 100 bp.	

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 bp, of all filtered and trimmed library reads reported in the barcode BAM file.	
Read Length Histogram	A thumbnail histogram of the read lengths for this barcode. Click the thumbnail histogram to open a larger image.	
Files	Provides links to download the UBAM, BAM and BAM index files (BAI) for this barcode. The BAM file contains aligned reads sorted by reference location.	

Output files

These links allow you to directly download the data and report files. Some files are compressed, using ZIP, to provide data integrity and to reduce download time.

Click a file type to save the file to your local computer. Most output files can be loaded into third-party viewers (such as IGV) for visualization. The barcode row only appears for runs on barcoded data.

Files in the barcode row are zips of one file per active barcode. To download only BAM and BAI files for a single barcode, go to the barcode section at the top of the run report.

Column	Description
Reads	Files with unaligned reads (before alignment)
Aligned Reads	Files with aligned reads

File type	Reads	Aligned reads
BAM	Unaligned reads in BAM format. In this release, the BAM file contains some flow space information.	Aligned reads sorted by reference location.
BAI		BAM index file

The BAM format

Binary Sequence Alignment/Map (BAM), is a compressed, binary form of the SAM format. BAM files can be indexed, using the BAM Index file, for fast access to sequence alignment data. See **http://samtools.sourceforge.net** for a more detailed description of the SAM/BAM file format. Many tools are available for working with SAM files.

FASTQ file format generation

The FASTQ file format is not produced by the default analysis pipeline.

The FileExporter plugin generates files that use the FASTQ format that contain data that is organized in a per-base basis, including quality scores. The reads contained in the file are unaligned reads.

IMPORTANT! The FASTQ files that are created by the FileExporter plugin can be downloaded after a sequencing run that uses the plugin. For details on how to download the files, see "FileExporter plugin" on page 144.

Rename your output files

You can rename your output files with the FileExporter plugin. This plugin also optionally create and download versions of the files that use BAM, VCF, XLS, or FASTQ formats. You can also download compressed versions of the results files. For details, see "FileExporter plugin" on page 144.

Test fragment report

The **Test Fragment Summary** section of the Analysis Report provides information about the performance of each Test Fragment included in the experiment.

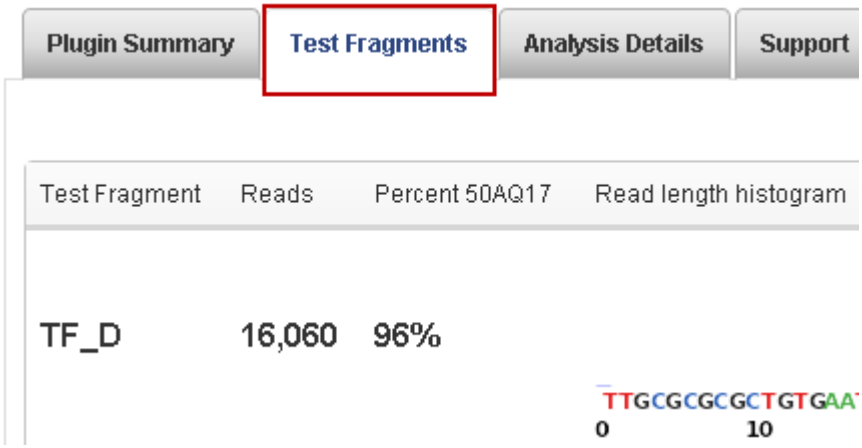
Test Fragments are used during analysis to predict the CF/IE/DR values for each Test Fragment, regionally. Analysis results for a Test Fragment are displayed when there are at least 1000 high-quality Test Fragments, where there is an 85% match against the

appropriate template in the Test Fragment list. This includes CF/IE/DR estimates and performance calculations.

IMPORTANT! The number of TFs reported includes lower quality TFs, down to 70% match, to better represent the run quality from all TF's.

Open the test fragment report

Click Test Fragments near the bottom of the run report to open the test fragment report:



Test fragment metrics

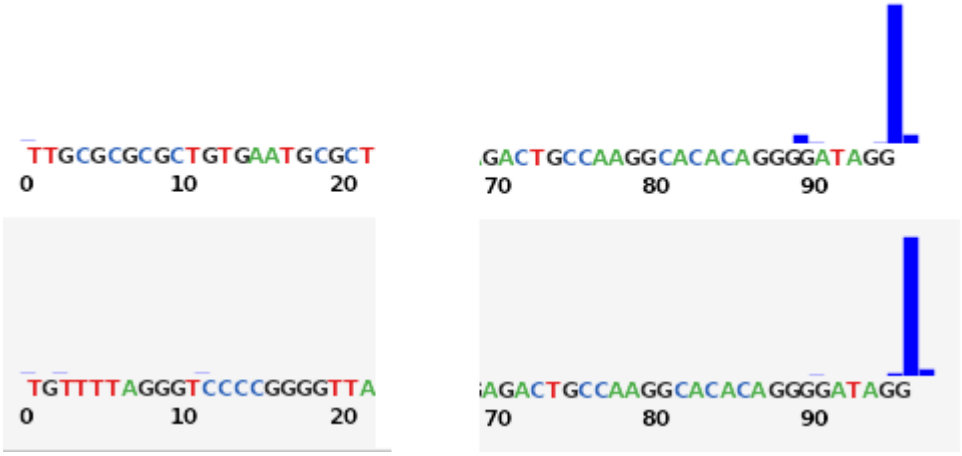
The Test Fragments report displays the following information:

Parameter	Description
Test Fragment	Test fragment name (defined in the Admin > References tab of Torrent Browser).
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.

The test fragment sequence is also shown in the read length histogram.

Read length histogram1

This is a histogram of read lengths, in *bp*units, that have a Phred-like score of 17 or better, 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 the histogram only displays a maximum size based on the length of the test fragment.

View Analysis Details of a report

To access the **Analysis Details** of a report:

1. In the **Data** tab, click **Completed Runs & Reports**.
2. Scroll to the bottom of the screen, then click **Analysis Details**.

Analysis details

The **Analysis Details** report displays the following information:

Parameter	Description
Run Name	Name of the run.
Run Date	Date and time the Ion PGM™ or Ion Proton™ run was started.
Run Cycles	Number of Ion PGM™ or Ion Proton™ cycles analyzed for this report. Note that this number can differ from the total number of cycles run on the sequencer.
Run Flows	Number of Ion PGM™ or Ion Proton™ nucleotide flows analyzed for this report. Note that this number can differ from the total number of flows occurring on the sequencer.
Project	Names of the projects the result set is a member of.

Parameter	Description
Sample	Name of the sample assigned to the run used to generate this analysis. This is assigned on theIon PGM™ or Ion Proton™ Sequencer.
Sample Tube Label	The label or written text on a sample tube used to track each sample through the sequencing workflow.
Reference	Name of the library assigned to the run used to generate this analysis. This library name is used to specify the reference genome used for alignment.
Instrument	Name of the sequencing instrument on which the run was performed.
Flow Order	Flow order selected on Ion PGM™ or Ion Proton™ 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 improve sequencing accuracy for longer reads by resisting phase errors.
Library Key	A short known sequence of bases used to distinguish the library fragment from the test fragment. Example: "TCAG"
TF Key	A short known sequence of bases used to distinguish the test fragment.
Chip ID	The ID number of the chip that appears on the chip barcode label.
Chip Check	A series of tests on reference wells (about 10% of the chip in non-addressable areas) is performed to ensure that the chip is functioning at a basic level. The value of this field is either **Passed** or **Failed**.
Chip Type	Type of chip used on the Ion PGM™ Sequencer. Usually, 314, 316, or 318 (for the Ion 314™ chip, Ion 316™ chip, and Ion 318™ chip.) A letter follows the numbers, indicating the chip version.
Chip Data	In this release, the value is **single**, for a forward run.
Chip Lot Number	The lot number of the chip as scanned by the Ion Proton™ Sequencer or Ion S5™ Sequencer. Not available for Ion PGM™ runs.
Barcode Set	The name of the barcode set assigned to the run. Blank for non-barcode libraries.

Parameter	Description
Analysis Name	Name of the analysis provided in Torrent Suite™ Software when the analysis was started. If the analysis was scheduled to auto-start, this is the default analysis name.
Analysis Date	Date the analysis was performed.
Analysis Flows	Number of Ion PGM™ orIon Proton™ nucleotide flows analyzed for this report. Note that this number can differ from the total number of flows occurring on the Ion PGM™ orIon Proton™ Sequencer.
runID	The run code that the Torrent Suite™ Software assigned to the planned run for this analysis.

(/section>

Software version

The **Software Version** report display includes version information for the modules installed on your Torrent Server.

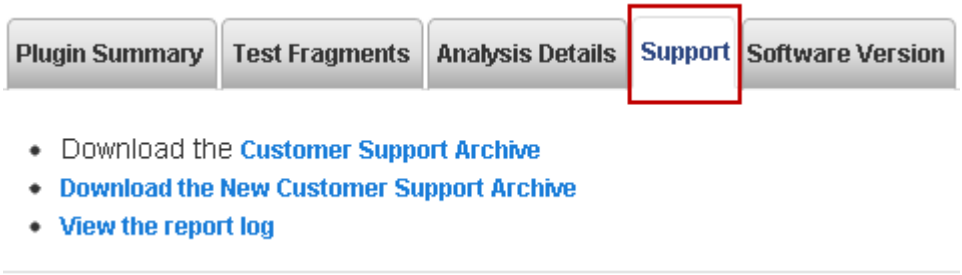
IMPORTANT! The version numbers shown in the example may be different from your current version of the software depending on the age of the analysis. See the About tab in the Torrent Browser for a complete list of modules and version on your server. See the Torrent Suite™ Release Notes for the package versions in a specific release.

Parameter	Description
Torrent Suite™	Version of Torrent Suite™ Software software used to generate the analysis.
Datacollect	Version of the Datacollect package.
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 used to generate the analysis.
ion-db reports	Version of the ion-dbreports package.
ion-gpu	Version of the NVIDIA® Tesla® 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.

Support

The Support button opens links to the following:

- **Download the Customer Support Archive** Download a ZIP archive containing the PDF and HTML version of the run report as well as useful logs in case troubleshooting is required. See Customer Support Archive for a description of the archive and its contents.
- **Download the New Customer Support Archive** Generate a new customer support archive and download it.
- **View the Report Log** View the error log for this run report.



An example report log is shown below (chopped for width considerations):

Report Error Log Refresh the page to see updates

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

Hostname = moorea13
Start Time = Sun Aug 19 17:07:12 2012
Version = 2.9-9+1 (40185) (201208171640)
Command line = Analysis --librarykey=TCAG --tfkey=ATCG --no-subdir --out
dat source = /results-nas9/2-4-RegressionTests/C25-336
SystemContext::SetUpAnalysisLocation... experimentName=/results2/analysi
SystemContext::SetUpAnalysisLocation... analysisLocation  =/results2/ana

SystemContext::SetUpAnalysisLocation... baseName      =sigproc_results
SystemContext::SetUpAnalysisLocation... runId         =MA72D
Found chip id 318
Use dud and empty wells as reference: no
Proton 1.wells correction enabled   : no
Empty well normalization enabled    : no
Per flow t-mid-nuc tracking enabled : no
Regional Sampling : no
Image gain correction enabled       : no
Col flicker correction enabled      : no
SystemContext::SetUpAnalysisLocation... experimentName=/results2/analysi
SystemContext::SetUpAnalysisLocation... analysisLocation  =/results2/ana

