ion torrent

Torrent Suite™ Software Help

HELP

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Revision B.0

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ThermoFisher SCIENTIFIC

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Revision	Date	Description
B.0	14 October 2016	Includes v5.2.1 new features. See release notes for details.
A.0	2 August 2016	New format and updated to include v5.2 new features.

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Contents

Welcome to the Torrent Suite™ Software Help System

This help system provides instructions for using the Torrent Suite™ Software to:

- Upload or create samples
- Execute and monitor the status of runs on an Ion S5™, Ion S5™ XL, Ion PGM™, or Ion Proton™ sequencer.
- View data, results, and QC reports after a run
- Download data files for all results from a run
- Use a variety of plugins to extend the capability of Torrent Suite™ Software
- Create and manage user accounts
- Manage and archive data for future use

Introduction

Torrent Suite™ Software provides an integrated environment to manage your sequencing instrument runs and the resulting sequencing data.

The Torrent Browser 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). Choose 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 issues.
- **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 sequencing application (and by product for some applications):

Table 1

Sequencing application	Description
AmpliSeq	For Ion AmpliSeq™ applications (DNA, RNA, and exome), including the Ion AmpliSeq™ Any Genome, and Custom Ion AmpliSeq™ panels.
DNA and Fusions	For Ion AmpliSeq™ applications such as Colon and Lung Research Panel v2.
Generic Sequencing	For your own applications that do not fit in the other categories. Here you provide all the choices for the experiment. Your choices are not restricted based on a common application workflow.
Oncology - Liquid Biopsy	For your liquid biopsy oncology research panels.
Pharmacogenomics	For Ion AmpliSeq™ Pharmacogenomics Research Panels imported from Ampliseq.com.

Sequencing application	Description
RNA Seq	For RNA sequencing applications.
TargetSeq	For TargetSeq™ applications, with parameters optimized for hybridization-based target enrichment.
Whole-Genome Seq	For whole genome sequencing applications, such as Ion ReproSeq™ Aneuploidy, which do not assume enrichment and do not require a target regions file.
16 S 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 Admin 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 tasks, see the "Software Administration and Data Management" on page 379.

Plan a run

These steps describe how plan templates and planned runs 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 you create your own template from scratch. You customize your template.
3. Create new planned runs from your templates, adding the names of the samples to be sequenced.

   The Torrent Browser assigns your new plan 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.

Plan templates and planned runs allow you to enter run information via the Torrent Browser rather than 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 planned run's short code or by selecting the planned run from a menu list of planned runs. You can optionally overwrite (change) planned run information directly on the sequencer.

Register for a new account

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

Follow these steps to register for a new user account:

1. On the login page, click **Register**.
2. Fill out the new user form and click **Submit**.
   Your account is created, pending administrator approval.

Samples and Sample Sets

Samples and Sample Sets can be set up prior to run planning or during run planning.

In the **Plan ▸ Samples** screen you can:

- Enter samples manually
- Create sample sets manually
- Import samples and sample sets
- Manage sample attributes

Enter new sample

1. In the **Plan ▸ Samples** screen, click **Enter Samples Manually**.
2. Click **Enter New Sample**.
3. In the **Add Sample** dialog, complete all required fields (marked "Required" in software); click **Done**.

Field	Description
Sample Name	Must follow Ion Reporter™ sample name restrictions: A unique identifier representing the sample, containing only alphanumeric characters (0-9 and A to Z0, periods(.), underscores(_), or hyphens(-). If the actual sample name already exists in Ion Reporter™ Software, a string such as _v1 or _v2, etc., is appended to the sample name.
Sample External ID	A field for your own use.
Gender	The gender of the sample: Male, Female, or Unknown. Do not leave empty. Select Unknown if the gender is not known.
Description	A field for your own use.
Type	The Ion Reporter™ Software relationship type for this sample.

Field	Description
Relationship Group	The Group number in the sample set. Defines related samples, the same as Set ID in the IonReporterUploader plugin.
Additional fields	If you create sample attributes, each attribute has its own field in the popup and in the CSV file. Attributes that are marked as mandatory must be entered for each sample. If you create an attribute of type Integer, only numeric characters (whole numbers) can be entered into the field for that attribute.

Your new sample is now available in the Samples list.

Create sample sets manually

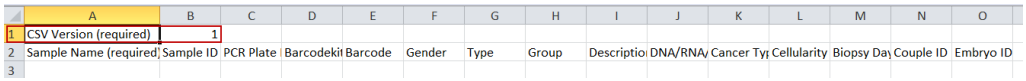
1. In the **Plan ▸ Samples** screen, click **Enter Samples Manually**.
2. Click **Enter New Sample**.
3. Click **Done**. Your attributes appear on the Enter Samples table.
4. Enter additional samples.
5. (Optional) If you have sample pairs, set Relationship Group numbers to reflect pairs. For example, DNA and RNA samples from the same sample would have the same Relationship Group number.
6. Click **Save Sample Set**.
7. Name Sample Set or add samples to an existing sample set.

Import samples to create a sample set

If you have a large number of samples, you can use the sample import feature to facilitate sample creation.

Note: New in version 5.2, the Sample File Format CSV template has a top row that indicates the version of the template. If you are using sample CSV files you created in past versions of the software, you will need to create a new CSV file with a new

template downloaded in v5.2. Just 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	Description	DNA/RNA	Cancer Type	Cellularity	Biopsy Day	Couple ID	Embryo ID
3															

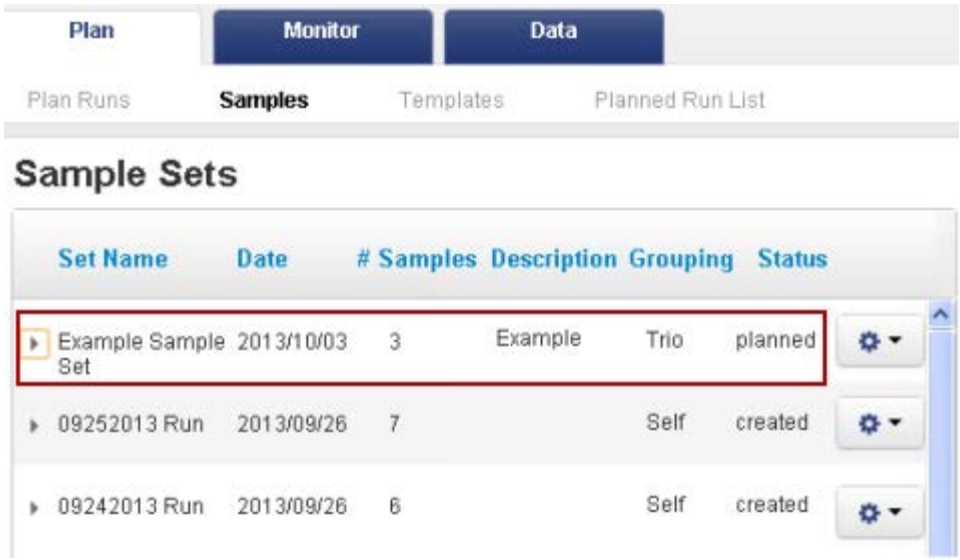
1. Click **Sample Sets ▸ Import Samples from File**.
2. Click the **Sample File Format** button to download a CSV template.
3. Fill out the CSV template fields: Sample Name, Sample ID, PCR Plate, Barcodekit, Barcode, and other relevant information, and save it to your computer.
4. Click **Select File** to upload it.
5. Click **Add Sample Set**, then enter a Sample Set Name, Group Type, and *(optional)* description. Click **Save & Finish**.
   The software automatically imports the samples into the Sample Sets table.

Example use of a sample set

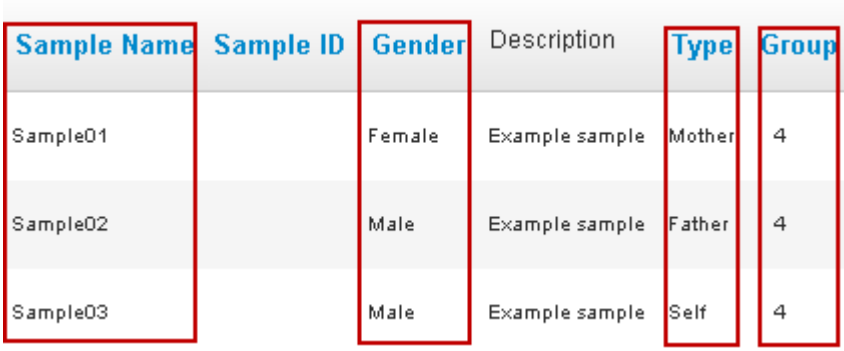
This example shows a trio sample set and how the run plan reads the sample set information for Ion Reporter™ Software users.

In the sample set

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



Click the expansion arrow to the left of the Example Sample Set entry to open the details for the sample set:



Sample Name	Sample ID	Gender	Description	Type	Group
Sample01		Female	Example sample	Mother	4
Sample02		Male	Example sample	Father	4
Sample03		Male	Example sample	Self	4

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

- The Type column contains 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

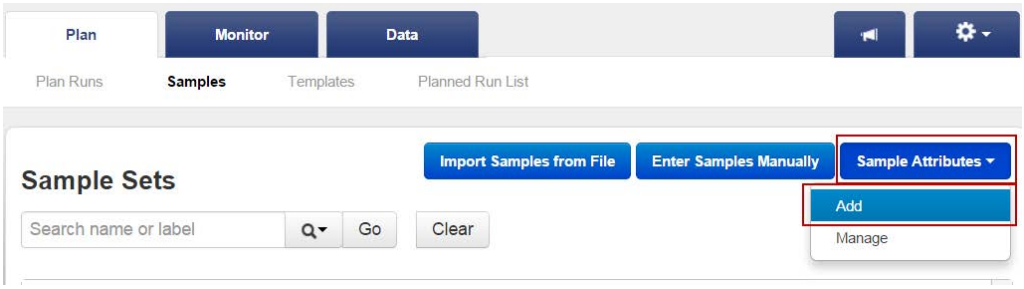
You can add fields to samples for sample management. Attributes that you create appear in the sample listing, in the Add Sample dialog, and in the CSV file used to import sample information.

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

Note: Even though you create an attribute on the Sample Set page, the attribute is applied to each individual sample that is currently a member of the sample set. The attribute is not applied to the sample set itself.

Add a sample attribute

To create a new sample attribute, select **Sample Attributes** ▸ **Add**.



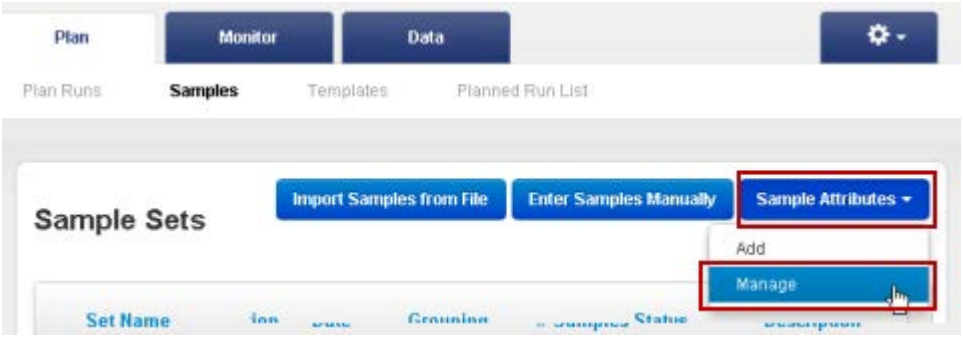
In the popup, enter the attribute name, type, and description. If you want the attribute to be required with every sample, select the **Is Mandatory** checkbox.

If the Attribute Type is set to Integer, you can only enter numeric characters (whole numbers) for this attribute.

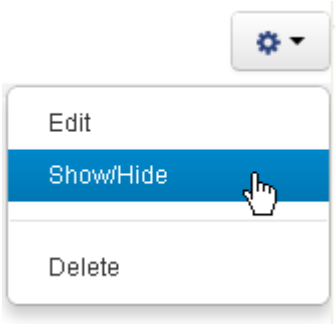
Hide a sample attribute

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

You hide a sample attribute in the attribute manage page. From the main Samples tab, select **Sample Attributes** ▸ **Manage**.



In the Sample Attributes page, select the **Show/Hide** option in the gear menu:



Sample information

This table describes the fields in the Add Samples dialog. The same fields are used in a CSV file that is imported to create samples.

Field	Description
Name	Must follow Ion Reporter™ Software sample name restrictions. If the actual sample name already exists in Ion Reporter™ Software, a string such as _v1 or _v2, etc., is appended to the sample name.
External ID	A field for your own use.
Gender	The gender of the sample: Male, Female, or Unknown. Do not leave empty. Select Unknown if the gender is not known.
Description	A field for your own use.
Role	The Ion Reporter™ Software relationship type for this sample.
Relationship Group	The Group number in the sample set. Defines related samples, the same as Set ID in the IonReporterUploader plugin.
Additional fields	If you create sample attributes, each attribute has its own field in the popup 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.

Volcabulary 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:

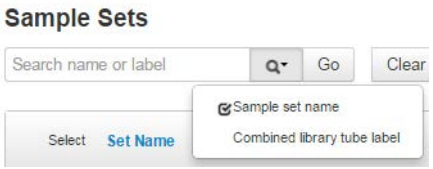
- Self
- Tumor, Normal
- Control, Sample
- Father, Mother, Self

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, in the **Plan** ▸ **Samples** screen:

1. Enter a search name or label in the text box.
2. Select Sample set name or Combined library tube label from drop-down box.

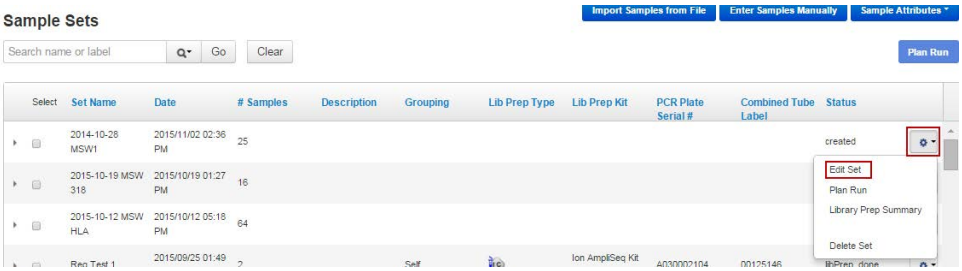


3. Click **Go**.

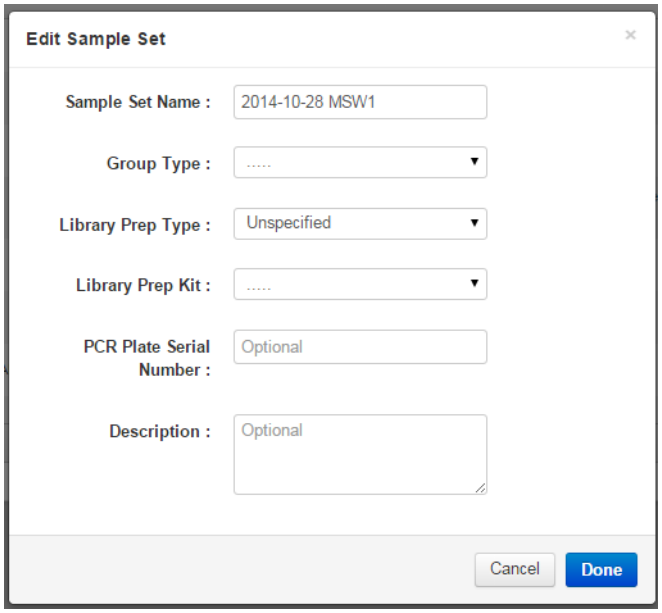
Edit samples

To edit samples:

1. From the tools drop-down menu, select **Edit Set**.

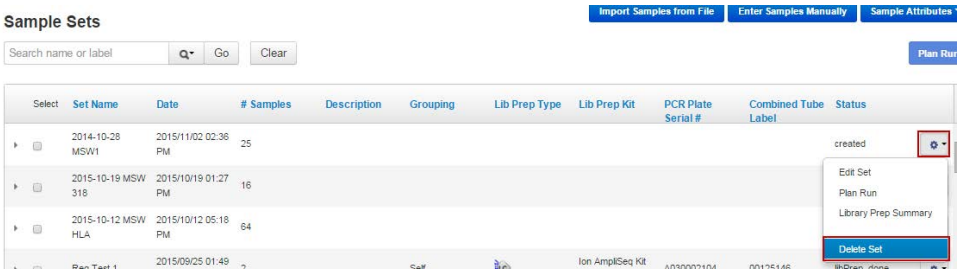


2. Make any desired changes, then click **Done**.



Delete samples

1. Click the Gear button in the row of the sample you wish to delete, then click **Delete Set**.

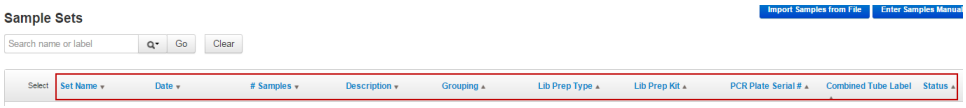


2. Click **Yes, Delete!**.

Sort samples

To sort samples:

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



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

Plan an instrument run

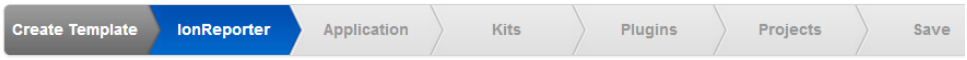
Differences between templates and planned runs

Templates and planned runs have much the same information.

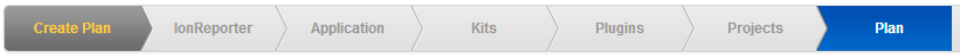
- Planned runs are created from templates.
- Templates do not have sample names and run names.
- Planned runs are executable on the sequencing instrument.
- Add a sample name and run name to a template to create a planned run.
- The planned run wizard opens in the last page, so that if you accept all 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 505 and “Plan Tab“ on page 508 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 using 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. Additionaly, you can edit the templates 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 Custom Analysis Parameters" on page 526.

1. Select **Plan ▸ 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 page.
3. Add samples, confirm the default settings, and enter a plan name, then click **Plan Run**.
4. Run the plan on your sequencing system.

Create a Planned Run using DNA and Fusions template

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 Custom Analysis Parameters" on page 526.

1. Select **Plan ▸ 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, and 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 will 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 Custom Analysis Parameters" on page 526.

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

   Note: If you are using the Ion PGM™ Hi-Q™ Chef Kit , select the **Ion Chef** option next to **Template Kit**, and select the kit name. Click on the **Details** button to select the **Ion PGM™ Hi-Q™ Chef for STR** 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 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 Custom Analysis Parameters" on page 526.

1. Select **Plan ▸ 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** chevron.

   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 Custom Analysis Parameters" on page 526.

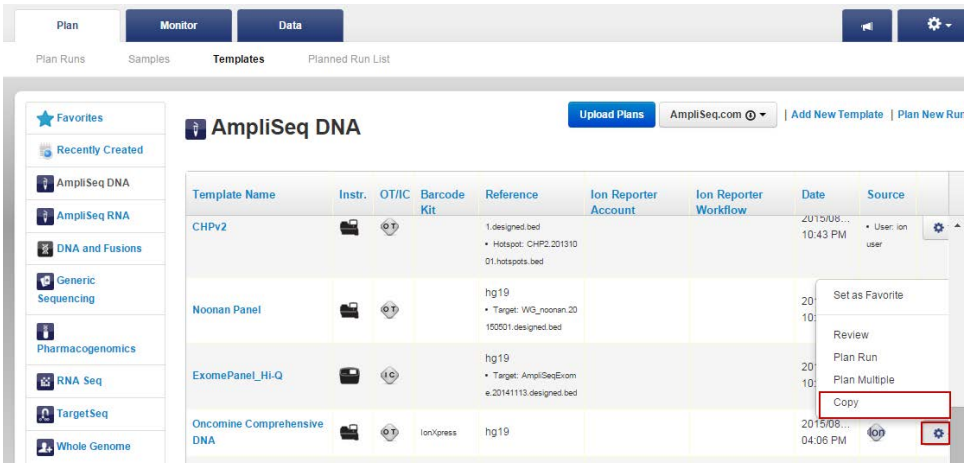
1. Select **Plan** ▸ **Templates**, then in the Favorites list, select **Generic Sequencing**.
2. Select a template that best matches your application. For instance, if you are using a MuSeek library, select the template with the same name from the Generic Sequencing category.
   The wizard launches 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 Custom Analysis Parameters" on page 526.

1. Click **Plan** ▸ **Templates**, then in the Favorites list, click the Gear button in the row of the template you want to start with. Click **Copy**.



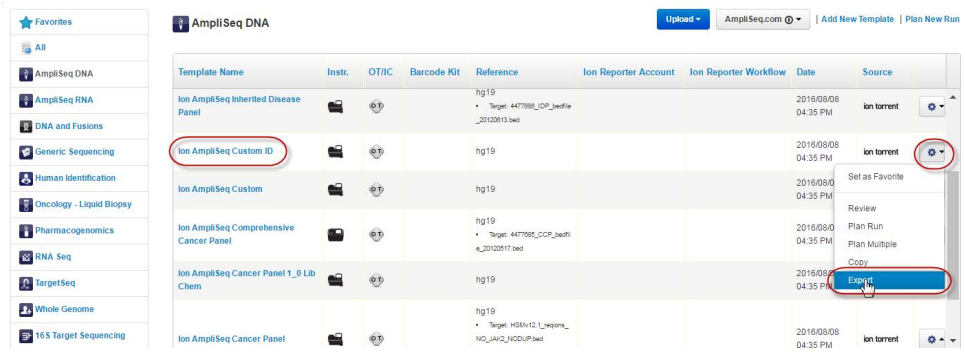
The wizard launches and displays the Save page.

2. Enter a name for the template.
3. If desired, go back to previous chevrons in the workflow 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. Click **Plan** ▸ **Templates**, then in the Favorites list, click the Gear button in the row of the template you want to start with. Select **Export**.



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.

Import a template

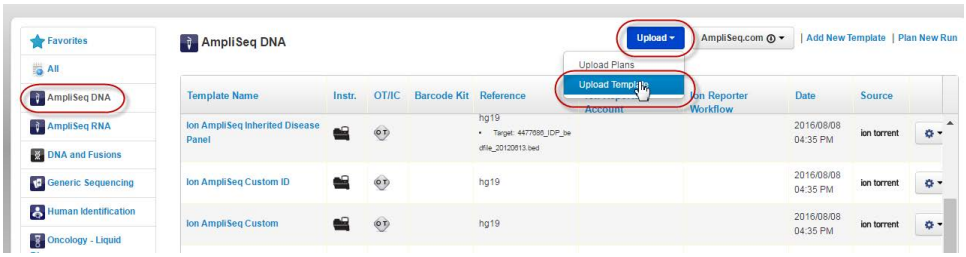
You can import all the parameters in a template in the form of a CSV file.

Note: The CSV file must be formatted correctly for import. We recommend exporting a template (see "Export a template" on page 33) 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. Click **Plan** ▸ **Templates**, then in the Favorites list, select the application group that you want to import the template into.

2. Click **Upload**, then select **Upload Template**.



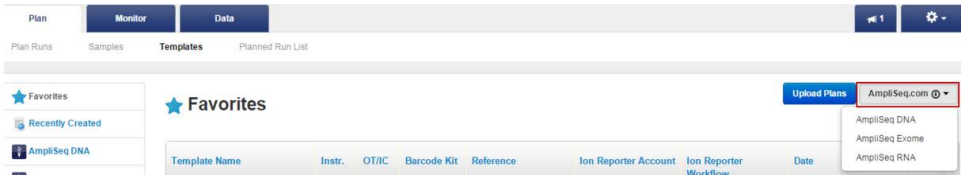
3. In the Import Plan Template dialog, click **Choose File**, select the CSV file to import, then click **Load**.
   The template appears listed in the application group.

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 TVC 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. Go to **Plan** ▸ **Templates**.

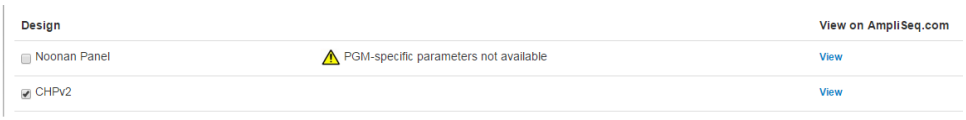
2. Click the **AmpliSeq.com** button and select the type of panel you want to import: AmpliSeq DNA, AmpliSeq Exome, or AmpliSeq RNA.



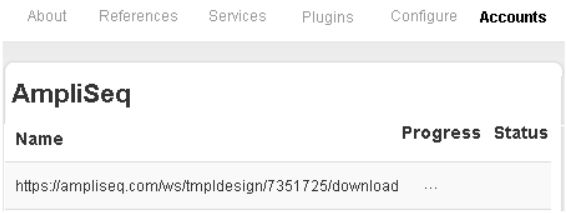
3. Enter your ampliseq.com username and password (if required).

4. Select your instrument and chip.

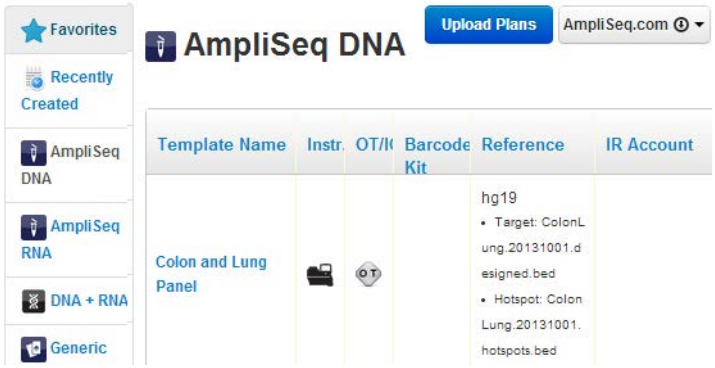
5. The Torrent Browser lists the available panels. Some panels do not have optimized Torrent Variant Caller parameter sets available for multiple chips and sequencers. A caution warning denotes choices for which optimized Torrent Variant Caller parameters have not be developed for the selected chip type, which may 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 page. Refresh your browser to track the progress and see 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 pages. 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.

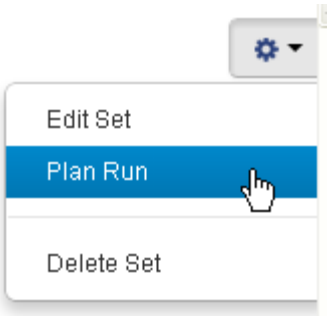
The plan-by-sample-set feature is recommended for the following:

- Ion Reporter™ Software users setting up multi-sample analyses
- Sample sets that include a large number of samples

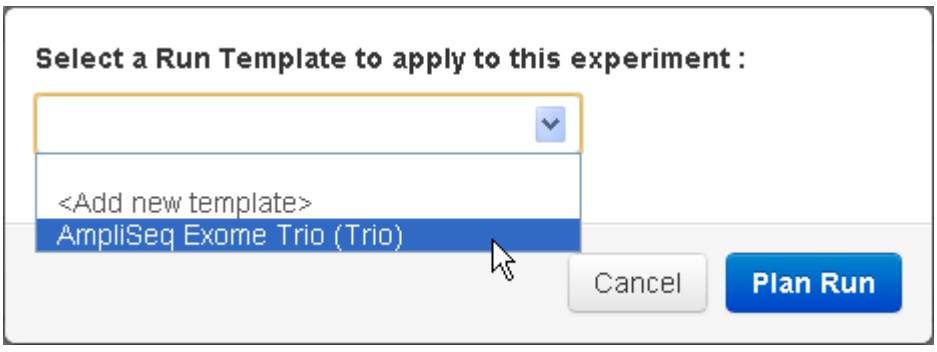
Follow these steps to initiate a run plan based on your sample set:

1. In the Sample Sets page (in the **Plan** ▸ **Samples** tab), find your sample set.

2. Click the gear menu for your sample set and use the **Plan Run** menu selection to begin a run plan for this sample set:



3. 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 you are looking for, check the **Show All Templates** box.

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

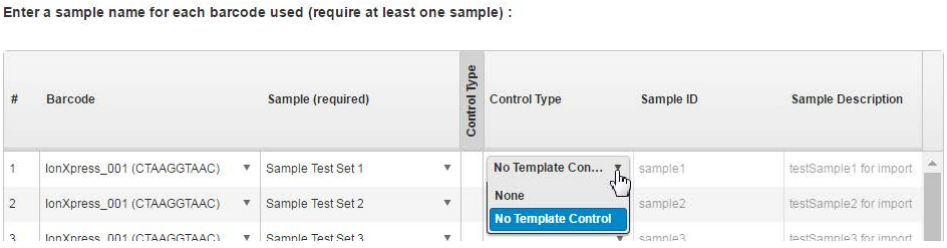
5. In the **Default Reference & BED Files** region of the screen, select the appropriate reference library and BED files for the target and hotspot regions covered by the selected panel.

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

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

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

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

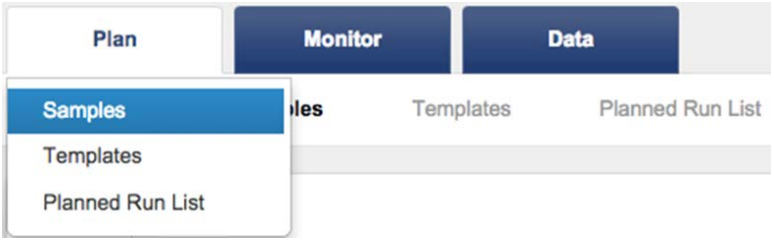


Note: The No Template Control option can be used to indicate negative control samples.

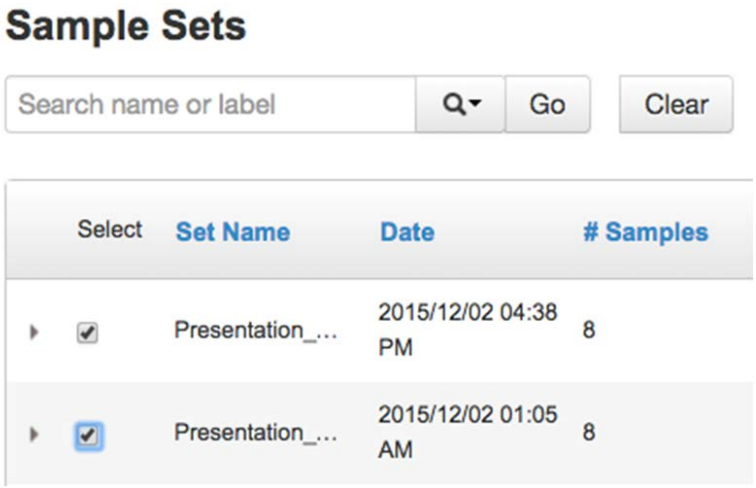
Include multiple sample sets in one planned run

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. Go to **Plan** ▸ **Samples**.

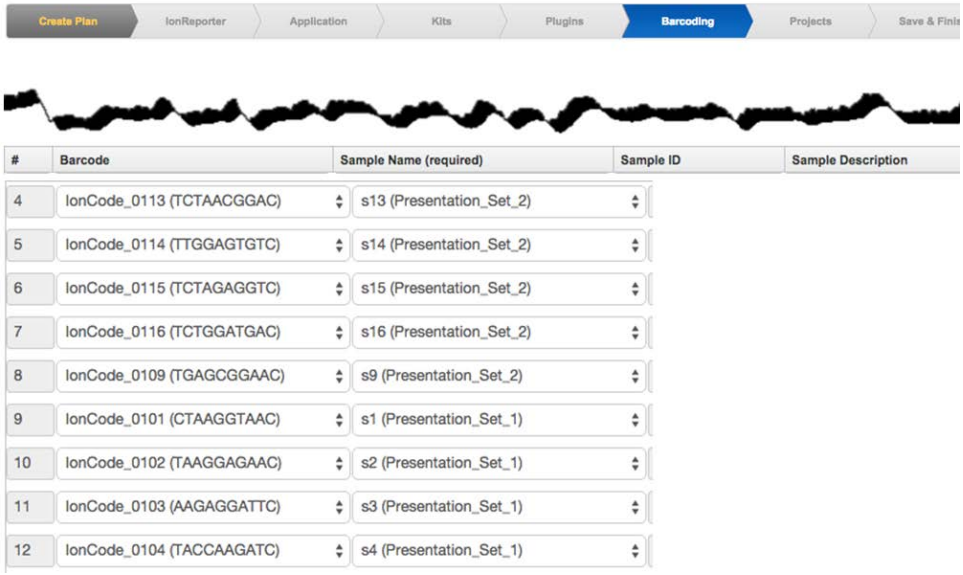


2. Select multiple samples sets, ensuring that they use the same barcode kit.



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

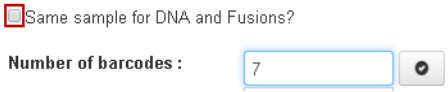
In the Barcoding page of the Planned Run wizard, you will now see the sample sets you added in the barcode table.



Create a planned run for mixed samples using a template

If you want to plan a run for DNA and Fusion sample pairs and several individual Fusion or DNA samples, you can start with a fusions template and then alter it to accommodate single samples on the same chip. The example below 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. Uncheck "Same sample for DNA and Fusions" option.



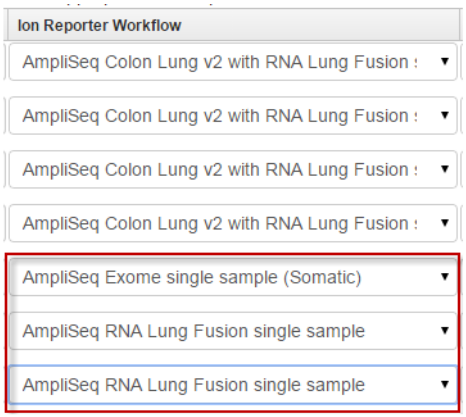
4. Renumber the samples.

#	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 via CSV upload

The Plan Multiple option for a barcoded template creates a CSV file with setup structure to create multiple planned runs. Each data line in the CSV file is a separate run plan.

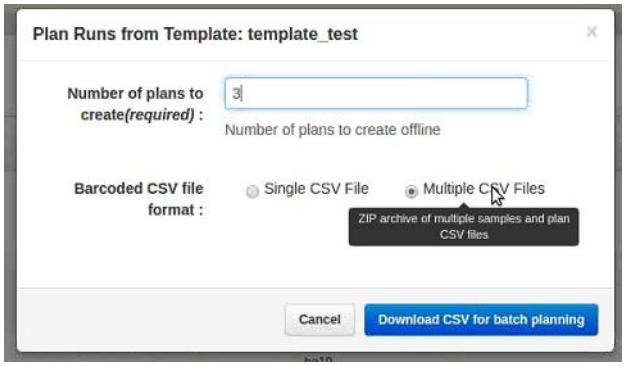
Note: New in version 5.2, the Download CSV for batch planning template has a top row that indicates the version of the template. If you are using CSV files you created in earlier versions of the software, you will need to create a new CSV file with a new template downloaded in v5.2. Check that the columns are the same between the current and old formats. If so, copy and paste the contents of your existing CSV file into the template under the version row. If the columns are different between the current and new template, copy one column at a time.

Follow these steps to use the Plan Multiple feature:

1. Go to **Plan ▸ Templates**, select a barcoded template, and in the row of the template you wish to use, click the gear icon.

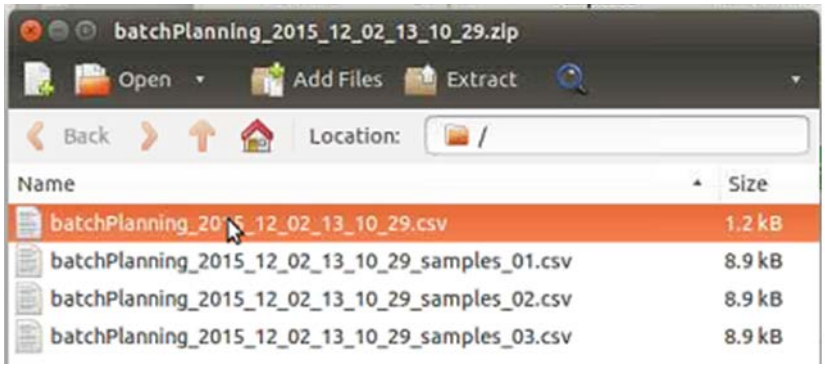
2. In the drop-down menu, select **Plan Multiple**.

3. In the Plan Runs from Template dialog, enter the number of plans and select **Multiple CSV Files**. Click **Download CSV for batch planning**.



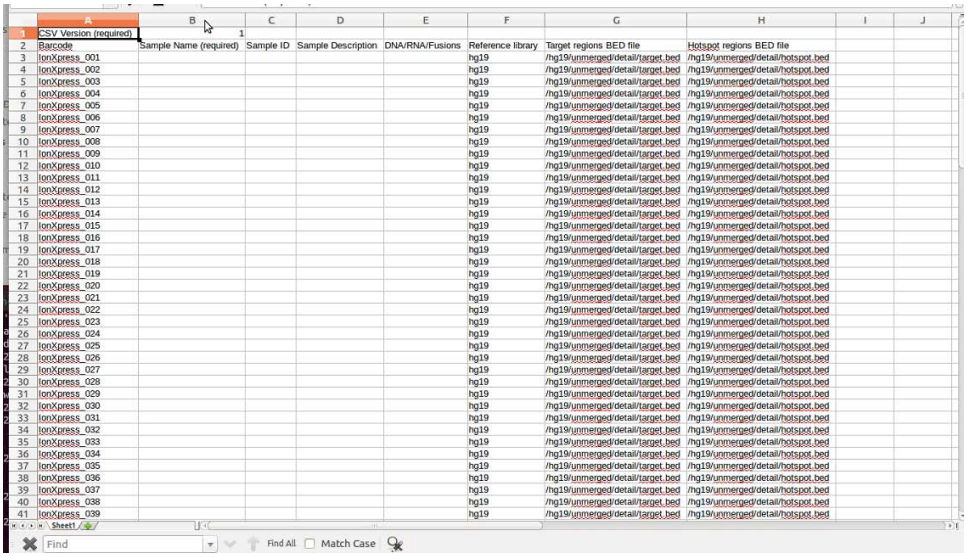
When Multiple CSV Files is selected, a Main CSV file and several sample CSV files are created in a zipped file.

4. Download the zipped file and unzip it.



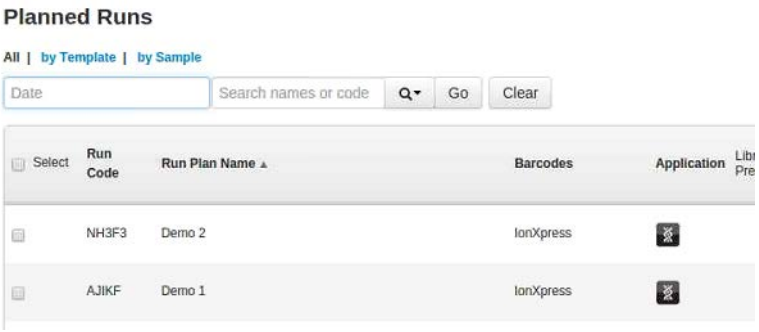
5. Enter the plan name(s) in the master .csv file named batchplanning....10-29.csv.

6. Enter sample names, chip types, barcodes, and other sample attributes.



7. Save and re-zip the CSV files.

8. Click **Upload Plan**. The system parses the files and creates the run plans.



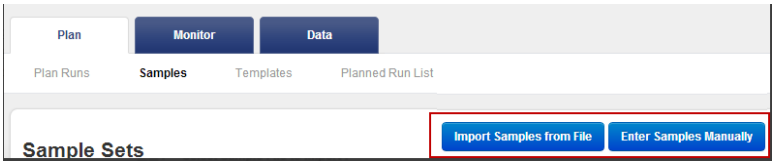
Analyze Ion AmpliSeq™ on Ion Chef™ samples

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

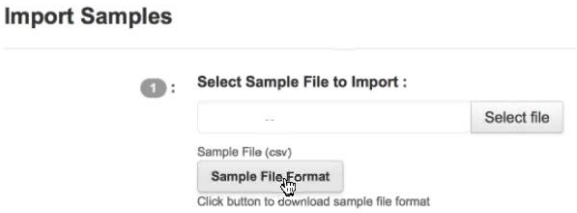
The process involves creating a Torrent Suite™ Sample Set, preparing an Ion Chef™ library, creating a Torrent Suite™ planned run, preparing an Ion Chef™ or OneTouch2 template, and sequencing on an PGM™, Ion Proton™, or Ion S5™ sequencer.

Create Sample Set

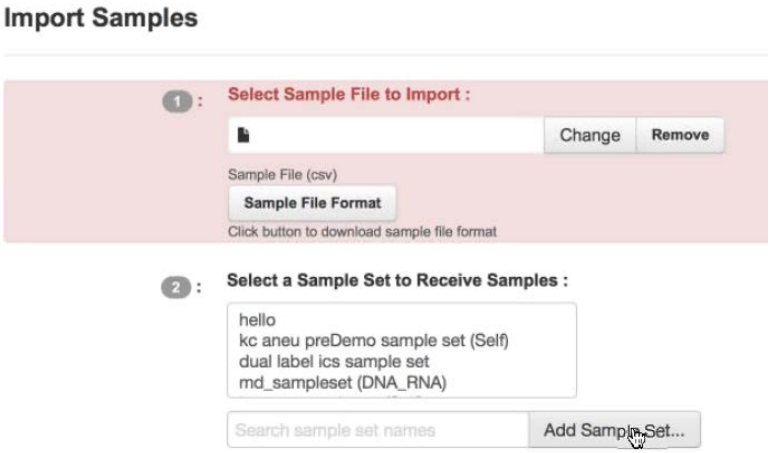
1. Import samples from a file or enter them manually.



2. For this use case, we've selected to import samples from a file.
3. Go to **Plan** ▸ **Samples** and click **Import Samples from File**.
4. On the Import Samples window, click **Sample File Format** button.

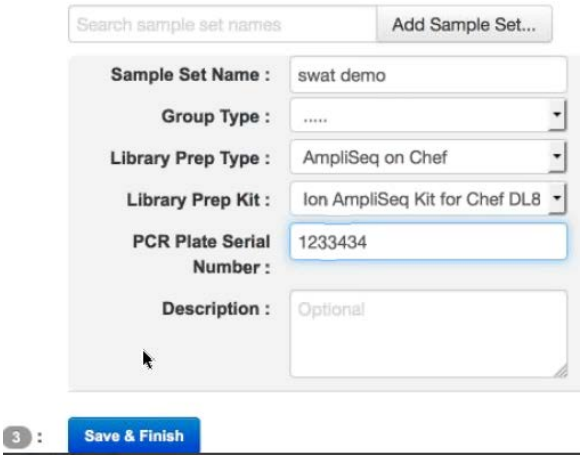


5. A CSV template downloads. Click on it and enter sample names, PCR Plate positions, and DNA or RNA at minimum. Save to your desktop.
6. Now, click the **Select File** button, choose your CSV file, and click **Open**.
7. Click **Add Sample Set**.

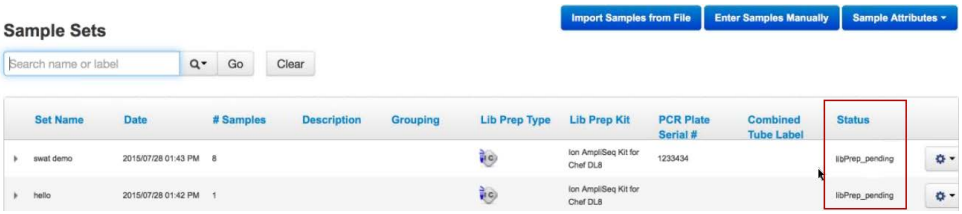


8. Name your new sample set.
9. Set Library Prep Type to **AmpliSeq on Chef**.
10. Set Library Prep Kit to **Ion AmpliSeq Kit on Chef DL8**.

11. Enter PCR plate serial number and click **Save & Finish**.

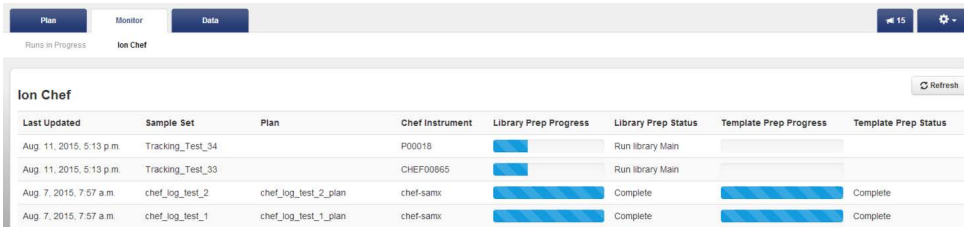


12. The software creates your new sample set. View it at **Plan** ▸ **Samples** ▸ **Sample Set**. Check its status in the Status column. Either libPrep_pending, libPrep_reserved, libPrep_done, planned, Voided, or Run are displayed.



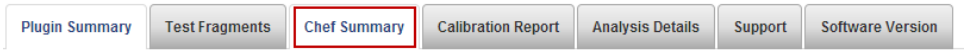
13. Notice a new icon for AmpliSeq on Chef in the Lib Prep Type column.

14. Monitor the Ion Chef library and templating steps from **Monitor** ▸ **Ion Chef**.



15. When the sequencing run is complete, view the Ion Chef run report. Go to **Data** ▸ **Completed Runs & Results,** and select your Ion Chef run.