SystemContext::SetUpAnalysisLocation... baseName      =sigproc_results
SystemContext::SetUpAnalysisLocation... runId         =MA72D
```

Applications

Introduction

Torrent Suite™ Software supports many research applications. In this section, we highlight some of the main ones.

Oncology – Liquid Biopsy

The Oncology – Liquid Biopsy application supports tumor and liquid biopsy oncology research applications, for the following sample types: lung, breast and colon. The corresponding Planned Run templates for related panels are named as follows:

Name
Oncomine™ Colon Tumor DNA
Oncomine™ Colon Liquid Biopsy DNA
Oncomine™ Breast Tumor DNA
Oncomine™ Breast Liquid Biopsy DNA
Oncomine™ Lung Tumor DNA
Oncomine™ Lung Liquid Biopsy DNA

The following instructions provide a basic overview of how to set up a planned run for the related panels.

Plan an Oncology – Liquid Biopsy run from template

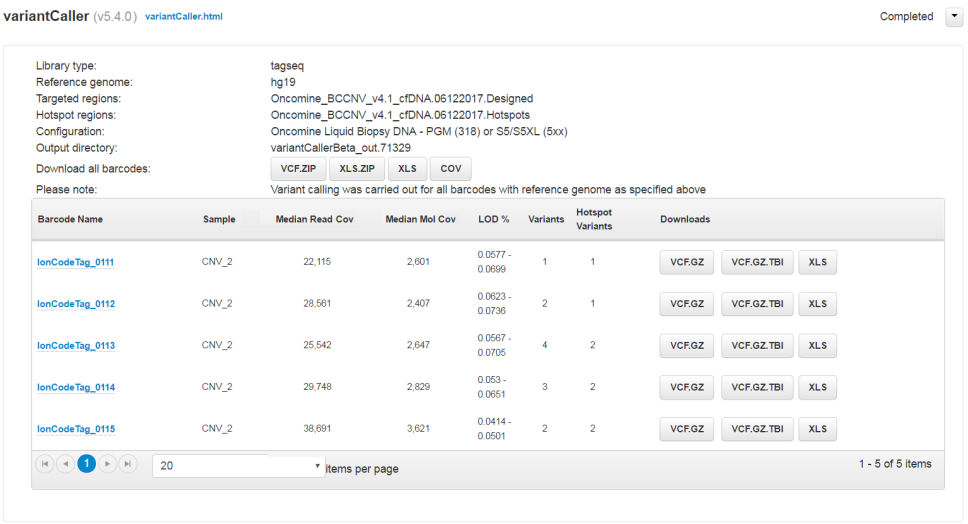
1. In the Template Name column, click on the template and the wizard opens on the Plan tab.
2. In the Ion Reporter tab, select **None**, and click **Next**.
3. In the Application tab, confirm **Oncology – Liquid Biopsy** and **Tag Sequencing** are selected. Click **Next**.
4. In the Kits tab, select **Oncomine cfDNA Assay**. Click **Next**.
5. In the Plugins tab, select **variantCaller_cfDNA**. Click **Next**.
6. (Optional) on the Projects tab, select a project. Click **Next**.
7. In the Plan tab, enter a name for your run and add samples. Click **Plan Run**.

Create an Oncology – Liquid Biopsy Planned Run template

1. In the **Plan** tab, click **Templates**, then select the **Oncology – Liquid Biopsy** category under **Favorites**.
2. Create a copy of the appropriate factory template, either **Oncomine™ Lung Tumor DNA** or **Oncomine™ Liquid Biopsy DNA**.
   a. Click **Settings** (⚙) ▸ **Copy** in the row of the appropriate template.
3. Define your template on the Copy Template page.
   a. Enter a name for the template.
   b. Verify the DNA Reference Library.
   c. Add DNA Target Regions .bed file.
   d. Enter a note about the template (if desired).
   e. Click **Copy Template**.
   Your new template appears under the Template Name column.

Review Oncomine™ cfDNA assay run results

1. After the run is complete, in the **Data** tab, click **Completed Runs & Reports**, then click the **Run Report** for your results.
2. To view a summary of the variant analysis, scroll down to the variantCaller section, then click the appropriate button to download variant calls in .vcf or .xls formats.

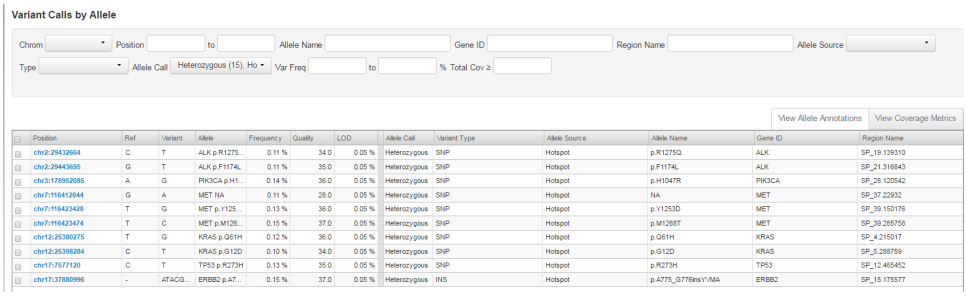


3. Review the results in the **Median Read Cov**, **Median Mol Cov**, and **LOD %** columns.

Column	Description
Median Read Coverage	Reports median coverage across targets. Median Molecular Coverage reports median number of individual interrogated DNA molecules across targets.
Median Molecular Coverage	Directly influences the limit of detection in a sample run. We always require two independent molecular families to identify a variant for it to be called. Lower median molecular coverage values result in less sensitive detection of variants at 0.1% frequency, although still sufficient for sensitive detection of variants with higher frequency. For example, Median Molecular Coverage of 700 is sufficient for accurate detection of variants at 0.5% frequency.
LOD %	A segment (e.g., 0.02–0.03) where 0.02 represents the median value across all targets, and 0.03 represents the limit of detection (LOD) for the 80th percentile targets. If both numbers are ⩽0.1% then the sequencing run is of acceptable quality for 0.1% LOD.

For sensitive variant detection down to 0.1% frequency, we see optimal results when targeting a Median Read Coverage >25,000, Median Molecular Coverage >2,500, and both numbers of the LOD % segment are ≤0.1.

4. Click a Barcode Name of interest to review Variant Calls by Allele.



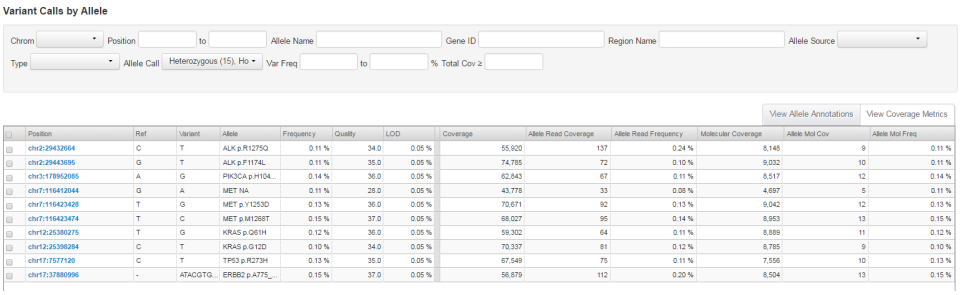
By default only hotspot alleles calls are shown in the variant table. We do not report hotspot alleles that did not meet our criteria for calling. However, we do provide at least one record for each hotspot position. This can include: novel allele call at hotspot position, hotspot allele call, or absent call when the first two are missing.

Column	Description
Frequency	Reports the observed frequency of hotspot allele.
LOD	Reports limit of detection at hotspot position, which is based on the number of interrogated DNA molecules (fragments) containing target. We use the term 0.1% LOD to mean we have data to support specific sensitivity and specificity claims (90% and 98%) at the 0.1% allelic frequency. By default, our analysis tool uses minimum alternative allele frequency threshold of 0.05% and we have a technical lower limit of detection of 0.03% for this method.

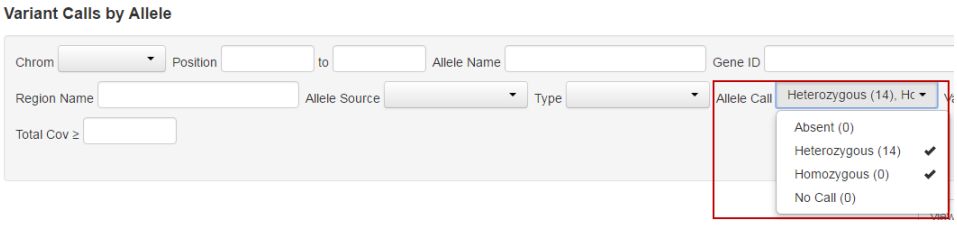
Observed frequency can be lower than LOD due to sampling nature of the assay.

If selected to display hotspot positions with absent variant call, then only one record per hotspot position is displayed and only one of the hotspot alleles at that position is displayed under "Allele Name".

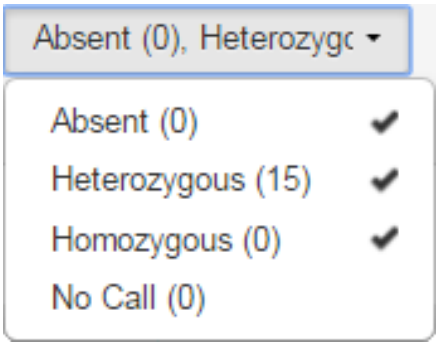
5. Click **View Coverage Metrics** to view the total number of interrogated DNA molecules at hotspot positions (Molecular Coverage), and the number of molecules containing the variant (Allele Mol Cov).



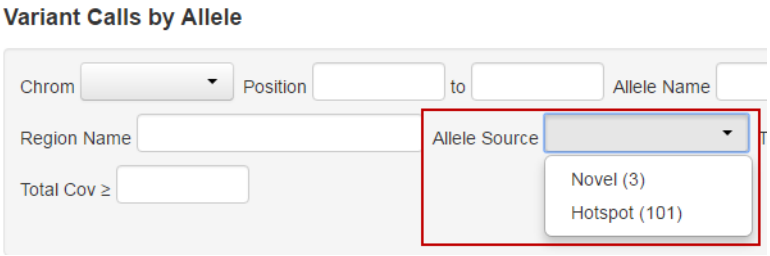
6. You can modify the types of calls that are displayed in the Allele Calls dropdown list, by selecting or deselecting Absent, Heterozygous, Homozygous, or No Call. No calls are variant calls that are classified as systematic errors.



7. Select **Absent** in the Allele Call dropdown list to visualize hotspot positions without a valid variant call that meets our analysis criteria. We report one record per hotspot position with missing alternative call, and the alternative allele is an arbitrary value distinct from reference. LOD and molecular coverage metrics at those positions are measurements for variant absence among many interrogated molecules.



8. To view novel alleles, select **Novel** (sequenced allele that is different from the expected allele defined in the panel hotspot file) in the Allele Source dropdown list.



16S Metagenomics application

Plan a run using Ion 16S™ Target Sequencing template

The Ion 16S™ Target Sequencing templates are used to create planned runs for the Ion 16S™ Metagenomics Kit. You can select your Ion Reporter™ account, kits, plugins, and parameter settings.

Note: To modify default parameters, see "Configure and select a custom analysis parameter set" on page 412.

1. In the **Plan**tab, click **Templates**, then in the **Favorites** list, select **16S Target Sequencing**.
2. Select the **Ion 16 S Metagenomics Template**.
   The wizard launches and displays the Plan page.
3. Add samples, confirm or change the default settings, and enter a plan name, then click **Plan Run**.
4. Run the plan on your sequencing system.

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 121.
- The RNASeqAnalysis plugin and smallRNA plugin. These plugins that 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. Enable the checkbox next 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, click **Upload and Install**.

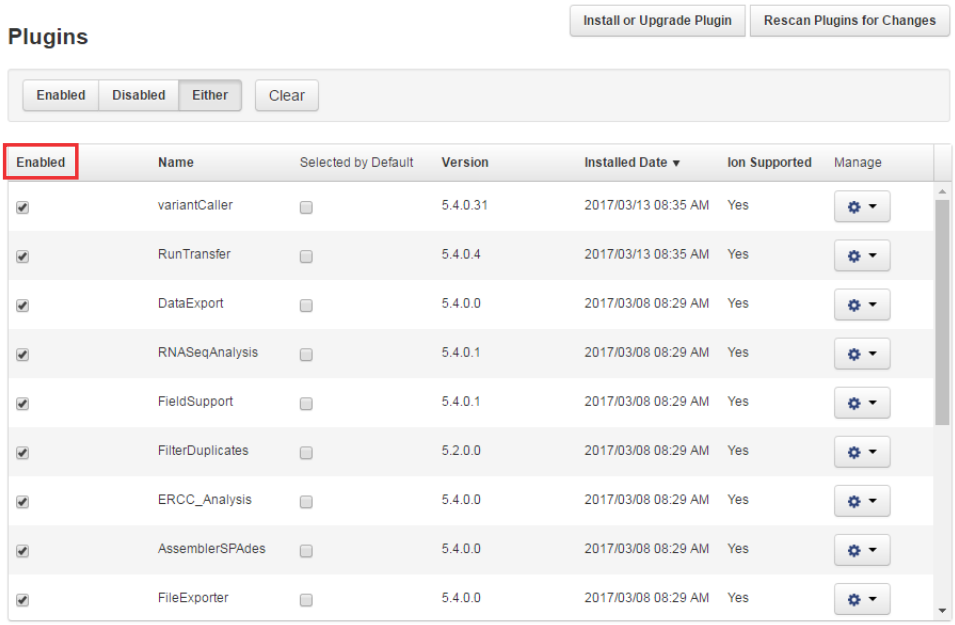
The plugin is now visible in Torrent Suite™ Software.

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. Click 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 **Settings** (⚙ ▾) ▸ **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 116). 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 will 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

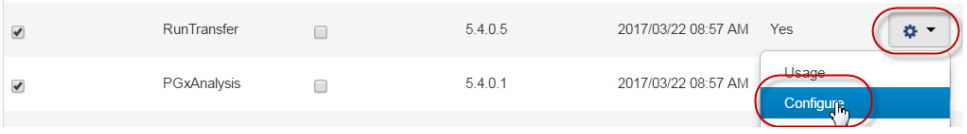
The following pre-installed plugins can be configured globally:

- 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 **Settings** (⚙) ▸ **Configure**.



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

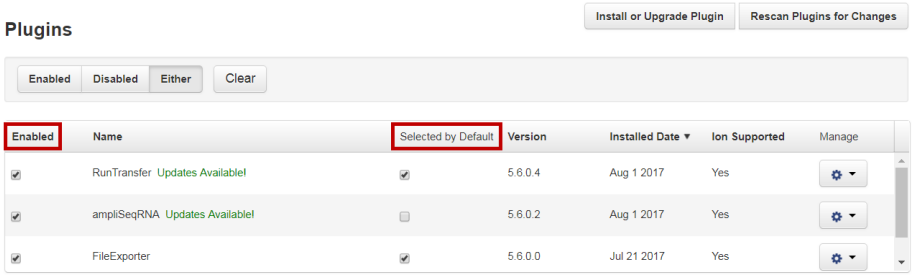
3. To save your changes, click **Submit** or **Save Configuration**.

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 118 .

- 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 121.

  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 121.

   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**.

View plugin run status

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

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
2. In the left navigation menu, click the plugin name, or scroll to the **Plugins** section of the run report.
   The plugin run status (Queued, Started, or Completed) is listed under the name of each plugin.

Stop a plugin run

You can stop a plugin run that is in progress.

1. In the left navigation menu, click the name of the plugin you want to stop, or scroll to the appropriate plugin section in the run report.
2. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** link for your completed sequencing run.
3. In the left navigation menu, click **Plugins**, or the name of the plugin results to be deleted.
4. Click **Stop** to cancel a plugin run that has started.

Open a plugin log

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

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

Delete a plugin result

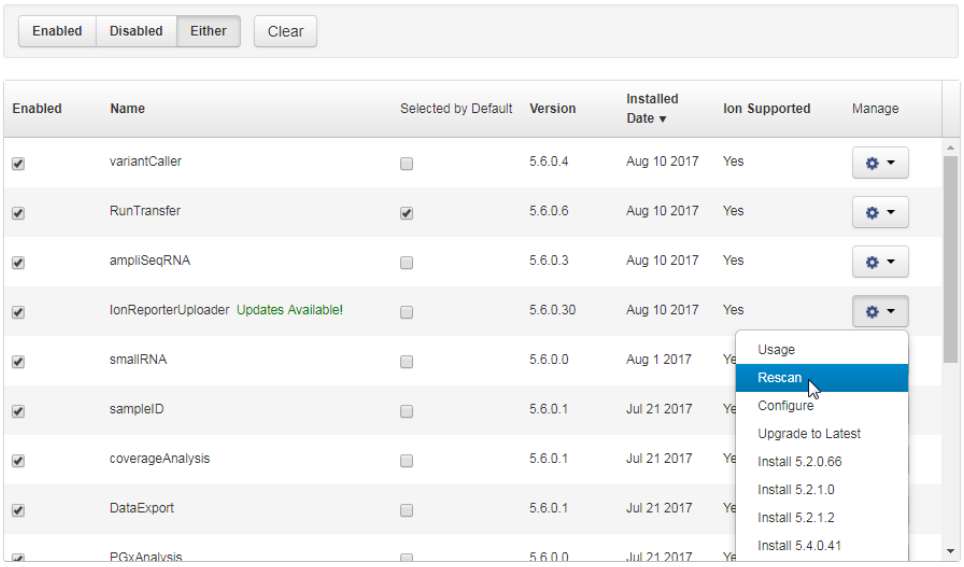
You can delete plugin results from the **Plugins** section of the run report.

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 **Settings ( )Rescan**.

   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 187.

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 122.
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 126.
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 130.
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 140.
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 141.
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.

Plugin name	Description
FilterDuplicates	Removes duplicate reads and creates BAM files that do not contain the duplicate reads. For details, see "FilterDuplicates plugin" on page 147.
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 171.
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 hg19_AmpliSeq_Transcriptome_ERCC_v1 reference (see "References Management Guide" on page 224) and appropriate targets panel, such as hg19_AmpliSeq_Transcriptome_21K_v1 (see "Manage Target Regions Files and Hotspot Files" on page 235).

ampliSeqRNA plugin configuration

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

Note: This plugin cannot be configured globally. The configuration options are noted in the table.

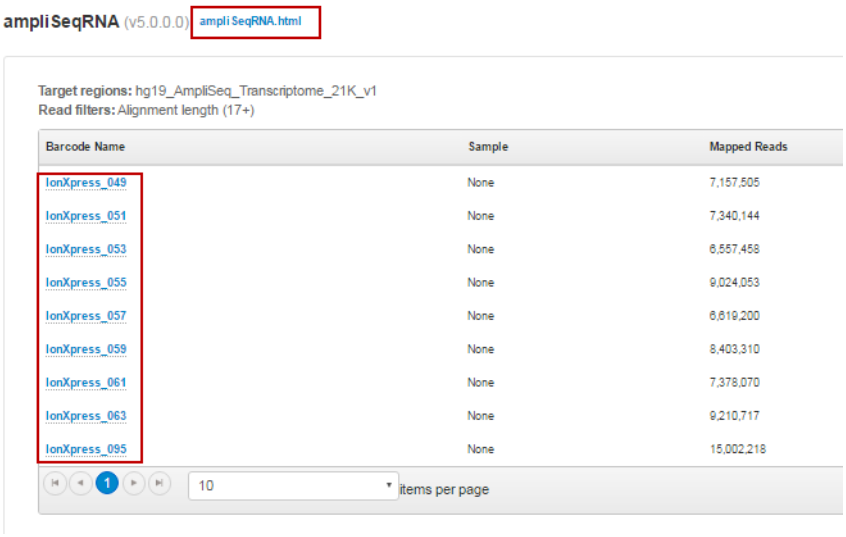
Setting	Description
The following settings can be configured when you run this plugin manually, or select it as part of a Planned Run or Planned Run template.	
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses if they have a relatively low number of reads, such as those that might 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.
Reference Genome	The short name of the reference genome (or DNA sequences) used to generate the current run report. It will be used for coverage analysis. See "References Management Guide" on page 224.
Library Type	Select **AmpliSeqRNA**, which is the library type that was sequenced.
Targeted Regions	Select the appropriate target regions file for your desired analysis. See "Manage Target Regions Files and Hotspot Files" on page 235. This setting can only be configured when you run this plugin manually.

Review ampliSeqRNA plugin results

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

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

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, locate the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **ampliSeqRNA** to view the plugin results.



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

Graphical report	Description
Distribution Plots	
Reads Alignment Summary	A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the **Barcode Summary** table.
Distribution of Gene Reads	Distribution of genes across barcodes showing the frequency of numbers of genes having similar log10 read counts. All curves are plotted on the same axis scale. The counts data are fitted to a Gaussian kernel using the default R 'density' function.

Graphical report	Description
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.

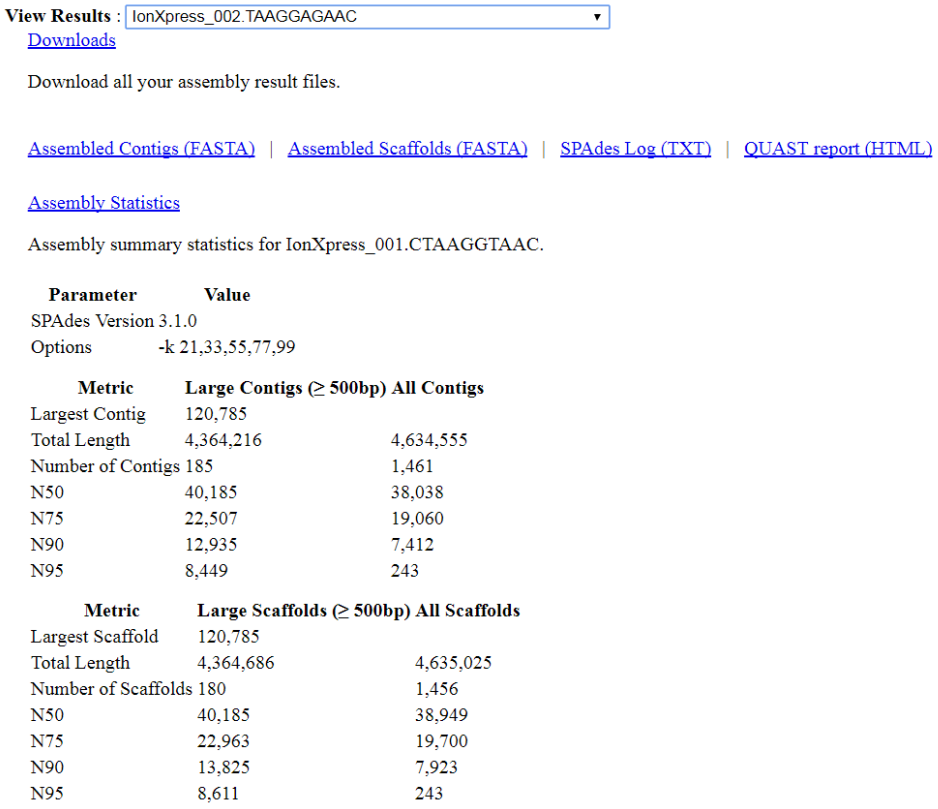
Setting	Description
Fraction of reads to use	The default setting of 100% is recommended, and handles most changes in coverage. If you enter a value of less than 100%, the reads are randomly sub-sampled.
Only process barcodes	By default, the plugin processes all barcodes in the analysis and produces a 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 the library type is changed, a different report will be 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	Determines type of report generated for individual barcodes. Barcode-specific targets are selected in the Planned Run, and can only be changed after the run is complete if the plugin is run manually. No reports are generated for individual barcodes that have target regions that are specified as **None** for targeted applications. 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 1–3 to associate additional barcodes with specific target region files. **Note:** Alternatively, you can copy and paste the barcode/target file pairs manually.
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.

Setting	Description
The following settings are available only with specific library types.	
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.
Use Only Uniquely Mapped Reads	Select the checkbox to enable the plugin to examine unique starts only.
Use Only 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 presented depend on the library type for the analysis.

The report lists the samples, the number of mapped reads, the percent of valid reads, and the percent 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 around 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**.

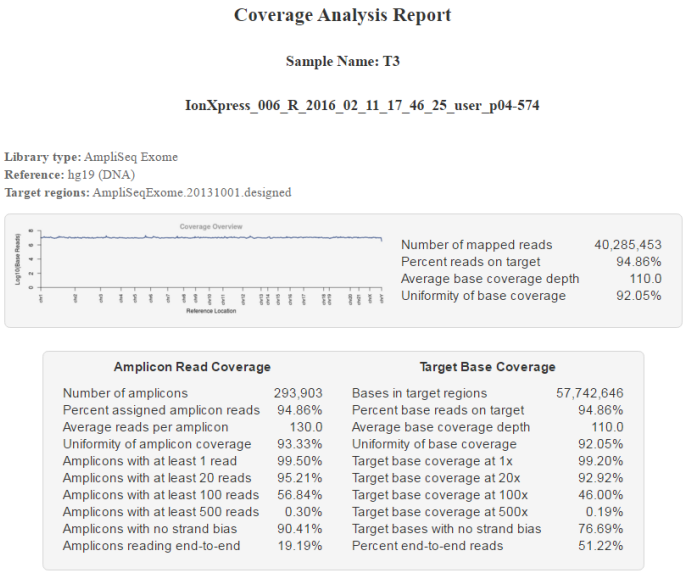
After the sequencing run completes, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, locate 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.

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

Coverage Overview

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.

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 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.
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.

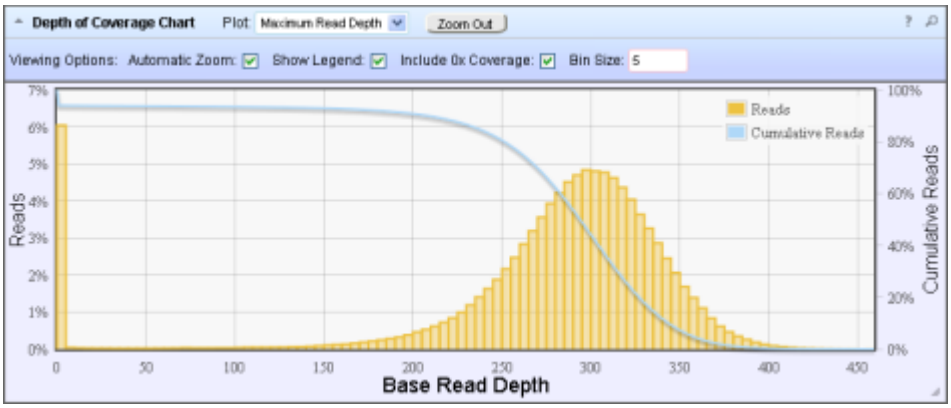
Statistic	Description
Percent of reads on target	The percentage of reads mapped to any targeted region relative to all reads mapped to the reference.
Total aligned base reads	The total number of bases covered by reads aligned to the reference.
Total base reads on target	The total number of target bases 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 once.
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 towards forward or reverse strand read alignments. An individual target is considered to have read bias if it has at least 10 reads and the fraction 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 will be 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) in to account.

Statistic	Description
Number of amplicons	The number of amplicons specified in the target regions file.
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 is considered to have read bias if it has at least 10reads and the fraction 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 G/C base pair content of their insert sequences. The value is relative to that in the center 50th percentile of amplicons and weighted by the standard deviation of representation over all amplicons.

Example charts

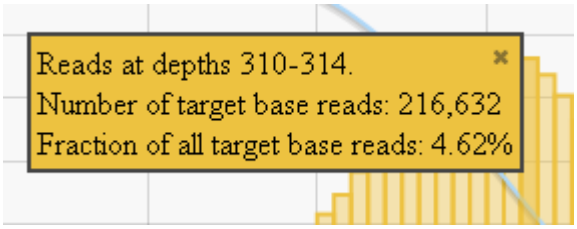
Many of the charts that are generated by the coverageAnalysis plugin include a **Plot** menu that allows you to change characteristics of the chart. For example, you can show both strands.

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

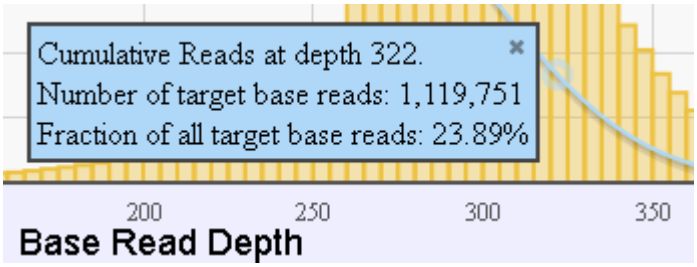


In the **Depth of Coverage** chart above, the left Y-axis (% reads) is the number of reads at a particular read depth (or bin of read depths) as a percentage of the total number of base reads. The right Y-axis (% cumulative reads) is the cumulative count of the number of reads at a given read depth count 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).

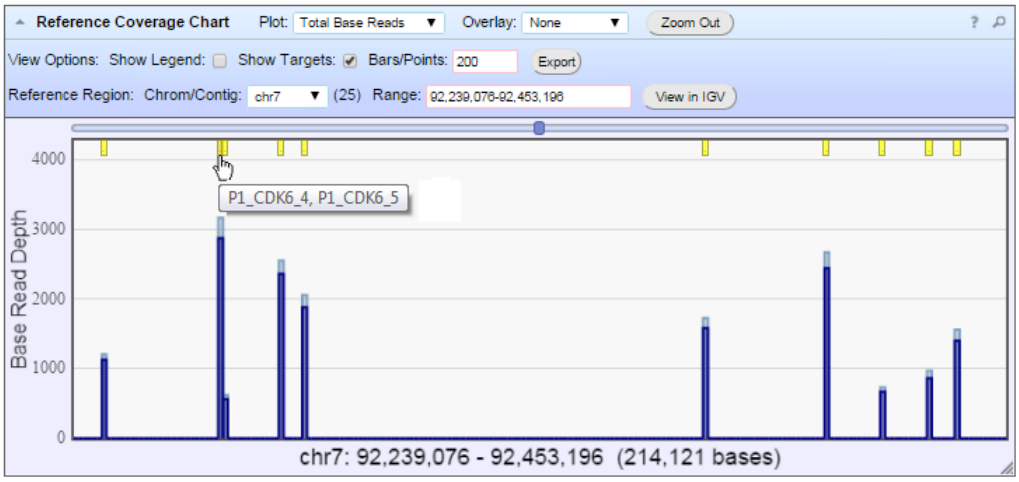
In most charts you click on a data point to open a detail panel for that data:



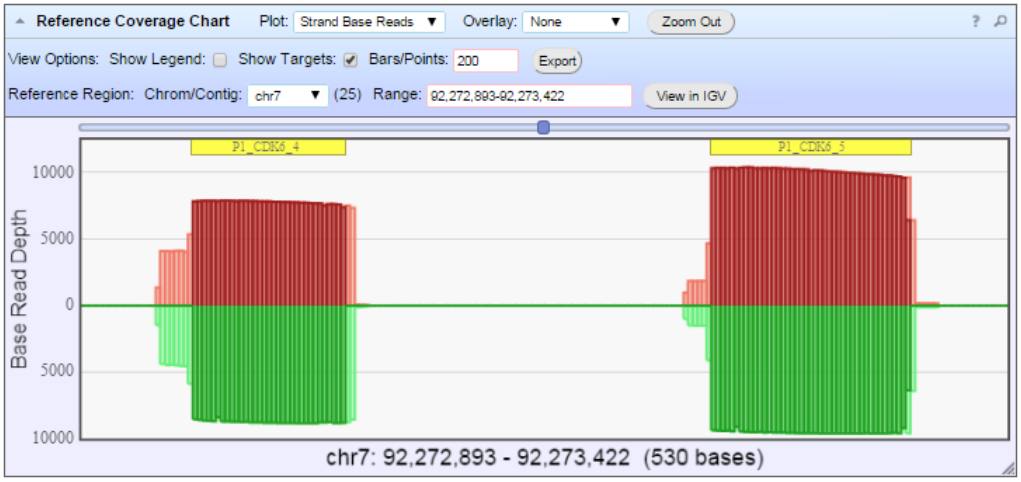
In this chart, the blue curve measures the cumulative reads at that read depth or greater. Click a point on the blue curve to open the blue detail panel for that read depth:



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



You can also zoom in on a region of interest.

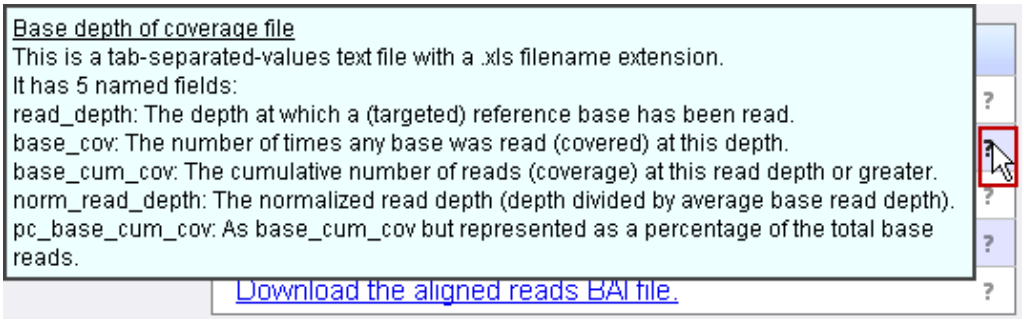


Output files

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

Note: Sometimes the file name may be too long to open in applications such as Microsoft™ Office Excel™. To resolve this issue, 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 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. Hence, %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.

File	Description
	• **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.
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). Refer to the current SAM tools documentation for more file format information.
Aligned reads BAI file	Binary BAM index file as required by some analysis tools and alignment viewers such as IGV. This is the same file that can be downloaded from the main report (for the specific barcode).

DataExport plugin

Use the DataExport plugin to export data from a sequencing run to a network drive, an external hard drive, or a removable media device, such as a USB drive. The exported data can be used to create backups, or to quickly transfer files to another system. When you configure the plugin, you select which files 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 to which the files will be exported
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, locate the run of interest, then click the link in the **Report Name** column.
3. In the **Summary**, click **DataExport** to view the plugin summary.
4. 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	Is the location to which the files are exported 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	To optionally 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	**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	To optionally 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:

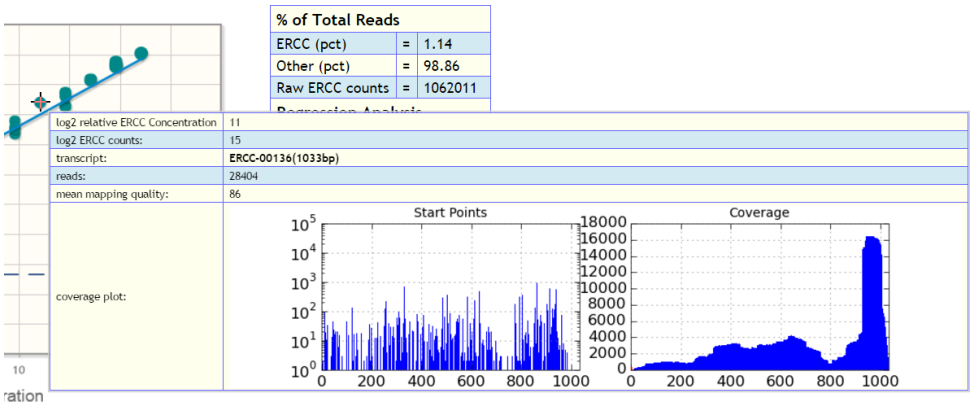
Interpret the ERCC Dose Response plot

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 about 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 about 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

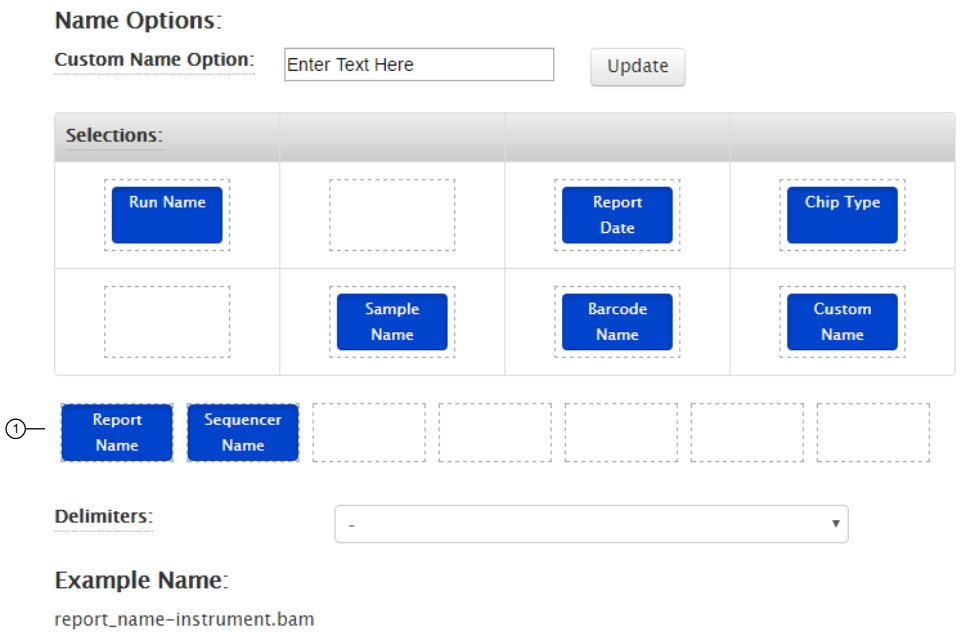
1. Select from the following options to choose the file types that you export:

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 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, locate 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. Verify 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 of the files that you selected when you configured the plugin.
5. When the list contains all of the files that you want to download, click on 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 Duplicate 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

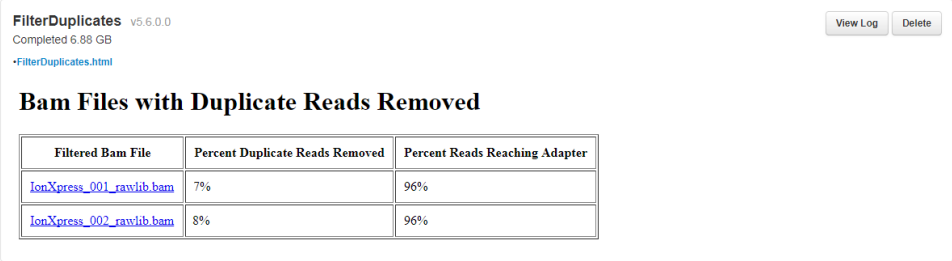
After the sequencing run completes, review the FilterDuplicates plugin results, and download the BAM files with duplicate reads removed.

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.

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

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


FilterDuplicates v5.6.0.0
Completed 6.88 GB
•FilterDuplicates.html
View Log Delete

Bam Files with Duplicate Reads Removed

Filtered Bam File	Percent Duplicate Reads Removed	Percent Reads Reaching Adapter
IonXpress_001_rawlib.bam	7%	96%
IonXpress_002_rawlib.bam	8%	96%

   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 will depend 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 223 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

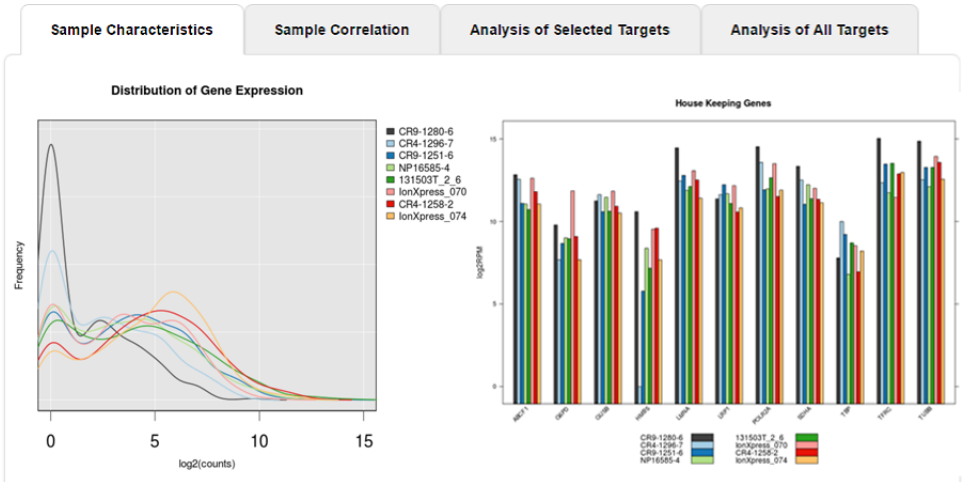
After the sequencing run completes, review the plugin results in the report summary.

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.
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.

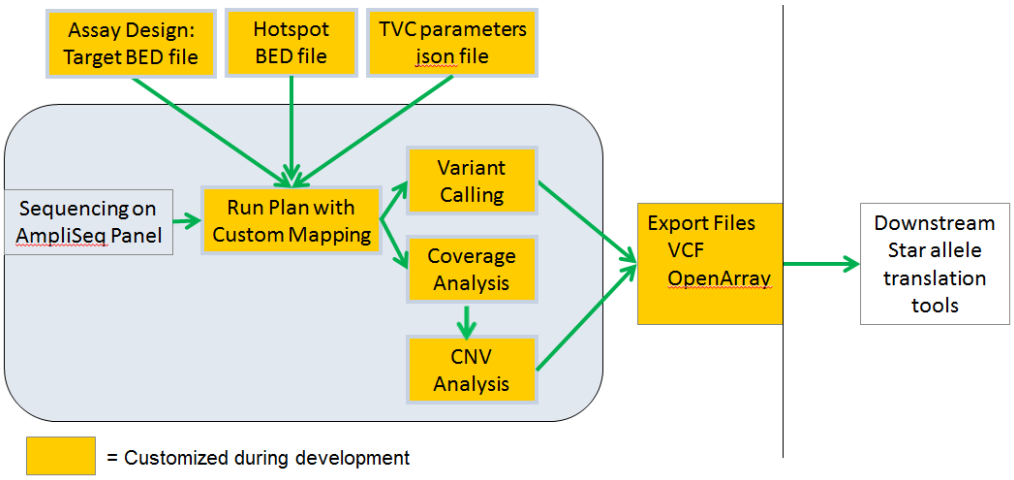
Ion Reporter™ Software uses the Torrent Suite™ Software output BAM file for analysis. The Ion Reporter™ Software annotation-only workflow also accepts the VCF output file of the variantCaller plugin. Use the IonReporterUploader plugin to transfer these BAM and VCF output files to Ion Reporter™ Software.

For details about the IonReporterUploader plugin, see "Integration with Ion Reporter™ Software" on page 171

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

Review PGxAnalysis plugin results

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

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, 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 193 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 131 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.

Setting	Description
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.
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 theIon Proton™ System and Ion 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 Proton™ System, Ion S5™ System, and Ion PGM™ 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 193.

Plugins available on only Thermo Fisher cloud

There are two plugins that are supported by Thermo Fisher Scientific and available on the Thermo Fisher Cloud. 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 121.

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	Analyzes small RNA reads with an emphasize on micro RNA molecules. Use with reference genom 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 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, locate 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 which 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 found on each barcode.

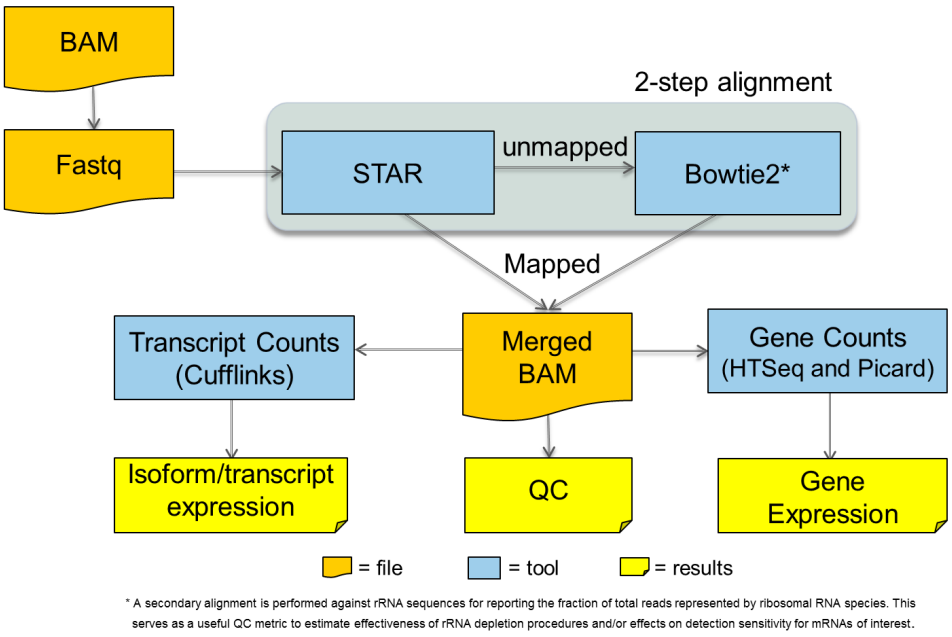
RNASeqAnalysis plugin

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 mm10 genome with this plugin, the mm10 genome reference must first be uploaded to Torrent Suite™ Software.

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 the either the hg19 or mm10 reference genome when you plan a run.

Note: This plugin requires the use of the Ion AmpliSeq™ application for sequencing Runs. When you run the plugin manually, you will receive an error if the Ion AmpliSeq™ application was not used.

Setting	Description
Reference Genome	Select from the dropdown list: **hg19** **mm10**

Note:
In order to use the mouse mm10 Reference Genome with this plugin, it must first be uploaded to Torrent Suite™ Software.

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 Reportaligned.	A table that lists the sample name for each barcode, total reads, aligned reads and percent
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.
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 167.
Correlation Plot	For details, see "Correlation plot" on page 168.
Gene Heatmap	For details, see "Gene heatmap" on page 168.
Isoform Heatmap	For details, see "Gene heatmap" on page 168.

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.

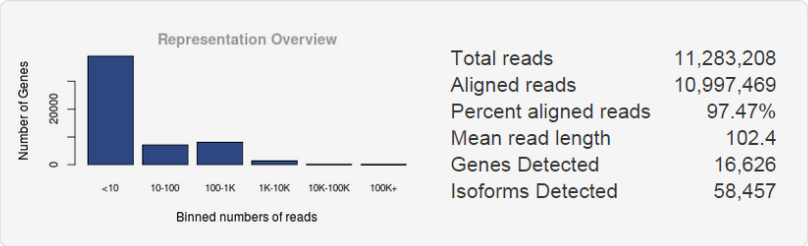
File Size	Date	File
5.5M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genes.fpkm_tracking
24M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.isoforms.fpkm_tracking
0	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_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

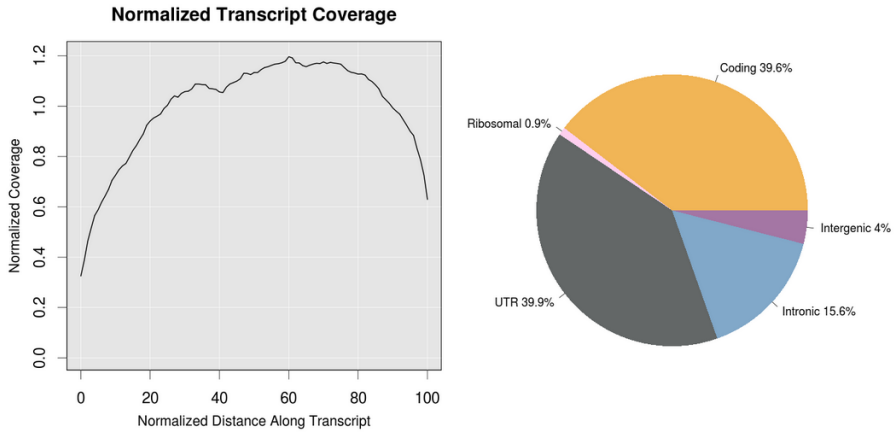
Reference genes	55,765
Reads mapped to genes	7,390,706
Genes with 1+ reads	26,969
Genes with 10+ reads	16,626
Genes with 100+ reads	9,531
Genes with 10,00+ reads	1,429
Genes with 10,000+ reads	35
Isoforms Annotated	230,756
Isoforms Detected	58,457

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

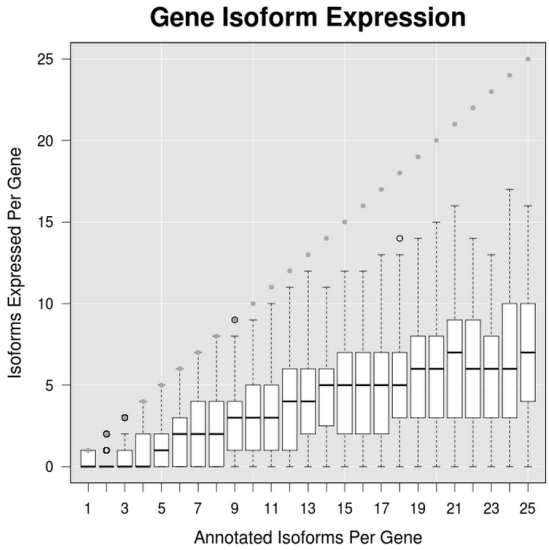
Base Mapping Summary

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.

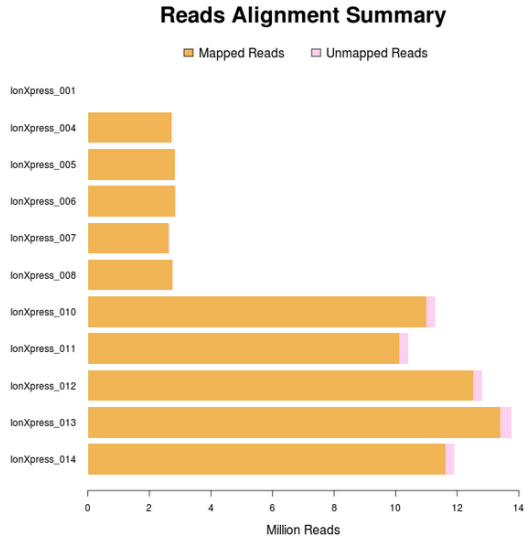


Gene Isoform Expression - Box plots showing variation of isoforms expressed at FPKM ≥ 0.3 for each set of genes grouped by the number of anticipated (annotated) isoforms. Whiskers are defined by points within Q1-1.5xIQR to Q3+1.5xIQR. Only genes with 25 or less isoforms are represented in this plot.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

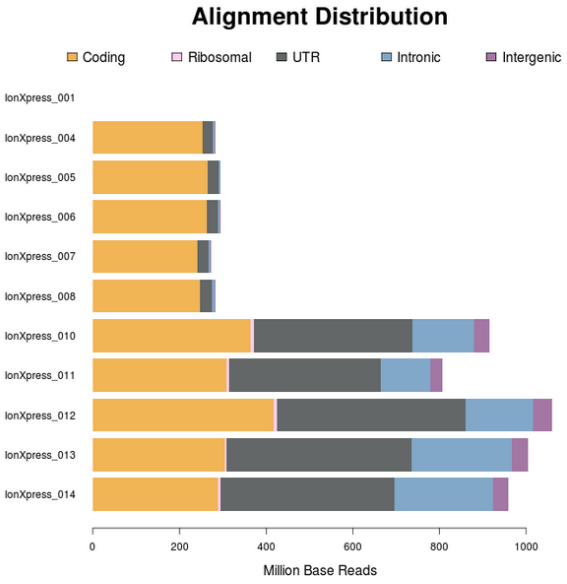


Distribution Plots

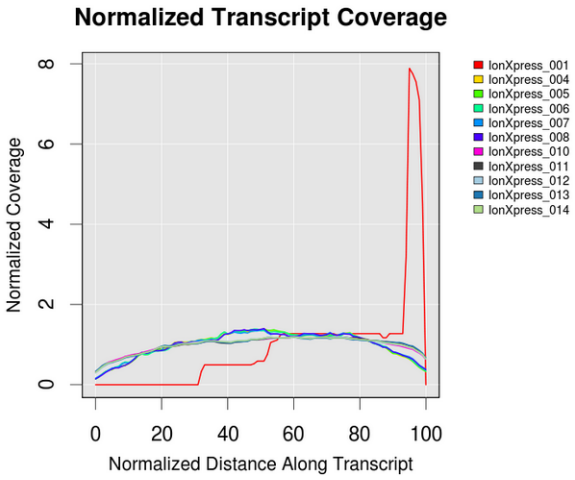
Reads Alignment Summary- A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the barcode summary table.



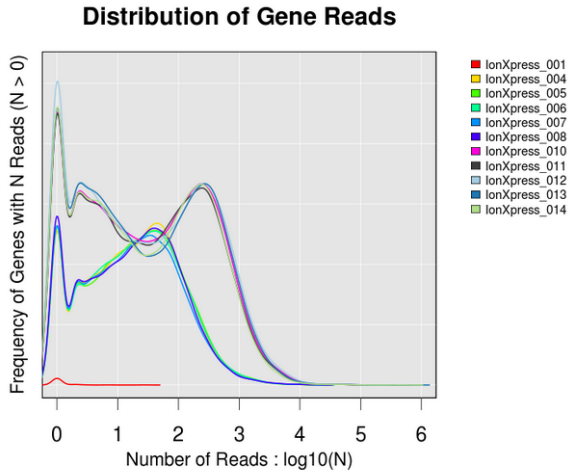
Alignment Distribution - A graphical summary of the distribution of reads to genomic features.



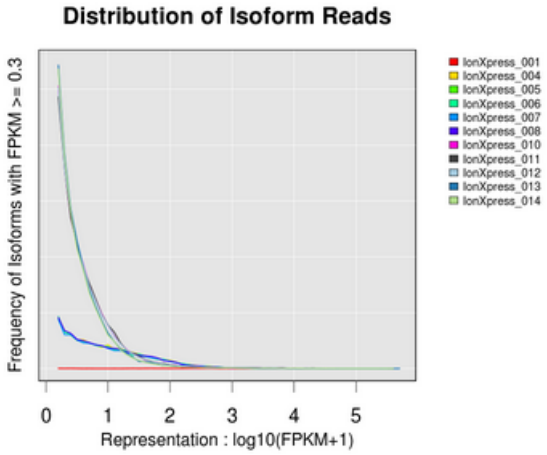
Normalized Transcript Coverage - An overlay of individual normalized transcript coverage plots for each barcode.



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.

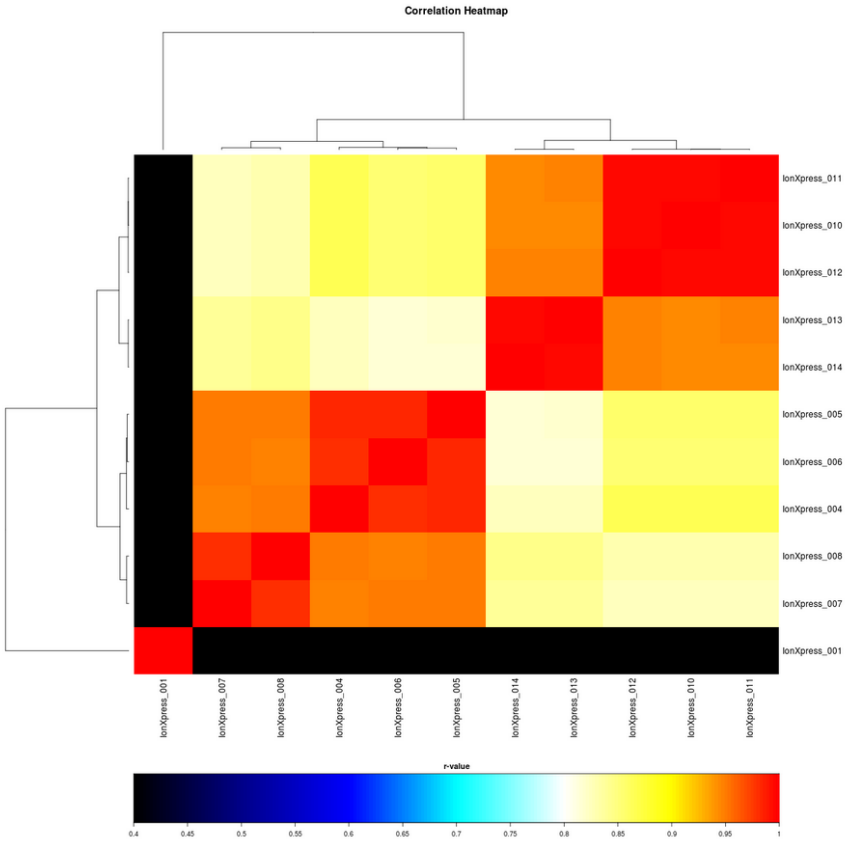


Distribution of Isoform Reads - Distribution of transcript isoforms across barcodes showing the counts of isoforms having similar FPKM values. All curves are plotted on the same y-axis, normalized to the highest count and scaled for FPKM values ≥0.3.



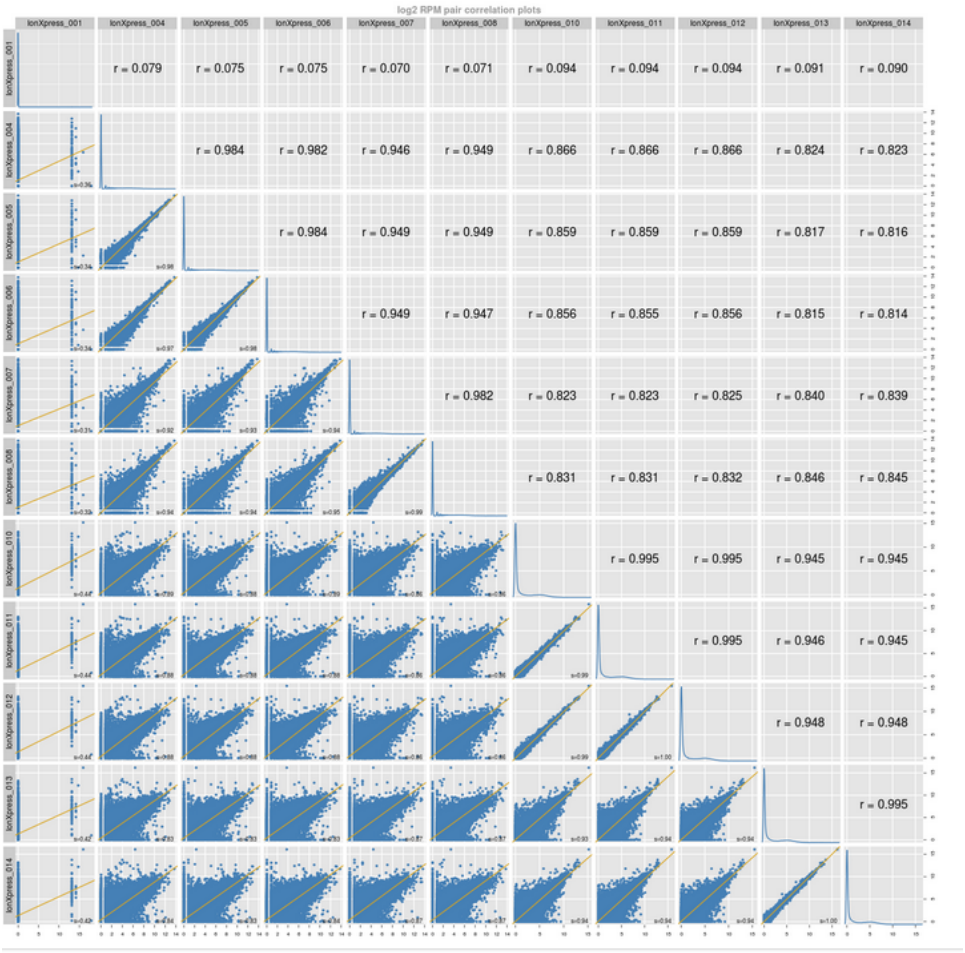
Correlation heatmap

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

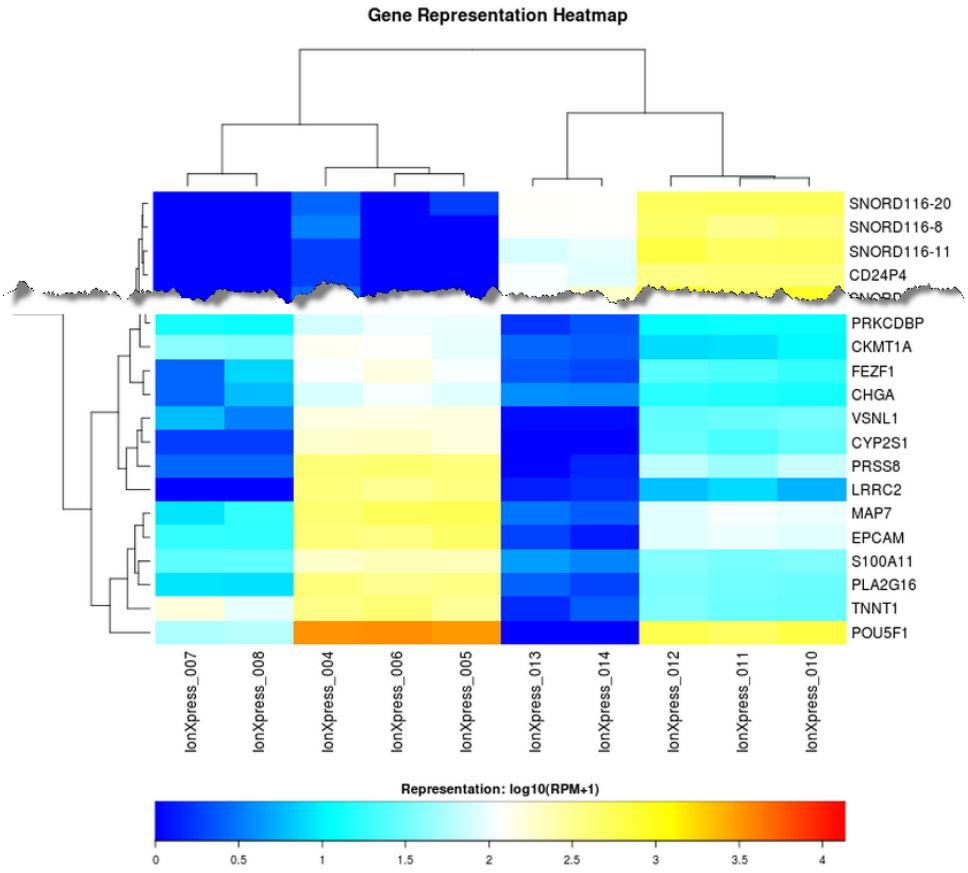
Barcode read pair correlation plot. Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show the frequency density plot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

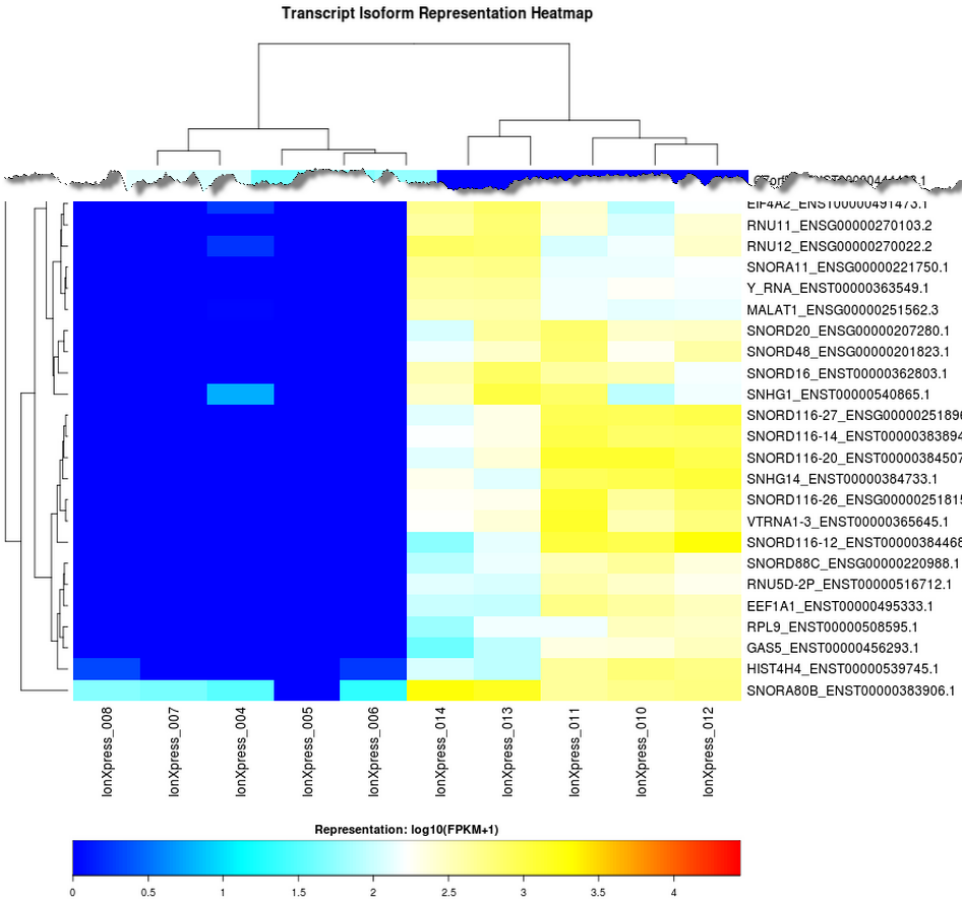
reads, plotted using log10 of those counts. For this plot, barcodes will be omitted if they have less than 100,000 total reads.



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

Analysis files that are generated in the Torrent Suite™ Software can be directly transferred to an organization 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.

There are two ways to run the IonReporterUploader plugin:

- Configure the IonReporterUploader plugin to run by default after every sequencing run. Torrent Suite™ Software results files are transferred to Ion Reporter™ Software and are defined as samples in Ion Reporter™ Software when the plugin is run manually.
- Run the plugin manually. Results files are also defined as samples in Ion Reporter™ Software when the IonReporterUploader plugin is run as part of a Planned Run or Run template. In addition, the workflow of your choice Ion Reporter™ Software is automatically launched on your newly transferred samples Ion Reporter™ Software.

The Ion Reporter™ Software is not included with Torrent Suite™ Software and is available under separate license. Before you run the IonReporterUploader plugin, you must add a valid Ion Reporter™ Software account.

Note: When the IonReporterUploader plugin defines samples in Torrent Suite™ Software for your newly-transferred files, the plugin also defines sample relationships for paired and trio samples and defines sample attributes. For details, see “Sample gender“ on page 182
.

Transfer limitations

The IonReporterUploader plugin transfers results files for a completed run plan that executed on the Torrent Server where the plugin is configured. The following limitations apply to the IonReporterUploader plugin:

- You cannot add supplemental files to the results files of a run, to have the plugin transfer those files.
- For barcoded runs:
  - For sequencing runs that use barcoded data, the IonReporterUploader plugin only transfers samples if the barcode kit selection is correct. If you correct or add the barcode kit selection on the sequencing instrument, the IonReporterUploader plugin still uses the original run plan information and the results file transfer fails.
  - For manual launches of the IonReporterUploader plugin on barcoded data, the IonReporterUploader plugin uses the barcode kit that you select on the sequencing instrument.

Install the IonReporterUploader plugin on your Torrent Server

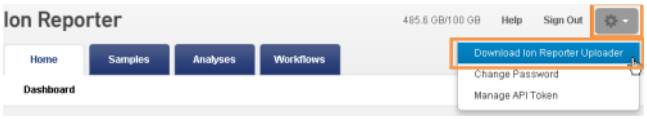
The IonReporterUploader plugin is automatically installed on Torrent Server when you update to a new release.

To update the IonReporterUploader plugin on a Torrent Server 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 305 and "Update off-cycle release plugins" on page 306.

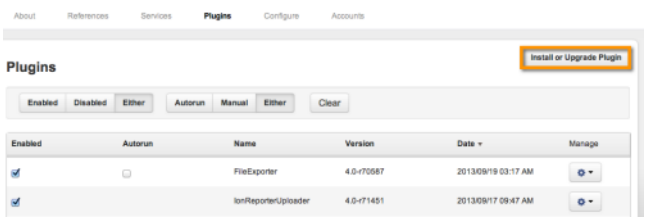
If you do not have an internet connection, then download and install the latest version that is named `IonReporterUploader_<version>.deb` from **http://iru.ionreporter.thermofisher.com/**.

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

1. Sign in to Ion Reporter™ Software, then click **Settings** (⚙) ▸ **Download Ion Reporter Uploader**.



2. Click the filename **IonReporterUploader.zip**, then download the file to your local machine.



3. Sign in to Torrent Suite™ Software, then click **Settings** (⚙) ▸ **Plugins**.

4. Click **Install or Upgrade Plugin**.
5. Click **Upload a Plugin file**, then browse to the **IonReporterUploader.zip** file that you downloaded. Click **Open**, click **Upload**, then **Install**.

Set up an account for IonReporterUploader plugin

Before you use the IonReporterUploader plugin, you must configure it with a valid Ion Reporter™ Software account. Torrent Suite™ Software uses the account information to transfer analysis 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. You can then upload the Torrent Suite™ Software output files to more than one Ion Reporter™ Software account.

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 (`ionadmin`) 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. In the **Add Ion Reporter account** screen:
   a. Enter your name and password. The Server and Port fields are pre-populated.

b. Enter this information for the hosted cloud Ion Reporter™ Software solution:

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	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

Note: For a local Ion Reporter™ Server, these entries depend on the system configuration. Ask your local Ion Reporter™ Server system administrator for values for: Server Type (HTTP or HTTPS), Server, and Port.

5. Select one of the following options:
   - **Default Account** The account that is configured by default in the run templates and run plans. If the main account is for file transfers, enable the Default Account checkbox. You can change the default account later when you use the run plan template wizard, or the **Upload to IR** link.
   - **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.

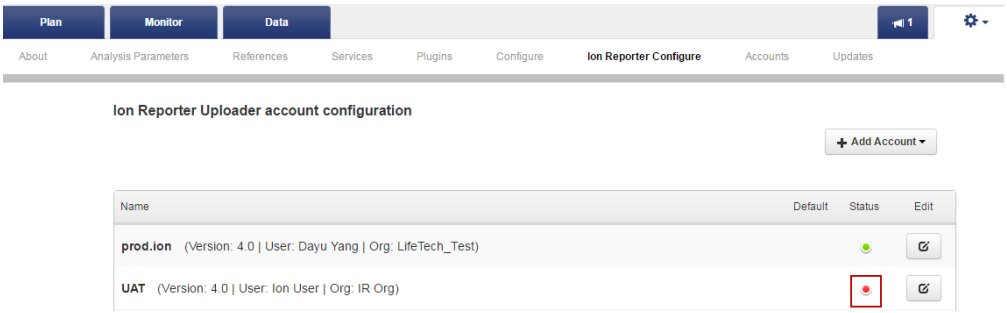
6. Click **Add**.

7. (*Optional*) The IonReporterUploader plugin can manage multiple configurations. To add another configuration, repeat the process.
   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 files and launch Ion Reporter™ Software analyses. If you set up multiple accounts, there is list of accounts for: data transfers, planned run creation, manual runs of the plugins, and the **Upload to IR** link in the Run Summary.

Red status on Ion Reporter™ Software account configuration screen

When you change your Ion Reporter™ Software account password or upgrade the account, you can see the status column of the Ion Reporter™ Uploader account.



If you changed your password, click **Edit** and enter your new password.

If you upgraded your account to a new version, click **Edit**, then delete your old account and create a new account for the new version.

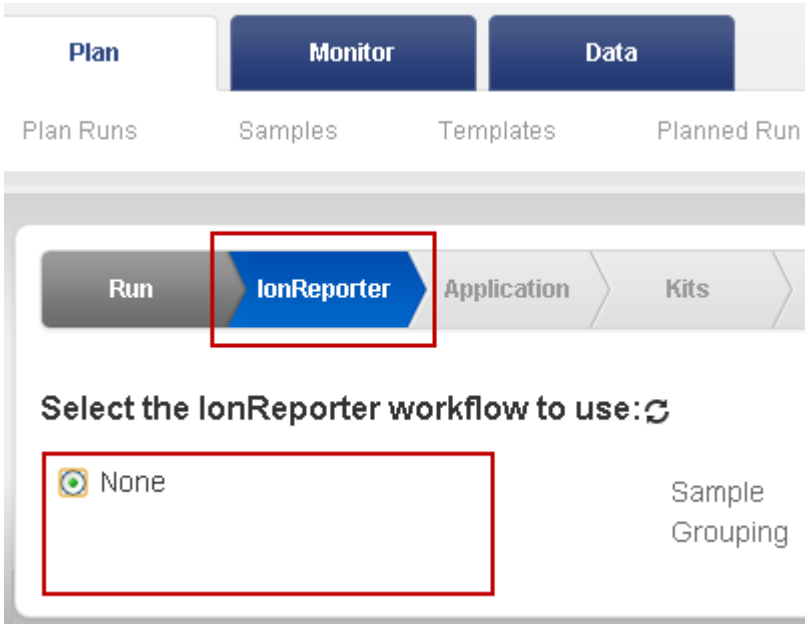
Accounts required for the IonReporterUploader plugin

If IonReporterUploader plugin is not yet configured to transfer to your Ion Reporter™ Software organization, you can add an account and configure the plugin.

If any of the following circumstances occur, you must add an Ion Reporter™ Software account:

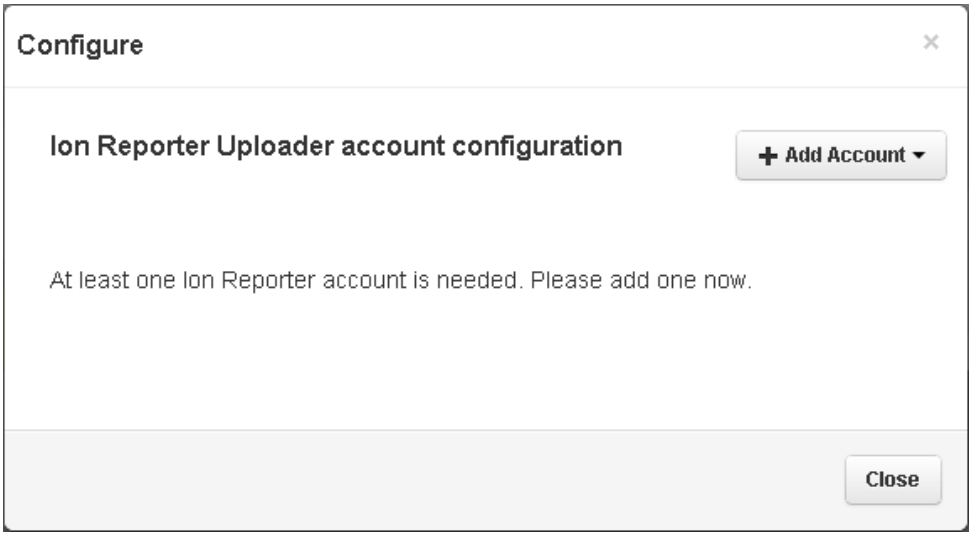
- There is no account to select when you add the IonReporterUploader plugin to a Planned Run, or a run template.

  If you click **Ion Reporter** in the workflow bar or if you select **None**, the Ion Reporter™ Software workflow selection menu does not appear. Also, Ion Reporter™ Software accounts are not listed.



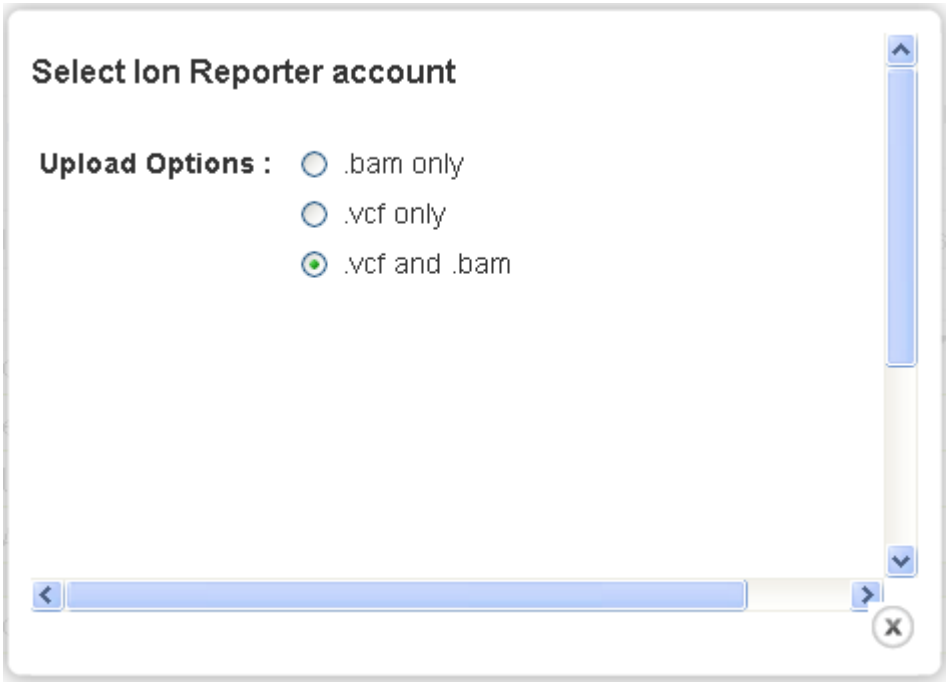
  For details on how to configure the account, see "Run a plugin manually from the sequencing run report" on page 118.

- If you try to configure it globally, you are prompted to add an Ion Reporter™ Software account.



For details on how to configure the account, see "Configure plugins globally" on page 116.

- If you click the plugin name for a manual run, there are no accounts listed under **Select Ion Reporter account**.



For details on how to configure the account, see "Run a plugin manually from the sequencing run report" on page 118.

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
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 178.

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

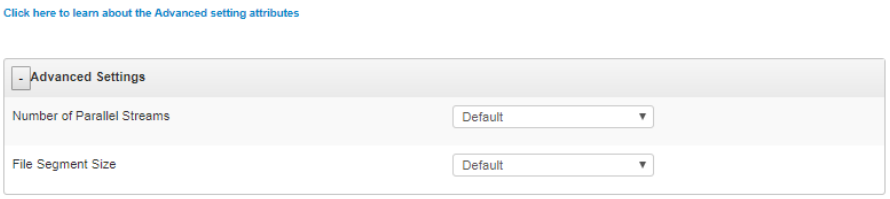
Setting	Description
Advanced Settings For details on these settings, see "Tune Ion Reporter™ Software speed parameters" on page 178.	
Number of Parallel Streams	Set the Number of Parallel Streams to **Default** (the recommended optimal speed) or select **1-5** to slow down upload
File Segment Size	Set File Segment Size to **Default** (recommended), or **16MB**, **32MB**, **64MB**, or **128MB**

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**.

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.

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 select **IonReporterUploader** .

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 **Select Ion Reporter account** dialog , 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.

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.

Your results files are transferred to Ion Reporter™ Software and defined as samples. Your selection of Ion Reporter™ Software workflow is automatically launched on your newly transferred samples.

Note: The IonReporterUploader plugin must be added to a Planned Run, or run manually.

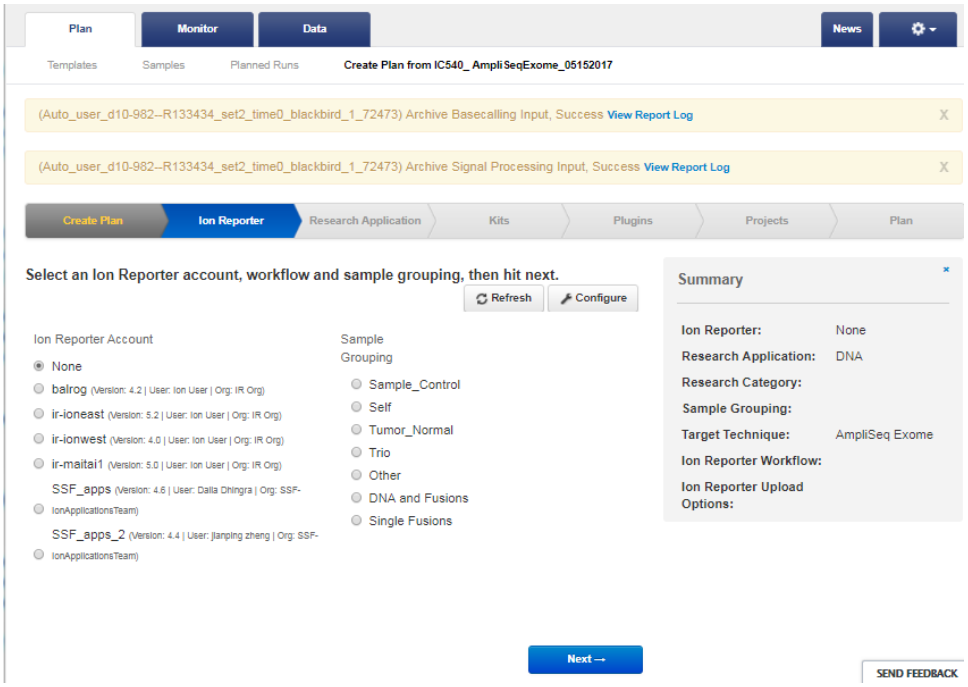
For sequencing runs that use barcoded data, select the correct barcode kit under **Kits** in the workflow bar. When you select a barcode kit, a sample name field for each barcode is generated.

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 33.

1. In the **Plan** tab, click **Templates**, then in the Favorites list, select an 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. If multiple Ion Reporter™ Software accounts are available, select the account that you want to use for the transfer of analysis files to Ion Reporter™ Software. The selected account is the one that you will use to view and further analyze the files in Ion Reporter™ Software.

   Note: To add another Ion Reporter™ Software account, click **Configure**, then use the steps in "Set up an account for IonReporterUploader plugin" on page 173.

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. In the **Existing Workflow** menu, select your Ion Reporter™ Software analysis type. When you select a workflow from this menu, the **Sample Grouping** sections show the sample relationship that is required by that workflow.

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. Click **Kits** in the workflow bar to select the following:
   a. Chip Type (required). If the template contains the chip type, that information is pre-populated in the **Chip Type** field. Otherwise, select the **Chip Type**.

   b. If your sequencing run uses a barcode kit, select that kit. Based on your barcode kit selection, a sample field for each barcode is added to **Plan** in the workflow bar.

9. If appropriate, enter the gender of a sample. For details, see "Sample gender" on page 182.

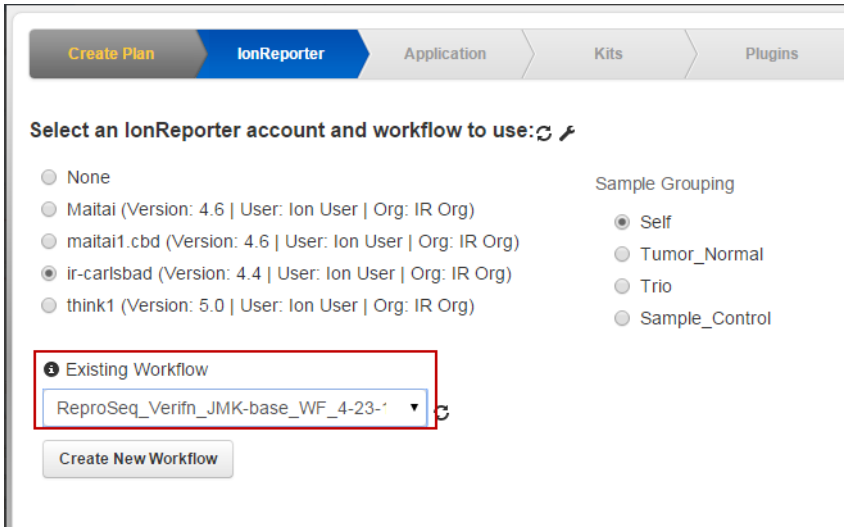
   Note: If the gender of a sample is not specified or if the sample gender is specified as "Unknown", IGV assumes that the gender of a sample is female.

10. 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.

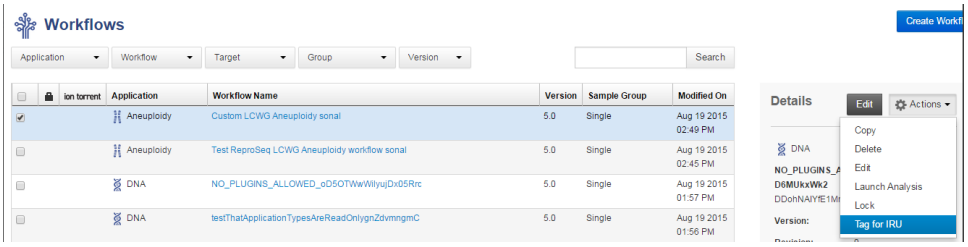
11. The Planned Run is ready to run on your sequencing system.

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 during in Torrent Suite™ Software. To do so, use the **Tag for IRU** label in Ion Reporter™ 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 the **Tag for IRU** workflows 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 genetic disease screening (GDS), are limited when the sample gender is unknown, and they return unexpected results when the gender is incorrectly specified.

For example, in the GDS 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:

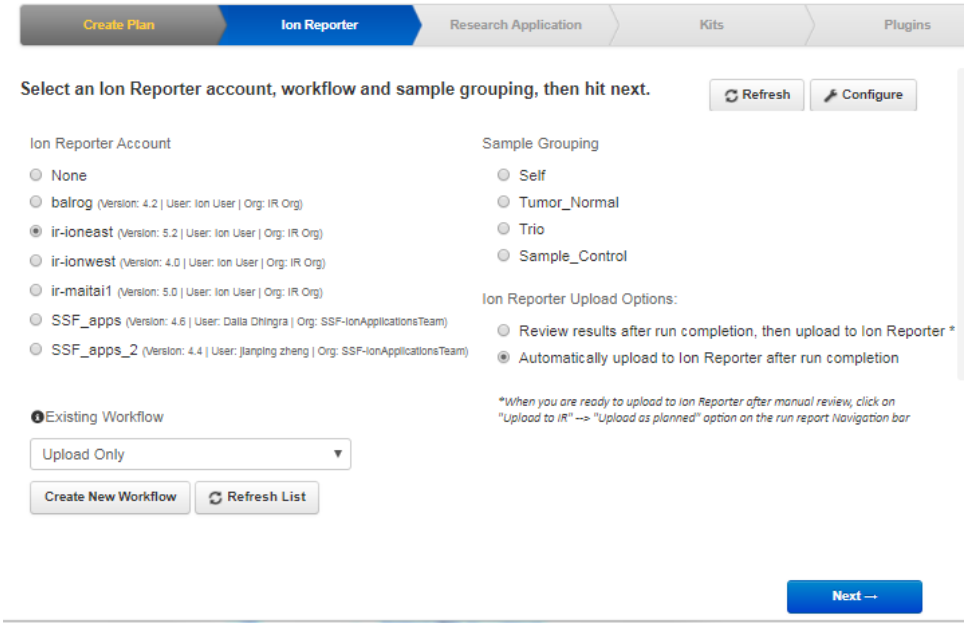
- You cannot edit samples that have been launched in an Ion Reporter™ Software analysis. Instead, define new samples from the raw data files, and add the correct gender metadata to the new samples.
- If the gender of the sample is not specified or specified as "Unknown", the Integrative Genomics Viewer (IGV) uses female as the gender.

Set **Review results** option in Run template

You can set an option to review the results of a IonReporterUploader plugin run, before data is transferred to Torrent Suite™ Software. When you set this option in the Planned Run template, you can review the details about the file transfer, then manually upload the data to Ion Reporter™ Software. Use this procedure to change the setting in a Planned Run template, then reanalyze the run and use the **Review results** option.

1. In the **Plan** tab, click **Templates**, then click the template of interest.
2. In the Ion Reporter workflow bar, select your Ion Reporter server or cloud account.

   Note: If no Ion Reporter account is listed, see "Set up an account for IonReporterUploader plugin" on page 173.
3. Select an existing workflow, then select **Review results after run completion, then upload to Ion Reporter**.



4. Make any other required changes, name your template, then click **Save**, then **Finish**.
5. In the **Plan** tab, click **Templates**, find your new template, click **Settings** (⚙) ▸ **Plan Run**.

6. Execute the run on the sequencer.

   After run is completed, the Plugin report indicates that the IonReporterUploader plugin status as completed. See "View plugin run status" on page 118 for details.

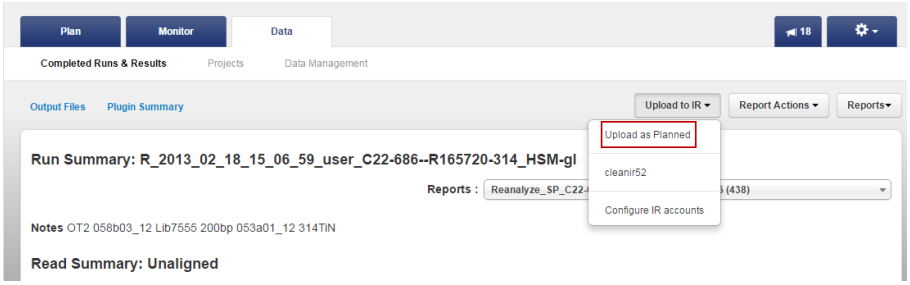
   IMPORTANT! To proceed with the IonReporterUploader upload, you must do the next steps.

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

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

   c. In the left navigation menu, click **IonReporterUploader** to view the plugin results.

   d. Review the Run results. If results are acceptable, click **Upload to IR ▸ Upload as Planned**.



   A confirmation dialog appears.

   - Select **Yes** to upload as planned.
   - Select **No** to cancel.
   - Select **Review-Plan** to look at the run results.

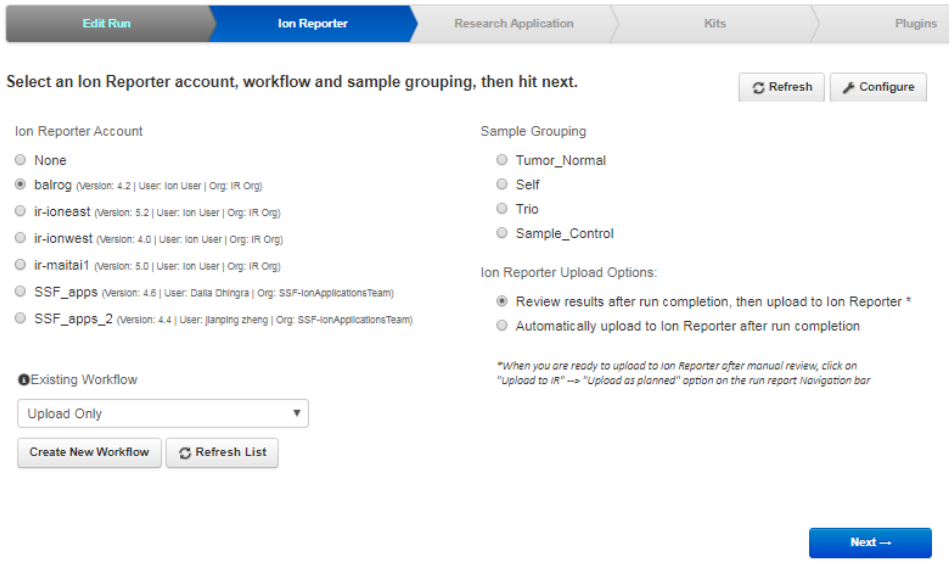
Set **Review results** option in Planned Run

You can set an option to review the results of a IonReporterUploader plugin run, before data is transferred to Torrent Suite™ Software. With this option, you can review the details about the file transfer, then manually upload the data to Ion Reporter™ Software. Use this procedure to change the setting in an existing Planned Run, then reanalyze the run and use the **Review results** option.

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. Click **Report Actions ▸ Edit Run Plan**.
4. Click **Ion Reporter** in the workflow bar, then select your Ion Reporter™ Server or cloud account.

5. **Note:** If no Ion Reporter account is listed, see "Set up an account for IonReporterUploader plugin" on page 173.

   Select an existing workflow, then select **Review results after run completion, then upload to Ion Reporter**.



6. Make any other necessary changes, then click **Update Run & Reanalyze**.

   After run is completed, the Plugin report indicates that the IonReporterUploader plugin status is complete. See "View plugin run status" on page 118 for details.

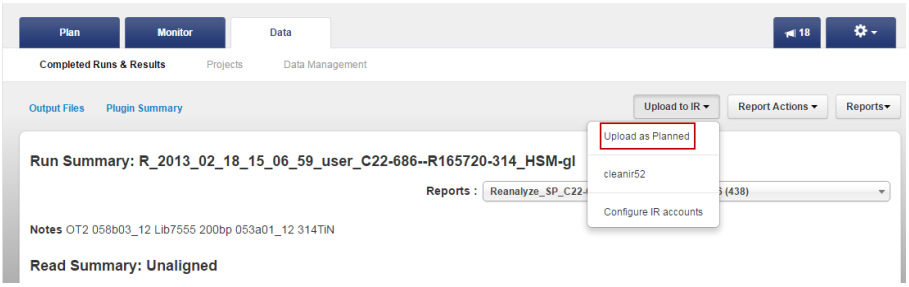
   IMPORTANT! To proceed with the IonReporterUploader upload, you must do the next steps.

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

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

   c. In the left navigation menu, click **IonReporterUploader** to view the plugin results.

   d. Review the Run results. If results are acceptable, click **Upload to IR ▸ Upload as Planned**.



A confirmation dialog appears.

- Select **Yes** to upload as planned.
- Select **No** to cancel.
- Select **Review-Plan** to look at the run results.

IonReporterUploader plugin file transfer progress

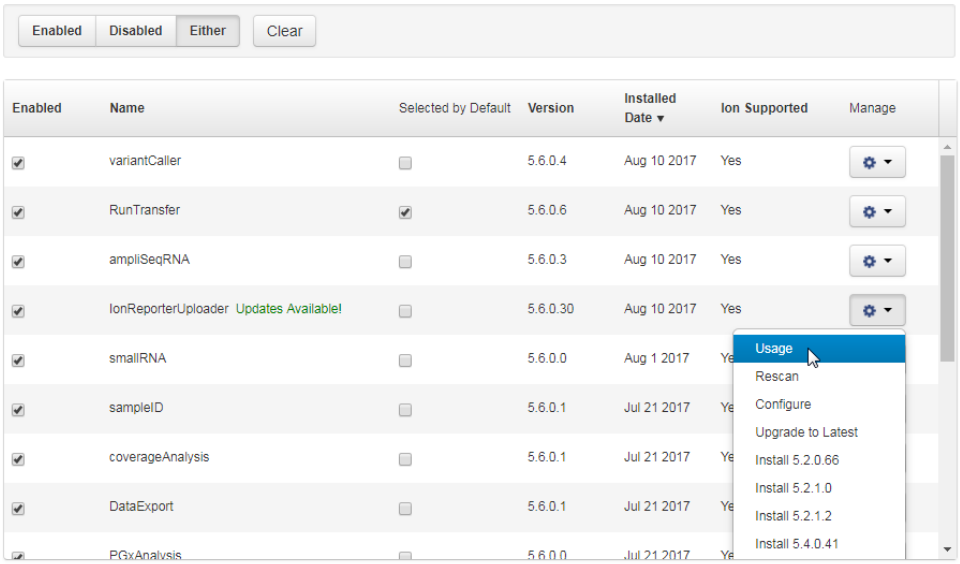
You can monitor the progress of the transfer of analysis results files from Torrent Suite™ Software to Ion Reporter™ Software.

To monitor IonReporterUploader plugin progress through the following	See the following
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	See the Torrent Suite™ Software Help
Log files	"Open a plugin log" on page 119

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 **Settings (⚙)Usage** for 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 119.

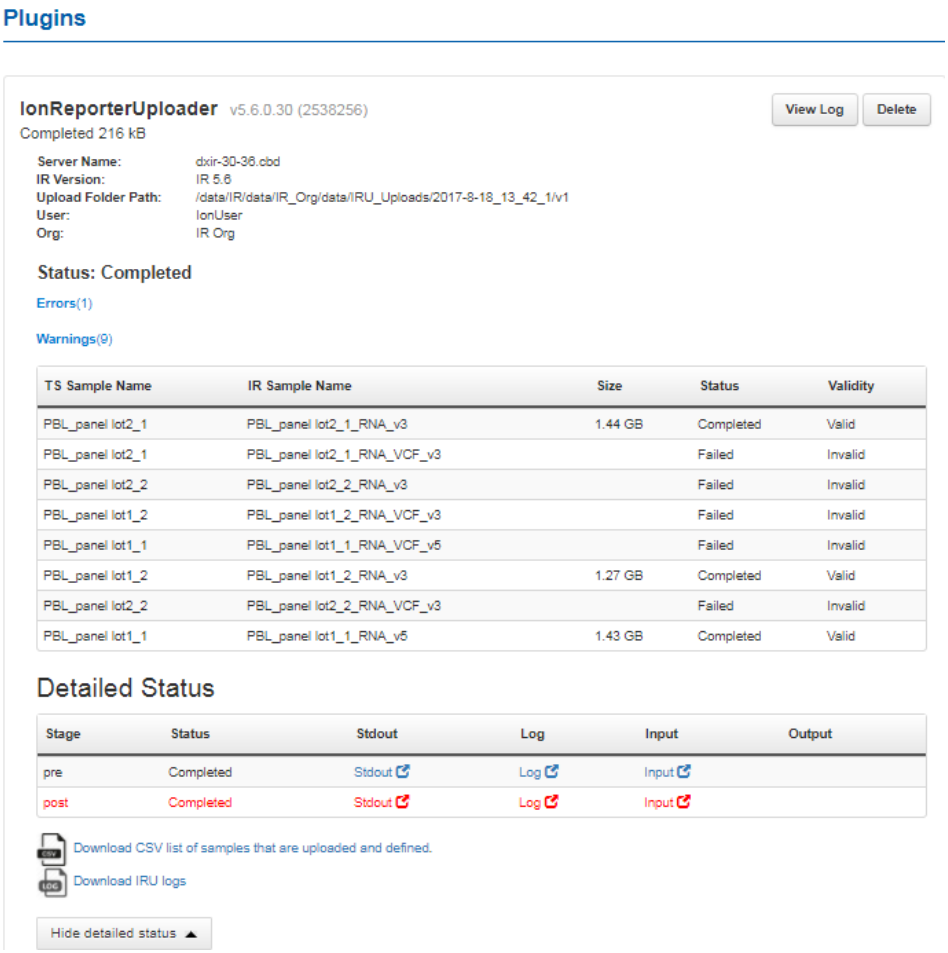
Review IonReporterUploader plugin results

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 errors 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

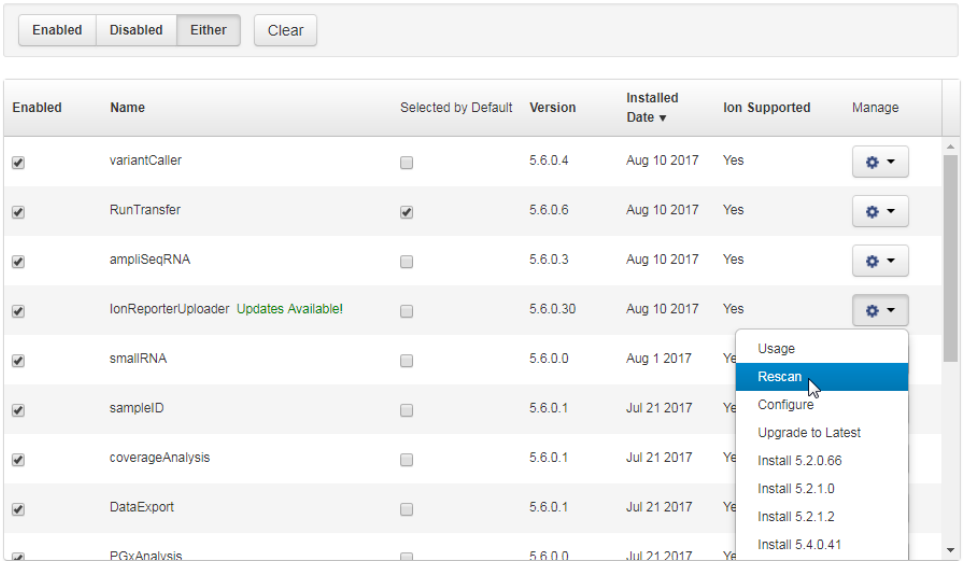
Each output file type is required as input to the next analysis phase. In almost all cases, the Ion Reporter™ Software analysis phases are performed in order.

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 a Ion Reporter™ Software analysis, a 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 **Settings ( )Rescan**.

   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 187.

Ion Reporter™ Uploader command-line utility

You can use IRU command-line utility to transfer files from a local machine (that is not an Ion Reporter™ Software server) to the Ion Reporter™ Software server. This utility can be used if you do not have access to Torrent Suite™ Software, and you have files on your local machine that you want to transfer. For example, if you have a BAM or VCF file on your local machine that you want to upload and then analyze the file in Ion Reporter™ Software.

You can also use IRU command-line utility if you have problems using the plugins in Torrent Suite™ Software.

The IonReporterUploader command-line utility is a stand-alone utility that is not part of either Ion Reporter™ Software or the Torrent Browser. This procedure is recommended only for users who are familiar with the command-line utilities.

The Command-line Uploader 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.

Download Ion Reporter Uploader command-line utility

This procedure explains how to download and install the Ion Reporter Uploader command-line utility from Ion Reporter™ Software.

Ideally, download the Ion Reporter Uploader command-line utility onto the machine where you run it. At a minimum, use a machine with the same 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 your target machine.
3. On your target machine, extract the downloaded **IonReporterUploader-cli.zip** file, then copy the **IonReporterUploader-cli** directory to a convenient location.

Run Ion Reporter Uploader command-line utility

The Ion Reporter Uploader command-line utility (irucli) is ready to run after you extract it. Run the Ion Reporter Uploader command-line utility from the IonReporterUploader-cli bin directory (with the **irucli.bat** or **irucli.sh** script). Instructions for using the command-line uploader are downloaded with the utility and with Ion Reporter documentation.

Variant calls in Torrent Suite Software

To call single-nucleotide polymorphisms (SNPs), multi-nucleotide polymorphisms (MNPs), insertions, deletions (INDELS), and block substitutions in a sample across a reference or within a targeted subset of that reference in Torrent Suite Software, use the variantCaller plugin.

This variantCaller plugin provides optimized pre-set parameters for many experiment types. The settings can also be customized. After you find a parameter combination that works well on your data and includes the balance of specificity and sensitivity that you want, you can save that parameter set and reuse it in your research.

Supported Ion AmpliSeq™ panels

The variantCaller plugin supports the various panels in the Ion AmpliSeq™ family of sequencing kits. See the **AmpliSeq.com** website.

Run the variantCaller plugin

There are two ways to run the variantCaller plugin:

- Configure the variantCaller plugin as part of the Planned Run or Run template to run it immediately after primary analysis is complete.
- Run the variantCaller plugin at any time from a completed Run report.

Note: To get variantCaller plugin results as quickly as possible, set up the plugin to run automatically.

variantCaller plugin configuration

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

Note: Changes to parameters can dramatically affect the behavior and sensitivity of the variantCaller plugin. If you are new to the variantCaller plugin, we recommend that you do not change parameters.

Note: The variantCaller plugin parameter settings change according to the selections you make. Data from the Ion PGM™, Ion Proton™, and Ion S5™ sequencers require different default settings. Select settings that are appropriate for both your sequencing instrument and your experiment.

Setting	
Configuration	If you run the the variantCaller plugin manually, you can apply the same settings to all barcodes, with a saved configuration. Use the Configuration dropdown list to apply the settings.  Click **Manage Configurations/Barcodes**to review or change the configurations and barcodes.
Chip Type	Select the chip type that is used in the sequencing run.
Library Type	Select Whole Genome, AmpliSeq or TargetSeq. When **Library Type** is set to AmpliSeq, read trimming will be automatically applied to remove the primers from reads.
Variant Frequency	Select one of the following choices: • **Somatic:** This option detects somatic variants at low allele frequencies. • **Germline:** This option optimize detection of germline variants that are expected to be present at allele frequencies of 50 to 100 percent.