16. Scroll to the bottom of the Run Summary page and select **Chef Summary**.

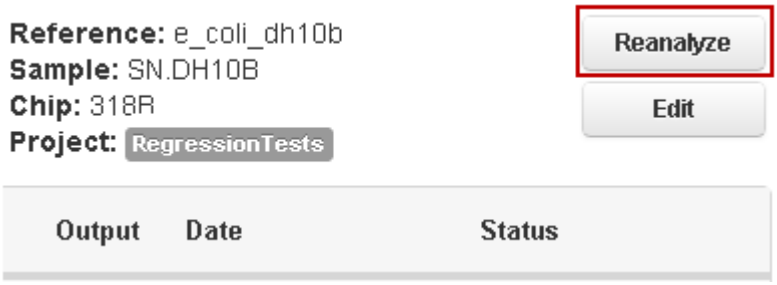


17. Review the Chef Library Prep Info and Chef Template Prep Info sections.

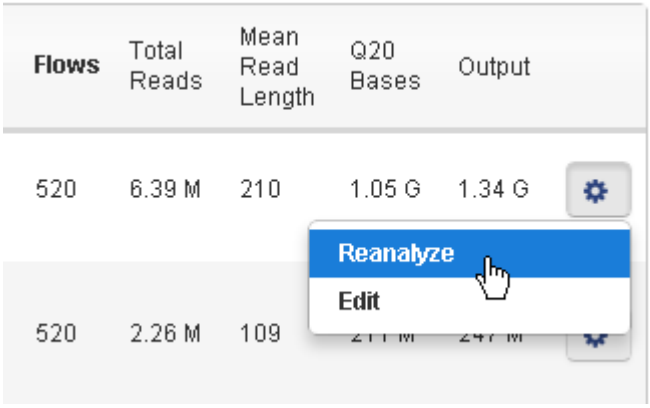
Restart a run

Follow these steps to restart an analysis run:

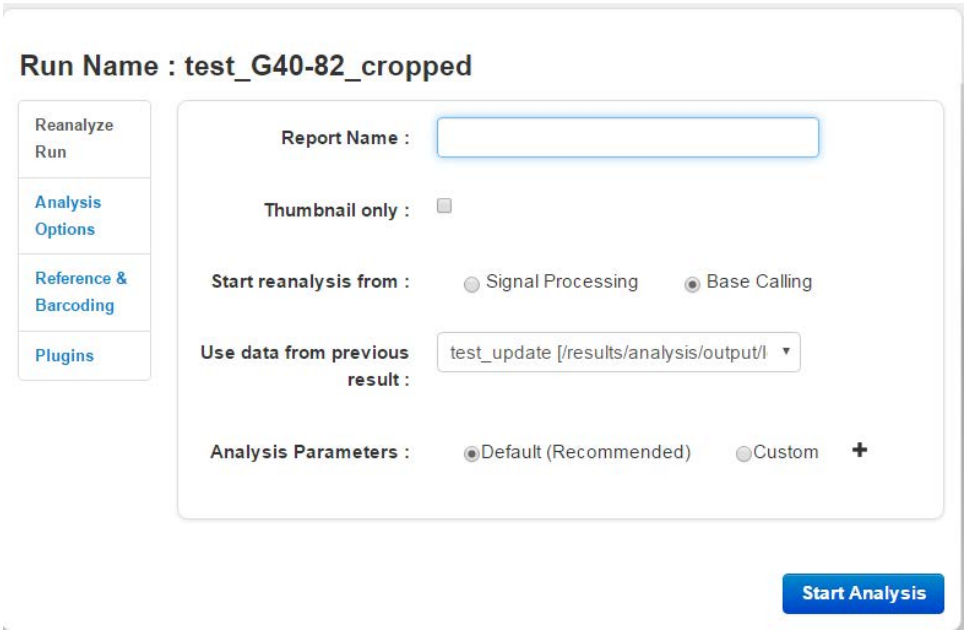
1. Open a run reports listing in the **Data > Completed Runs & Results** tab to access run management functions. In the list view, click the Analyze button on the right side of the run listing:



In the table view, click the gear menu on the right side of the run report listing and click the **Reanalyze** option:

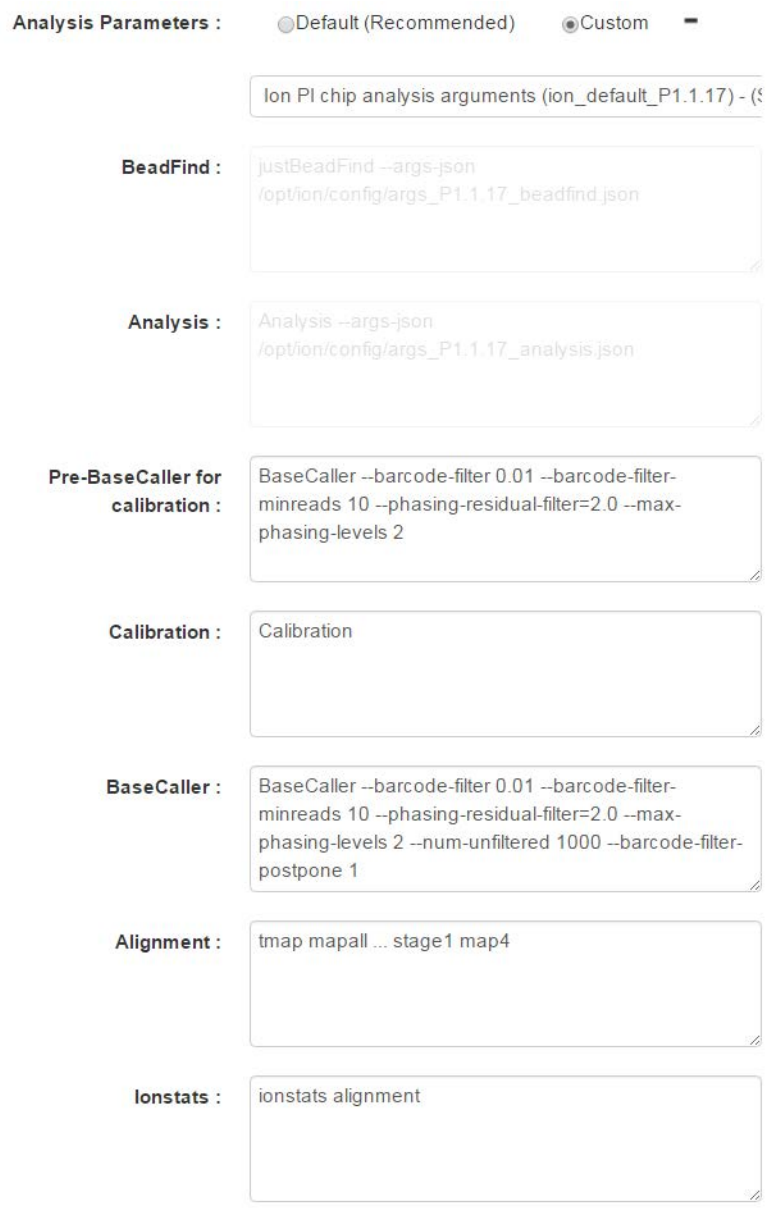


2. The Start Analysis window opens:



To rerun with the same run settings, click **Start Analysis**. To modify settings, click options as appropriate. To modify Analysis Parameters, click the **Custom** button.

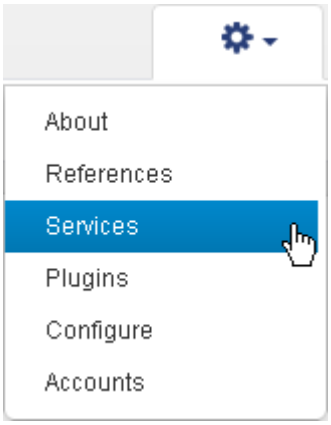
3. Modify the advanced options, if needed.



Note: You can also modify

4. Click the **Start Analysis** button.

5. Verify that the run has started by clicking the Admin gear menu **Services** option to view your job:



Realign a run to a different reference genome

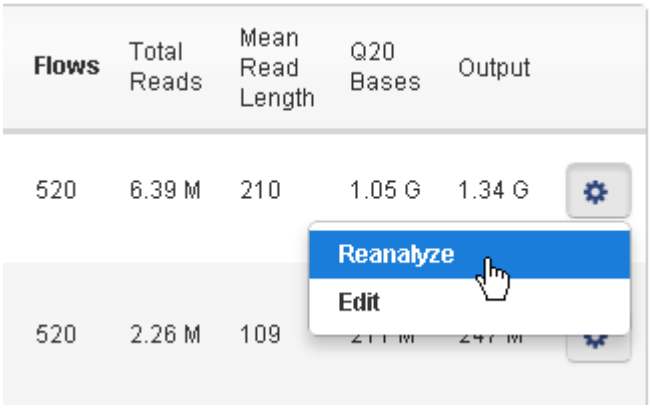
This section describes how to rerun an analysis with alignment to a different reference genome.

These steps create a new run report.

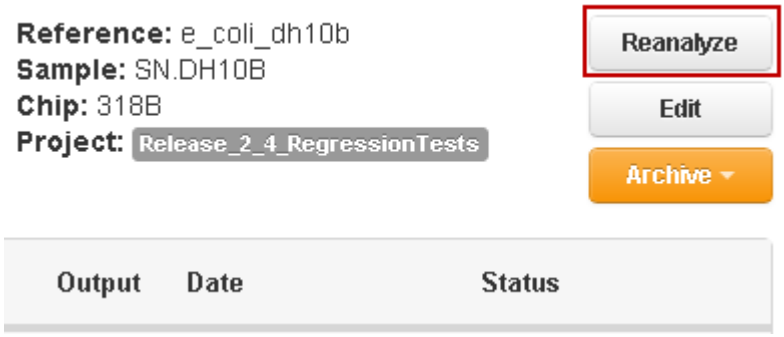
The Alignment plugin also realigns to a different reference genome, but does not generate a new run report.

1. Log in to Torrent Browser and click the **Data** tab **Completed Runs & Results** page. Go to the **Data > Completed Runs & Results** tab and find your run name.

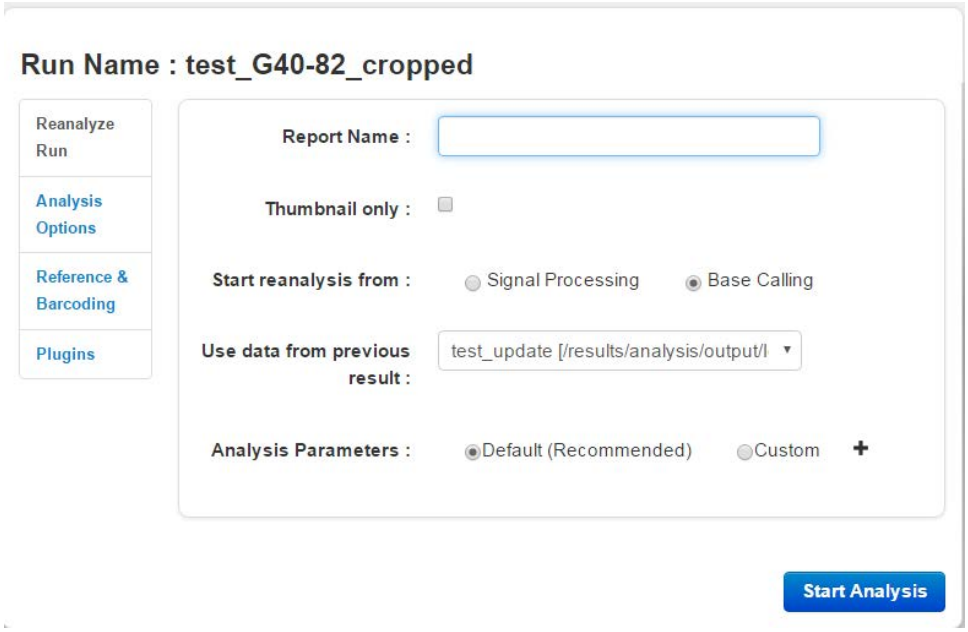
   In the table view, click the **Reanalyze** option in the gear menu on the right of the run entry:



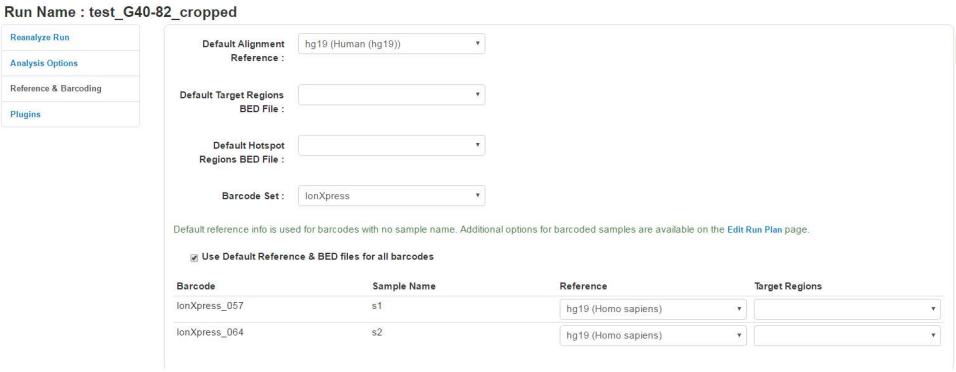
In the list view, click the **Reanalyze** button on the right of the run entry:



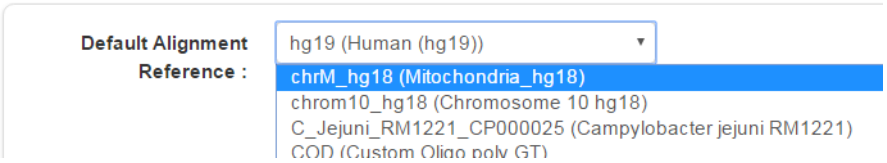
The main run analysis dialog opens:



2. 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. Click the Alignment Reference pull-down menu and select the reference for this run:



   This menu shows the references available in your Torrent Browser. Your list is different from the list shown here.

4. (*Optional*) Change other advanced options if required.

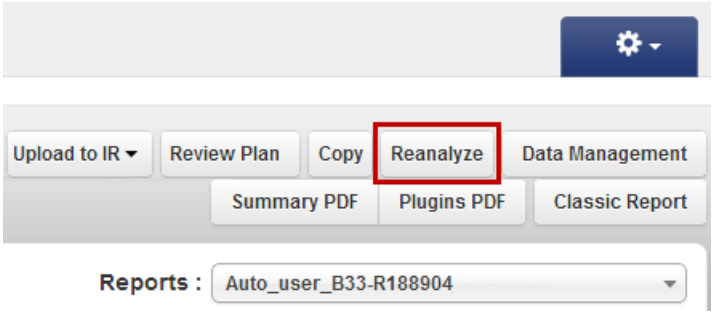
5. Click the **Start Analysis** button.

Reanalyze a run

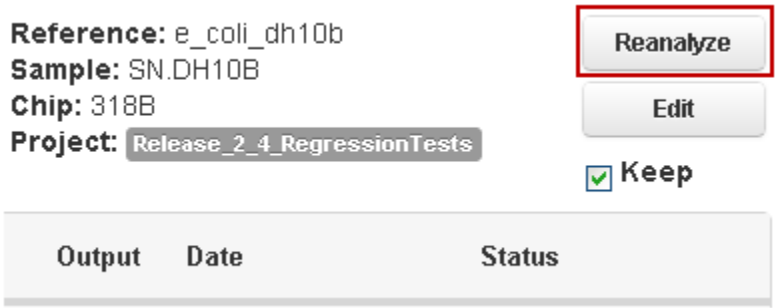
When you reanalyze, any setting changes you make affect only the current run (when you click the **Start Analysis** button). If you instead click **Edit**, your changes are saved for each subsequent reanalysis.

Start your reanalysis

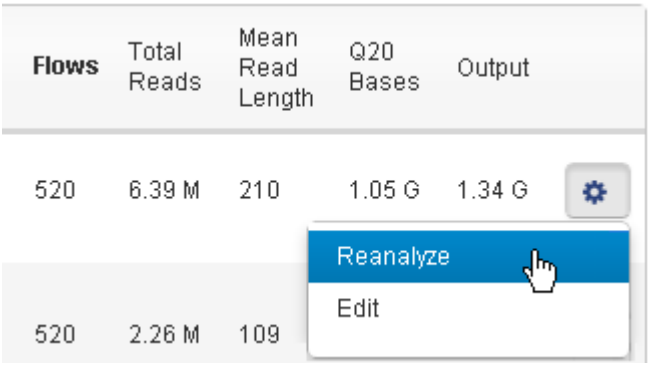
1. You select the Reanalyze option either from the Completed Runs & Results table or from a run report:
   a. In the run report of a completed run, click the **Reanalyze** button in the report header:



   b. In the **Data > Completed Runs & Reports** tab list view, click the **Reanalyze** button on the right of the run entry:

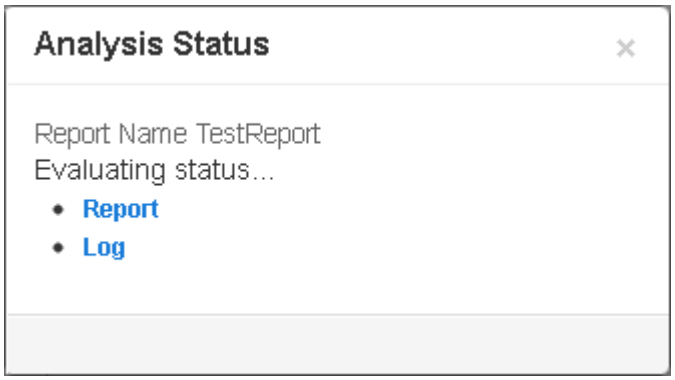


c. In the **Data > Completed Runs & Reports** tab table view, click the **Reanalyze** option in the gear menu on the right of the run entry:



2. Follow these steps to reanalyze your run:
   a. Use one of the Reanalyze buttons or menu options.
   b. Enter the Report Name for the new run (required).
   c. To rerun the analysis *with the same settings*, fill in the report name and click **Start Analysis**.

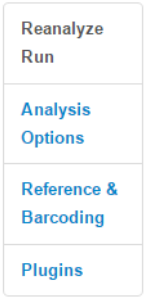
      To rerun the analysis *with changed settings*, click the tabs on the left panel to display the analysis options and make your changes.
   d. Click **Start Analysis** to start the run, which checks the analysis status before displaying the run confirmation message:



   e. View run progress by clicking **Report** or by selecting the run name in the **Monitor** tab.
   f. Click **Log** to view run progress details in text form. Click your browser refresh button to update the log.

Settings tabs

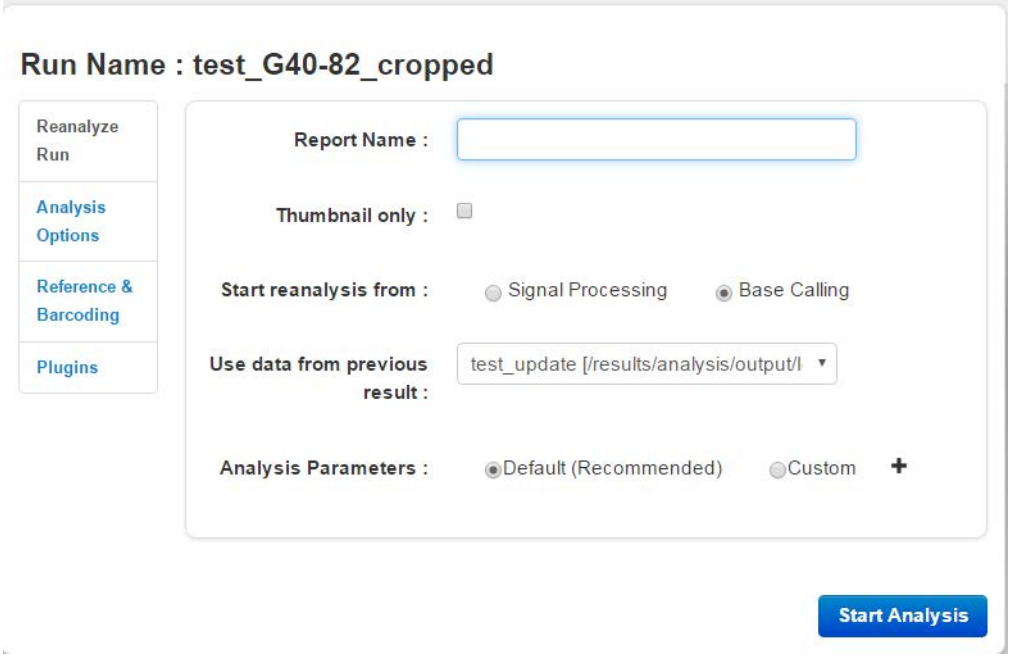
When you reanalyze a run, you have to option to change the run's settings. Settings are organized by tab in the left panel:



The settings for your original run are displayed when you open these tabs.

The Reanalyze Run tab

In the Reanalyze tab, you enter a name for the new run report and the reanalysis starting point:

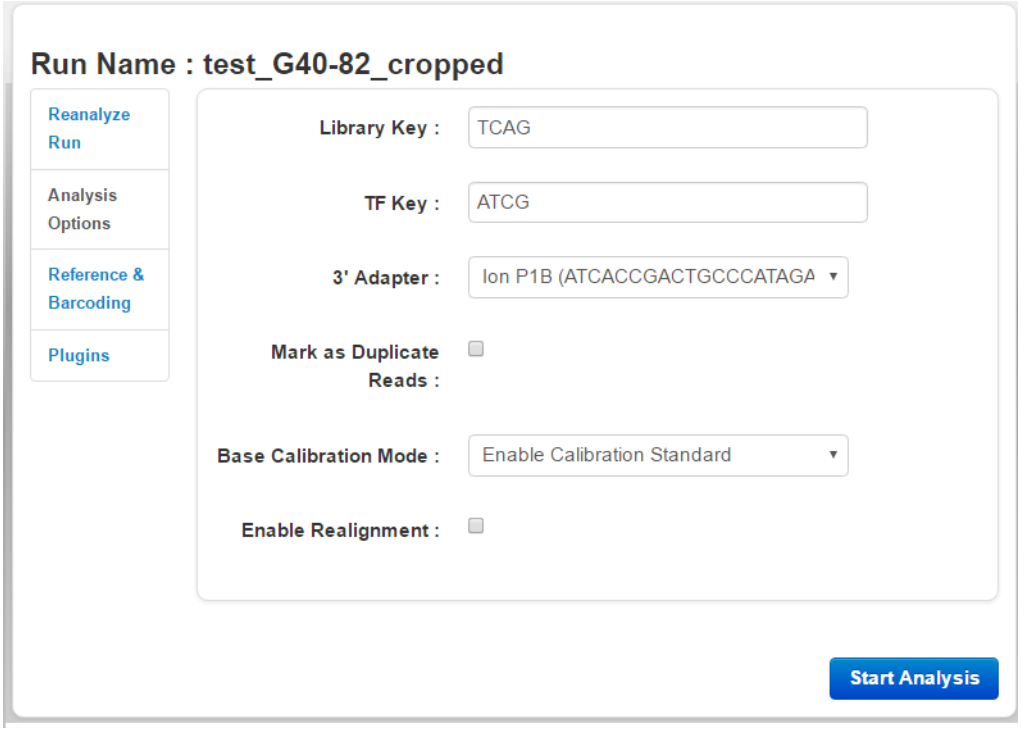


Setting	Description
Report Name	The name of the new run report (the result of the reanalysis).
Thumbnail only	Displays thumbnail view of report.

Setting	Description
Start reanalysis from	The Analysis Pipeline proceeds through three stages: Signal Processing, Base Calling, and Alignment. Normally report generation proceeds through all three steps, but 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 may wish to change the genome that is used for Alignment. After changing the genome for the experiment on the Runs page using the Edit field, you need to reanalyze data to produce a new report using the new genome. But since 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, and the report will be completed much more quickly. You can restart the analysis from these points: • **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 Custom Analysis Parameters" on page 526

The Analysis Options tab

An example Analysis Options page is shown here:



The Analysis Options tab contains these settings:

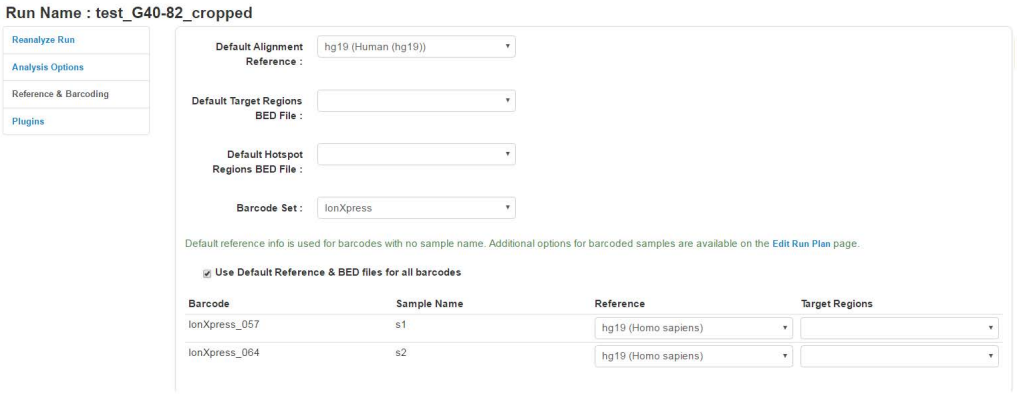
Setting	Description
Library Key	Sequence used to identify library reads. Example: "TCAG".
TF key	Sequence used to identify test fragment reads. Example: "ATCG".
3' Adapter	The name and sequence of the 3' adapter.
Mark as Duplicate Reads	Filter out PCR duplicates. Useful when reanalyzing combined BAM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

The Reference tab

The References tab contains these settings:

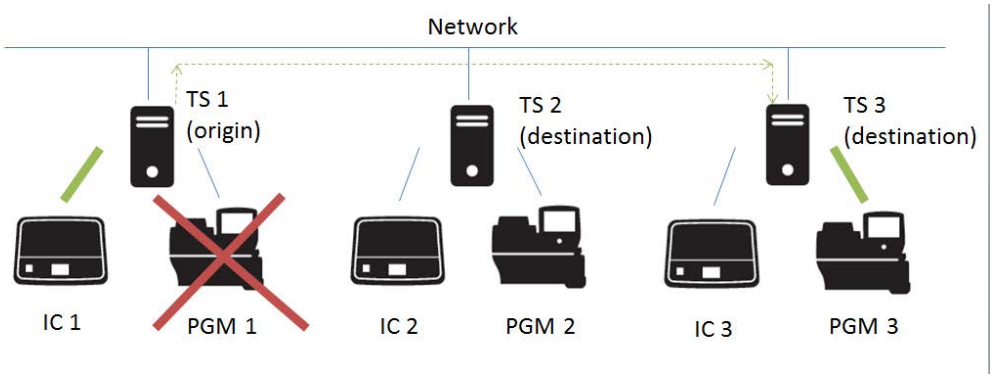
Setting	Description
Default Alignment Reference	The genomic reference to align to. Use this menu to change the reference used for alignment in the new analysis.
Default Target Regions BED File	Targeted regions of interest file. Analysis is restricted only to regions listed in this file.
Default Hotspot 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.

You can now select specific references for specific sample barcodes.



Planned run sharing among Torrent Servers

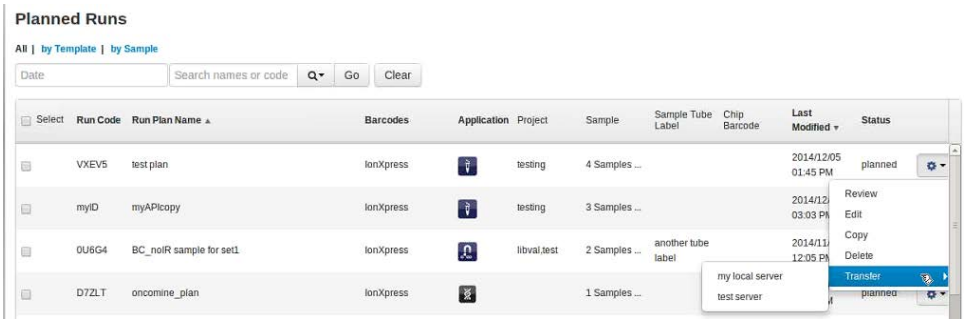
Planned run sharing among Torrent Servers was implemented in version 4.4. Previously, run planning was limited to the Torrent Server networked to your sequencer. Now, if you have multiple Torrent Servers and multiple sequencers, you can create a run plan on one Torrent Server and if the dedicated sequencer is offline, you can now transfer your planned run to another Torrent Server and run it on a different sequencer. However, this networking capability must first be set up by an administrator or by an Ion Torrent™ field service representative.



Using the diagram above 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). But if Ion PGM™ 1 (PGM 1) is offline, you can transfer the planned run to TS 3 and run it on PGM 3 (or transfer to TS 2 and run it on PGM 2, if also networked).

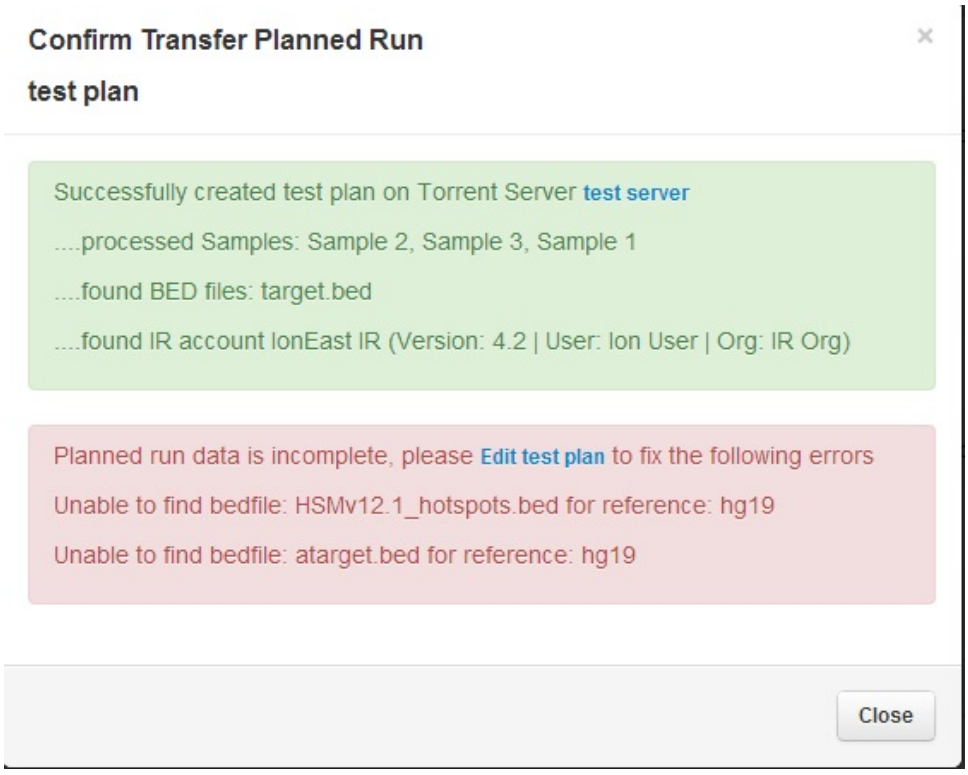
To transfer a planned run:

1. On the *origin* Torrent Server, create a plan for Ion Chef™, execute Ion Chef™ plan, and 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. From the Gear menu of the selected planned run, select **Transfer**. Then click the *destination* Torrent Server.



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

Note: You can no longer access this planned run on the origin server once 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 will be transferred if the copy is successful. However, you will need to edit the transferred run on the destination server and add the BED files or other missing data in order to have a successful sequencing run. Click the Edit [plan name] link in the confirmation page to fix 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).

Undo planned run transfer

If you find you transferred the planned run in error or else need to move the planned run back to the origin server, you can transfer it back to the origin server or have an administrator delete it from the destination server and restore it on the origin server.

- To transfer the planned run back to the origin server (user action):
  a. On the *destination* Torrent Server, browse to **Plan > Planned Run List** and locate the transferred planned run.

  b. From the Gear menu of the selected planned run, select **Transfer**. Then click the Torrent Server you wish to transfer the run back to.

- To undo the transfer of a planned run (administrator action):
  a. On the *destination* server, delete the transferred planned run from either Planned Run or admin pages.

     Note: If deleted from the Admin page, there will not be an audit trail for the transfer back, which could potentially make customer support efforts more time consuming.

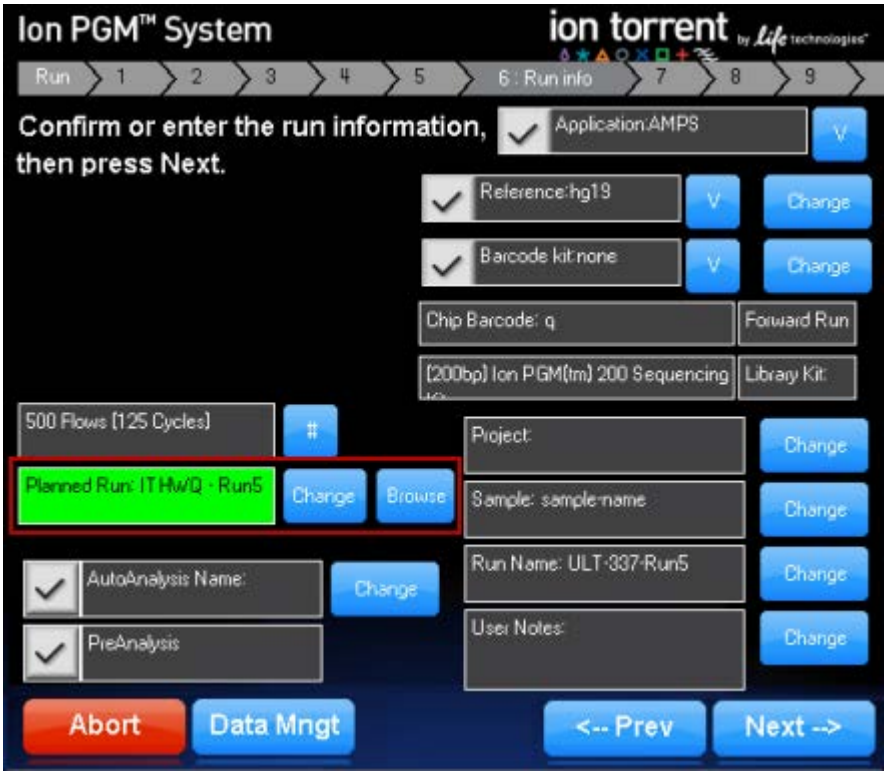
  b. On the *origin* server, located the plan on /admin/rundb/plannedexperiment/page:
     – Uncheck **PlannedExecuted**.
     – Change PlanStatus to **Planned**.

  This method does not require the destination server to be set up for plan sharing. Any changes made to the plan on the destination server are discarded.

Execute a run plan on your sequencer

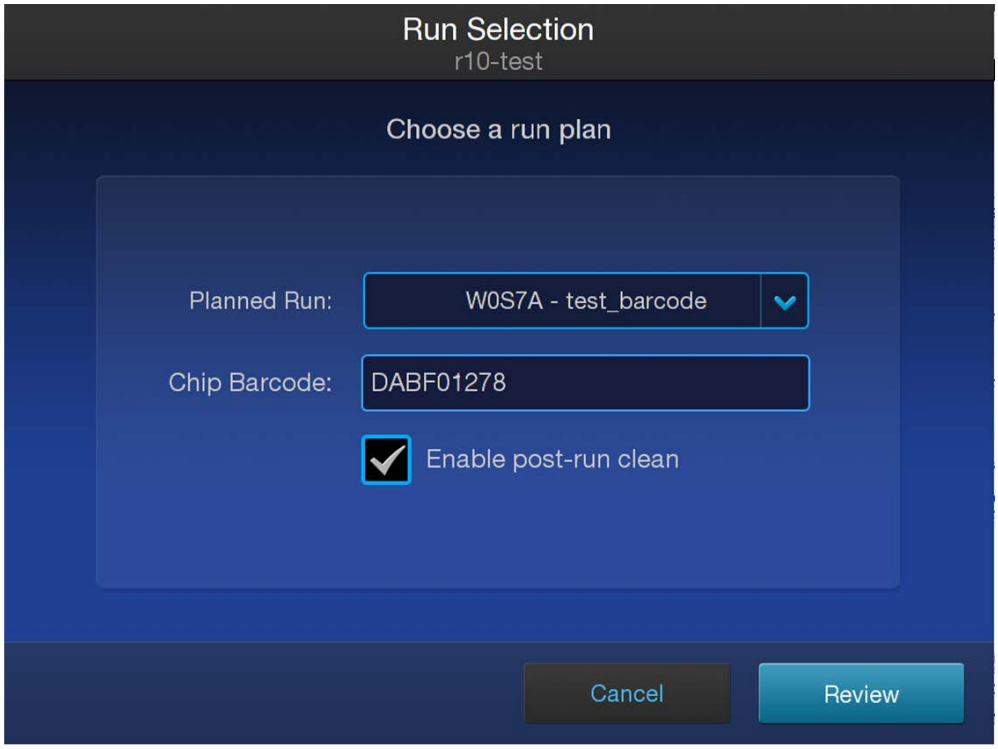
A planned run that you create in the Torrent Browser is executed on the Ion Torrent sequencer by selecting it from the sequencer's Run Info screen. With the **Browse** button you can select a planned run from a list of runs previously created on the Torrent Browser. The **Change** button allows you to select a planned run via its run code.

The pending run information is populated into the Run Info screen. 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 Info screen:

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



Monitor runs

Monitor tab

The **Monitor** tab provides you with information, optionally in thumbnail graphs as well as numerical format, to help you confirm that your current runs are working.

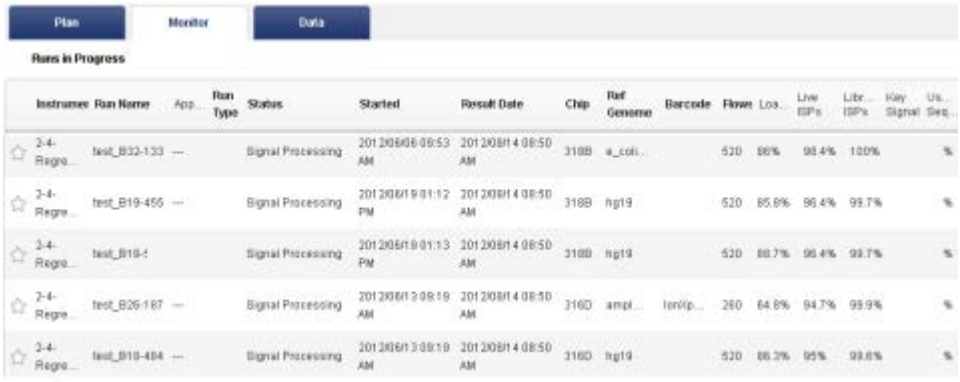
This tab provides data for you to know whether an in-progress run is working or not, through the following metrics:

- Beads loading
- Key signal
- Usable sequence

In the Monitor tab, you can also review the planned run settings for a run that is currently in progress on the sequencing instrument or the Ion Chef™ instrument.

Example monitoring metrics

Below is an example entry in Progress in the **Monitor ▸ Runs** screen:



With the Monitor tab List View you can see at a glance if any run quality metrics fall below the thresholds that you define in your template. Any metrics below threshold are shown in red in the thumbnail graphs.

Other information shown in Run in Progress entries are:

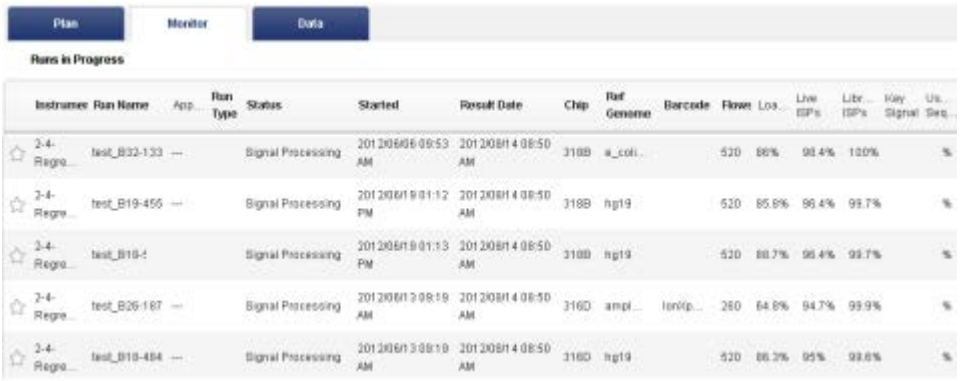
- The Sequencing run name
- Run information: started date, chip type, run type, and run notes
- A link to the run report
- Run status: In progress, completed, or terminated
- A link to the run plan for this sequencing run

- The number of flows transferred
- A flow transfer progress bar

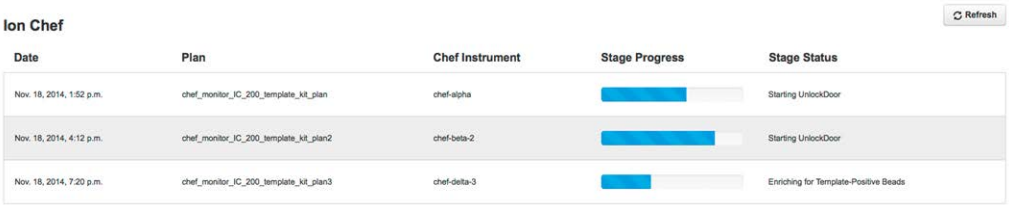
The Monitor tab also allows you to monitor Ion Chef templating runs.

Views

The **Monitor ▸ Runs** screen in Progress page supports a list view and a table view. The list view display 3 or 4 runs per page. The table view displays 1 run per row.



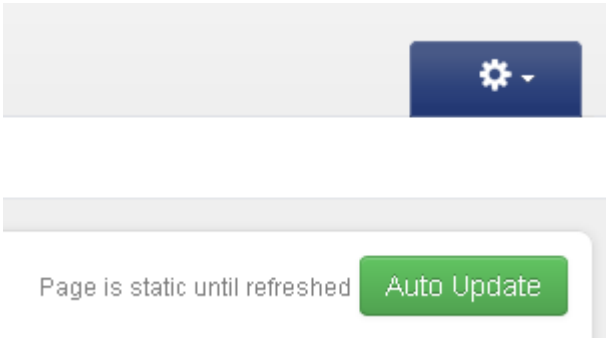
The **Monitor ▸ Ion Chef**™ screen supports a table view.



When you use the table view, you can sort the table by any of the columns in bold type. Click a column header to sort the table, and click the header a second time to reverse the sort.

Auto Update

Auto Update refreshes your page display whenever a new run is available to display. With Auto Update off, the page is a static display of information at the time you opened the page:



When Auto Update is on, the button changes to **Stop Updates**:

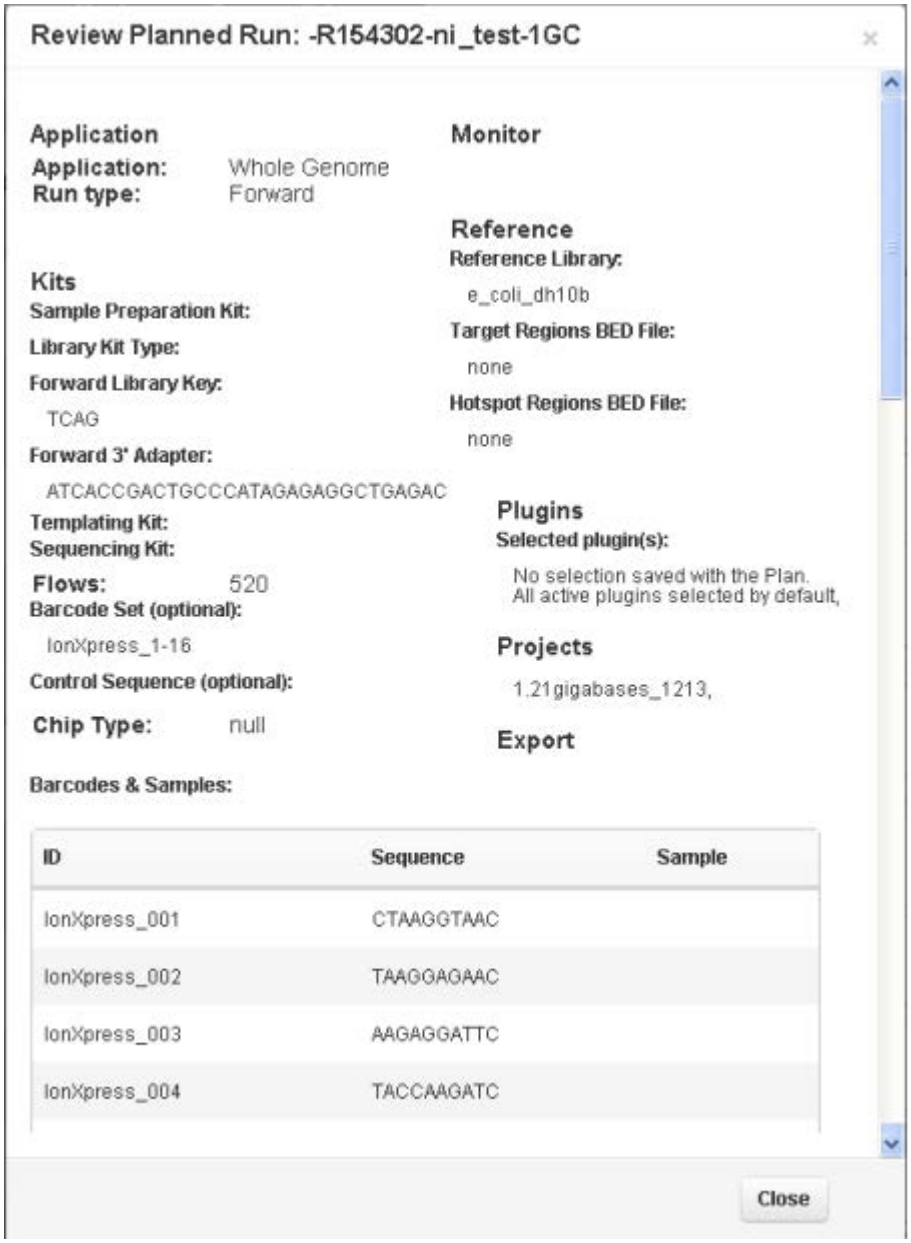
Review the planned run settings

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

In the table view, open the planned run review from the gear menu on the right of a table entry. The gear menu is only available on this page while the run is still in progress.

In the list view, click the Review Run Plan link. This link is only available while the run is still in progress.

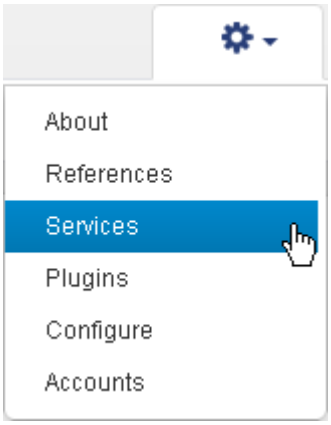
The following is an example of the display when you review planned run settings:



Terminate an analysis run

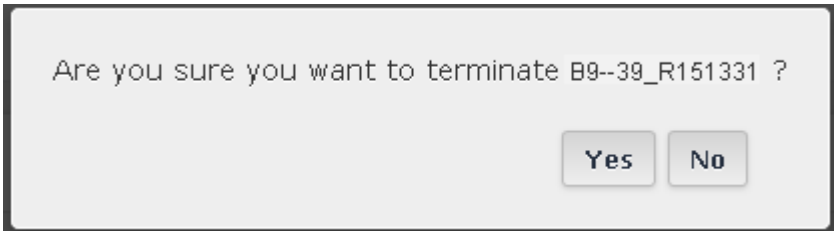
Use the following procedure to terminate an analysis job for a run that has started but not completed:

1. In the Torrent Browser, near the top right, click the Admin gear menu and click the **Services** option:

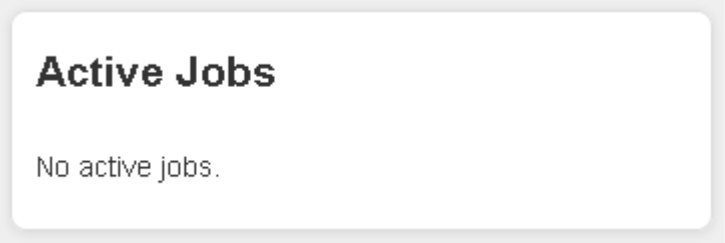


2. Scroll down to the **Active Jobs** panel. Find the run **Name** you want to terminate and click the **Terminate** button associated with the job (the **Status Message** column indicates **job is running**).

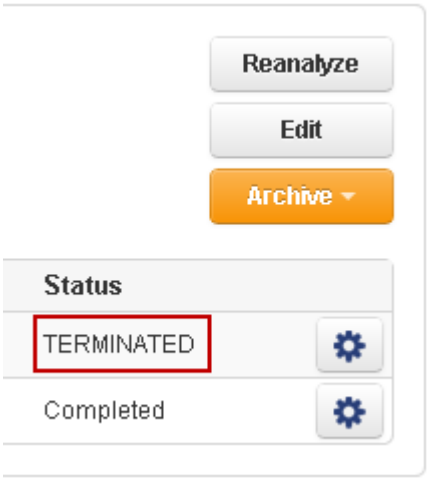
3. In the confirmation dialog, click **Terminate** to end the run or click **Cancel** to let the analysis job continue:



4. 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:



On the **Data> Completed Runs & Reports** list view, the deleted report shows a **TERMINATED** status:



You can always start a new analysis run.

Review data

Review data

From the **Data** tab, access the following functions.

"Manage completed runs and results" on page 67

"Create and manage projects" on page 75

"Data Management tab" on page 404

Manage completed runs and results

Navigate to the **Data** tab and click **Completed Runs & Results** to access the following functions.