AmpliSeq Panel	If the sequencing run used an AmpliSeq panel, you can select a panel here to automatically add the selections for Reference Genome, Target Regions, Hotspot Regions, and Parameter Settings.
Reference Genome	Select a reference genome that the current Run report was generated against and that is for use with variant calling. The files that you upload when you configure the variantCaller plugin must be uploaded to a specific reference, such as hg19. For details, see "Upload a new reference file" on page 226.

Setting	
Targeted Regions	Variant calling is restricted to only the regions of interest that you specify in the targeted regions file. **Note:** Before you can use a targeted regions file, it must be uploaded in the Torrent Browser References and associated with a specific reference. See "Target Regions Files and Hotspot Files" on page 231 for details. If a target regions file is not provided, the variantCaller plugin analyzes every position of the reference genome, which typically takes longer. In the case of a whole genome analysis, variantCaller plugin processes every position that corresponds to the reference genome.
Hotspot Regions	A hotspots regions file contains a list of alleles on the genome. Before you can use a Hotspot Regions file, it must be uploaded in the Torrent Browser References and associated with a specific reference. See "Target Regions Files and Hotspot Files" on page 231 for details. When a hotspot regions file is used, the alleles listed in the hotspots regions file at the position will be generated as variant candidates, then be evaluated. variantCaller plugin output files include these alleles whether or not a variant is called, and include evidence for a variant and the filtering thresholds that disqualified a variant candidate. The filtering metrics for each allele are reported in the output VCF file, including for NOCALL.

Setting	
Parameter Settings	You can use advanced parameter settings that are pre-configured in the variantCaller plugin, or you can customize parameter settings. • The variantCaller plugin provides these defaults that are optimized for several experiment types. Select **Generic** to use pre-configured parameter settings for Chip Type, Library Type and Variant Frequency. • Select **Custom** to change the advanced parameter settings. For details see "variantCaller plugin advanced parameters" on page 212. • **Germ Line - Low Stringency** Optimized for high frequency variants and minimal false negative calls for Ion AmpliSeq™ experiments. • **Somatic - Low Stringency** Optimized for low frequency variant detection with minimal false negative calls Ion AmpliSeq™ experiments. • **Germ Line - TargetSeq Low Stringency** Optimized for high frequency variants and minimal false negative calls. (Ion Proton™ data only). For Ion AmpliSeq™ experiments, when you import your template from AmpliSeq.com, your template and run plans are already pre-configured with parameters that are optimized for your panel.
Load external parameter file	You can import a file that contains parameter settings that are optimized for fixed panels and community panels in ampliseq.com. (Optimized parameter sets for on-demand panels are not supported in this release.) Click **Custom Settings Choose File** to upload a custom parameters file. You can also download the parameters used in a variantCaller run and then either customize those parameters or reuse them in future variantCaller runs. The parameters file is a JSON text file of the variantCaller parameter values.
Configuration Name	You can name and save a configuration if you have made changes to the pre-configured settings, or have used the **Advanced Settings**.

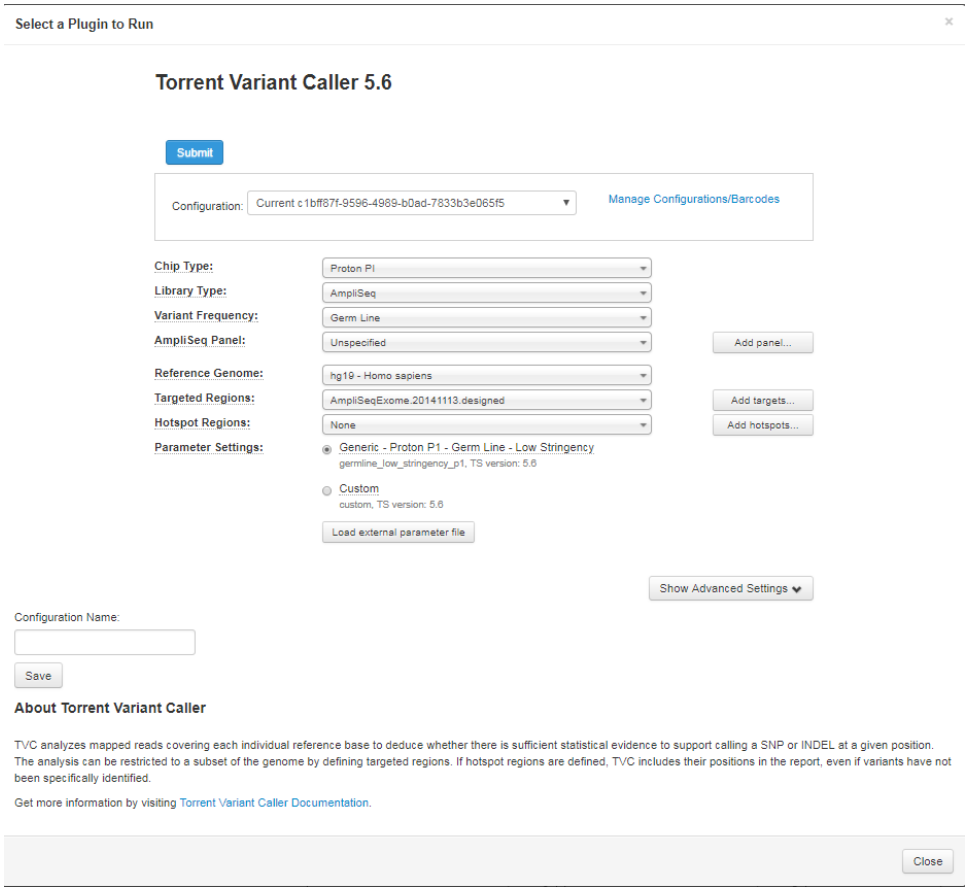
Manage configurations for the variantCaller plugin

You can save configurations settings for the variantCaller plugin and apply the configuration later when you run the plugin manually. For barcoded runs, you can save a variantCaller plugin configuration that applies to all barcodes. You can also temporarily apply one or more saved configurations to individual barcodes. When you apply a saved configuration to individual barcodes, you must run the plugin when you apply the configurations. Configurations applied to individual barcodes cannot be saved.

Create or modify a configuration for the variantCaller plugin

When you run the variantCaller plugin manually, you can configure and save a barcode configuration setting so that you can reuse the configuration for other sequencing runs.

1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** for the sequencing run of interest.
2. Click **Plugins ▸ Select Plugins to Run**.
3. Select the variantCaller plugin. The variantCaller plugin configuration screen opens.



4. Click **Manage Configurations/Barcodes**. In the **Configuration** tab, you can add a new configuration or edit an existing configuration:
   - Click **Add** to add a new plugin configuration. Name the configuration, then select settings for that configuration.
   - Click the **Edit** link to modify an existing plugin configuration. Change the configuration settings. You can modify Chip and Library types, variant frequency, reference genome, targeted, and hotspots regions and parameter settings. You can also click **Show Advanced Settings** and adjust variant detection and alignment parameters.
5. Click **Save**.

If you create a new configuration, it is added to the list on the Configuration tab. An existing configuration reflects the changes that you made. You can now apply the configurations when you run the variantCaller plugin manually. See "Run the variantCaller plugin manually" on page 199 for details.

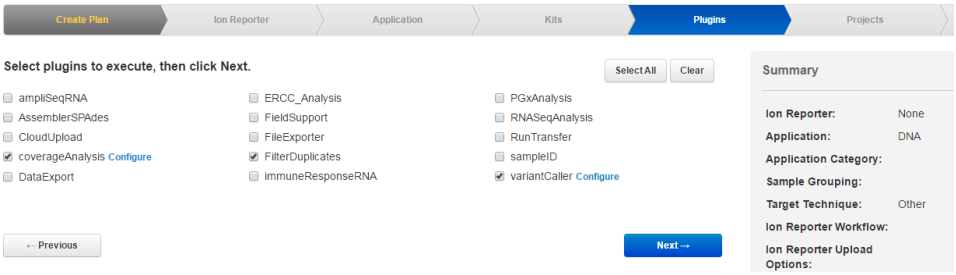
Configure the variantCaller plugin in a Planned Run or Planned Run template

To run the variantCaller plugin automatically after the Torrent Suite™ analysis completes, configure the vvariantCaller plugin when you create a Planned Run or Run template.

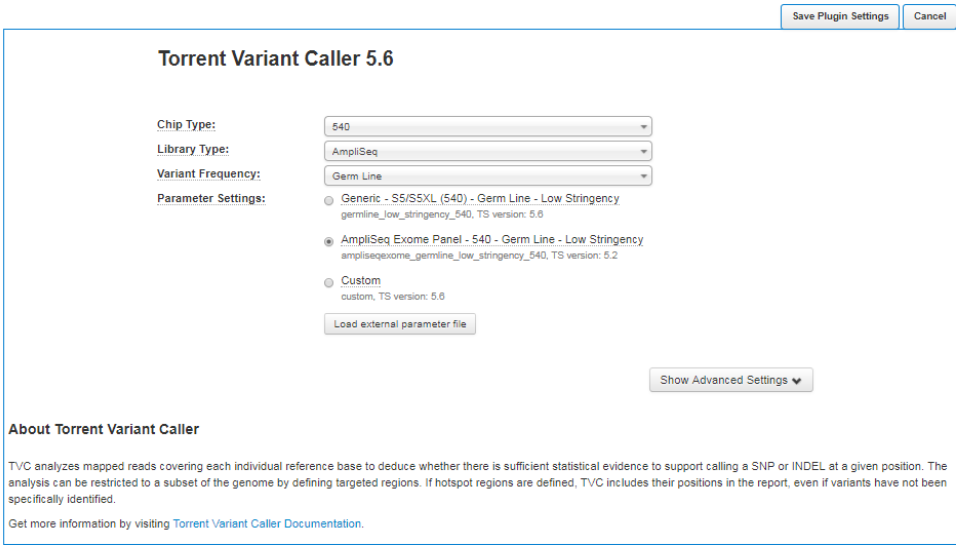
IMPORTANT! The variantCaller plugin parameter settings are saved in Run templates but *are not saved* in Planned Runs. Parameter changes that you make in a Planned Run affect only that specific run. When you change variantCaller plugin parameter settings in a Run template, your changes affect all users who create run plans from that template.

---

1. During the plugin step in the Workflow bar of the Planned Run or Planned Run template, select the **variantCaller** checkbox, then click the **Configure** link next to the **variantCaller** listing:



2. Make your changes to the parameter values available in the variantCaller configuration screen. For details, see "variantCaller plugin configuration" on page 194.



3. *(Optional)* Click **Show Advanced Settings**, then customize additional parameters.

4. Click **Save Changes**.

   Note: The settings for the reference genome file, target regions file, and hotspots file that are used in the Planned Run are used for all barcoded samples on the chip when the variantCaller plugin is run automatically. When you configure the variantCaller plugin in a Planned Run, you cannot change the reference genome file, target regions file, or hotspots file for any barcoded sample. The parameter file for the Planned Run will be applied to all barcodes.

   To use a different reference genome file, target regions file, or hotspots file, for one or more barcodes, or if you want to apply different parameter files for each barcode, you must run the variantCaller plugin manually.

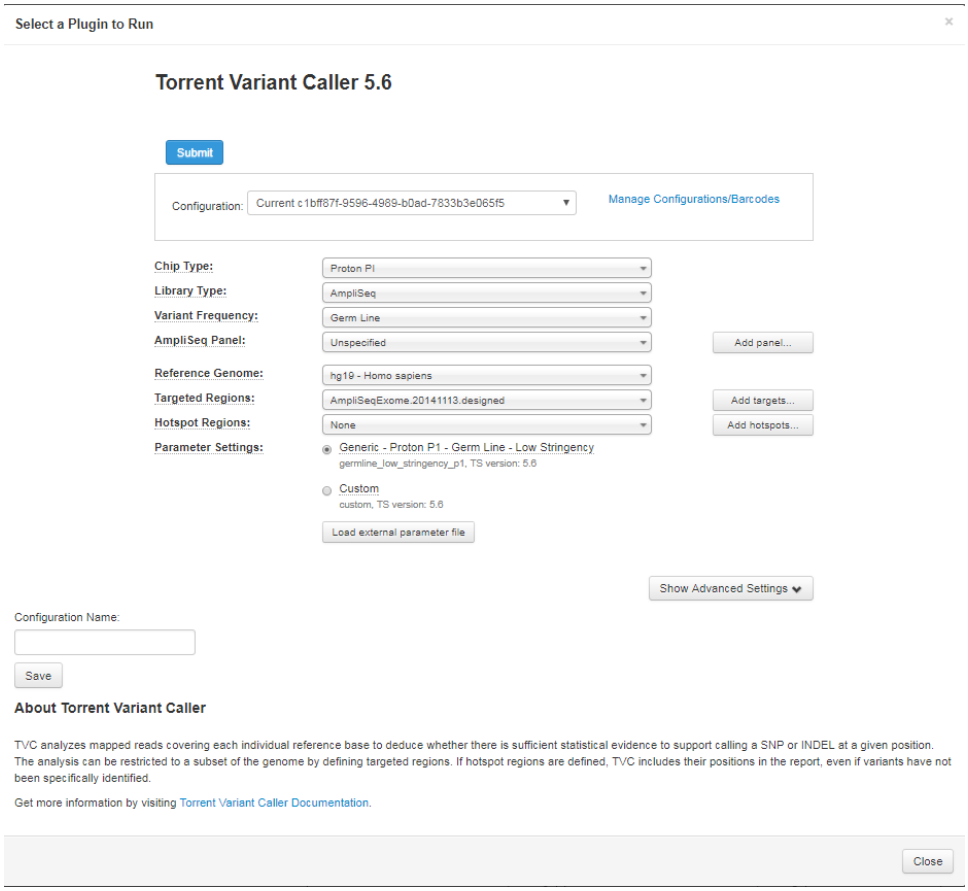
Run the variantCaller plugin manually

You can start the variantCaller plugin from a completed run report. If the sequencing run includes barcodes, you can apply one configuration that you saved previously all barcodes, or you can apply a different configuration individual barcodes.

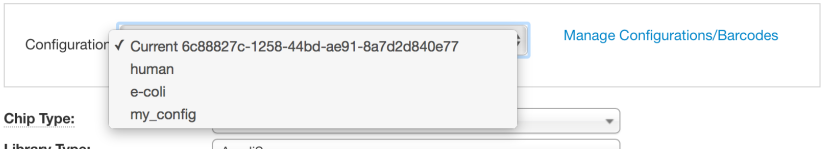
1. In the **Data** tab, click **Completed Runs & Reports**, then click the **Report Name** for the completed sequencing Run of interest.

2. Click **Plugins** ▸ **Select Plugins to Run**.

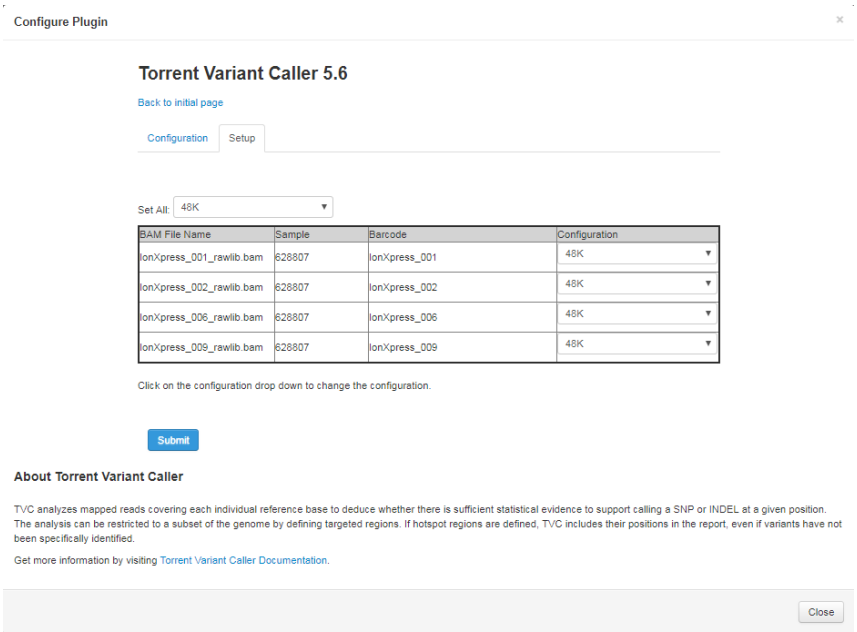
3. Select the variantCaller plugin. The variantCaller plugin configuration screen opens.



4. Apply one or more configurations to barcodes:
   - If you want to apply the same configuration to all barcodes:
     a. Select the configuration in the **Configuration** dropdown list.



     b. *(Optional)* Make any other changes to the configuration settings.
   - If you want to apply a different configuration to individual barcodes:
     a. Click **Manage Configurations/Barcodes**, then click **Setup** and apply a saved configuration to for one or more barcodes.



5. When your changes are complete:
   - Click **Submit**. The variantCaller plugin reruns, then applies the changes that you made.
   - If you want to save the configuration for later use, click **Close** to close dialog without running the plugin.

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

Review variantCaller plugin run results

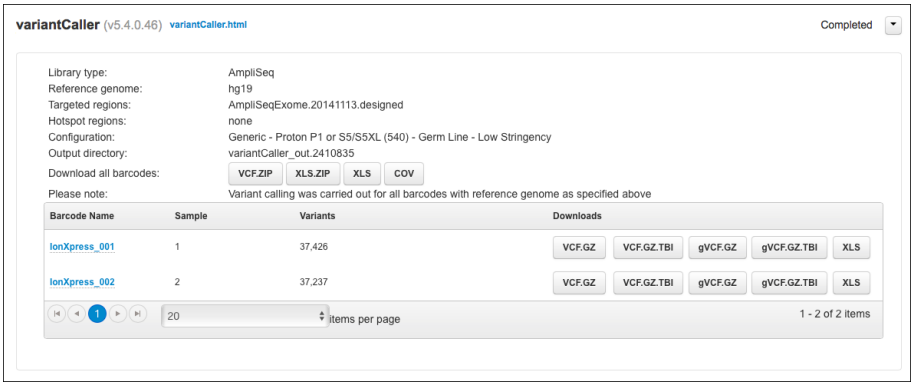
After your variantCaller plugin run completes, review results on the run summary page.

Variants that pass all the set filters are reported to a single output VCF file. All variants, not at the hotspots regions, that fail any one of the set filters are reported to a filtered output VCF file. The filtered variants have an associated filter reason tag in the

VCF file, which the users can query to identify the filters that the candidate variant failed to pass.

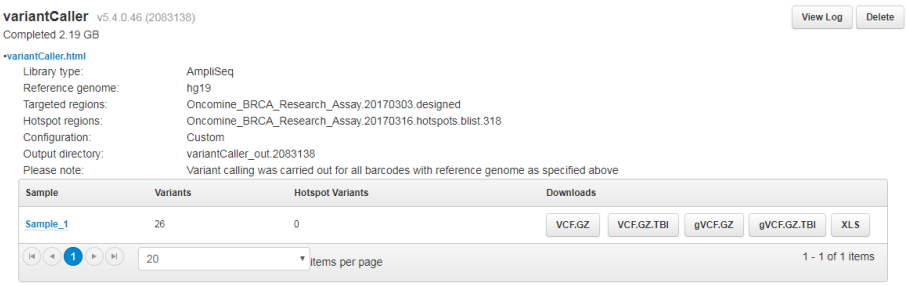
1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, find the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **variantCaller** to view the plugin results.

   The variantCaller plugin summary includes information about the analysis type, targeted regions and hotspot files, and variantCaller parameter settings and the total number of variants called.
4. Click **variantCaller.html** link to open the **variantCaller** plugin report in the browser tab.
   - If the sequencing run contains barcodes, the plugin report includes a list of the barcodes that were used and file download options.



Download option	Description
VCF.ZIP	A compressed directory that contains the VCF files of each barcode.
XLS.ZIP	A compressed directory that contains the tables of alleles for each barcode in tab-separated format, which can be opened in Microsoft™ Excel™.
XLS	The table of alleles of all barcodes in tab-separated file, which can be opened by Microsoft™ Excel™.
COV	This a file for 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 the sample used in the and file download options.



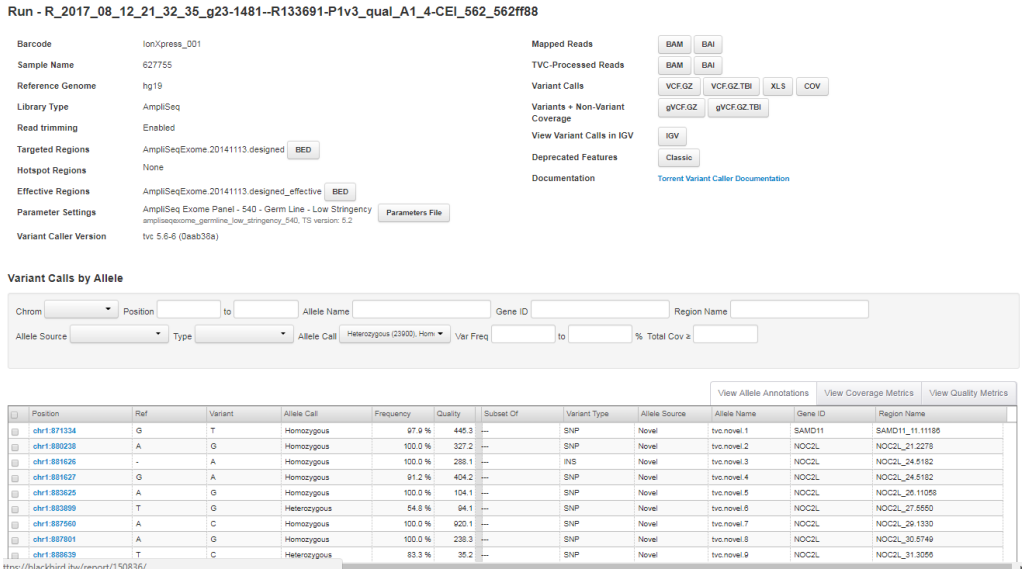
5. To open the detailed variantCaller plugin report for each barcode, click one of the following links in the **variantCaller** section of the plugin summary:

   - `Barcode name` link in a report from a barcoded sequencing run. For example, **IonXpress_301**.
   - `Sample name` link in a report from a sequencing run that does not contain barcodes. For example **Sample_1**.

   You can download files for all barcodes and files for individual barcodes. See "Detailed variantCaller plugin summary report" on page 202 for more information.

Detailed variantCaller plugin summary report

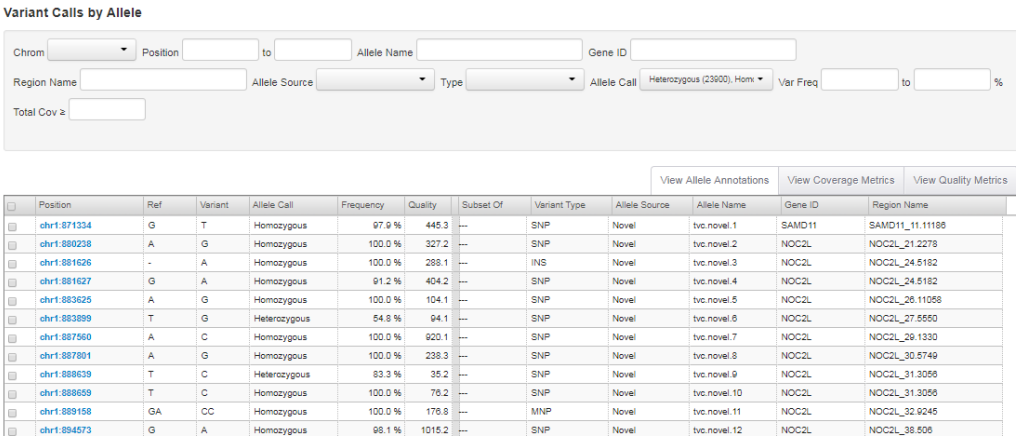
You can view details about individual barcodes and download files in the **variantCaller** section of the plugin summary. Click the **Barcode Name** link in a report from a barcoded sequencing run; Click the **Sample Name** link in a report from a sequencing run that does not contain barcodes.



Download option	Description
VCF.GZ and **VCF.GZ.TBI**	Compressed directory of variant call results files in VCF file format
gVCF.GZ and **gVCF.GZ.TBI**	Compressed directory of files for the variant call results and the coverage in the genome VCF (gVCF) file format.
XLS	The table of alleles in the tab-separated file format, which can be opened in Microsoft™ Excel™.

Variant Calls by Allele table

The Variant Calls by Allele table in the variantCaller plugin detailed report includes the following features:



- Each position is a link to open the variant in Integrative Genomics Viewer (IGV). In some browsers, you save the `igv.jnlp` file to your local system, and then click on `igv.jnlp` to open the IGV browser.
- Click on a column header to order the table by the contents of that column.
- For candidates that are filtered out, the filtering reason is highlighted in the table. For example:

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

The main columns are described in the following table. Use the tabs on the right of the table (View Allele Annotation, View Coverage Metrics, and View Quality Metrics) to change the display of the columns on the right.

Column	Description
Position	The chromosome (or contig) name in the reference genome, and the one-based position in the reference genome.
Ref	The reference base(s).
Variant	Variant allele base(s).
Var Freq	Frequency of the variant allele.
Quality	Phred-scored quality field. [1] Larger values mean more certainty in the call. 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 or there is insufficient depth to resolve, or the observed allele frequency is near the cutoff. Filters to compensate for the cases in which the model assumptions are not true are found in the INFO tags. Computed by posterior probability that the sample variant allele frequency is greater than the min-allele-frequency specified for the variant type (if a variant), or posterior probability that the variant allele frequency is below this threshold (if a reference call). Posterior probability computed conditional on the reads observed, includes sampling variability.

[1] For variants found by the indel assembler, this value is always set to 50.

View allele annotations

These columns are displayed in the run report in the View Allele Annotations tab:

Column	Description
Variant Type	• SNP Single nucleotide polymorphism • IND Insertion • DEL Deletion • MNP Multiple nucleotide polymorphism, the substitution of a block sequence by the block of another length • COMPLEX Block substitution of sequence by a block of unequal length

Column	Description
Allele Source	• Hotspot if called only because of its entry in a hotspots file • Novel all others
Allele Name	The Allele name as given in the hotspot regions file, if it is an hotspot allele. Since Torrent Suite™ Software 5.6, the name of an novel allele is given by `tvc.novel.#`.
Gene ID	The Gene ID as given in the target regions file
Region Name	The regionname as given in the target regions file

View coverage metrics

These columns are displayed in the run report in the View Coverage Metrics tab:

Column	Description
Coverage	Total coverage at this position, after downsampling [1]
Coverage +	Total coverage on the forward strand, after downsampling
Coverage -	Total coverage on the reverse strand, after downsampling
Allele Cov	The number of reads that contain this allele, after downsampling
Allele Cov +	Allele coverage on the forward strand, after downsampling
Allele Cov -	Allele coverage on thereverse 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**.

View quality metrics

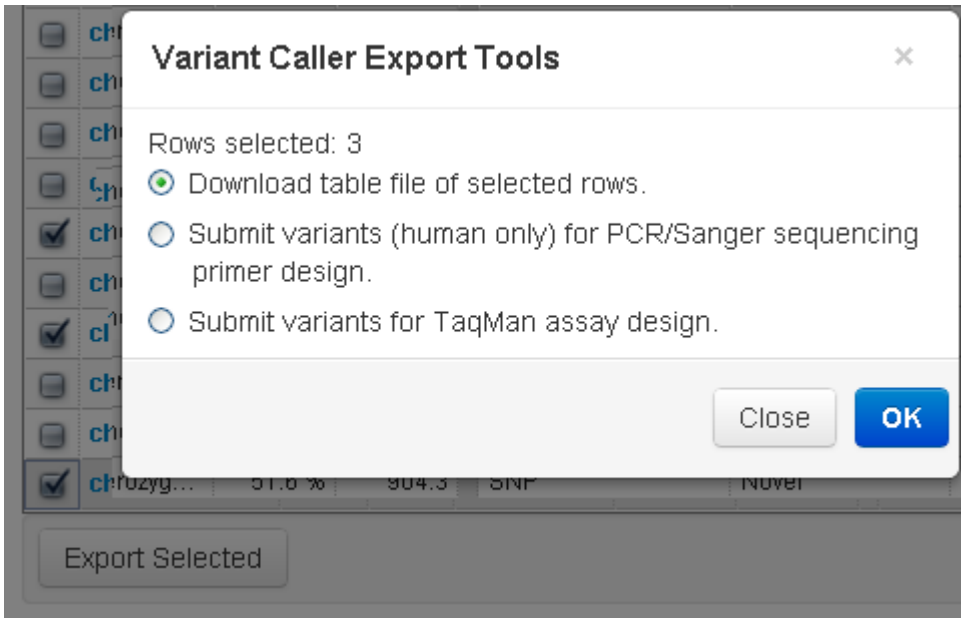
These columns are displayed in the run report in the View Quality Metrics tab. Associated filtering codes are given in brackets.

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 under the 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).

Export variant calls to a file

This option exports your variant calls to a tab-separated file. The exported file is named `subtable.xls` and has the same columns that are included in the Variant Calls table (including columns for all three display options: View Allele Annotations, View Coverage Metrics, and View Quality Metrics).

Click the left column checkboxes to select your variants, then click the **Export Selected** button:



Troubleshoot the variantCaller plugin results

Click **Show Troubleshooting** in the variantCaller plugin detailed report to troubleshoot the variantCaller plugin results for missing variants (false negatives) or false positives. You can also export the results to share them with a field bioinformatics specialist.

Find false negatives

When a variant expected to be present is not called by the variantCaller plugin, an alignment viewer, such as Integrative Genomics Viewer (IGV) or Ion Reporter™ Genomic Viewer (IRGV) in Ion Reporter™ Software, can help you ensure the presence of the variant in the sample at the position where it is expected.

- IGV can reveal problems, such as mismapping or low coverage.
- Visually inspect the coverage of the region where the variant is expected, paying special attention to the depth of coverage and the quality of the bases covering the position of the variant. Low coverage or low base quality might explain the no-call.
- The variant could be slightly misplaced (especially for indels).

Optionally, the variantCaller plugin has built-in tools that can be used to display call details.

If a hotspots regions file was used:

1. Check that the position of the variant is included in the hotspots file.
2. 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

3. Adjust parameters.
4. Rerun the variantCaller plugin.

If no hotspots regions file was used:

1. Navigate to the variantCaller plugin results directory on the Torrent Server and open the file small_variants_filtered.vcf. On Linux, 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. In the Torrent Suite™ Software, you can access the variantCaller plugin results directory by opening the variantCaller plugin results for the sample or barcode of interest, removing the final 'variantCaller.html' from the URL, and press **Enter**.
2. If the location of the variant is found, look at the FR field (filtered reason).
3. Relate the reason to parameters using the table Filtering Codes variantCaller v4.x.
4. Adjust parameters.
5. Rerun the variantCaller plugin.

If the location of the SNP is NOT in the filtered.vcf file, create a hotspots regions file that includes this location.

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:

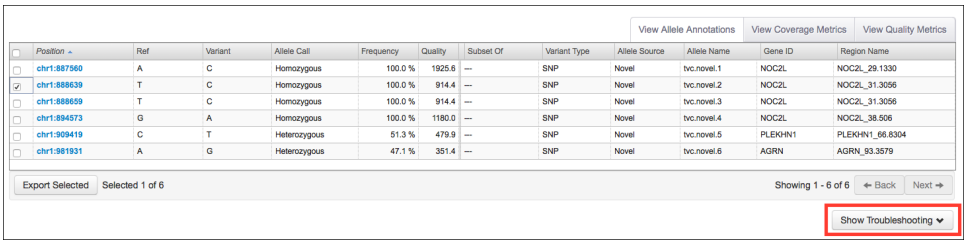
- Adjust parameters that control the homopolymer calls. This can increase the report of false negatives.
- If you are repeatedly running a panel, manually curate specific sites (positions) since the false positive tends to occur in the same positions.

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

Export files for troubleshooting

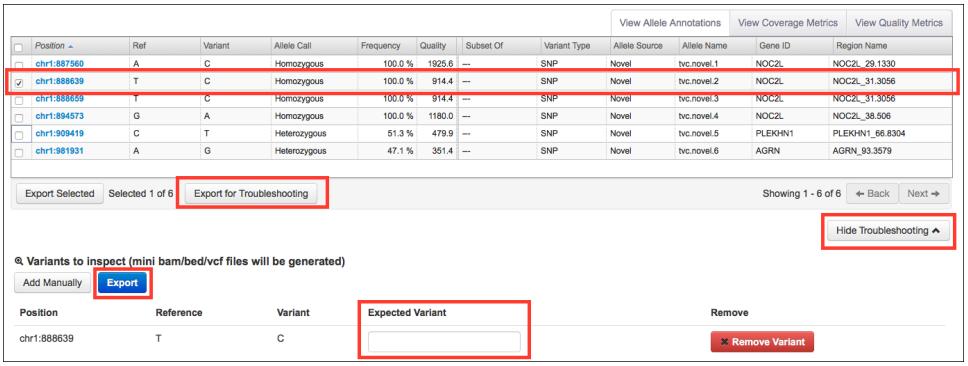
Torrent Suite™ Software includes a tool that helps you determine why variant calls are unclear in analyses. You can use the Slicer tool to select one or more variant calls, then export the related data as miniature BAM, BED, and VCF files. You can then share these files with a field bioinformatics specialist for further review.

1. In the variantCaller plugin output table, click **Show Troubleshooting**.



2. Select the variants of interest.

3. Click **Export for Troubleshooting**.



4. Enter the **Expectant Variant**.

5. Click **Export**.

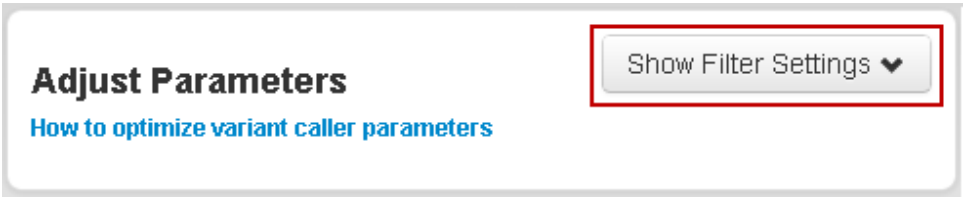
6. Click **Download the .zip**.
   The compressed directory of miniature BAM, BED, and VCF file is downloaded to the directory, according to your browser settings.

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.

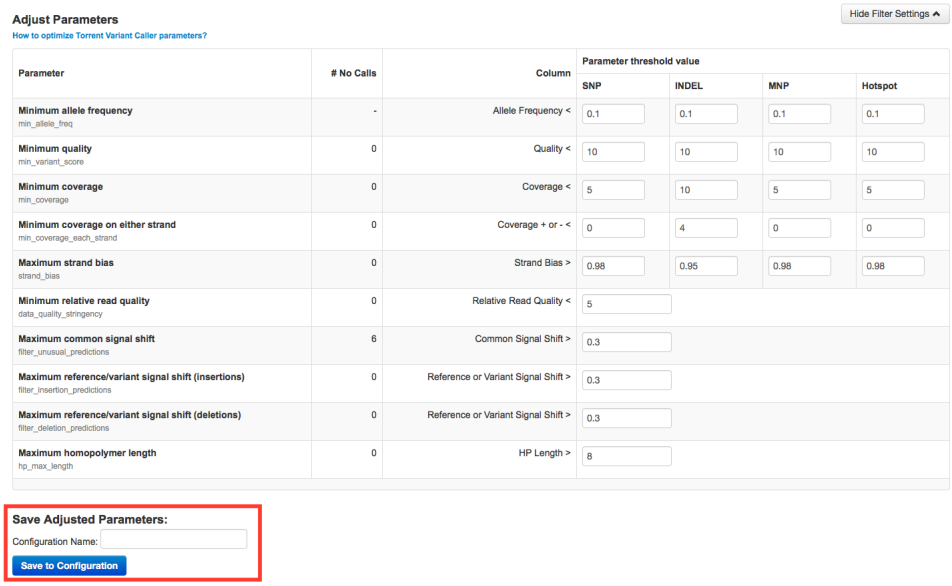
Note: The reference genome, target regions, and hotspots files in the saved configuration inherit the files that are used to obtain the variantCaller plugin results for this barcode.

1. Scroll to the **Adjust Parameters** section at bottom of the detailed results page, then click **Show Filter Settings**:



2. In the parameter list, change the parameter settings:

   Note: Only the parameters that are most commonly changed available.



3. Enter the Configuration Name that stored the adjusted parameters, then click **Save to Configuration**.

4. To add barcodes to the configuration, run the plugin manually, then assign the barcode to the saved configuration with the adjusted parameters.

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.

In general, you can safely customize parameters for SNP calling. For indel calling, changes to the parameters tend to have a significant effect in the number of indels called. With indels, the tradeoff between sensitivity and specificity becomes too large.

Both the long indel assembler and the FreeBayes module generate lists of variant candidates. The list is then passed on to other modules that evaluate the candidates. The assembly module attempts to call any indel longer than 3 bp, but only reports indels that fail to be called by the FreeBayes module.

Parameter	Description
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)
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
min_detail_level_for_fast_scan	A variant candidate evaluating parameter: The minimum detail-level to trigger the fast scan algorithm that considerably speeds up the evaluator. **Allowed values:** >=0 (0 = always apply the fast scan algorithm) **Suggested trial value:** 0
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 (20 if the Hotspots file contains long variants)
suppress_recalibration	A variant evaluation parameter: Homopolymer recalibration values should not be used when set **Allowed values:** 0 = allow recalibration, 1 = do not allow 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

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, moderate FD, FD) allele applies the (INDEL, SNP, MNP) parameters, respectively. If powered on, the (non-FD, moderate FD, FD) allele applies the (INDEL, SNP, MNP) parameters, respectively. **Allowed values:** 0: do not use FD parameters, 1: use FD parameters.
min_ratio_for_fd	A filter parameter: Claim flow-disruption if the portion of reads that are flow-disrupted >= this value
indel_as_hpindel	A filter parameter: A flag indicating whether INDEL filters or SNP filters should be applied to non-HP indels
X_min_allele_freq	X is one of the allele type 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 and 0.2
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 **Related VCF fields:** QUAL

Parameter	Description
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
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 **Filter reason:** STDBIAS and STDBIASPVAL **Related VCF field:** STB **Allowed values:** Decimal numbers between 0.5 and 1.0 **Suggested trial value:** 0.95

Parameter	Description
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
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 **Related VCF field:** MLLD **Filter reason:** STRINGENCY **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 **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**

Parameter	Description
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
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
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:** []

Parameter	Description
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
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, POSBIASPVAL **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.05

Parameter	Description
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 **Related VCF field:** PPA **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.02
report_ppa	(Torrent Suite Software 5.4) Report Possible Polyploidy Alleles (PPA) in the INFO FIELD of the vcf lines. **Related VCF field:** PPA **Allowed values:** 1 = report PPA, 0 = do not report

Long indel assembly advanced settings

The Long indel assembly 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

Parameter	Description
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
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 generates a list 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:**1 = generate indel candidates, 1 = generate indel candidates **Suggested trial value:** 1
allow_snps	Candidate generation parameter: Allow SNP candidates to be generated when set **Allowed values:** 1 = generate indel candidates, 1 = generate indel candidates **Suggested trial value:** 1
allow_mnps	Candidate generation parameter: Allow MNP candidates to be generated when set **Allowed values:** 1 = generate MNP hypotheses , 1 = generate indel candidates **Suggested trial value:** 1
allow_complex	Candidate generation parameter: Allow complex variant candidates to be generated when set **Allowed values:** 1 = generate MNP hypotheses, 0 = don't generate **Suggested trial value:** 1
gen_min_alt_allele_freq	A candidate generation parameter: A non-HP-indel candidate needs to have an allele frequency greater than this value in the pileup **Allowed values:**Decimal numbers between 0 and 1 **Suggested trial value:** 0.02 to 0.15
gen_min_indel_alt_allele_freq	A candidate generation parameter: An HP-indel candidate needs to have an allele frequency greater than this value in the pileup **Allowed values:** Decimal numbers between 0 and 1 **Suggested trial value:** 0.02 to 0.15

Parameter	Description
gen_min_coverage	A candidate generation parameter: A variant candidate location needs to have coverage depth greater than this value **Allowed values:** Integers >= 0 **Suggested trial value:** 6
min_mapping_qv	A candidate generation and variant evaluation parameter: Minimum mapping quality value required for a read to be considered (for both candidate generation and variant evaluation) **Allowed values:** >= 0 **Suggested trial value:** 4
read_snp_limit	Do not use reads with number of snps above this **Allowed values:** Integers >= 0 **Suggested trial value:** 10
read_max_mismatch_fraction	A candidate generation parameter: Ignore reads with fraction of mismatch greater than this value **Allowed values:**Decimal numbers between 0 and 1 **Suggested trial value:** 1.0

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

GRCh38 human reference

New in Torrent Suite™ Software 5.4, you can start using the Ion GRCh38 human reference in custom run plans. The new Ion GRCh38 Reference Genome is based on the latest GRC human reference assembly. Highlights include:

- Changes to chromosome coordinates
- Corrected errors in the former sequence
- Addition of Mitochondria
- Multiple loci for some highly variable genes.

Add the Ion GRCh38 Reference to Torrent Suite™ Software

AmpliSeq™ Designer currently offers one custom AmpliSeq™ panel and related target and hotspot regions files for GRCh38 experiments. Optionally, you can also convert existing coordinates to GRCh38 by using a publicly available lift-over tool, such as **CrossMap**.

To use the GRCh38 human reference in Torrent Suite™, you must import it.

1. Log into Torrent Suite™ as administrator.
2. Go to the **Reference** page and click **Import Preloaded Ion References**.
3. Select **GRCh38** and click **Import**.

Now the reference is available and can be selected in run plan.

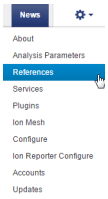
AmpliSeq™ Designer preloaded reference genomes

AmpliSeq™ Designer includes many preloaded reference genomes, including:

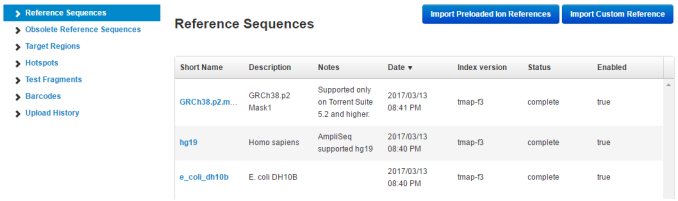
- Human (GRCh38)
- Human (hg19)
- Mouse (mm10)
- Cow (boxTau7)
- Chicken (galGal4)
- Pig (susScr3)
- Sheep (oviAri3)
- Maize (AGPv3)
- Rice (IRGSP-1.0)
- Soybean (Glyma1.1)
- Tomato (SL2.40)

References Management Guide

1. In the **Plan** tab, click **Settings (⚙)**, then click **References**.
   The main reference management page opens:



   The main reference management page opens:



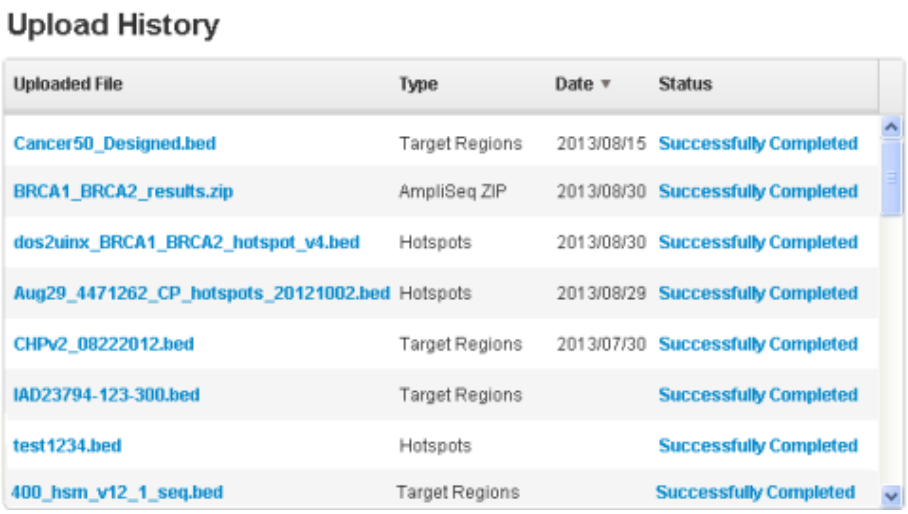
2. Enter the following:

   - Nucleotide sequence **Test Fragments**
   - **Reference Genomes** for aligning reads
   - **DNA Barcodes** for barcode set management

   In this page you can select reference details, download a reference file from your Torrent server, add a new reference, or use the navigation tabs on the left:

   - **Reference Sequences**. The main reference management page.
   - **Obsolete Reference Sequences**. Lists references that need to be reindexed before use. Reindexing is required only on releases that involve a TMAP index change.
   - **Target Regions**. Analysis is restricted to only the regions of interest that you specify in this file.
   - **Hotspots**. Variant Caller output files 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**. Known sequences used to monitor system characteristics.
   - **Barcodes**. Work with Ion barcode sets or your own custom barcodes sets.

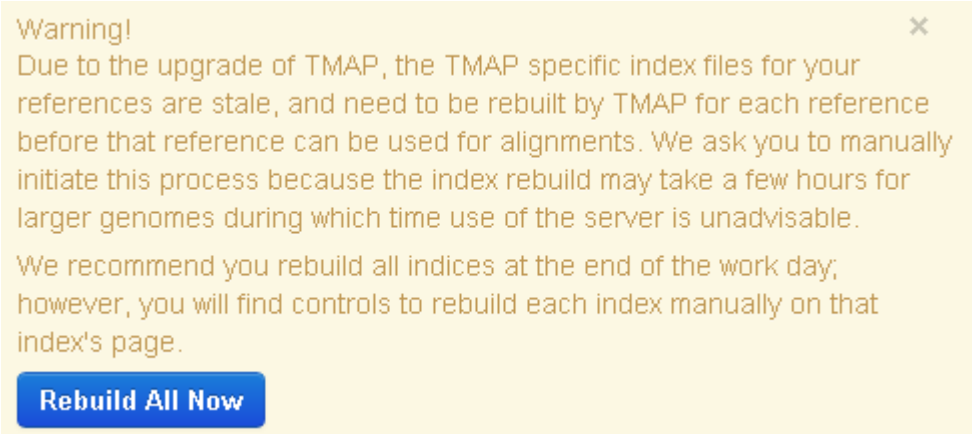
- **Upload History**. Shows the recent uploads of target regions, hotspots, and ampliseq.com zip files:



The Status column shows any error results.

Rebuild warning

This warning often appears in the References tab:



This warning appears if your server has references listed in the Obsolete References section. Your action in response to this warning depends on your particular upgrade scenario and obsolete references situation:

- If you upgrade from release 2.2 or higher to 4.x, you do not need to rebuild your reference indices.
- If you upgrade from a release earlier than 2.2, you must rebuild your reference indices *after* the first upgrade to a 3.x or 4.x release (and *before* using the upgraded server for analyses).

Reference pages

The following pages describe how to manage your references and related files.

Upload a new reference file

As part of the standard analysis process, reads are aligned to a genomic reference, using the TMAP aligner that comes pre-installed on the Torrent Server.

Note: Currently, the variantCaller plugin does not support IUPAC base codes other than A, C, T, G, and N. When Torrent Suite™ software uploads a genome containing other IUPAC characters, each such character is replaced with N.

For a new genome sequence, use the **Admin ▸ References** tab to add the new reference genome. (These reference sequences are also displayed on the Ion PGM™ or Ion Proton™ Sequencer when you load a sample.)

Prerequisites

The following are prerequisites to uploading a new reference file:

- Create a **FASTA** format reference sequence file (on your client machine).

  Note: FASTA files can be found at: **http://www.ncbi.nlm.nih.gov/sites/genome** download the FASTA file to your local client machine.

  IMPORTANT! It is important that the format of your FASTA file conform to Ion Torrent™ requirements.

  IMPORTANT! When working with larger genomes, performance improves if you first zip the FASTA file. The create index tool supports a zip archive, provided the file contains only a single FASTA file.

- Prepare a descriptive name for the genome.
- Prepare the short name for the genome.
- Prepare a version for the genome.
- Know the number of reads to randomly sample for alignment.
- Prepare a regions of interest file or hotspots file (on your client machine).

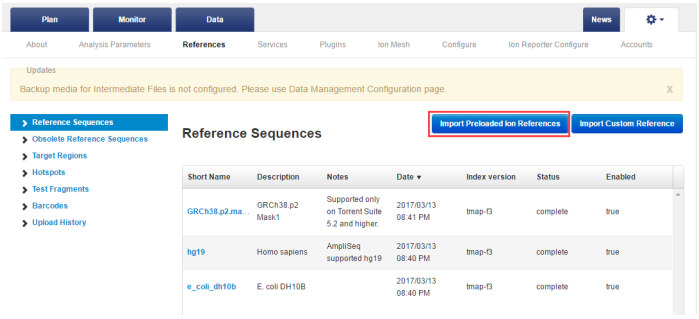
  Note: To provide a better uploading experience, Adobe® Flash® or Microsoft™ Silverlight® plugins are required to be installed for your browser. You may need to contact your local system administrator for assistance.

  – Silverlight® can be downloaded from **http://www.silverlight.net/getstarted/**.
  – Adobe® Flash® can be downloaded from **Flash® player/**.

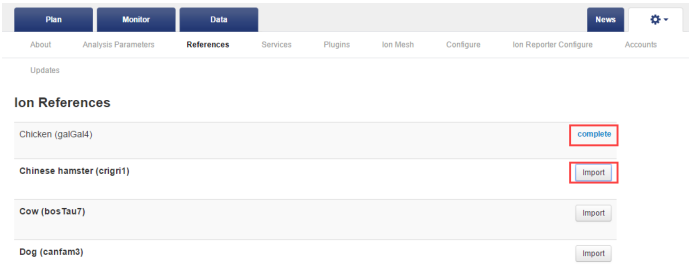
Import preloaded ion references

1. Click **Settings ( )References**:

2. Click the **Import Preloaded Ion References** tab.



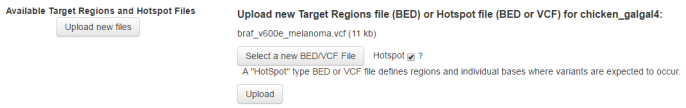
The following screen opens:



3. Click **Import** to download the genome. **Complete** appears when the download is finished.

4. (Optional) Click **complete** to edit the data. You have the following choices:

   - Edit the fields, then click **Save Changes**.
   - Click **Delete Genome**.
   - In the **Available Target Regions and Hotspot Files**. click **Upload New Files**. The following section appears:

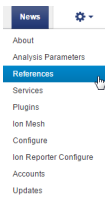


Click Select a new BED/VCF. The file appears under **Upload new Target Regions file**.

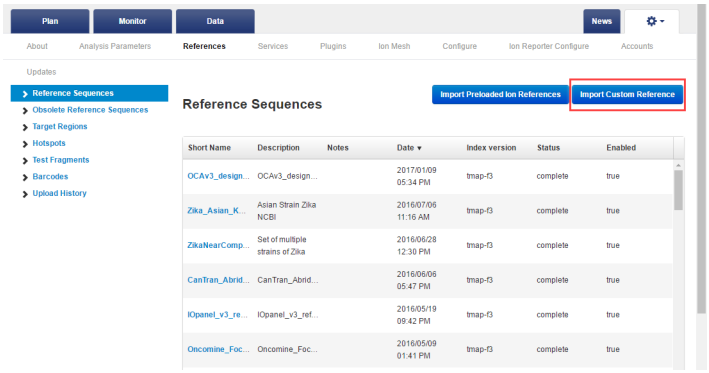
Import custom reference

Follow these steps to import a reference genome:

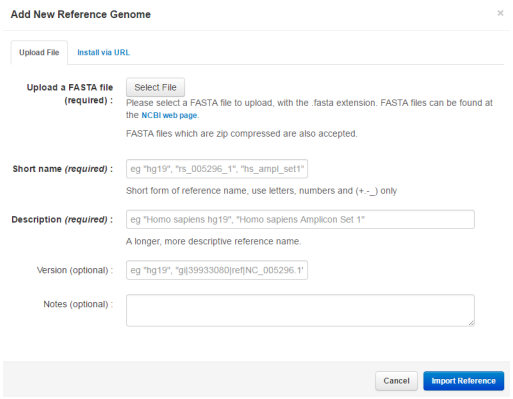
1. Click **Settings (⚙)References**:



2. In the **References Sequences** section, click **Import Custom Reference**:



3. Fill out the **Add New Reference Genome** form. Required fields are noted on the form.



Field	Description	
Upload FASTA file	[required] This entry must have a .fasta extension. You can upload a FASTA file from your local machine or click the link to the website and upload one from there.	
Short name	[required] A shortened form of the genome name, the short form of the genome name may be any alphanumeric character and the underscore (_) character. The name should not match any existing references installed in the `/results/referenceLibrary/<index_type>/<genome_shortname>/` directory, including previous unsuccessful attempts at creating reference sequences. Undesired sequences can be removed. Deletion allows the short name to be used for a new genome.	
Description	[required] This entry may be any text string. The description usually includes the genus-species, version, and other identifying information. The description entered here is displayed in various report output, and is listed	

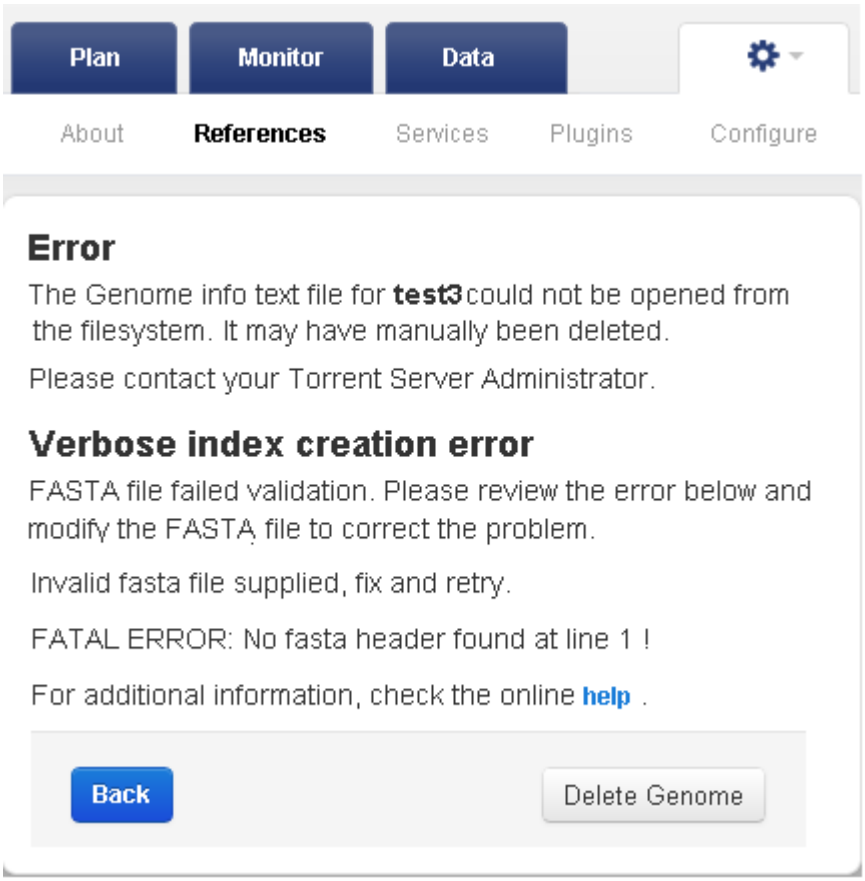
Field	Description	
	in the Reference Sequences section of the Admin > **References** tab.	
Version	[required] Enter any string for the genome version number. The accession number, if there is one, is a good choice. The version entered here is displayed in various report outputs.	
Notes	[optional] Use this field to record any notes about the reference genome	

4. Click **Select File**, then browse to the genome file (on your local machine).
5. Click the **Upload file and create reference** button.
6. (Optional) Click the **Install via URL** tab, fill out the form, then click **Import Reference**.

   After the reference is created, you can optionally add target regions BED files and hotspots BED or VCF files to the reference.

Error handling

If you uploaded an invalid FASTA file, the following error displays when you attempt to view the reference sequence associated with the file:



To recover from the error:

1. Delete the existing reference sequence entry.
2. Identify and correct formatting errors in the FASTA file.
3. Retry uploading the reference.

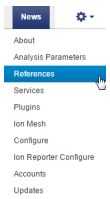
Target Regions Files and Hotspot Files

Browser Extensible Data (BED) files and Variant Call Format (VCF) files supply chromosome positions or regions. When applied to a reference genome in the Torrent Browser, these files perform these two functions:

- **Targeted regions of interest** Specifies your regions of interest, for instance the amplified regions that are used with targeted sequencing. Analysis in the complete Torrent Suite™ Software analysis pipeline, including plugins, is restricted to only the specified regions. (BED file only)
- **Hotspot** Instructs the Variant Caller to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. Only affects the variantCaller plugin, not other parts of the analysis pipeline. (Either a BED or VCF file)

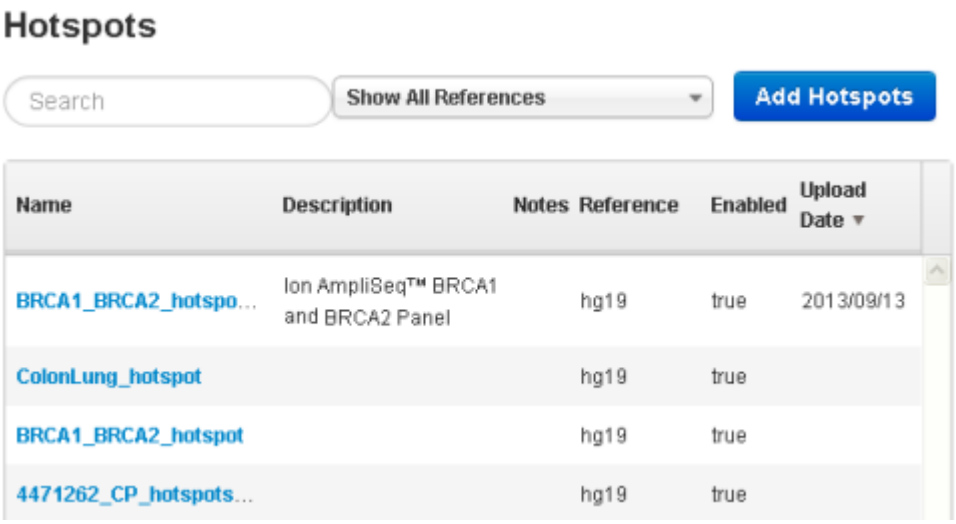
Target regions files and hotspot files are listed in the admin References tab. These files are uploaded to a specific reference and available for use only when that reference is used for an analysis.

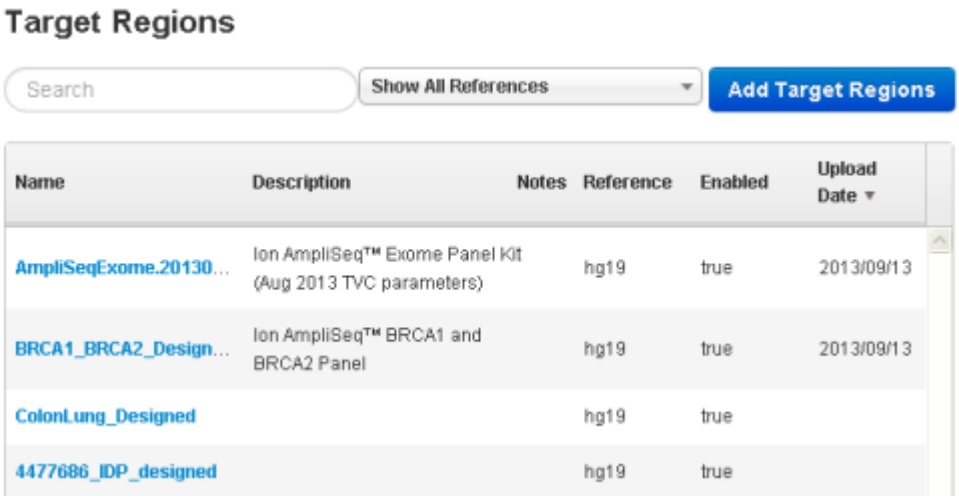
To view the target regions files and hotspot files on your system, click **Settings** (⚙) ▸ **References**:



In the References tab left navigation panel, click the **Target Regions** or **Hotspots** tab:

The left navigation tabs openHotspots or Target Regions pages, which are very similar:





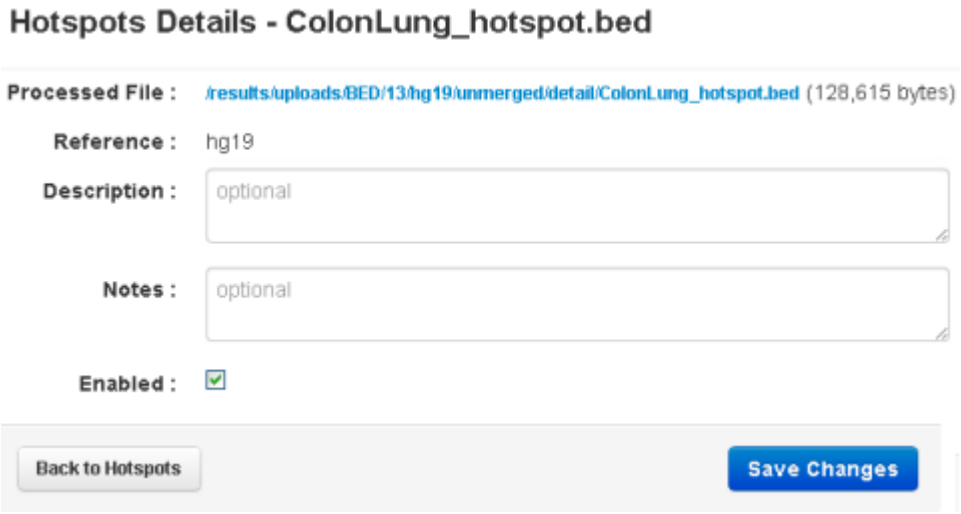
Both Hotspots and Target Regions pages offer the following actions:

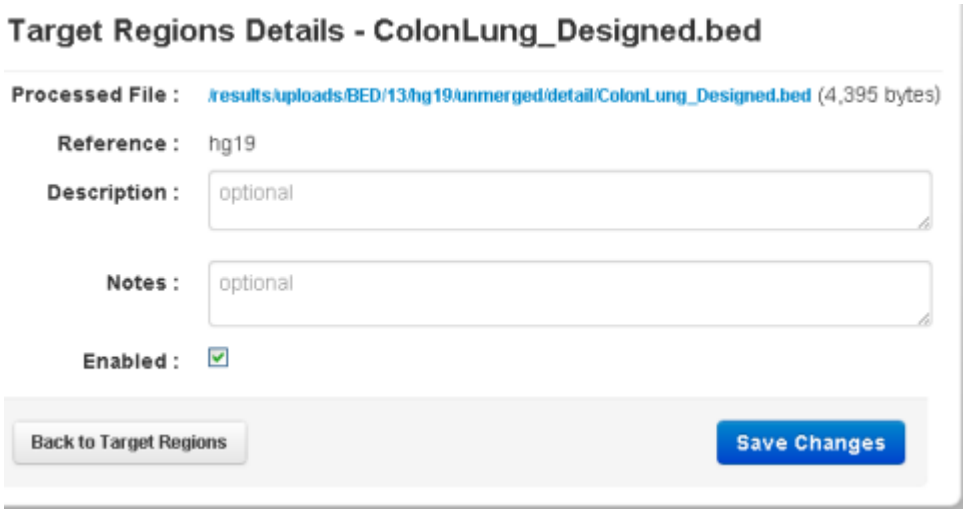
- Click the file name to open its details page.
- Use the references selection menu (default Show all References) to display only files of one reference.
- Click the **Add Hotspot** or **Add Target Regions** button to upload a new file (to associate with any reference).

Details page

File details and download

In either the Hotspots or Target Regions page, when you click on a hotspot file name or a target regions file name, a details page opens with details of both the hotspot file and the related target regions file (provided both are available):





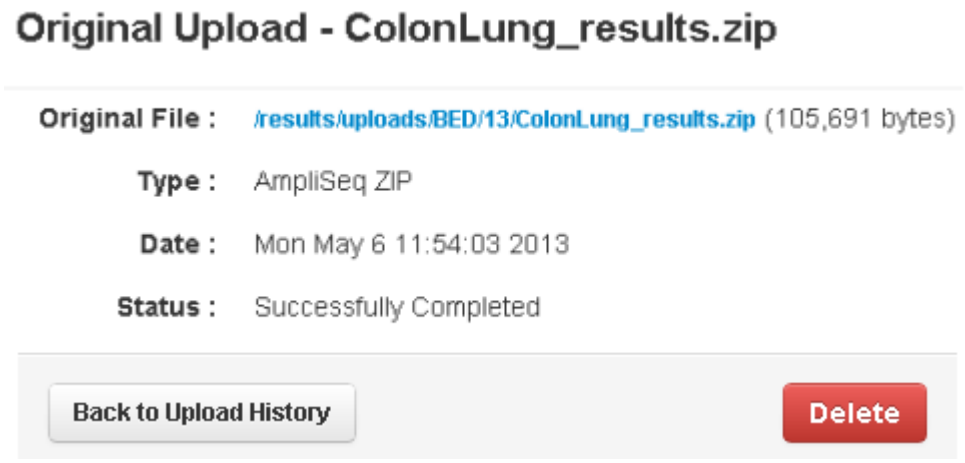
In these details sections, you can do the following:

- Click on the **Processed File** link to download the hotspot or target regions file.
- Add a description or notes.
- Uncheck the Enable check box to prevent the file from being used in an analysis.

Click the **Save Change** button to save your description, notes, or Enable status.

Zip file details and download

For files imported for ampliseq.com, the details page also shows the zip file that was imported from ampliseq.com:



Note: The **Delete** button in the Original Upload section removes the hotspot or target regions file from the system. The file is not available to be used in analyses.

Upload log file

The details page also has a section with the validation log from when the hotspot and target regions files were originally uploaded.

Manage Target Regions Files and Hotspot Files

This page describes how to add, download, and remove target regions files and hotspot files.

Overview

Browser Extensible Data (BED) files and Variant Call Format (VCF) files supply chromosome positions or regions. When applied to a reference genome in the Torrent Browser, these files perform these two functions:

- **Targeted regions of interest** Specifies your regions of interest, for instance the amplified regions that are used with targeted sequencing. The complete Torrent Suite™ Software analysis pipeline, including plugins, is restricted to only the specified regions. (BED file only)
- **Hotspot** Instructs the 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 hotspots file affects only the variantCaller plugin, not other parts of the analysis pipeline. (Either a BED or VCF file)

With the Torrent Browser, you add BED and VCF files to an existing reference. The reference must be listed in the Torrent Browser Admin > References tab before you can upload our BED or VCF files.

Your uploaded BED and VCF files are then available as an option when you create a new template or planned run in the Plan tab. In the template and planned run wizard, menus on the Reference chevron page offer the BED and VCF files that you uploaded to a reference.

You can optionally upload multiple BED and VCF files to a reference. In the template and planned run wizard, you specify the BED or VCF files used for each template or each run.

Notes about hotspot files:

- By default the variantCaller plugin calls variant candidates at hotspot positions with more sensitivity than candidates at other positions. You can customize certain variantCaller parameters separately for hotspot candidates.
- The Torrent Browser also accepts VCF files as hotspot files.

IMPORTANT! Target regions BED files provide an option to restrict the analysis of the entire reference genome. Whole genome analysis is supported by the run type Whole Genome Analysis. Do not specify a target regions BED file on the Planning tab run registration page if the variants are to be called over the whole genome.

IMPORTANT! All regions specified in your t arget regions BED files are analyzed. Follow the instructions in "Modify a BED file" on page 237 (before uploading your Target regions BED file) to delete lines representing regions that span variants that you do not wish to call.

IMPORTANT! 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.

IMPORTANT! BED files used with Ion AmpliSeq™ workflows define the internal segment only, and do not include the primer sequence.

IMPORTANT! A BED or VCF file is tied to specific reference. The coordinates within a BED or VCF file must match coordinates and the coordinate sorting in the reference genome. Torrent Suite™ Software reference genomes are sorted alpha-numerically (not by a chromosome sort). The BED files and VCF files that you use with Torrent Suite™ references must also use an alpha-numeric sort. If you upload your own reference genome, the BED and VCF files that you use with that reference must be sorted by the same method as your reference file.

Summary of steps to add a target regions or hotspots file

Before your analysis run or run registration (on the Planning page), you can add BED or VCF files to your genome reference:

1. Use the Torrent Browser to upload the BED or VCF file from your local client machine to Torrent Suite™ Software.
2. During file upload, the Torrent Browser validates the BED or VCF file, and ensures that the BED or VCF file's coordinate regions are valid for the genome reference.
3. The new BED or VCF file is then available as an option when you create a new run registration in the Planning tab. Your new file also appears in the Target Regions or HotSpots menus in the template and planned run wizard References step in the Workflow bar.

Modify a BED file

You can optionally modify a BED file *before* adding it to your reference genome. You can use this technique to avoid regions for which you do not want variants called (even if the variants appear in your sample).

You can modify a BED file only *before* uploading the file with the Torrent Browser.

Follow these instructions to modify a BED file:

1. Make a copy of your BED file. Rename the two files 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.

   If the region (or regions) appear in both your targeted regions BED file and in your hotspots BED or VCF file, you must delete the line for those regions from both types of BED file.

Supported file types

- **Targeted regions of interest** BED file only. Supported file extensions are .bed, .zip, and bed.gz.
- **Hotspot** BED file or VCF file. Supported file extensions are .bed, .vcf.gz, .zip, bed.gz, and .vcf.gz.

Upload a BED or VCF file

These instructions upload a BED or VCF file from your local client machine to Torrent Suite™ Software. These instructions apply to both targeted regions of interest files and hotspot regions files.

IMPORTANT! You must upload only BED or VCF files that both match the reference and are for the correct reference version. The uploader attempts to validate the BED or VCF files, but cannot always detect the errors listed below.

You have the responsibility to avoid the following mismatch errors. The uploader does not always detect these errors:

1. Upload a BED or VCF file to a reference genome of a different version (for example, an hg18 BED or VCF file with an hg19 reference).

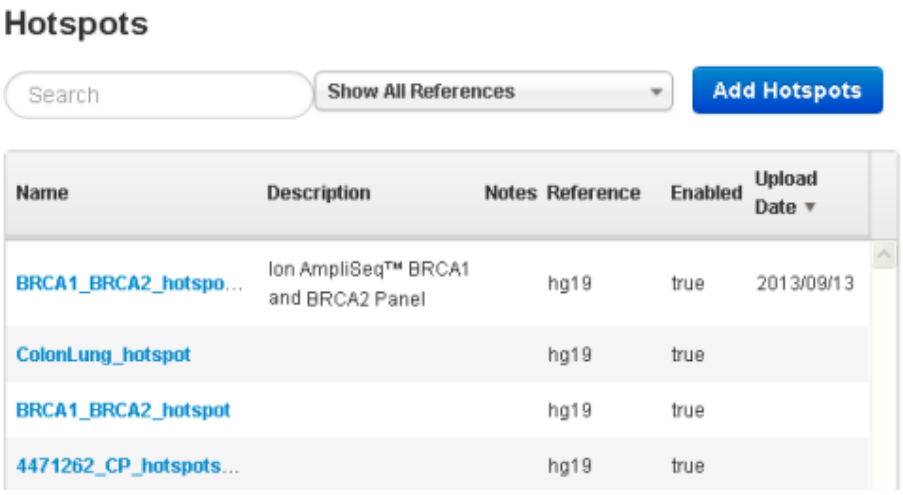
2. Upload a BED or VCF file for a different species.

3. Upload a hotspots BED file as a targeted regions BED file, or upload a targeted regions BED file as a hotspots BED file.

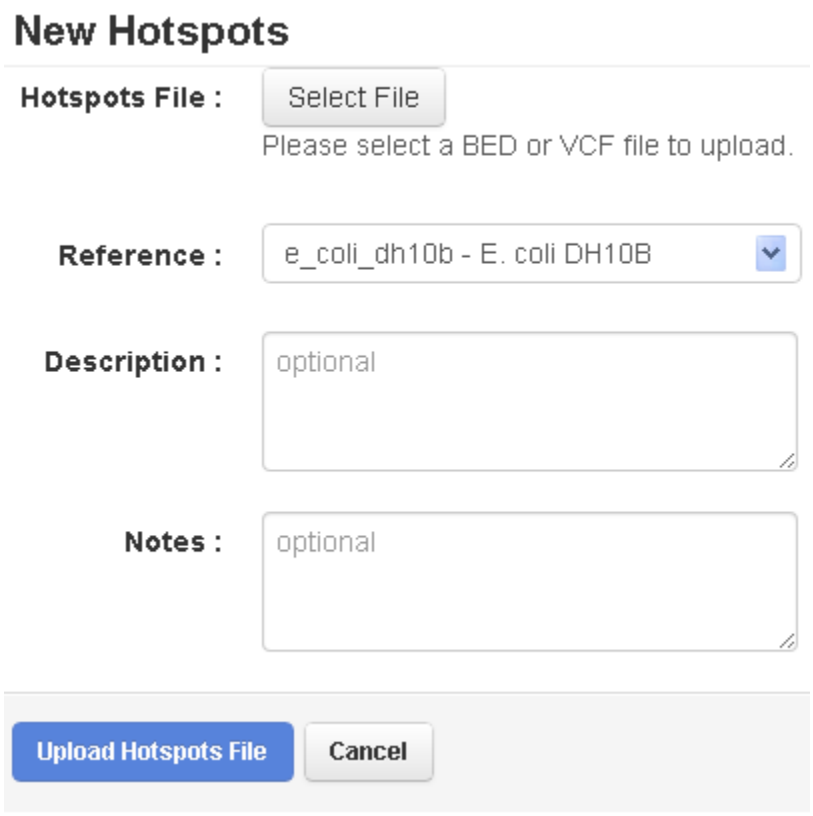
   Follow these steps to upload a target regions BED file or hotspots BED or VCF file to a reference:

   a. In the **Reference** tab, click either the Hotspots or Target Regions tab in the left navigation panel:

   The Hotspots (or Target Regions) page opens:



b. Click the **Add Hotspots** (or **Add Target Regions**) button in the top right corner. The New Hotspots (or New Target Regions) page opens:

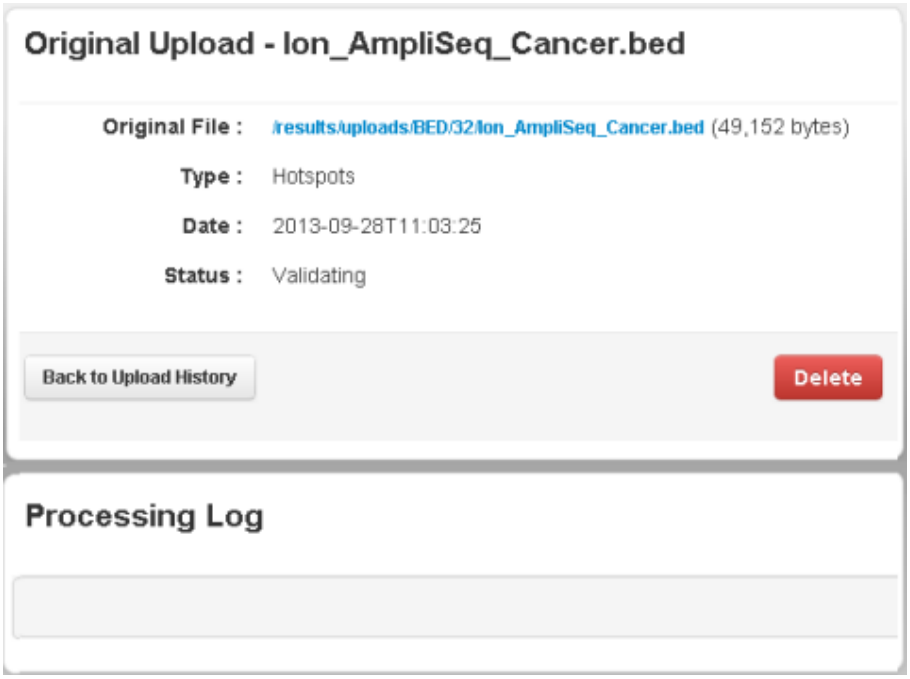


c. Click the **Select File** button and browse to the file to be uploaded.

d. In the Reference menu, be careful to select the correct reference. The new file can only be used with this reference.

e. Add the optional (but recommended) description and notes.

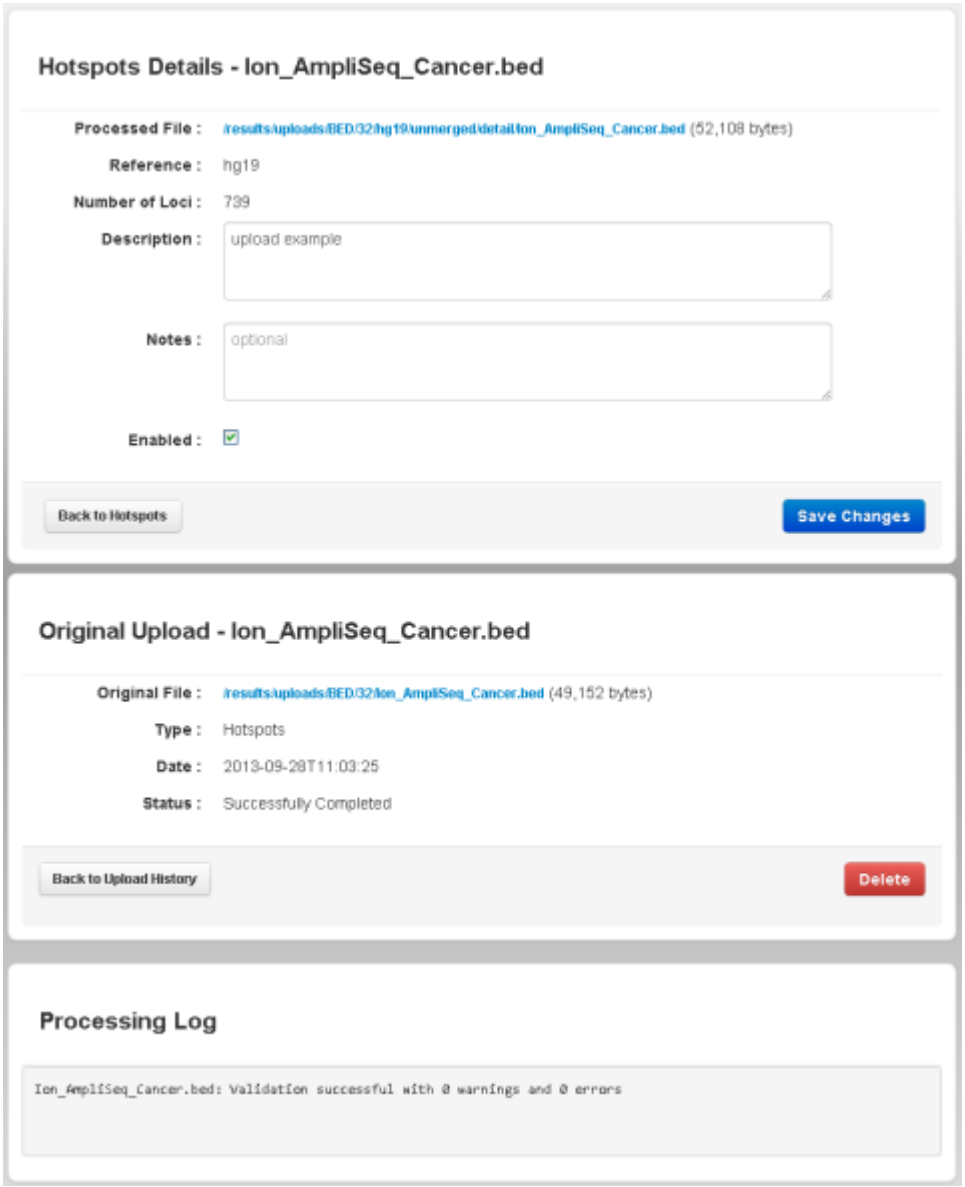
f. Click the **Upload Hotspots File** (or **Upload Target Regions File**) button. Wait while the file is validated:



For large files, validation can take a couple minutes. Refresh your browser to check that validation is complete.

After upload

After validation, the Torrent Browser opens to the Hotspots detail page for your new file:



From this page, you can download the hotspots file or target regions file, remove the file from the system, and view the validation log.

Uploading errors

Validation errors appear in the Processing Log section of the details page.

Some types of error do not appear in the Processing Log section. There are major problems that prevent validation from being attempted:

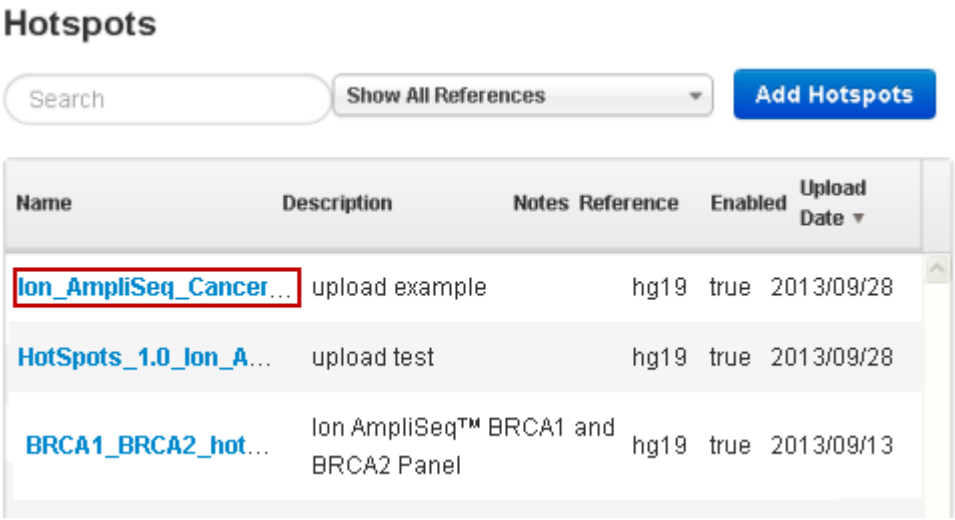
- Incorrect file format
- Incorrect file extension
- Zip contains 0 or multiple files
- A corrupted .zip .gz file

Download a hotspots or target regions file

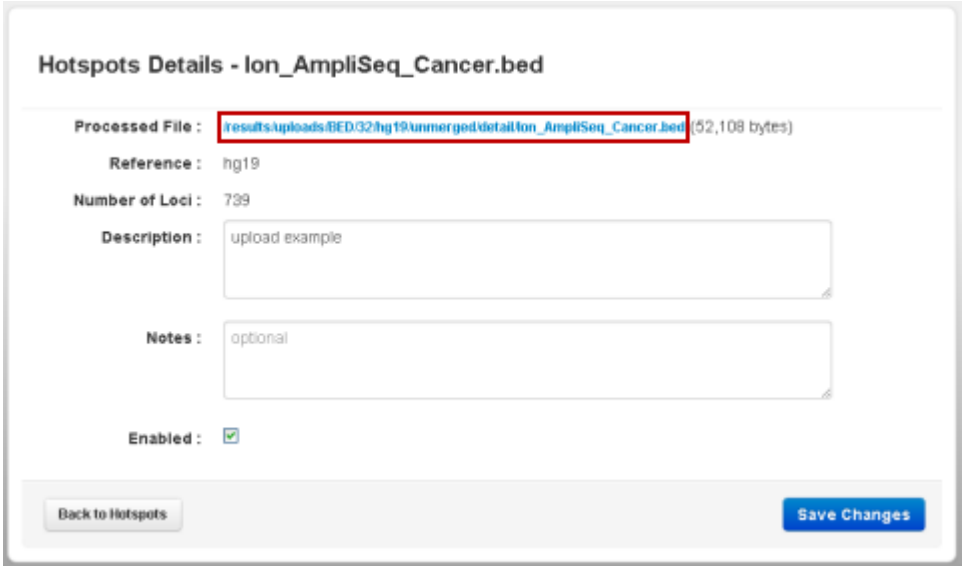
Follow these steps to download a hotspots BED or VCF file, or a target regions BED file:

1. Go to the admin References tab and click either the Hotspots or Target Regions tab in the left navigation panel:

2. In the Hotspots (or Target Regions) page, click the name:



3. In the details page, click the link in the Processed File field:



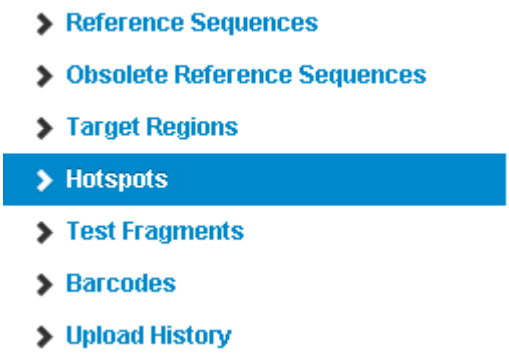
The Original File link in the Original Upload section also downloads the same file.

Delete a hotspots or target regions file

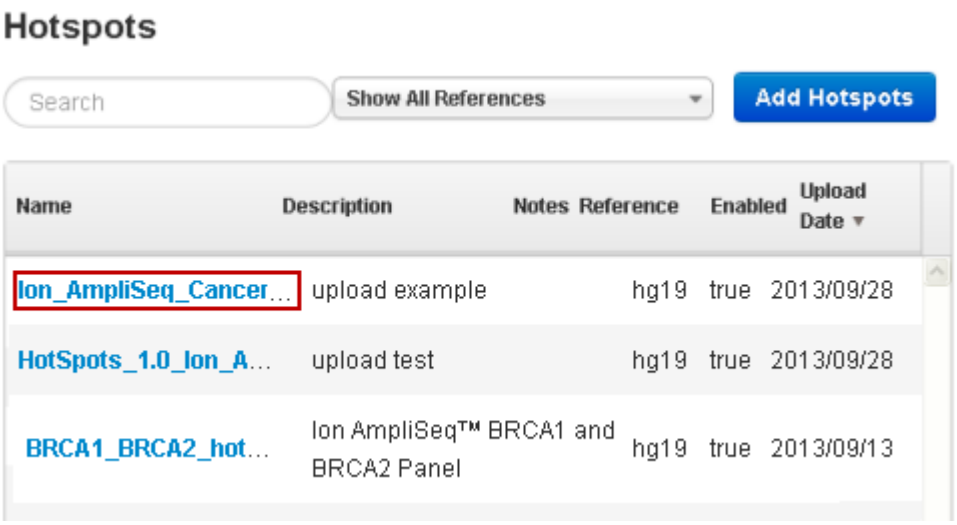
Note: This step removes the file from the system. There is no recovery or undo. Consider first downloading the file as a backup.

Follow these steps to delete a hotspots or a target regions file:

1. Go to the admin References tab and click either the Hotspots or Target Regions tab in the left navigation panel:



2. In the Hotspots (or Target Regions) page, click the name:



3. In the details page, go to the Original Upload section and click the **Delete** button. If you are sure, click **Yes** in the confirmation popup.

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 File Formats

Target regions BED files use 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 File 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

Beginning with the 3.0 release, AmpliSeq.com uses this format for the following fixed panels:

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

New fixed panels introduced after the AmpliSeq.com 3.0 release 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 mandatory for Fusions designs files, but most of these are optional for other White Glove designs.

Note that the Genomic (hg19) coordinates provided in the Key-Value pairs must represrent the entire Amplicon sequence. If we want to generate the fusions mapping reference fasta file from the BED file, all the information needed to do that should be available in the Bed file.

These 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
```