- Click on the report name to access a detailed run report.
- Toggle between **Table View** and **List View** for alternative displays of the run information.
- *(Table view only)* Click a header to sort the table by that column. Click a second time to reverse the sort.
- Click **Download CSV** at the bottom of the page to obtain a spreadsheet of run information to your local machine.
- "Search for a run" on page 68
- "View the Data Management log" on page 69
- "Edit a run plan" on page 70
- "Reanalyze a run" on page 70
- "Add a run to a project" on page 72
- "Terminate an analysis run" on page 73
- "Change the analysis reference" on page 74
- "Change run metadata" on page 74
- "Add barcoding to a completed run" on page 75
- "Edit run metadata" on page 75

Search for a run

Navigate to the **Data** tab and click **Completed Runs & Results** to search for a run.

1. Enter a **Date**, **Run Name**, or **Sample Tube Label** in the fields provided.
   - Click in the **Date** field, and select from **Today**, **Last 7 day**, **This month**, or select a **Date Range** using pop up calendars.
   - Select **Run Name** or **Sample Tube Label** from the **Search names** drop down, then enter text in the **Search names** field
2. *(Optional)* Use the filters to narrow results by project, sample, reference, flow, chip, instrument, or results status.

   Click the **X** in a selected filter box to remove that filter and restore the search results that were filtered.
3. *(Optional)* Select the blue star checkbox to restrict the search results to starred runs.

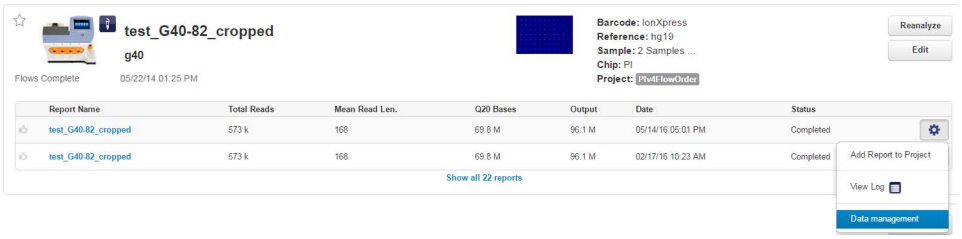
   Star any run by clicking the empty star next to the **Run Name**.
4. *(Optional)* Use the drop-down menu to order the results list by name, date, number of flows, etc.
5. Click **Go**.
6. *(Optional)* Click **Clear** to remove filters and restore all results.

Delete, archive, or export run data

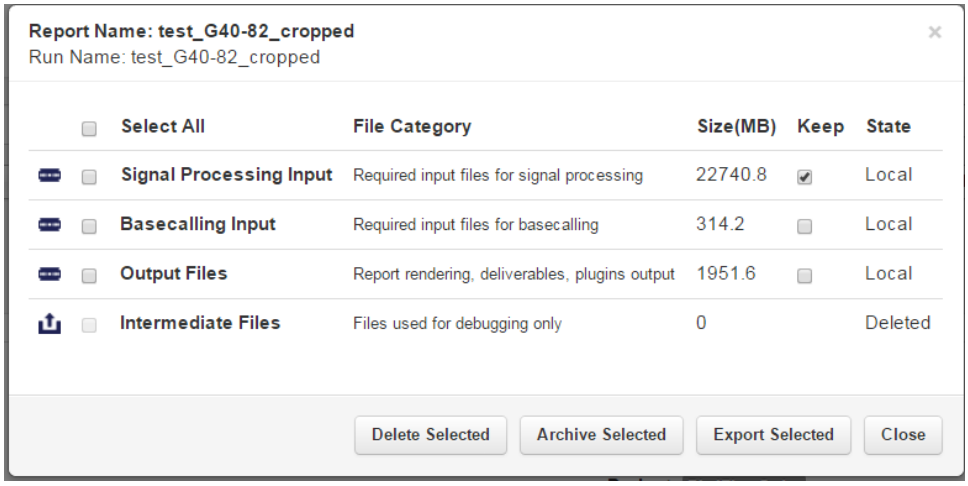
Navigate to the **Data** tab and click **Completed Runs & Results** to access the following functions.

1. Click **List View**.
2. Navigate to your record of interest.

   See “Search for a run“ on page 68 for help finding an individual record.
3. Click ⚙, then select **Data management**.



4. Select the file category or categories that you are interested in.
   These categories include:
   - Signal Processing Input - Required input files for signal processing
   - Basecalling Input - Required input files for basecalling
   - Output Files - Report rendering, deliverables, plugins output
   - Intermediate Files - Files used for debugging only



5. *(Optional)* Select the **Keep** checkbox next to any category.

6. Delete, archive, or export the selected files.
   - Click **Delete Selected**, add an optional comment, and click **Confirm**.
   - Click **Archive Selected**, choose either to use the configured directories or browse to a new one, add an optional comment, and click **Confirm**.
   - Click **Export Selected**, choose either to use the configured directories or browse to a new one, add an optional comment, and click **Confirm**.

View the Data Management log

Navigate to the **Data** tab and click **Completed Runs & Results** to access the following functions.

1. Click **List View**.

2. Navigate to your record of interest.
   See "Search for a run" on page 68 for help finding an individual record.

3. Click ⚙, then select **View log**.

A history log of Data Management actions taken on this run report displays, along with the associated date and user for each action.

Auto update runs and results

Auto Update refreshes your **Completed Runs & Results** page whenever a new run is available to display. Without Auto Update, the page is a static display of information at the time you opened the page. This feature is available in the **Data ▸ Completed Runs & Results** screen.

1. Click **Auto Update**.
2. Click **Stop Updates** to turn Auto Update off.

Reanalyze a run

If you want to reanalyze a run to correct a setup mistake or optimize parameters, navigate to the **Data** tab and click **Completed Runs & Results**.

1. Click **Table View**.
2. Navigate to your record of interest.
   See "Search for a run" on page 68 for help finding an individual record.
3. Click ⚙, then select **Reanalyze**.
4. Enter a name for a new run report.
5. Change any parameters of the run as appropriate. Possible items to change, include:
   - Start the analysis from **Signal Processing** or **Base Calling**.
   - Change Analysis Parameters. See "Configure Custom Analysis Parameters" on page 526 for more detail.
   - Add plugins or re-run plugins.

Edit a run plan

If you want to edit a run plan to correct a setup mistake or optimize parameters for all future re-analyses, navigate to the **Data** tab and click **Completed Runs & Results** to access the following function. This feature is available in **Table View** only.

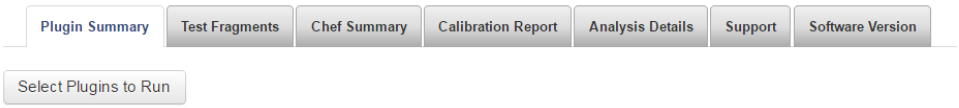
1. Click **Table View**.
2. Navigate to your record of interest.
   See "Search for a run" on page 68 for help finding an individual record.
3. Click ⚙, then select **Edit**.
4. Correct or improve any of the editable features, including:
   - Name
   - Custom Analysis Parameters
   - Reference
   - Ion Reporter account
   - Plugins

Manually run a plugin

You can manually run a plugin in the run report of a completed analysis run, with the **Select plugins to run** button. Only enabled plugins are listed. Follow these steps to manually run a plugin:

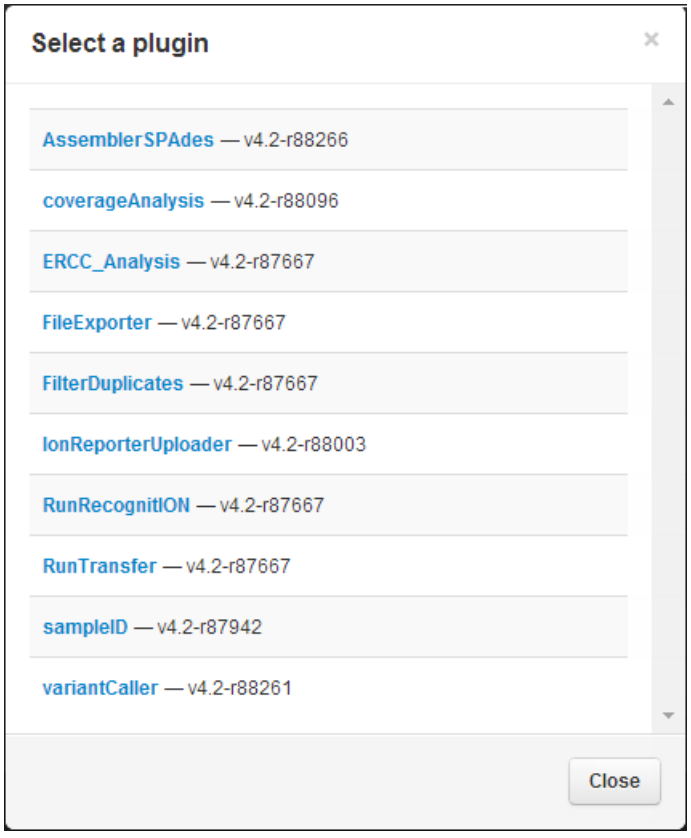
1. Go to the **Data > Completed Runs & Reports** tab, then click the link for your completed analysis run.

2. In the run report, scroll down to Plugin Summary tab.



   The Plugin Summary also lists any plugins that executed on your run (not shown in this example).

3. Click **Select plugins to run** to see the list of plugins available in your Torrent Suite™ Software.



4. Click the desired plugin name to run a plugin. If the plugin does not require user input, it starts immediately, without a confirmation screen.

5. Click **Close** to close the Plugin List without running a plugin.

Add a run to a project

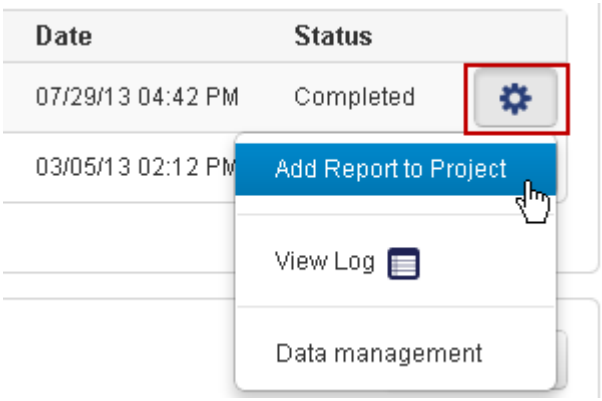
In the **Data** tab **Completed Runs & Reports** page table view, you can add a completed run to a project.

The following list describes advantages of grouping your results sets into projects:

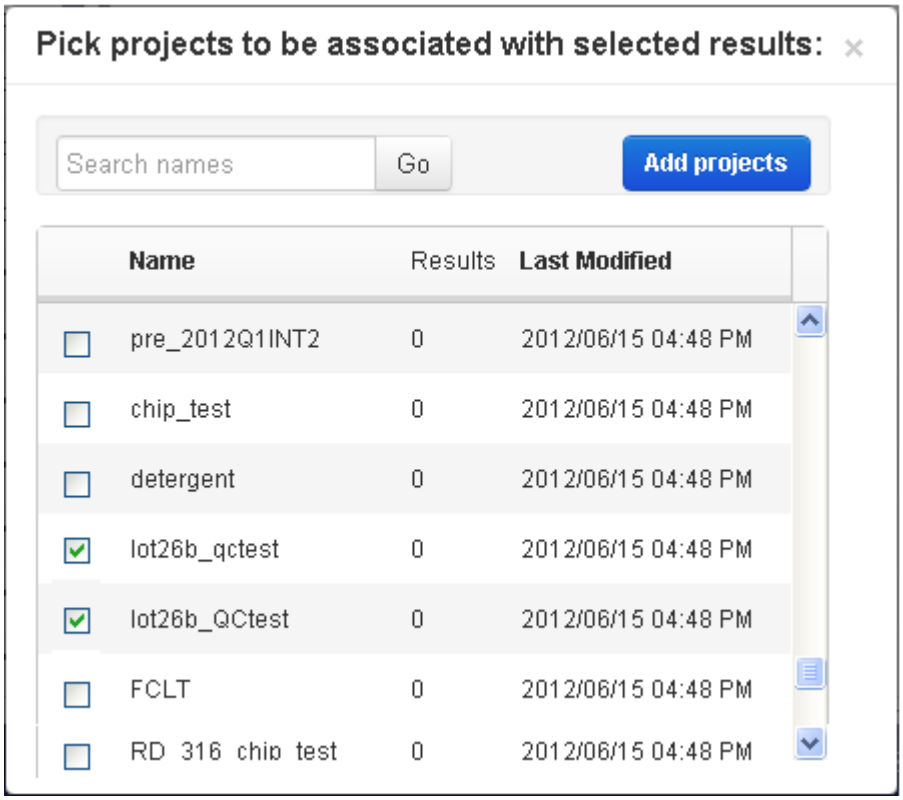
- Combine multiple result sets into one (useful to later analyze as a single run)
- Export result sets to another system for additional analysis
- Group result sets into projects for convenient tracking and bulk data management

Follow these steps to add a completed run to a project:

1. Go to the **Data > Completed Runs & Reports** page and click **List View**.
2. Find the run report you want to add to a project. For that run, click the gear menu on the right and select **Add Report To Project**:



3. The Pick Projects page opens:



4. Enable the checkbox for the project (or projects) you want the run report added to. Click the **Add projects** button. The run report is added to the selected project or projects.

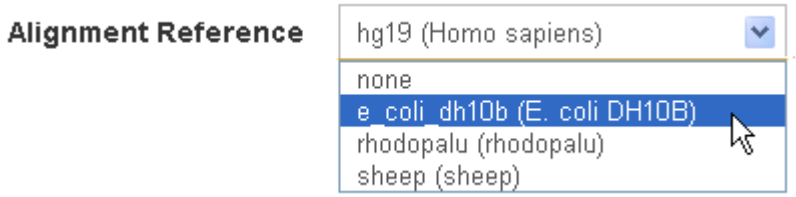
Terminate an analysis run

To terminate a run that has not yet completed, go to the **Services** option in the Admin menu and click **Terminate** to the right of the run name.

Change the analysis reference

Use the following procedure to change the reference for an analysis:

1. Follow the instructions in "Reanalyze a run" on page 70 to open the run analysis dialog advanced options.
2. Use the Alignment Reference menu to select the new reference:



   Note: If various references were selected per barcode in the initial run, use the lower per-barcode reference selection utility here as well.

3. Follow the instructions in "Reanalyze a run" on page 70 to save your selection and to redo the analysis.

Change run metadata

Select **Data ▸ Completed Runs & Results ▸ List View**, then click **Edit** on an individual run to edit the following run metadata.

- Sample name
- Application type (run type)
- Library kit
- Sequencing kit
- Chip identifying barcode
- Library key
- Notes
- Alignment reference
- Target regions BED files
- Hotspot regions BED or VCF files
- Plugins
- DNA barcode set (index)
- 3' adapter

IMPORTANT! You must restart or re-analyze your run for analysis-related changes to take effect. Changes to the sample name and to the notes take effect immediately.

When you change the metadata, you change the information in the run database. Because the analysis pipeline is initialized with the run database information at the time that an analysis starts, changing metadata does not affect an analysis that is in progress. For a running analysis, you must terminate the run and start analysis manually. For a completed analysis, you must re-analyze the run. The run report (in the Completed Runs & Reports tab) always shows the metadata in effect for the run. If

your changes are not shown in the run report, the changes were not in place at the time the report was generated. If you add or change an entry in the Notes field, that note does not appear in the run report unless you restart or re-analyze the run (even though the note does not affect the analysis results).

Add barcoding to a completed run

Use the **Edit** option to do either of the following:

- 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.

Edit run metadata

Follow these steps to change metadata for a run:

1. In the table view, click the Edit option in the run entry's gear menu. In the list view, click the edit button in the run entry.
2. In the Edit Run page, make your corrections to the metadata.
3. When you are done, click **Save**.
4. Restart the run:
   a. If the run is in progress, terminate the run and restart it.
   b. If the run is completed, re-analyze the run.

IMPORTANT! Your changes in the Edit Run page do not affect a run that is in progress.

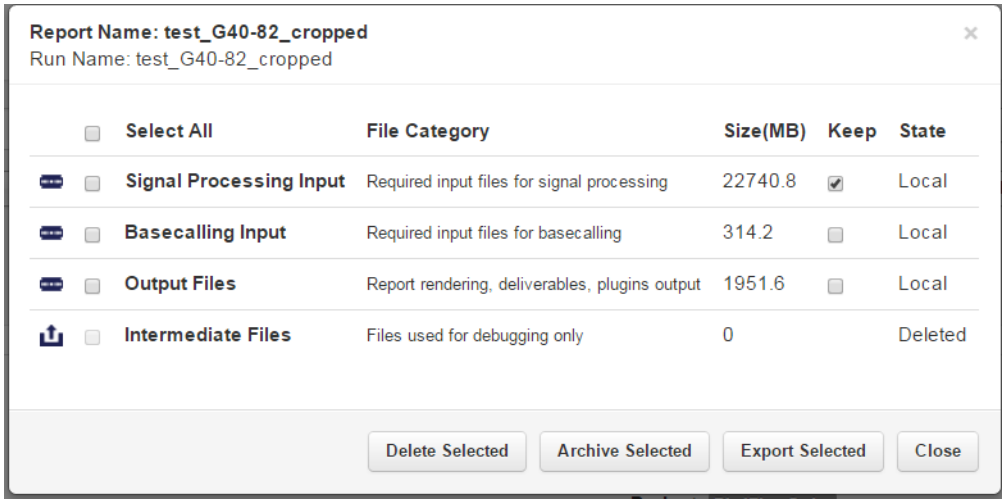
Note:

- The ChipBarCode field contains a chip identifier. The chip barcode should not be confused with chemical barcodes and barcode sets.

Create and manage projects

Data Management

Opens the Data Management on-demand menu so that you can archive, delete, or export files for the selected members of the project:



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, 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 login to see these files. Opening a report and removing the report name gives you a directory listing of all of 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.
`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 duration. The command-line passed to the Analysis program is also included, which is useful if you want to re-run the same analysis. These files are in subdirectories named `sigproc_results/block_*`.

Filename	Description
`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 pages. 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.
`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>/
```

Work with the database

User configuration information and experiment descriptions can be accessed and modified directly in the database, provided you have administrator privileges.

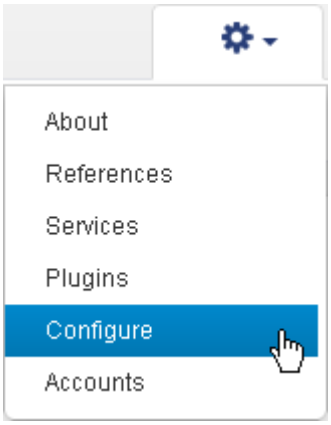
IMPORTANT! Care must be taken when modifying database items. Setting fields to incorrect values may corrupt the database or produce unpredictable results.

Access the database

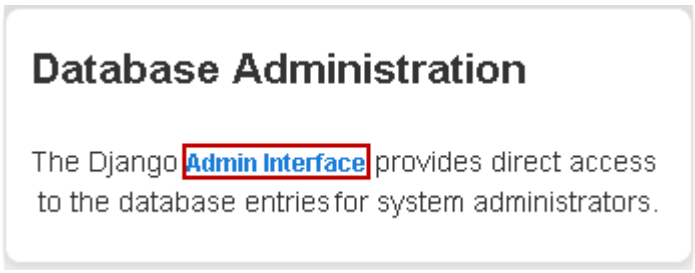
For all of the common database operations, you must begin by logging in to the database interface:

WARNING! Torrent Suite™ Software administrator-level user access is required to make changes to the database.

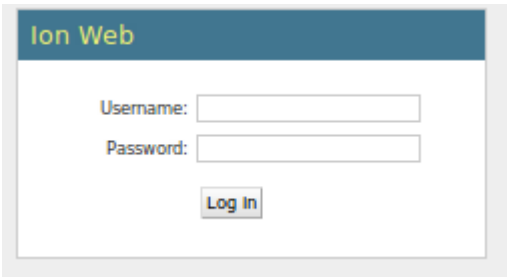
1. In the Torrent Browser, near the top right, click the Admin gear menu and click the **Configure** option:



2. In the Admin **Configure** tab, scroll down to the Database Administration section. Click **Admin Interface** to access database administration functions:



3. If you are prompted to log in, enter the administrator (`ionadmin`) **Username** and **Password**, and click **Log In**:

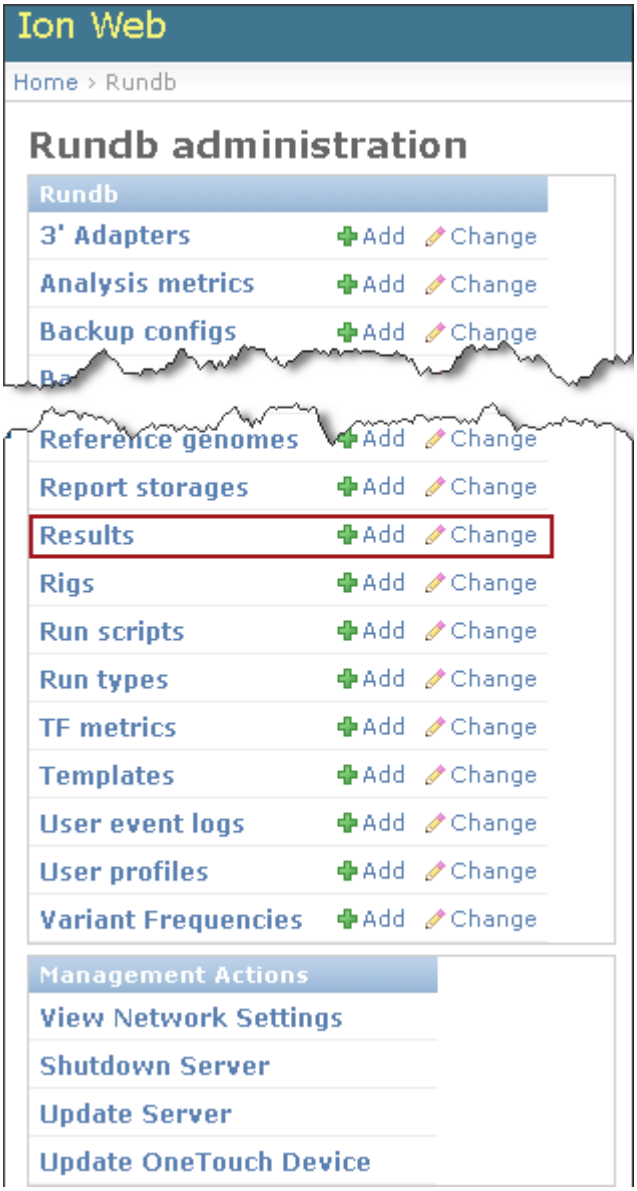


Change the report name

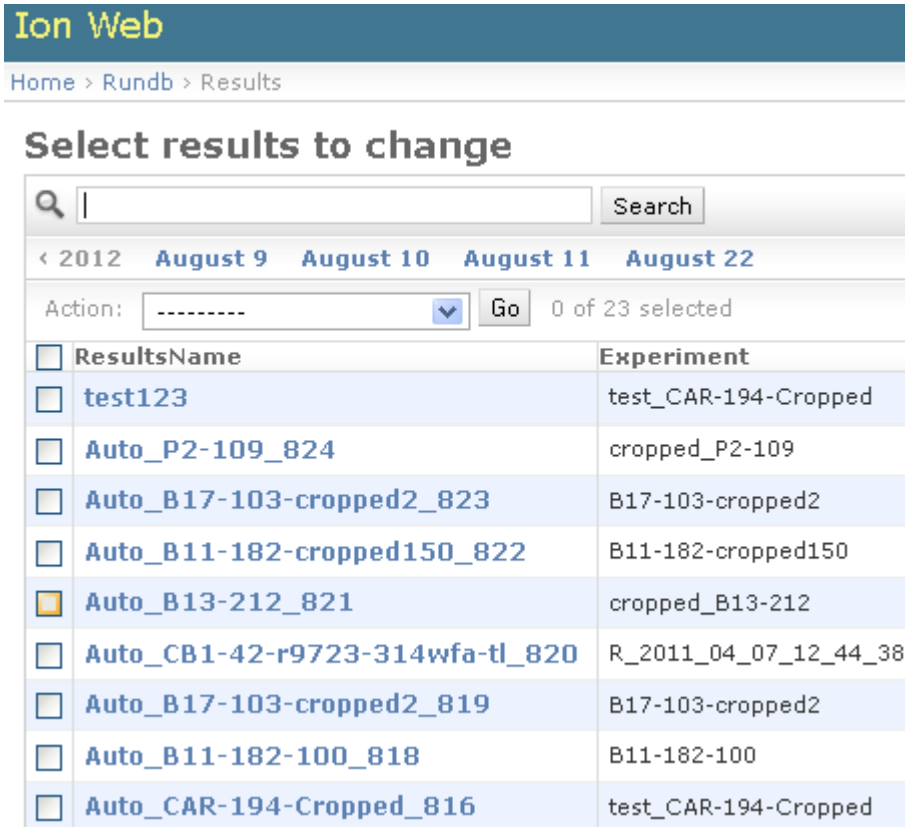
If you manually started an analysis and realize that you typed the report name incorrectly, you can change the report name using the following procedure. These steps require admin login.

IMPORTANT! It is not safe to change the report name while the report is being processed.

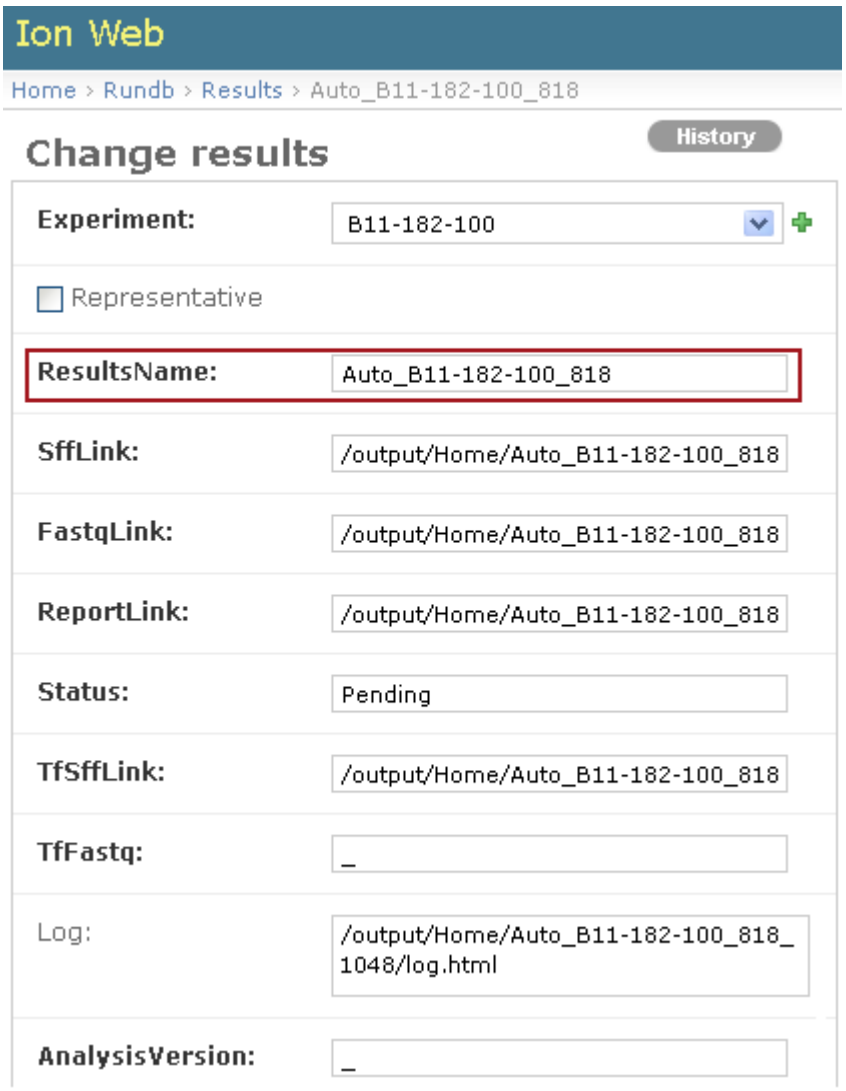
1. Select the **Results** dialog.



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.

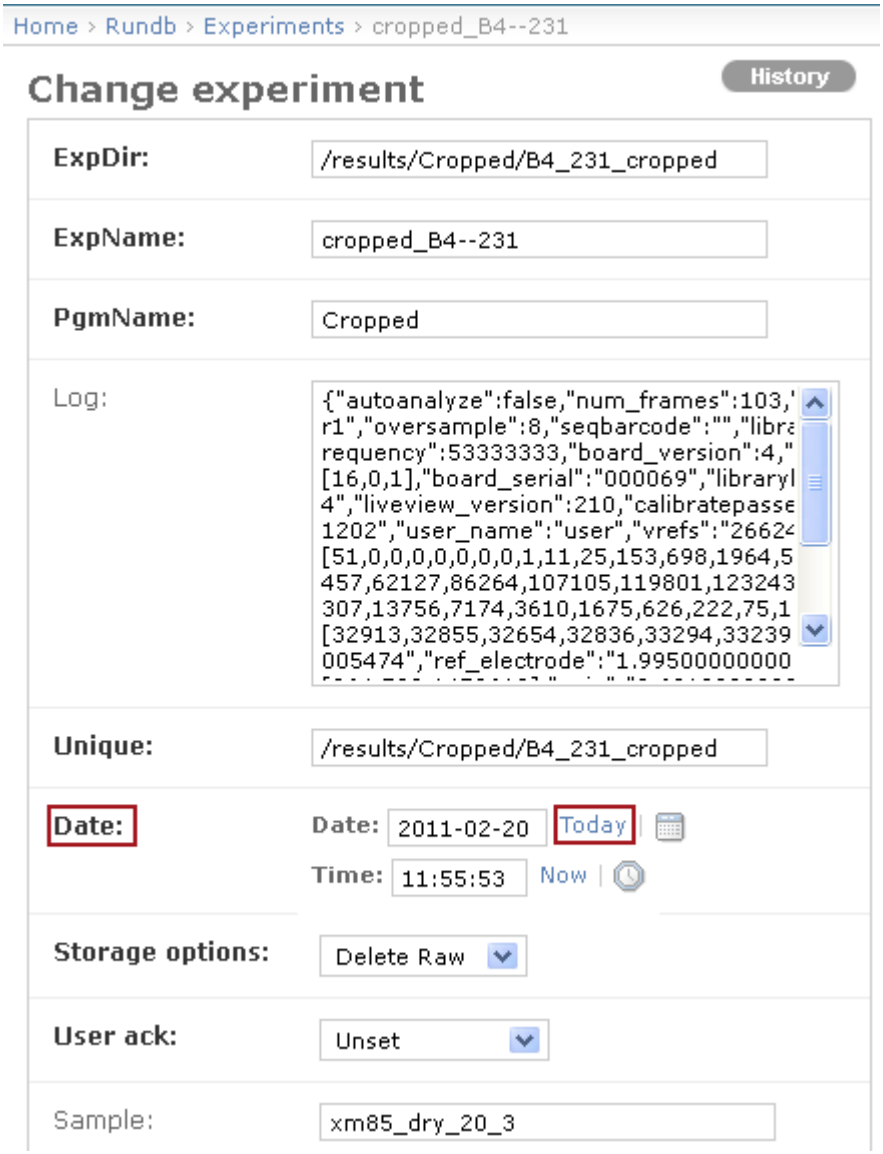
Use the following procedure to change the date for this run:

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:



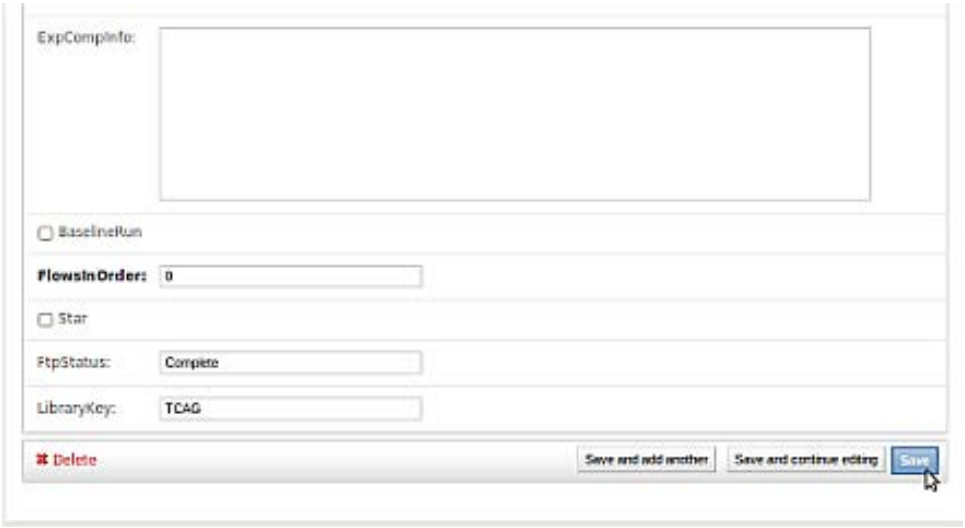
5. Use one of the following two options to change the date: a) Click the **Today** and **Now** buttons to set the **Date** and **Time** values to the current date and time in one click.

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.

Add or change a sequencer

Do not add or change a sequencer through the Site administration **Rigs** configuration item. During initial setup, sequencers automatically register themselves with the Torrent Browser. Changes to a sequencer are also communicated directly to the Torrent Browser.

Never delete a Rig using the Site administration **Rigs** configuration item. That action could corrupt the database.

The Site administration **Rigs** configuration item is *not* to be used:

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

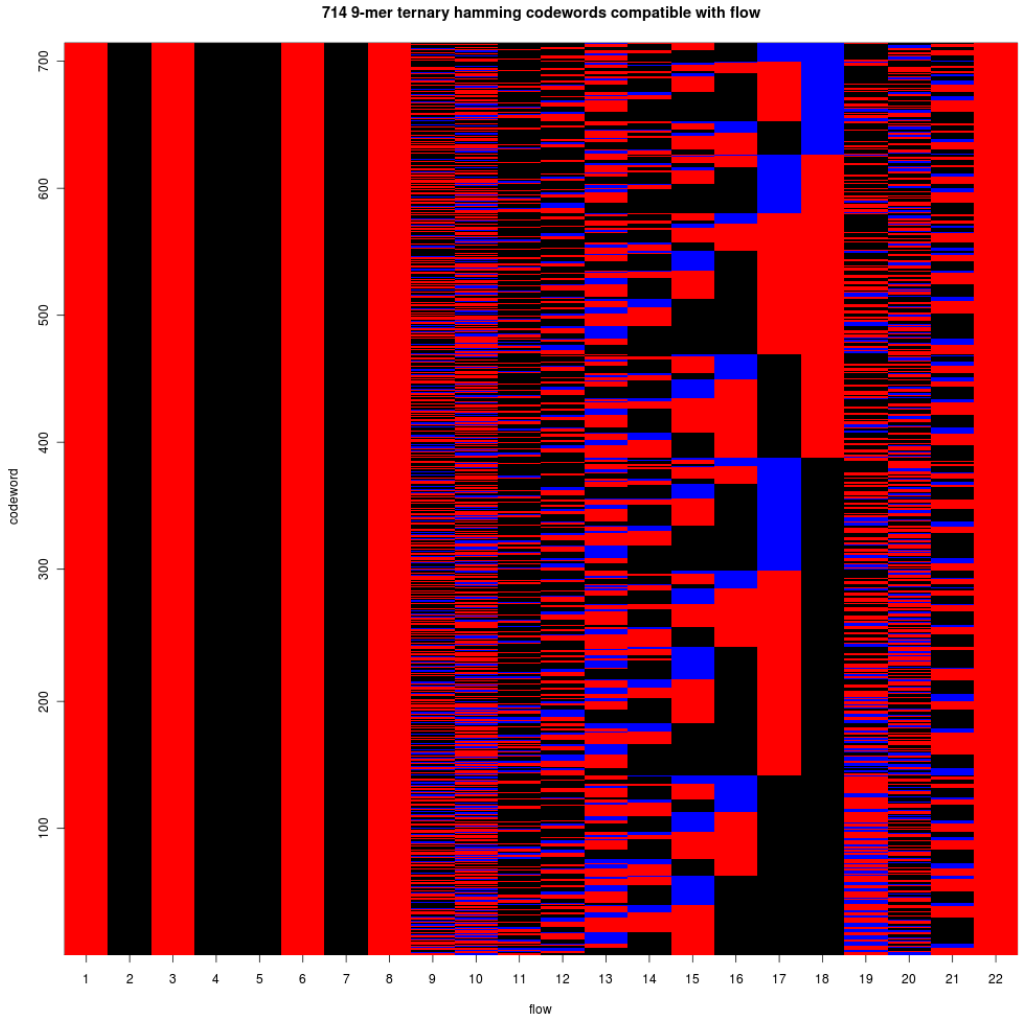
We designed Ion Torrent™ barcode sets to provide at least 1-error correction (hamming distance 3) in flow space for a large set of barcodes, and 2-error correction (hamming distance 5) for a usefully sized subset of such codes. This goal is accomplished by taking the ternary hamming code on 13 characters 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.



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.

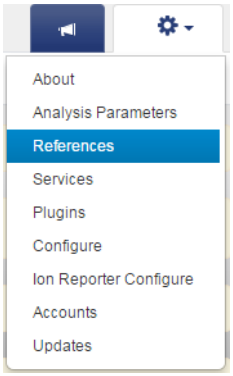
Your Torrent Suite™ Software comes pre-installed with several DNA barcode sets, including: Ion Code, ionSet1, ionXpress, ionXpressRNA, MuSeek_5prime_tag, and RNA_Barcode_None. These barcode sets are available for use on the Ion S5™, Ion PGM™, and Ion Proton™ Sequencers.

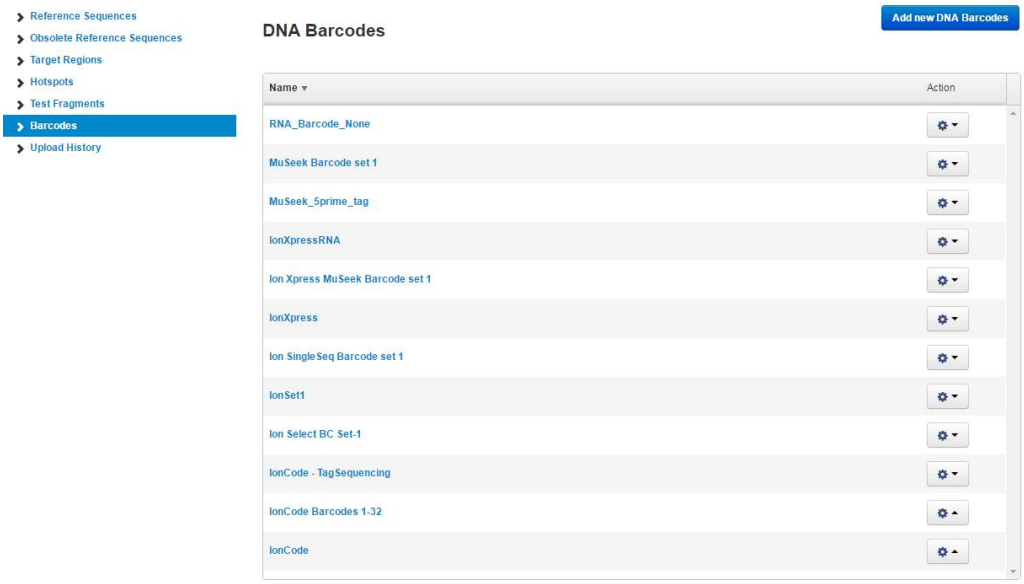
A barcode run on the Ion sequencer requires a sample-prep kit such as the IonSet1 or Ion Xpress barcode adapter kits. You select a DNA barcode adapter kit when you set up your Ion sequencer run. The barcode sequences for the IonCode, IonSet1, Ion Xpress, and Ion Xpress RNA barcode adapter kits are included 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 using the Torrent Browser Admin gear menu References option:





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

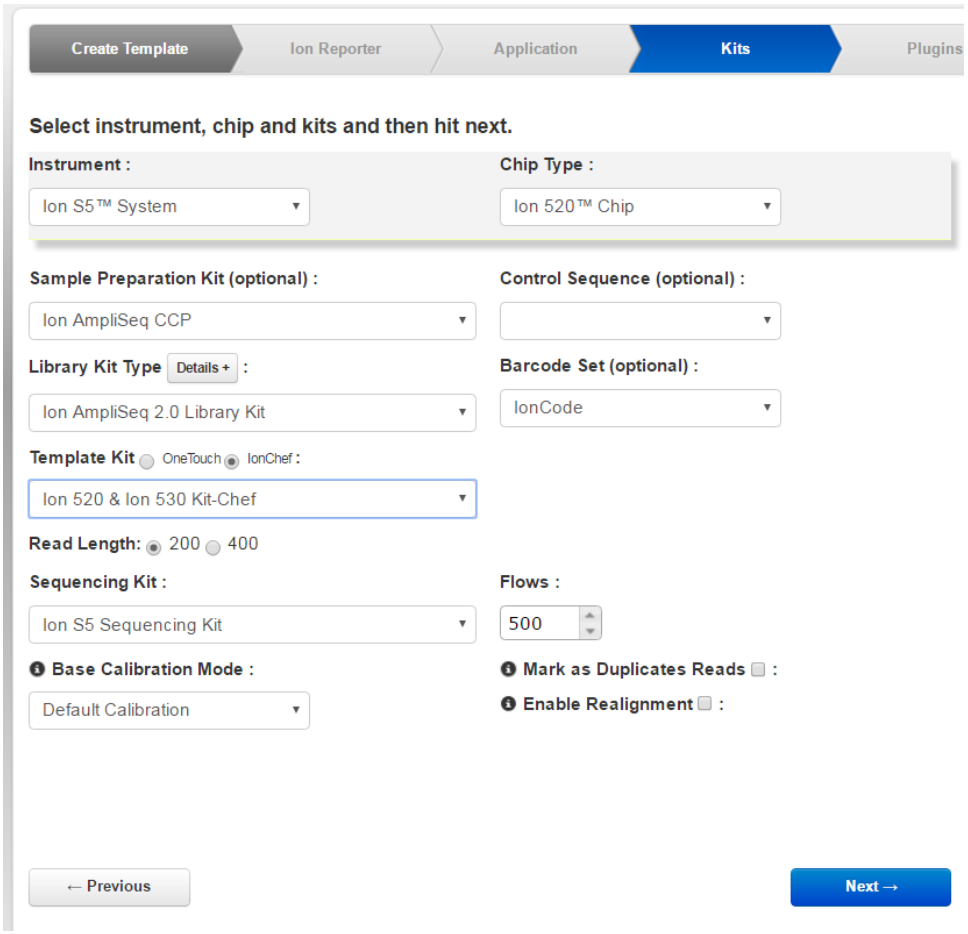
The same steps apply to a planned run (which is created from a template).

Follow these steps to set up a barcoded run in a template:

1. Click the **Plan** ▸ **Template** page, click **Add New Template** for the application group appropriate to your experiment.
   The Template wizard opens.

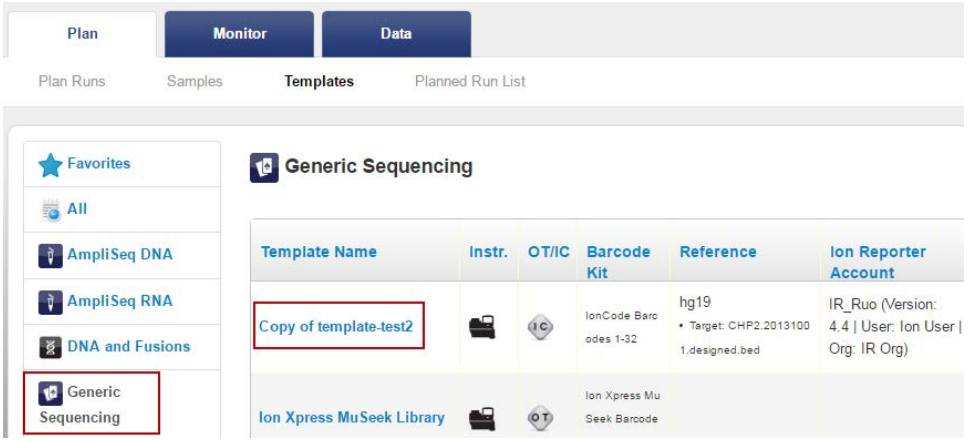
2. Select the correct application group and click **Next**.

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**.

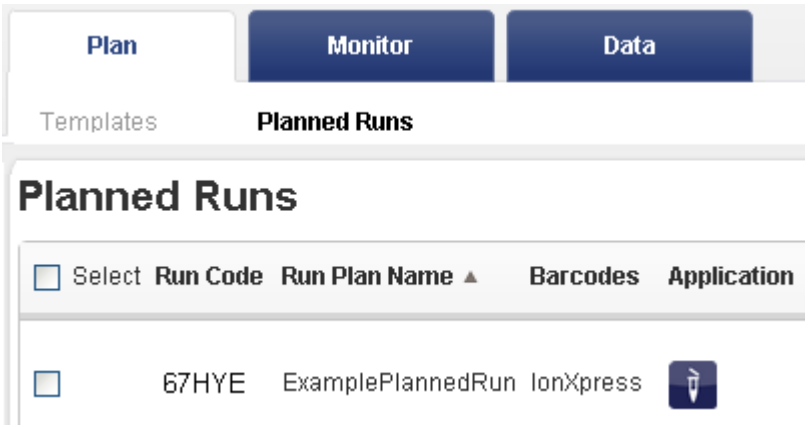
5. Your new template appears in the **Plan** ▸ **Templates** page, in the application group you selected.



6. To run on the Ion sequencing instrument, create a planned run from your new template. Click the Gear button > Plan Run option 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`

.

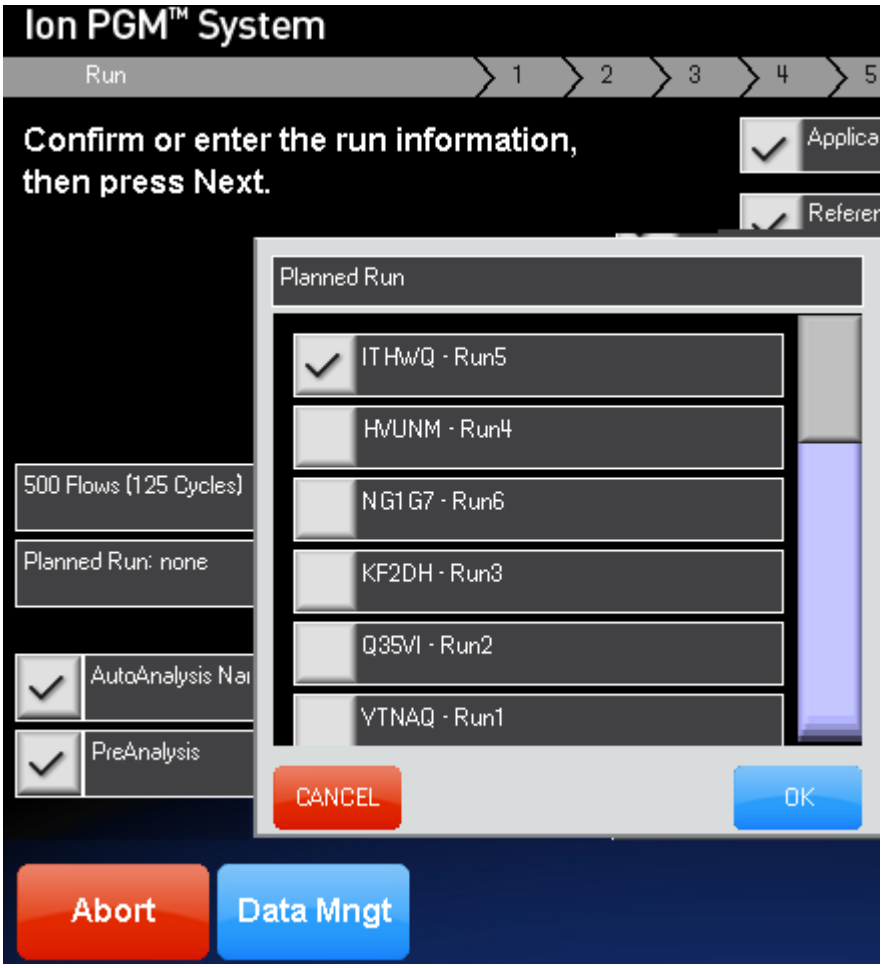
Start your planned run on the Ion sequencer

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.

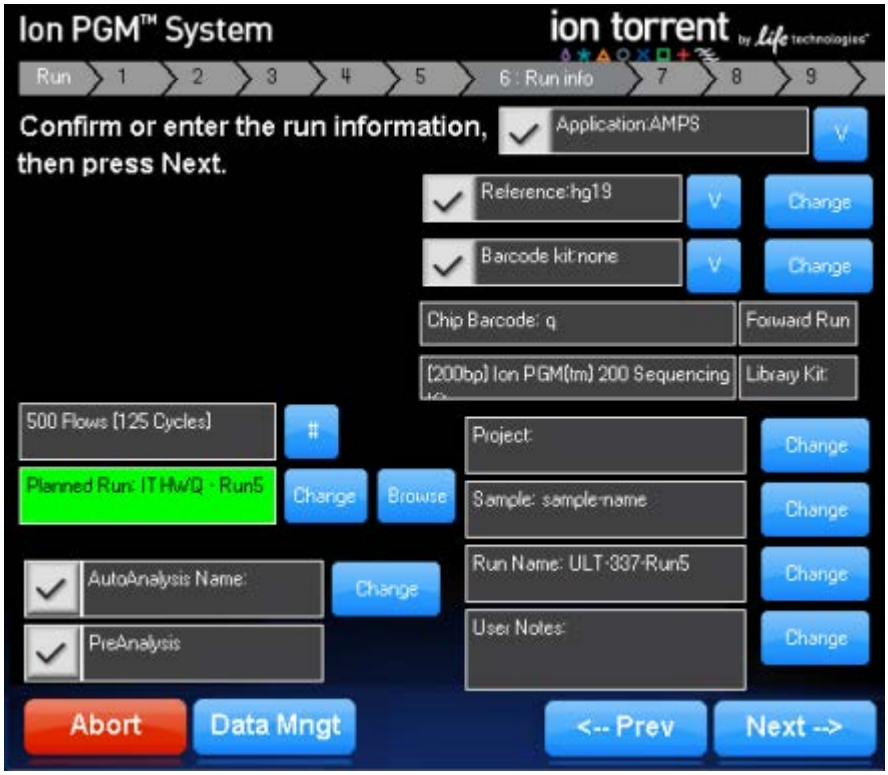
2. Click on the Browse button (near the middle of the screen, to the right of the Planned Run field).

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.

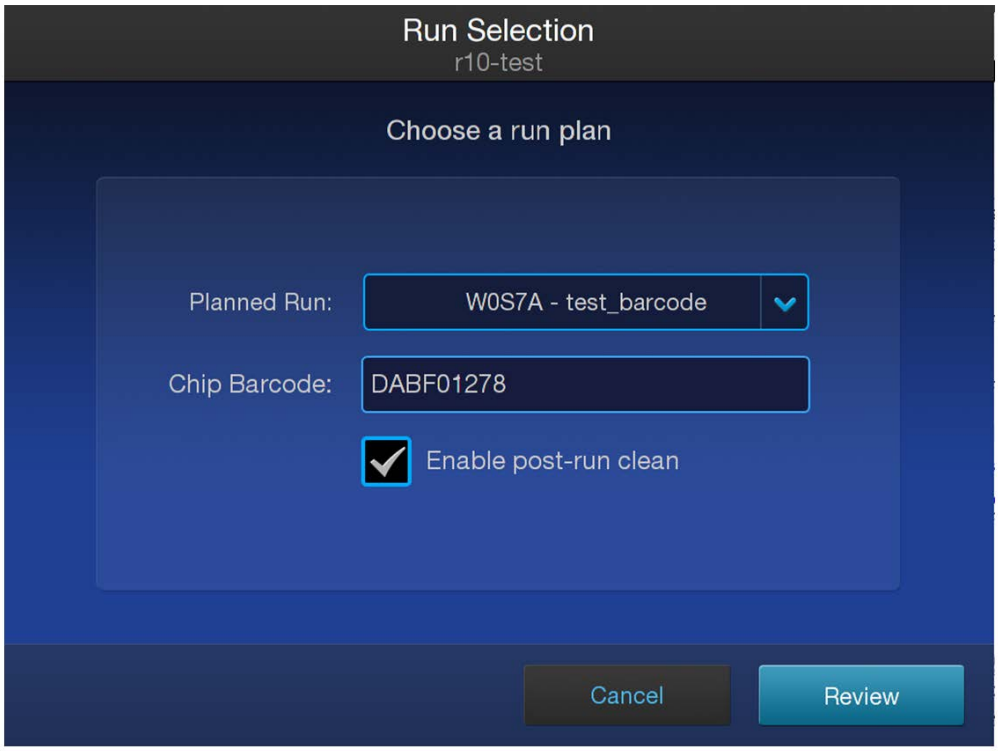
4. Click **Next -->** to start your Ion PGM™ Sequencer run, as usual. Approve your run on the confirmation screen.

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 zipped 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 zipped together.

IMPORTANT! The SFF and FASTQ file formats are not produced by the default analysis pipeline.

Plugin Support for Barcodes

The following plugins supports barcode libraries:

- Coverage Analysis
- Torrent Variant Caller

The following plugin does not support barcode libraries:

- Alignment

Scan your sequencing kit

Scanning sequencing kits is a part of the Ion S5™, Ion PGM™, and Ion Proton™ instrument setup workflow. The Torrent Browser template wizard (under the Plan tab) also supports specifying the sequencing kit when you create a template or a planned run.

Here is an example of the kit scanning page and keyboard during the Ion PGM™ system setup:

IMPORTANT! Scanning the sequencing kit is preferred to selecting a checkbox, because scanning provides more detailed kit information that can be used for troubleshooting or other purposes.

The choice of sequencing kit affects the nucleotide flows on the Ion sequencer.

The template wizard

Enter the sequence kit in the Torrent Browser template wizard, under the Kits chevron.

The Projects tab

With the **Data > Projects** tab, you control your data analysis and data management tasks.

Projects are simply groups of runs. You create and use these groups in a way that makes sense for your research. Projects are useful to hold runs for instance for the same laboratory project or runs that you will later handle in the same way (for data export or archival).

Projects are intended to be a convenience:

- You do not have to repeatedly search through the completed runs table to find related runs.
- You can perform data management tasks on many members of a project at a time.

In the Projects tab, you can access the main projects listing page and also the detail page for a single project (called called the project result sets page).

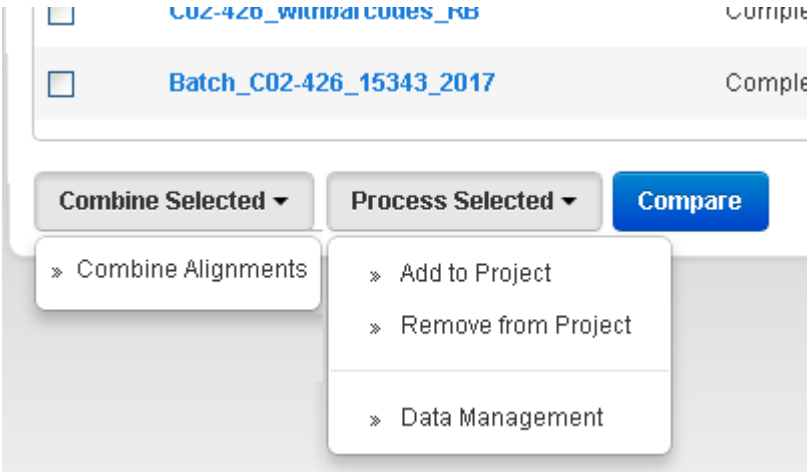
In the main projects listing page, under **Data > Projects**, you can do the following:

- Search the listing by project name or partial name
- Filter the listing by date (date range, current month, current week, current day, or specific date)
- Rename a project
- Delete a project
- View a history log for a project
- Open the result sets page for a project

In a project result sets page, under **Data > Projects > *projectname***, you can manually perform the following data management tasks. In one action, you can do these to a single result set (a single run), or to some or all of the result sets in the project.

- Combine multiple result sets into one (useful to later analyze as a single run)
- Archive result sets
- Prune results sets (remove some data from a result set)
- Export result sets to another system for additional analysis
- Group result sets into projects for convenient tracking and bulk data management
- Copy result sets to other projects
- Remove result sets from the current project
- Search the result sets for by name or partial name
- Filter the project display by date (date range, current month, current week, current day, or specific date)
- Download a CSV file of metrics for one or more analyses in the project
- Compare metrics for multiple run reports

These menus show the actions you can take on members of a project, from the project result sets page, under Data > Projects > *projectname*:



You can think of these options as acting on either the run report or the run's result set (or both).

The Projects Listing Page

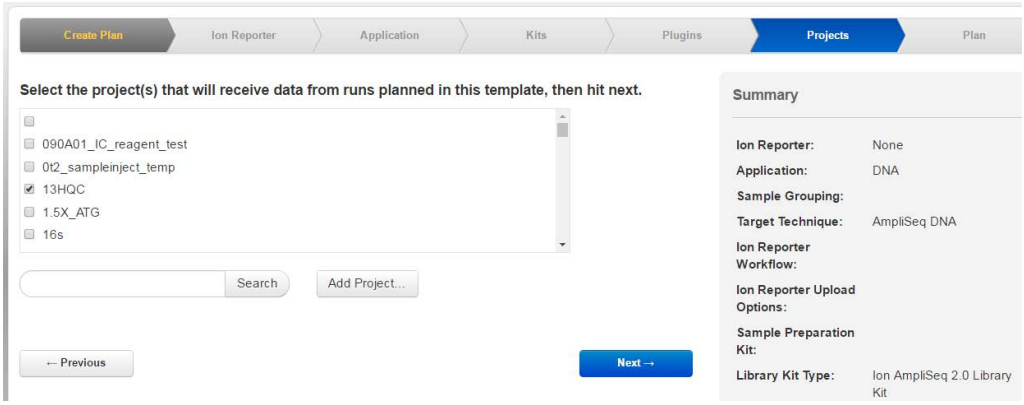
The **Data > Projects** page lists all projects.

Add a report to a project

Below are three ways to add a report to a project.

Before analysis

In the run plan wizard, you specify project names in the Projects chevron:

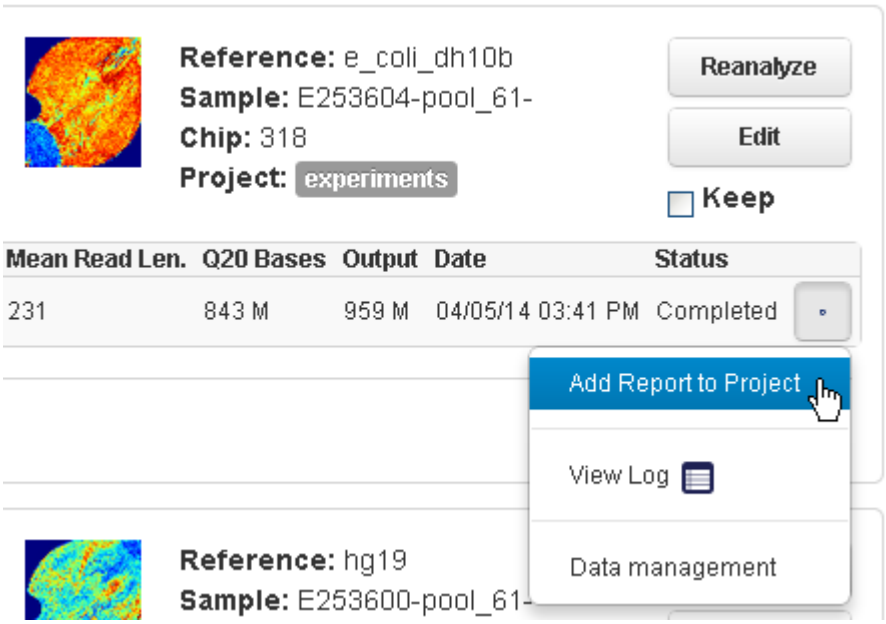


From a completed run report

After analysis, you add a completed run report to a project from the **Data > Completed Runs & Reports** page or from an existing project.

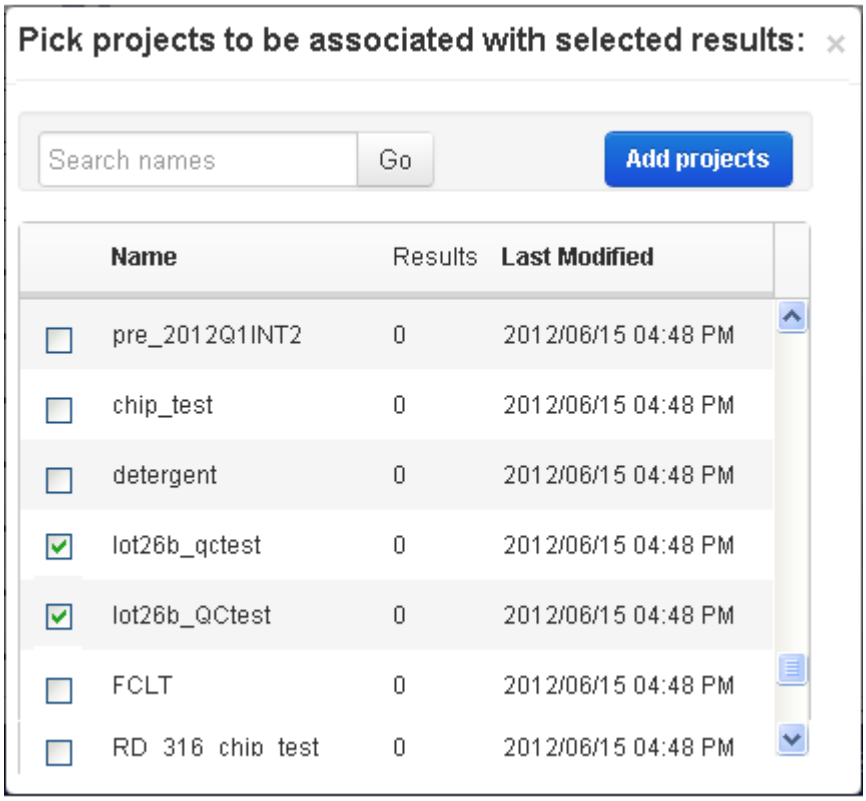
Follow these steps to add a report to a project from the **Data > Completed Runs & Reports** page:

1. In the list view, click the gear menu for the report and select **Add Report to Project**:



"Add to Project" is not available in the table view.

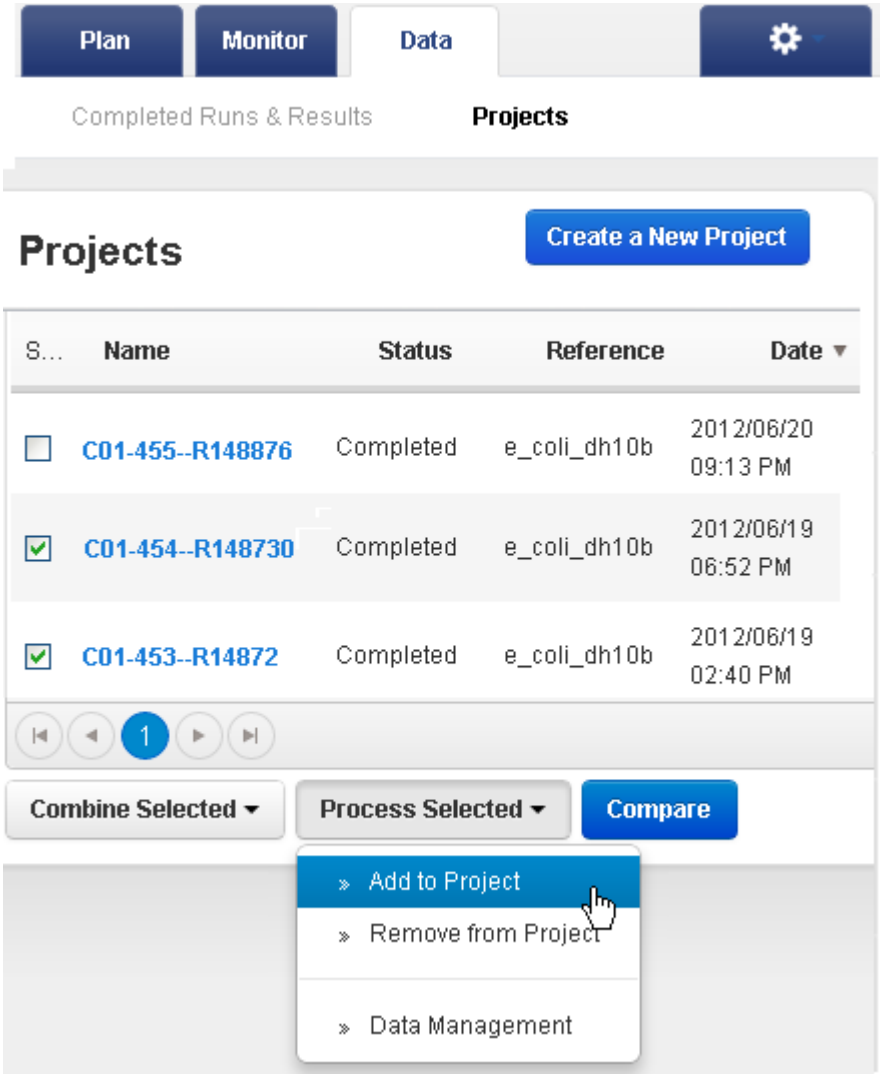
2. The Pick Project popup opens:



Enable the checkbox for the project or projects and click the **Add projects** button. The report becomes a member of the project or projects that you select.

From an existing project

From an existing project, click the **Process Selected...** button and select **Add to Project**. Then follow the Pick Project popup step described above.



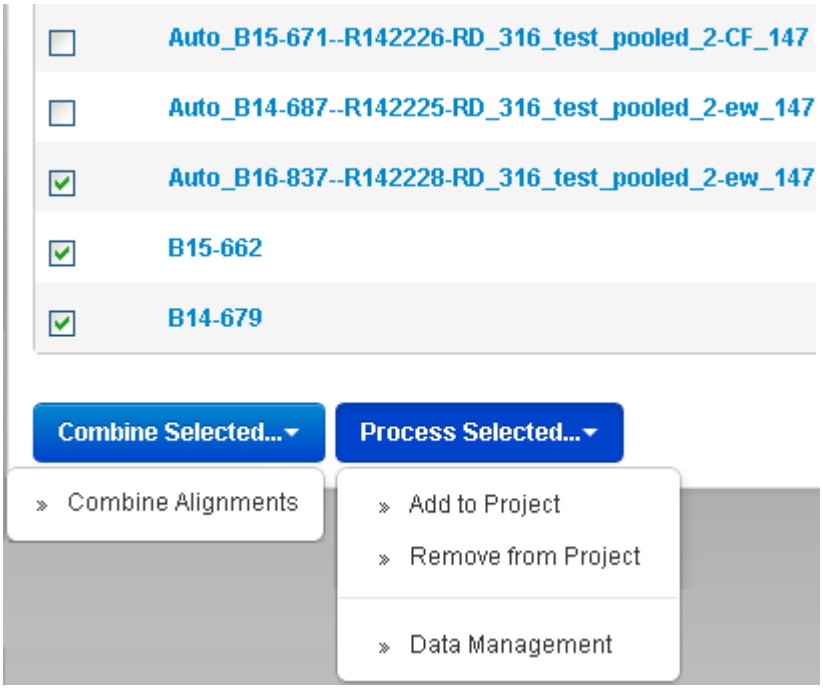
Download a CSV file of metrics

In a project's Results Sets listing, you can generate a CSV file of analysis metrics and compare results across analyses.

To generate the CSV file, first select the checkboxes for the analyses, then click the **Download Selected CSV** button. The button is inactive until at least one analysis checkbox is enabled.

Project menus and actions

These menus show the actions you can take on members of a project:



You can think of these selections as acting on either the run report or the run's result set (or both).

In each case, you first select the reports' checkboxes, then select the menu action:

- **Combine Alignments.** Combines reads aligned from multiple run reports. The resulting data set can be treated the same results from a single analysis run, for instance to export or to use as input to a plugin. Intended for use 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.
- **Add to Project.** Adds the selected result sets to other projects.
- **Remove from Project.** Removes the selected result sets from the current project. (Does not delete the run report.)
- **Data Management.** Opens a data management popup you can use to archive, delete, or export files from the selected runs.

Actions on members of a project

The **Combine Selected...** and **Process Selected...** menus show the actions you can take on members of a project. You first select the reports' checkboxes, then select the menu action.You can think of these selections as acting on either the run report or the run's result set (or both).

Mark as Duplicates Read

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

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

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

Add to Project

Use the **Process Selected... > Add to Project** option to copy the selected result sets to one or more other projects. In the next screen, the project selection screen, enable the checkbox for each project that the selected result sets are to be copied to. Use the page 1 navigation at the bottom right to scroll through the list of project names.

The **Add to Project** option adds the selected result sets to the other project (or projects). The result sets are not removed from the current project.

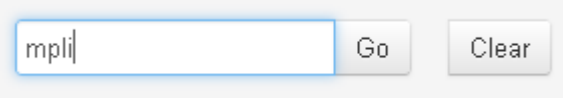
Remove from Project

Use the **Process Selected... > Remove from Project** option to remove the selected result sets from the current project. Be careful when using this option it does not give you a confirmation screen before removing the projects.

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

Search

In the Data > Projects page, you can search for project names and in a project details page you can search for run report names. The search field behavior is the same in both cases. After you press **Go**, the displayed information is limited to only names which match or contain the search string.



The search field takes a complete or partial name. For example, the following project names match the search string "mpli": amplicon, amplicon33, AmpliSeq, Samplier.

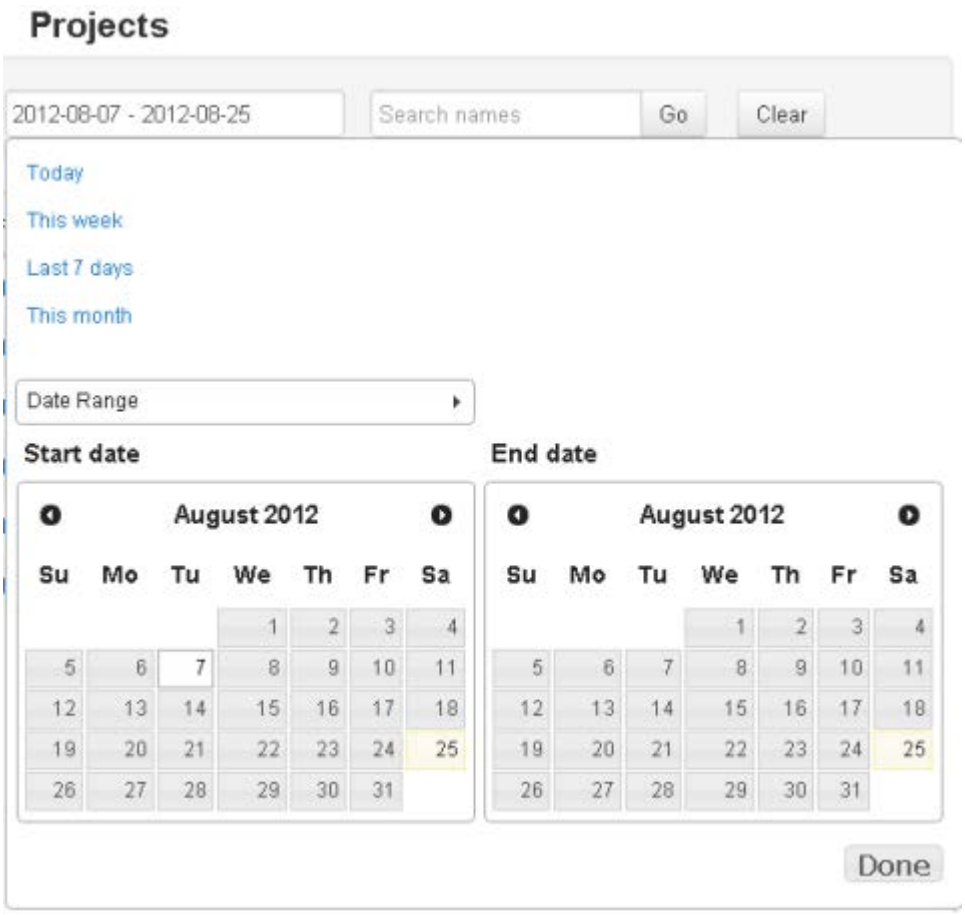
The search is not case-sensitive. Wildcards are not supported in the search string.

Click the **Clear** button to cancel the search and display unfiltered results.

Filter by date

The date filter controls are the same on both the Data > Projects tab and in a project details page.

The date field opens a menu with preset choices or a date range picker:



The selection you make in the Date field takes effect immediately (depending on server load). You do not need to click the search Go button.

- The **Today** selection enters the current date in the Date field and limits the run table display only to jobs with a run date from the current date.
- The **This week** selection uses a date range from Monday to the current day.
- The Date Range selection opens two calendar pickers, one for the range start date and one for the range end date. The current date is shown in pale yellow. Dates that you select are shown in white, as are the dates of the current date range (if any).
- You can optionally edit the date range directly in the Date field.

Use the **Clear Selection** button to cancel filtering and return to the full results listing.

Sort projects

You can sort the project's run reports by clicking on any of the column heading that are in bold type. Click the heading a second time to reverse the sort.

Run Reports

Introduction

A Torrent Browser 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 (see the example run report below):

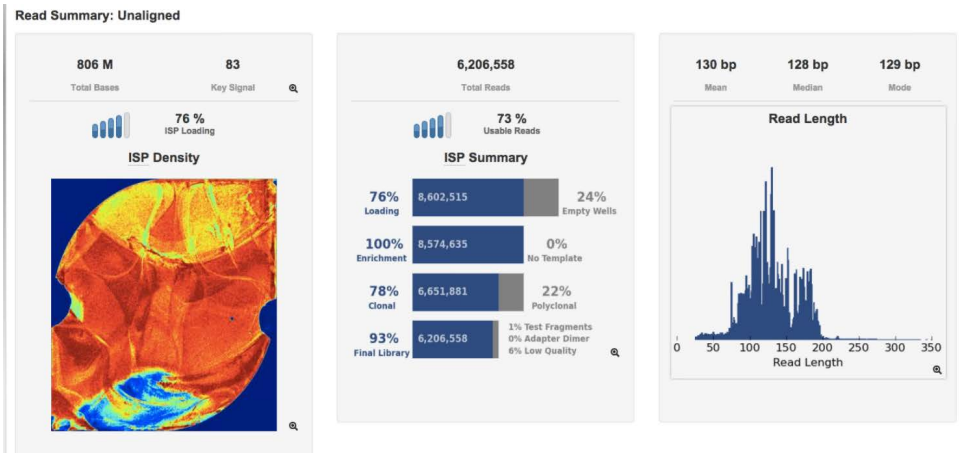
- **Report header** - Buttons to download the run report or summary in PDF format, to review the planned run settings for the run, to reanalyze the run, and to upload the run's output files to Ion Reporter™ Software; a menu to change to a different result set for the same sample; and a navigation bar to jump to the Output Files or Plugin Summary sections.
- **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 buttons for reads 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 a button to select another plugin to run on this analysis
- **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 (run date, sample name, chip type, instrument name, barcode set, etc.)
- **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 a run's quality, first look at the un-aligned 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. Go to **Data ▸ Completed Runs & Results**, select a report of interest.
2. In the Unaligned section, review Total Bases, Total Reads and Mean and Median Read Length to determine the run's quality.

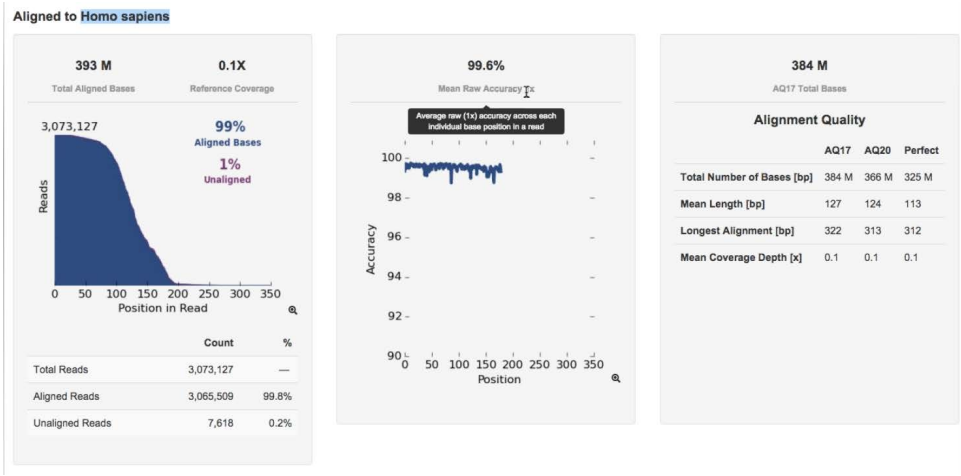


For more information on these metrics see “Run report metrics before alignment“ on page 120.

Review alignment metrics

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

1. Go to **Data ▸ Completed Runs & Results**, and select a run of interest.
2. Scroll down to the Aligned to reference section and review Total Aligned Bases, Reference Coverage, Mean Raw Accuracy, and Total Bases Alignment Quality.

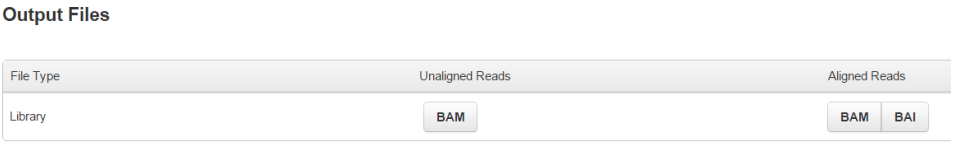


For more information on these metrics, see “Run report metrics on aligned reads“ on page 130

Download results set

You can download the run results in several formats.

1. Go to **Data ▸ Completed Runs & Results** and select a run of interest.
2. Scroll down to the Output files selection and choose your output type: unaligned reads BAM, or aligned reads BAM or BAI.



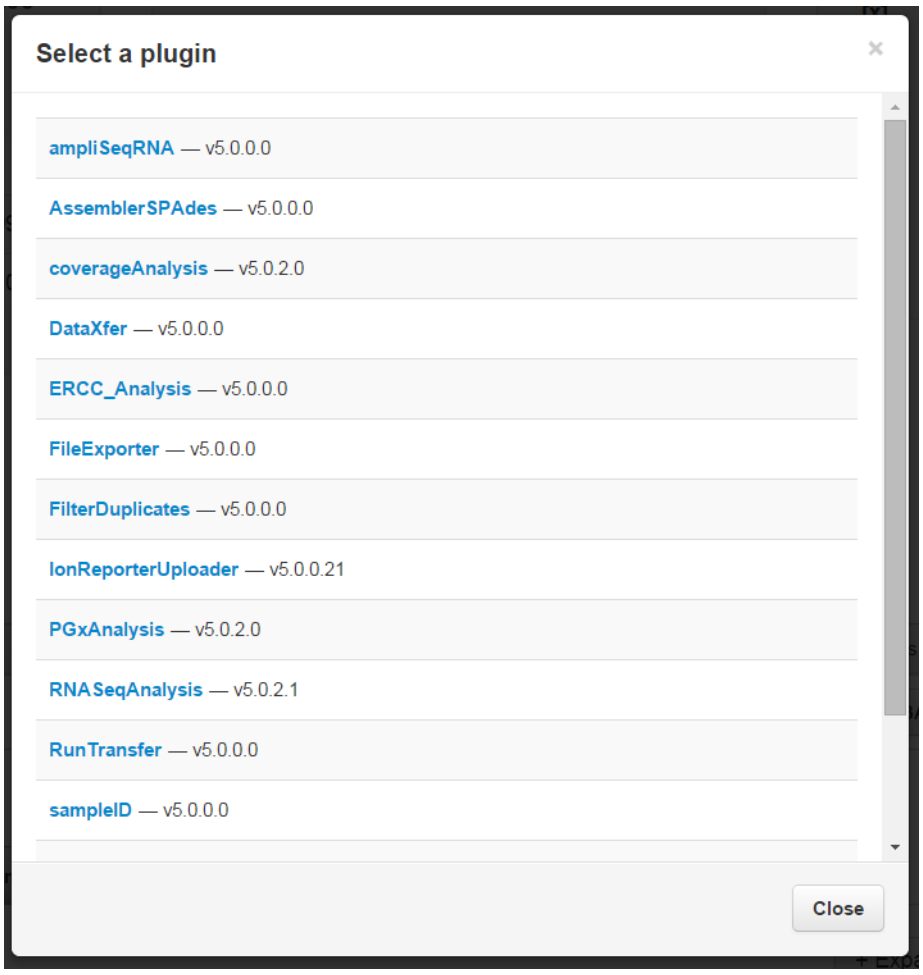
For more information, see the “Output files“ on page 135 section.

Manually run a plugin on the run results

After your run has completed, you can further your analysis by running various analysis plugins.

1. Go to **Data ▸ Completed Runs & Results** and select a run of interest.
2. Scroll down to the very bottom of the run report and click **Select Plugins to Run**.

3. Select the plugin you wish to run.



If the plugin does not require user input, it is immediately queued for execution. Other plugins display a user interface in which you will need to select options. When finished, click **Submit**.

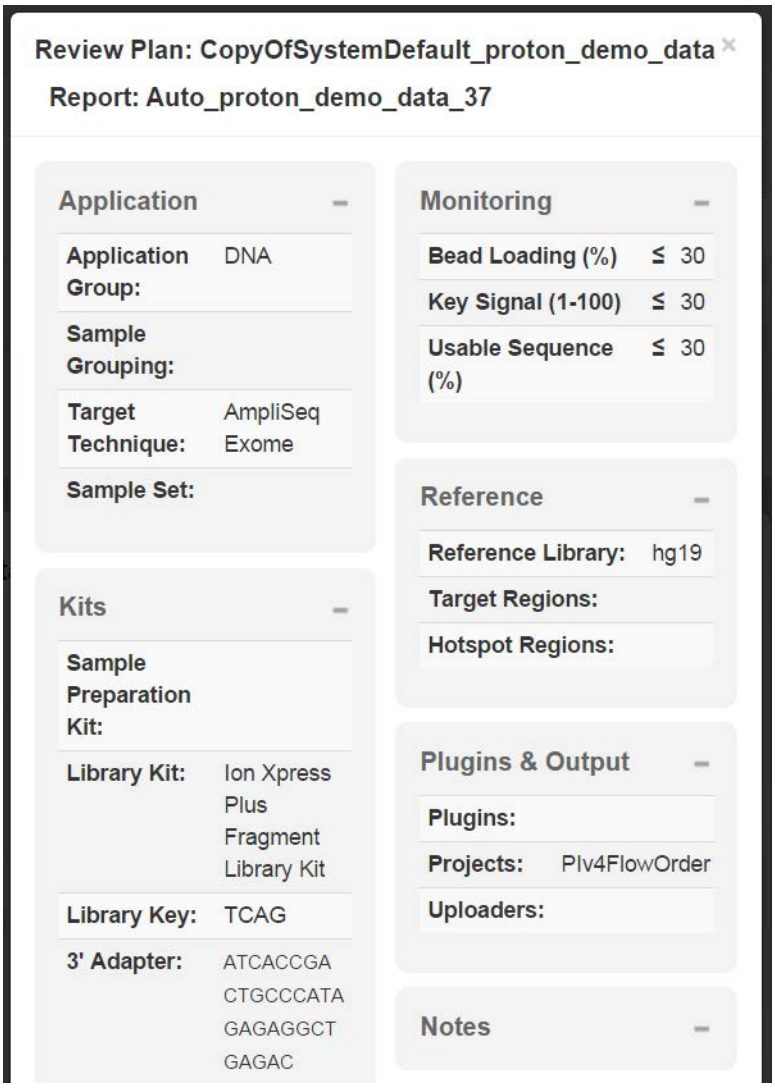
4. View results in the Plugin Summary section.

   For more information, see "Available plugins" on page 178.

Review the planned run settings

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

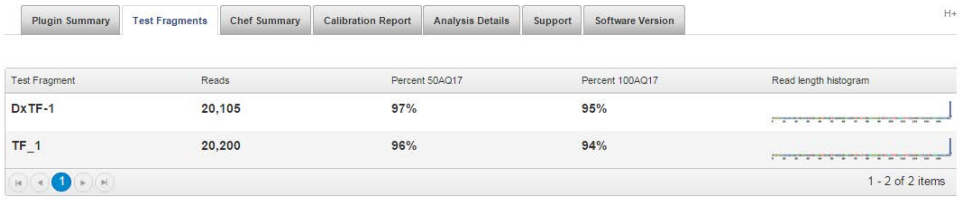
1. Go to **Data** ▸ **Completed Runs & Results** and select a run of interest.
2. Click **Report Actions** ▸ **Review Plan**.
   A Review Plan window appears.



Review the test fragments and their quality metrics

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