Manage DNA Barcodes and DNA Barcode Sets

This section describes how to manage barcode sets.

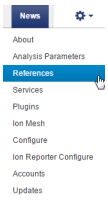
With the pre-installed Ion Torrent™ barcodes, you can view the barcode sets and the barcodes, incuding the barcode sequences.

With your own barcodes sets, you can do the following:

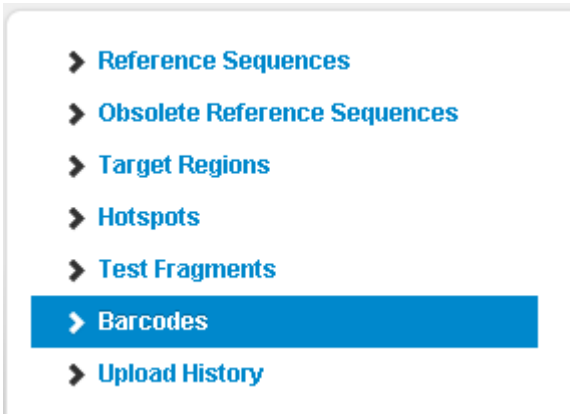
- View a DNA barcode or barcode set
- Add a custom DNA barcode set
- Delete a DNA barcode set
- Add a barcode to an existing DNA barcode
- Edit or delete an individual barcode

Access the DNA barcode set pages

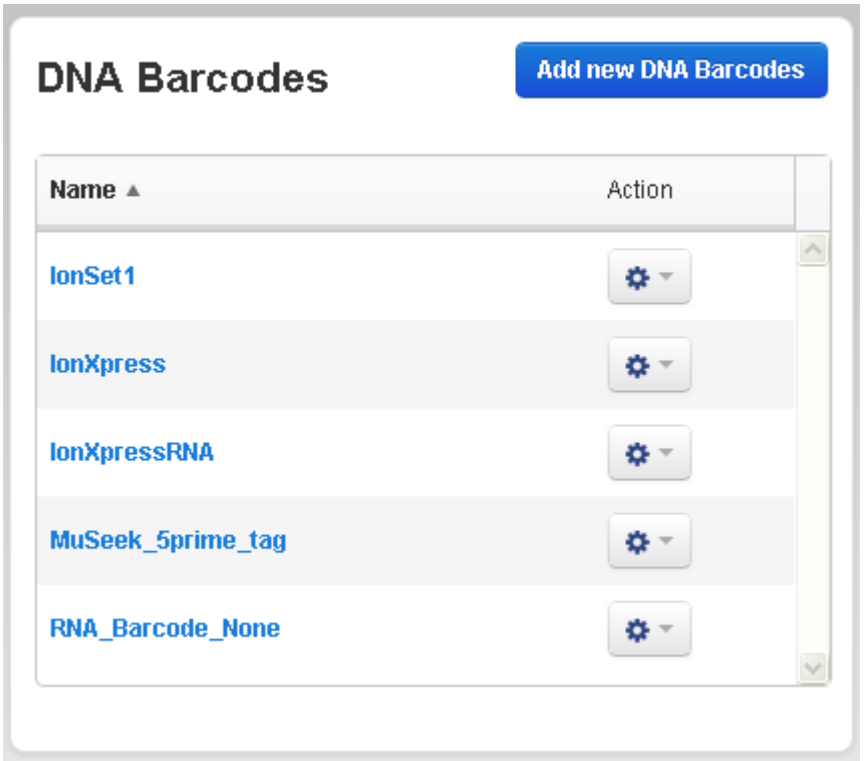
1. Click **Settings** (⚙) ▸ **References**.



2. In the **Admin References** tab, click the **Barcodes** option in the left navigation panel:

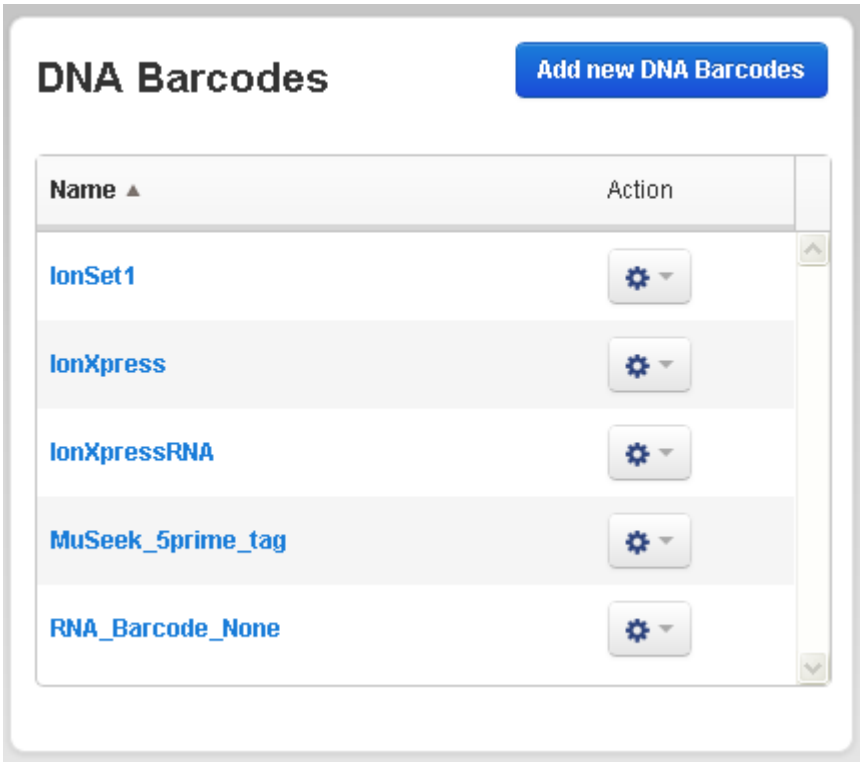


The DNA Barcodes page opens:



Pre-installed DNA barcode sets

The pre-installed DNA barcode sets are seen under the Admin References tab:

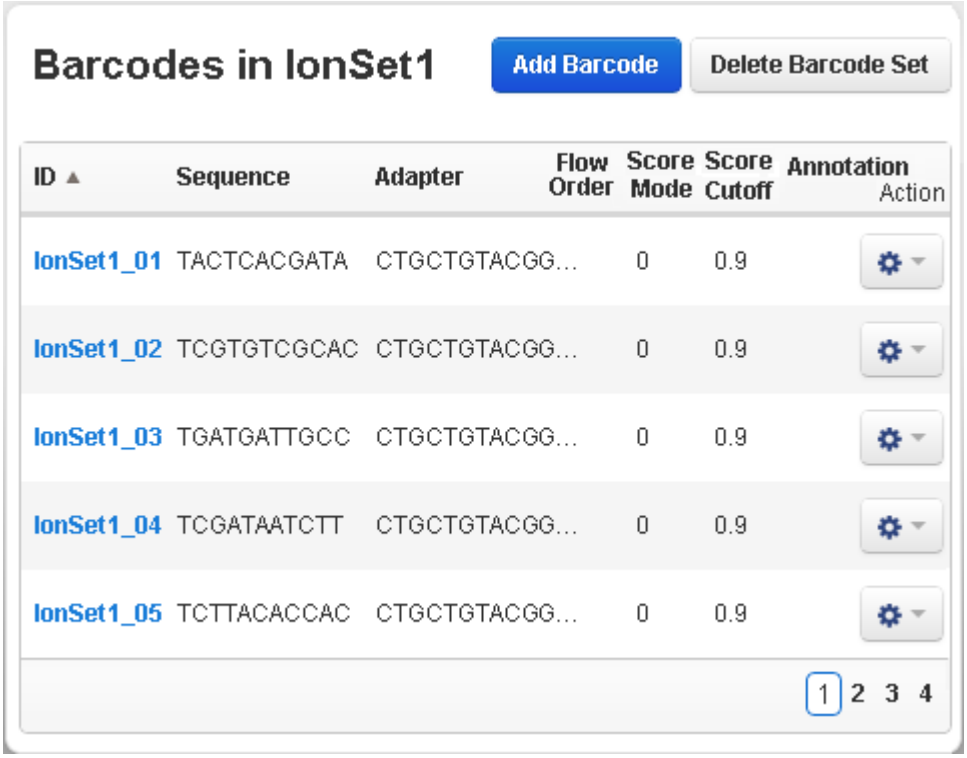


View a DNA barcode or barcode set

Follow these steps to view a DNA barcode or barcode set:

1. Click **Settings** (⚙) ▸ **References**, then scroll down to the DNA Barcodes panel.
2. Do one of the following:
   - Click the name of the barcode set to view.
   - Click **Settings** (⚙) ▸ **Edit** for that barcode.

This displays the barcodes in the set:



Note the page number controls to view other pages:

You can click any column header in bold to sort the display by that column.

The **Settings (⚙)** menu provides **Edit** and **Delete** options:

The **Settings (⚙)** menu Edit option is the same as double-clicking the barcode name.

Dialog buttons are displayed to add a new barcode to this set and to delete the entire barcode set. The barcode edit and delete feature is only for custom barcode sets that you install.

IMPORTANT! Do not edit, delete, or modify the pre-installed barcode sets `IonSet1`, `IonXPress`, `IonXPressRNA`, `RNA_Barcode_None`, or `MuSeek_5prime_tag`.

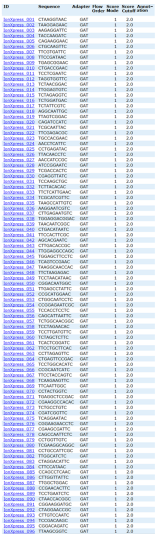
IonSet1 barcodes

Here are the barcodes in the IonSet1 barcode set:

ID	Sequence	Adapter	Flow Order	Score Mode	Score Cutoff	Annotation
IonSet1_01	TACTCACGATA	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_02	TCGTGTCGCAC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_03	TGATGATTGCC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_04	TCGATAATCTT	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_05	TCTTACACCAC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_06	TAGCCAAGTAC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_07	TGACATTACTT	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_08	TGCCTTACCGC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_09	TACCGAGGCAC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_10	TGCAAGCCTTC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_11	TACATTACATC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_12	TCAAGCACCGC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_13	TAGCTTACCGC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_14	TCATGATCAAC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_15	TGACCGCATCC	CTGCTGTACGGCCAAGGCGT		0	0.9	
IonSet1_16	TGGTGTAGCAC	CTGCTGTACGGCCAAGGCGT		0	0.9	

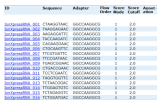
IonXpress barcodes

Here are the barcodes in the IonXPress barcode set:



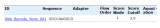
IonXpressRNA barcodes

Here are the barcodes in the IonXPressRNA set:



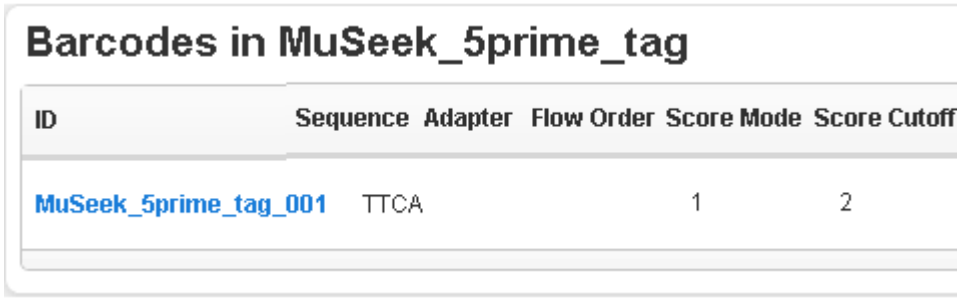
RNA_Barcodes_None barcode

Here is the barcode in the RNA_Barcodes_None barcode set:



Museek barcode

Here is the barcode in the MuSeek_5prime_tag barcode set:



Barcodes in MuSeek_5prime_tag

ID	Sequence	Adapter	Flow Order	Score Mode	Score Cutoff
MuSeek_5prime_tag_001	TTCA			1	2

For custom DNA barcode sets

For your own barcode sets, you can do the following:

- View a DNA barcode or barcode set.
- Add a custom DNA barcode set.
- Delete a DNA barcode set.
- Add a barcode to an existing DNA barcode.
- Edit or delete an individual barcode.

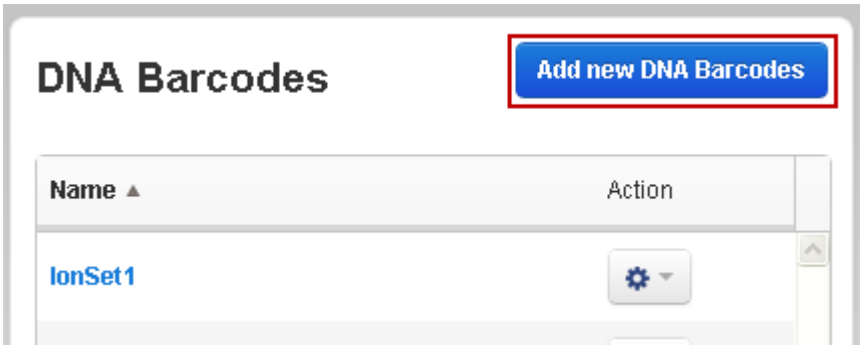
Add a custom DNA barcode set

To add a barcode set, packaged as a list of barcodes in a Comma-separated Variable (CSV) text file, create the CSV file then select the file to add it to the barcode set list.

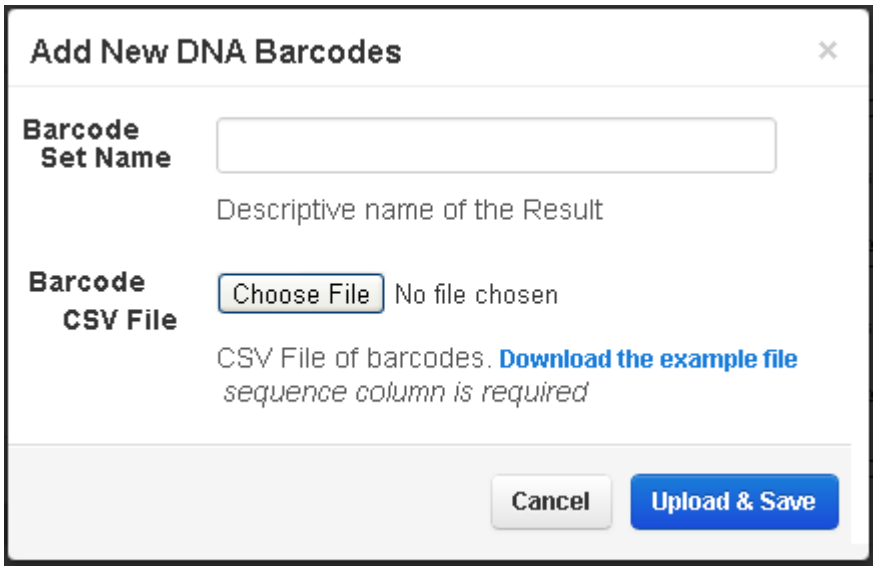
1. If needed, create the CSV file containing a maximum of 96 barcodes, using Microsoft™ Office Excel™, OpenOffice.org Calc, or an equivalent program. Save the file with a `.csv` extension.

2. Click **Settings (⚙) ▸ References**, then scroll down to the DNA Barcodes panel.

3. Click **Add new DNA Barcodes** on the right side of the **DNA Barcodes** panel:



4. In the **Add New DNA Barcodes** dialog, enter the required **Barcode Set Name** in the edit window and browse to find the **Barcode CSV File**:



5. To view an example CSV file, click **Download the example file**: The example CSV file contains column headers only. The following table describes the column headers:

Name	Type	Description
id_str	String	The unique name for this barcode entry.
sequence	String	The barcode sequence. G, C, A, and T (always upper-case) are allowed.
adapter	String	The portion of the barcode adapter not used to identify this barcode. Often referred to as the "stuffer sequence". G, C, A, and T (always upper-case) are allowed.
flow order	--	Not used.
annotation	--	Not used.

6. Click **Upload & Save** to add the new barcode set.

7. When you return to the DNA Barcodes section, click the Name column header to sort the column and have your new barcode set appear.

Note: In previous releases, the CSV file used score_mode and score_cutoff fields. These are now entered as BaseCaller parameters (--barcode-mode and --barcode-cutoff) during reanalysis of a run.

Delete a DNA barcode set

This feature is only for your own custom barcode sets.

IMPORTANT! Do not delete the pre-installed barcode sets IonSet1, IonXPress, IonXPressRNA, RNA_Barcode_None, or MuSeek_5prime_tag.

1. At the top of the page, click **Delete Barcode Set**. This displays a delete confirmation prompt:

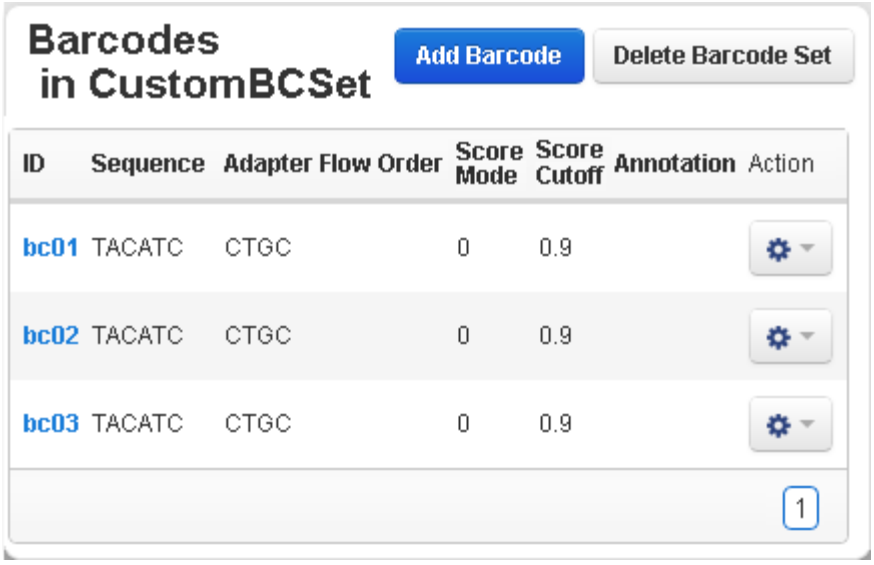
2. Click **Yes, Delete!** to delete the entire barcode set. Click **Cancel** to keep the displayed barcodes.

Add a barcode to an existing DNA barcode set

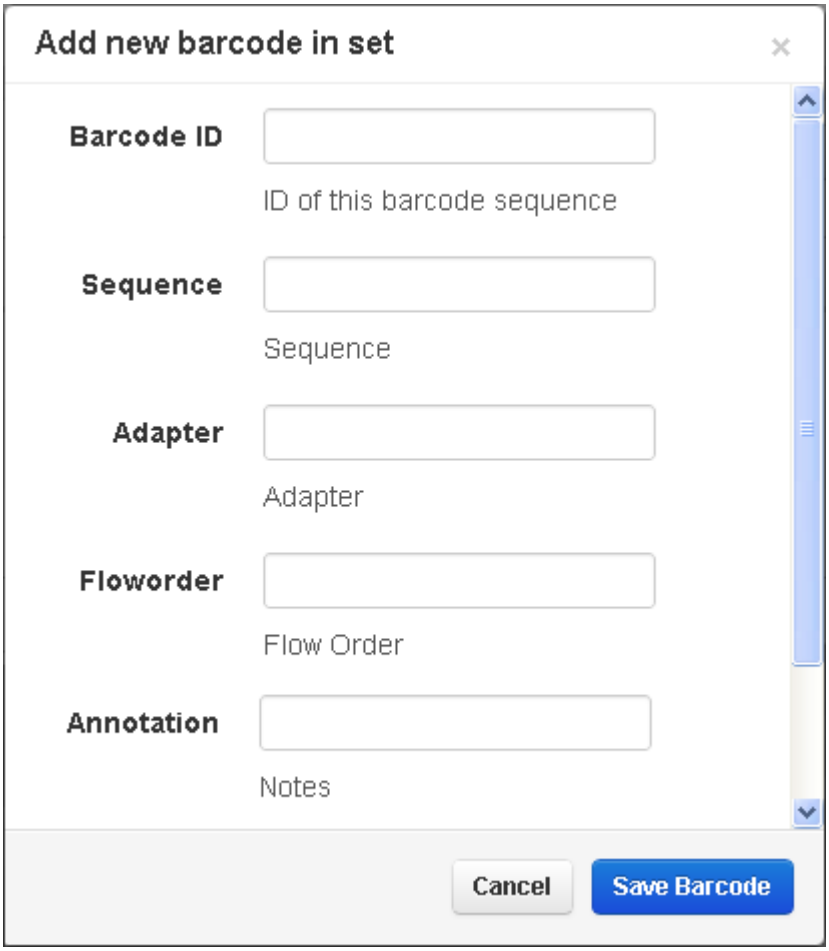
This feature is only for custom barcode sets that you install.