1. Go to **Data ▸ Completed Runs & Results** and select a run report of interest.
2. Scroll down to the bottom of the report and click **Test Fragments**.

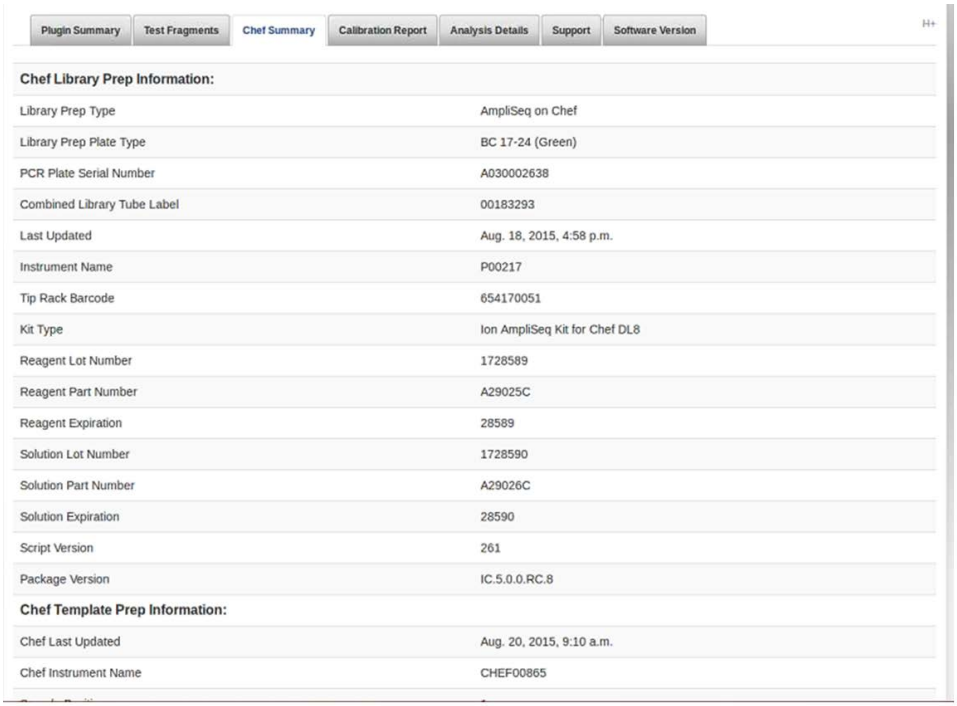


For more information, see "Test fragment report" on page 137

Review Chef Summary

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

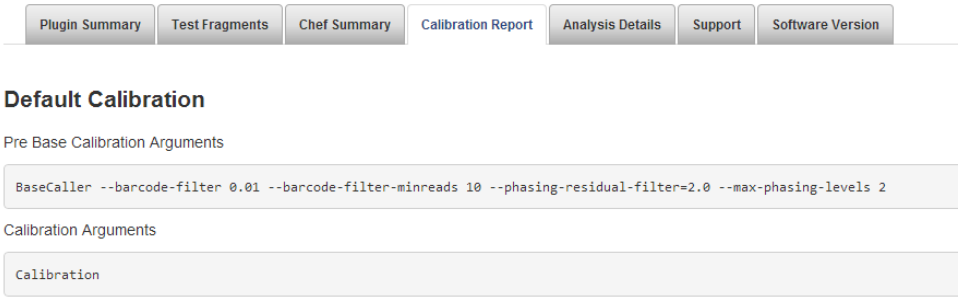
1. Go to **Data ▸ Completed Runs & Results** and select your run which incorporated an Ion Chef™ instrument.
2. Scroll to the bottom of the run report and click **Chef Summary**.
   Here you can review the library preparation and templating information.



Review Calibration Report

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

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



For more information, see "Base Calibration mode options" on page 503

Review analysis information

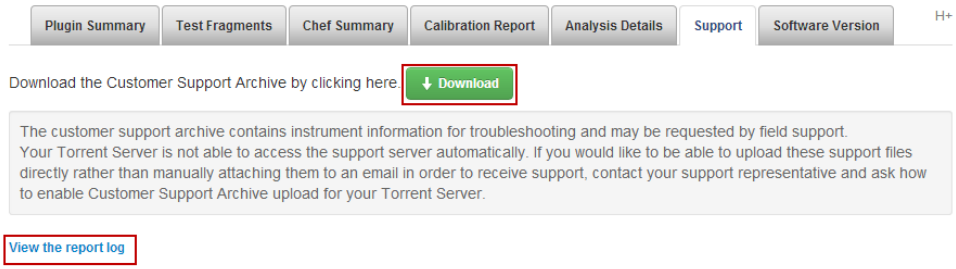
You can review the analysis details of a completed run.

1. Go to **Data ▸ Completed Runs & Results** and 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 138

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 & Results** 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.

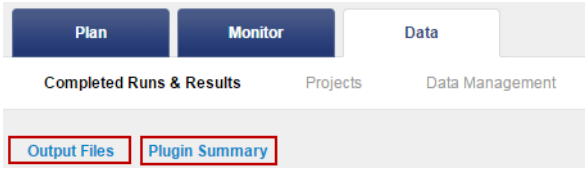


For more information, see "Support" on page 141.

Report header

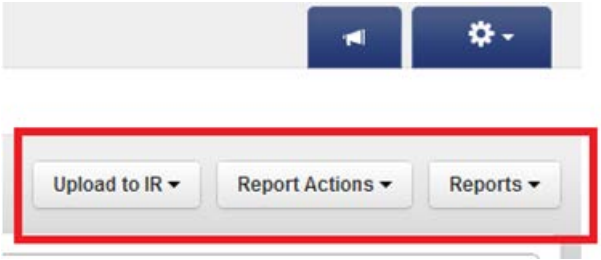
The left side of a run report header contains the following navigation links:

- **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)

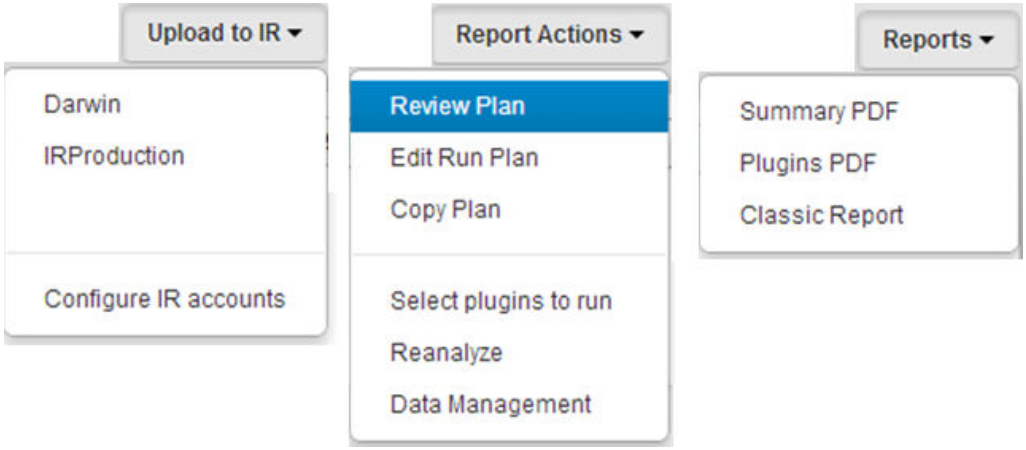


The right side of run report header contains buttons for the following:

- **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 page 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 initial 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)

Read alignment can be used to assess 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 permitted. 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. So for example the AQ20 length is the greatest length at which the error rate is 1% or less. The "perfect" length is simply the longest perfectly aligned segment. For all of these calculations the alignment is constrained to start from position 1 in the read - in other words, no 5' clipping is permitted.

The underlying assumption is that the reference to which the read is aligned represents the true sequence that should have been seen. Suitable caution should 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 computed as follows:

1. Every base in the read is classified as being correct or incorrect according to the alignment to the reference.
2. At every position in the read the total error rate is computed up to and including that position.
3. 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 100bp 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 lengths are 80 and 100 bases, respectively.

Alignment

Within Torrent Browser, the objective is to provide you with a view on alignment that helps determine run and library quality.

There are many alignment algorithms available within 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 of the commercial software tools available within the Ion Torrent™ Web store. You are also encouraged to experiment with these tools.

Alignment within Torrent Browser is performed using TMAP. TMAP is currently an unpublished alignment algorithm, created by the authors of the BFAST algorithm. Please, contact your Ion Torrent™ representative or Technical Support for more information about 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 the user is willing to wait. So for such circumstances the Torrent Suite™ Software also has the capability to define on a per-reference basis the maximum number of reads that should be 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 enough information to be able to judge the quality of the sample, library and sequencing run for quality assessment purposes.

The outputs 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.

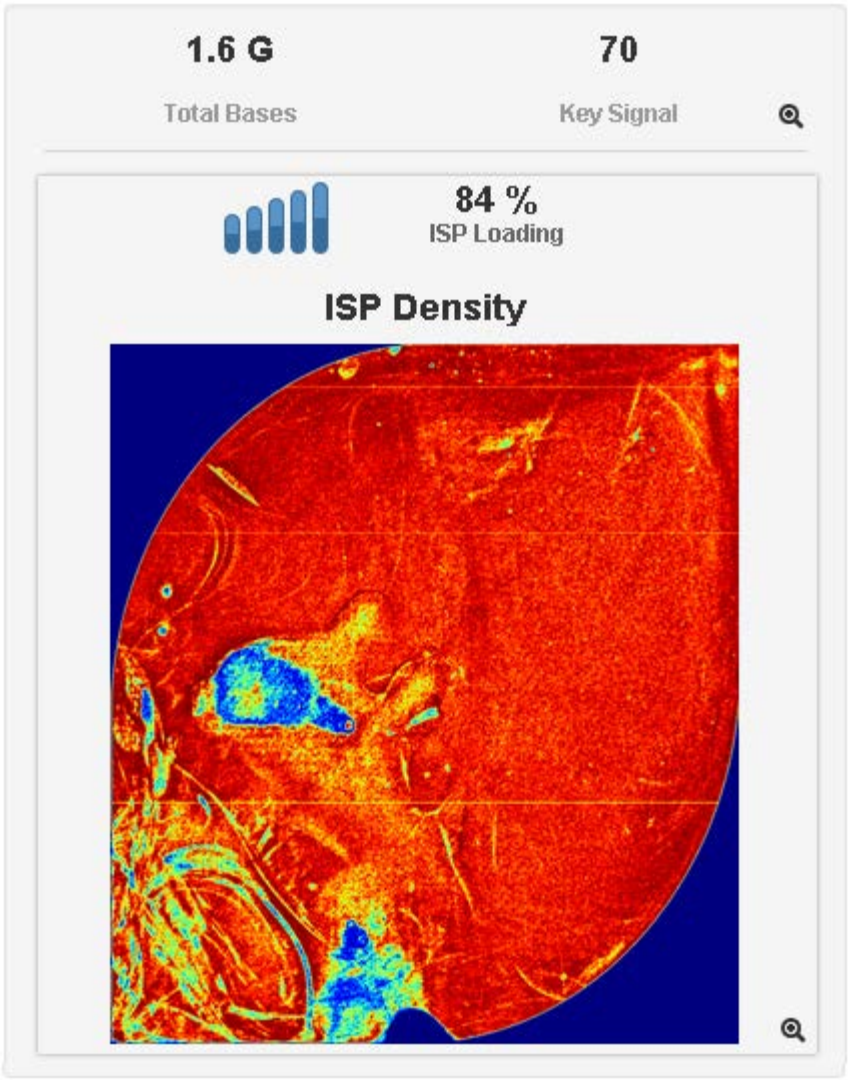
Run report metrics before alignment

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:

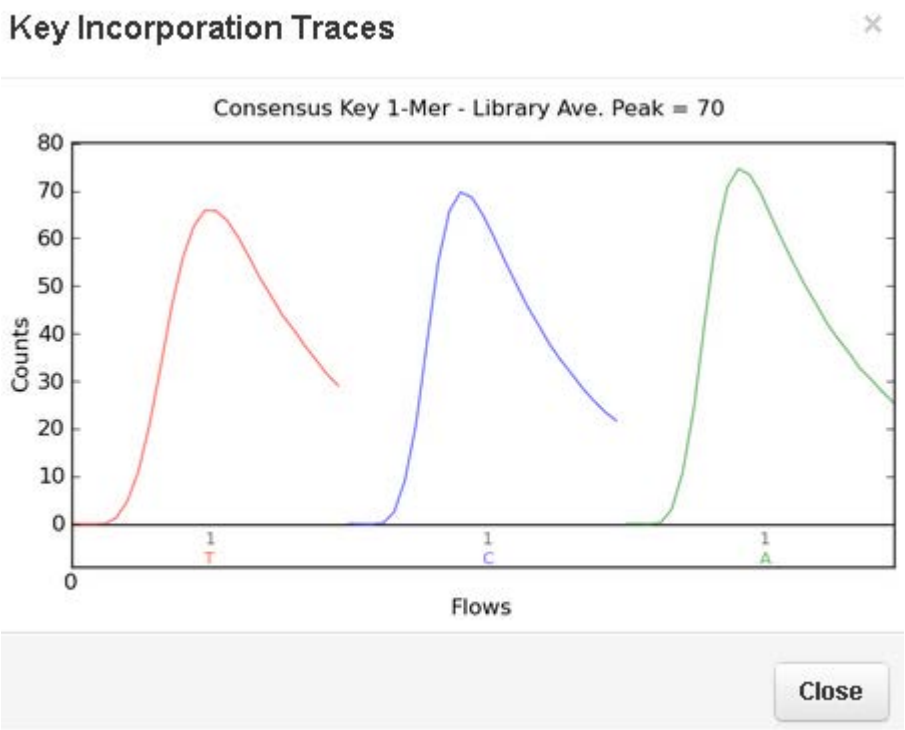
Metric	Description
Total Bases	Number of filtered and trimmed base pairs reported in the output BAM file.
Key Signal	Percentage of Live ISPs with a key signal that is identical to the library key signal.
Bead Loading	Percentage of chip wells that contain a live ISP. (The percentage value considers only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

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

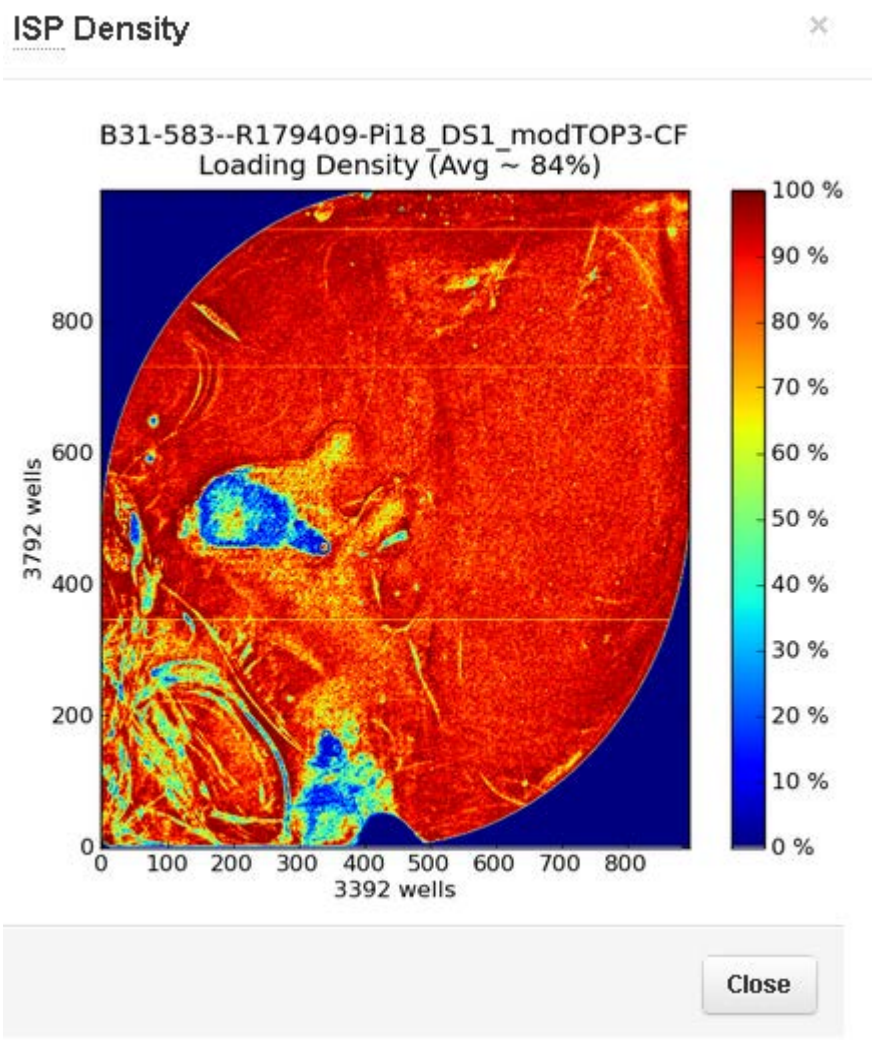
Click on the image (or the magnify icon) to open a larger version:

Key signal

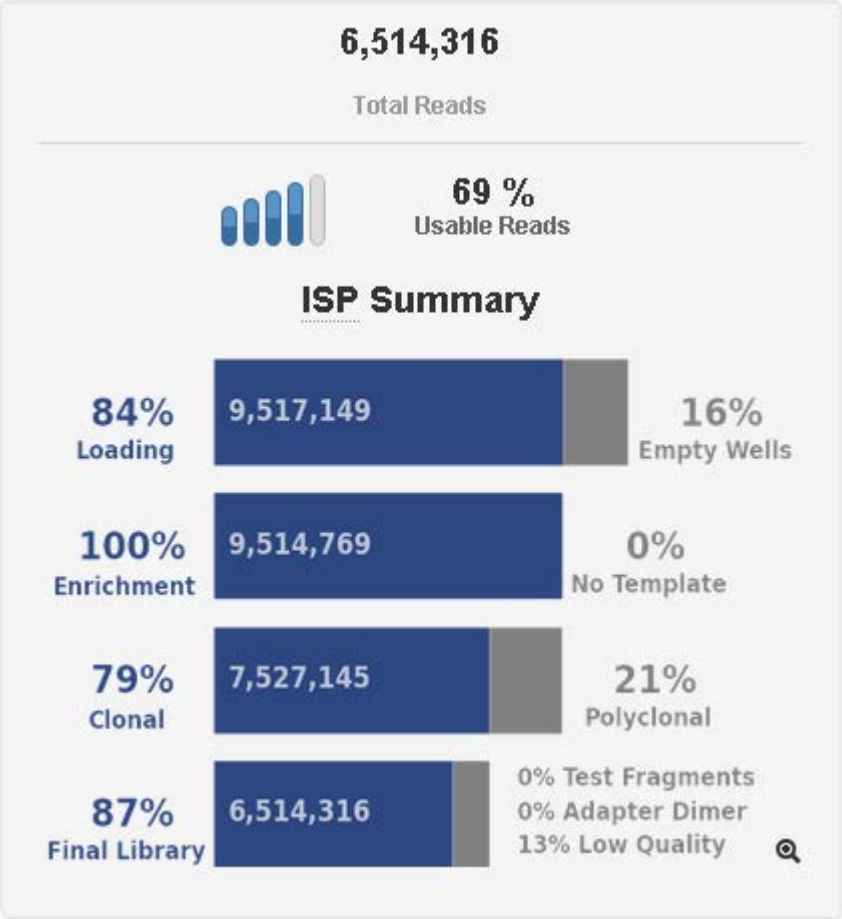
Click the magnify icon in the Key Signal area  to open the key incorporation graphs:



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:

Chip well details

Addressable Wells	11,303,834	
With ISPs	9,517,149	84.2%
Live	9,514,769	100.0%
Test Fragment	22,789	00.2%
Library	9,491,980	99.8%

Library ISP details

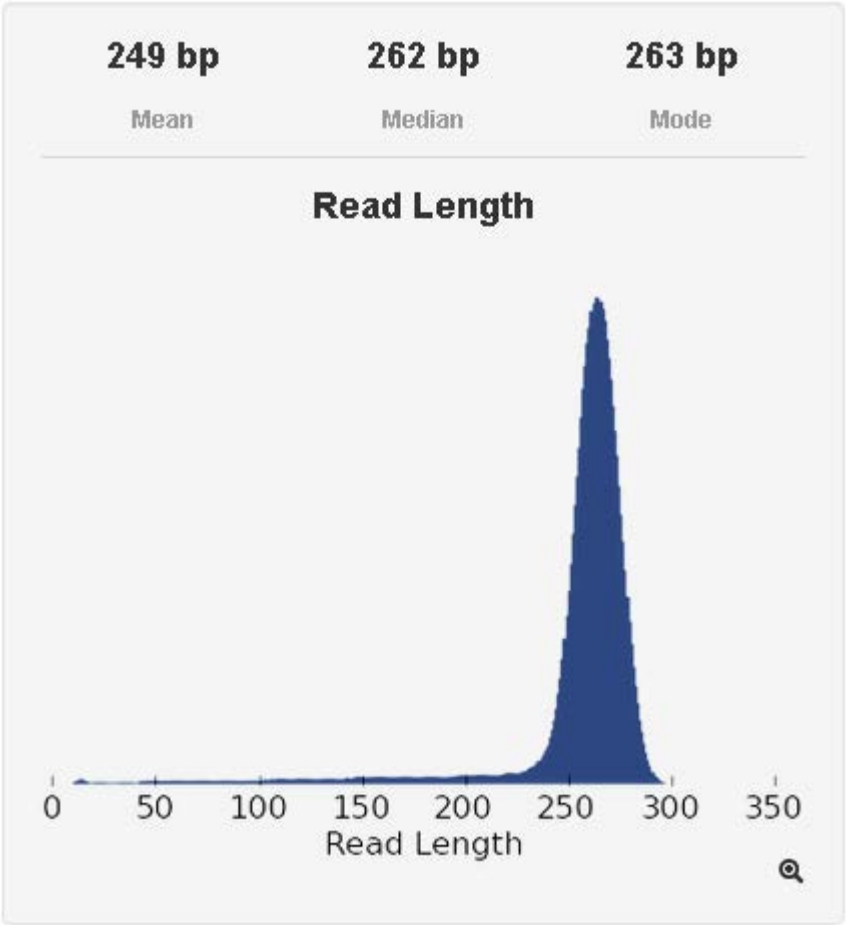
Library ISPs	9,491,980	
Filtered: Polyclonal	1,987,624	20.9%
Filtered: Low Quality	989,726	10.4%
Filtered: Primer Dimer	314	00.0%
Final Library ISPs	**6,514,316**	**68.6%**

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 within 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
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

Metric	Description	Calculation
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** located 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

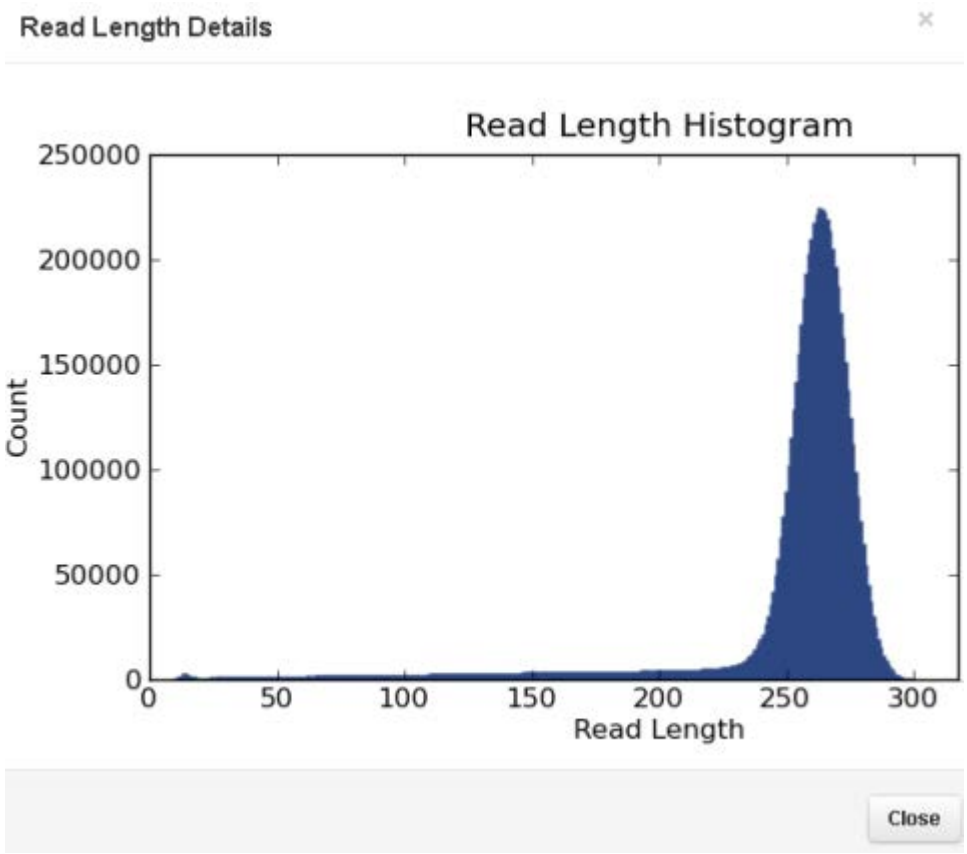


This table describes the read length metrics:

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.

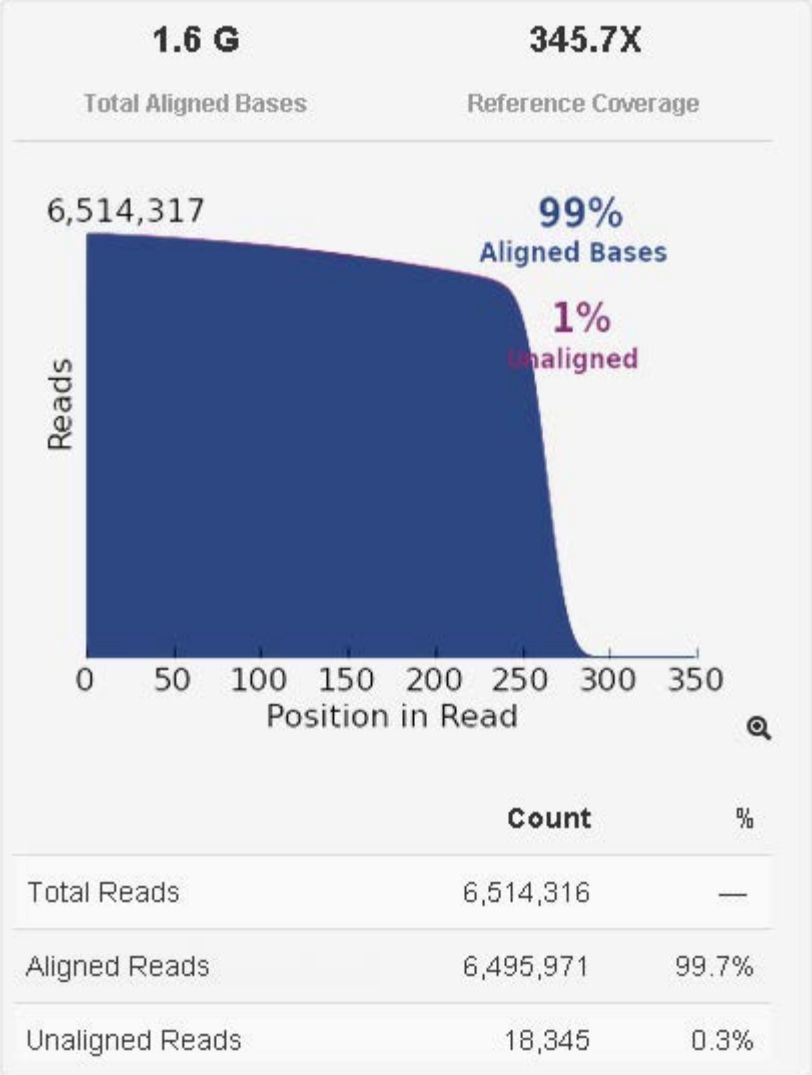
Click on the histogram to open a larger version:



Run report metrics on aligned reads

The run report provides metrics on aligned reads.

Total aligned bases

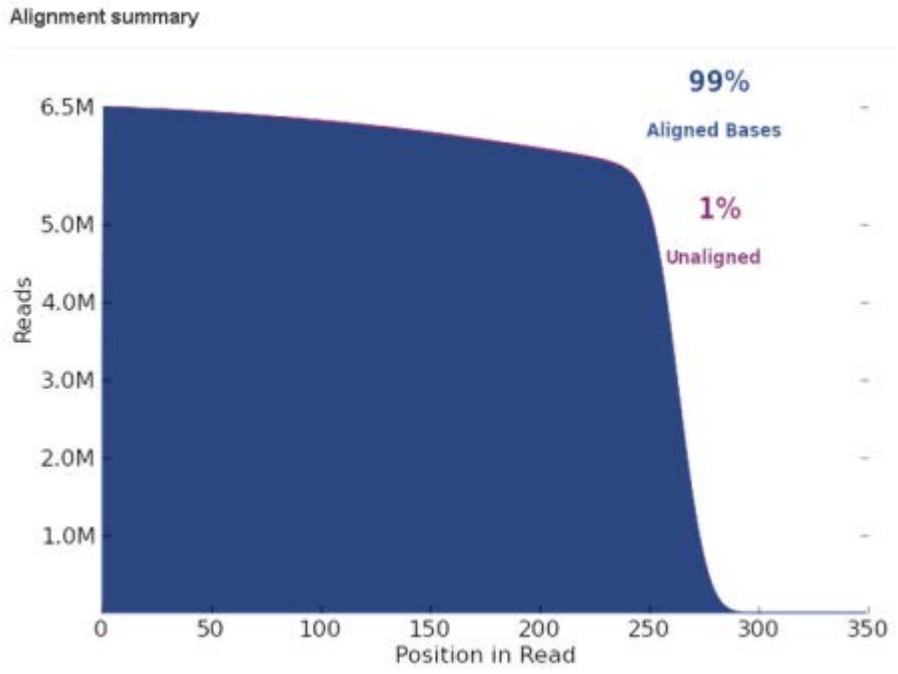


	Count	%
Total Reads	6,514,316	—
Aligned Reads	6,495,971	99.7%
Unaligned Reads	18,345	0.3%

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.
% **Aligned Bases**	Percentage of Total Aligned Bases out of all reads.
% Una ligned	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.

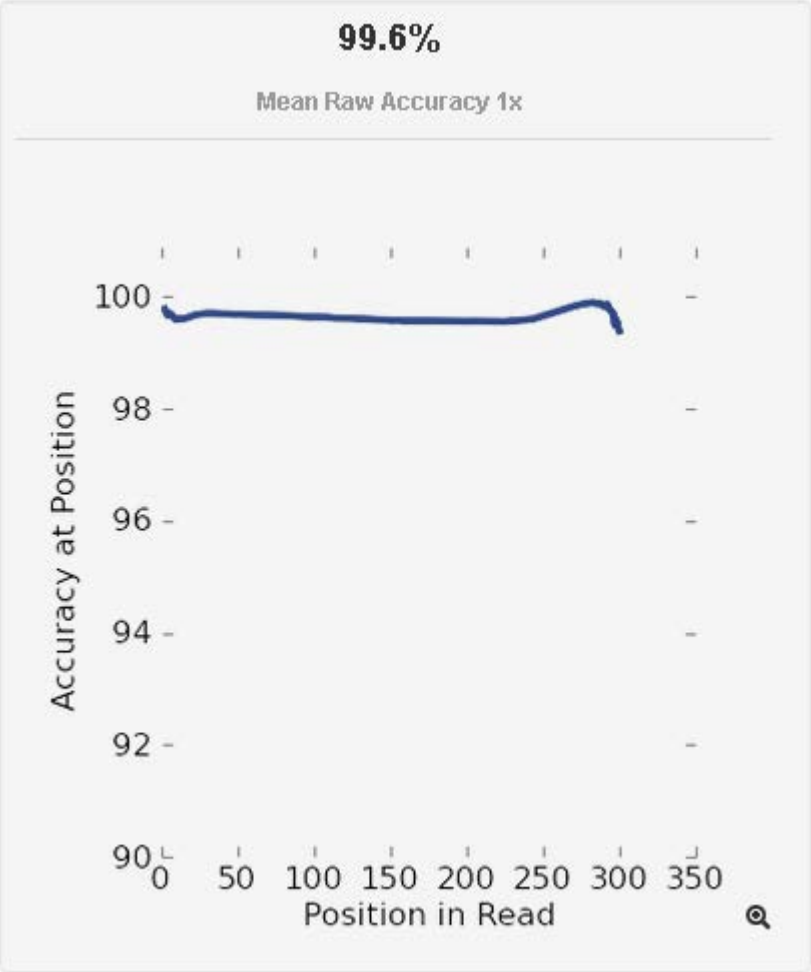
The graph in the Total Aligned reads column plots number of aligned (in blue) and unaligned (in purple) bases byposition in an aligned sequence. (The purple area cannot be seen easily when it is under 3 or 4 percent.)



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

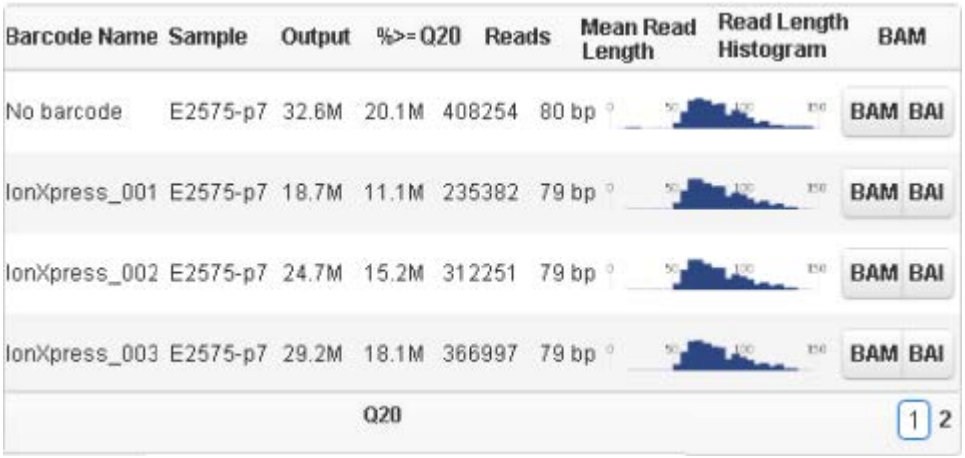
Alignment Quality

	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 thequality level.

Barcode reports

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



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

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.
Output	Total number of reads.
% >= Q20	The percentage of reads that have a predicted quality score of Q20 or better. A Q20 score is the predicted quality ofa Phred-like score of 20 or better, or one error in 100 bp.
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 on the thumbnail histogram to open a larger image.
BAM	Buttons to download the BAM and BAM index file (BAI) for this barcode. The BAM file contains aligned reads sorted by reference location.

The number of barcodes shown in the barcode section varies according to the barcode set used in your run and on the barcodes actually present in the sample. Only data for barcodes present in the run are displayed in the run report.

Output files

These links permit you to directly download the data and report files. Some files are compressed, using the `.zip` format, to provide data integrity and to reduce download time.

Click on a file type button 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 quick access to sequence alignment data. See **http://samtools.sourceforge.net**.for more a more detailed description of the SAM/BAM file format. Many tools are available for working with SAM files.

Deprecated file formats

The following file formats are deprecated and not produced by the default analysis pipeline. The FileExporter Plugin optionally generates these files.

IMPORTANT! The SFF and FASTQ files created by the FileExporter Plugin are generated in the plugin directory, not in the main analysis directory.

File type	Description
SFF	Compressed (.zip) Standard Flowgram Format (SFF) -formatted file that contains "flow space" data. The bases called in a run are stored in two formats: SFF and FASTQ. Both files contain the nucleotide calls and associated quality values, the SFF files, additionally, contain signal values in flow space and a mapping between sequence and flow spaces. The data are organized on a per flow basis, and contain information about nucleotide flows that both did and did not result in base incorporation. **Note:** The SFF file format is deprecated and not produced by the default pipeline.
FASTQ	Compressed (.zip) FASTQ-formatted file containing data organized in a per-base basis, including quality scores. The reads contained in the file are unaligned reads. **Note:** The FASTQ file format is deprecated and not produced by the default pipeline.

Rename your output files

See the FileExporter Plugin supports renaming your output files. (This plugin also optionally creates SFF or FASTQ formats, or zips your output.)

Test fragment report

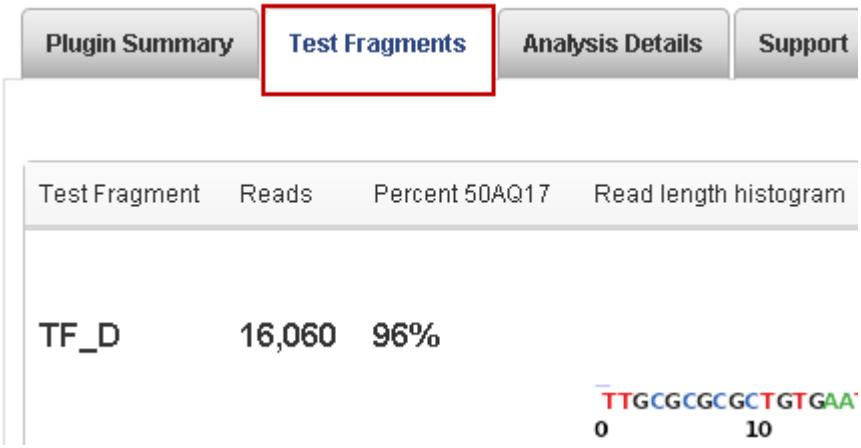
The **Test Fragment Summary** section of the Analysis Report provides information about the performance of each Test Fragment included in the experiment.

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

Open the test fragments report with the Test Fragments button, near the bottom of the run report:



Test fragment metrics

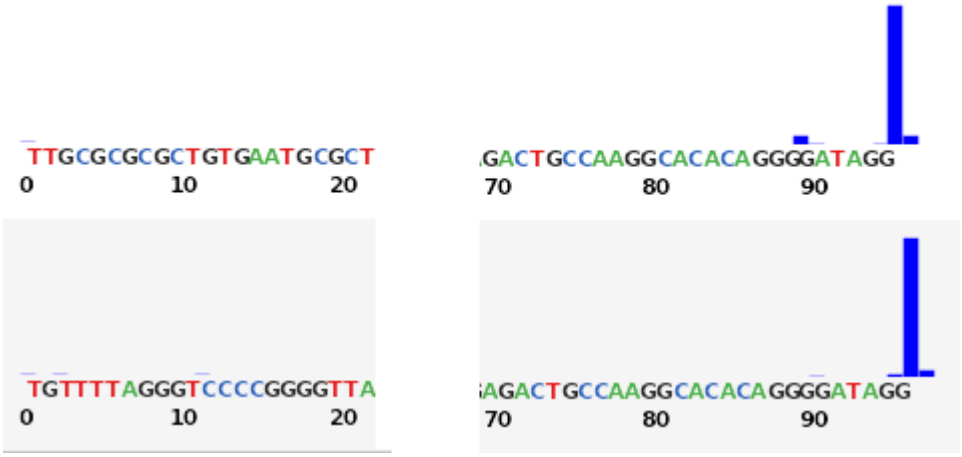
The Test Fragments report displays the following information:

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.

Report information

This section describes the following run report buttons.

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™ orIon Proton™ run was started.
Run Cycles	Number of Ion PGM™ orIon 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™ orIon 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.
Sample	Name of the sample assigned to the run used to generate this analysis. This is assigned on theIon PGM™ or Ion Proton™ Sequencer.

Parameter	Description
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™ 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 IonPGM 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.
Analysis Name	Name of the analysis provided in Torrent Browser when the analysis was initiated. If the analysis was scheduled to auto-start, this is the default analysis name.
Analysis Date	Date the analysis was performed.

Parameter	Description
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 Browser assigned to the planned run for this analysis.

Software version

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

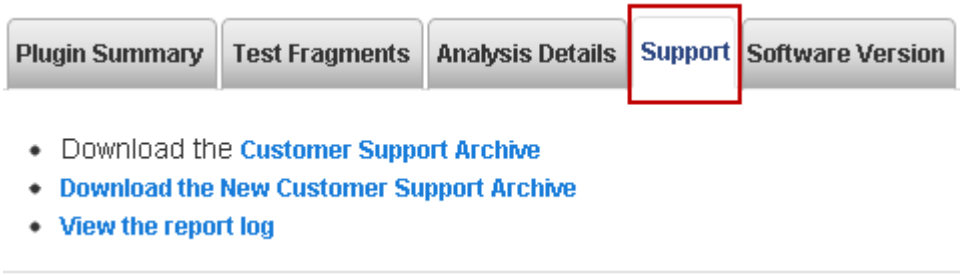
IMPORTANT! The version numbers shown in the example may be different from your current version of the software depending on the age of the analysis. See the About tab in the Torrent Browser for a complete list of modules and version on your server. See the Torrent Suite™ Release Notes for the package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

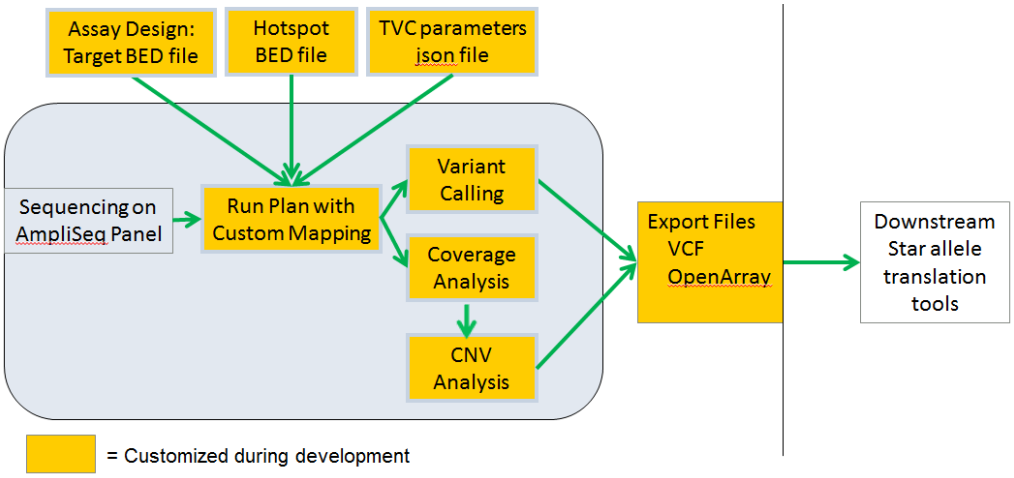
Applications

Introduction

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

Pharmacogenomics Research Analysis Plugin

The Pharmacogenomics (PGx) Research Analysis plugin is designed to be 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. The figure below describes the pipeline of analyses.



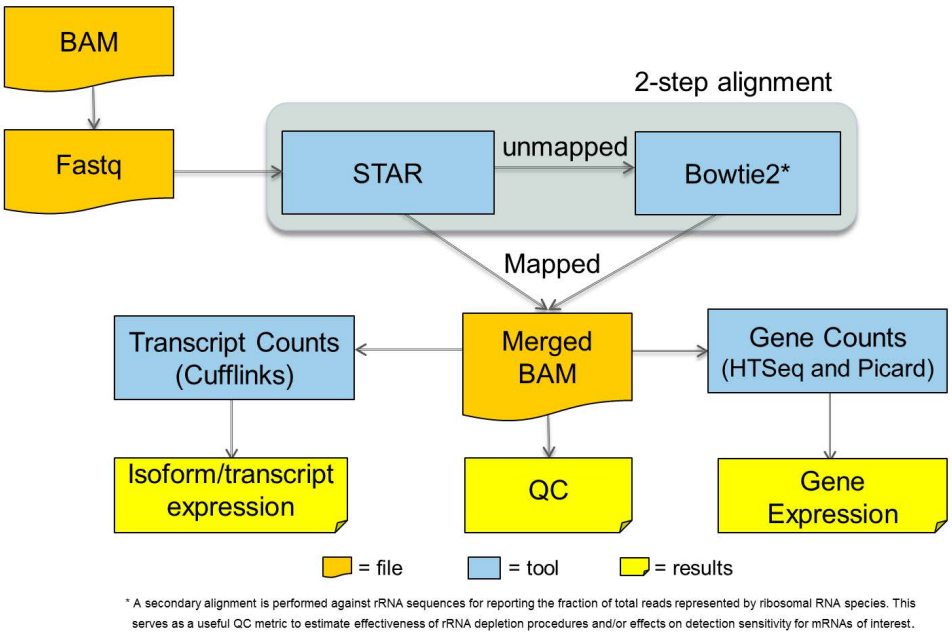
The following instructions are intended to assist Pharmacogenomics (PGx) research panel customers in setting up Torrent Suite™ (v5.0.2 and later) planned runs incorporating the PGx research panel template and plugin.

- **https://tools.thermofisher.com/content/sfs/manuals/MAN0013730_CreatePlannedRun_IonAmpliSeqPharm_UB.pdf**
- **https://tools.thermofisher.com/content/sfs/manuals/MAN0014300_CustomizGuidelines_IonAmpliSeqPharm_UB.pdf**

Ion RNASeq Plugin

The following instructions are intended to assist RNA Seq plugin customers set up Torrent Suite planned runs incorporating the RNA Seq plugin.

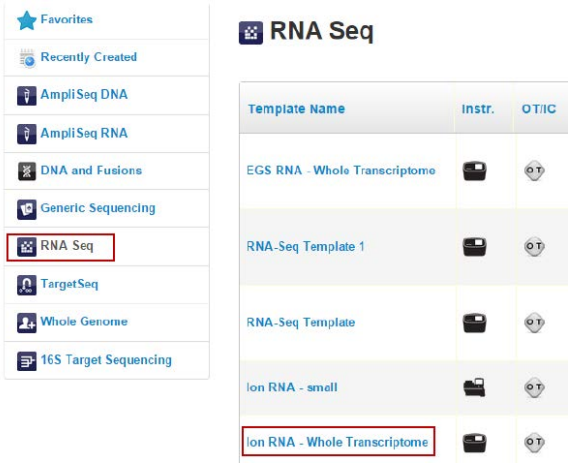
The Torrent RNASeqAnalysis Plugin is an RNA Transcript Alignment and Analysis tool for use with reference genomes hg19 and mm10. Use this plugin to analyze cDNA reads, as produced by RNA-Seq. Reads are aligned to the reference genome using STAR and bowtie2 aligners to find full and partial mappings. 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.



Create an RNA Seq analysis run from factory template

1. Log into Torrent Suite™ Software.
2. Click on the **Plan** Tab.
3. Click on the **Templates** link to view Templates.

4. Click the **RNA Seq-Whole Genome** option under Favorites in the left side navigation list.

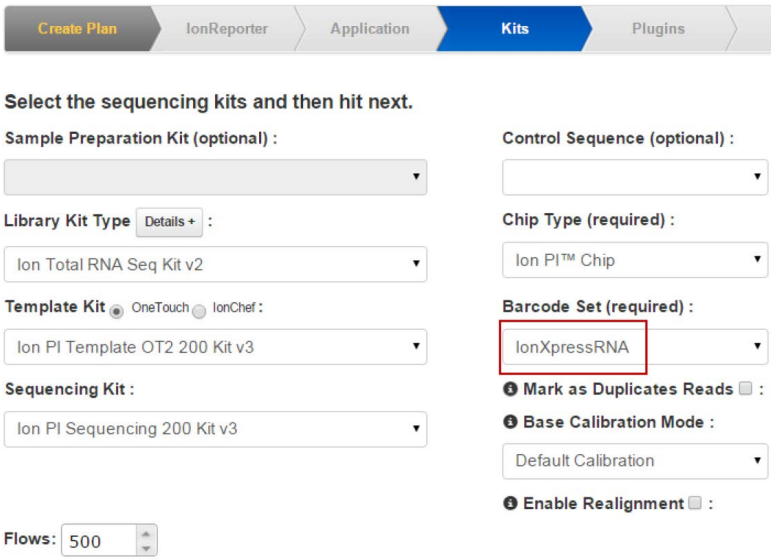


The Plan tab appears.

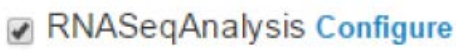
5. Click on the Ion Reporter tab, and click **None** and then click **Next**.

6. Click on the Application tab, select **RNA** and then click **Next**.

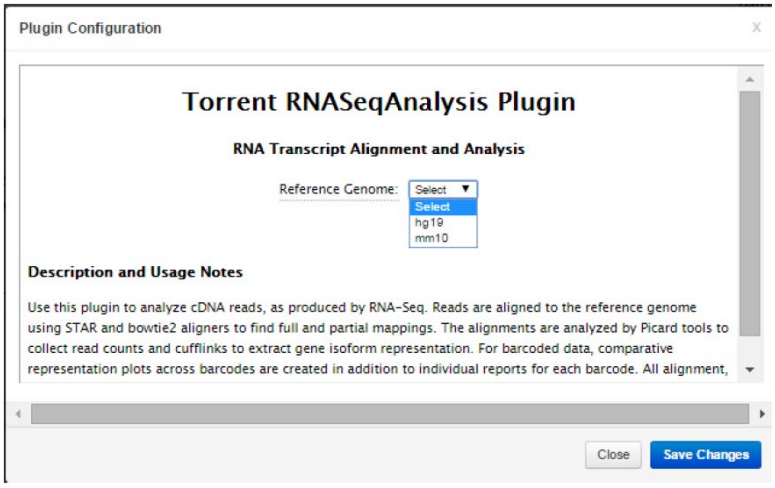
7. On the Kits tab, change Barcode Set to **IonXpressRNA**. Click **Next**.



8. On the Plugins tab, check **RNASeqAnalysis** and click **Configure**.



9. Select human or mouse reference and click **Save Changes**.

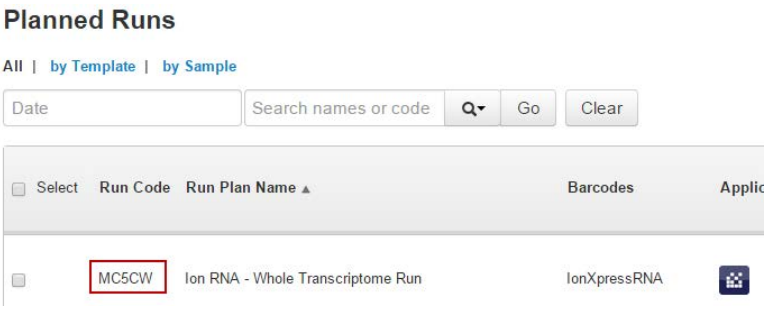


10. Click **Next**.

11. (Optional) On the Projects tab, select a project and click **Next**.

12. On the Plan tab, select **None** as the reference, enter a name for the run and add samples. Click **Plan Run**.

13. Run plan on your sequencing system.
Each planned run contains complete instructions for its sample, from sequencing on instrument to export of the results files to Ion Reporter software. Here is what a planned run from this example looks like on the **Plan ▸ Planned Run** page:



A planned run is ready to execute on the sequencing instrument and is executed by entering the 5-digit run code on the instrument. From the run code, all the plan run's settings are available on the instrument and to the Torrent Suite software. All of your selections, from original template and the planned run that you saved, are known to the Torrent system and software. The system carries out your instructions from sequencing to data export.

Modify RNASeq Template

If you wish to customize the template, create a copy of the template.

1. Click the **Gear** button in the **Ion RNA - Whole Transcriptome** row and select **Copy**.

2. Enter a name for the template and modify settings as required.
3. Click **Copy Template**.

Review run results

After your sequencing run completes, review results on the Run Summary page.

1. Go to **Data** ▸ **Completed Runs & Results** and search for your run. The Run Summary page appears.
2. Scroll down to the **RNASeqAnalysis** section and you'll see the Barcode Summary table. This is an overview table that includes columns for Barcode Name, Sample, Total Reads, Aligned Reads, Percent Aligned Mean Read Length, Genes Detected, and Isoforms Detected.

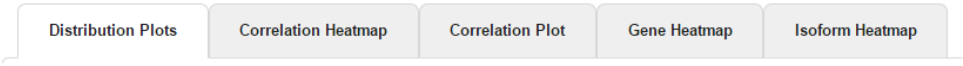
Barcode Summary

Reference: hg19

Barcode Name	Sample	Total Reads	Aligned Reads	Percent Aligned	Mean Read Length	Genes Detected	Isoforms Detected
IonXpress_001	Sample 1	222	222	100%	101	1	131
IonXpress_004	None	2,727,892	2,713,002	99.45%	107.9	9,451	17,723
IonXpress_005	None	2,839,827	2,828,560	99.6%	108.2	9,455	17,346
IonXpress_006	None	2,842,945	2,824,513	99.35%	108	9,336	17,355
IonXpress_007	None	2,642,234	2,623,932	99.31%	107.8	8,860	16,831
IonXpress_008	None	2,757,938	2,737,906	99.27%	108	9,232	17,578
IonXpress_010	None	11,283,208	10,997,469	97.47%	102.4	16,626	58,457
IonXpress_011	None	10,408,573	10,127,505	97.3%	96.7	16,095	57,737
IonXpress_012	None	12,817,747	12,521,818	97.69%	102.2	16,843	58,273
IonXpress_013	None	13,774,036	13,398,953	97.28%	92.4	16,261	58,927
IonXpress_014	None	11,895,869	11,614,056	97.63%	102.9	15,842	57,181

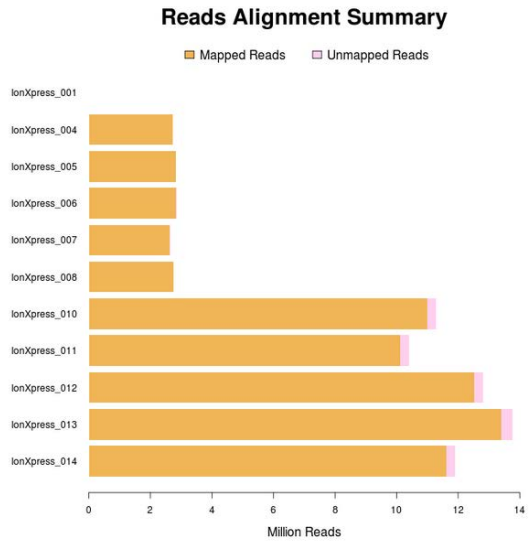
1 20 items per page 1 - 11 of 11 items

3. Click on the **RNASeqAnalysis.html** link to view other components of the report. Below the Barcode Summary are tabs to view the results graphically.

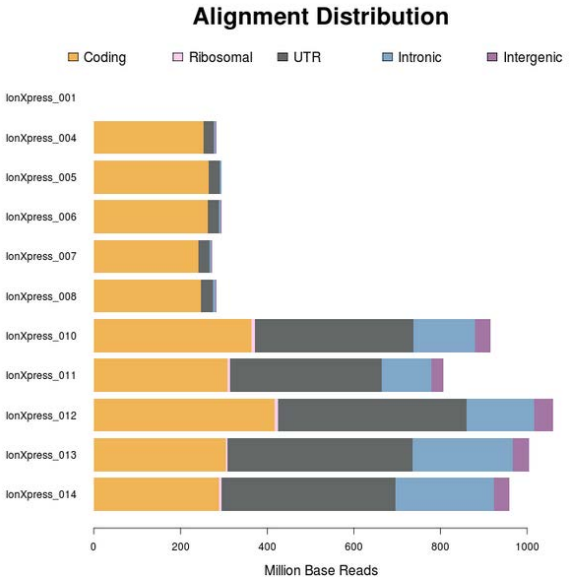


Distribution Plots

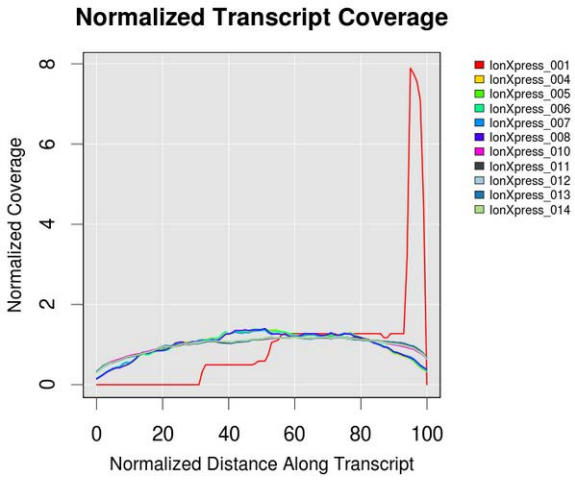
Reads Alignment Summary- A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the barcode summary table.



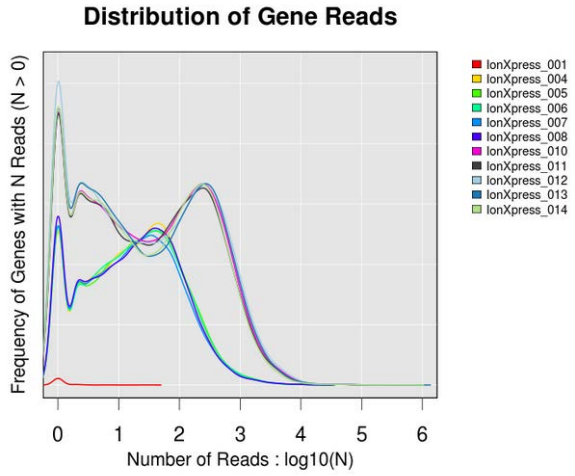
Alignment Distribution - A graphical summary of the distribution of reads to genomic features.



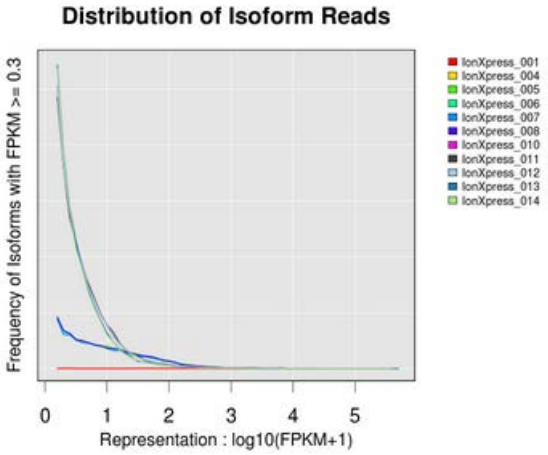
Normalized Transcript Coverage - An overlay of individual normalized transcript coverage plots for each barcode.



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.

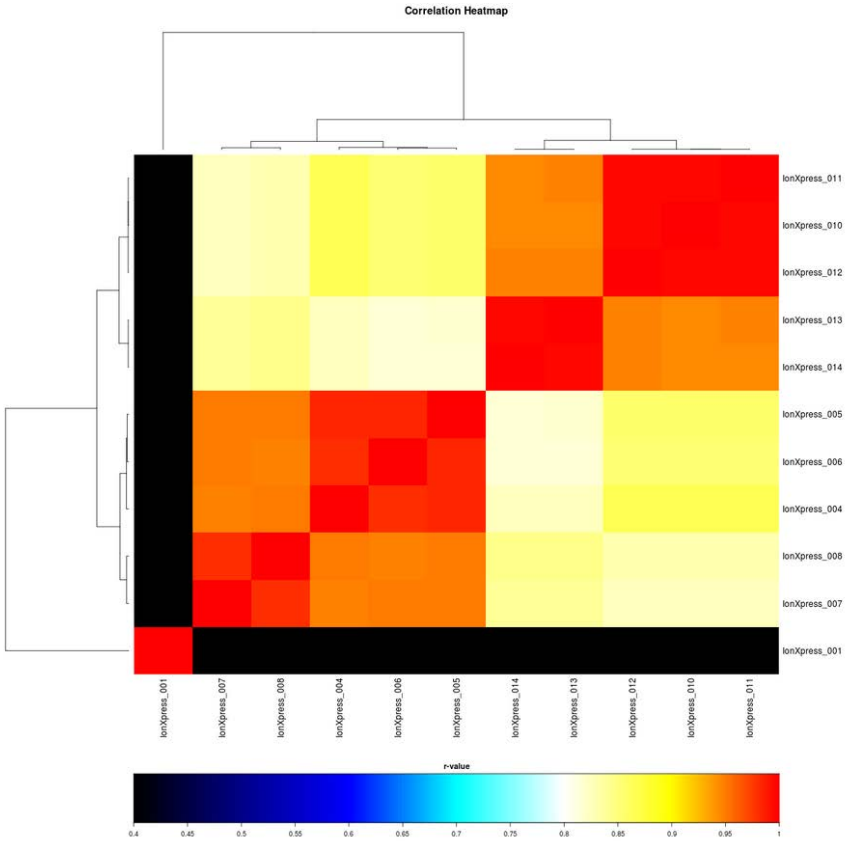


Distribution of Isoform Reads - Distribution of transcript isoforms across barcodes showing the counts of isoforms having similar FPKM values. All curves are plotted on the same y-axis, normalized to the highest count and scaled for FPKM values ≥0.3.



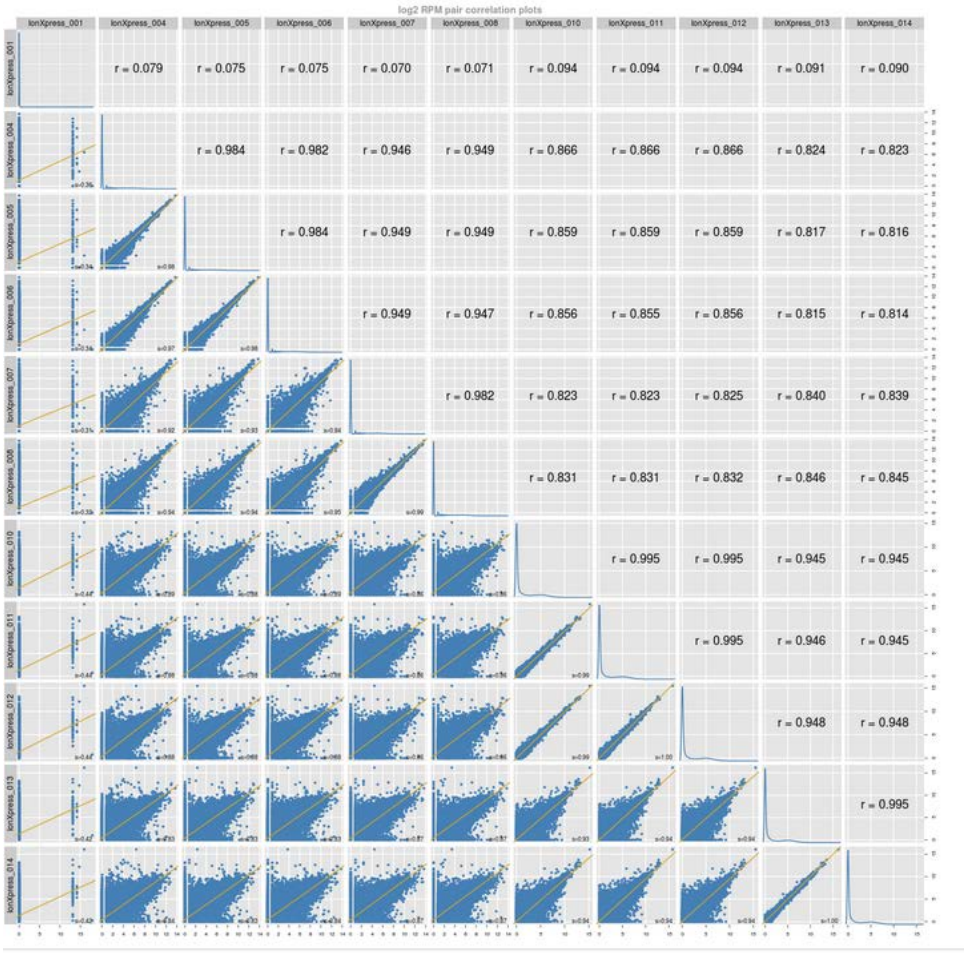
Correlation heatmap

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

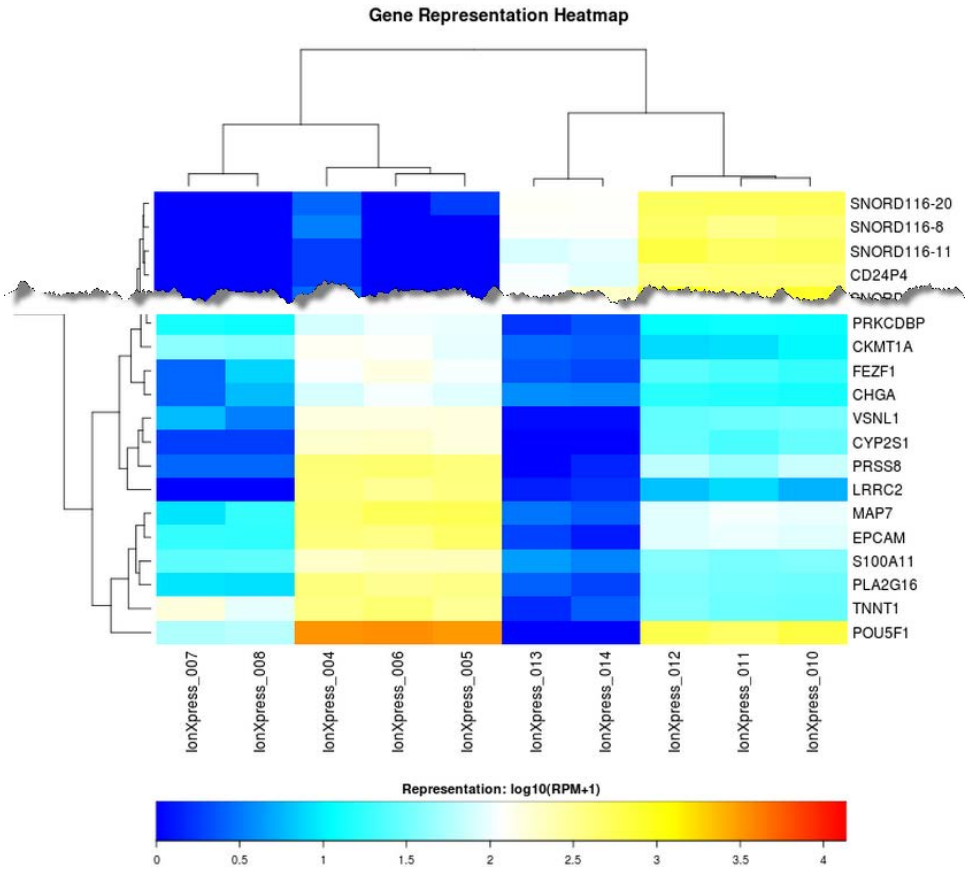
Barcode read pair correlation plot. Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show the frequency density plot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

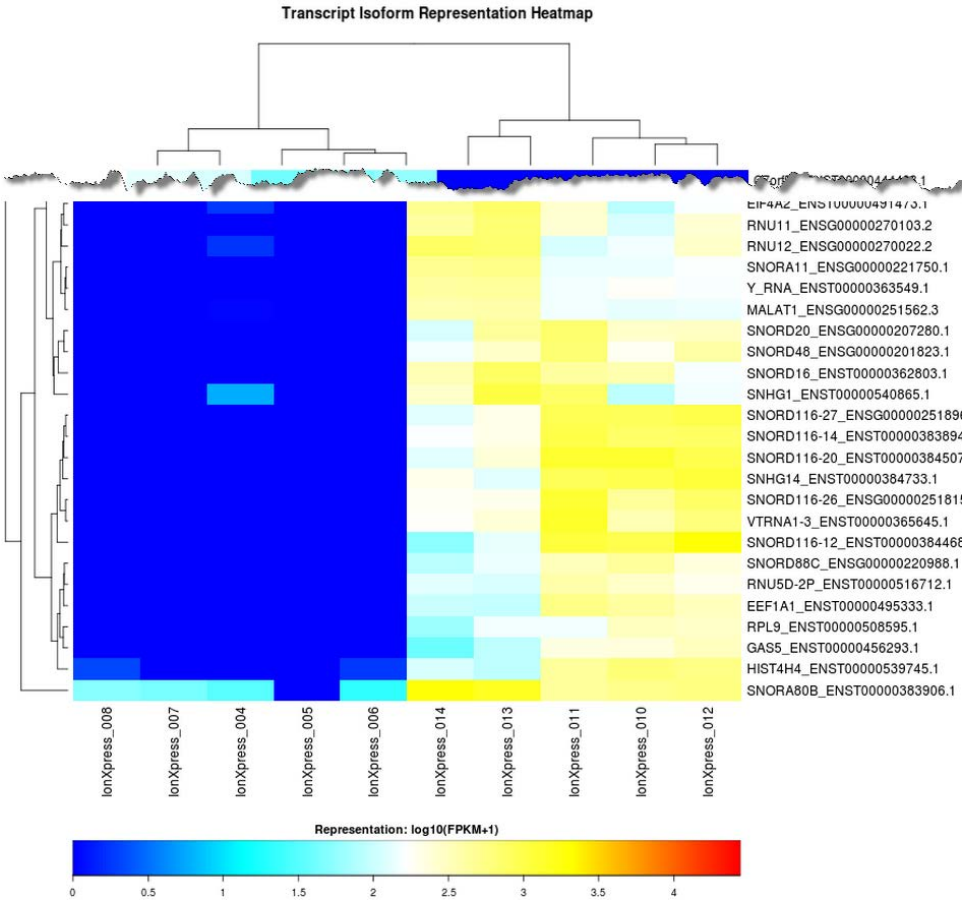
reads, plotted using log10 of those counts. For this plot, barcodes will be omitted if they have less than 100,000 total reads.



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.



Downloadable reports

At the bottom of the screen are links for downloading raw analysis output files:

Download Barcode Summary Report
Download absolute reads table
Download absolute normalized reads table
Download aligned reads distribution table
Download isoform FPKM values table

Barcode Summary Report - This report produces a Microsoft™ Excel™ table listing each barcode's sample name, total reads, aligned reads and percent aligned.

Absolute Reads Table - This Microsoft™ Excel™ table lists absolute reads for the genes found on each barcode.

Absolute Normalized Reads Table - This Microsoft™ Excel™ table lists absolute normalized reads for the genes found on each barcode.

Aligned Reads Distribution Table - Distribution of genes across barcodes showing the frequency of numbers of genes having similar log10 read counts.

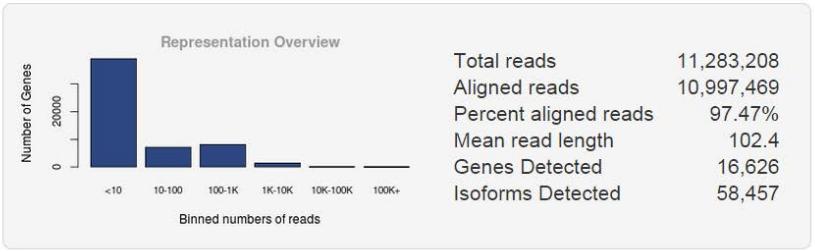
Isoform FPKM Values Table - Table format of the Isoform gene heatmap.

Individual barcode view

Click on any barcode of interest to see similar graphs of the barcode alone.

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

Reference: hg19



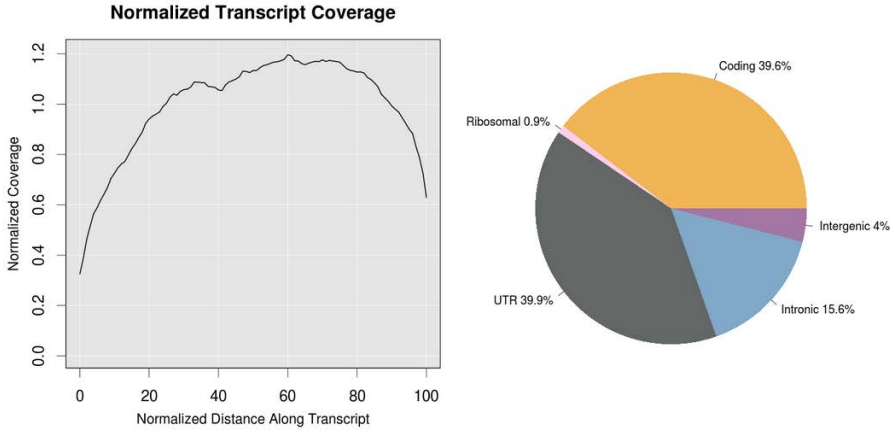
Gene Mapping Summary - Summary of reads mapped to genes of annotated reference.

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

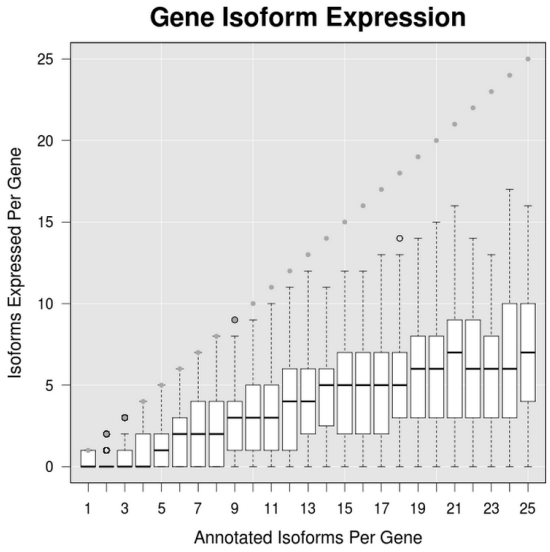
Base Mapping Summary - Summary of base reads aligned to genetic features of an annotated reference.

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.



Downloadable reports

At the bottom of the screen are links for downloading raw analysis output files for the individual barcodes:

- 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.mareads.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.mareads.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	maseq.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.

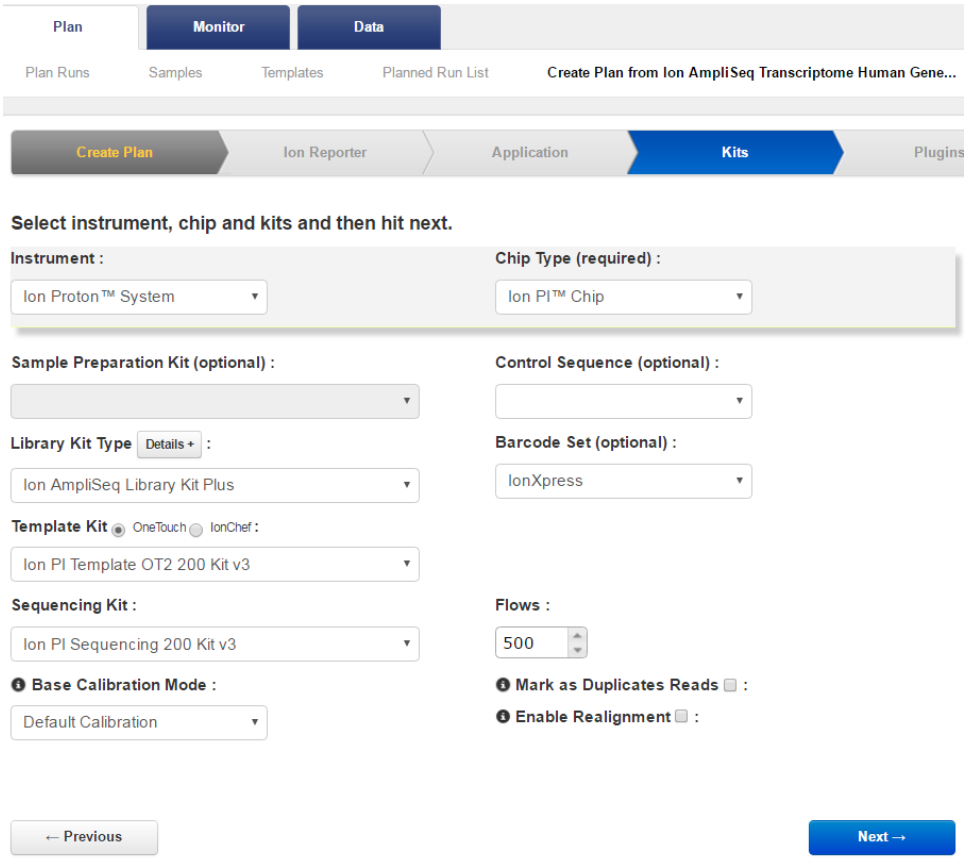
File Size	Date	File
5.5M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.genes.fpkm_tracking
24M	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Update_for_less_barcodes.isoforms.fpkm_tracking
0	2015-06-02	IonXpress_010_R_2015_02_12_15_16_34_sc_P19-753-P2bead_on_p1--R79599_Upd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

ampliSeqRNA

The ampliSeqRNA plugin is used with the AmpliSeq™ Transcriptome Human Gene Expression Kit, AmpliSeq™ RNA panel, and custom RNA panels.

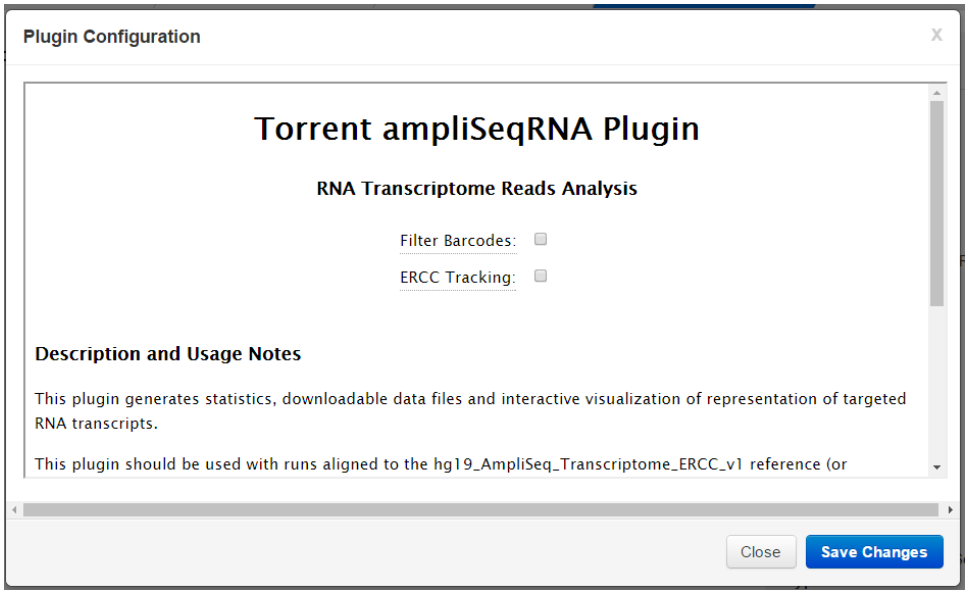
Create an ampliSeqRNA planned run with factory template

1. Click **Plan** ▸ **Templates** link to view Templates.
2. Click the **AmpliSeq RNA** option under Favorites in the left side navigation list.
3. Choose the **Ion AmpliSeq Transcriptome Human Gene Expression Panel** template.
   a. In the Reference Library drop-down box, select **hg19_AmpliSeq_Transcriptome_ERCC_v1**.
   b. In the Target Regions drop-down box, select **hg19_AmpliSeq_Transcriptome_21K_V1 BED** file.
4. Click on the Ion Reporter tab, and click **None** and then click **Next**.
5. Click on the Application tab, confirm **RNA** and **AmpliSeq RNA** are selected and then click **Next**.
6. On the Kits tab, confirm the following are selected, then click **Next**.



7. On the Plugins tab, check **ampliSeqAnalysis** and click **Configure**.

8. (Optional), consider the Filter Barcodes and ERCC Tracking options as described in the **Plugin Configuration** screen. Click **Save Changes** then click**Next**.



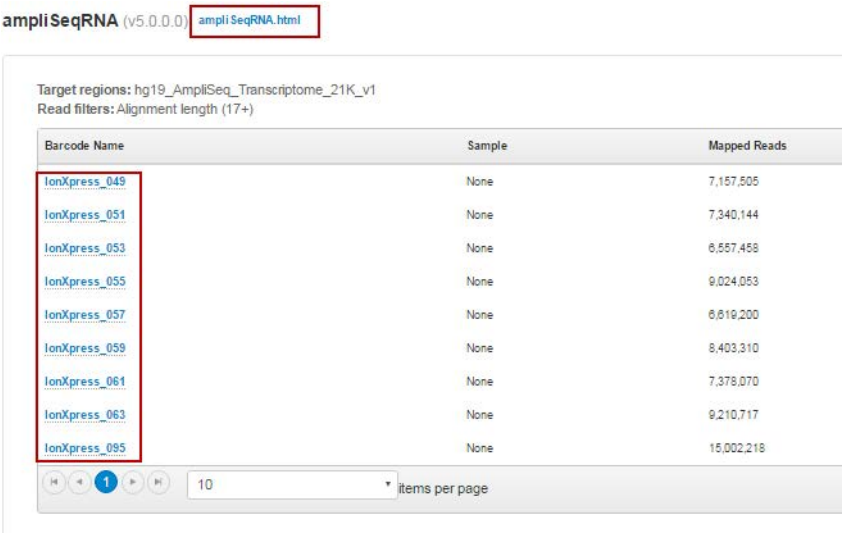
9. (Optional) On the Projects tab, select a project and click **Next**.
10. On the Plan tab, click **Plan Run**.
11. Run plan on your sequencing system.

Review run results

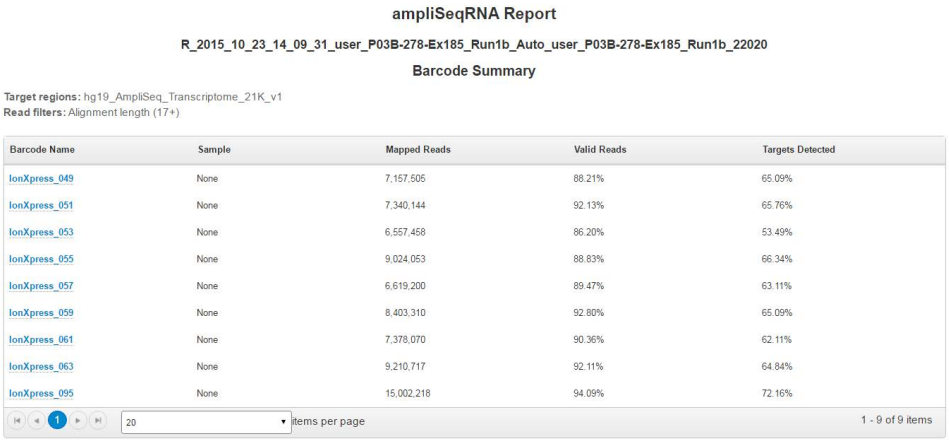
After your sequencing run completes, review results on the Run Summary page.

1. Click the **Data tab ▸ Completed Runs & Results**, then search for your run.
2. Click the **Report Name** link to open the **Run Summary** page for your run.

3. Scroll down to the **AmpliSeqRNA** section, then click the **ampliSeqRNA.html** link to open the **ampliSeqRNA Report – Barcode Summary** for all barcodes. Alternatively, click individual barcode names to see the results for an individual barcode.



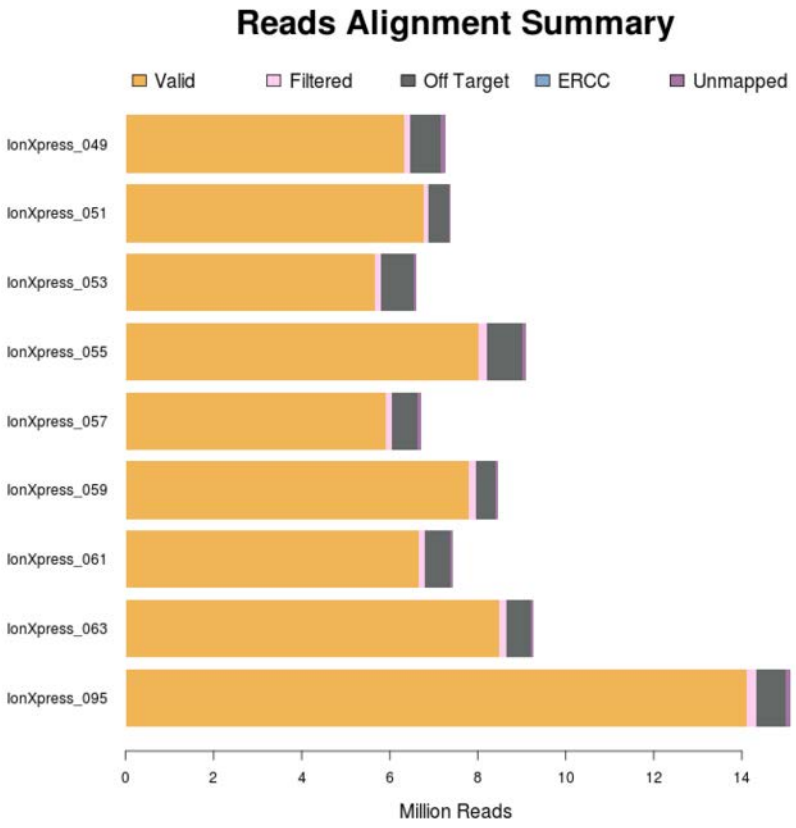
4. In the Barcode Summary table, review your **Mapped Reads**, **Valid Reads**, and **Targets Detected** by barcode.



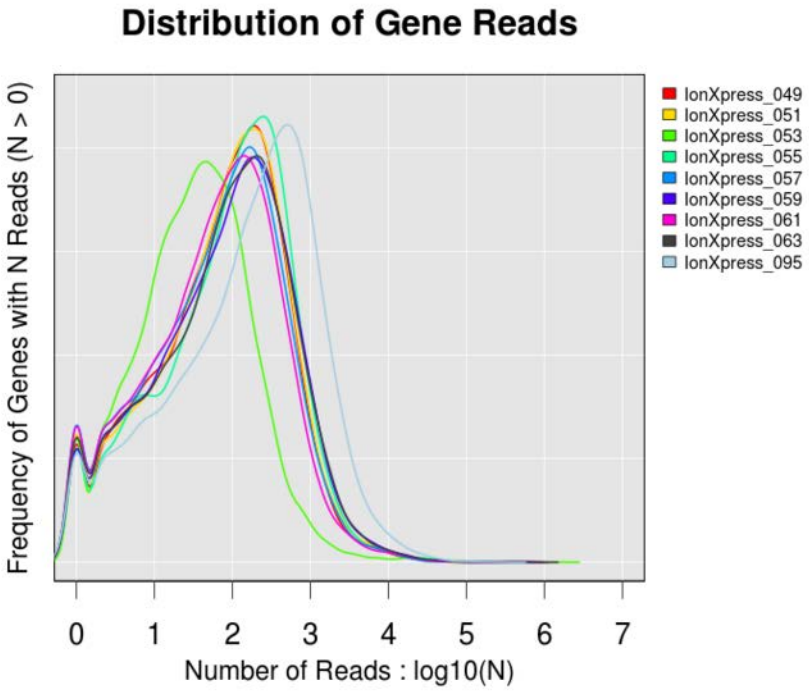
5. At the bottom of the report, click the **Distribution Plots**, **Correlation Heatmap**, **Correlation Plot**, and **Gene Heatmap** tabs to review the data graphically.

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.

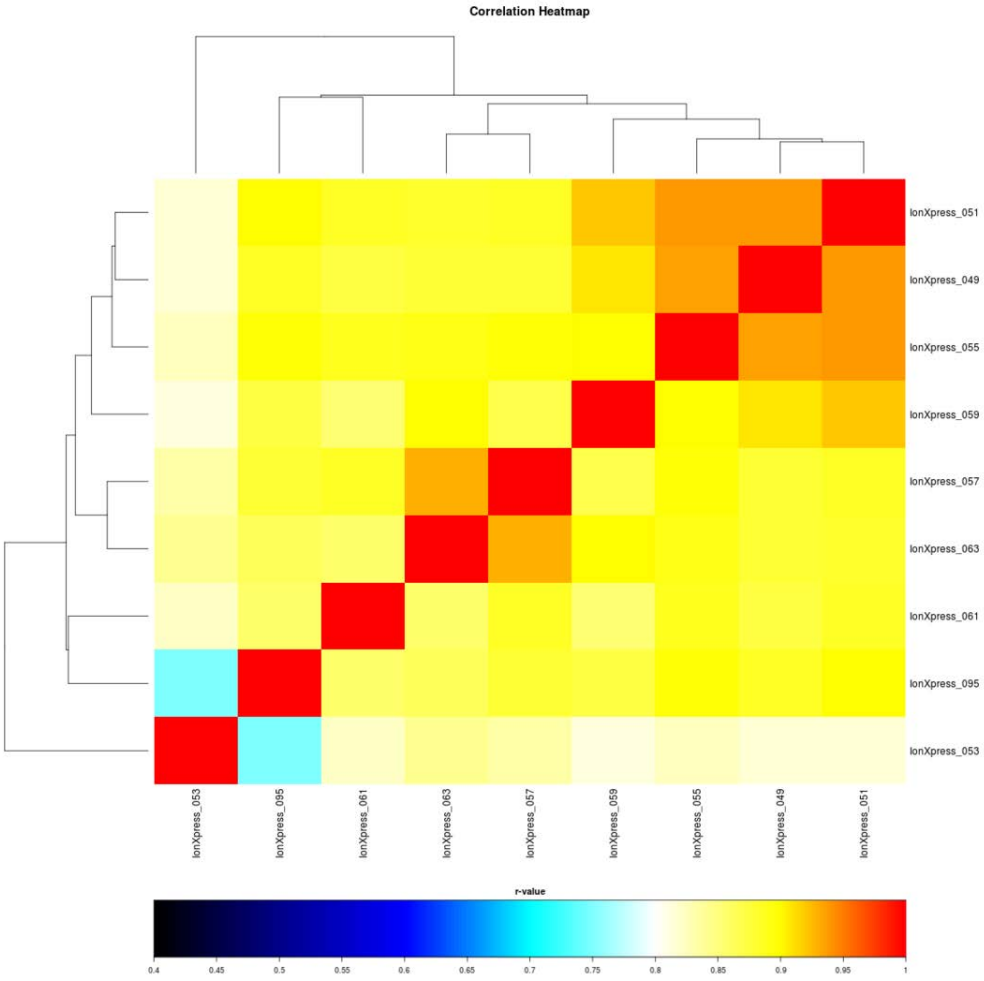


Distribution of Gene Reads – Distribution of genes across barcodes showing the frequency of numbers of genes having similar log10 read counts. All curves are plotted on the same axis scale. The counts data is fitted to a Gaussian kernel using the default R 'density' function.



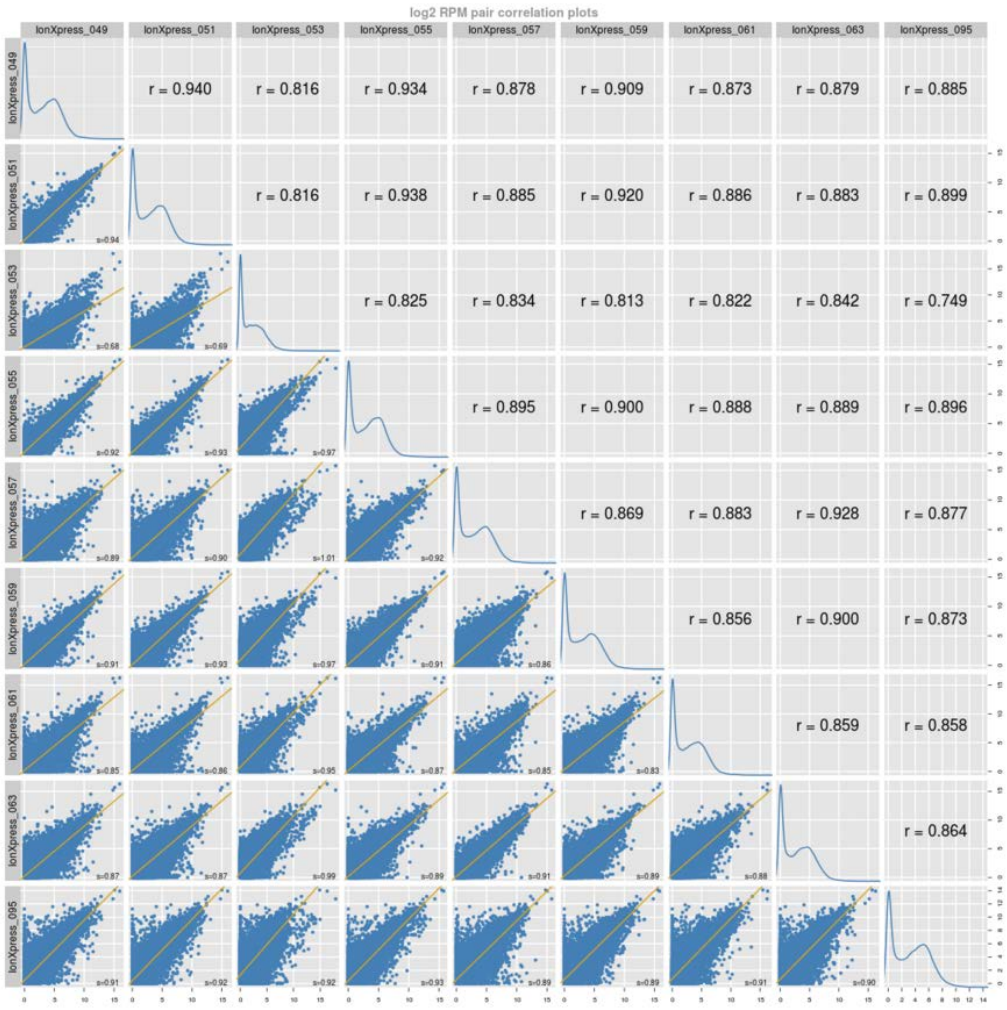
Correlation Heatmap

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



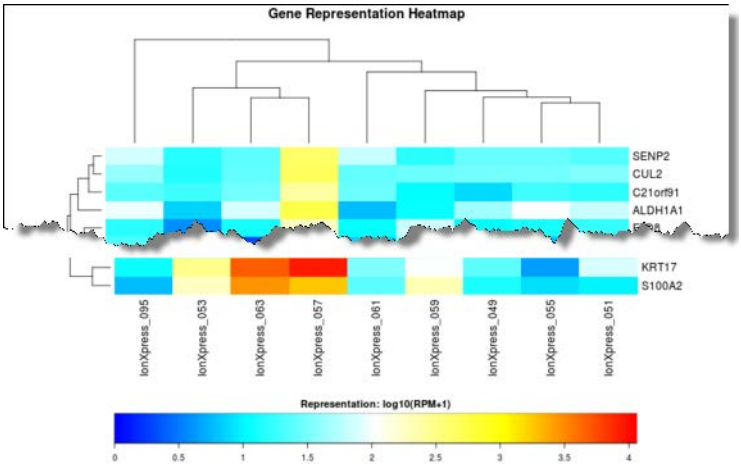
Correlation Plot

Barcode read pair correlation plot – Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show the frequency density plot for the individual log(RPM+1) values for each barcode. (If only one barcode has reads, a density plot is displayed.) Click the plot to open an expanded view.



Gene heatmap

Gene Representation Heatmap – Displays 250 genes showing the most variation in representation across barcodes as measured by the coefficient of variation (CV) of normalized read counts for genes that have at least one barcode with at least 100 RPM reads, plotted using log10 of those counts. For this plot, barcodes are omitted if they have $<10^5$ total reads.



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:

- **Barcode Summary Report** – A table listing each barcode's sample name, total reads, aligned reads, and percent aligned.
- **Absolute Reads Matrix** – This table lists absolute reads for the genes found on each barcode.
- **Absolute Normalized Reads Matrix** – This table lists absolute normalized reads for the genes found on each barcode.
- **Differential Expression for Barcode Pair** – Is 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.

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 ▸ Templates** screen, select **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 the Gear button to access the Copy function 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).

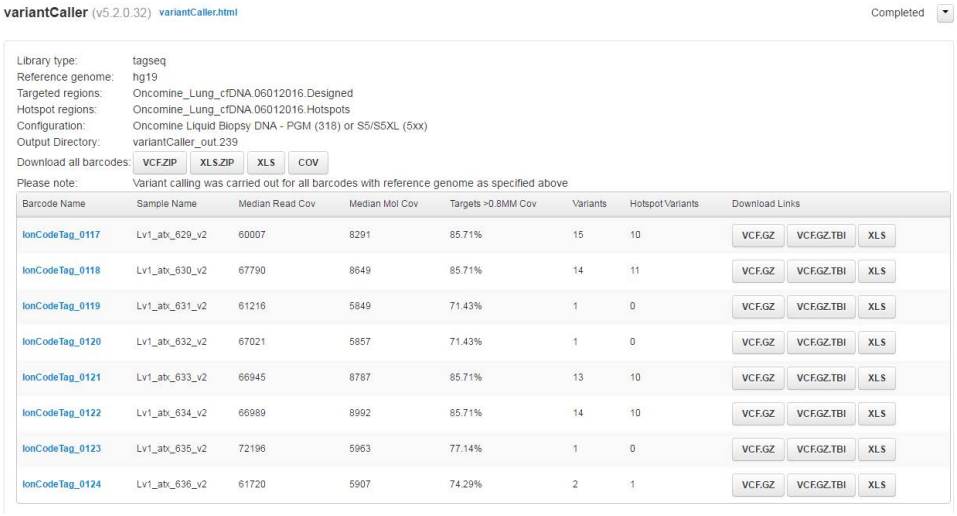
e. Click **Copy Template**.
Your new template appears under the Template Name column.

Review Oncomine™ cfDNA assay run results

The Completed Runs Report from an Oncomine™ cfDNA Assay run is similar to variantCaller plugin reports. The following outputs have been added.

1. After the run is complete, click **Data ▸ Completed Runs and Results**, 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.

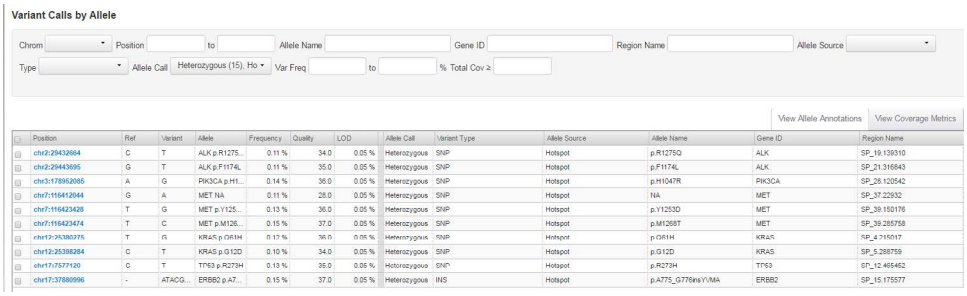


3. Review the results in the **Median Read Cov**, **Median Mol Cov**, and **Targets > 0.8MM** columns.

Column	Description
Median Read Coverage	Reports median coverage across targets. Median Molecular Coverage reports median number of individual interrogated DNA molecules across targets.
Targets >0.8 Median Molecular Coverage	Reports percent of targets with molecular coverage within 80% of the median coverage value. This is a new stricter definition of panel uniformity.
Median Read Coverage and Targets >0.8 Median Molecular Coverage	Measures the quality of the sequencing run and library performance, while Median Molecular Coverage measures the amount and quality of the input DNA sample.
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.

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 Targets >0.8 Median Molecular Coverage >60%.

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



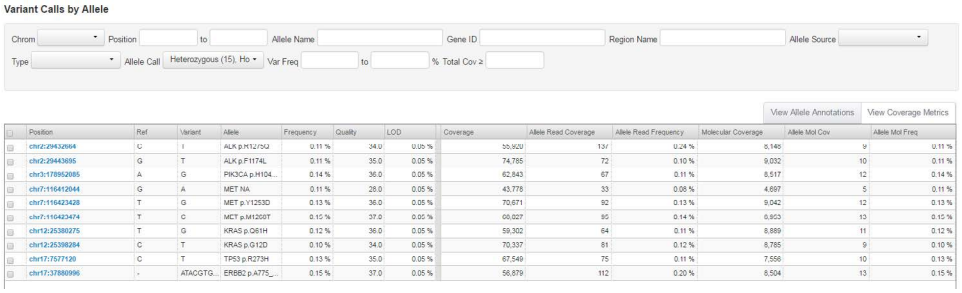
By default only hotspot alleles calls are shown in the variant table. We do not report hotspot alleles that did not meet our criteria for calling. However, we do provide at least one record for each hotspot position. This can include: novel allele call at hotspot position, hotspot allele call, or absent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

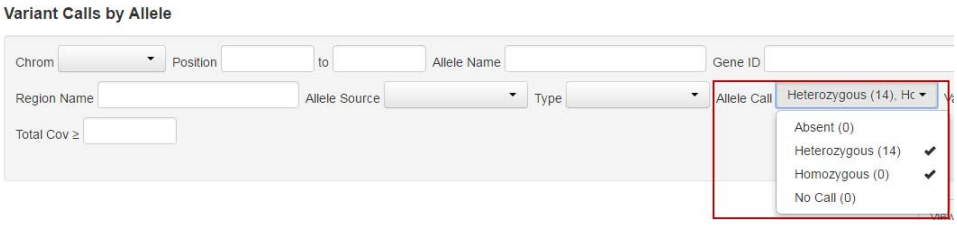
Observed frequency can be lower than LOD due to sampling nature of the assay.

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".

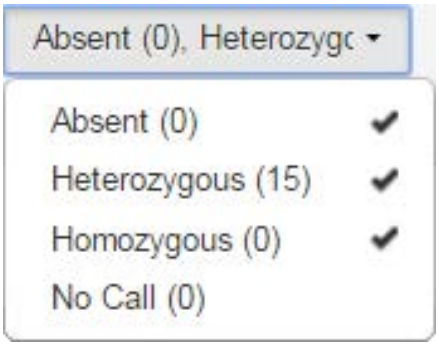
5. Click **View Coverage Metrics** to view the total number of interrogated DNA molecules at hotspot positions (Molecular Coverage), and the number of molecules containing the variant (Allele Mol Cov).



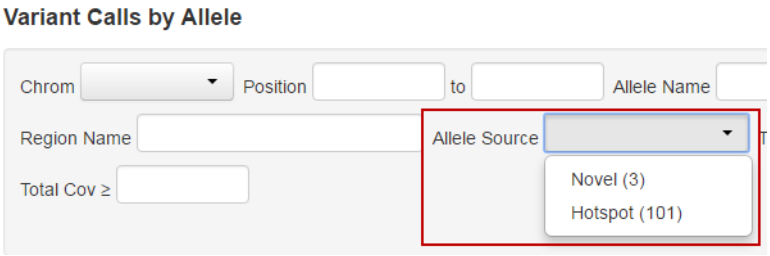
6. You can modify the types of calls that are displayed in the Allele Calls dropdown list, by selecting or deselecting Absent, Heterozygous, Homozygous, or No Call. No calls are variant calls that are classified as systematic errors.



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 Custom Analysis Parameters" on page 526.

1. Select **Plan** ▸ **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.

Plugins

The Torrent Browser Plugin Store

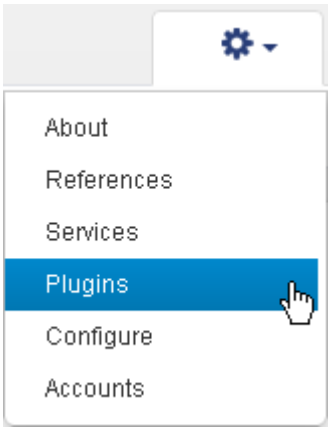
In the **Torrent Browser Plugin Store** you can search for plugins of interest, download them in your Torrent Browser, and run them on your analyses.

You can install a plugin from the store automatically or manually. Each plugin page provides an XML link for automatic installation and a zipped offline installer for manual installation. These links are shown in the example plugin page below.

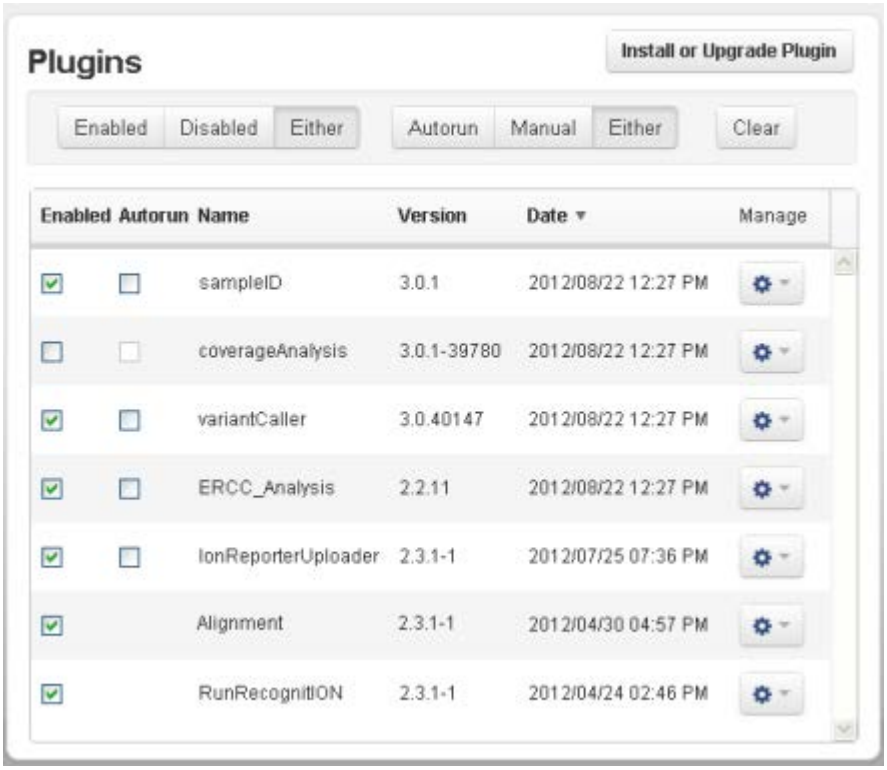
Automatic installation from the Torrent Browser Plugin Store

Follow these steps for automatic installation of a plugin from the Torrent Browser Plugin Store:

1. In the **Torrent Browser Plugin Store** go to the page for the plugin to be installed.
2. Scroll to the bottom of the page, and find the XML link with these directions: "Copy and paste this link into your Torrent Browser under the 'Config' tab after clicking the 'Add' button in the plugin section to auto install the plugin."
   An example XML link is the following: `http://torrentcircuit.iontorrent.com/warehouse/download/feedfile/Ex.xml`
   Copy this link.
3. In your Torrent Browser, click the gear menu (near the top right) and select **Plugins**:



4. In the Plugins tab, click the **Install or Upgrade Plugin** button:



5. In the **Install via URL** tab, paste the plugin's XML link into the empty field, and click **Download**.

After installation is complete, you must click the **Enabled** checkbox to make the plugin available to users.

Manual installation of a Torrent Browser Plugin Store package

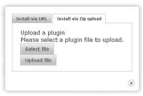
Follow these steps to download a plugin's installation package for manual installation:

1. In the **Torrent Browser Plugin Store** . go to the page for the plugin to be installed.

2. Scroll to the bottom of the page, and find the table with this explanation: "Links to the plugin archives are provided below for manual installation." Click theOffline Installer link.

3. Save the download to your machine.

4. In your Torrent Browser, go to the Admin Plugin section, and click the **Install or Upgrade Plugin** button.
5. In the **Install via Zip upload** tab, click the **Select file** button. Browse to the Offline Installer you just downloaded, and select it.

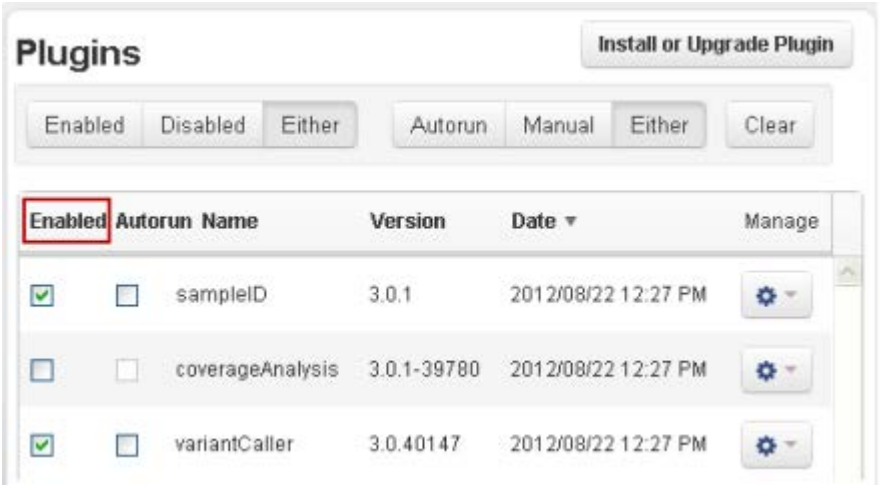


6. Click the **Upload file** button. The installation begins.
7. After installation is complete, you must click the **Enabled** checkbox to make the plugin available to users.

Enable an installed plugin

Follow these steps to enable an installed plugin:

1. Click the Torrent Browser Admin gear menu, and select **Plugins**. The installed plugins are displayed.



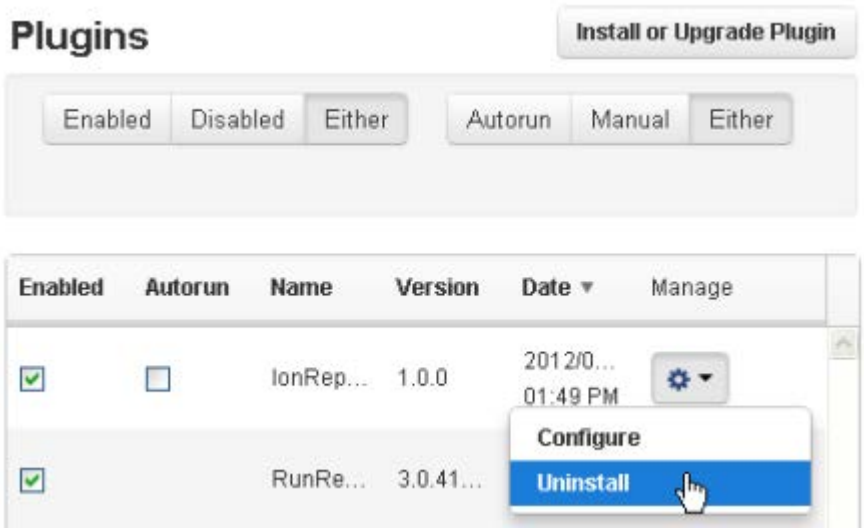
2. Click the **Enabled** checkbox for new plugins, to make them available to users.Click the Auto-Run checkbox for plugins that you want to be run on *every* analysis run.

IMPORTANT! You must click the **Enabled** checkbox for new plugins, to make them available to users.

Delete a plugin

To delete a plugin:

1. Click the Torrent Browser Admin gear box and select **Plugins**.
2. For the plugin you want to remove, click the gear box menu for that plugin and select **Uninstall**:



Not all plugins offer a Configure option.

Enable or disable automatic plugin execution

1. Click the **Enabled** checkbox for the plugin to enable the plugin to run automatically after pipeline analysis processing. Uncheck the checkbox to disable automatic plugin exection.
2. When the plugin **Auto-Run** status is enabled, the plugin is run at the end of *every* analysis job.
3. Plugins that require manual input, for instance for options or user information, cannot be **Auto-Run**.

Plugin Summary

The **Plugin Summary** section lists the plugins associated with the analysis, and provides an interface for running and monitoring your plugin(s).

Each plugin has one of the following behaviors:

- **Run without user input** Plugins without a user interface display a confirmation message that the plugin has been submitted to run. These plugin launch immediately (without confirmation) when you click them in the **Select Plugins to Run** list.
- **Run with a user interface** Plugins requiring input parameters display a user interface dialog and are launched after you click **Submit**.

The Combine Alignment and IonReporterUploader functionality

In previous releases, Combine Alignment was a plugin available through the **Select plugins to run** button. Combine Alignment is now available in a project result set page, Data > Projects > *projectname*, with the **Combine Selected...** button. The result sets to be combined must be members of the same project.

The IonReporterUploader plugin is available on a completed run report to launch manually through the **Select plugins to run** button and can also be specified in the template and planned run wizard, under the IonReporter chevron, to run automatically (after the plugin is configured).

Run a plugin

IMPORTANT! The following plugins are pre-installed.

- ampliSeqRNA
- AssemblerSPAdes
- coverageAnalysis
- DataXfer
- ERCC_Analysis
- FileExporter
- FilterDuplicates
- PGxAnalysis
- RNASeqAnalysis
- IonReporterUploader
- Run Transfer
- sampleID
- variantCaller

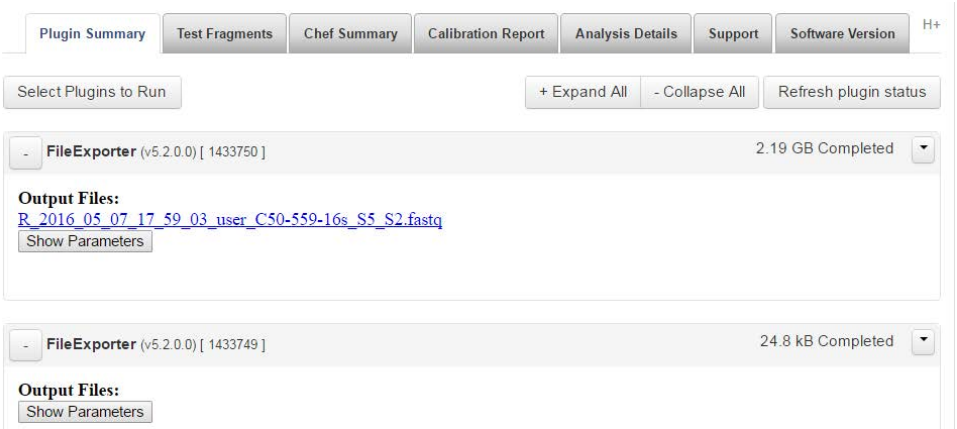
To manually run a post-analysis plugin on the report data:

1. Click **Select Plugins To Run**.
2. The Plugin List pops up and displays a list of available plugins:
3. Click the plugin you want to run. If the plugin does not require user input, it is immediately queued for execution.
4. Select the desired plugin options and click **Submit**. This runs your plugin, listing the run status in the **Plugin Summary** panel.
5. For plugins that take a long time to run, click **Refresh Plugin Status** to update the plugin display status.

   Note: You can stop a plugin from the status screen.

The Plugin Summary list

After a plugin runs, it is listed in the **Plugin Summary** panel:



Some plugins, such as Alignment, display a preview results window in the Plugin Summary list.

Plugin status and information

Each plug includes a summary line:

The summary line includes the following information:

- The size of the plugin report and output files.
- A trash icon

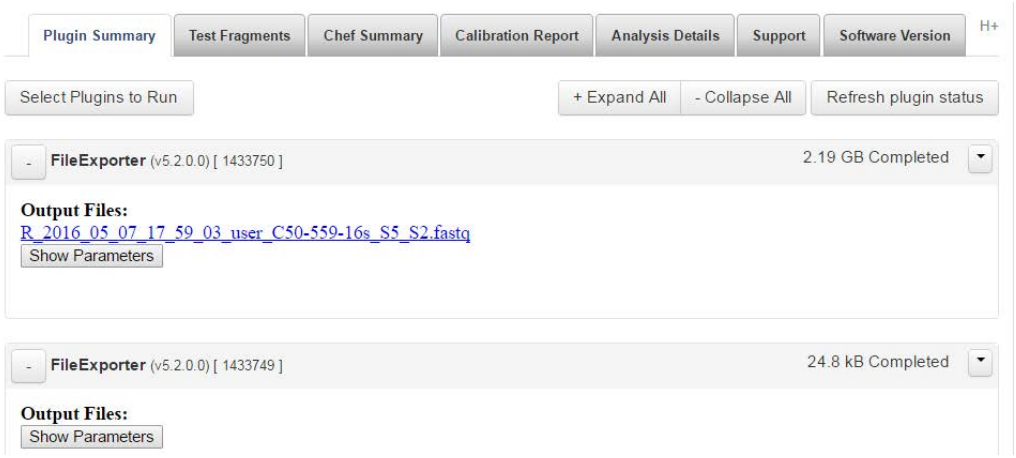
  to delete the plugin report and results. (Careful, there is no confirmation dialog.)
- The plugin status, such as Queued, Started, Completed, or Error.
- The scroll icon

  for the plugin's log file.

Plugin reports

Plugin results, results summaries, links to output files, and other information are available in the plugin report pages.

Click the plugin html link in the Plugin Summary section to open that plugin's report page:



Run the Installed Plugins

You customize your Torrent Suite™ Software analysis by running one or more plugin applications at the end of each run. Your Torrent Browser includes several of these plugins, such as for variant calling and realignment. The Torrent Browser Plugin Store offers other plugins written by Ion Community members. The **Torrent Browser Plugin Store** is on the Ion Community (registration required).

Available plugins

This table lists the pre-installed and officially supported plugins.

Plugin	Description	
ampliSeqRNA	Performs primary analysis from the sequencing output of the AmpliSeq Transcriptome Panel. The plugin generates an initial summary report listing 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.	
AssemblerSPAdes	Allows for an initial level analysis on assembly, with metrics such as number of contigs, N50 and other analysis metrics. The plugin is ideal for genomes less than 50 megabases in size.	
Coverage Analysis	Provides statistics and graphs describing the level of sequence coverage produced for targeted genomic regions.	
ERCC Analysis	Helps with ERCC RNA Spike-in Controls: enables you to quickly determine whether or not the ERCC results indicate a problem with either the library preparation or the sequencing instrument run.	

Plugin	Description	
FileExporter	Customizes the output file names of an analysis run. This plugin allows you to rename output files. The plugin also offers these options: • Generates a Fastq format file of the analysis output • Generates an SFF format file of the analysis output • Renames Variant Caller plugin output files (when available) • ZIps output files	
FilterDuplicates	Removes duplicate reads. BAM files with duplicate reads removed are saved in the FilterDuplicates plugin directory.	
IonReporterUploader	Transfers run results files to your organization in Ion Reporter™ Software (available under a separate license). Please read the Ion Reporter™ Software release notes for instructions about the Uploader plugin.	