IMPORTANT! Do not edit, delete, or modify the pre-installed barcodesets `IonSet1`, `IonXPress`, `IonXPressRNA`, `RNA_Barcode_None`, or `MuSeek_5prime_tag`.

1. Click **Add Barcode**.



2. The **Add new barcode in set page** opens:



3. Add the barcode information and click **Save Barcode**. The new barcode is added to the set displayed in the current barcode set list.

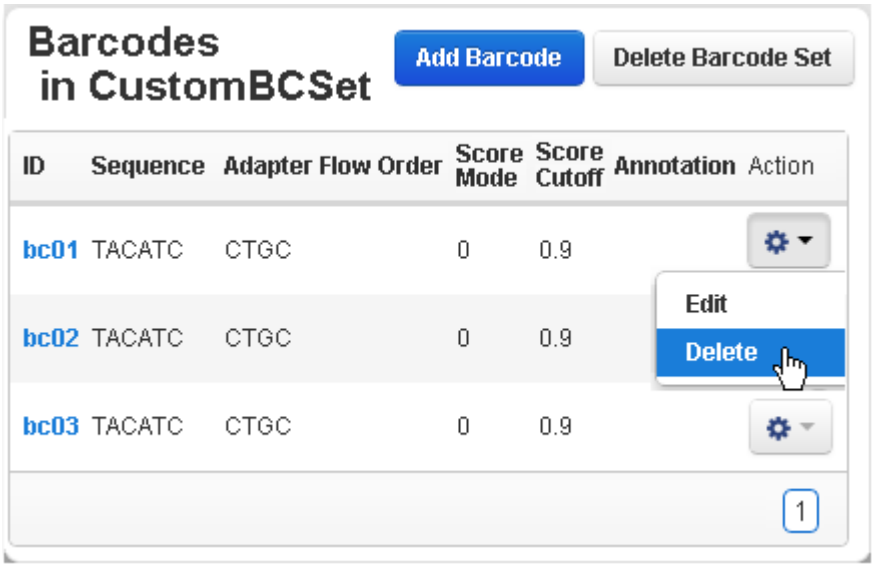
Delete an individual barcode

The barcode delete feature is only for custom barcode sets that you install.

IMPORTANT! Do not edit, delete, or modify the pre-installed barcode sets `IonSet1`, `IonXPress`, `IonXPressRNA`, `RNA_Barcode_None`, or `MuSeek_5prime_tag`.

Follow these steps to remove a single barcode from a custom barcode set:

1. For the barcode to be deleted, click **Settings (⚙) ▸ Delete**:



2. In the confirmation window, if you are sure, click **Yes, Delete!**:

The barcode is removed for the barcode set.

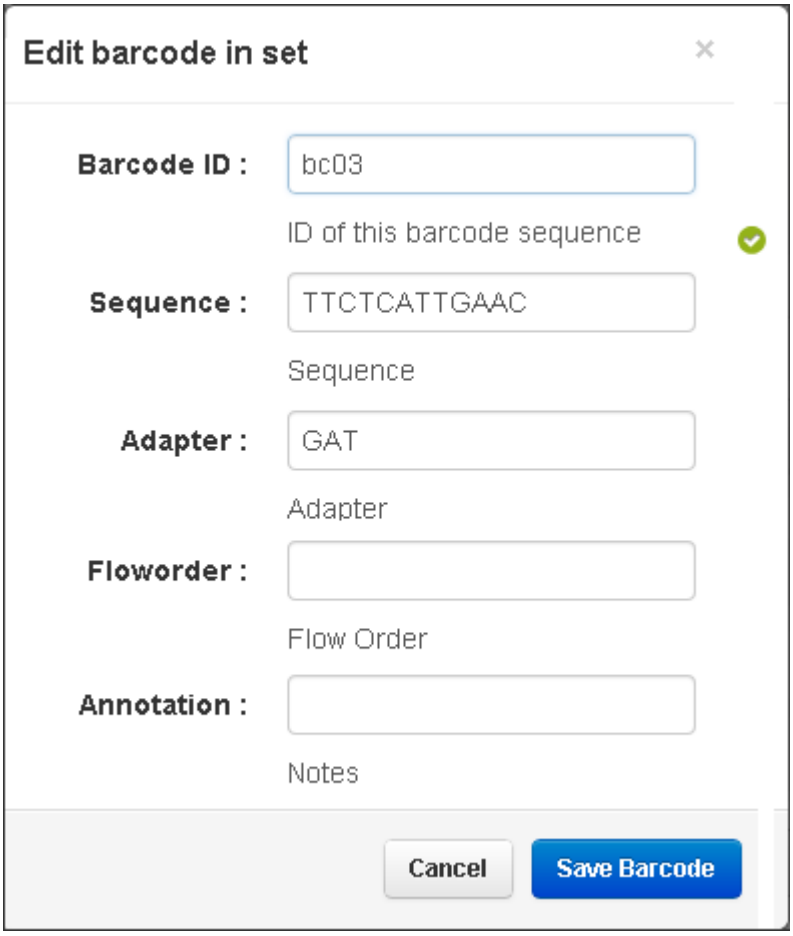
Edit an individual barcode

The barcode edit feature is only for custom barcode sets that you install.

IMPORTANT! Do not edit, delete, or modify the pre-installed barcodesets `IonSet1`, `IonXPress`, `IonXPressRNA`, `RNA_Barcode_None`, or `MuSeek_5prime_tag`.

Follow these steps to edit a single barcode in a custom barcode set:

1. Click on the ID of a barcode, such as bc03. The **Edit barcode in set** page opens:



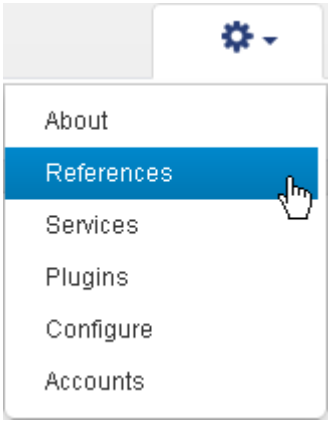
2. To edit the barcode details, make your changes and click **Save Barcode**.

Update Reference Library Indices

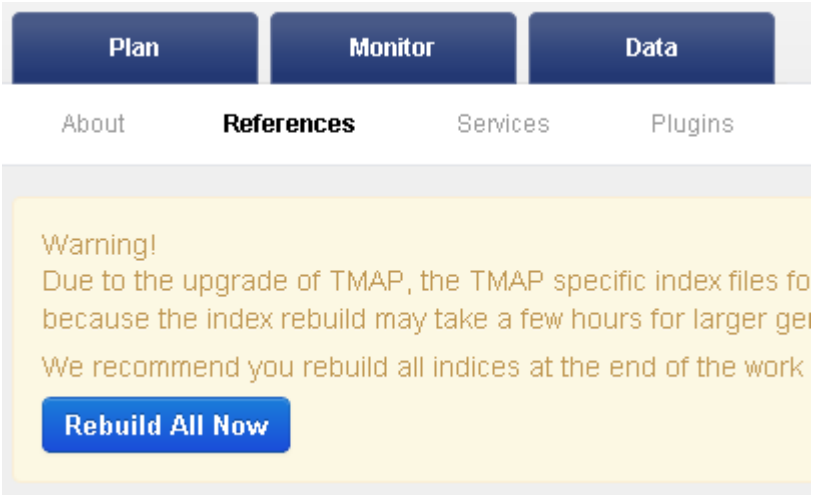
Note: When you upgrade your Torrent Suite™ Software from a version earlier than 3.0, you must rebuild your reference indices. This process can take a few hours for larger reference genomes. Your users should not submit data analysis jobs while the reference indices are being rebuilt.

Follow these steps to rebuild your reference genome indices:

1. Log in with an `ionadmin` account.
2. Ensure that users ***do not submit analyses*** while the rebuild is in progress.
3. Click **Settings ( )** ▸ **References** option:



4. Click the **Rebuild All Now** button:



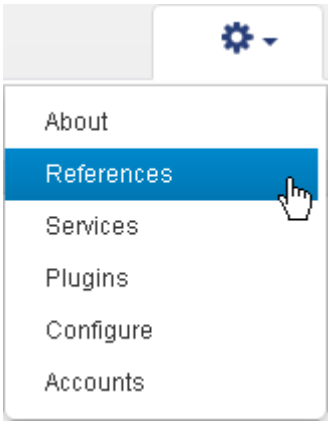
The TMAP index version used in 3.x and 4.x releases is tmap-f3.

Work with Test Fragments

Use the Admin **References** tab to enter the test fragment nucleotide sequence to search for within the sequenced nucleic acids. You can give a **Name** label and **Key** to your test fragment sequence.

Ion Torrent™ Software provides four test fragments by default.

1. Click **Settings (⚙)** ▸ **References**:



2. In the Admin References tab, click the **Test Fragment** option in the left navigation panel:

The Test Fragment listing page opens:

3. Click on a test fragment name to see its complete sequence.

   Be sure to enter the test fragment sequence using only the uppercase letters: A, T, C and G. If you enter an invalid character or duplicate test fragment, you are not be able to save your changes.

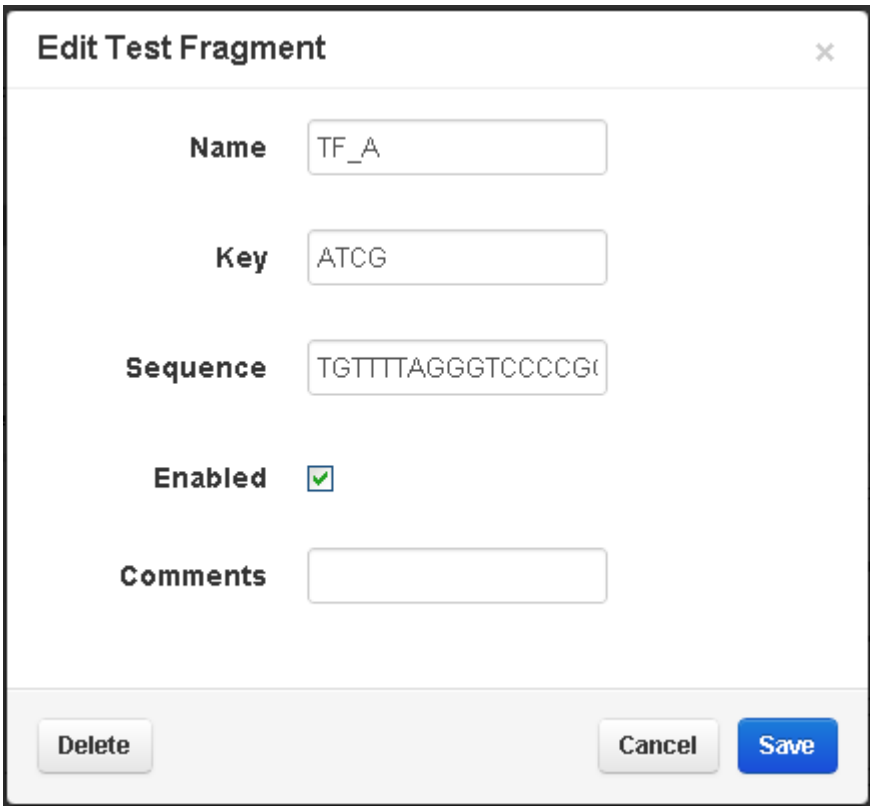
Contact your Ion Torrent™ representative if you have questions about the test fragment templates installed in your Torrent Browser.

Edit a test fragment

If Ion Torrent™ provides new test fragments as part of an updated protocol, it will be necessary to carefully cut and paste this information into the fields.

⚠ **WARNING!** Do not modify the test fragment sequences for the test fragments that are supplied by Ion Torrent™ Software: TF_A, TF_B, TF_C, and TF_D

1. Click the **Name** column label to display test fragment details. This example showstest fragment **TF-C** selected for editing:

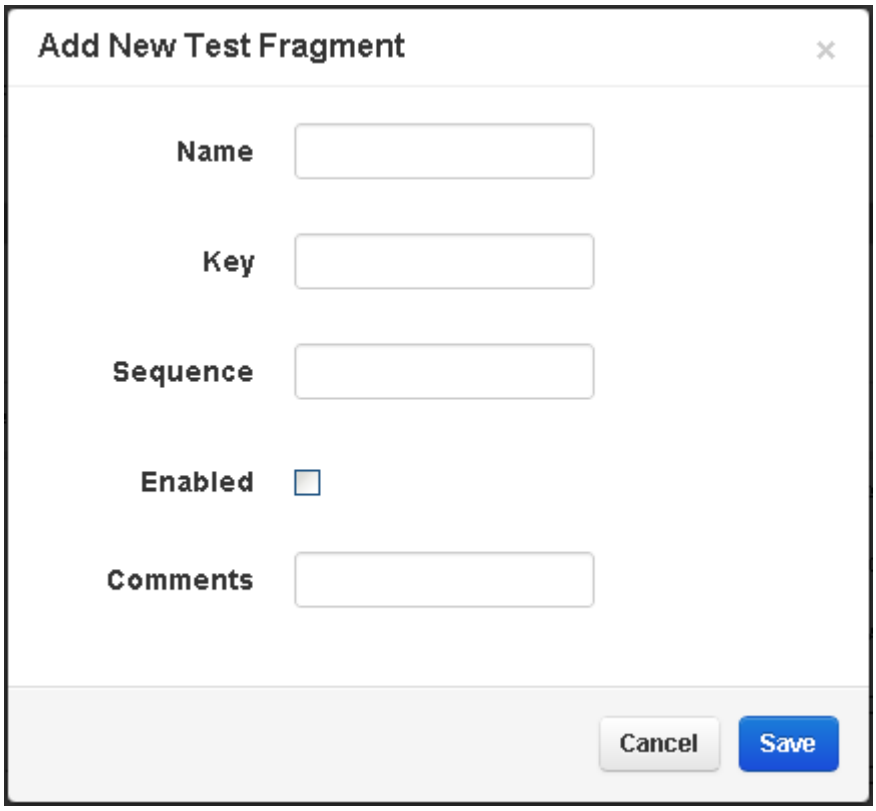


2. On your own test fragment (not test fragments supplied by Ion Torrent™), you can make the following edits:
   - Change the test fragment name, key, or comments.
   - Change the test fragment nucleotide sequence in the Sequence field.
   - Change whether or not the test fragment is enabled.

3. Click **Save** to save your changes or click **Cancel** to end your edit session without modifying the test fragment.

Add a test fragment

1. Click the **Add Test Fragment** button at the upper right corner to add a new test fragment.



2. Choose a unique name for your test fragment.
3. Be sure to enter thetest fragment Key and Sequence using only the uppercase letters: A, T, C and G. If you enter an invalid character or duplicate test fragment, you are not be able to save your changes.
4. Click **Save** to save your changes. Your new test fragment is displayed in the test fragment list.(Or click **Cancel** to end your session without adding a new test fragment.)

Download an Ion Reference File

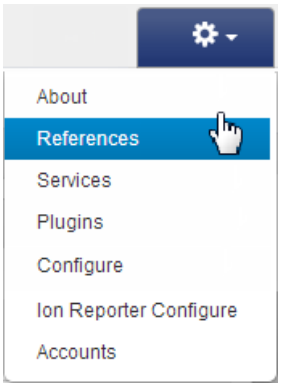
In the admin References tab, you can download a GHRC38, MM10, hg19, or *E. coli* reference file.

The hg19 reference available here is the same as what is used for Torrent Suite™ analyses.

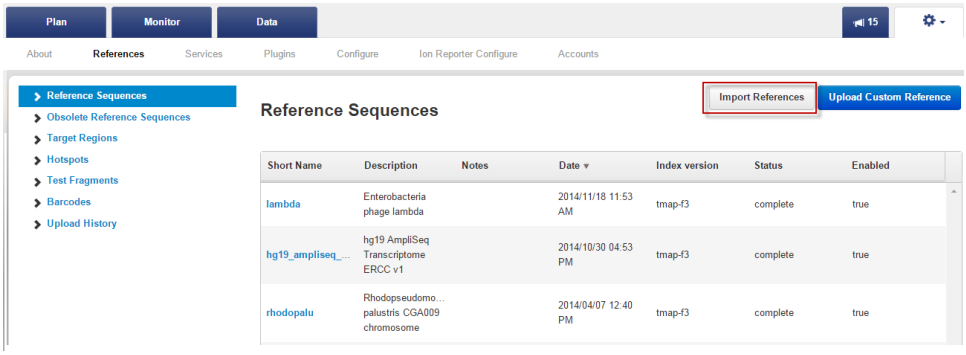
Download a reference file

Follow these steps to download a reference file:

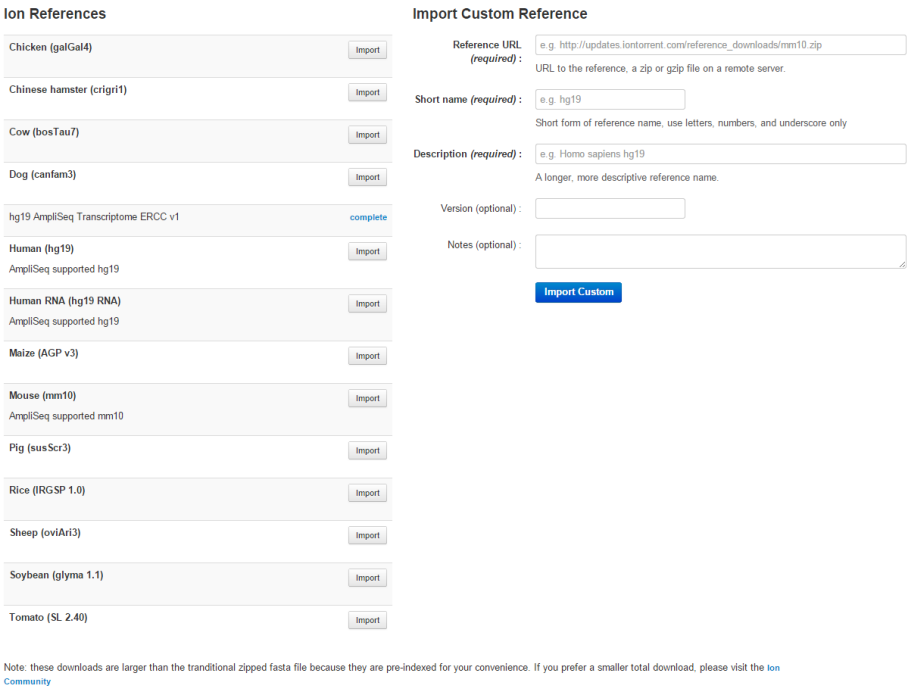
1. Click **Settings** (⚙) ▸ **References**:



2. Click the **Import References** button:



3. Click the **Import** button for the reference:



4. Wait while the file downloads. You can click the **Refresh** button to update the progress percentage:

5. Alternatively, you can also import a custom reference from this page. Enter relevant information in the Import Custom Reference pane and click **Import Custom**.

Details about the Ion hg19 Reference

This human reference is based on the GRCh37.p5 version of the human genome assembly. The GRCh37.p5 version is described at this web site: **http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/data/index.shtml**.

The remainder of this section lists differences between GRCh37.p5 and the Ion Reference hg19 versions of the human genome.

Three positions with ambiguity codes

Three positions on chromosome 3 are marked with `'N'` in the UCSC version of the genome. These positions have IUPAC ambiguity codes in our version:

Position	IUPAC Ambiguity code in Ion reference	Hard masked character in UCSC hg19
60830534	M	N
60830763	R	N
60830764	R	N

Hard masked PAR regions in chromosome Y

The chromosome Y sequence has the Pseudo Autosomal Regions (PAR) hard masked. This practice is consistent with the 1000 Genome Consortium's decision to hard mask these regions inchromosomeY in order to prevent mis-mapping of reads and issues in variant calling on the gender chromosomes.

The masked Y pseudoautosomal regions are chrY:10001-2649520 and chrY: 59034050-59363566. (A related file can be downloaded from **ftp://ftp.ensembl.org/pub/release-56/fasta/homo_sapiens/dna/Homo_sapiens.GRCh37.56.dna.chromosome.Y.fa.gz**)

The following background information is from the UCSC site **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**

"The Y chromosome in this assembly contains two pseudoautosomal regions (PARs) that were taken from the corresponding regions in the X chromosome and are exact duplicates:

chrY:10001-2649520 and chrY:59034050-59363566 chrX:60001-2699520 and chrX: 154931044-155260560"

Chromosome M

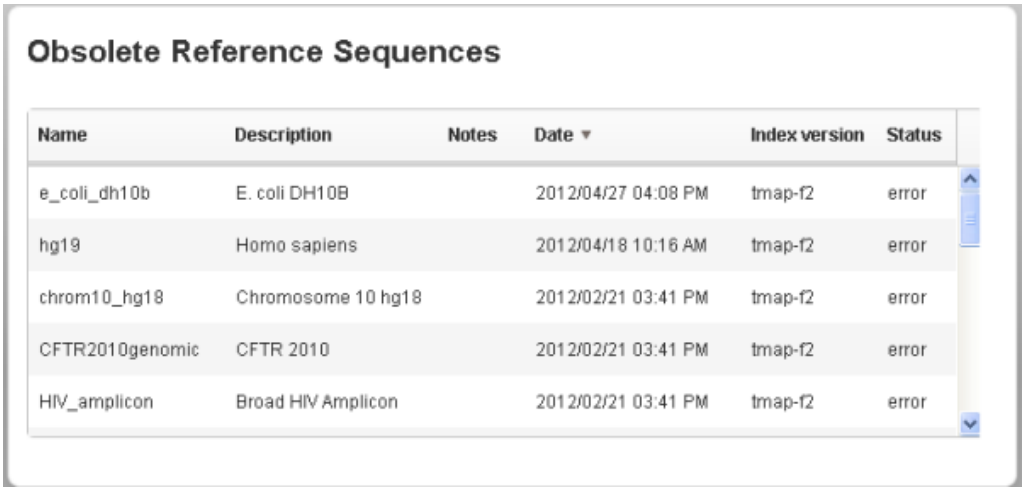
We use the Cambridge Reference Sequence (rCRS) for chromosome M with the GenBank accession number NC_012920. UCSC has announced that they also are using this version in the next human assembly release.

The following background information is from the UCSC site **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**

*"Note on chrMSince 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."*

Work with Obsolete Reference Sequences

The Obsolete References Sequences section provides a checklist of the libraries that need to be upgraded after an update to a Torrent Suite™ Software release that uses a new TMAP index. (Your list will be different.)



The Torrent Browser aids you in identifying the obsolete sequences by automatically recording the libraries that were installed before the upgrade. You need to upgrade these obsolete reference sequences using the **Rebuild All Now** button. (However, the Rebuild All Now process does not remove the references from the obsolete table. If you previously upgraded to 2.2, you rebuilt your references indices at that time, and you do not need to rebuild them again.)

The only reference library available after upgrade is *E. coli* DH10B, which is displayed in the **Reference Sequences** panel of the Admin **References** tab and on the Ion PGM™ Sequencer genome choice list menu. The previous default Ion Torrent™ reference library, *E. coli* K12, is permanently removed.

Why are my references obsolete

Only when a Torrent Suite™ Software upgrade requires that reference indices be rebuilt, the upgrade involves these steps:

1. The upgrade installs only *E. coli* DH10B and moves other references into the Obsolete Reference Sequences table.
2. When you do **Rebuild All Now** and the previously obsolete references are copied back to the main Reference Sequences section.
3. The previously obsolete references also remain in the Obsolete Reference Sequences table.

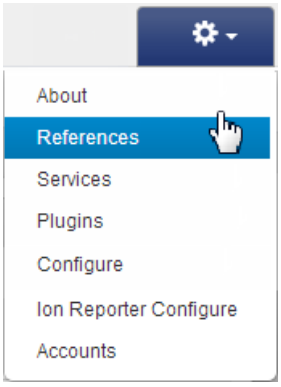
Delete a Reference Sequence

The section provides instructions to delete a reference sequence.

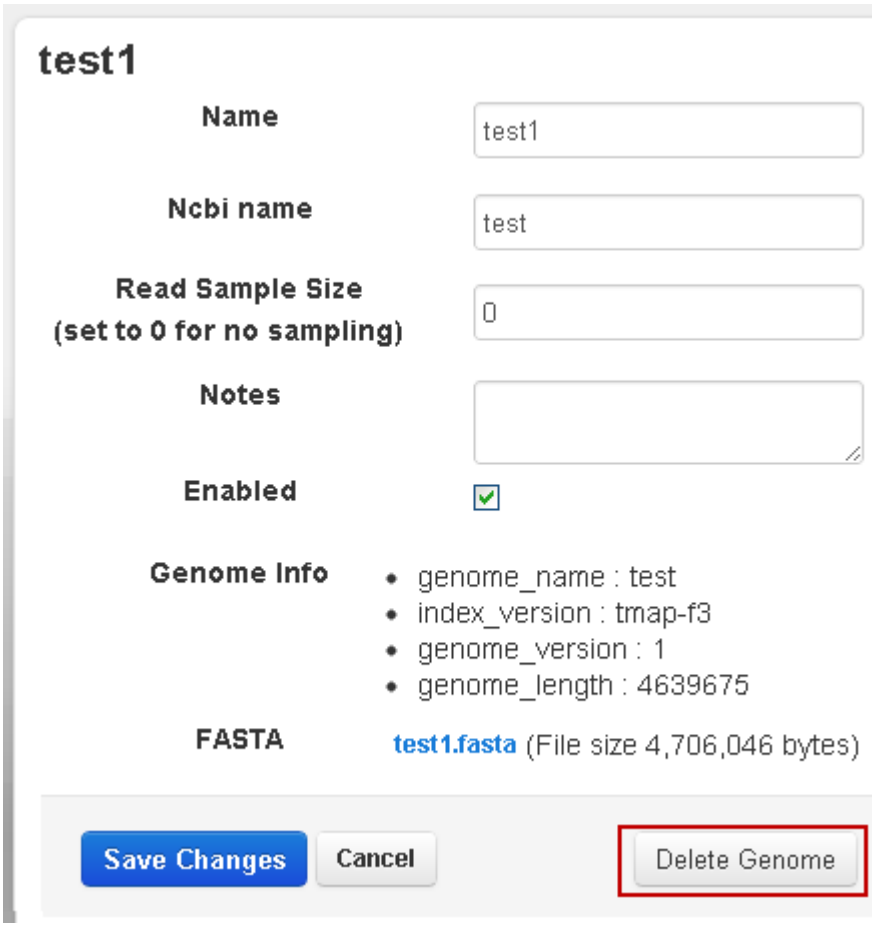
Recovery of a deleted reference sequence is not supported.

Delete a Reference Sequence

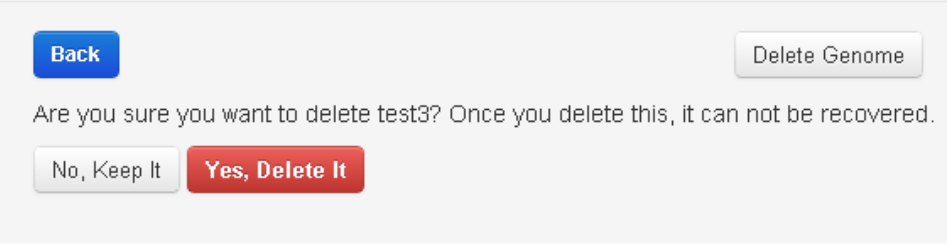
1. Click **Settings** (⚙) ▸ **Reference**:



2. In the **Reference Sequences** section, click the **Name** of the reference sequence you want to delete. Click **Delete Genome** to delete the reference sequence:



A confirmation box appears:



3. Click **Yes, Delete It** only if you are sure this genome should be deleted. Click **No, Keep It** to exit the dialog without deleting the reference sequence: The deleted reference sequence is removed from the **Reference Sequences** list.

In this release you cannot delete a reference from the Obsolete Reference Sequences section.

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 417.

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™ or Ion S5™ Systems.
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™ and Ion S5™ Systems, Basecalling Input data are transferred to the Torrent Server and are available for reanalyses.

File type	Details
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.

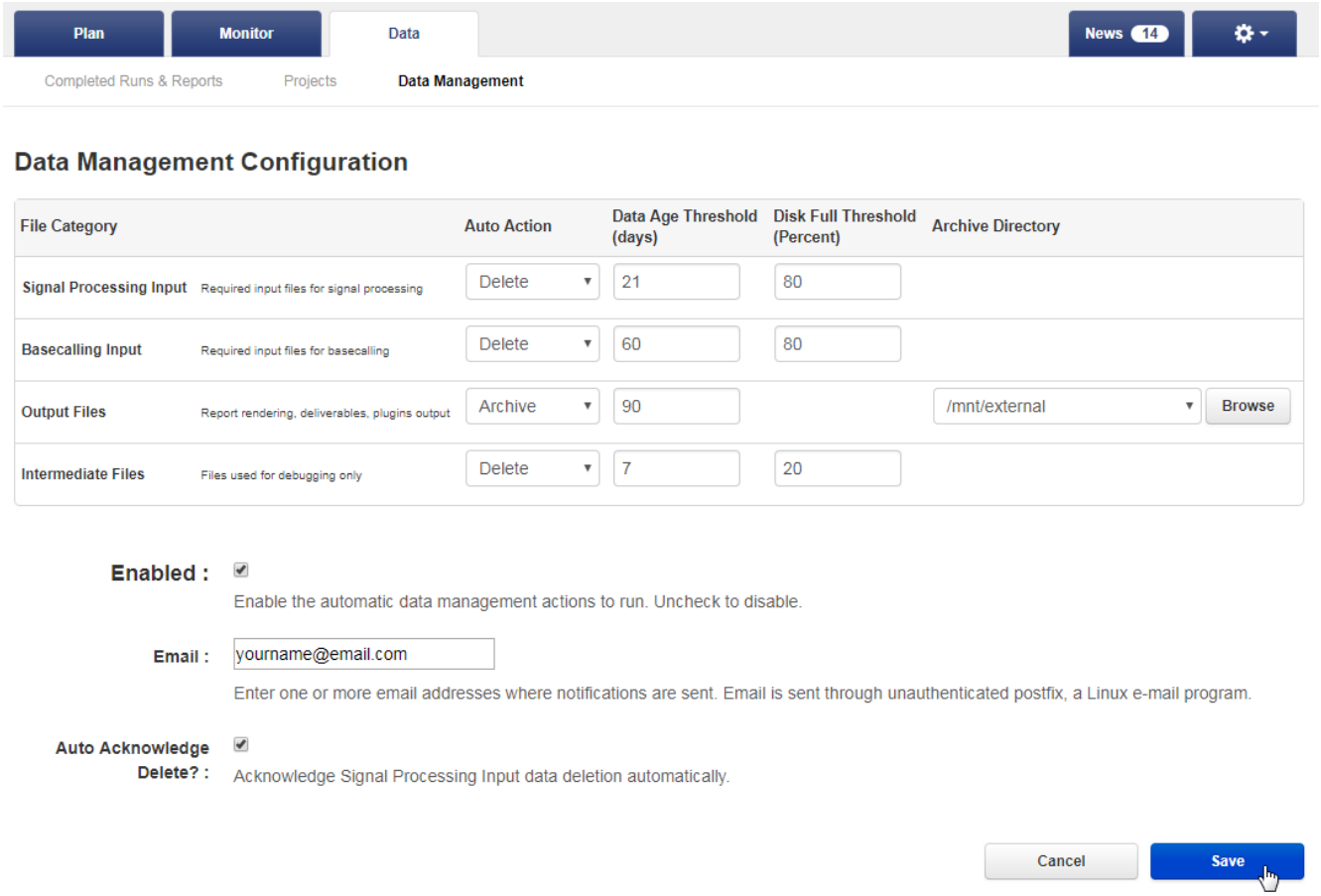
- You can configure your Torrent Server to archive data to a mounted drive automatically after a data age threshold is met. Data that you assign to be automatically archived are copied to the designated location, then deleted from the Torrent Server. Automatic archiving helps to maintain available disk 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 admin 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.

Note: Data deletion is triggered when the **Disk Full Threshold** for that file category is met. Data that exceeds the **Data Age Threshold** for that file category is then 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 293.

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.

Parameter	Definition
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 about automatic deletion and archive creation, see "Archive or delete data automatically" on page 283.

View category statistics

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

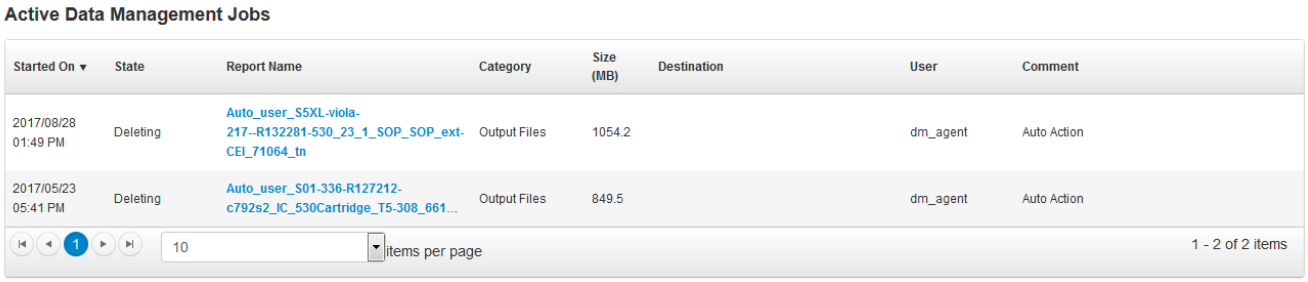
Parameters of the Category Statistics section

Parameter	Definition
File Category Group	File type (see "Ion instrument data types" on page 282 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 system by data management archival.
Deleted	Number of data sets in each file category that have been removed from your system 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, then click **Data Management**, and scroll to the **Active Data Management Jobs** section.



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

2. *(Optional)* Click on 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 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 293 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.

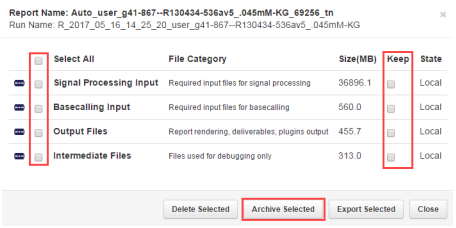
Manually archive run data

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 293.

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.

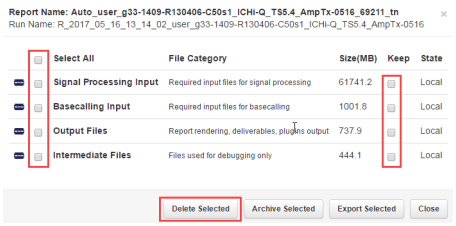
Note: For details about automatic deletion of run data, see "Archive or delete data automatically" on page 283.

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 system.

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 289 for details about how to search for a run in the **Disk Space Management** section.

4. Click **Settings (⚙) ▸ 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 or Ion S5™ Instrument server, 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 296.
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 server. After installation, it must be mounted on the local server as described in "Mount a USB drive" on page 293.

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
I/O size (minimum/optimal): 512
bytes / 512 bytes
Disk identifier: 0x0004366b

Device Boot  Start    End
Blocks   Id  System
/dev/sda1   *    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/sda1   *    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 297.
- If the device connects over a network to the Torrent Server, see "Connect over a network to a Torrent Storage™ NAS device" on page 298.

Connect directly to a Torrent Storage™ NAS device

If your Torrent Server or Ion S5™ Instrument 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 303.

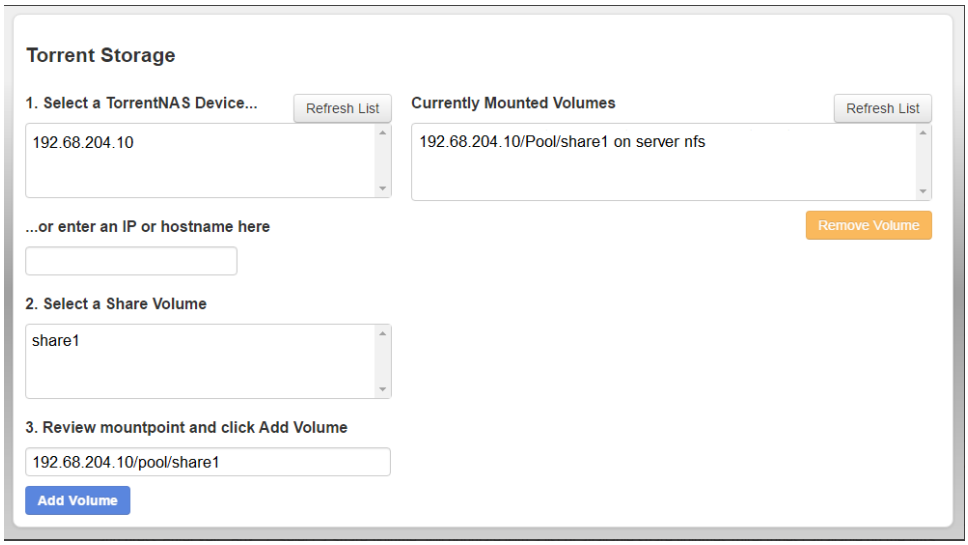
2. Click **Settings (⚙) ▸ Configure**, then scroll to **Torrent Storage**.

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 or Ion S5™ Instrument 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.

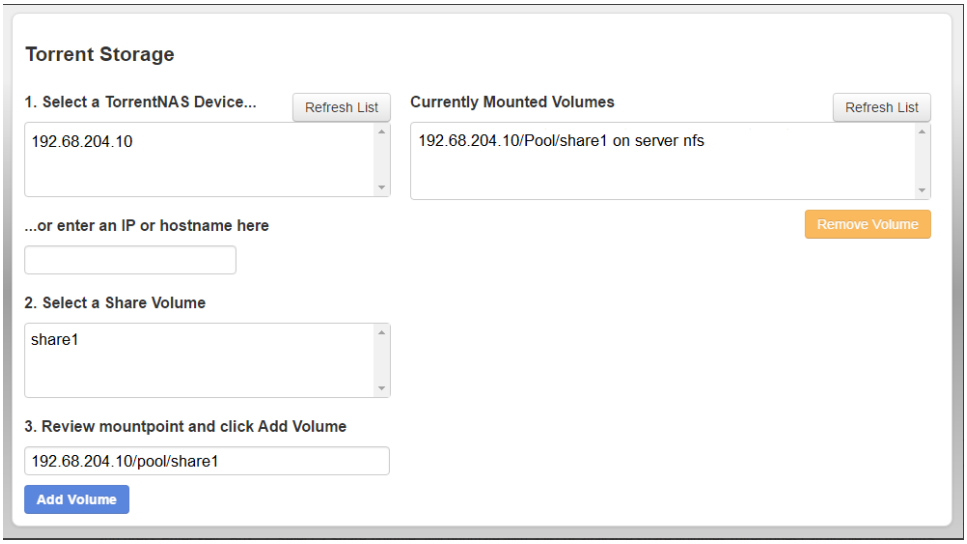
1. In Torrent Suite™ Software, click **Settings (⚙) ▸ About**, then confirm that the Torrent Suite™ Software version is 5.2 or later.

   Note: To update your software, see "Update Torrent Suite™ Software" on page 303.

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**.

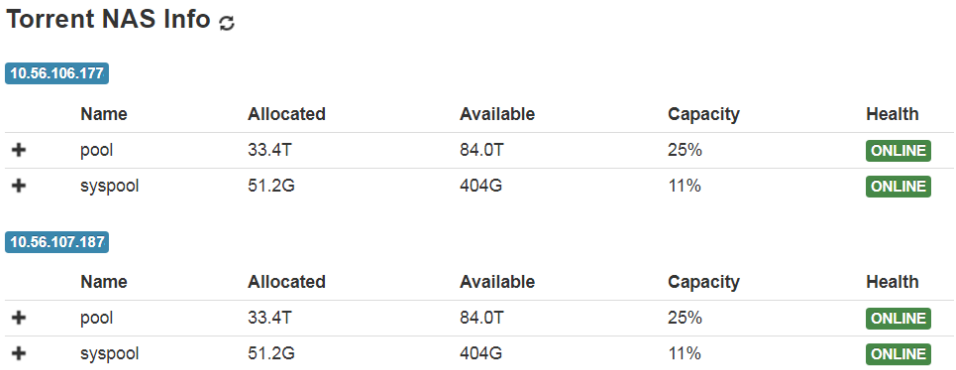


The storage volume is connected to the server and is listed in **Currently Mounted Volumes**.

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

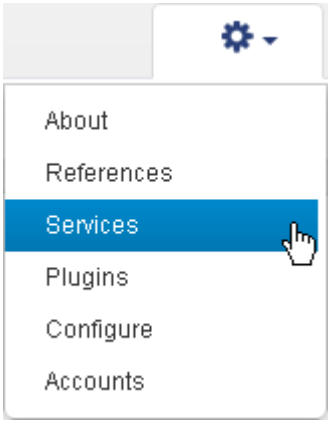
Administrative privileges allow you to configure Torrent Suite™ Software and administer Torrent Server databases. An `ionadmin` account is required for the procedures in this section.

Note: An `ionuser` account does not include sufficient privileges for these procedures.

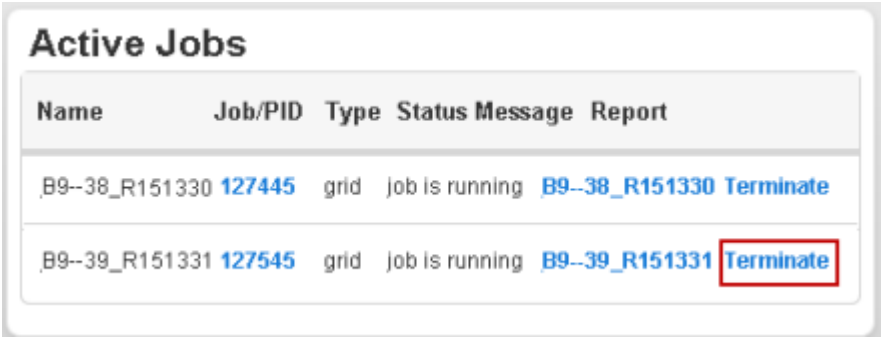
Stop a sequencing Run

Use the following procedure to stop an analysis job for a run that has started but not completed.

1. Click **Settings (⚙) ▸ Services**.

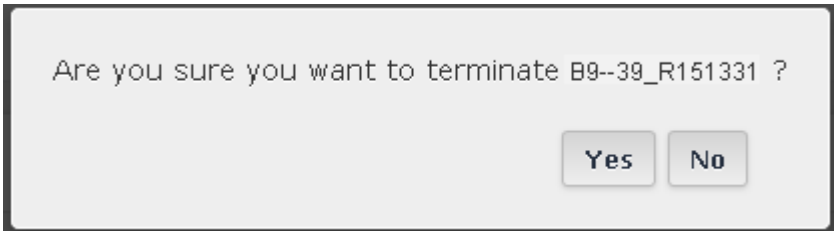


2. Scroll down to the **Active Jobs** section, find the **Name** for the sequencing Run that you want to stop. The **Status Message** column indicates **job is running**.

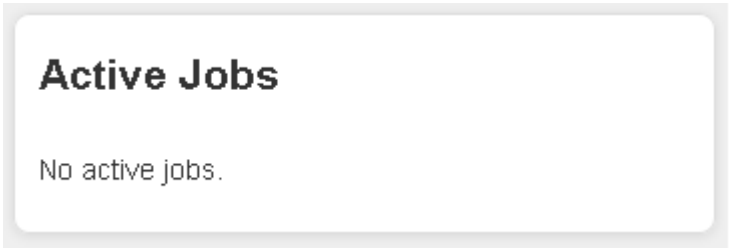


3. Click **Terminate** for the sequencing Run that you want to stop.

4. In the confirmation dialog, click **Yes** to end the run, or click **No** for the analysis job continue.

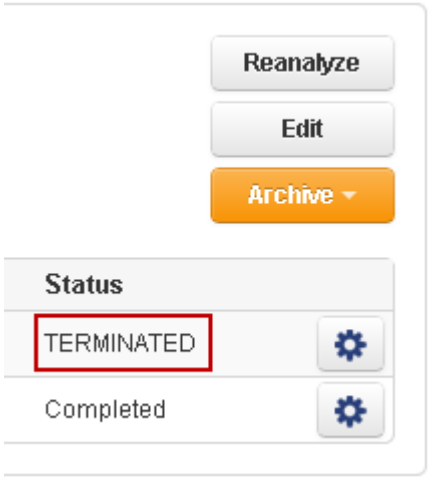


5. Refresh your browser to update the information in the **Active Jobs** section. The run is removed from the **Active Jobs** list, which displays **No active jobs** if no other runs are active:



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.

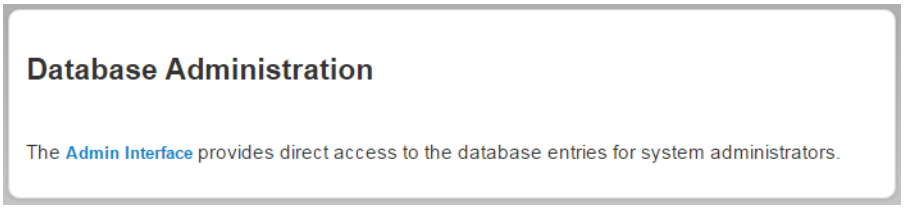
IMPORTANT! To ensure compatibility between the software and instruments, you must also upgrade your instruments after the Torrent Suite™ Software upgrade is complete.

Update Torrent Suite™ Software

Updates to Torrent Suite™ Software cause the software web services to restart. Ensure that no analysis jobs are running on the server or are queued to run.

IMPORTANT! These procedures require an administrative (`ionadmin`) account. A user account such as `ionuser` does not include sufficient privileges for these procedures.

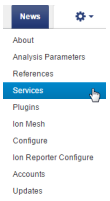
1. Sign in to Torrent Suite™ Software with your `ionadmin` account.
2. Click **Settings (⚙) ▸ Configure**.
3. Scroll to the **Database Administration** section, then click **Admin Interface**.



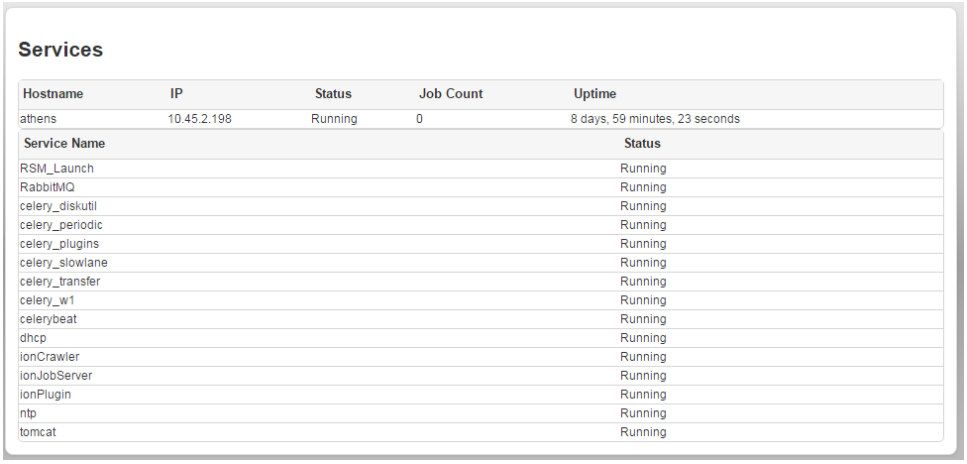
   If you are prompted to Sign in, use your `ionadmin` account.
   The Site administration page opens.
4. Click **Update Server** in the **Management Actions** section:

   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 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.



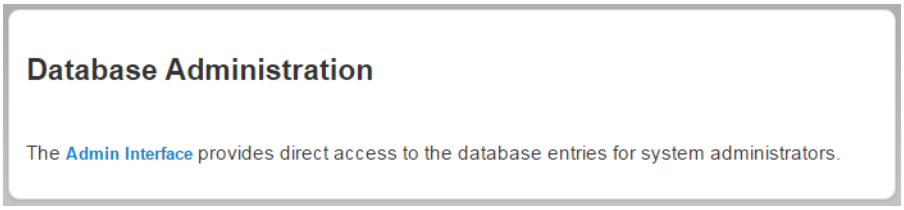
IMPORTANT! To ensure compatibility between the software and instruments, you must also upgrade sequencing instruments after the Torrent Suite™ Software upgrade is complete.

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 `ionadmin` account.
2. Click **Settings (⚙) ▸ Configure**.

3. Scroll to the **Database Administration** section, then click the **Admin Interface** link.

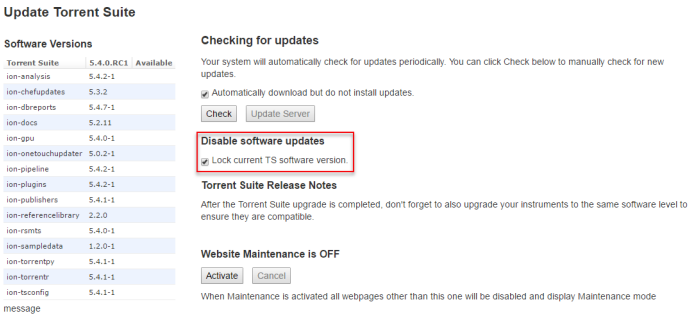


   If you are prompted to Sign in, use your `ionadmin` account.

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

Beginning in Beginning in Torrent Suite™ Software 5.2, 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

Beginning in Torrent Suite™ Software 5.2, you can add new plugins that are released outside of the regular software release cycle.

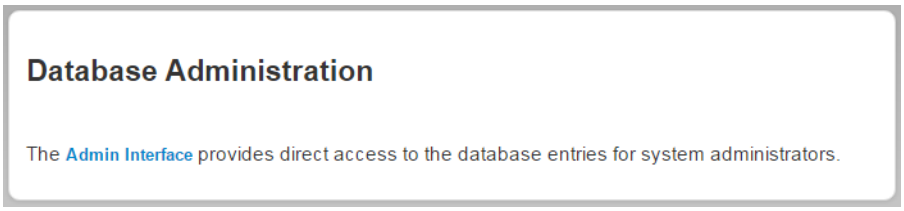
When you learn of a new plugin that you would like to use, check to see if an update is available.

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.

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 `ionadmin` account.

   The **Users** dialog allows you to create and modify user accounts to access the Torrent Suite™ Software.

Approve a new user account

An administrator must approve new accounts that users register for on the sign-in screen. When a new user approval is pending, you see a message when you log in as an administrator.

1. In the message for the new pending registration, click **Account Management**.
2. Scroll to the **User Registrations** section.

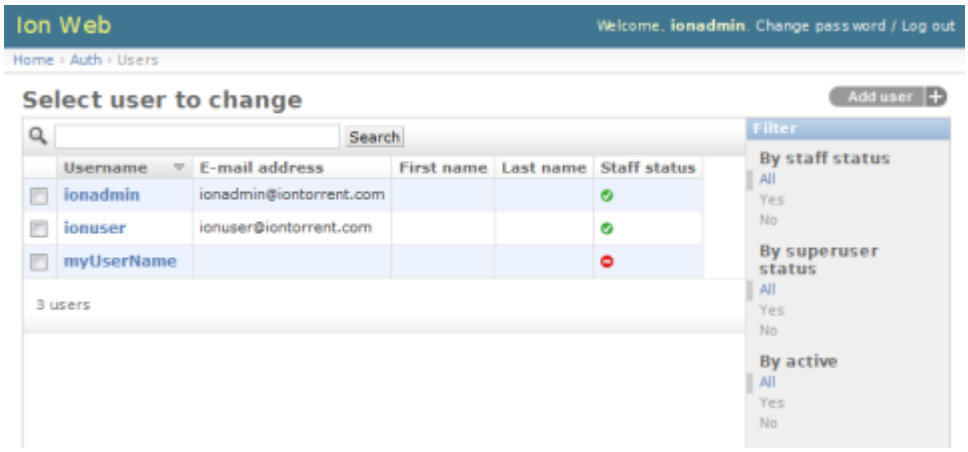
Option	Description
Approve the registration.	Click **Approve** to approve the account for the new user, then click **Yes, Approve**.
Reject the registration.	Click **Reject** to approve the account for the new user, then click **Yes, Reject**.

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 306.

Modify a user

Use the following procedure to modify the information and permissions for an existing user:

1. On the **Users** line of the main **Site administration** menu, click **Change**.
2. On the **Select user to change** screen, click the **Username** of the user you want to change. Usernames can be filtered, selected to the right, according to: **By staff status**, **By superuser status** or **By active** status.

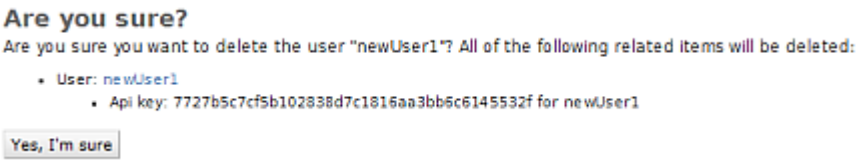


3. Use the **Change user** dialog to modify user information. To log in to the server, it is important to select the **Staff status** checkbox in the **Permissions** dialog, which is shown in the following figure:

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

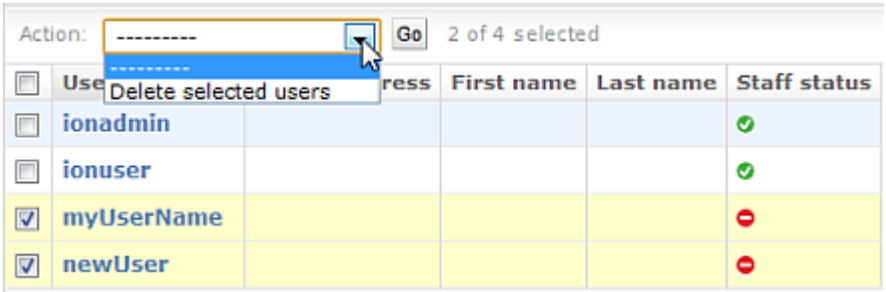
Delete a single user account

1. In the **Users** line of the main **Site administration** menu, click **Change**.
2. On the **Select user to change** page, click the **Username** of the user to be deleted.
3. At the bottom-left of the **Change user** page, click **Delete**.
4. Ensure that you want to delete the user by clicking **Yes, I'm sure**:

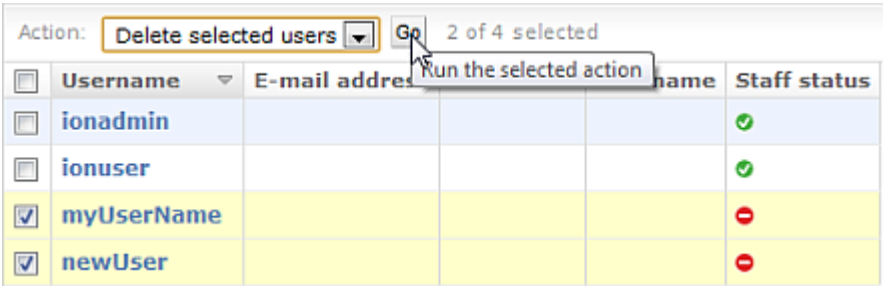


Delete multiple user accounts

1. In the **Users** line of the main **Site administration** menu, click **Change**.
2. On the **Select user to change** page, check the checkbox for each users you want to delete.
3. Click the dropdown menu, then select **Delete selected users**:



4. Click **Go**:



5. 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.

6. On the **Select user to change** page, the list of users confirms your deletions.

Approve requests for new accounts

New users can request accounts on the Torrent Suite™ Software login page. An admin must approve each request in the **Site administration** page before the new account is active.

An administrative account (`ionadmin`) is required to approve a user account request.

Approved accounts are created with `ionuser` permissions.

Torrent Suite™ Software user password changes

An administrative user (ionadmin) can change the password that they use to log in to the Torrent Suite™ Software and can be locked out of the administration menu, or locked out of Torrent Suite™ Software.

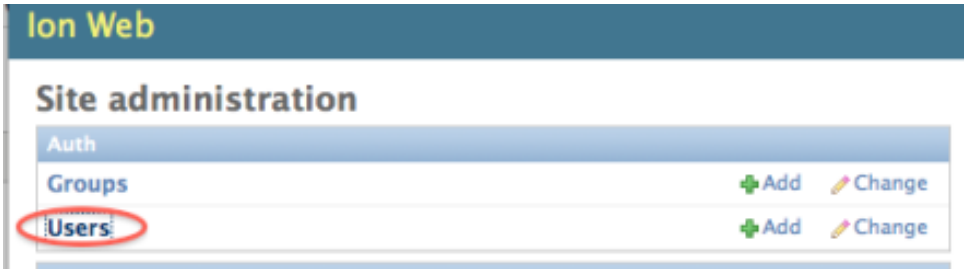
This password is stored in a database field. Use one of these two methods to access the database and change the administrative user password:

- "Create a new superuser account to change a password" on page 310
- "Change a password in the Torrent Suite™ Software database" on page 312

Create a new superuser account to change a password

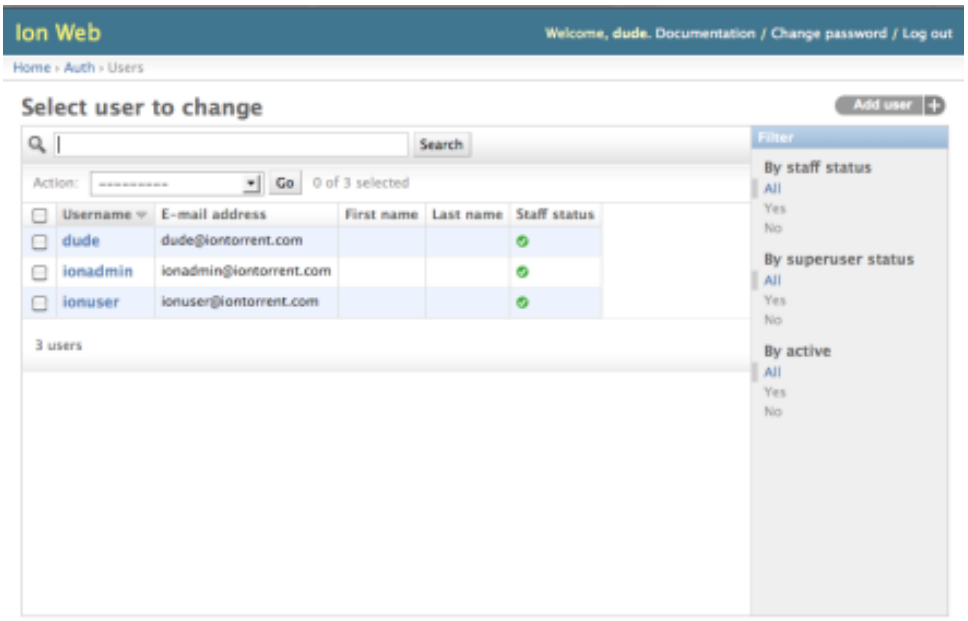
The first way to change a username with minimal terminal interaction is to create a new super user account.

1. Run the following commands: `cd /opt/ion/iondb ./manage.py createsuperuser`
2. After the new superuser account has been created, Sign in to the admin page with the newly created username and password.
3. Select the Users section under Auth:

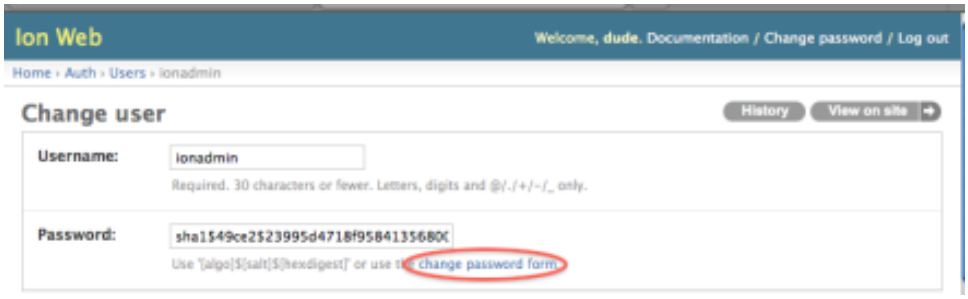


Note: If you Sign in with an `ionuser` account, the Auth section does not appear .

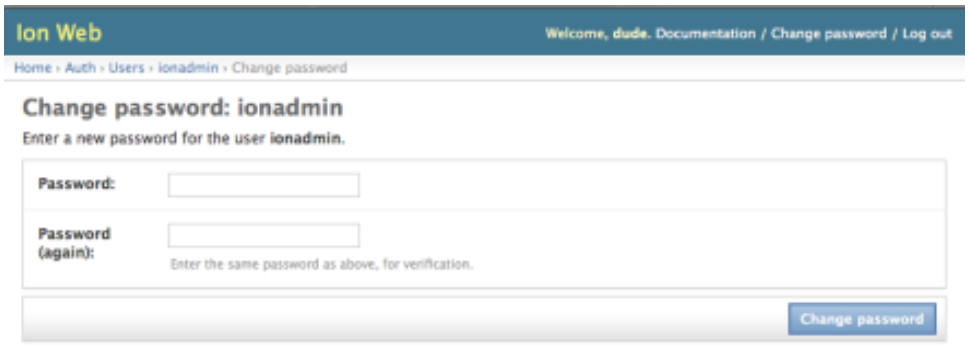
4. Select the account that you want to change the password for:



5. Click **Change password form**:



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



Change a password in the Torrent Suite™ Software database

IMPORTANT! This process updates the database directly, and cannot be undone or recovered in case of error. Do not proceed unless you can confidently execute SQL commands with a command-line utility.

1. Login to the database

   Sign into your Torrent Server host and get an interactive postgres database command prompt:

```
ionadmin@my-torrent-server:~$ psql -U ion -d iondb psql
(8.4.5) Type
                          "help" for help. iondb=>
```

2. Display the user list

   In our example, the user `ionadmin` forgot the password, but we know the `ionuser` password. This command provides a list of users and passwords:

```
iondb=> SELECT username, password from auth_user; username
|
                          password ----------
+-----------------------------------------------------
                          ionuser |
sha1$7e254$476582a5fa365cdd6081a80ac161c1904cc9c374
ionadmin |

sha1$93099$b7da0df453d8db1c7715cabef9651c73003de849 ion |
                          sha1$7798b
$c025c463682f84b66cf3b5168356a04e3ce3b899 (3 rows)
```

3. Copy the password from another user

   The passwords are hashed in the database, so we do not know what the actual password is. But we know the `ionuser` password is `ionuser`, so we can copy that hashed password to `ionadmin`, and that will change the `ionadmin` password to `ionuser`.

   IMPORTANT! The UPDATE command modifies the database. Do not proceed with this step if you are not comfortable with SQL commands.

```
iondb=> UPDATE auth_user set

password='sha1$7e254$476582a5fa365cdd6081a80ac161c1904cc9c3
74' where
                          username='ionadmin';
```

4. Check that the password has been changed

   Query the database one again to verify that the password has been changed. See that `ionadmin` and `ionuser` now have the same password

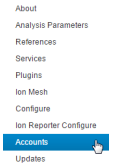
```
iondb=> SELECT username, password from auth_user; username
|
                          password ----------
+-------------------------------------------------
                          ionuser |
sha1$7e254$476582a5fa365cdd6081a80ac161c1904cc9c374
ionadmin |

sha1$7e254$476582a5fa365cdd6081a80ac161c1904cc9c374 ion |
                          sha1$7798b
$c025c463682f84b66cf3b5168356a04e3ce3b899 (3 rows)
```

5. Reset the password

   Now you can Sign in via the UI as `ionadmin`, and reset the password. Remember to change the password via the **Change password** form.

Check user account notification

Click **Settings (⚙) ▸ Accounts**.

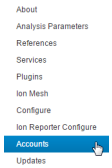


When user account requests are pending, the **Accounts** tab contains notifications such as the following:

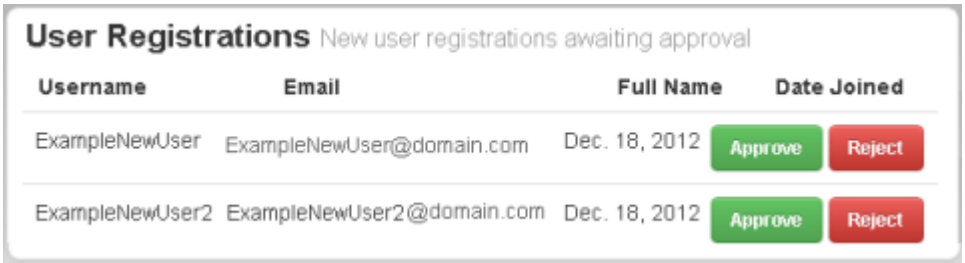
New pending user registration for 'ExampleNewUser'. See “Manage Torrent Suite™ Software user accounts“ on page 306 to review.

Approve and reject new accounts

1. Click **Settings (⚙) ▸ Accounts**.



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



2. To approve or reject a new account, do one of the following:

Option	Action
Approve	Click **Approve** in the **User Registrations** section, then confirm.
Reject	Click **Reject** in the **User Registration** section, then confirm.

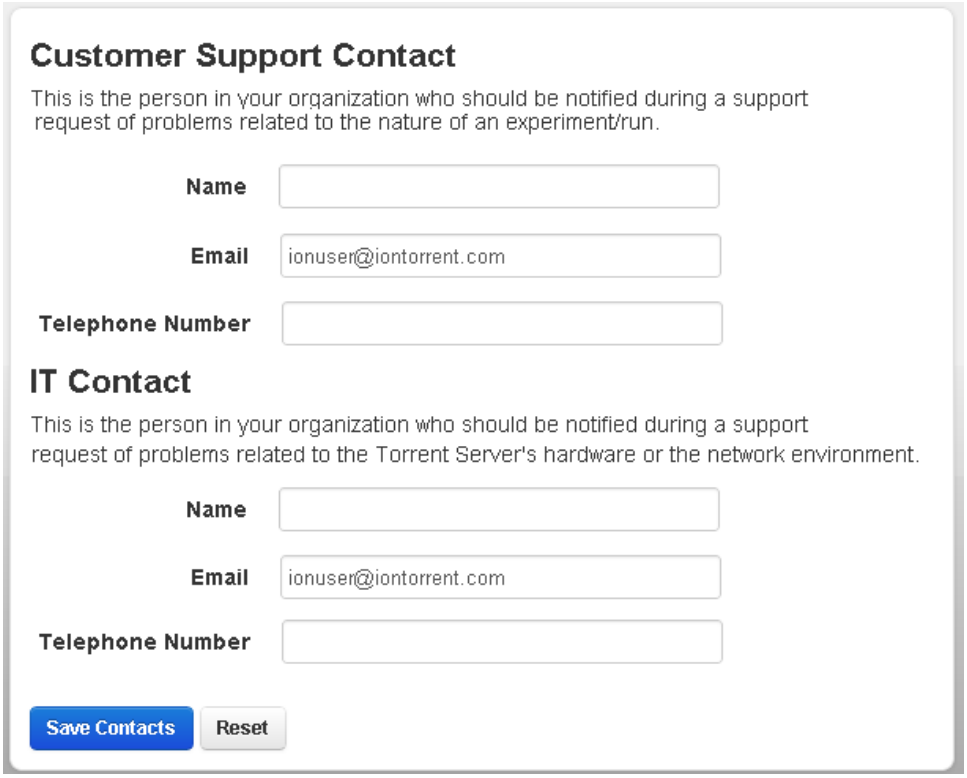
When you approve an account request, the account status is changed to **Active** in the user database and the user can Sign in to the Torrent Browser.

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 286.

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

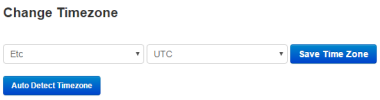
You can change the server name that appears in the Torrent Suite™ Software. By default, this name is `Torrent Server`. This change affects only the server name that is shown in the Torrent Suite™ Software, and the default bookmark name that appears in the browser when a bookmark is created.

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**.

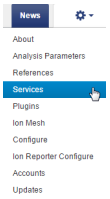


3. () Click **Auto Detect Timezone**, then click **Save Time Zone**.

The new time zone takes effect immediately on the Ion Torrent™ Server.

Monitor your Ion Torrent™ Server

Click **Settings (⚙) ▸ Services**.

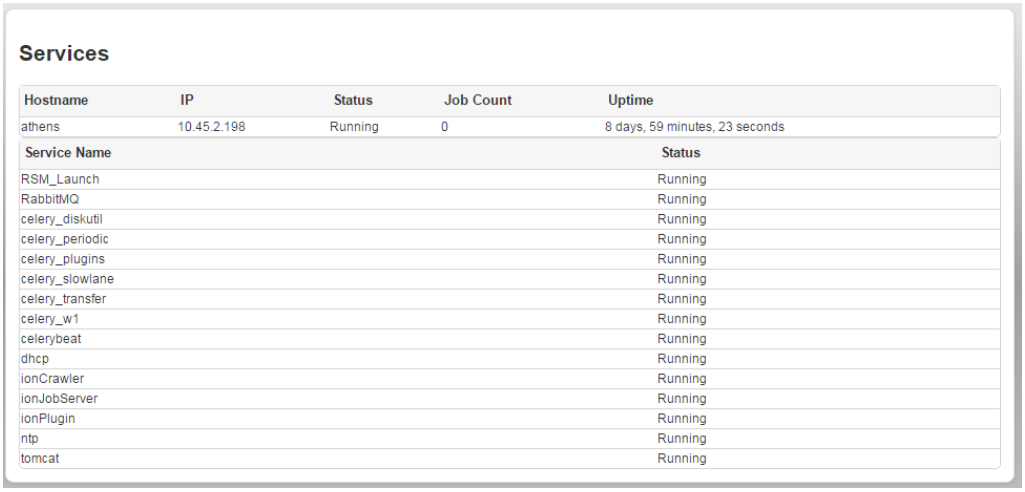


The following information appears:

- **Services**
- **Active Jobs**
- **ionCrawler Service Details**
- **RAID Info**

Jobs Server service

The **Services** panel lists services used by Torrent Suite™ Software.



During normal operation each service's status is "Running". A status of "Down" indicates the service should be restarted.

Start a job request

There are 2 ways to start a job request.

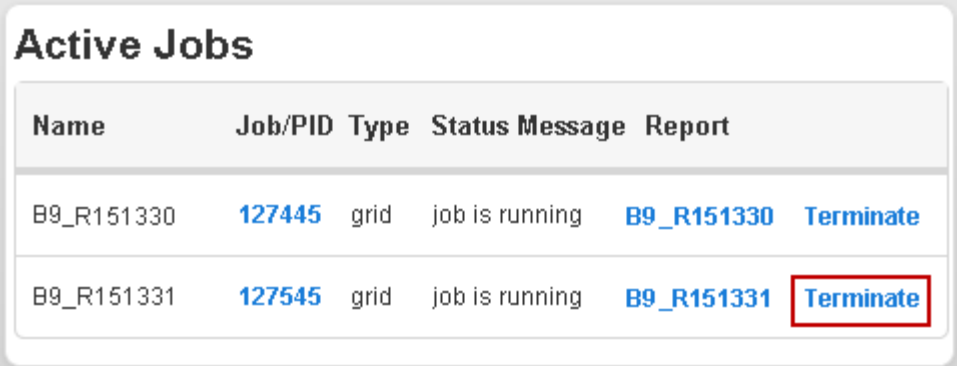
1. (*Optional*) Click **Analyze** to start a job request for a given run.
   An analysis job starts automatically for that run after data is transferred when an auto-analysis completes on aIon PGM™ or Ion Proton™ Sequencer.

2. (*Optional*) **Click auto-analysis** on aIon PGM™ or Ion Proton™ Sequencer. After data is transferred when an auto-analysis completes, an analysis job starts automatically for that run.

Stop a run that is in progress

You can end a sequencing run that has started but is not yet completed.

1. Scroll to the **Active Jobs** section and click **Terminate** to the right of the run name.
2. Click **Terminate** to stop a job in **Active Jobs**.



ionCrawler service

The **ionCrawler** panel displays information about processes that transfer data from Ion PGM™ and Ion Proton™ Sequencers to the 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):

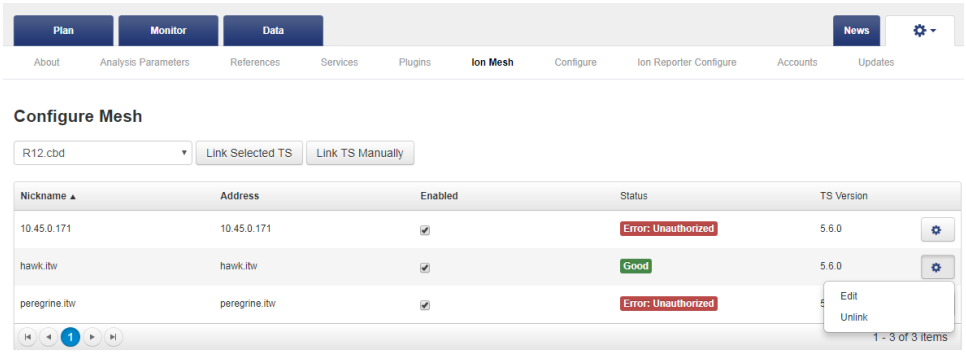
Slot 0	
Media Error Count	0
Other Error Count	0
Predictive Failure Count	0
Firmware state	Online, Spun Up
Inquiry Data	SEAGATE ST3200044SS KS679WM0L47T
Needs EKM Attention	No
Foreign State	None
Port-0	
Port status	Active
Port-1	
Port status	Active
Drive has flagged a S.M.A.R.T alert	No
Drive Temperature	30C (86.00 F)

Refresh your browser to see changes in status. This information is not updated automatically.

Set up Ion Mesh

Follow these steps to connect (link) or disconnect (unlink) your Torrent Server (TS) to another Torrent Server(TS).

1. In the any tab, click **Settings** (⚙), then click **Ion Mesh**.



2. Select one of the following options.

Option	Action
Unlink a TS from another TS	Click **Settings** (⚙) in the **TS Version** column of the row of the TS being unlinked, then click **Unlink**.
Edit the connection settings of a linked TS	Click **Settings** (⚙) in the **TS Version** column of the row of the TS being edited, then click **Edit**.
Link a selected TS	Click **Link Selected TS**.
Manually link a TS	Click **Link TS Manually**.

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

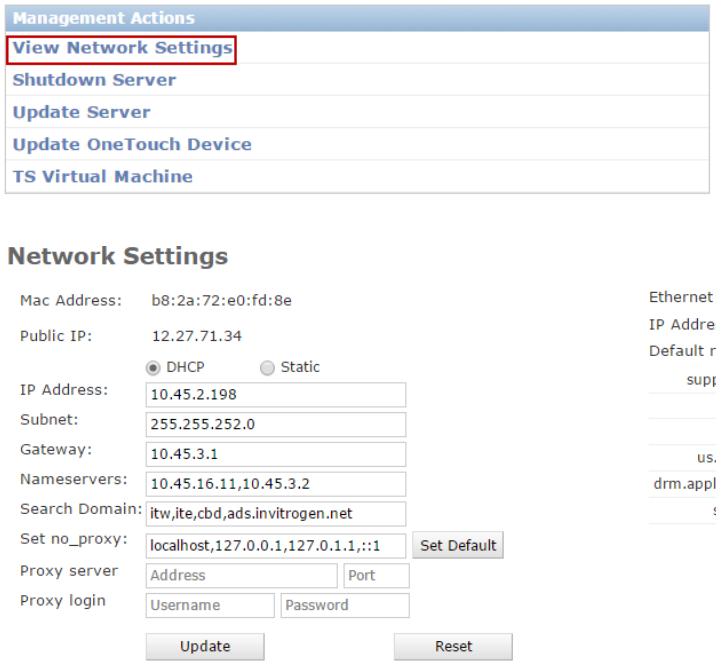
Parameter	Definition
Hostname/Address	Host name or address of the server. **Note:** If linking a selected TS, this field is automatically populated and cannot be edited.
Nickname	A common name that is assigned to the TS.
Username	Your username.
Password	Your password.

4. Click **Setup**.

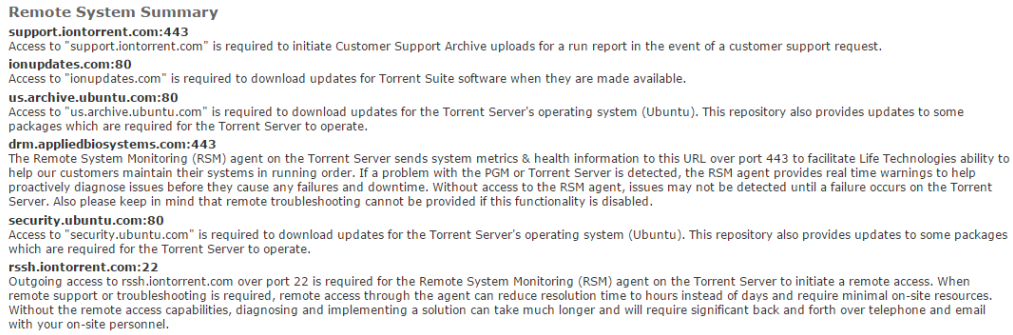
View network settings

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 data	`/results/< PGM_Name/S5 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™ Sequencers via the software update process.

Approximately every 60 seconds, this agent sends a heartbeat message to Thermo Fisher. 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 technical support personnel can review the information that agents collect. Because the heartbeat message is sent many times an hour, Tech 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 Ion Torrent™ to log in remotely to the Ion S5™, Ion PGM™, andIon Proton™ systems and the Torrent Suite™ Software, which is required for system support. Without remote access, Thermo Fisher 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™, andIon Proton™ Sequencers 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	

Event Name	Type	Sample Value	
TS.Server.ionPlugin	String	ok/offline/error	
TS.Server.ntp	String	ok/offline/error	
TS.Server.RabbitMQ	String	ok/offline/error	
TS.Server.RSM_Launch	String	ok/offline/error	
TS.Server.tomcat	String	ok/offline/error	
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

Event Name	Type	Sample Value
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
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™ and Ion Proton™ data

Ion S5™ and Ion Proton™ sequencer data is 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

Data Item Name	Type	Sample value
Event.DatacollectStarted	String	Datacollect Started
Event.InstrumentMustBeInitialized	String	Instrument must be initialized
Event.PostRunCleanHasNotBeenRun	String	Post Run Clean has not been run
InstrumentState	String	Idle
RunData.a1a2	String	R_2016_02_17_13_01_08_user_F4--145 W1.dat dfffe cntArry 9 0 0 9
RunData.AutoPhFinal	Analog	7.660635
RunData.AutoPhInitial	Analog	6.321023
RunData.AutoPhIterations	Analog	4
RunData.AutoPhResult	String	Pass
RunData.AutoPhTotalW1Volume	Analog	1.0
RunData.ChipGain	Analog	1.066389
RunData.ChipPixelAverage	Analog	8241
RunData.ChipPixelsInRange	Analog	164698460
RunData.ChipPixelsPinnedHigh	Analog	0
RunData.ChipPixelsPinnedLow	Analog	676
RunData.ChipTemp	Analog	81.826172
RunData.CpuTemp0	Analog	53
RunData.CpuTemp1	Analog	74
RunData.efuse	String	**********L:Q6C841,W:4,J:WC2012C00086-C00272,P:16,C:PT4,F:F6,Y:4,X:0,B:3,SB:31,B:1P,N:343**********
RunData.FpgaMasterTemp	Analog	113
RunData.FpgaSlaveTemp	Analog	118.4
RunData.GpuTempC	Analog	82
RunData.LastAutoPhRealPh	Analog	766

Data Item Name	Type	Sample value
RunData.LastAutoPhRef	Analog	745
RunData.LastAutoPhTarget	Analog	770
RunData.R1pH	Analog	7.00
RunData.R2pH	Analog	7.00
RunData.R3pH	Analog	7.00
RunData.R4pH	Analog	7.00
RunData.W1pH	Analog	8762
RunData.W2pH	Analog	7619
RunData.W3RefpH	Analog	7.45
Status.HDPctFull	Analog	0.823612
Status.SsdPctFull	Analog	6.220454
System.CpuUsagePct	Analog	7
System.Date	String	2013-01-0
System.FreeMemoryKB	Analog	129951948
System.Hostname	String	d1.ite
System.IpAddress	String	10.25.3.150
System.PhysMemTotalGB	Analog	128
System.Time	String	03:42:58 PM GMT
TYPE	String	Proton1
Version.Datacollect	String	3371
Version.DiskImage	String	2015_06_04
Version.Graphics	String	80
Version.KernelRelease	String	3.13.9-ionrt1
Version.LiveView	String	2166
Version.OIA	String	5203
Version.OS	String	17
Version.Reader FPGA	String	3d400109
Version.Reader FPGA1	Analog	33400109
Version.Reader Woddr FPGA	String	3400043
Version.Reader Woddr FPGA1	String	340004b

Data Item Name	Type	Sample value
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, this agent allows Thermo Fisher support personnel to remotely:

- Collect log files from the systems 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™ system, an Ion PGM™ system, an Ion Proton™ system, or Torrent Suite™ Software is reported, the Thermo Fisher 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 service and support requires authorization from the technical contact to initial remote connection. Only after getting authorization does Thermo Fisher 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 suggestions apply to system level issues such as networking, disk space, and system load.

For investigations of an individual failed analysis run, see instead “Handle a failed analysis run“ on page 43.

Check crawler and job server status

Access the Crawler and Jobs Server page:

Click **Settings (⚙) ▸ Services**.

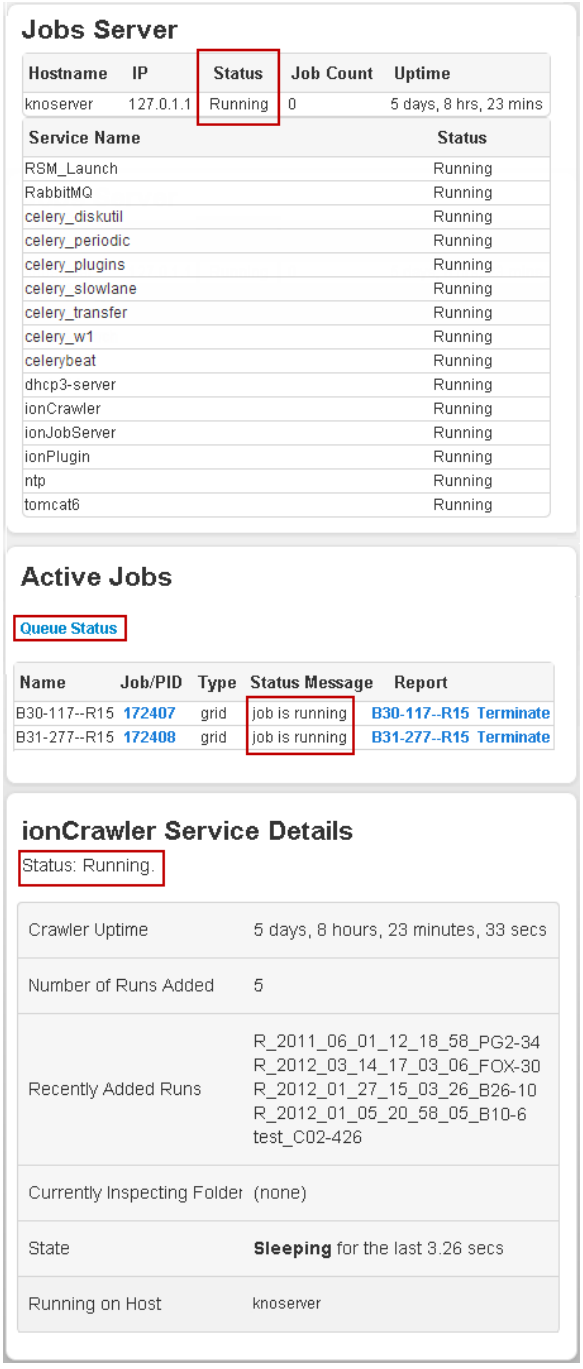
Note: Startup scripts for each process can be found in the `/etc/init.d` directory.

Note: Log file for each process can be found in the `/var/log/ion` directory. They are:

- `crawl.log`
- `iarchive.log`
- `celery_w1.log`
- `ionPlugin.log`

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.

Process status is displayed in the Admin **Services** tab, as shown in the following figure:



Jobs Server

Hostname	IP	Status	Job Count	Uptime
knoserver	127.0.1.1	Running	0	5 days, 8 hrs, 23 mins

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
dhcp3-server	Running
ionCrawler	Running
ionJobServer	Running
ionPlugin	Running
ntp	Running
tomcat6	Running

Active Jobs

Queue Status

Name	Job/PID	Type	Status Message	Report
B30-117--R15	172407	grid	job is running	B30-117--R15 Terminate
B31-277--R15	172408	grid	job is running	B31-277--R15 Terminate

ionCrawler Service Details

Status: Running.

Crawler Uptime	5 days, 8 hours, 23 minutes, 33 secs
Number of Runs Added	5
Recently Added Runs	R_2011_06_01_12_18_58_PG2-34 R_2012_03_14_17_03_06_FOX-30 R_2012_01_27_15_03_26_B26-10 R_2012_01_05_20_58_05_B10-6 test_C02-426
Currently Inspecting Folder	(none)
State	**Sleeping** for the last 3.26 secs
Running on Host	knoserver

If a process is not running, a **Down** or **Offline** reason is displayed in the Admin **Services** tab. An example is "The crawler is offline".

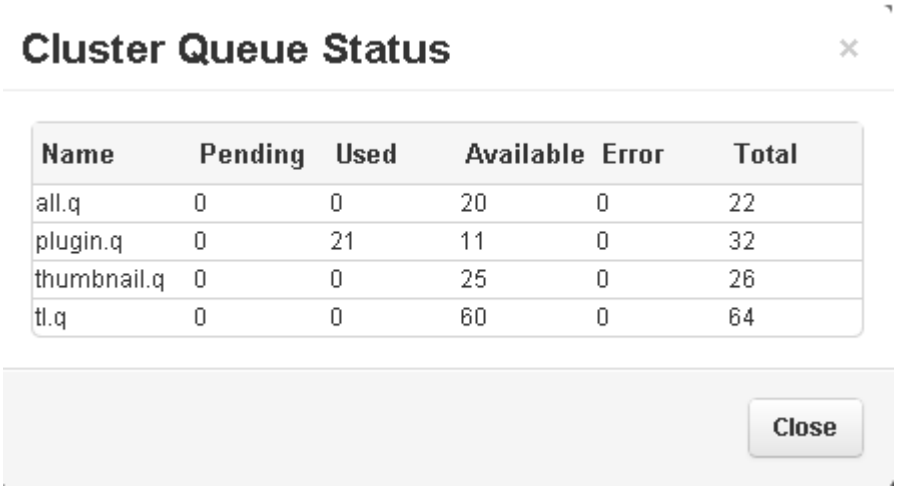
Click **Settings (⚙) ▸ Services**.

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™ orIon Proton™ 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.

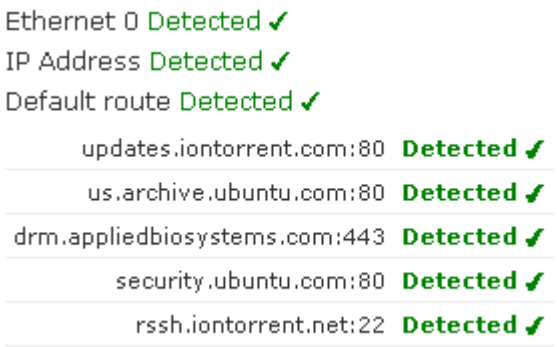
   Note: If the processes do not continue to run after being restarted, contact your Ion Torrent™ representative for assistance.

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 ▸ Admin interface ▸ Management Actions ▸ Network Settings**. The Torrent Browser performs several network checks:

2. Verify that the Torrent Server is configured correctly by reviewing the Torrent Server deployment instructions.
3. Find the IP address of the Torrent Server as described in “Verify Torrent Server IP address“ on page 334.

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™ Sequencers. However, if the server is not able to synchronize with the trusted time service, it does not act as a time server for the sequencers (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™ Sequencers 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:

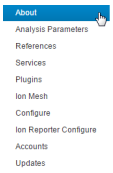
The files in this folder contain background model parameter values based on the location of the well in the chip.

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
```

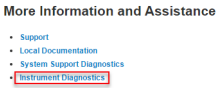
Data

```
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.

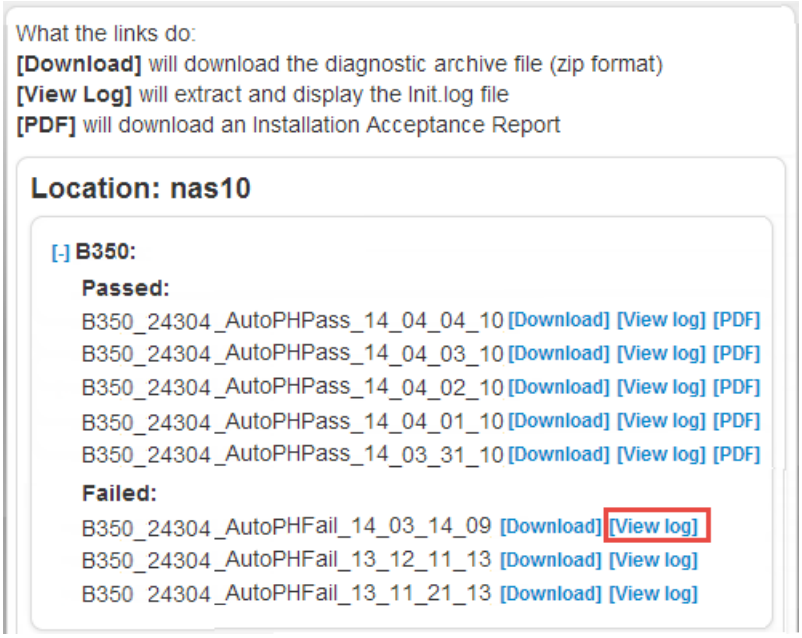
1. To access the **Instrument Diagnostics** information, click **Settings (⚙) ▸ About**:
2. Scroll down to the **More Information and Assistance** section, then click the **Instrument Diagnostics** link:



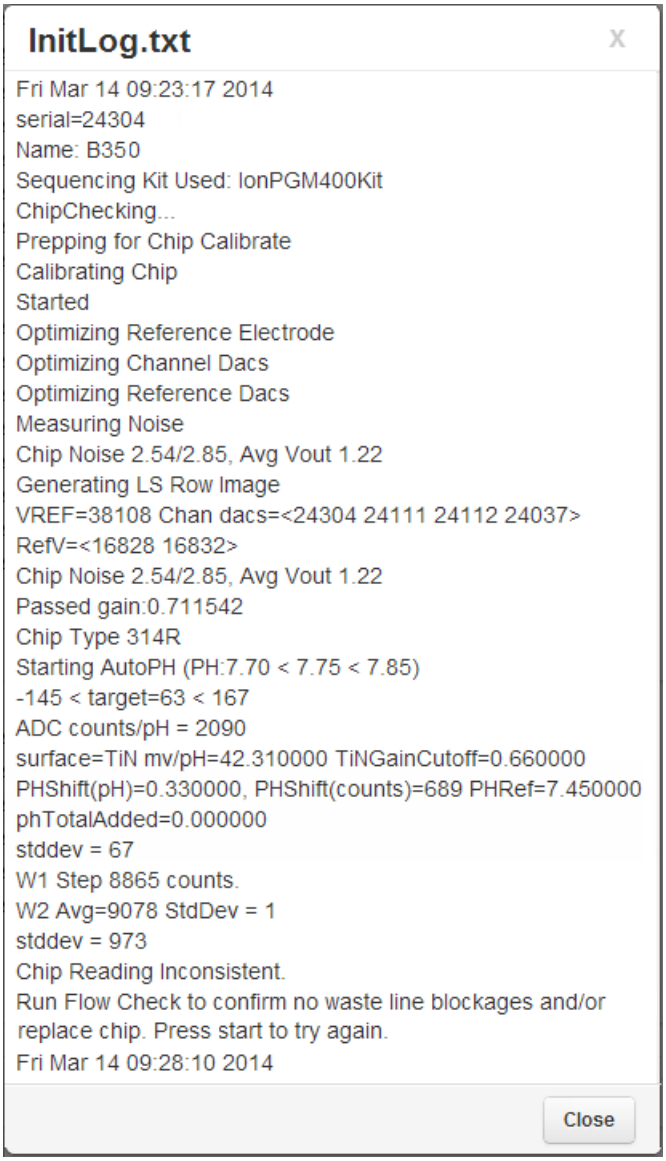
The **Instrument Diagnostics** page lists the sequencing instrumentsthat are associated with each of your results partitions. Passed and failed analysis runs are shown for each instrument.

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 286.

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.

Manage sequencer settings from Torrent Suite™ Software

Work with analysis files

Analysis results file location

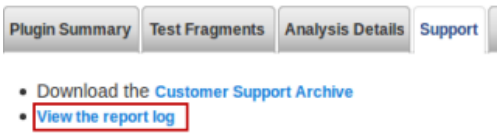
For a standard Torrent Server configuration, analysis results files are located in the following directories:

Type of Data	Directory Name
Raw	`/results/<Sequencer_name>/<Run_name>/`
Processed	`/results/analysis/output/Home/<Report_name>/`

Log files in the results folder

Many log files, which are shown in the following table, are generated for different parts of the Analysis pipeline. Some files only appear when a problem occurs. You do not need to log in to see these files. Opening a report and removing the report name gives you a directory listing of all the files, which you can open directly as text files. Be careful that you do not open a large file using the web browser.

Filename	Description
`version.txt`	Lists the versions of the Ion software packages that were installed at the time the report was generated and the host name of the server. This information is also displayed on the default report.
`DefaultTFs.conf`	Lists all of the Test Fragment Templates that were used for generating this report. If the file size is zero and there are no data in the file, either no templates are installed or none are flagged `isofficial`. Analysis only checks against the templates that are marked `isofficial`, which is set using the **Templates** tab in the browser.

Filename	Description
`uploadStatus`	Lists problems uploading data to the database. If analysis results are not being displayed in the browser, check this file. **Normal results:** Updating AnalysisAdding TF MetricsAdding Analysis MetricsAdding Library MetricsAdding Quality Metrics **Error examples:** Failed addAnalysisMetricsFailed addLibMetrics
`status.txt`	Analysis run status. If the analysis completed successfully, the contents of this file are a 1. A value of 0 indicates a failure occurred, requiring that you check other log files to determine the cause. No specific error information is provided in this file.
`processParameters.txt`	Run events and length. The command-line passed to the Analysis program is also included, which is useful to re-run the same analysis. These files are in subdirectories named `sigproc_results/block_*`.
`sigproc_results/sigproc.log basecaller_results/ basecaller.log alignment.log`	Analysis pipeline log files. Always check for errors in these files, especially the first and the last windows. The contents of these log files (without HTML formatting) are available in the Torrent Browser with the run report Support tab **View the report log** link: 
`drmaa_stdout.txt`	Post-analysis events.
`drmaa_stderr.txt`	Error messages related to processes called after the primary analysis. This has a value of zero if the analysis completed successfully.
`analyzeReads_err.txt`	Useful troubleshooting information generated during the alignment process. This file is only created when there is a problem.

Filename	Description
`core`	A memory dump listing, usually caused by a critical fault. You should see a related `exception` or `core dump` message in an analysis pipeline log file.
`alignmentQC_out.txt`	Errors related to TMAP. If the file is not present, it is likely that TMAP was not called.These files are in subdirectories named `basecaller_results/block_*`.

Standard reference file location

Standard reference files are stored in the following location:

```
/results/referenceLibrary/<index_type>/<genome_shortname>/
```

Design custom barcodes

Cautions

Custom barcode design involves certain technical challenges:

- Calculation of the your barcodes' hamming distances in flow space
- Adjustment of basecaller parameters to match your barcodes' distances

Custom barcode design is for advanced users only and only if you have a compelling need for a custom barcode set.

If are considering creating your own custom barcode set, we recommend that you first contact your FBS.

IMPORTANT: The default Basecaller parameter settings are optimized for the IonXpress barcode set. The use of a different barcode set, especially a custom barcode set, requires custom Basecaller parameter settings.

Barcode overview

The Torrent Suite™ Software supports barcoded runs, in which multiple barcoded samples are processed on the ION Chip during an Ion sequencing run. A barcode run typically involves sample-prep with an Ion barcode adapter kit (or compatible kit) such that two or more barcode adapters are present in a run. The user selects the barcode set in the run Planning tab of the Torrent Browser. This barcode set information is used during analysis to separate out reads by barcode, remove the barcode and adapters from the read, and output reads by barcode into separate BAM files. Reads are aligned against the reference genome, and results stored in BAM and BAM index (BAI) files for each barcode. Reads that can not be classified as being one of the barcodes in the designated set are grouped into a "no-match"' group, and alignment against the reference also performed on the no-match group.

Alignment metrics for each barcode are available in the Output Files section of the analysis run report. The run report shows Q20 performance metrics for all barcodes in the run, providing a quick glance at the high-level quality of each barcode. The

barcode section in the run report also shows the following metrics *for each barcode* in the run:

- The number of bases
- The number of bases at Q20 (or better) accuracy
- The number of mapped reads
- The mean read length
- A read length histogram

The Torrent Suite™ Software includes barcode sets for the latest available barcode kits. These barcode sets are selected in the run Planning tab. Advanced users optionally can add additional barcode sets in the References section of the Torrent Browser admin tab, either by uploading a CSV file of all barcodes or by manually adding each barcode.

Barcode set design considerations

Barcode sets are designed to efficiently separate reads from each other in the presence of errors. Ion Torrent™ sequencing technology produces raw data in flow space. These reads are best described as having a homopolymer run of length 0, 1, 2, etc., ... in flow 1, 2, 3, etc. Because of this characteristic, the most typical error patterns involve either over- or under-estimation of a homopolymer signal in a flow. The most effective barcodes designs for Ion Torrent™ technology are those with distinctive flow-space representations.

Hamming distance

One way of describing the separation of two sequences in flow space is by the hamming distance between them for relevant flows. Hamming distance is the number of flows in which the expected homopolymer length is different between the two sequences. For example, if two barcodes differ in 5 flows in flows 9-22, those two barcodes have hamming distance 5.

Hamming distance corresponds naturally to the ability to detect and correct errors. When two sequences have hamming distance 5, 2 errors can occur on one of the sequences and that sequence is still 3 errors away from the other sequence. Sequences separated by hamming distance 5 can tolerate 2 errors and still be classified correctly.

Ternary encoding

One side effect of operating in flow space is that barcodes are not limited to binary sequences. For example, each flow can correspond to 0, 1, or 2 bases in a ternary encoding scheme. This scheme allows for a greater number of codewords occupying the same number of flows. However, a flowspace representation must correspond to a legitimate sequence that yields these flow-space values. For example, we cannot have a flow of T, C, T with values 1, 0, 1. For a flow of T, C, T, both T bases are consumed in the first flow, and the sequencing reaction yields the incorrect values 2, 0, ?.

Ion Torrent™ barcode design

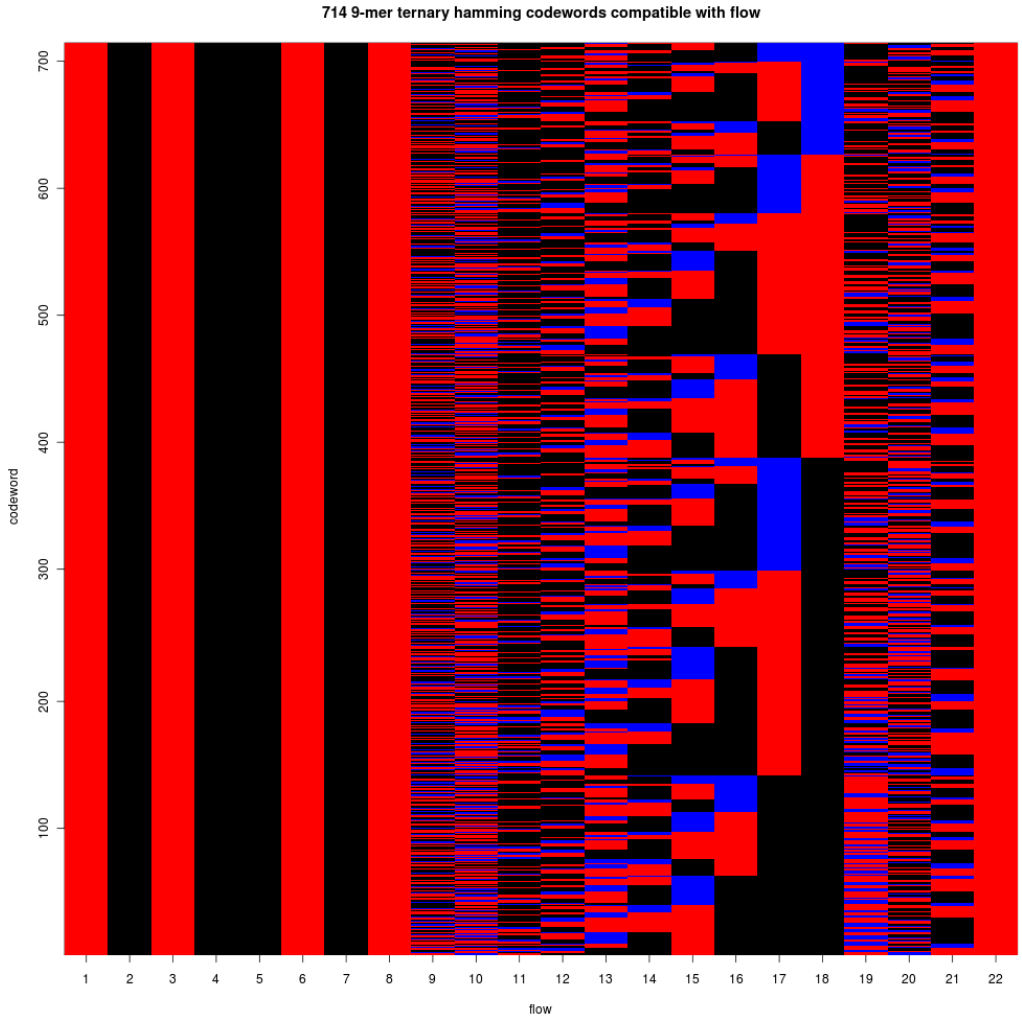
We designed Ion Torrent™ barcode sets to provide at least 1-error correction (hamming distance 3) in flow space for a large set of barcodes, and 2-error correction (hamming distance 5) for a usefully sized subset of such codes. This goal is accomplished by taking the ternary hamming code on 13 characters and assigning codewords to flows 9-22 to generate flow sequences (flows 1-8 are used for the library key and are not considered here). These flow sequences then have hamming distance 3 and are 1-error correcting. The codewords are further reduced by the constraint of requiring that they correspond to legitimate flow sequences. We also apply the constraint that the flow sequences must correspond to base sequences that are 9 to 11 bases in length. Finally, within the set that satisfies all these constraints, a subset is chosen (by greedy aggregation) such that any pair of flow sequences has hamming distance 5.

To insulate these sequences from the target sequences, a ligation adaptor CGAT is added. The ligation adaptor performs two functions. First, the C in flow 22 provides a synchronized flow that both marks the end of the barcodes and ensures that barcodes ending with "0" do not have sequence overwrite those flows. Secondly, this adapter mitigates any sequence-specific biases caused by the differing barcode sequences.

We provide a tool that classifies barcode reads by finding the flow-space representation of the read and comparing it to the flow-space representation of the barcodes. Classification standardly occurs after the last flow of the key (G), and continues to the end of the barcode sequence provided in flowspace. IonTorrent barcode sets are designed to be synchronous so that they all are classified using the same set of flows.

For flow space classification of custom barcodes, the barcodes should be designed to be compatible with the flow order, be synchronized at a final flow, and be well separated. However, the Torrent Suite™ Software attempts to classify any reasonable set of sequences that are separated in flow space. Many standard software packages

also classify usefully in sequence space, and have been found to work well with Ion Torrent™ data.



Scan your sequencing kit

The sequencing kit that you use affects the nucleotide flows on the Ion sequencer.

You can scan the sequencing kits for the Ion S5™, Ion PGM™, and Ion Proton™ instruments. You can also enter sequencing kit information in the Torrent Browser when you create a template or a planned run.

IMPORTANT! Use of the sequencing kit scanner is preferable for this procedure, because the scanner provides more detailed kit information that can be used for troubleshooting or other purposes.

The template wizard

Enter the sequence kit in the Torrent Browser template wizard, under the Kits step in the Workflow bar.

Use DNA barcodes with the Ion Torrent Sequencers

Overview

The Torrent Suite™ Software supports barcoded runs, which allow you to process multiple barcoded samples in a single run on the Ion S5™, Ion PGM™, or Ion Proton™ Sequencer.

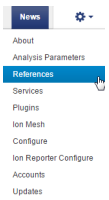
Your Torrent Suite™ Software comes pre-installed with several DNA barcode sets, including: Ion Code, ionSet1, ionXpress, ionXpressRNA, MuSeek_5prime_tag, and RNA_Barcode_None. These barcode sets are available for use on the Ion S5™, Ion PGM™, and Ion Proton™ Sequencers.

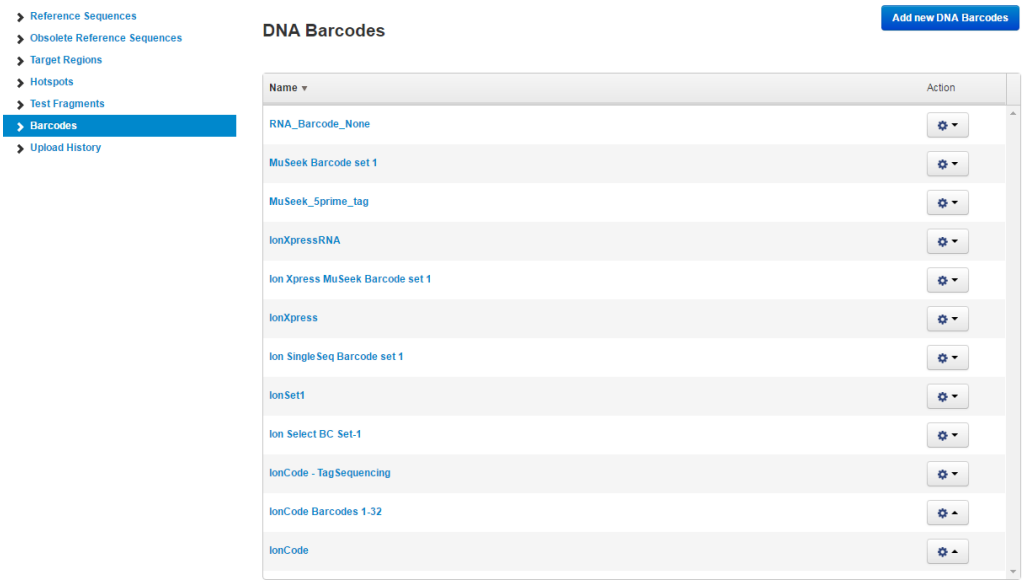
A barcode run on the Ion sequencer requires a sample-prep kit such as the IonSet1 or Ion Xpress barcode adapter kits. You select a DNA barcode adapter kit when you set up your Ion sequencer run. The barcode sequences for the IonCode, IonSet1, Ion Xpress, and Ion Xpress RNA barcode adapter kits are included with the Torrent Suite™ Software.

This barcode set information is used during analysis to separate out reads by barcode, to remove the barcode and adapters from the read, and to output reads by barcode into BAM files. Reads are aligned against the reference genome, and the results stored in BAM and BAM index (BAI) files for each barcode. Reads that can not be classified as being one of the barcodes in the designated set are grouped into a "no-match" group, and alignment against the reference also performed on this group. The new barcode results files are available in the run report File Links section.

Alignment metrics for each barcode are available in the run report page for the given run.

You can add additional DNA barcode sets by clicking **Settings (⚙) ▸ References**:





Workflow

The standard workflow for a barcoded sample is similar to a normal Ion S5™, Ion PGM™, or Ion Proton™ run and analysis. This section provides an overview of the workflow, with the new steps involved on a barcoded run.

Summary of the recommended workflow

Here is an overview of the recommended workflow for a barcode run. Screenshots and more details are provided below.

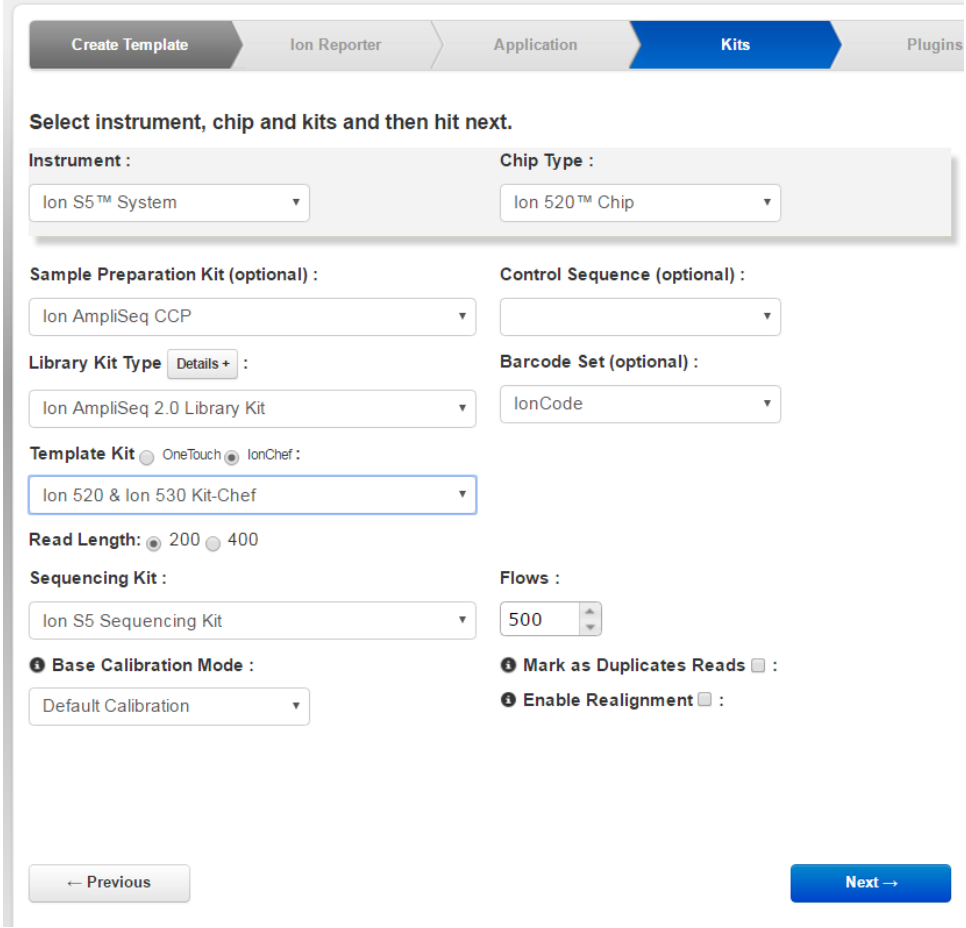
1. Create a template for your runs in the Plan tab Template page. In the template wizard Kits page, select one of the available barcode sets from the drop-down Barcode Sets menu, and fill out the other run information. Save your template.
2. When you have the actual sample name, click the **Plan Run** button for your template. Enter you run name and sample name, then click **Plan**.
3. The Torrent Suite™ Software assigns a name to your planned run, and generates a 5-character code for your planned run name. Your run information is stored in the Torrent Suite™ Software as a planned run until you are ready to start the run on the sequencer.
4. When you are ready to start the run, on the Ion S5™ Run Selection screen you select your run from a list of planned runs. Torrent Suite™ Software populates the Ion S5™ Detail screen with the information you entered in the Planning tab. (You may optionally change information on the Run Info screen.)
5. You start the Ion S5™ sequencer run as usual.
6. When the run and report are complete, you can review the performance of the barcoded reads in the default report page. The following additional barcode-specific files are available for download from the File Links download section:
   - A zip of BAM and BAM index (BAI) files for each barcode
   - A csv-style spreadsheet summarizing the barcode performance for each barcode

Set up a barcode run in a template

The same steps apply to a planned run (which is created from a template).