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. See Pharmacogenomics section in the Applications chapter for more information.	
RNASeqAnalysis	Used to analyze cDNA reads. This plugin is an RNA transcript alignment and analysis tool for use with the reference genomes hg19 and mm10. See RNASeqAnalysis section in the Applications chapter for more information.	
RunTransfer	Transfers the signal processing output of a completedTorrent Suite™ Software run from one Torrent Server to another Torrent Server. On the new Torrent Server, a analysis is launched on the newly-transferred files.	

Plugin	Description	
sampleID	Uses sample fingerprinting to identify any cross-contamination between samples or between barcodes in a run.	
variantCaller	Calls SNP and indel variants across a reference or within a targeted subset of that reference. With low-frequency variant options, the plugin can call variants down to a 5% level of variant frequency. The plugin can also show which variants coincide with predefined HotSpot positions on the reference sequence.	

This table lists functionality that previously shipped as a supported plugin and that now is available only on the Plugin Store.

Previous plugin	Description
Alignment	Performs a new alignment to the reference you specify. (The main Torrent Suite™ Software analysis pipeline automatically performs alignment to hg19, unrelated to the Alignment plugin.)

This table lists functionality that previously shipped as a supported plugin and that now is replaced by the RunTransfer plugin.

Previous plugin	Description
TorrentSuiteCloud	Replaced by the RunTransfer plugin.

This table lists functionality that previously shipped as supported plugins and that are replaced by the FileExporter plugin.

Previous plugin	Description
FastqCreator	Creates a FASTQ format file from the BAM results file of a completed analysis run.

Plugin configuration

This section describes the various ways plugins are configured.

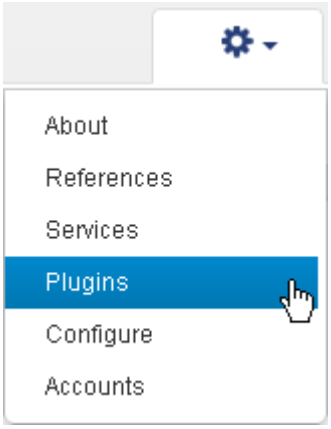
- **No configuration** These plugins (sample ID and FilterDuplicates) do not take an user options. These plugins are ready to use as-is on new systems. When selected for manual launch on a completed run report, these plugins launch immediately.
- **The admin Plugins tab** Several plugins can be configured in the admin Plugins tab. In this case, the one configuration is used for all runs of the plugin by all users (unless overridden by a template configuration or manual launch configuration). If someone changes an existing plugin configuration here, those settings become the new configuration that is used by everyone.
- **Manual launch page** Many plugins can be configured on a manual launch in the run report for a completed run. Each run can be configured with different options.
- **Plan template wizard** Some plugins, IonReporterUploader andvariantCaller, also can be configured in a run plan template. Each run using that template has the same plugin configuration. (The IonReporterUploader has its own chevron in the wizard. The variantCaller plugin is configured in the Plugins chevron.)

Plugin	Accepts configuration	Admin Plugin tab configuration	Plan template wizard	Manual launch configuration	
ampliSeqRNA			**Yes**		
Assembler SPAdes	**Yes**	No	**Yes**	**Yes**	
Coverage Analysis	**Yes**	No	**Yes**	**Yes**	
DataXfer					
ERCC Analysis	**Yes**	**Yes**	**Yes**	**Yes**	
FileExporter	**Yes**	**Yes**	**Yes**	**Yes**	
FilterDuplicates	No	No	**Yes**	No	
IonReporterUploader	**Yes**	**Yes**	Yes (IonReporter chevron)	**Yes**	
PGxAnalysis					
RNASeqAnalysis					
RunTransfer	**Yes**	**Yes**	**Yes**	**Yes**	

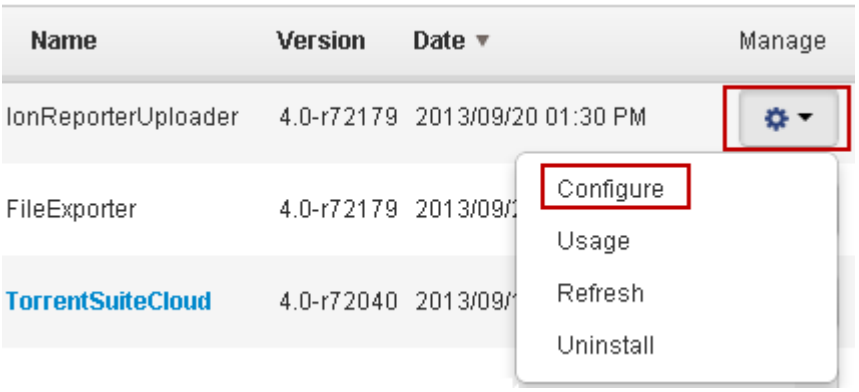
Plugin	Accepts configuration	Admin Plugin tab configuration	Plan template wizard	Manual launch configuration	
SampleID	No	No	**Yes**	No	
variantCaller	**Yes**	No	**Yes**	**Yes**	

Admin plugin configuration area

Access the admin plugin configuration area from the gear menu **Plugins** option:



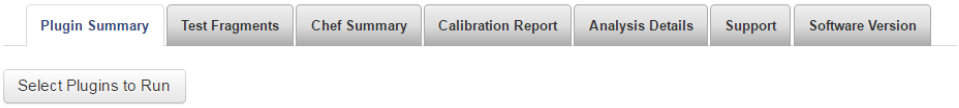
Then click the Manage column gear menu for the specific plugin and select **Configure**:



Manually run a plugin

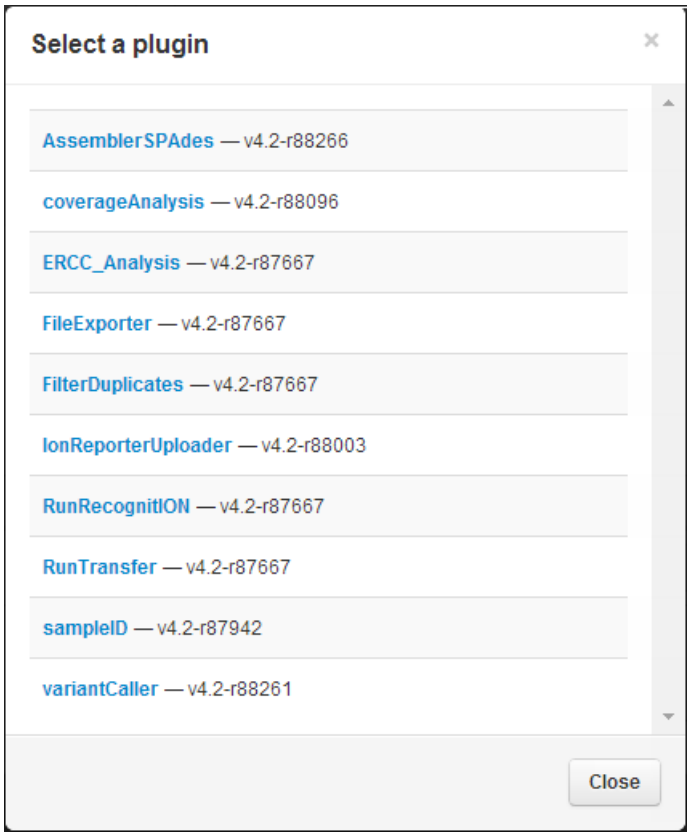
You can manually run a plugin in the run report of a completed analysis run, with the **Select plugins to run** button. Only enabled plugins are listed. Follow these steps to manually run a plugin:

1. Go to the **Data > Completed Runs & Reports** tab, then click the link for your completed analysis run.
2. In the run report, scroll down to Plugin Summary tab.



   The Plugin Summary also lists any plugins that executed on your run (not shown in this example).

3. Click **Select plugins to run** to see the list of plugins available in your Torrent Suite™ Software.



4. Click the desired plugin name to run a plugin. If the plugin does not require user input, it starts immediately, without a confirmation screen.
5. Click **Close** to close the Plugin List without running a plugin.

Automatically run plugins

When you design your sequencing protocol in the **Plan ▸ Template** page, you specify which plugins to execute. You can select any plugin that is installed and configured on your Torrent Suite™ Software. You are not limited to the pre-installed plugins.

Assembler SPAdes Plugin

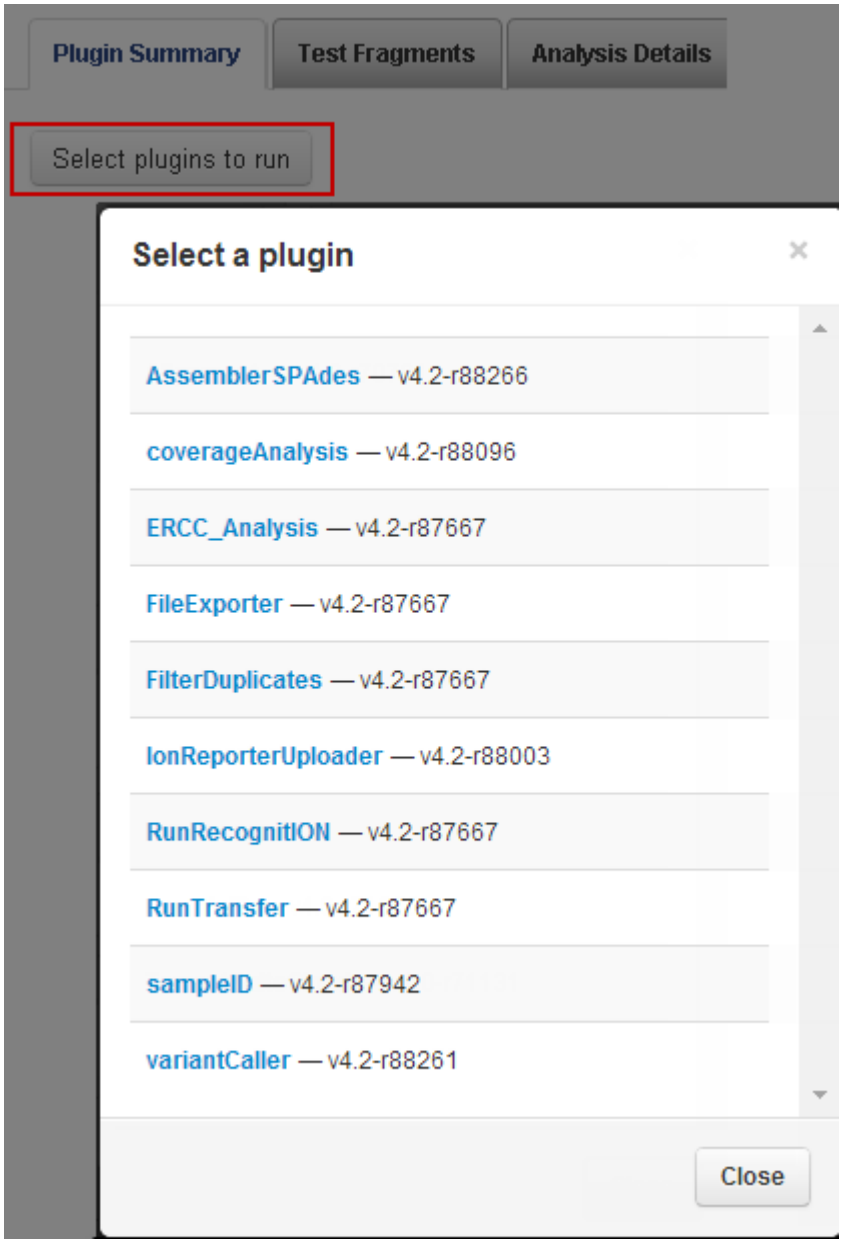
This plugin assembles reads into long sequences (contigs) and allows for basic level analysis, with metrics such as number of contigs, N50, and other analysis metrics. The plugin is ideal for genomes less than 50 megabases in size. The plugin assumes a haploid genome. For multiploid genomes, reads from different copies of a chromosome tend to assemble into different contigs.

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

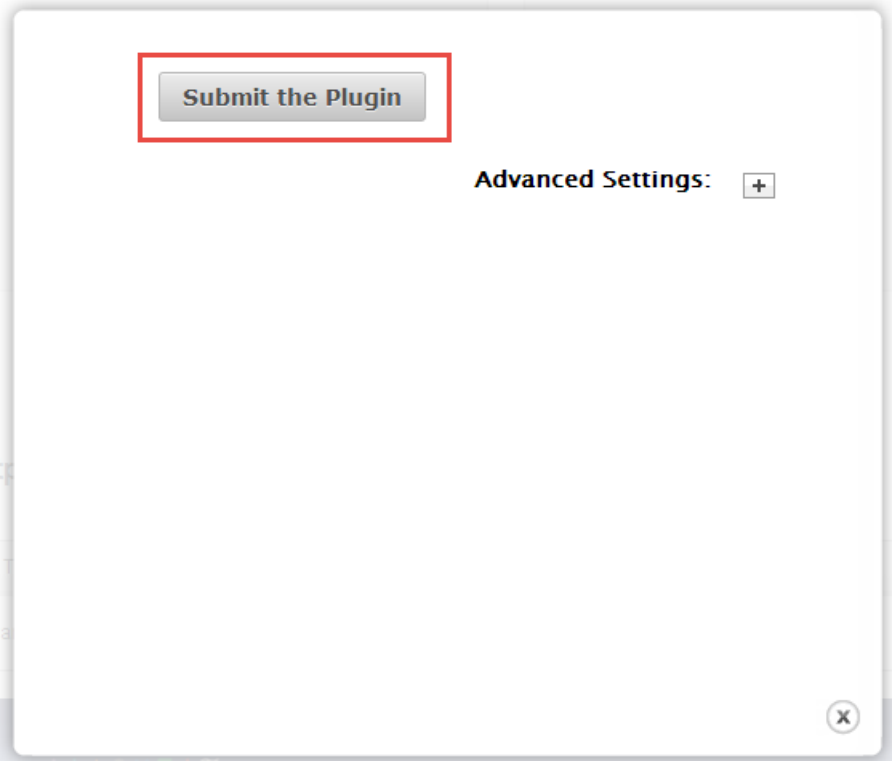
Run the AssemblerSPAdes plugin on a completed run

Follow these steps to run the plugin and to review the plugin output report.

1. To run this plugin, in the report for your run, scroll down to the Plugin Summary section, and click **Select plugins to run**.



2. In **Select a plugin**, select **Assembler SPAdes**. The plugin displays the plugin user interface:

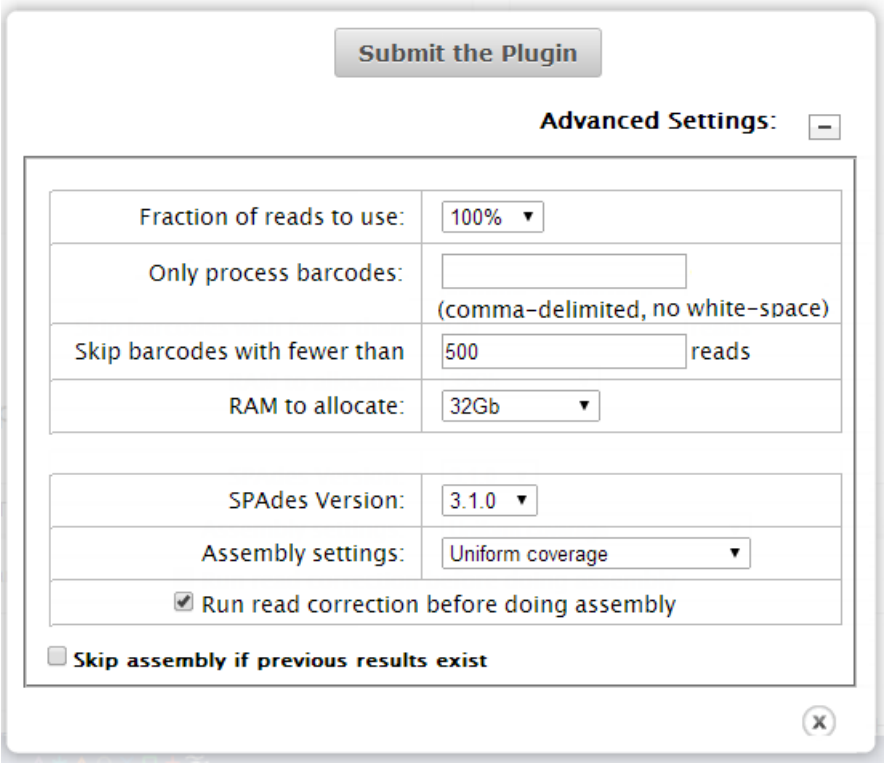


For most uses, you can take the defaults and click **Submit the Plugin**.

Advanced settings

This section describes the optional advanced settings. To open the advanced parameters fields, click the **Advanced Settings +** button.

These are the default advanced settings:

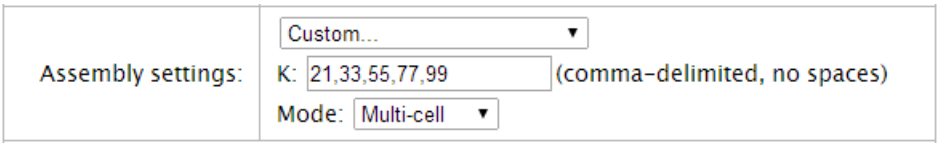


Field	Description
Fraction of reads to use	If less than 100%, reads are randomly sub-sampled. 100% is recommended the plugin automatically handles most changes in coverage.
Only process barcodes	By default the plugin processes all barcodes in the analysis and produces separate set of contigs for each barcode. To limit plugin analysis to only specific barcodes, list those barcodes here (separated by commas, no spaces). Example:
Skip barcodes with fewer than	Ignores barcodes whose number of reads do not meet this threshold. Intended to filter out barcode classification problems with noisy data.

Field	Description
RAM to allocate	The plugin attempt to allocate the amount of RAM specified here. 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 if you do not know.
Assembly settings	Set this menu as follows: • **Uniform coverage** Choose this setting for data with average or low GC content. This setting uses the default kmers. • **Non-uniform coverage** Choose this setting for data with high GC content. This setting uses the default kmers. • **Highly non-uniform coverage** Choose this setting for data with high GC content. This setting uses a different set of kmers.
Run read correction before doing assembly	Recommended.
Skip assembly if previous results exist	Recommended.

Custom assembly settings

The K and Mode fields are displayed when the Assembly settings menu is set to Custom:



The K field

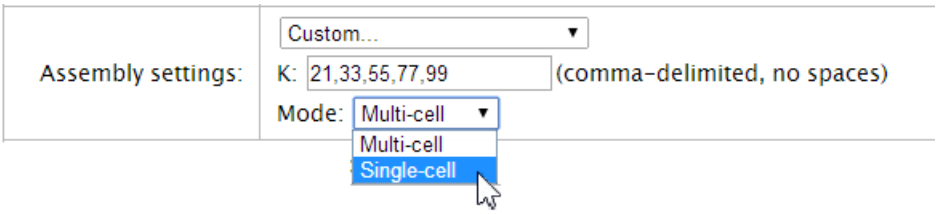
The SPAdes plugin is a De-Bruijin graph assembler. The plugin breaks reads in kmers, makes a connected graph, and traverses through that graph to produce contigs.

The K field determines the kmer size and how many kmers are used.

Notes about the kmers setting:

- Use a smaller kmer if your data contians many errors.
- If your data contains many repeats, use a larger kmer.
- Each additional kmer adds a fixed amount to the processing time (using 2 kmers takes twice as long as one kmer).

The Mode menu

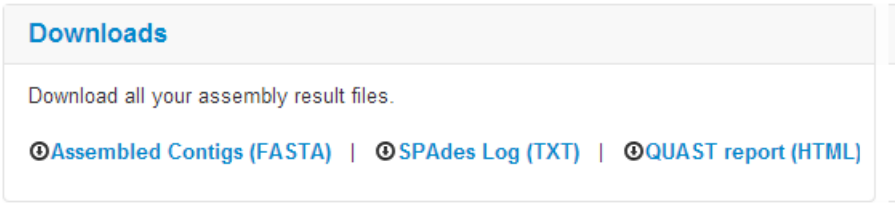


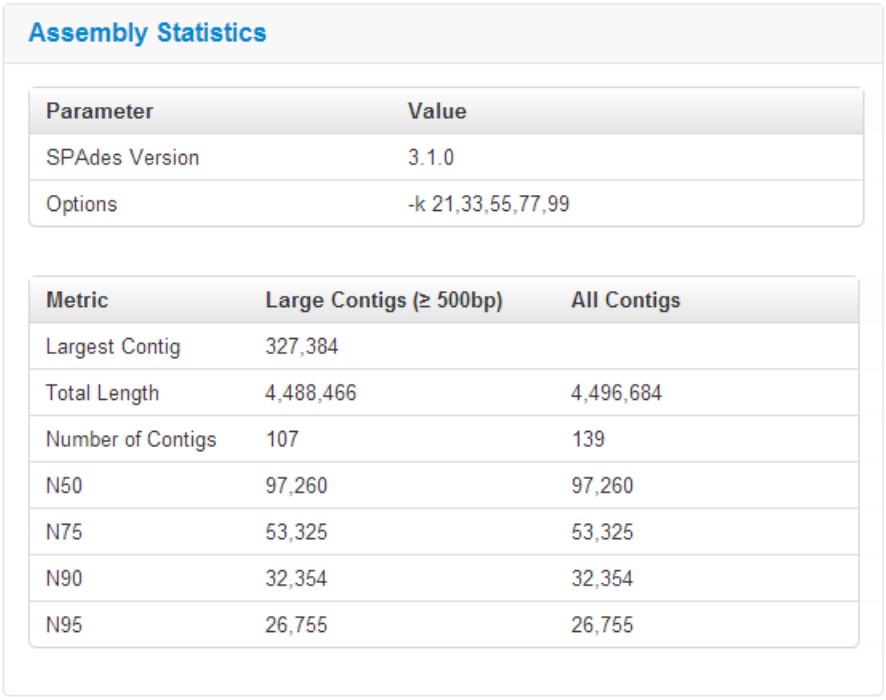
Select the **Single-cell** option for data with high (> 68%) GC content.

Select the **Multi-cell** option for data with average or low GC content.

Output

The plugin output includes sections for downloads and statistics:





Downloads

- **Assembled Contigs** Download or open a FASTA file of the assembled sequences in the FASTA format.
- **SPAdes log** Download or open a text file of log messages from SPAdes. (For more information, see the SPAdes site **bioinf.spbau.ru/spades**.)
- **QUAST report** Download or open an HTML file of assembly statistics generated by QUAST. (For more information, see SPAdes documentation **http://spades.bioinf.spbau.ru/release2.1.0/quality.htm**.)

Assembly Statistics

Parameter section

The Parameter section shows the version of SPAdes that generated this run and the set of kmers that was used:

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

Metric section

The Metric section shows the following information:

Metric	Large Contigs (≥ 500bp)	All Contigs
Largest Contig	327,384	
Total Length	4,488,466	4,496,684
Number of Contigs	107	139
N50	97,260	97,260
N75	53,325	53,325
N90	32,354	32,354
N95	26,755	26,755

Metric	Description
Largest Contig	Length of the longest assembled contig
Total Length	Total number of base pairs contained in all assembled contigs that are at least 500 bp in length.
Number of Contigs	N umber of assembled contigs that are at least 500 bp in length.

Metric	Description
N50	The contig length such that using longer or equal length contigs produces half (50%) the bases of the assembly. Usually there is no value that produces exactly 50%, so the more technical definition is the minimal length x such that using contigs of length at least x accounts for at least 50% of the total assembly length.
N75	The contig length such that using longer or equal length contigs produces 75% of the bases of the assembly.
N90	The contig length such that using longer or equal length contigs produces 90% of the bases of the assembly.
N95	The contig length such that using longer or equal length contigs produces 95% of the bases of the assembly.

Note: SPAdes only reports contigs that are 500 bp or longer.

Coverage Analysis Plugin

The Coverage Analysis plugin provides statistics and graphs describing the level of sequence coverage produced for targeted genomic regions. The report produced depends on the Library Type set in the plan. AmpliSeq™ DNA reports contain the most information, but Whole Genome runs will not have as many plots, for example. On some charts, such as the Amplicon and Reference Coverage charts, you can click an Export button and export the charts as graphics.

The plugin's documentation is embedded in its output page. Access the documentation through help and options icons in the top right corner of a chart:

You can also zoom in and out on many of the plots and graphs.

Run the Coverage Analysis plugin

You can run the Coverage Analysis plugin automatically or manually.

Include the Coverage Analysis plugin in a run plan

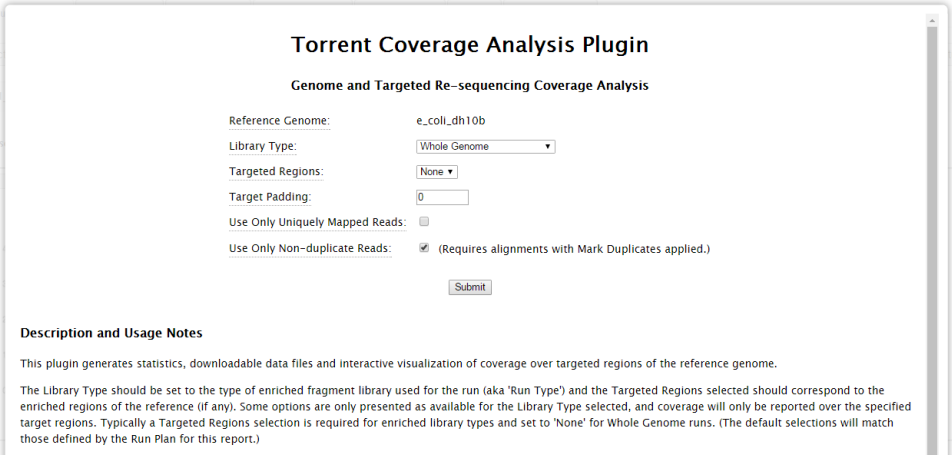
To run the Coverage Analysis plugin automatically, you select Coverage Analysis plugin during template setup. Refer to the Plan Tab and Templates pages sections for information about how to set up a template and create a planned run.

Manually launch the Coverage Analysis plugin

To run the Coverage Analysis plugin manually, perform the following steps:

1. In the Torrent Browser, select a run report by clicking a run link, then clicking a report from the drop-down area. The run report opens.
2. On the run report page, scroll about halfway down the screen to the Plugin Summary area. Click **Select plugins to run**. The **Select a plugin** popup appears:

3. Select **coverageAnalysis**. The Coverage Analysis Plugin interface appears.



4. Select a library type.
5. If you have one and would like to use it, select a targeted regions file.

6. Fill out the other plugin options. These options vary depending on your Library Type selection:
   - **Target Padding** If you would like to pad the target by a number of bases, enter the desired number. If you do not enter a number, the default of 0 is used.
   - **Use Only Uniquely Mapped Reads** If you would like the plugin to examine only unique starts, select the checkbox.
   - **Use Only Non-duplicate Reads** Select the checkbox to avoid duplicates. The Torrent Suite™ analysis must have been run with Mark Duplicates enabled.
   - **SampleID Tracking** Check this only if the Ion AmpliSeq™ library employed sampleID tracking amplicons.

7. When you are satisfied with your selections, click **Submit**.

   The analysis runs and a group of output reports is created.

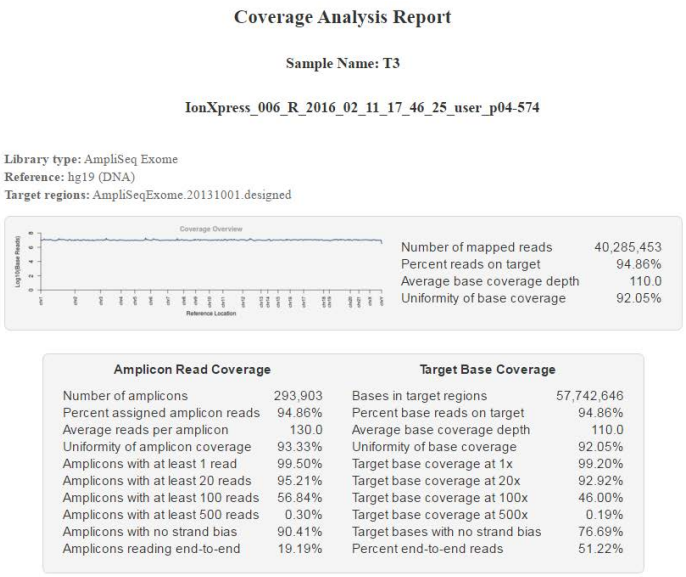
   The following sections of this document describe the output reports generated by the Coverage Analysis plugin.

Coverage Analysis Plugin output

The 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. In addition, in the File Links section at the bottom of the Coverage Analysis Report, you can download statistics files and the aligned reads BAM file.

Example statistics

The following is an example of the plugin statistics for an AmpliSeq Exome run. Most fields names offer hover help.



Coverage Analysis Report

Sample Name: T3

IonXpress_006_R_2016_02_11_17_46_25_user_p04-574

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

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

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

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

Reads statistics

The library type determines which statistics are presented.

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.
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 may 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.

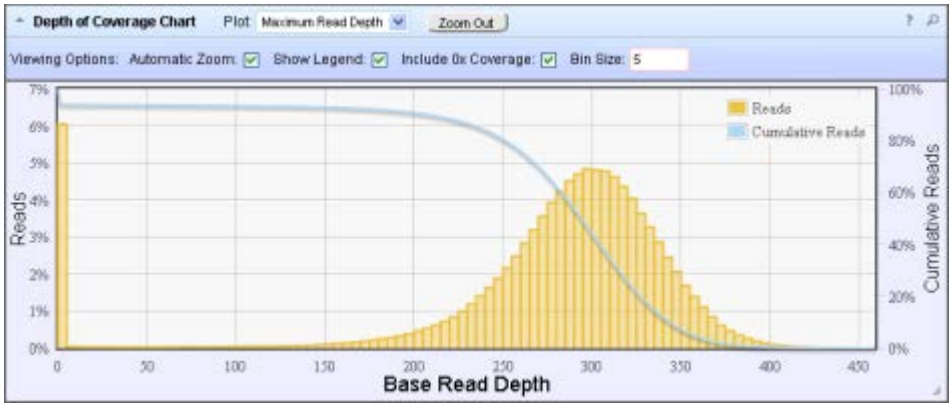
Statistic	Description
Uniformity of base coverage	The percentage of bases in all targeted regions (or whole genome) covered by at least 0.2x the average base coverage depth.
Maximum base read depth	The maximum number of times any single target base was read.
Average base read depth	The average number of reads of all targeted reference bases that were read at least once.
Std.Dev base read depth	The standard deviation (root variance) of the read depts 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 sufficientlyclose to the end of the amplicon region, taking the read direction (mapping strand) in to account.
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.

Statistic	Description
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 strandread 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 ofassigned reads (70%) that covered the whole amplicon target from 'end-to-end'. To allow for errorthe effective ends of the amplicon region for read alignment are within 2 bases of the actualends of the region.

Example charts

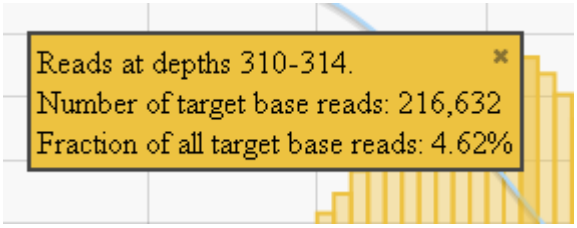
This section shows a couple example charts. Many charts have a Plot menu that allows you to change characteristics of the chart, for instance, to show both strands.

Click a chart's options icon (in the top right corner of a chart) to open the chart s viewing options panel.

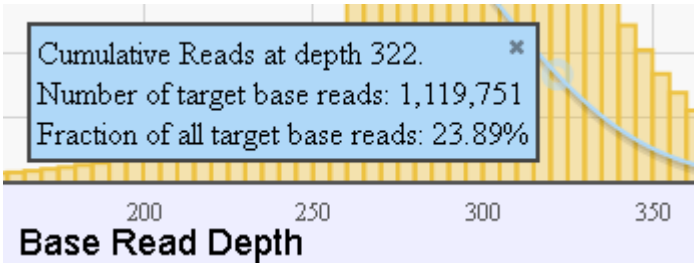


In the Depth of Coverage chart above, the left Y-axis (% reads) is the number of reads at a particular read depth (or bin of read depths) as a percentage of the total number of (base) reads. The right Y-axis (% cumulative reads) is the cumulative count of the number of reads at a given read depth *or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

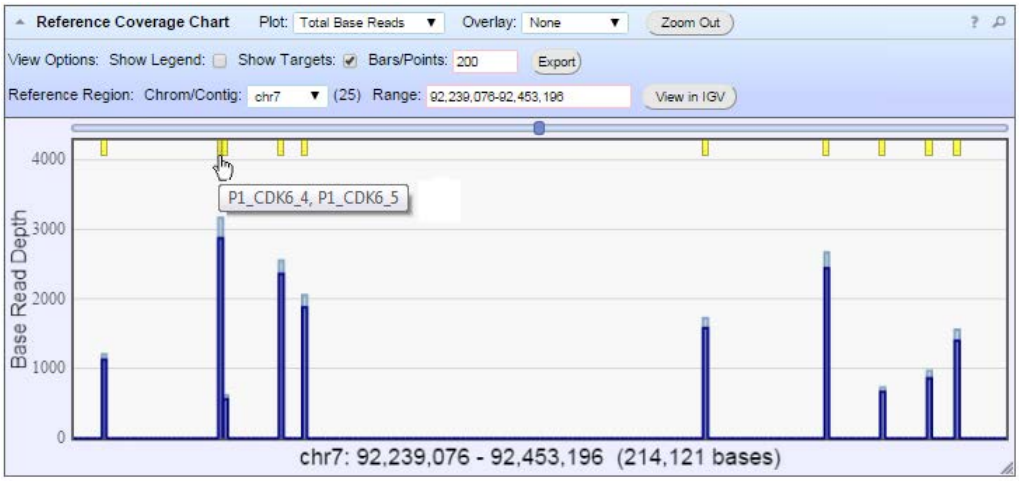
In most charts you click on a data point to open a detail panel for that data:



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:

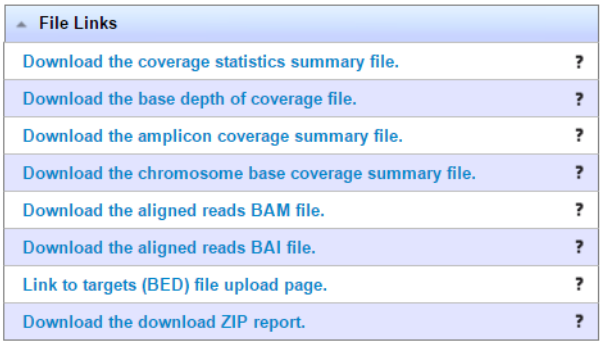


You can also zoom in on a region of interest.

Note: The Viewing options panel is revealed or hidden with the chart's options icon . The help icon opens a description of the chart.

Output files

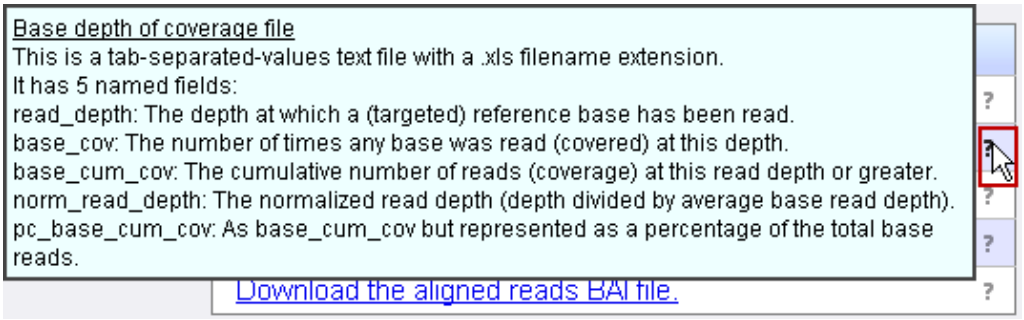
Download plugin results file from links in the File Links section. This example is from a generic sequence run:



TargetSeq™ analyses also offer the option "Download the TargetSeq coverage summary file".

Ion AmpliSeq™ analyses also offer the option "Download the amplicon coverage summary file".

Click a file's question mark icon ? to open a description of the file:



The following table lists the output files with a description of each. Not all output files are generated on every type of analysis.

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** 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 ofassigned 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.

File	Description