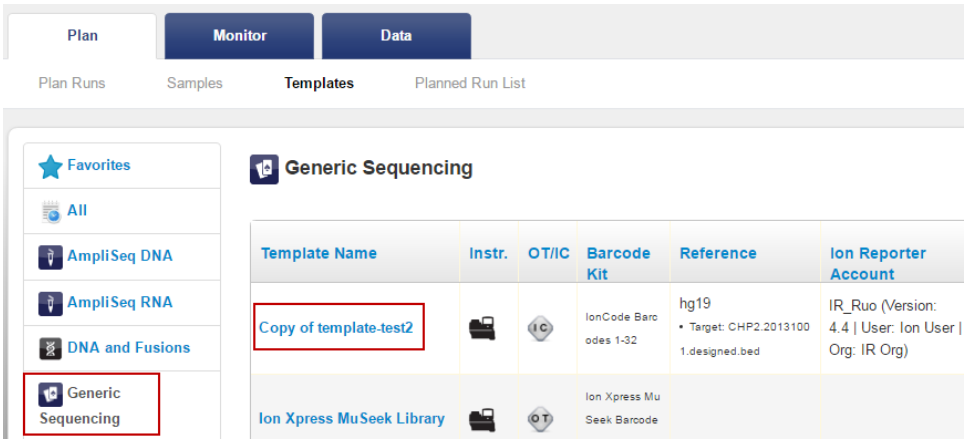
Follow these steps to set up a barcoded run in a template:

1. Click **Plan** ▸ **Template**, then click **Add New Template** for the application group appropriate to your experiment.
   The Template wizard opens.
2. Select the correct application group and click **Next**.
3. On the Kits page, click the Barcode Set menu. Select the barcode set that corresponds to your barcode kit.

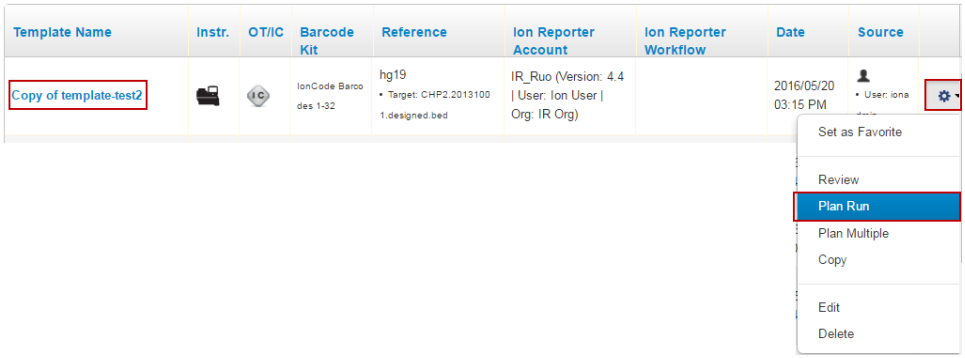


4. Click **Next** and complete the rest of the wizard. On the last page, click **Save**.

5. Your new template appears in the **Plan** ▸ **Templates** page, in the application group you selected.

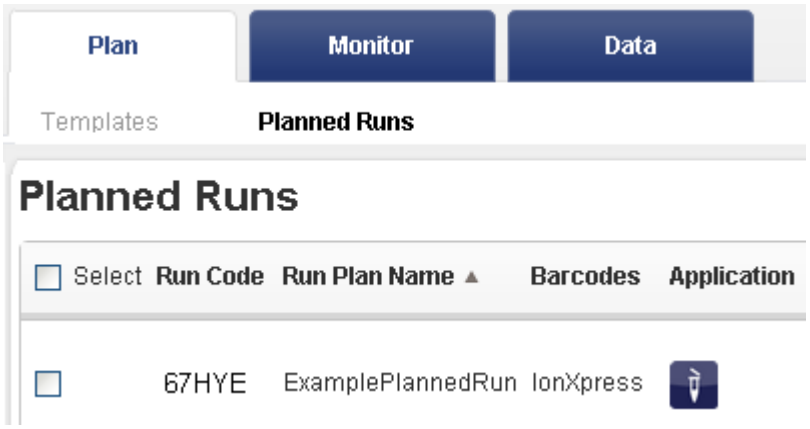


6. To run on the Ion sequencing instrument, create a planned run from your new template. Click **Settings (⚙)** ▸ **Plan Run** for the template you just created.



7. The planned run wizard opens, in the wizard Plan page. Enter a descriptive run name and enter the sample name for each barcode you want to use click **Plan Run** to save and finish.

8. The Planned Runs page opens with your planned run at (or near) the top of the table:



The Torrent Browser assigns a short code name to your planned run. The example short code here is `67HYE`

.

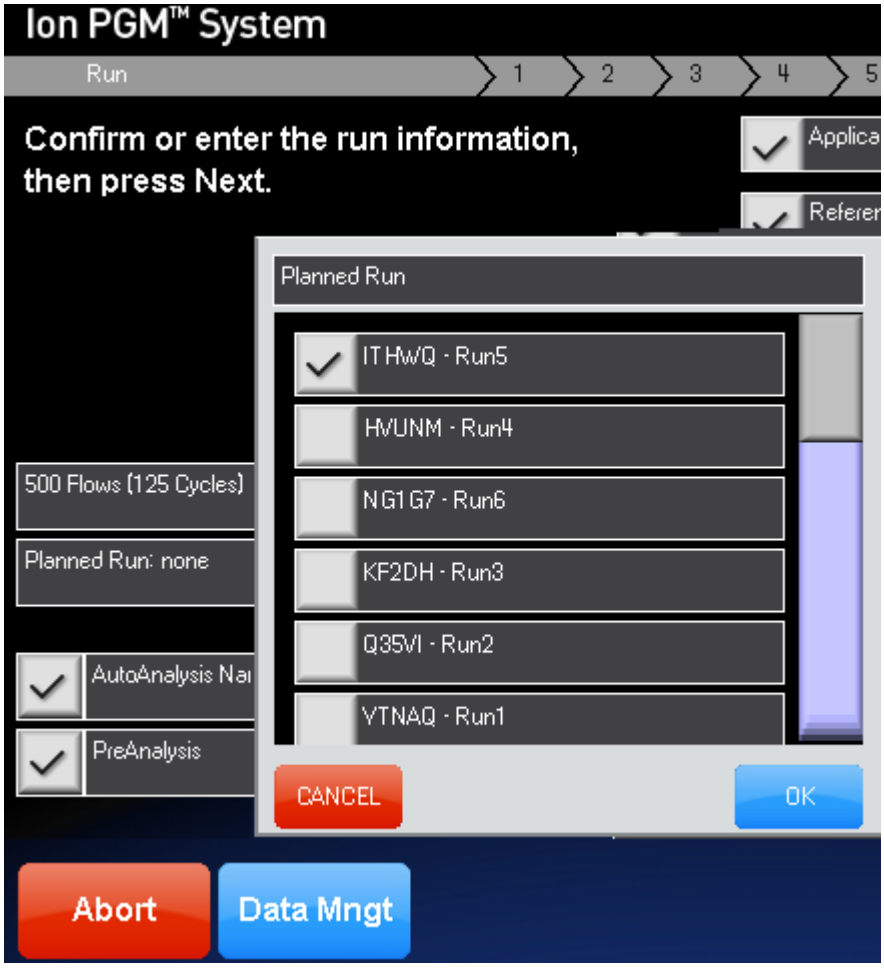
Start your planned run on the Ion sequencer

This section describes how to go from a planned run to an actual run on the Ion PGM™ or Ion Proton™ Sequencer. You must first create a planned run, as described in Set up a barcode run in a template before using the instructions in this section.

1. Open the Run Info screen on the Ion PGM™ Sequencer.

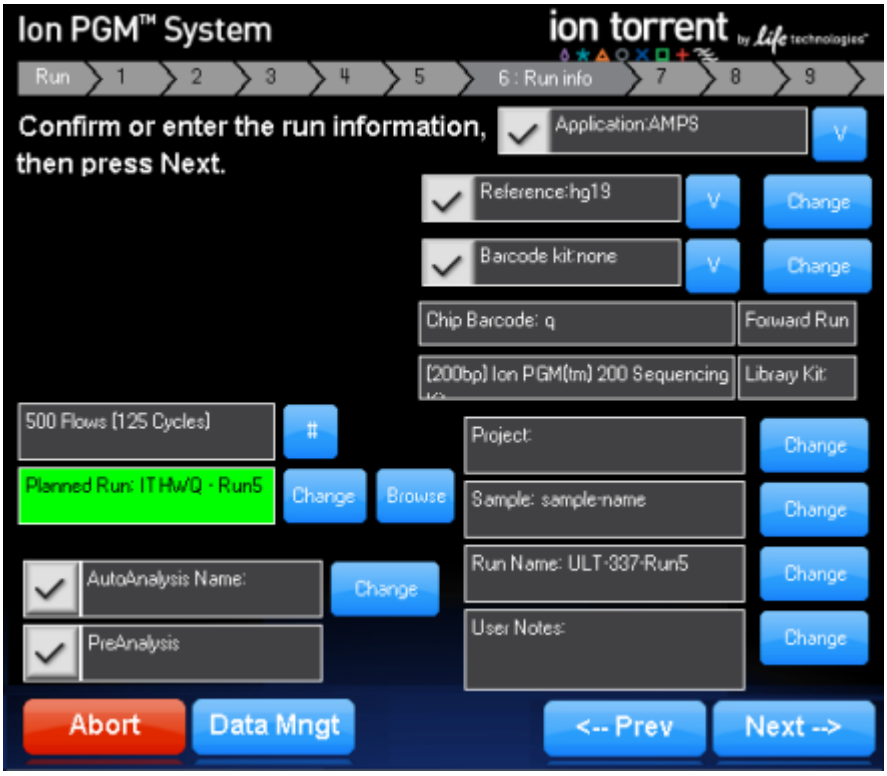
2. Click on the Browse button (near the middle of the screen, to the right of the Planned Run field).

3. The Planned Run pop-up opens with a list of available planned runs. Your planned run is identified by short code and plan name (as listed under the Plan tab). Select your run and click **OK**.



Your selection appears in the Planned Run field:

The Ion PGM™ Sequencer Run Info fields, including your barcode set, are populated with information from your planned run.



If required, you can manually update any Run Info fields now.

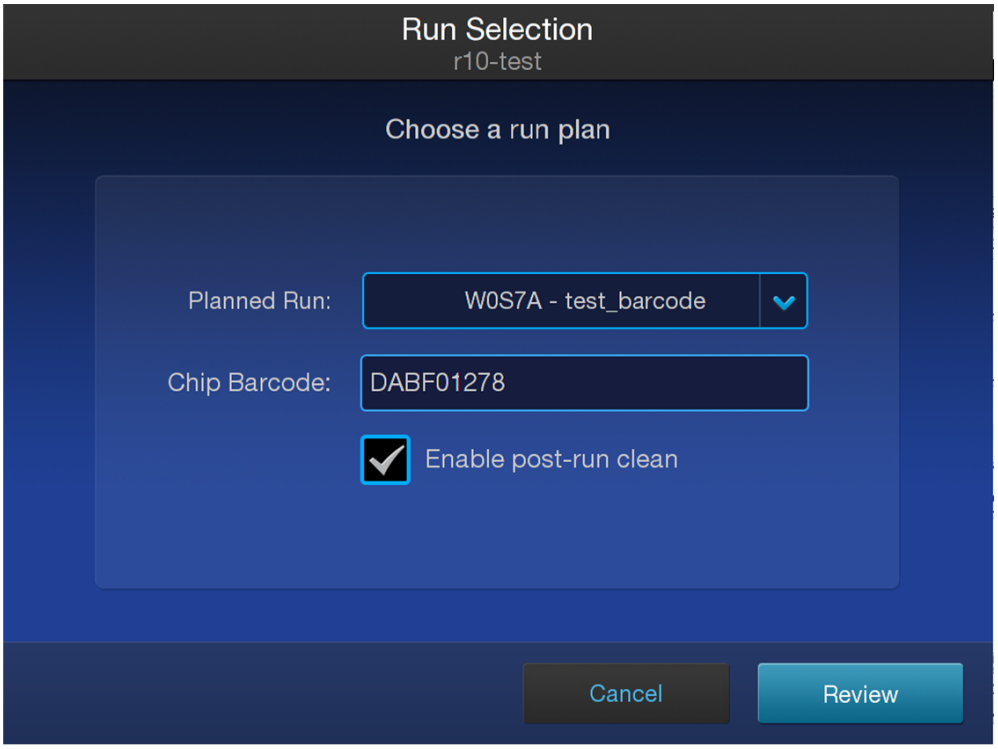
4. Click **Next -->** to start your Ion PGM™ Sequencer run, as usual. Approve your run on the confirmation screen.

IMPORTANT! When you accept the confirmation screen, your planned run information is deleted from the Data tab Planned Runs page. If you terminate your Ion PGM™ Sequencer run and at a later time want to start the run, you must either enter the run information on the Ion PGM™ Sequencer Run Info screen or re-create the planned run again under the Torrent Browser Planning tab. The new planned run has a different short code.

Start your planned run on the Ion S5™ or Ion S5™ XL sequencer

To initiate a plan on the Ion S5™ or Ion S5™ XLsequencer:

Select the appropriate plan when you are setting up the run on the sequencer.



Other methods to import your planned run

This section describes the ways to import your planned run information into the Ion PGM™ Sequencer Run Info screen. These are all done on the Ion PGM™ Sequencer Run Info screen, and are all different ways to populate theIon PGM™ Sequencer Run Info screen with the run information previously entered in the Planning tab. Choose the method which best fits your work environment.

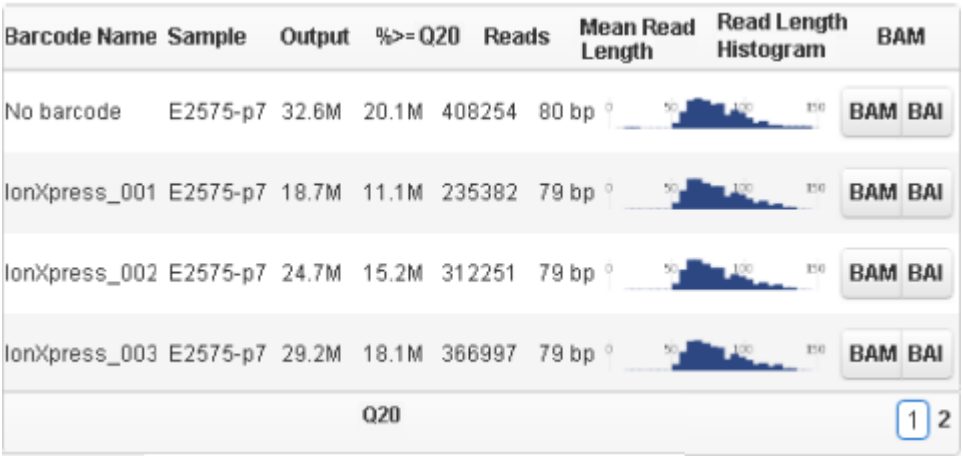
Planned run run code

You can type the run code for your planned run into the **Planned Run:** text field. An example run code is ITHWQ.

A run code is assigned to your planned run when you enter the run information in the **Plan > Template** page planned run wizard and is listed in the **Plan > Planned Runs** page.

Barcode reports and output files

This section describes output and reports for barcode runs. The barcode reports section appears at the top of a run report for a barcode run and shows key performance metrics for each barcode in the run. The category named "No barcode" contains barcodes that could not be matched to known members of the barcode set being used.



Barcode Name	Sample	Output	%>= Q20	Reads	Mean Read Length	Read Length Histogram	BAM
No barcode	E2575-p7	32.6M	20.1M	408254	80 bp		BAM BAI
IonXpress_001	E2575-p7	18.7M	11.1M	235382	79 bp		BAM BAI
IonXpress_002	E2575-p7	24.7M	15.2M	312251	79 bp		BAM BAI
IonXpress_003	E2575-p7	29.2M	18.1M	366997	79 bp		BAM BAI

Q20 1 2

The BAM and BAI links in the barcode report download files for only that barcode.

The Output Files section of the Torrent Browser run report includes barcode-related results files available for download. The links in the Barcodes row download compresses files of all barcodes for the run. The data in the Reads column are before alignment.

File Type	Description
Barcode-specific Library Alignments (BAM and BAM Index)	Binary Sequence Alignment/Map (BAM), is a compressed, binary form of the SAM file. The BAM index (BAI) file speeds up the access time for a coordinate-sorted BAM file. The BAM and BAI files for each barcode are added to a single compressed file.

IMPORTANT! The FASTQ file format is not produced by the default analysis pipeline.

Plugin Support for Barcodes

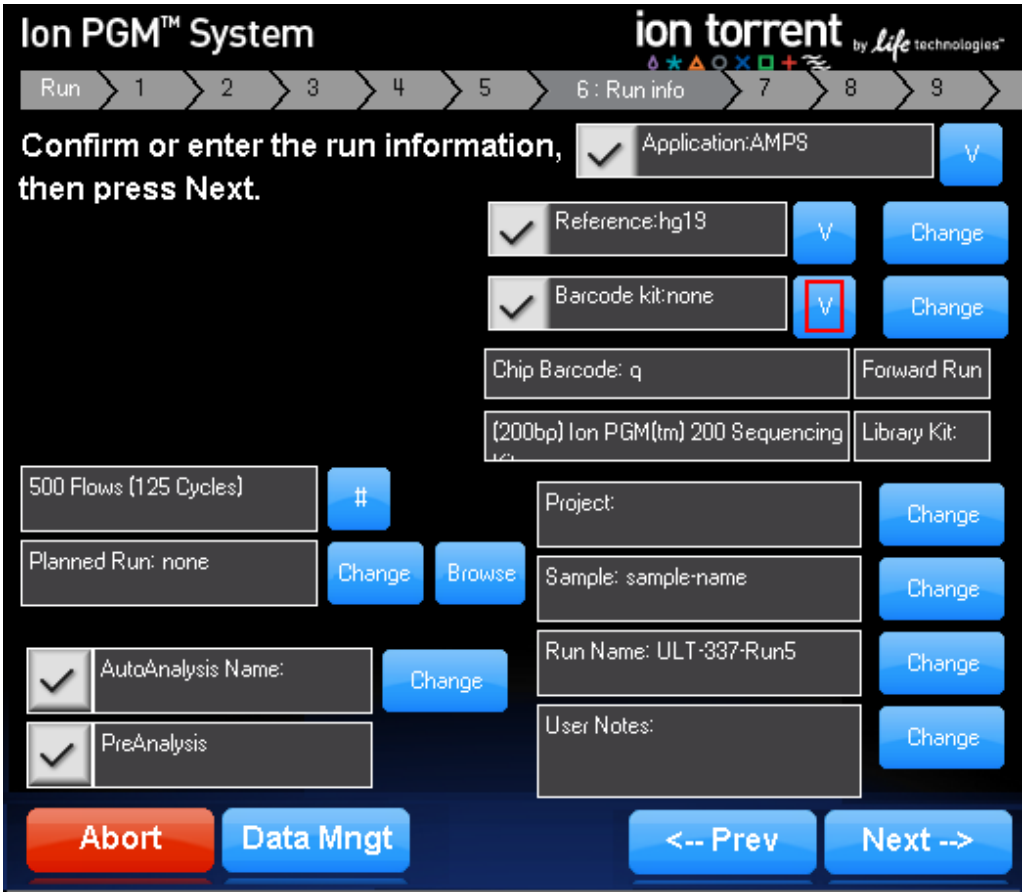
The following plugins supports barcode libraries:

- Coverage Analysis
- Torrent Variant Caller

Reference library and barcode

On the Ion PGM™ or Ion Proton™ instrument, during a run, you can enter information about the experiment, or run, on **Run Info** screen. The Ion PGM™ or Ion Proton™ instrument gets the lists of reference library and barcode set from the Torrent Browser. The information is queried in real time.

For example, while at this **Run Info** screen on theIon PGM™ or Ion Proton™ instrument, you realize the reference library has not been added on Torrent Browser. Click **Settings (⚙) ▸ Reference** and add a new reference library. Back at the sequencing instrument, you see the new reference library when pressing the drop-down menu (in red below, shown on an Ion PGM™ instrument):

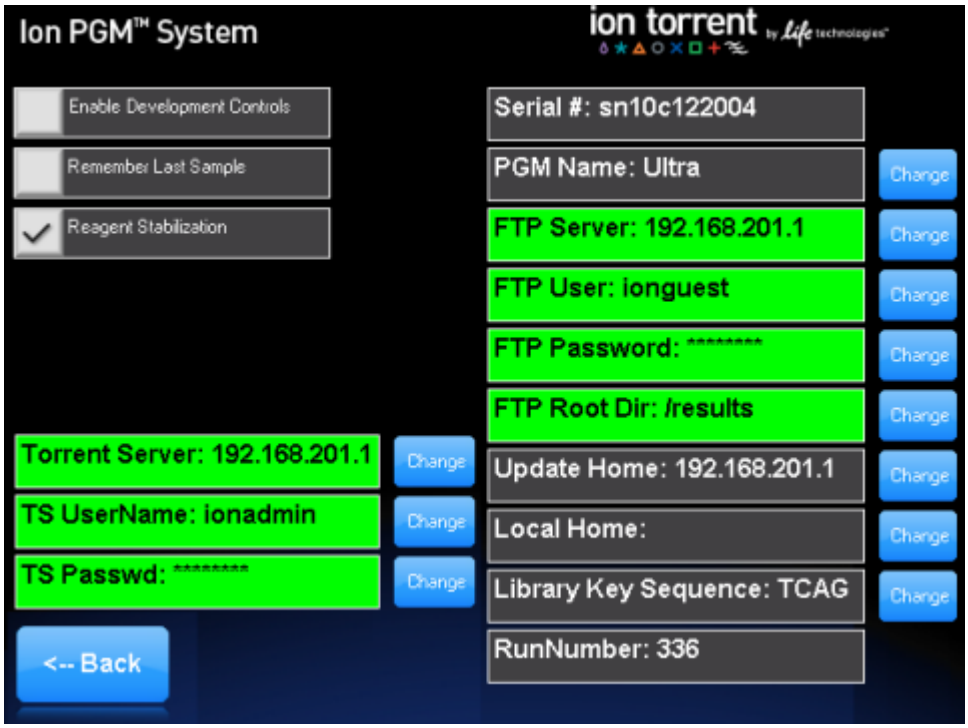


Connect the sequencer instrument to the Torrent Server

This section uses the Ion PGM™ Sequencer as an example of how to connect an Ion sequencer to the Torrent Server.

On the Ion PGM™ Sequencer Advanced screen, you can set Torrent Server login information, for example, server address (**Torrent Server**), username (**TS UserName**), and password (**TS Passwd**), to connect to the Torrent Server. The **Torrent Server** field turns green to indicate that the login information is correct.

IMPORTANT! The Ion PGM™ Sequencer uses the Torrent Browser API to communicate with Torrent Suite™ Software. The username and password are the ones used to log on to Torrent Browser. The Torrent Server `ssh` login can be different from Torrent Browser login.



Default settings for experiments

Default settings for experiments can be accessed and modified through Torrent Browser, provided you Torrent Suite™ Software administrator privileges. However, any changes made to these settings will affect all subsequent sequencing runs, or might lead you inadvertently corrupt a database or permanently delete experiment data. Therefore, such changes should only be made under special circumstances by administrators who are knowledgeable about the potential such consequences.

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.

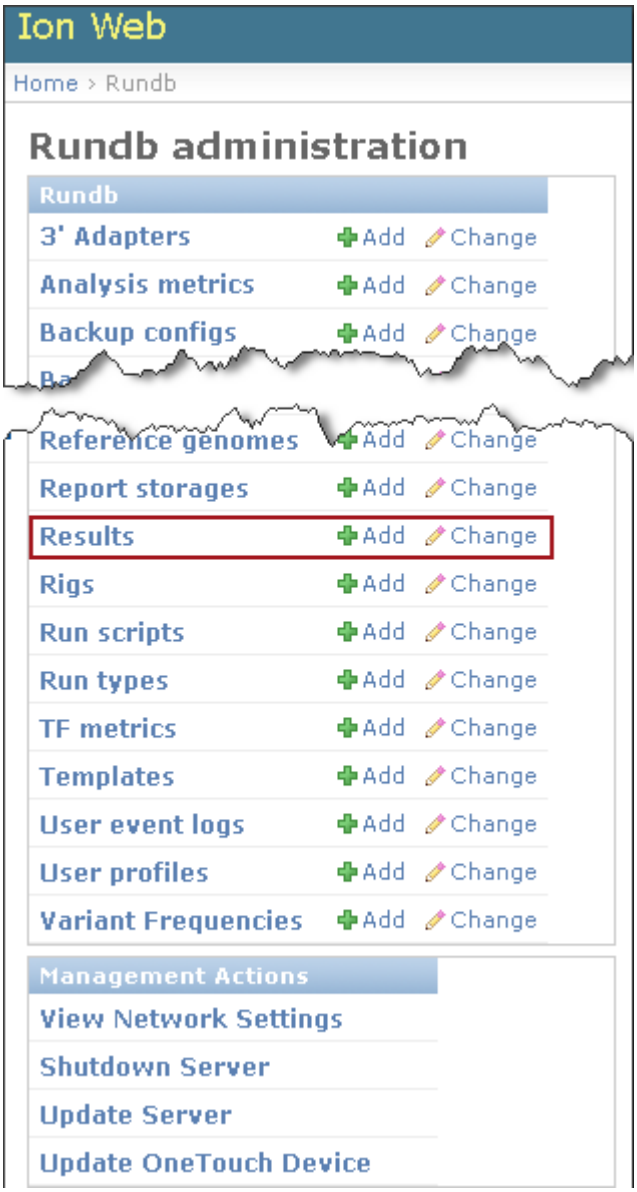
Change the report name

If you manually started an analysis and realize that you typed the report name incorrectly, you can change the report name using the following procedure. These steps require admin login.

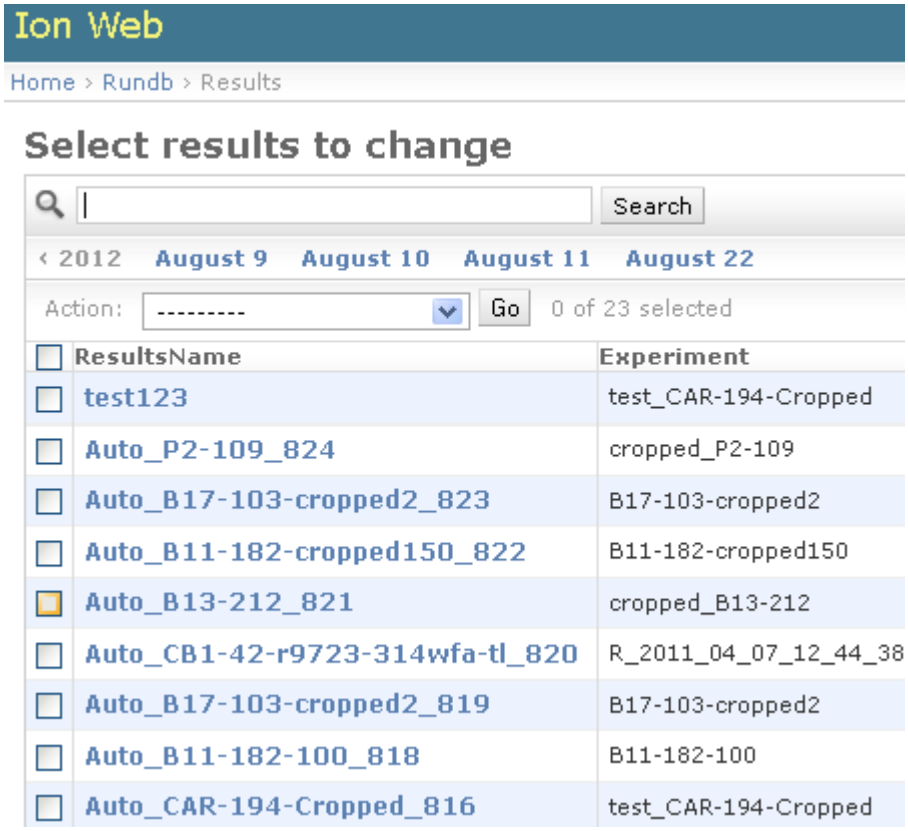
IMPORTANT! It is not safe to change the report name while the report is being processed.

---

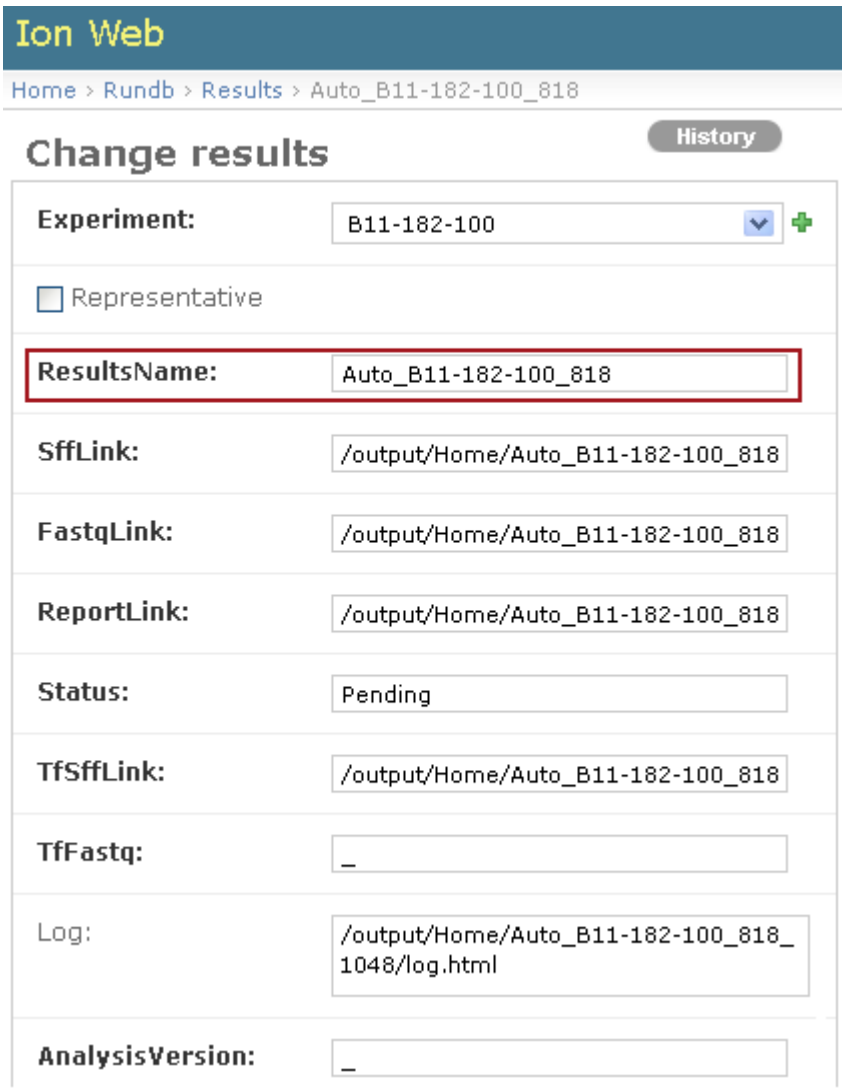
1. Select the **Results** dialog.



2. On the **Select results to change** page, click the name of the run you want to change, in the **ResultsName** column:



3. Enter the new report name in the **ResultsName** field:



4. Click **Save** (on the bottom right) to save your change.

Change the run date

Occasionally, the Ion PGM™ or Ion Proton™ Sequencer cannot get a date/time from the internet time server. When this occurs, the sequencer date is set to January 1, 1969.

The date of the run is encoded in the folder name, which is parsed and used as the **Run Date** in the database. This causes the new run to be displayed with the incorrect date. With a date of January 1, 1969, the run is the last item on the last page of run reports listings in the **Data** tab.

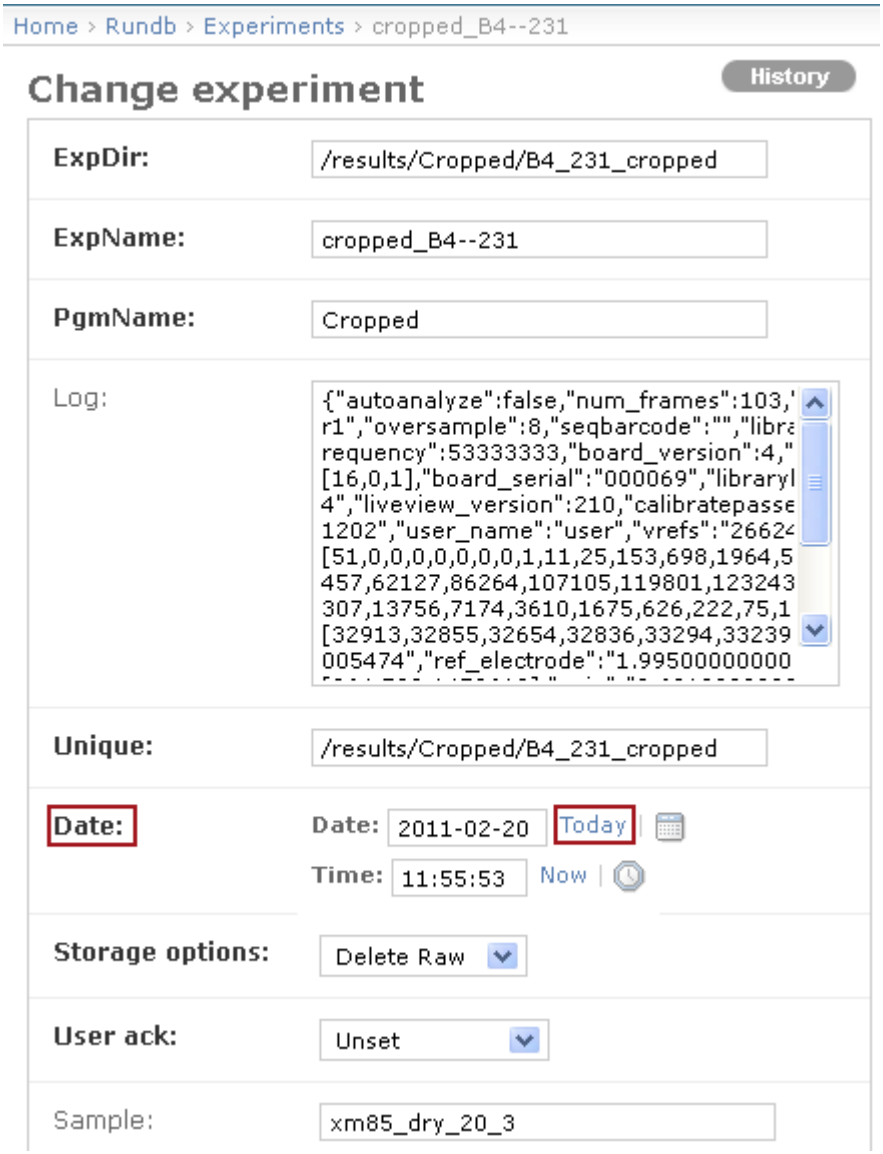
Use the following procedure to change the date for this run:

1. In the Torrent Browser **Config** tab, click **Admin Interface** and login, if prompted.

2. Click to open the **Experiments** database item for modification:

3. Find your run in the experiment name list. The list is sorted by date, starting with the newest runs in the database. Because the run from 1969 is at or near the end of the list, it is convenient to re-sort by date, in ascending order (oldest at top). Re-sort by clicking the **Date** column heading:

4. Click the **ExpName** for your run to select it and display the following run information:



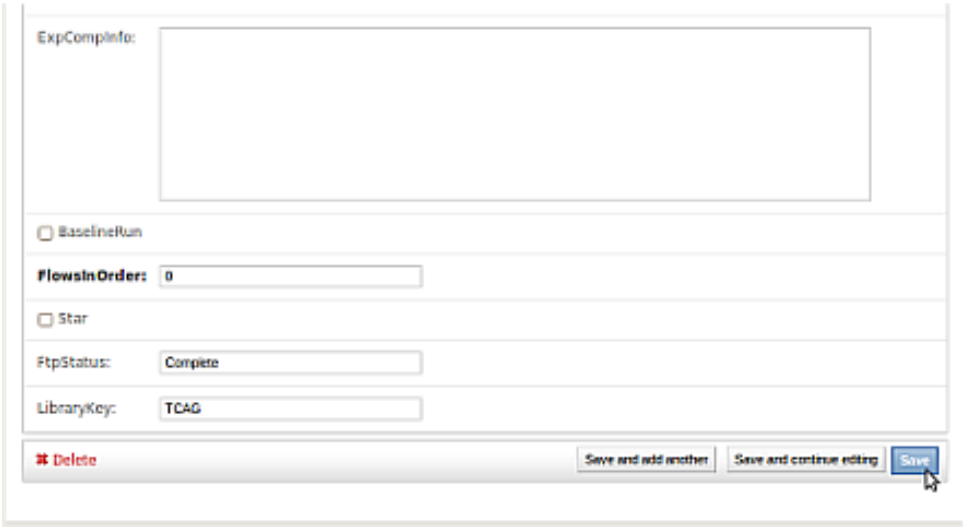
5. Use one of the following two options to change the date: a) Click the **Today** and **Now** buttons to set the **Date** and **Time** values to the current date and time in one click.

IMPORTANT! The automatic method is recommended because it places this run at the top of the run report lists, in both the **Data > Completed Runs & Reports** tab and the **Data > Projects > projectname** tabs.

---

b) Manually edit the date/time strings.

6. Click **Save**, on the bottom right to save the new date:



7. Return to the **Data** tab when done.

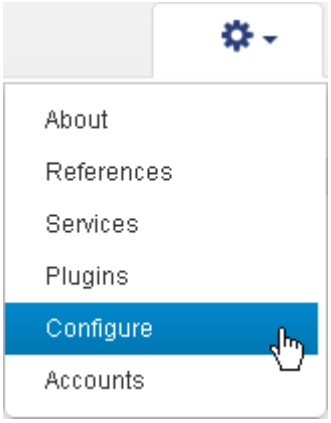
Update the Ion Ion OneTouch™ Device

IMPORTANT! These procedures require your `ionadmin` account. (Do not use your `ionuser` account.)

This procedure requires actions on both the Ion OneTouch™ sequencing instrument and in the Torrent Browser.

Follow these steps to update the Ion OneTouch™ instrument software:

1. Connect the Ion OneTouch™ device and the Torrent Server with an Ethernet connection.
2. Sign in to Torrent Browser with an administrator (`ionadmin`) account.
3. Click **Settings (⚙) ▸ Plugins**, in the Torrent Browser (near the top right).



4. Get the updated IP address of the Ion OneTouch™ device. Follow *either one* of the following steps: As `ionadmin`, in the Torrent Browser Config tab Management Actions section, click the link **Update OneTouch Device**.
   - Power cycle the Ion OneTouch™ device, or
   - Wait for the IP address to update (takes one or two minutes).To check for the IP address, press the **About** button on the Ion OneTouch™ device.

     IMPORTANT! This page does not refresh. To refresh, go to a different screen and then go back.

   More than one update may appear for the optional download.
5. Click **Update**.
   On the Ion OneTouch™ device, a splash screen appears with update progress.
6. After update is complete, the Ion OneTouch™ device reboots itself.

Update Ion Chef™ scripts

Ion Chef™ scripts can be updated between software releases and you can elect to update them. When an Ion Chef™ script is updated, you will see an announcement at the top of your Torrent Suite™ screen.

1. Click on the new Ion Chef™ script announcement and click **Upgrade**.
   The system installs the new script.
2. If you find you need to revert back to the old script, click **Revert**.
3. Next, upgrade the Ion Chef™ instrument.

Handle a failed analysis run

If an analysis run fails, determine the cause of the failure and, possibly, restart the run.

Determine the fault cause

If an analysis run fails, make the following checks:

1. Has the Ion PGM™ or Ion Proton™ Sequencer completely transferred the data for the run? Go to the sequencer Data Management screen to ensure complete data transfer. If you are not sure the data was transmitted. you can retransfer it.
2. In the **Data** tab, click **Completed Runs & Reports tab** to ensure that the file transfer was complete. Also, check if there are any error messages, such as **User Aborted**. Look for a status of Error or Pending.
3. If the report was generated, check if there are any messages on the report itself.
4. Click the **Support** link towards the bottom of the run report (above the **Plugin Summary** row of buttons). Click **View the Report Log** or **Download the Customer Support Archive**. You can send the customer support archive to your Ion Torrent™ contact for review.
5. If you cannot determine the cause of the fault, try restarting the run.

Screen descriptions

Planned Runs screen

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"Create a planned run with AmpliSeq™ DNA template" on page 26	"Plan Tab" on page 394
"Plan by sample set" on page 33	"Templates " on page 391
"Create multiple planned non-barcoded planned runs" on page 37	"Wizard Plan or Save step in the Workflow bar" on page 387
"Create a Planned Run with DNA and Fusions templates" on page 27	
"Plan a run using Generic Sequencing template" on page 29	
"Plan a run using Ion 16S™ Target Sequencing template" on page 113	
"Copy a template" on page 29	
"Create a template with Ion AmpliSeq.com Import" on page 31	

Samples screen

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"Create sample sets manually" on page 17	"Sample attributes" on page 19
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"Plan a run using Generic Sequencing template" on page 29	"Wizard Plan or Save step in the Workflow bar" on page 387
"Plan a run using Ion 16S™ Target Sequencing template" on page 113	
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Planned Run List screen

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	"Example Planned Runs page" on page 401

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	"Monitor the sequencing run" on page 60
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"Review the planned run settings" on page 80	"Key signal" on page 88
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Barcoded libraries

This appendix describes how to create and select barcode sets in the software for sequencing barcoded libraries.

Pre-installed barcode sets

Torrent Suite™ Software includes pre-installed barcode sets such as “IonXpress”, “IonXpressRNA” and "IonCode".

When setting up a Planned Run or performing a run, select the appropriate barcode set for your library type as follows:

- **DNA libraries:** Select the **IonXpress** barcode set, which includes all barcodes in the Ion Xpress™ Barcode Adapters 1–96 Kits, or the **IonCode** barcode set, which includes the 384 barcodes in the IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751).
- **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select the **IonXpressRNA** barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01–16 Kit (Cat. No. 4475485).

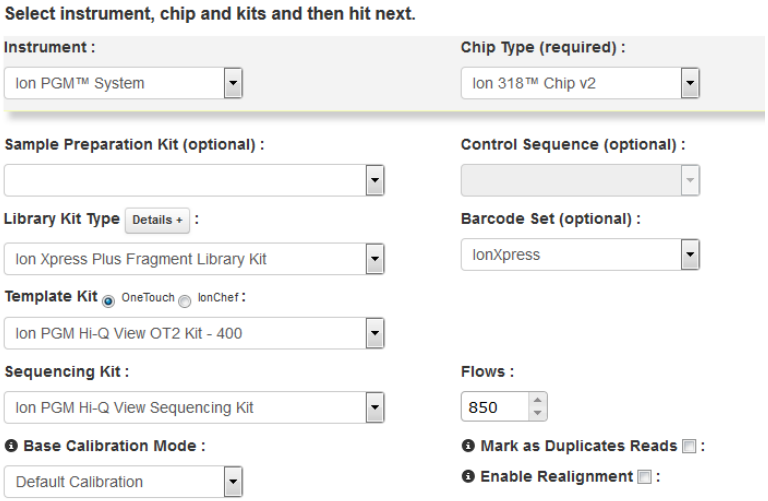
If you are not using barcodes:

- **DNA libraries:** Leave the Barcode field blank.
- **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select **RNA_Barcode_None** from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.

IMPORTANT! Do not edit, delete, or modify the pre-installed barcode sets.

Select a barcode set for a sequencing run

Select the barcode set in the Torrent Browser when planning the run.



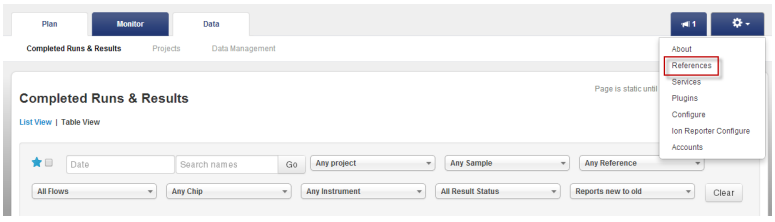
Custom barcode sets

You can create custom sets of barcodes as **comma-separated value (.csv) files**, then load these sets onto the Torrent Server for use during sequencing runs.

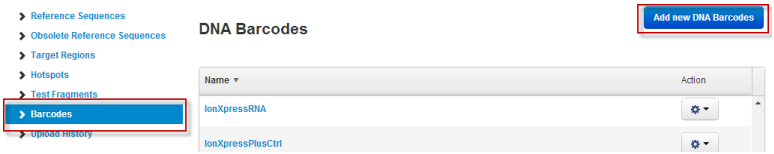
To access the Torrent Server, you must have a username and password. For more information on working with custom barcode sets, see the *Torrent Suite™ Software Help*.

Create and add a custom barcode set on the Torrent Server

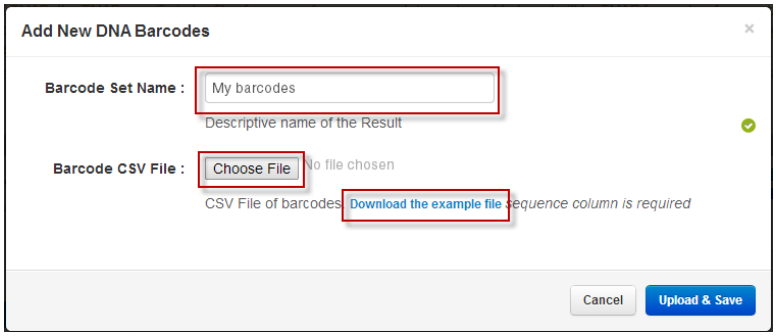
1. Create a comma-separated variable (CSV) text file for your custom barcode set. The CSV file can contain up to 384 barcodes.
2. To add the file to the Torrent Server, open the software and click **Settings** (⚙) ▸ **References**.



3. In the left navigation bar, select **Barcodes**.
4. Click the **Add new DNA Barcodes** button.



5. In the popup dialog, click on the **Download the example file** link for an example file showing the correct CSV format. Edit your own CSV barcode list to match this format, and save the CSV file on your computer.



6. Enter the **Barcode Set Name** and click on **Choose File** to select your formatted barcode CSV file. Then click **Upload & Save**.

7. The barcode set file name is displayed in the list.

Other barcode set operations

View a barcode set

1. To view a barcode set, go to the Torrent Browser and click the **References** tab.

2. Scroll down to the Barcodes section and click on the barcode set name to display the list of barcodes in the set.

Delete a custom barcode set from the Torrent Server

1. To view the barcode set names, click the **References** tab in the Torrent Browser.

2. Scroll down to the Barcodes section and click the name of the barcode set that you want to delete.

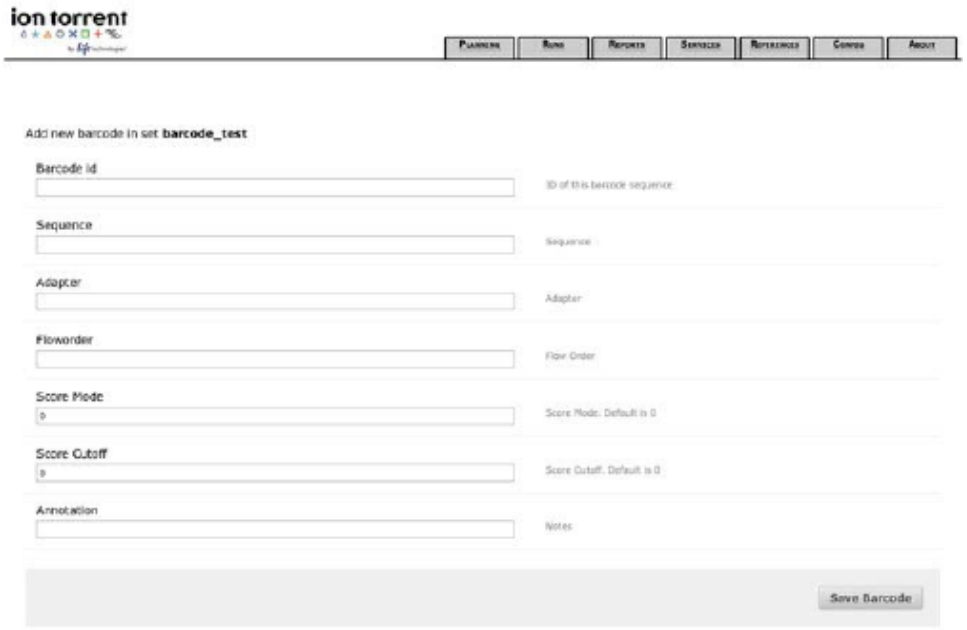
3. In the barcode set page, click **+ Delete Barcode Set** then click Yes to confirm the deletion.

Add a barcode to a custom barcode set

1. Open the Torrent Browser and click the **References** tab.

2. Scroll down to the Barcodes section and click the name of the barcode set to be edited.

3. Click **+ Add Barcode.** You see the new barcode window:



4. Complete the fields, then click **Save Barcode**.

Edit or delete a barcode from a set

1. Open the Torrent Browser and click the **Settings** button on the right side of the window, then select **References**.
2. In the Barcodes panel, click the file name of the barcode set to be edited.
3. Click the button under Action to edit or delete the panel.
   - To edit a barcode, change the barcode in the edit window, then click **Save Barcode**.
   - To *delete* a barcode from a set, click **Delete Barcode**, then click **Yes** to confirm the deletion.

Reference

Wizard Plan or Save step in the Workflow bar

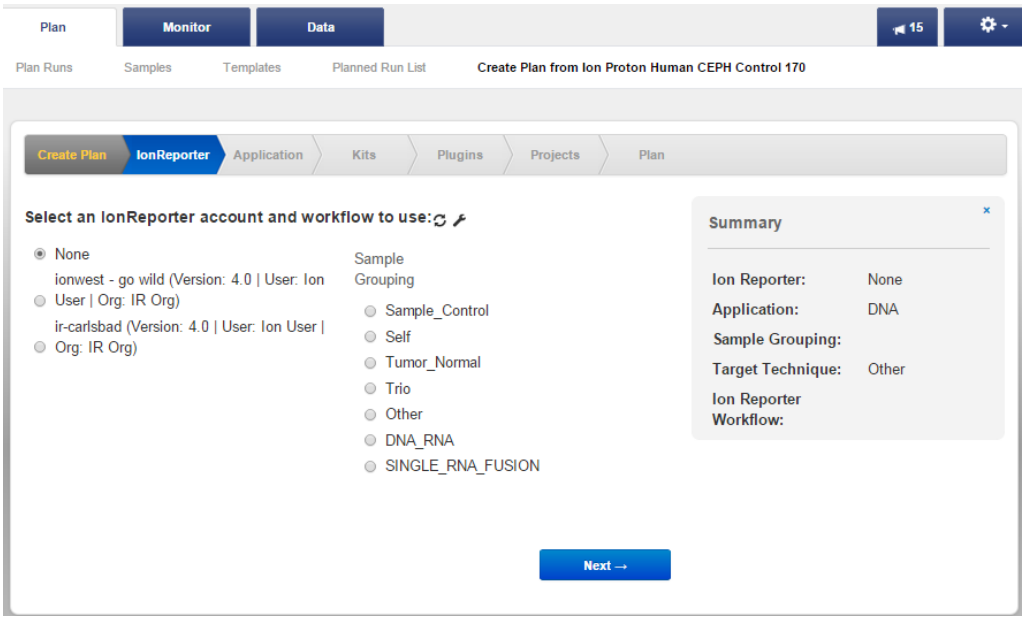
The title for last step in the Workflow bar of the wizard is different for templates, run plans, and run plans that are planned by sample set:

- **Templates** The step in the Workflow bar is Save. Here you enter the new template name and optionally mark it as a favorite.
- **Run plans** The step in the Workflow bar is Plan. Here you enter the new run plan name and sample information.
- **Plan by sample set** The step in the Workflow bar is Save & Finish. Here you enter the new run plan name. (Sample information is automatically entered into the Barcoding step in the Workflow bar.)

Note: Templates that are marked as favorites are listed in their own section at the top of the Templates tab.

Wizard Ion Reporter Page

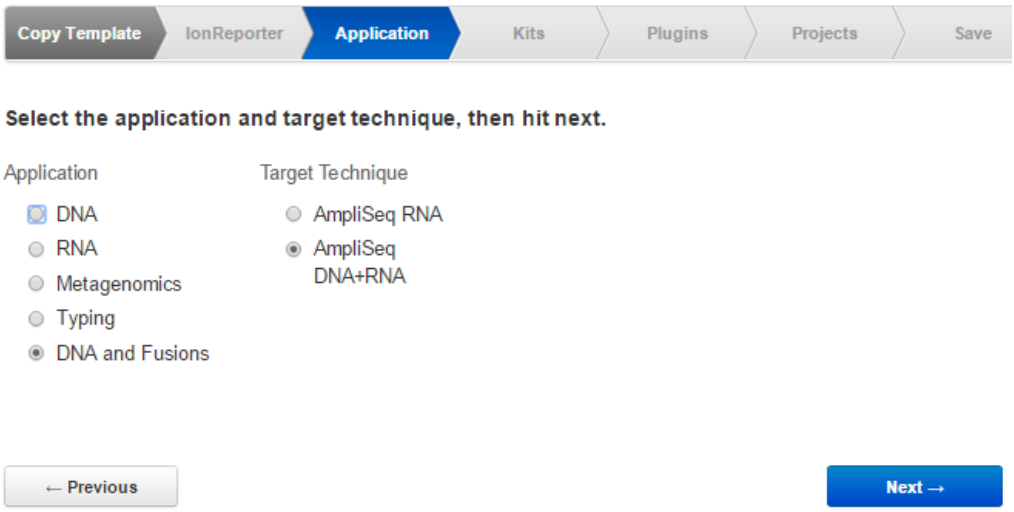
Ion Reporter is the first page in the Torrent Browser run template wizard. When you select an Ion Reporter account in this page, features related to Ion Reporter™ Software appear in the other wizard pages.



These selections on this page are only for Ion Reporter™ Software users.

Wizard Application Page

In the Application page you select your experiment type:



Based on the information that you specify here, the Kits page is set with the appropriate selections.

Notes about the Application choices:

- Metagenomics is reserved for future use with Ion Reporter™ Software.
- Typing is used for molecular fingerprinting to detect single strains of viral or bacteria for research purposes.

Wizard Kits Page

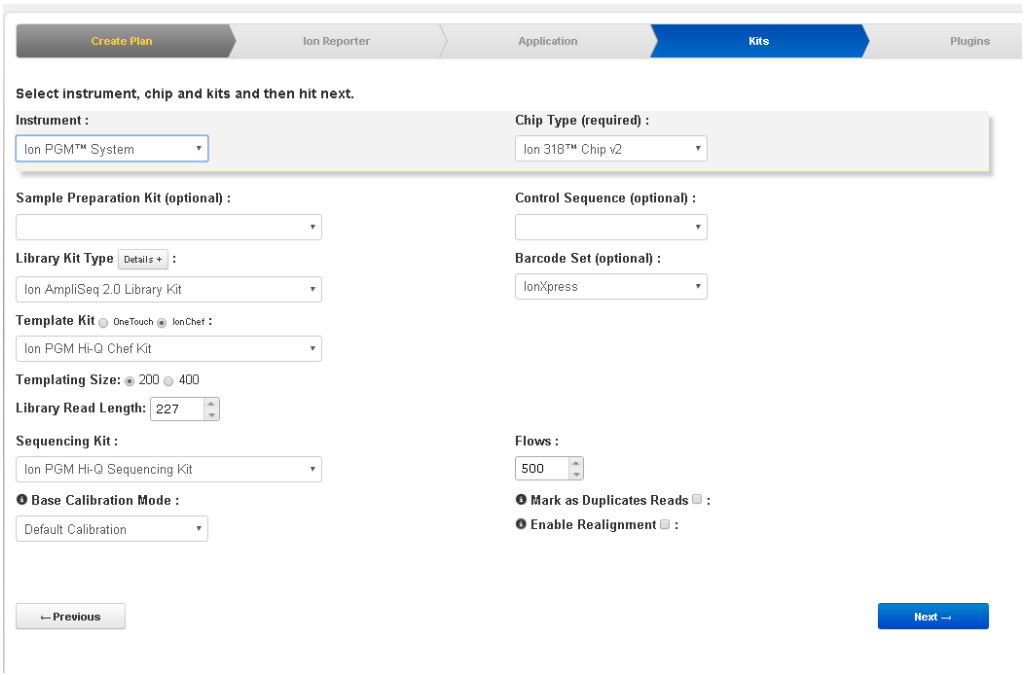
On the Kits wizard page, enter the following information about laboratory kits and other sequencing parameters:

- (*Optional*) Sample preparation kit
- Library kit type, including the forward library key and the forward 3' adapter
- Templating kit type
- Sequence kit
- Number of flows
- Barcode set ***Required*** for barcoded runs
- Base calibration mode
- Control sequence ***Required*** for RNA runs
- Chip type ***Required***
- Mark PCR Duplicates Not recommended for Ion AmpliSeq™ data

Chip type is required. As with all fields, if you enter chip type in your templates, then it is automatically entered in your run plans.

New in version 5.2, smart filtering is enabled on the Kits screen. When you select an instrument, the Chip Type options are filtered so that you cannot select an incompatible chip in error.

Example Kits page:



Note: The value entered for number of flows represents the maximum possible for a run using a planned run based on this template. Instrument conditions such as the availability of consumables might cause fewer flows to be completed.

Base Calibration mode options

Beginning in Torrent Suite 4.4, there is a base calibration mode drop-down menu. For Torrent Suite™ Software v5.2, this menu contains four options: Default Calibration, Enable Calibration Standard, Blind Calibration, and No Calibration. (Previously, in Torrent Suite™ Software v4.2, you could choose to Enable Base Recalibration or not.) You can select the base calibration method during run planning and in the reanalysis menu.

Default Calibration – allows a random subset of wells to be used for base calibration. (This is equivalent to the default setting for Torrent Suite™ Software v4.2 and earlier, i.e., a checked Enable Base Recalibration check box). This option uses TMAP to align the training subset of wells and is recommended if a good reference for the template is available.

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.

Enable Calibration Standard – allows wells belonging to the Calibration Standard to be selected as training subset.

The Calibration Standard is a small panel consisting of known sequence content with comprehensive and uniform representation of long homopolymers (up to 10-mers). The calibration standard can be spiked into Ion S5™, Ion PGM™, and Ion Proton™

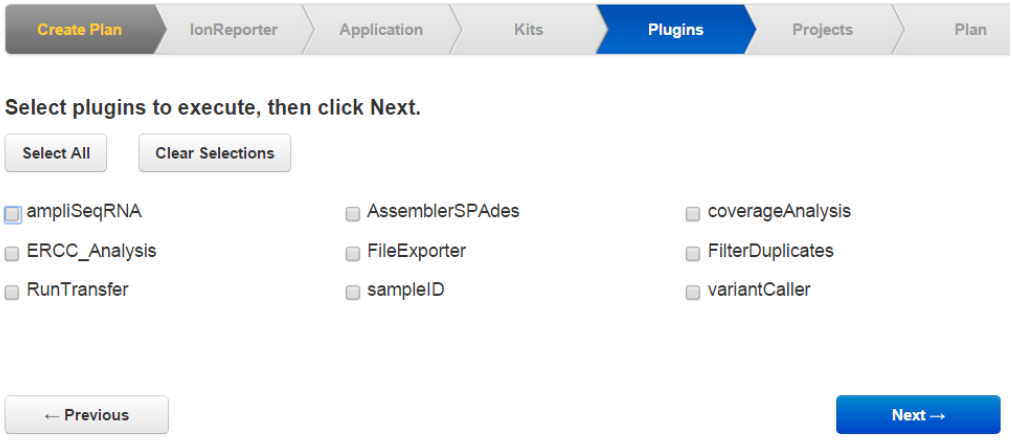
runs as a quality control for higher homopolymer performance and as a known reference for base recalibration.

The Calibration Standard is designed for use in combination with IonXpress or IonCode barcoded libraries. The calibration standard sequences are around 200 base pairs in length. Dor best results, the DNA templates should have similar read lengths.

Please note that this method of base calibration only works if calibration standard beads were spiked into the run. A summary of the number of calibration standard beads found can be viewed under the Calibration Report tab on the run page.

Wizard Plugins Page

In the Plugins page, you select plugins to run with this run plan or to run every time a run plan is created from this run template:

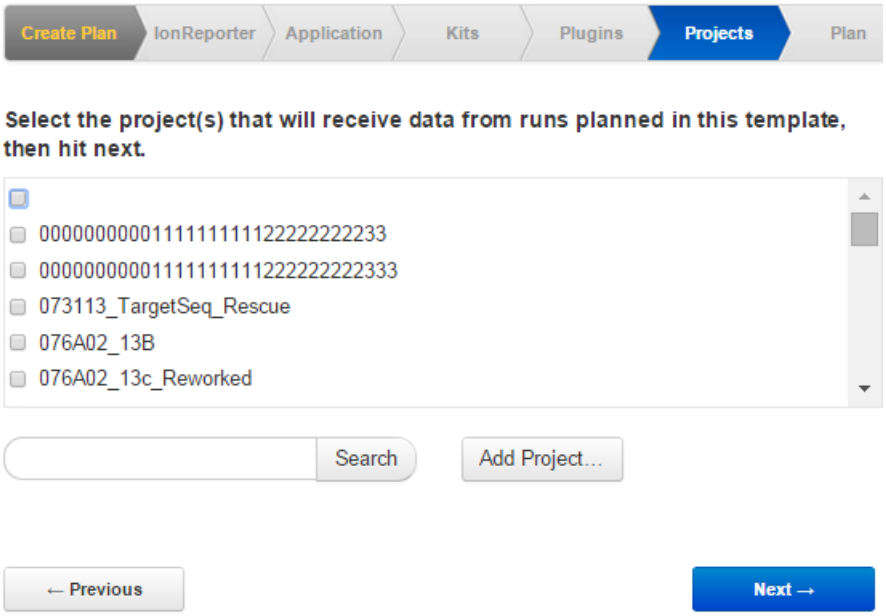


Note:

- The plugins available to you depend on what is installed and configured in your Torrent Browser.
- All active plugins (those installed, configured, and enabled on your Torrent Browser) are available in this menu.
- The IonReporterUploader plugin does not appear on this page.
- When you enable the variantCaller plugin, a **Configure** link appears for that plugin. For information on configuring the variantCaller (variantCaller) plugin.

Wizard Projects Page

In the Projects page, you select projects the will receive the completed analysis from this run plan or from every time a run plan is created from this run template:



You can also create a new project in this page.

Templates

Torrent Suite™ Software includes many planned run templates to simplify your sequencing. Most templates have a corresponding Ion AmpliSeq™ panel. The following describe a template:

- A canned set of instructions for both your sequencing run and your post-sequencing data analysis.
- A digital protocol with specifications for almost your entire experiment, from sample preparation through sequencing, data analysis, and data export to other systems for additional analysis. (A plan template is missing only the sample name, from your experiment information.)
- A sample planned run that you can copy to quickly create actual planned runs with known defaults and settings.
- A reusable set of laboratory, sequencing, data analysis, and data management instructions.

These steps describe how a plan template fits into your Ion S5™, Ion S5™ XL, Ion PGM™ or Ion Proton™ sequencing workflow:

- Decide what sequencing application and sequencing product (such as an Ion AmpliSeq™ panel) you will use.
- Select a pre-installed template with defaults for your application and sequencing product, or create your own template from scratch. Then, customize your template.
- Copy the template to a new planned run, adding the name of the tissue sample to be sequenced. The Torrent Browser assigns your new plan a run code.
- Enter the run code directly on the Ion sequencing instrument to initiate the sequencing. The planned run automates the process from sequencing through data analysis and data handling.

With the planned run wizard, you can create a new planned run with only a few clicks and the entry of the sample name. With the Plan Multiple feature, you download a CSV and customize it to create multiple planned runs without using the planned run wizard.

Plan templates play an important role in enabling rapid throughput across your sequencing instrument. Templates also help reduce the chance of error, by listing the reagent kits used on the instrument.

The **Plan ▸ Templates** screen contains your experiment templates. These include pre-installed product templates (for instance for products such as the Ion AmpliSeq™ panels) and as well as templates that you create, and areas for recently-used templates and ones you mark as favorites. Product templates contain the appropriate defaults for a product, including the default kits, BED files, and reference.

Plan > Template screen organization

Templates are organized by sequencing application (and by product for some applications):

- **AmpliSeq DNA** Ion AmpliSeq™ applications, including the Ion AmpliSeq™ Comprehensive Cancer Panel, Ion AmpliSeq™ Inherited Disease panels.
- **AmpliSeq RNA** Ion AmpliSeq™ RNA applications, including the Ion AmpliSeq™ Transcriptome Human Gene Expression Panel and Ion AmpliSeq™ RNA Panel.
- **DNA and Fusions**Ion AmpliSeq™ fusion applications, including the Ion AmpliSeq™ Colon Lung v2 with RNA Lung Fusion Panel and Ion AmpliSeq™ Lung Fusion Panel.
- **Generic Sequencing** Your own applications that do not fit in the other categories. With a generic sequencing template, you provide the settings for the experiment. Your choices are not restricted based on the logic of an application workflow, and it is theoretically possible to create a flawed template.
- **Pharmacogenomics** Ion AmpliSeq™ Pharmacogenomics Research Analysis Panel.
- **RNA Seq** RNA sequencing applications.
- **TargetSeq** TargetSeq™ products and other targeted resequencing applications, with parameters optimized for hybridization-based target enrichment.
- **Whole-Genome Seq** Whole genome sequencing applications, which do not assume enrichment and do not require a target regions file.
- **16S Target Sequencing** Ion AmpliSeq™ 16S metagenomics applications.

The template page also has groups for recently-used templates and for templates that you mark as your Favorites.

You can also create a template from your Ion AmpliSeq™ Designer.

Template customization

You can create your own template in order to have specific customization that is not available in the pre-installed templates. Examples of customization include the following:

- Custom plugin usage.
- Use of custom BED file for regions of interest or hotspot locations.
- Automatic inclusion of result sets into one or more projects, for convenient data management step later on.
- Automatic export of results sets to other analysis systems, such as to the Ion Reporter™ Software system.

In general, you start with the product template or application template that most closely matches your research requirements, copy that template, make your custom changes in the template wizard, and save your new template under a new name.

Your new template appears in the same application group as the original template. You optionally can also mark the new template to appear in your Favorites template group.

IMPORTANT! Valid characters in a template or plan name are the following: alpha-numeric, dashes, underscores, spaces, and periods.

Commas are not allowed in a plan or template name.

Plan > Template screen organization

Templates are organized by sequencing application (and by product for some applications):

- **AmpliSeq DNA** Ion AmpliSeq™ applications, including the Ion AmpliSeq™ Comprehensive Cancer Panel, Ion AmpliSeq™ Inherited Disease panels.
- **AmpliSeq RNA** Ion AmpliSeq™ RNA applications, including the Ion AmpliSeq™ Transcriptome Human Gene Expression Panel and Ion AmpliSeq™ RNA Panel.
- **DNA and Fusions**Ion AmpliSeq™ fusion applications, including the Ion AmpliSeq™ Colon Lung v2 with RNA Lung Fusion Panel and Ion AmpliSeq™ Lung Fusion Panel.
- **Generic Sequencing** Your own applications that do not fit in the other categories. With a generic sequencing template, you provide the settings for the experiment. Your choices are not restricted based on the logic of an application workflow, and it is theoretically possible to create a flawed template.
- **Pharmacogenomics** Ion AmpliSeq™ Pharmacogenomics Research Analysis Panel.
- **RNA Seq** RNA sequencing applications.
- **TargetSeq** TargetSeq™ products and other targeted resequencing applications, with parameters optimized for hybridization-based target enrichment.
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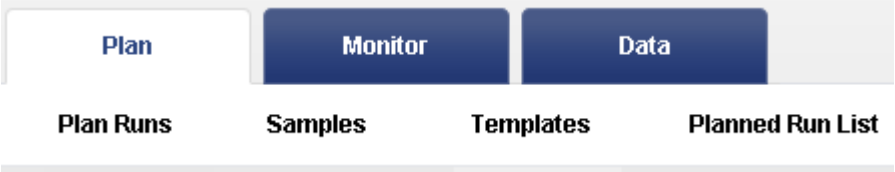
- **16S Target Sequencing** Ion AmpliSeq™ 16S metagenomics applications.

The template page also has groups for recently-used templates and for templates that you mark as your Favorites.

You can also create a template from your Ion AmpliSeq™ Designer.

Plan Tab



The **Plan** tab offers several routes for starting your sequencing experiments. The preferred way is to use a plan template in the **Plan ▸ Template** tab to create a digital protocol with specifications for almost your entire experiment, from sample preparation through sequencing, data analysis, and data export to other systems for additional analysis. From the template, you create one or more planned runs, which execute directly on your Ion S5™, Ion S5™ XL, Ion PGM™ or Ion Proton™ sequencing instrument.

Other ways to begin a sequencing run include:

- In **Plan ▸ Plan Runs**, you can plan a sequencing run by sample type or template run.
- In **Plan ▸ Samples**, you can start a run by clicking **Settings (⚙) ▸ Plan Run**.
- In **Plan ▸ Plan Run List**, click **Settings (⚙)** in the appropriate row in the **Run Plan Name** list, then select **Copy** to make a copy of an existing run.

The workflow below describes how templates and planned runs fit into your sequencing workflow:

1. Determine 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. Customize your template.
3. Copy the template to a new planned run, adding the name of the tissue sample to be sequenced. The Torrent Browser assigns your new planned a run code.
4. Enter the run code directly on the Ion sequencing instrument to initiate the sequencing. The planned run automates the process from sequencing through data analysis and data handling.

Typically, you create and organize templates, and create planned runs in the **Plan ▸ Templates** tab. You review planned run settings, edit, delete, or copy planned runs in the **Plan ▸ Planned Runs** tab.

Planned Runs

The **Plan ▸ Planned Runs** page contains planned runs which are ready to execute on your sequencing instrument. A planned run is an electronic protocol of everything required for a sequencing run, from reagent kits to sample name to genome reference, data analysis, and data management. You create each planned run from an application template (either from a product template or from your own template).

Templates and planned runs provide alternate methods (and timing) of entering the same data that is otherwise entered on the Ion sequencing instrument, for example on the Ion PGM™ Run Info screen. With templates and planned runs, you can enter the information in advance, and have an opportunity to print and review your entries. Use of templates and planned runs reduces your hands-on time on the instrument. If

you do not create planned runs here in the Plan tab, you must enter the run information directly on the Ion sequencing instrument.

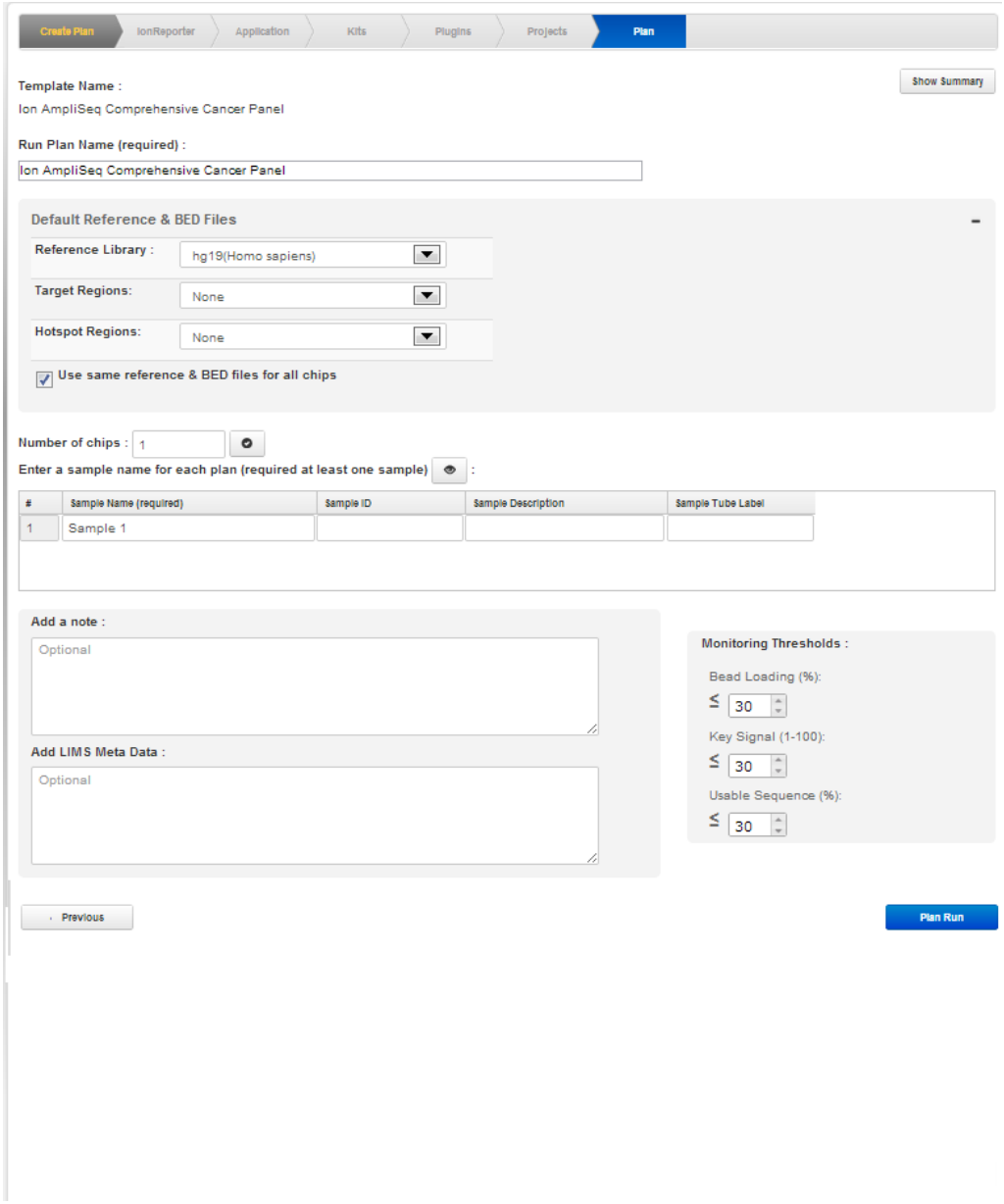
You can your run plans based on your sample sets or on run plan templates.

When you create a planned run, the run plan wizard walks you through each aspect of your new planned run, using pre-populated defaults based on the application template or product template you choose. The example below shows the defaults in the reference selections page. The chevrons across the top show the different pages of the wizard.

To execute a planned run, you select it directly on the sequencing instrument, for instance on the Ion PGM™ Run Info screen.

Wizard

When you create a new template or a planned run (from a template), the template wizard walks you through each aspect of your new template or planned run, using pre-populated defaults based on the application template or product template you choose. The example below shows the defaults in the Create Plan page. The chevrons across the top show the different pages of the wizard.



Start the wizard

For both templates and planned runs, you start the wizard from the **Plan ▸ Templates** page. The steps to start the wizard depend on whether you want to create a planned run from generic application template or an existing template, or create a template from generic application template or an existing template.

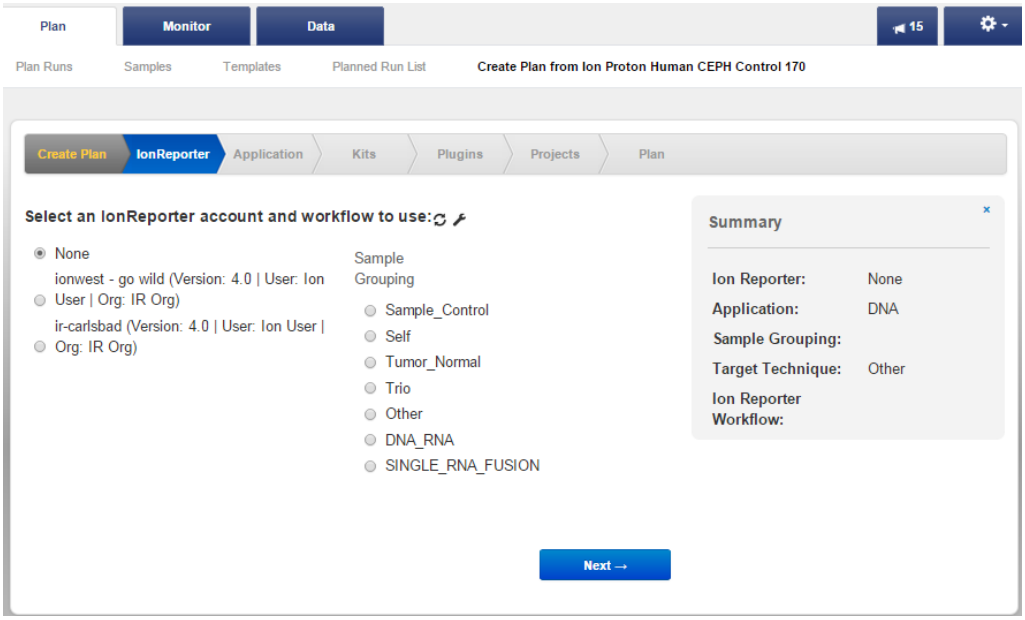
How you start the wizard is important, especially if your sequencing workflow uses common sequencing products. Pre-installed templates are available for these common sequencing products:

- Ion AmpliSeq™ Cancer Hotspot Panel v2.0
- Ion AmpliSeq™ Comprehensive Cancer Panel
- Ion AmpliSeq™ Inherited Disease Panel
- Ion AmpliSeq™ Cancer Panel
- Ion AmpliSeq™ Any Genome Panel

If you start with a pre-install product template, your new template or planned run has the correct settings for the product.

Wizard Ion Reporter Page

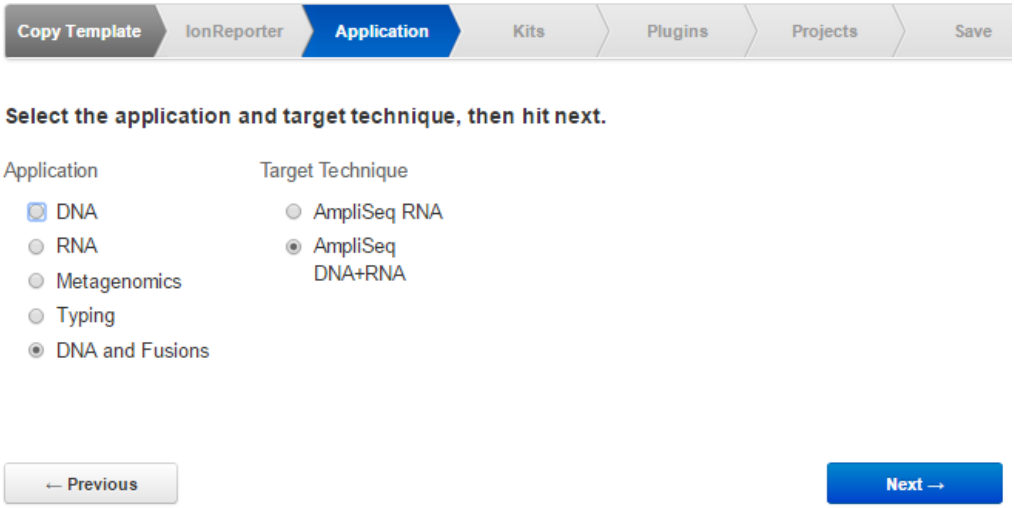
Ion Reporter is the first page in the Torrent Browser run template wizard. When you select an Ion Reporter account in this page, features related to Ion Reporter™ Software appear in the other wizard pages.



These selections on this page are only for Ion Reporter™ Software users.

Wizard Application Page

In the Application page you select your experiment type:



Based on the information that you specify here, the Kits page is set with the appropriate selections.

Notes about the Application choices:

- Metagenomics is reserved for future use with Ion Reporter™ Software.
- Typing is used for molecular fingerprinting to detect single strains of viral or bacteria for research purposes.

Wizard Kits Page

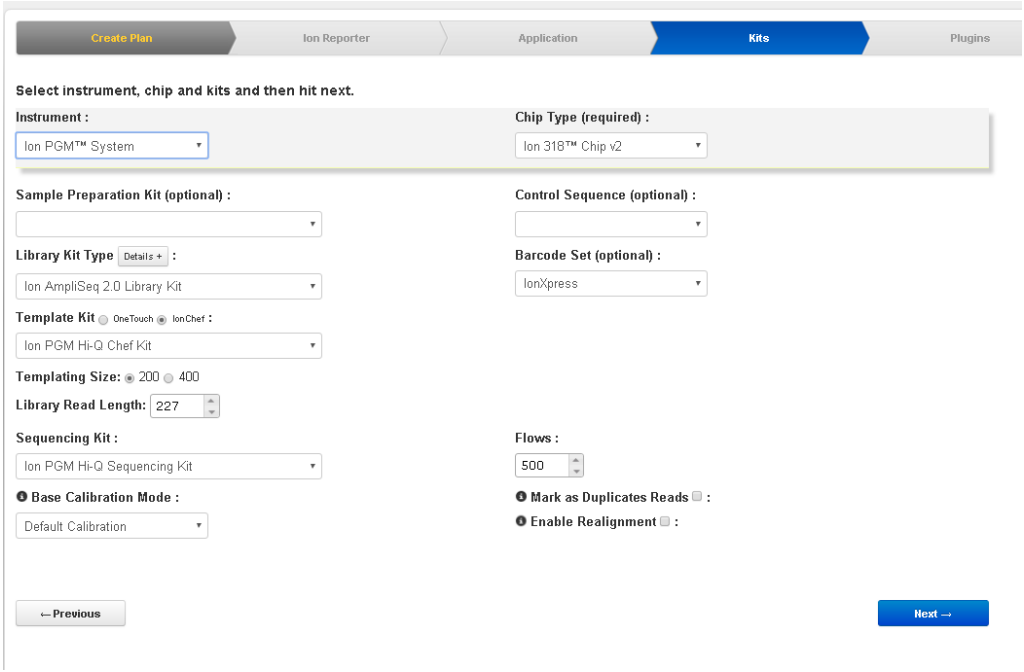
On the Kits wizard page, enter the following information about laboratory kits and other sequencing parameters:

- (*Optional*) Sample preparation kit
- Library kit type, including the forward library key and the forward 3' adapter
- Templating kit type
- Sequence kit
- Number of flows
- Barcode set ***Required*** for barcoded runs
- Base calibration mode
- Control sequence ***Required*** for RNA runs
- Chip type ***Required***
- Mark PCR Duplicates Not recommended for Ion AmpliSeq™ data

Chip type is required. As with all fields, if you enter chip type in your templates, then it is automatically entered in your run plans.

New in version 5.2, smart filtering is enabled on the Kits screen. When you select an instrument, the Chip Type options are filtered so that you cannot select an incompatible chip in error.

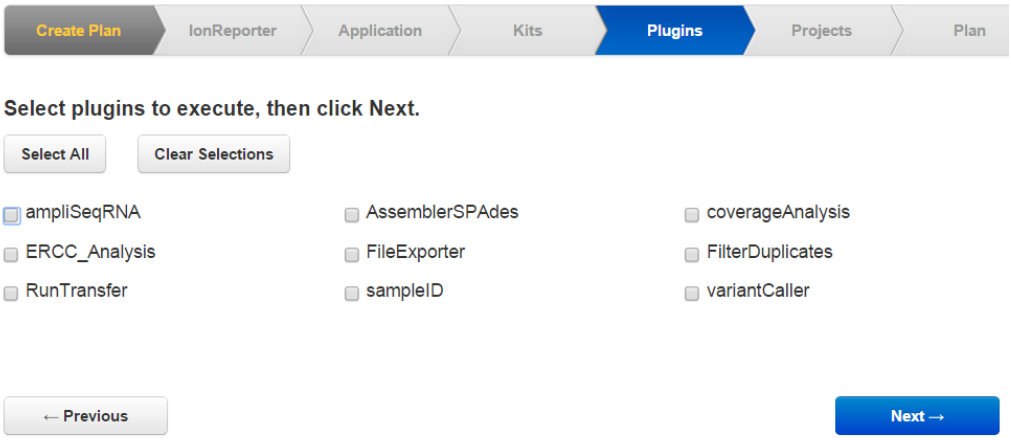
Example Kits page:



Note: The value entered for number of flows represents the maximum possible for a run using a planned run based on this template. Instrument conditions such as the availability of consumables might cause fewer flows to be completed.

Wizard Plugins Page

In the Plugins page, you select plugins to run with this run plan or to run every time a run plan is created from this run template:

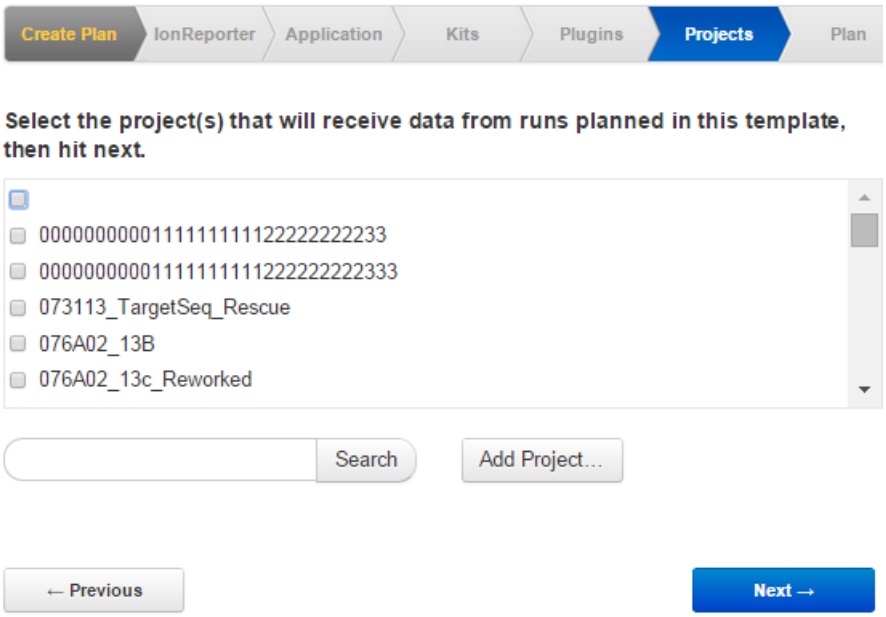


Note:

- The plugins available to you depend on what is installed and configured in your Torrent Browser.
- All active plugins (those installed, configured, and enabled on your Torrent Browser) are available in this menu.
- The IonReporterUploader plugin does not appear on this page.
- When you enable the variantCaller plugin, a **Configure** link appears for that plugin. For information on configuring the variantCaller (variantCaller) plugin.

Wizard Projects Page

In the Projects page, you select projects the will receive the completed analysis from this run plan or from every time a run plan is created from this run template:



You can also create a new project in this page.

Wizard Plan or Save step in the Workflow bar

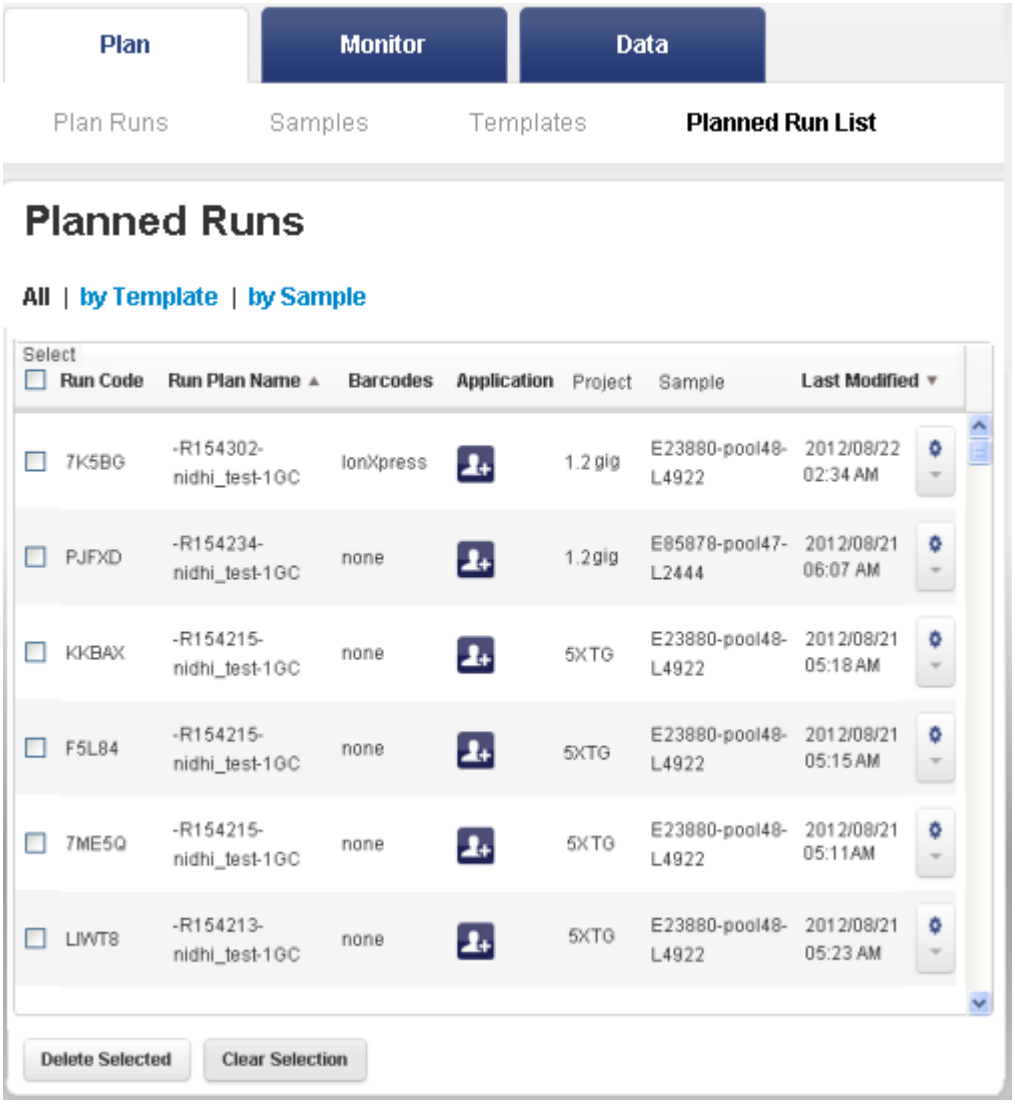
The title for last step in the Workflow bar of the wizard is different for templates, run plans, and run plans that are planned by sample set:

- **Templates** The step in the Workflow bar is Save. Here you enter the new template name and optionally mark it as a favorite.
- **Run plans** The step in the Workflow bar is Plan. Here you enter the new run plan name and sample information.
- **Plan by sample set** The step in the Workflow bar is Save & Finish. Here you enter the new run plan name. (Sample information is automatically entered into the Barcoding step in the Workflow bar.)

Note: Templates that are marked as favorites are listed in their own section at the top of the Templates tab.

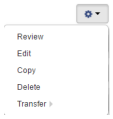
Example Planned Runs page

The following is an example of a Planned Runs page with several planned runs.



The following table describes the Planned Runs page contents.

Column heading	Description
Run Code	A short code identifying the planned run.
Run Plan Name	Name of the planned run.
Barcodes	Name of the DNA barcode set, if any.
Application	An icon identifying the sequencing application (such as whole genome, RNA Seq, etc.)

Column heading	Description
Project	Name of the project to contain the output result sets. **Note:** You can automate result sets going to more than one project. Only one project is shown here.
Sample	Name of the sample to be sequenced.
Sample Tube Label	Name of sample's tube.
Chip Barcode	Chip's barcode.
Library	Name of the reference library used.
Last modified	Time stamp of the last time the planned run was created or changed.
Status	Only runs with status of "planned" can be selected on the sequencing instrument. A new planned run for the Ion Chef™ System is first set to "pending". The instrument updates the plan to "planned" when the plan is ready to be selected on instrument.
Settings ( ) menu	The **Settings ( )** menu on the right side of a planned run allows you to review, edit, copy, delete, or transfer the planned run: 

CSV Metrics File Format

A Comma-Separated Value (CSV) file is a universal text file format for storing data. You can download an analysis metrics CSV file that contains analysis-level information for one or more Torrent Suite™ Software analysis runs, in the Torrent Browser **Projects** > **ProjectsName** > **Results Sets in ProjectName** page.

In the CSV file, each line represents a Torrent Suite™ Software analysis run, and within each line information fields are separated by a comma. These files are easily opened using spreadsheet software, such as Microsoft™ Office Excel™ or OpenOffice.org Calc,

where each comma-separated field is listed in a separate column. The Torrent Browser CSV file has many CSV 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
Library_Q10_Alignments	Number of alignments from reads with 90% or greater accuracy

Field	Description
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
Library_100Q47_Reads	Number of perfect reads of length at least 100bp

Field	Description
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
Number_Ambiguous	NA

Field	Description
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

* Columns 4-11 contain test fragment metric. There is one row of metrics for each test fragment: A through D. The other columns contain library read metrics.

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:

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 Sequences (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.
- 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

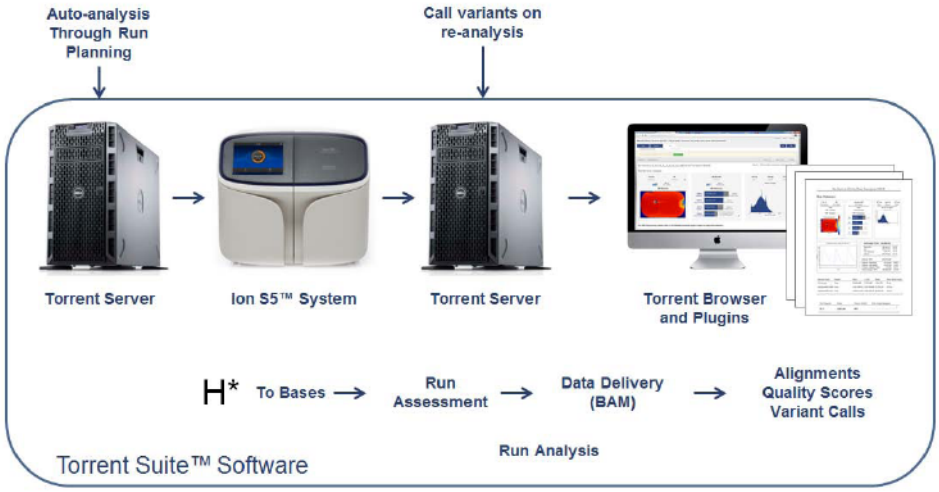
Ion Torrent uses a collection of custom tags to store sequencing and alignment information useful for downstream processing. In general, custom BAM tags starting with Z or Y are written by the BaseCaller and BAM tags starting with X stem from TMAP. As a consequence, tags starting with Z or Y are present 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 key and barcode adapter, and before the 3' adapter. (Only present if a 3' adapter was found.)
ZB	i	Number of overlapping adapter bases. (Only present if a 3' adapter was found.)

Tag	Type	Description
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)
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.

Dataflow file sizes

The Ion Torrent™ dataflow involves the transfer of raw sequencing data from the Ion S5™, 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 5.2/5.0 and 400 bp kit on the 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 520™ Chip	Ion 530™ Chip	Ion 540™ Chip
Read Capacity	--	5 M	15-20 M	60-80 M
Signal Processing Input	DAT	210 GB	530 GB	2 TB
Signal Processing Output	WELLS	30 GB	75 GB	180 GB
Base Calling Output	Unaligned BAM	55 GB	75 GB	85 GB
Aligned Output	Aligned BAM	10 GB	25 GB	55 GB

Ion Proton™ dataflow with 4.x software and 400 bp kit

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.

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Ion Proton™ dataflow with 4.x software and 200 bp kit

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.

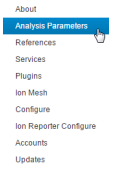
Step	Resulting file type	Ion Proton™ Chip	Ion 318™ Chip	Ion 316™ Chip	Ion 314™ Chip
Flows	--	500	500	520	520
Raw image acquisition	DAT	2.7 TB	225 GB	135 GB	30 GB
Image processing	WELLS	219 GB	16.4 GB	9.0 GB	2.0 GB
Signal processing and base calling	BAM	44 GB	4.2 GB	3.1 GB	0.5 GB

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.

Configure and select a custom analysis parameter set

To create and select a custom analysis parameter set:

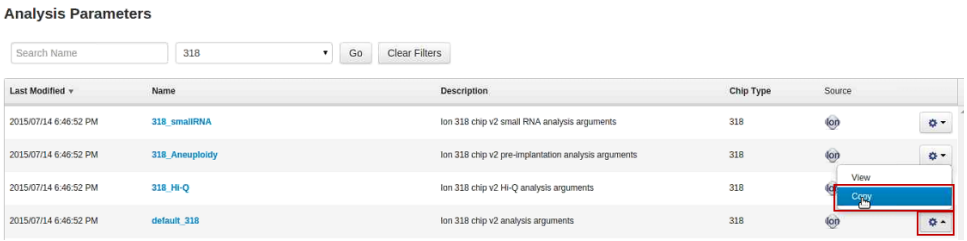
1. Click **Settings (⚙) ▸ Analysis Parameters**:



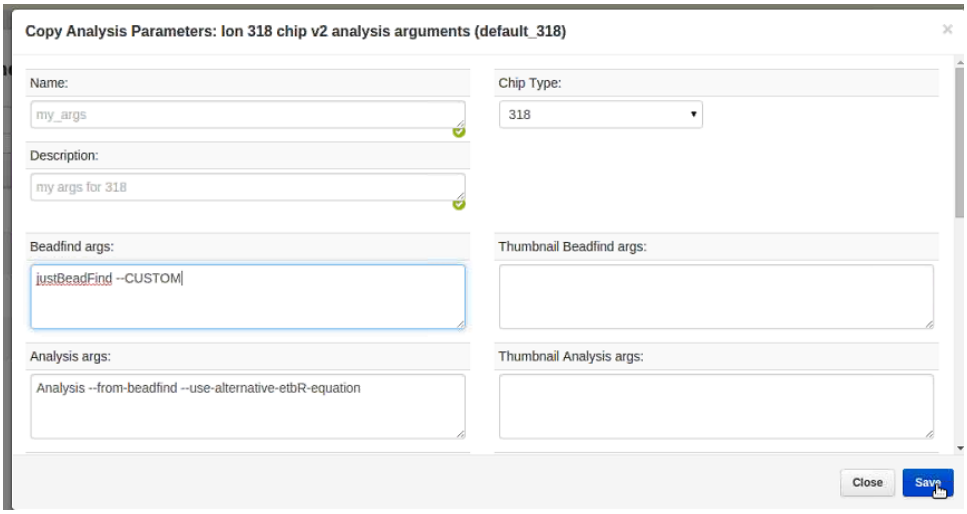
   In the **Analysis Parameters** screen, factory parameters are denoted by "Ion Torrent" in the **Source** column.

2. To filter the parameter sets by chip type, select your chip type from the **All Chips** menu.

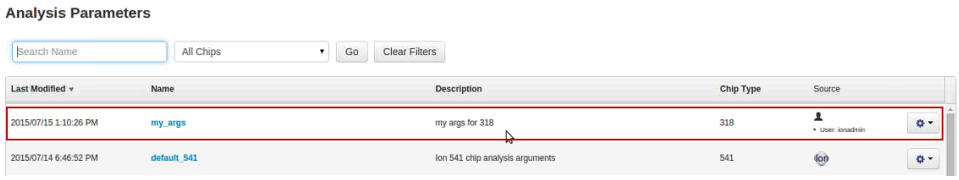
3. Identify the parameter set you want to copy, then click **Settings (⚙) ▸ Copy** in the table row for that set.



4. In the **Copy Analysis Parameters** dialog, enter a parameter name and description, and make any changes. Click **Save**.

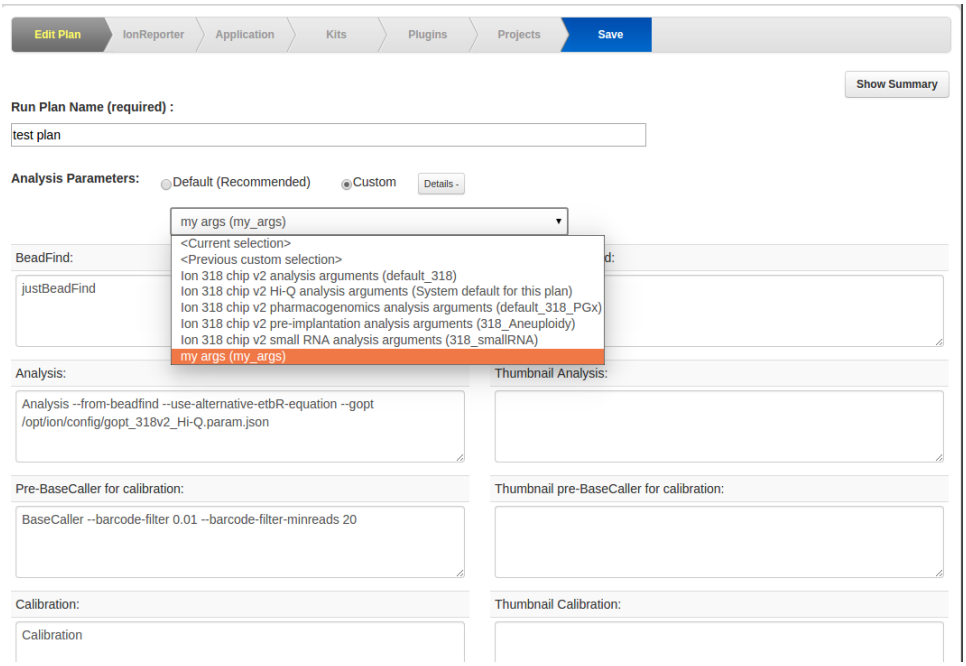


Your new analysis parameter set is available on the **Analysis Parameters** table. The **Source** column lists the name of the user that created it.



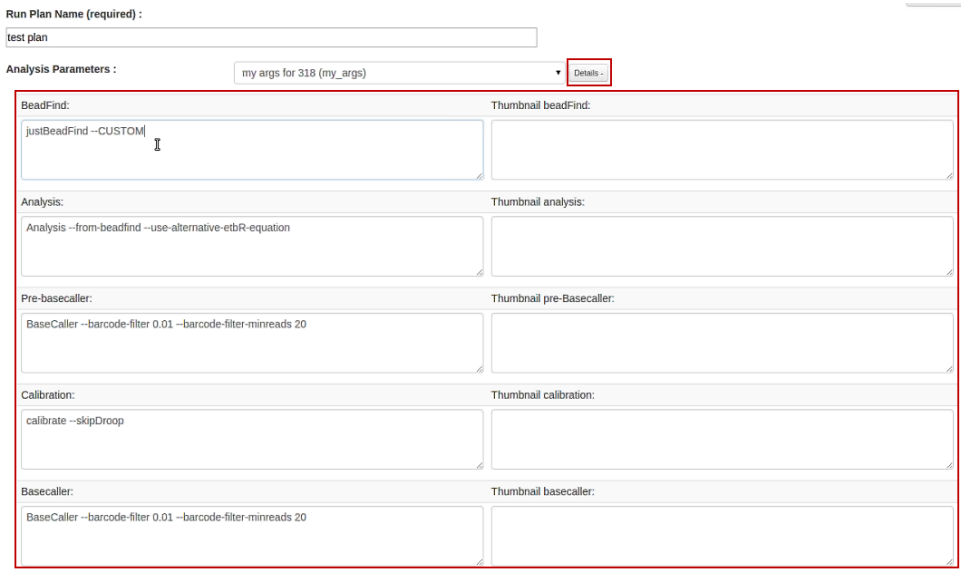
5. Click **Settings (⚙)** to **View** or **Copy** this parameter set.

6. You select the custom analysis parameter set when you create a Planned Run. Create a new Planned Run. In the **Plan** tab of the workflow bar, under the **Analysis Parameters** section of the screen, select the **Custom** button, then select your custom analysis parameters from the dropdown menu.

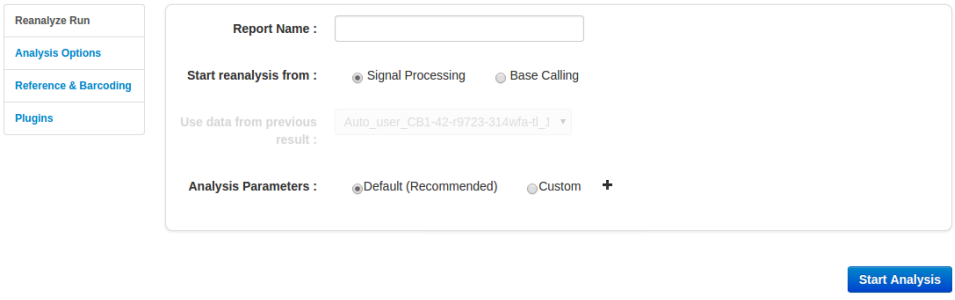


Note: You must first specify a chip type for the Planned Run (under **Kits** in the workflow bar) before you can select the custom parameters.

7. Click **Details+** to review the parameters.



Note: You can also access the custom analysis parameters from the **Reanalyze Run** screen.



Find the TMAP command for a specific analysis

See “TMAP examples“ on page 429 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.

Setting	Description
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.

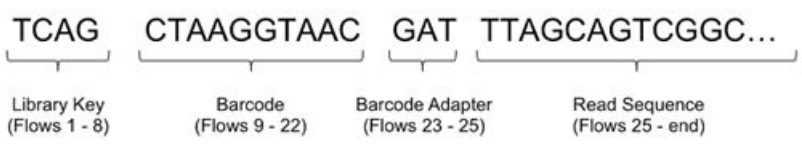
About barcodes

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.

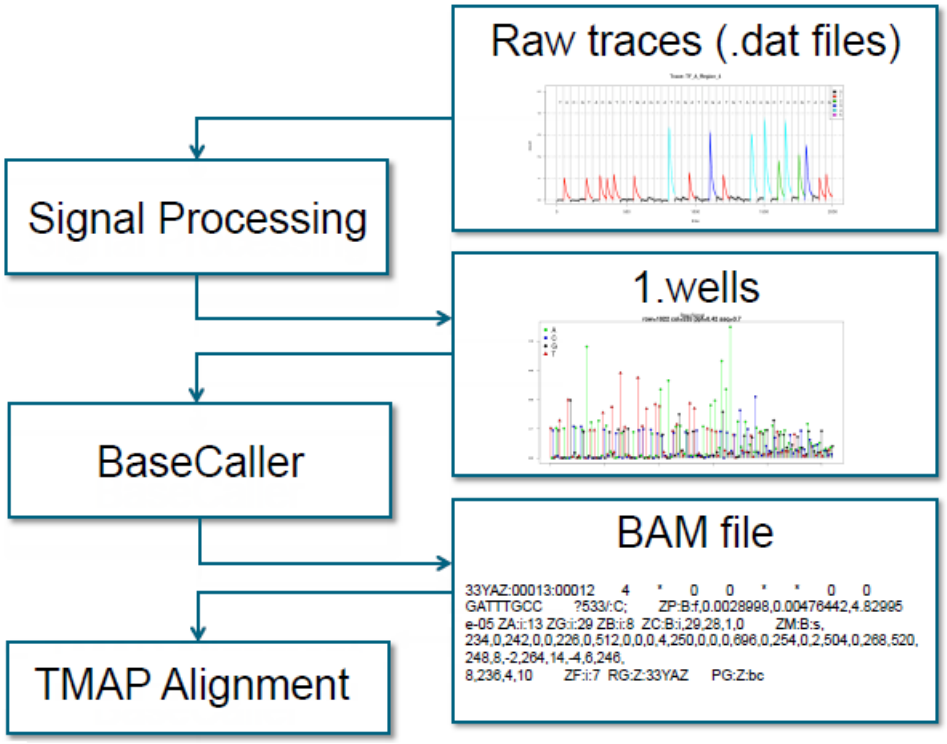
This diagram shows the placement of the barcode sequence, as well as the library key and adapters, with the read sequence (which is labeled "Template Bases"). The key is on the 5' end.

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.

Parameter	Default	Description
--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).

Parameter	Default	Description
--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-adpter-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).	

Parameter	Default	Description	
--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 contains several mapping algorithms, each with its own best application. TMAP's current default is `map4`.

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 429 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	

Option	alternate option	Type	Default	Description
		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
--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

--seed-length	INT	32	The k-mer length to seed CALs (-1 to disable)
--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

Option	Type	Default	Description
--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

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 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
--context	--	off	Modifies the gap penalty in homopolymers to achieve more accurate alignments
--do-repeat-clip	--	off	Clips repetitive sequence ends of aligned reads
--hit-frac	FLOAT	0.2	The fraction of seed positions that are under the maximum
--end-repair	INT	0	Rescues false negatives by selectively forcing alignment at the 3' end of the read. The recommended value is 15.
--J	INT	off 2147483647	Rescues false negatives by selectively forcing alignment at the 3' end of the read. The recommended value is 25.
--seed-step	INT	8	The number of bases to increase the seed for each seed increase iteration (-1 to disable)

Option	Type	Default	Description
--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.
--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)

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