Target coverage summary	Coverage summary data used to create the Target Coverage Chart. This file contains fields: • **contig_id** The name of the chromosome or contig of the reference for this target. • **contig_srt** The start location of the 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 target region. **Note:** The length of the target is given as tlen = (contig_end - contig_srt + 1). • **region_id** The ID for this target 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** The number of G and C bases in the target region. Hence, %GC = 100% * gc / tlen. • **covered** The number of bases of this target that were covered by at least one read. Hence the percentage coverage of this target is calculated as %cov = 100% * covered / tlen. Note that this might also not 100% because of base deletions in the sample vs. the reference genome. • **uncov_3p** The number of bases that are not covered at the 3' (downstream) end of the forward DNA strand. For TargetSeq™ analyses,this may indicate poor probe coverage at this end of the target. • **uncov_5p** The number of bases that are not covered at the 5' (upstream) end of the forward DNA strand. • **depth** The average target base read depth. This value equals (fwd_reads + rev_reads) / tlen 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 target.

File	Description
	• **rev_reads** The number of reverse strand reads assigned to this target.
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. • **rev_ontrg** (if present) Total number and reverse 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 alignedreads used to generate this report page, in BAM format. BAM is the binary form of the SAM format file that records individual reads and their alignment to the reference genome. 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.
Primer-trimmed reads BAM file.	Binary primer-trimmed aligned reads. Created from the original alignment file by trimming reads to specific amplicons regions they are assigned to, where necessary to resolve overlaps with multiple amplicon target regions.
Primer-trimmed reads BAI file.	Binary BAM index file as required by some analysis tools and alignment viewers such as IGV.

ERCC Analysis Plugin

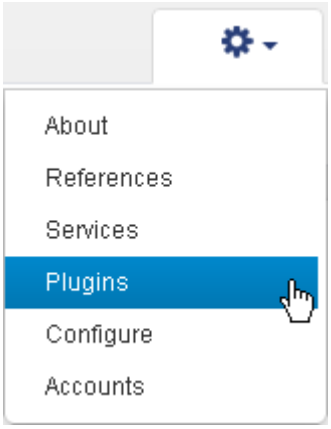
This section describes the ERCC Analysis plugin. This plugin helps with analyses that use ERCC RNA Spike-in Controls. The plugin enables you to quickly determine whether or not the ERCC results indicate a problem with either the library preparation or the sequencing instrument run.

Enable the ERCC Analysis plugin

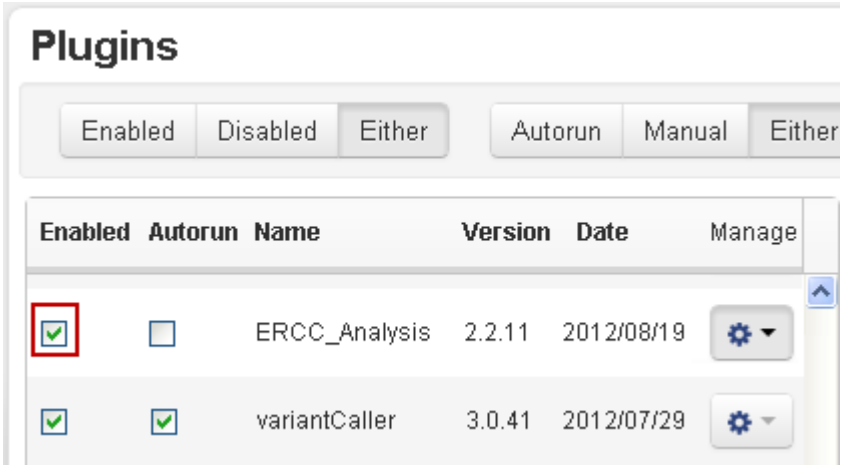
A plugin must be enabled before it can run. Your Torrent Suite™ Software administrator may have already enabled ERCC Analysis plugin and then the plugin appears in the run report Plugin Summary Select Plugin to Run list.

Follow these steps if you need to enable the plugin:

1. Scroll to the top of the Torrent Browser and click **Plugins** in the gear menu on the right:



2. If the ERCC_Analysis plugin does not appear on your plugin page, click the **Name** column to sort by name and scroll to the plugin. In the ERCC_Analysis row, click the Enable column checkbox:

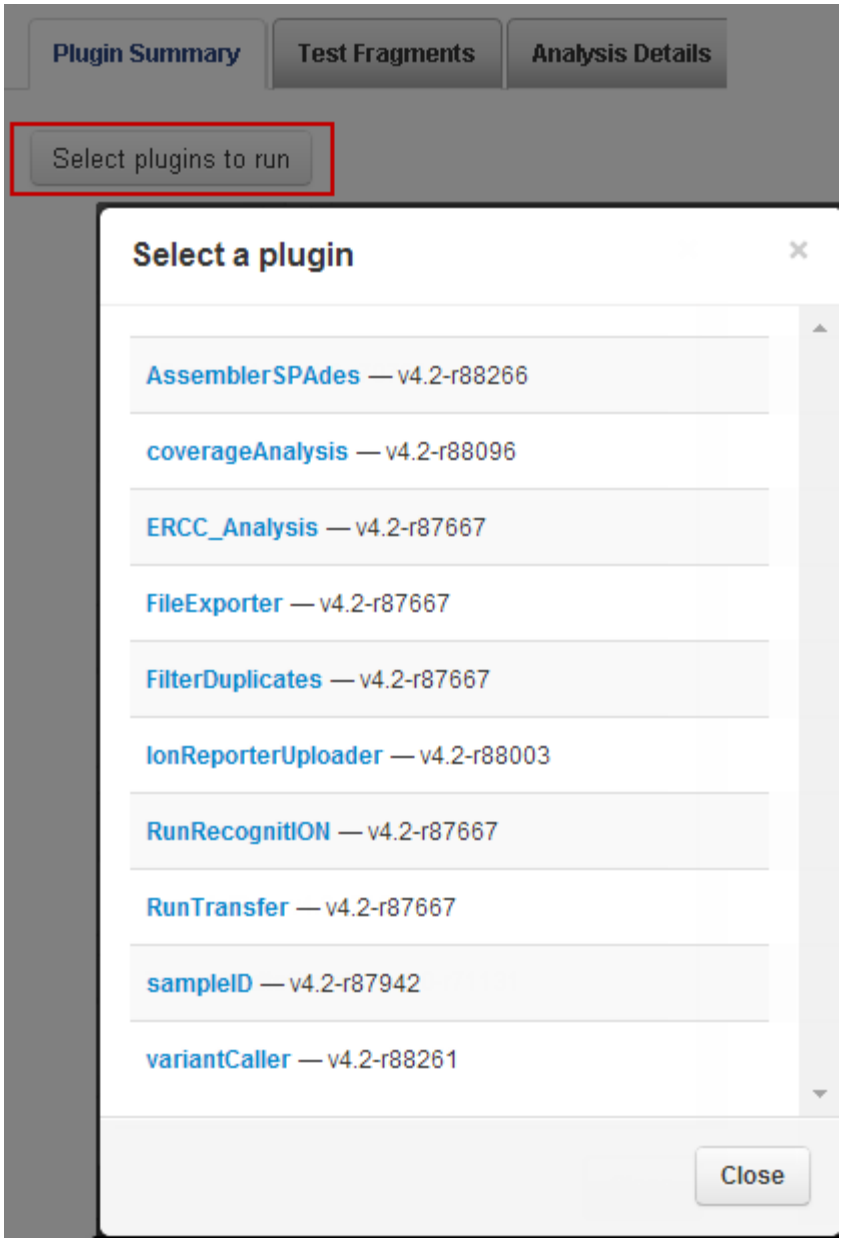


Note: Auto-run is not recommended for the ERCC_Analysis plugin unless most analyses on this Torrent Server use ERCC controls.

Manually run the ERCC Analysis plugin

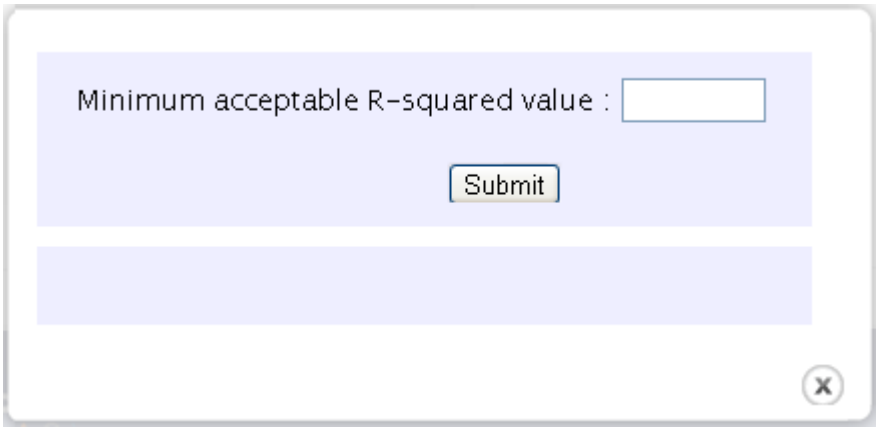
Follow these steps to manually launch the ERCC_Analysis plugin. You can use the default minimum R-squared value or enter you own value.

1. Open the run report for the analysis. Scroll down to the Plugin Summary section.
2. Click **Select plugin to run** to open the plugin list:



3. Click **ERCC_Analysis**. (The version number on your system might be different from the version shown here.)

4. A popup window with a minimum acceptable R-squared value appears. You can use the default value or enter your own value. The value should be in the range from 0 to 1.



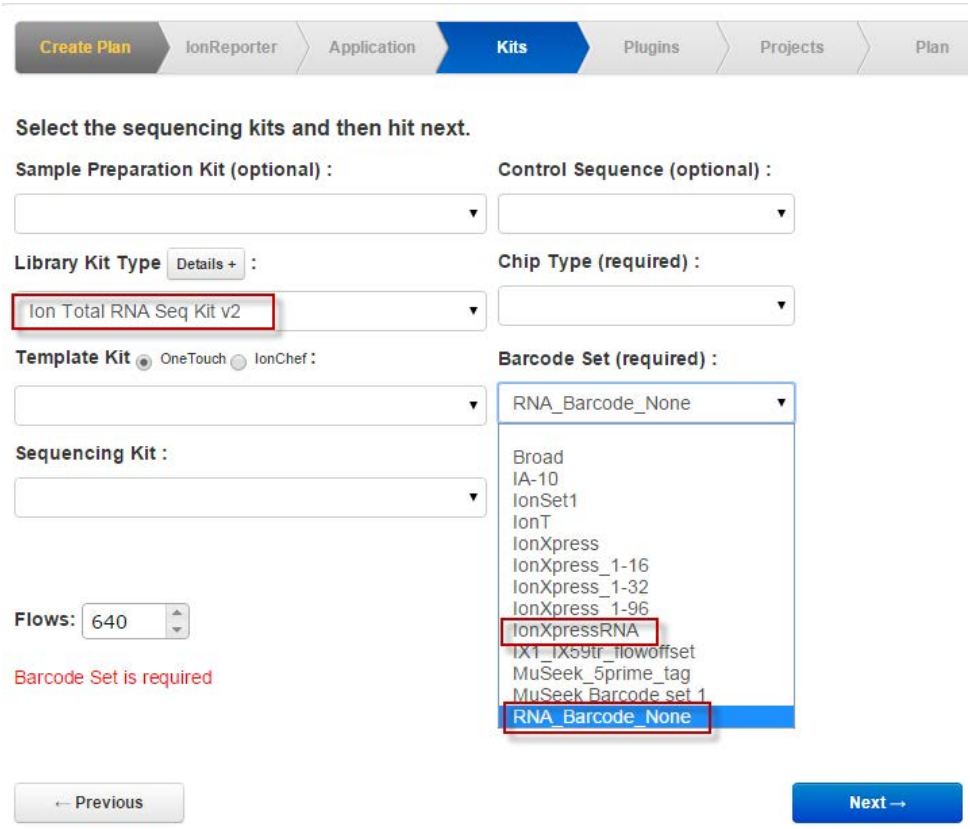
5. Click **Submit** to start the plugin.

Configure a template to run the ERCC plugin

If you configure a template or planned run to execute the ERCC Analysis plugin, *and* your experiment uses the Ion Total RNA-Seq Kit V2, then on the wizard Kits page, you must make a Barcode Set selection. Select either one of the following in the Barcode Set menu:

- **IonXpressRNA** Select this if your experiment uses this kit.
- **RNA_Barcode_None** Select this if your experiment does not use a barcode kit. This selection is required for the correct trimming.

  The following wizard image shows the two barcode kit selections. (Make only one selection per run.)



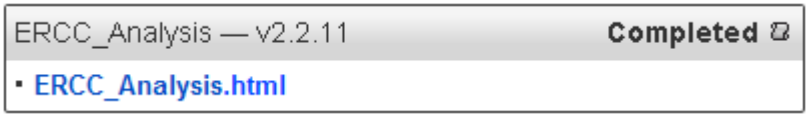
Plugin run times

For analysis runs with total reads under 1,000,000, the plugin normally takes 2-3 minutes to run (on supported hardware). For larger runs, the plugin takes approximately an additional 1-2 minutes per million total reads. For example, a run with 5 million reads may take 10-15 minutes. These run times are offered only as a guideline. If your Torrent Suite™ Software is busy with other processing, plugin run times are longer.

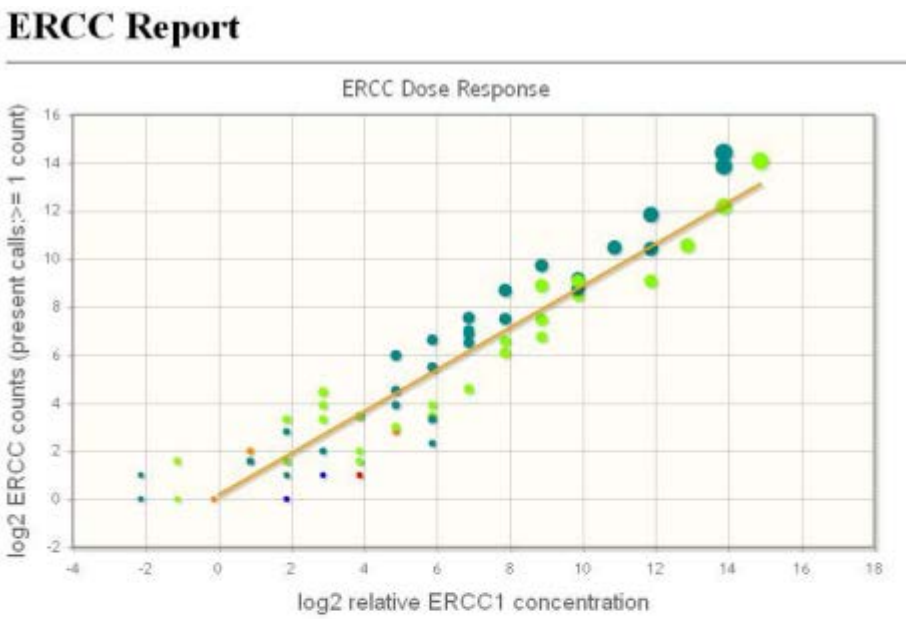
After the ERCC analysis is completed, you can view the analysis results.

View analysis results

Plugin analysis results appear in the plugin summary area:



After the status of the ERCC plugin has changed to Completed, click the ERCC_Analysis.html link in the Plugin Summary section to open the ERCC Report and view analysis results:



% of Total Reads		
ERCC (pct)	=	1.14
Other (pct)	=	98.86
Raw ERCC counts	=	72541
Regression Analysis		
R-squared	=	0.88
Slope	=	0.87
Y-intercept	=	0.17
N	=	65
Map Quality Legend		
Mean Mapq	=	Dot color
more than 90	=	
less than 90	=	
less than 80	=	
less than 70	=	
less than 60	=	

Interpret the data

The ERCC Report screen (shown on page 5) displays the ERCC Dose Response plot. The points are color-coded, based on mapping quality. There is also a trendline, based on the parameters shown in tabular form to the right of the graph.

The y-axis of the plot is the log (base 2) of the raw counts found for the transcript in question. The x-axis is also logarithmic, but represents the known relative concentration of the ERCC transcripts. Ideally, the points all fall on a straight line.

More realistically, in the good case, the raw counts and relative concentration should at least correlate with a high R-squared (for example, 0.9 or higher). The table to the right of the plot (shown on page 5) shows the R-squared value found for this plot, as

well as the Slope, Y-intercept, and N (number of transcripts found) values. Although there are 92 transcripts in the ERCC mix, it is not expected that all 92 will be detected. The number of transcripts detected depends on the sequencing depth.

View transcript details

If you want to look at the details regarding a particular transcript, there are two methods you can use:

- Hover your mouse-cursor over a point on the ERCC Dose Response plot to display a popup window that shows details about that transcript (the name, reads, and coverage plots).If several points are very close together on the plot and it is difficult to hover over the point you are interested in, you can zoom in on the plot to more easily distinguish points:
- – Use your mouse to draw a box around the point of interest and magnify it.
  – To zoom out to the full view of the ERCC Dose Response plot, either doubleclick the plot, or click the **Reset Zoom** button.
- Scroll to the particular transcript, and click the [+] next to the transcript name.This method shows the same information, plus a few additional pieces. See Definitions if the meaning of any of these pieces is unclear.

Definitions

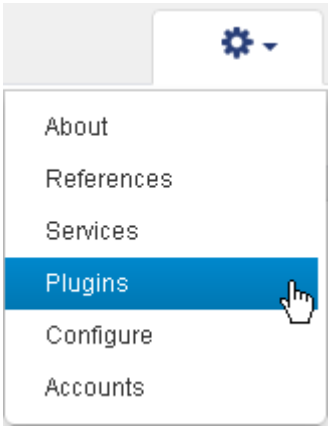
This section defines terms used in the plugin output.

- **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.

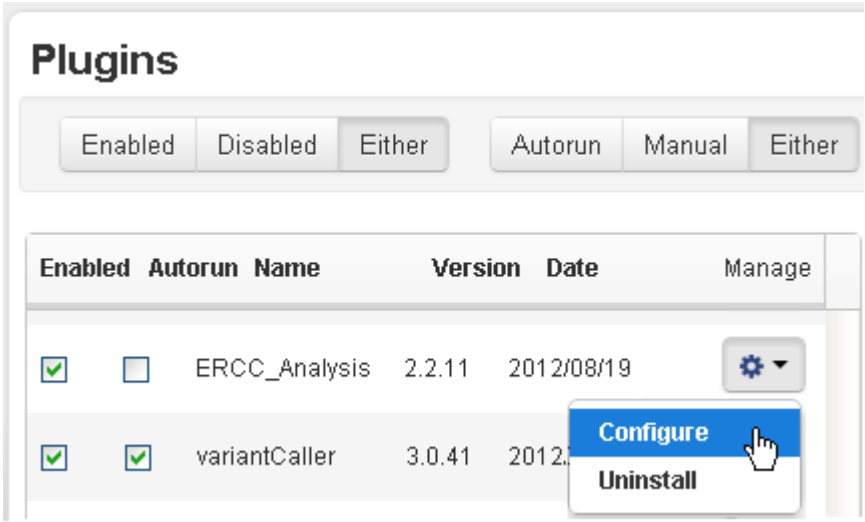
(Optional) Configure the ERCC Analysis plugin

You can optionally change the R-squared value to set a default value for the summary report screen:

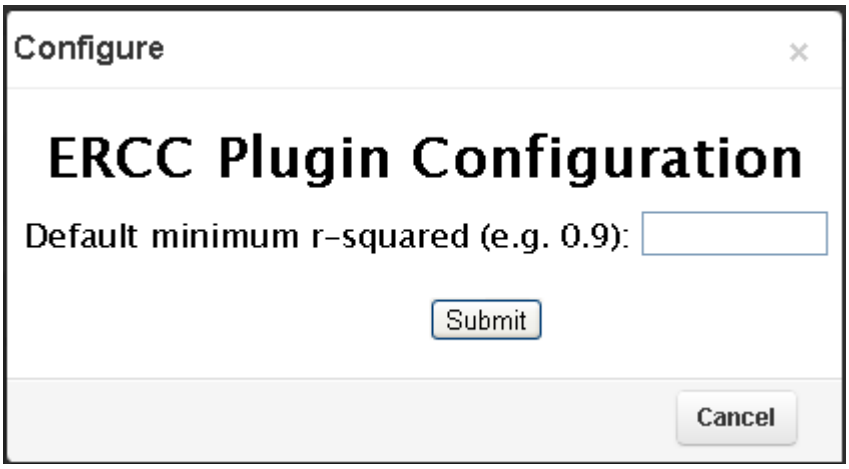
1. If the window showing the minimum acceptable R-squared value is open, close it. Then scroll to the top of the Torrent Browser and click **Plugins** in the gear menu on the top right:



2. If the ERCC_Analysis plugin does not appear on your plugin page, click the **Name** column to sort by name and scroll to the plugin. In the ERCC_Analysisrow, click the Manage column gear menu, then select **Configure**.



3. The ERCC Plugin Configuration screen opens:



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). Then click **Submit**.

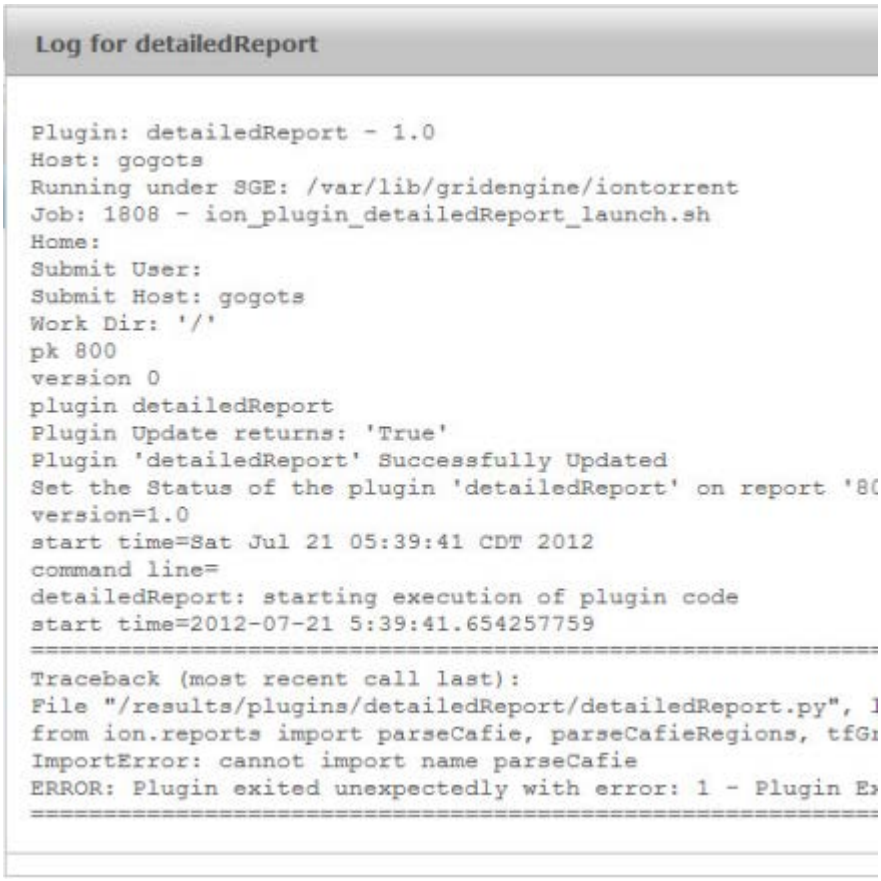
The value you enter on the ERCC Plugin Configuration screen is used when the plugin is auto-run and when a user manually launches the plugin without entering a value. Users can override this value on a per-run basis when they manually launch the plugin.

View plugin error information

If the ERCC_Analysis plugin status changes to Error after you click the ERCC_Analysis.html link, then something went wrong during the running of the plugin. In this case, look at the error log:

1. Return to the Plugin Summary, then click the log file icon to see the error log:

2. Scroll to the bottom of the log to see error messages:



An Error status for the ERCC_Analysis plugin should be a rare event and indicates that the ERCC_Analysis plugin itself failed to run. A plugin error does not indicate that the ERCC_Analysis results are bad.

ERCC resources

The External RNA Controls Consortium (ERCC) is hosted by the U.S. National Institute of Standards and Technology. The ERCC Analysis plugin is for experiments that use ERCC RNA Spike-In Control Mixes, set of RNA controls derived from the ERCC plasmid reference library.

For information on ERCC RNA Spike-In Control Mixes, please refer to the ***ERCC RNA Spike-In Control Mixes User Guide*** (Pub no. 4455352).

For more information on ERCC analysis, refer to the following resources: Figure 2, Analysis of ERCC read counts, in
Sensitivity of RNA-Seq using Ion semiconductor sequencing a comparison to microarrays and qPCR The Ion Torrent™ white paper
Methods, tools, and pipelines for analysis of Ion PGM™ Sequencer miRNA and gene expression data The information on the **ERCC ExFold RNA Spike-In Mix** product page.

The FileExporter Plugin

You use the FileExporter plugin to rename Torrent Suite™ Software results files and optionally to create FASTQ, SFF, or zipped versions of the results files.

This plugin replaces the FastqCreator and SFFCreator plugins.

Plugin output

The plugin output section shows the input options and provides links to the files that the plugin created or renamed:

The SELECTIONS area shows the file naming pattern used for this run. This example uses the naming pattern Run Name, Sample Name, and Report Date:

Run Name Auto_user_B22-884--26953

Sample Name E129294--L-

Report Date 2013-09-17

Configuration scope

You can configure the FileExporter plugin in two places.The configuration options are the same in both places. Only the scope of the configuration is different.

- **In the manual launch page** The configuration affects only that specific run.
- **In the admin Plugins tab** The configuration affects all automatic FileExporter runs by all users.

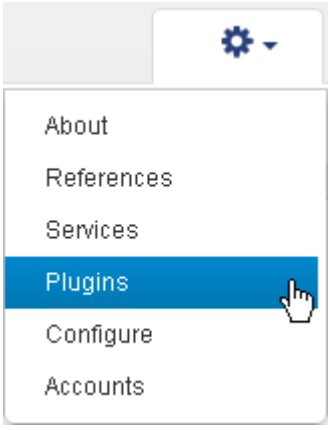
Manual launch

On a manual launch, the **Select plugins to run** button opens the plugin configuration page.

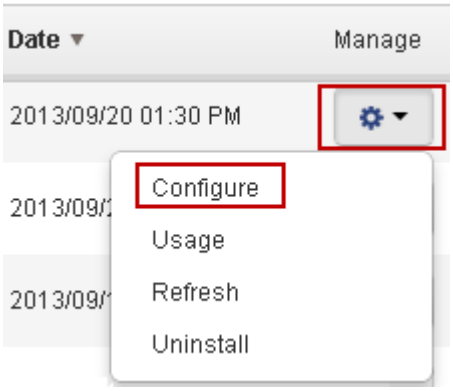
Admin Plugin tab

Configure FileExporter here to set up a file naming pattern and output file options to be used with all automatic FileExporter runs. This configuration affects FileExporter runs that are set up in the run plan and template wizard.

To access the admin plugin configuration area, click the Torrent Browser gear menu **Plugins** option:



Then click the Manage column gear menu for the specific plugin and select **Configure**:



Output file options

In the Output File Options section, you select the following options:

Option	Comments
Create SFF?	Creates an SFF-format file of your Torrent Suite™ results.
Create FASTQ?	Creates a FASTQ-format file of your Torrent Suite™ results.
Include Variant Caller Files?	Creates TVC output files named according to your file naming pattern. If the variantCaller plugin has not been run on this run report, this option has no effect. TVC output files are no included in the zipped file.
Zip results?	Zips the renamed results files (except for TVC results). The zipped file is downloaded from a link in the Files Created section: **Files created.** Link

In this release, if launched with no file options selected, the plugin creates FASTQ and SFF files.

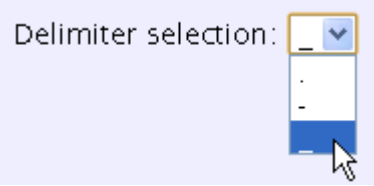
Plugin naming pattern (required)

This section describes how to set a file naming pattern in the FileExporter plugin. The naming pattern is used for the results BAM and BAI files, the SFF and FASTQ files, and the VCF results files (depending on the output file options selected).

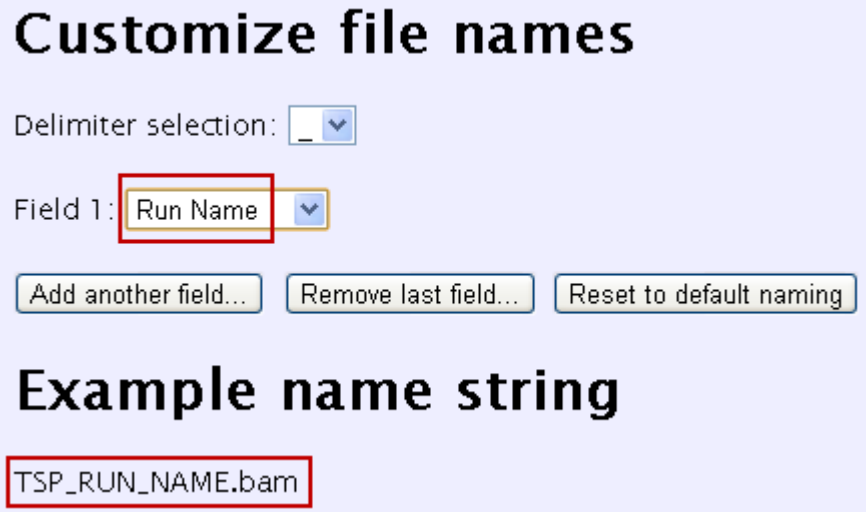
You can use the following run metadata in your file naming patterns:

- Run Name
- Report Name
- Report Date
- Chip Type
- Sequencer Name
- Sample ID
- Barcode Name

You also can choose the delimiter used between metadata fields. Support delimiters are dot, dash, and underscore (a naming pattern uses only only one delimiter):

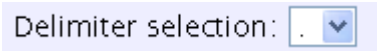


As you build your naming pattern, the **Example name string** section shows you the current pattern:

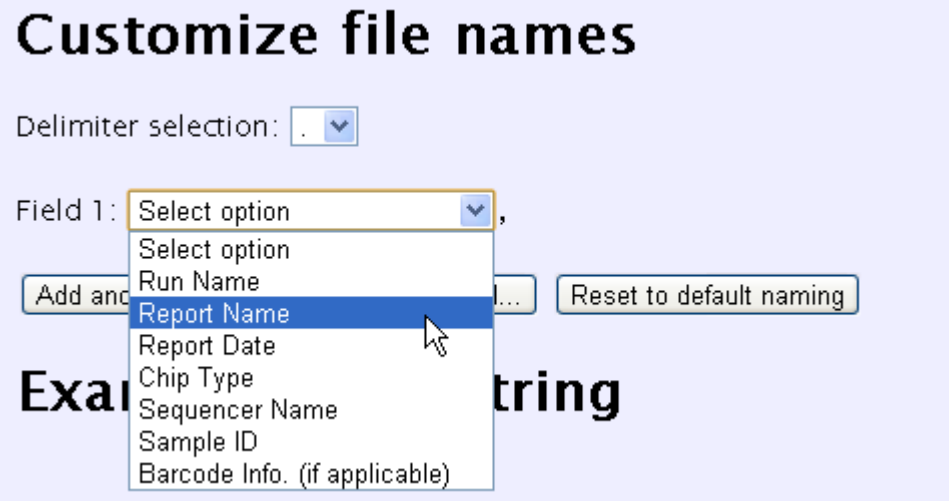


Follow these steps to create your file naming pattern in the **Customize file names** section. This example uses the following file naming pattern: Report Name.SampleID.Report Date.

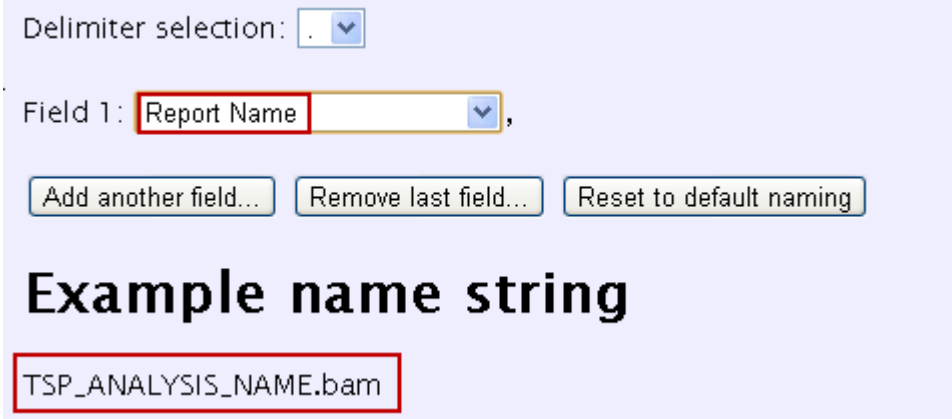
1. Select the delimiter dot:



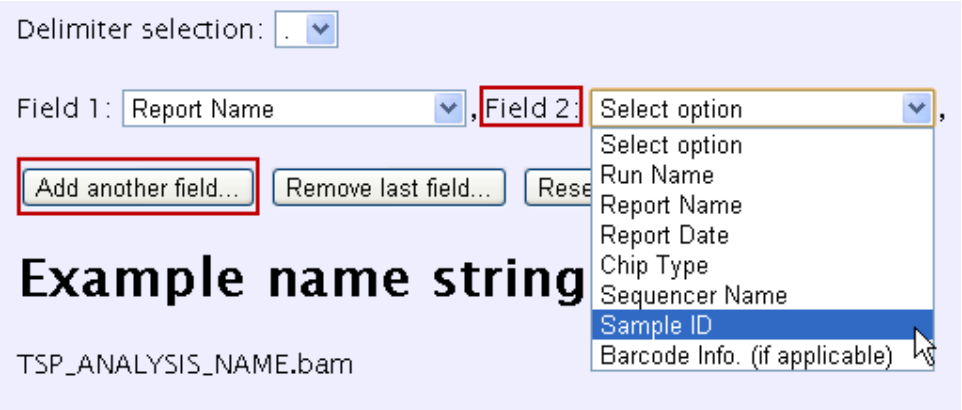
2. In the Field 1 menu, select the first part of the naming pattern. This example uses Report Name:



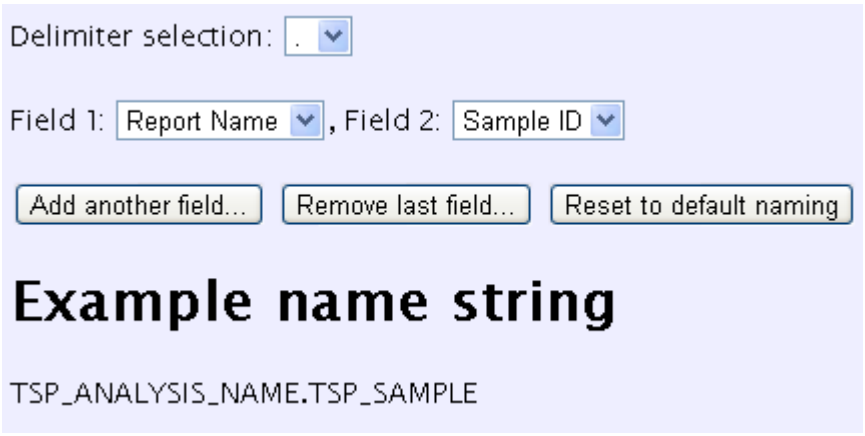
Both Field 1 and the example name string show Report Name (TSP_ANALYSIS_NAME.bam is an internal name for Report Name):



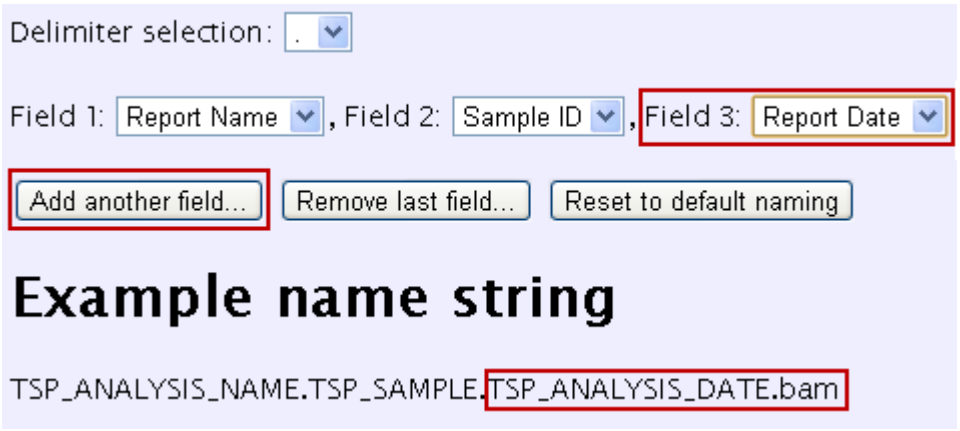
3. Click the **Add another field** button. In the Field 2 menu, select **Sample ID**:



Field 2 shows Sample ID and the example name string shows Report Name.SampleID (using internal names):



4. Click the **Add another field** button. In the Field 3 menu, select **Report Date**:



Your file naming pattern is ready to use.

Note: The FileExporter plugin takes no action if you do not specify a file naming pattern.

Run the plugin automatically

You can set up the plugin to run automatically when you configure your template or run plan. In the Plugin chevron of the template wizard, select which plugins run automatically on planned runs created from that template.

Important: Before you use the plugin in a planned run, configure the plugin in the admin Plugins page. A run template and planned run accept the plugin when it is not configured, but your plugin fails at run time.

The FilterDuplicates Plugin

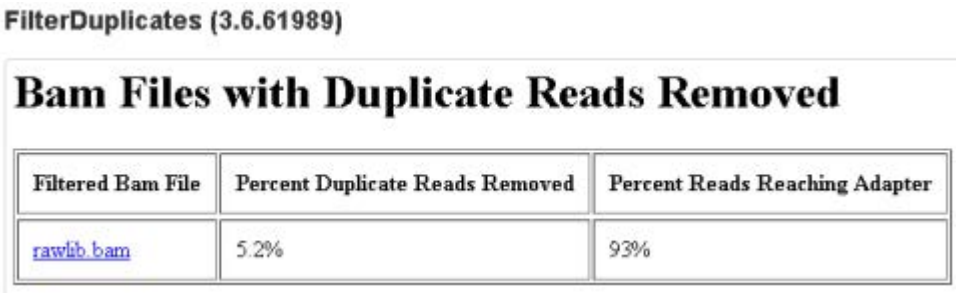
The FilterDuplicates plugin allows you to remove duplicate reads after a run is completed. BAM files with duplicate reads removed are saved in the FilterDuplicates directory. The original BAM files in the main analysis directory are not modified.

FilterDuplicates is applied only to merged data.

Note: The Mark Duplicate feature in the main analysis pipeline marks reads as duplicates but does not remove them from the BAM files.

Plugin output

The plugin output section contains links to the BAM files that have duplicate reads removed:



FilterDuplicates (3.6.61989)

Bam Files with Duplicate Reads Removed

Filtered Bam File	Percent Duplicate Reads Removed	Percent Reads Reaching Adapter
rawlib.bam	5.2%	93%

This section also shows the percentage of reads that were removed and the percentage of all reads that reached the adapter.

Barcoded runs

For barcoded runs, FilterDuplicates runs on each barcode separately.

Run the plugin automatically

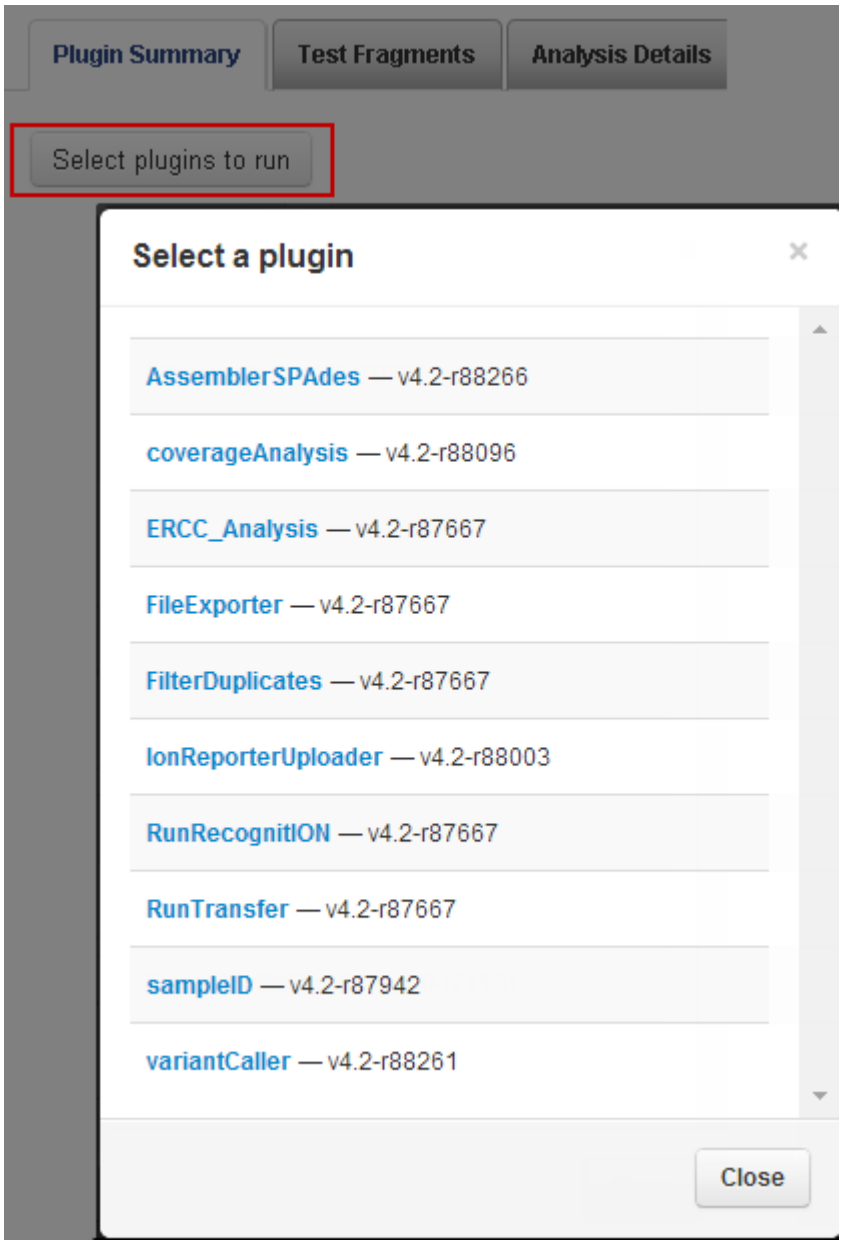
You set up the plugin to run automatically when you configure your template or run plan. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template.

Run the plugin manually

You can launch the plugin manually from a completed run report.

Follow these steps to run the plugin manually:

1. Open the run report and scroll down to the Plugin Summary button. Click **Select plugins to run**.



2. In the **Select a plugin** list, click **FilterDuplicates**. The plugin does not take user input.

ZC tag

The BAM header ZC tag is produced by the base caller to indicate the flow index of the last base of the template. FilterDuplicates plugin uses the ZC tag's information about adapter position, when possible, to improve the resolution of duplicate marking on fragment reads.

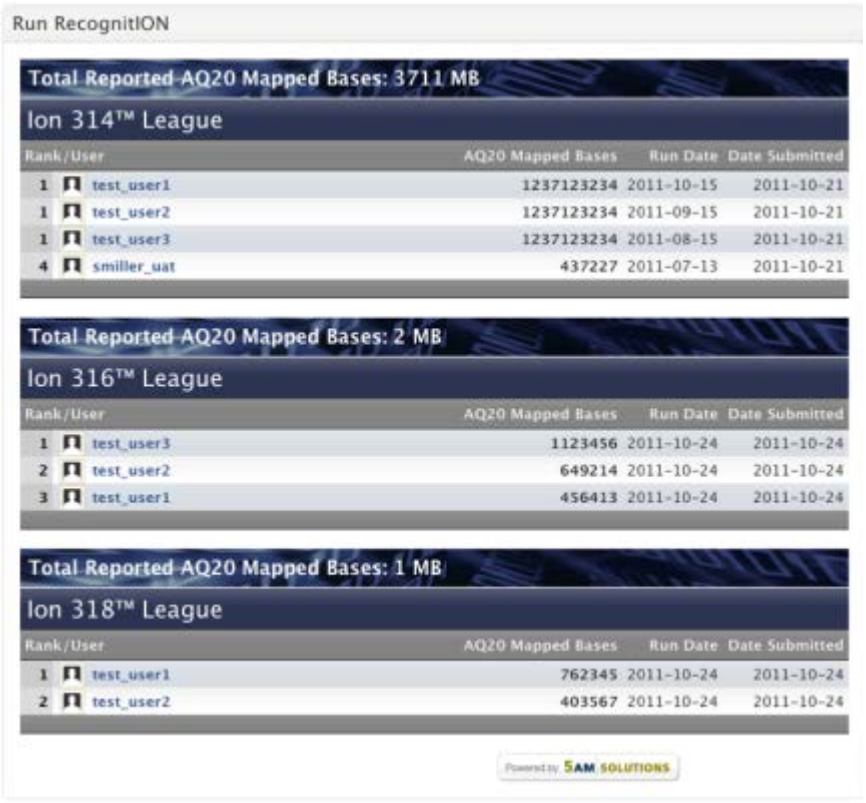
The base caller only creates ZC tag for reads in which the adapter is found.

The FilterDuplicates plugin groups reads with matching start coordinate, strand, and ZC tag value, and selects one read from each group to not be a duplicate read:

1. For every read, the 5' sequencing strand coordinate, mapping strand, and ZC value (if present) are recorded.
2. The plugin groups together reads that have the same 5 start coordinate, strand, and ZC tag value.
3. The plugin also adds to this group those reads that do not have a ZC tag but have the same start and strand coordinates as the group.
4. The next step depends on the longest read in the group:
    a. If the longest read in the group has a ZC tag, the plugin does not mark the first read from the group as a duplicate. The rest of the reads in the group are marked as duplicate.
    b. If the longest read in the group does not have a ZC tag, that read is not marked as a duplicate. The rest of the reads in the group are marked as duplicate. (In this case the base caller did not find the adapter in the read that is not a duplicate and that remains in the plugin output BAM file while the other reads are deleted.)

Run RecognitION Plugin

The Run RecognitION plugin allows you to submit your best runs to the Ion Community leaderboards. The leaderboards are available on the Ion Community, and are organized in leagues according to chip type:

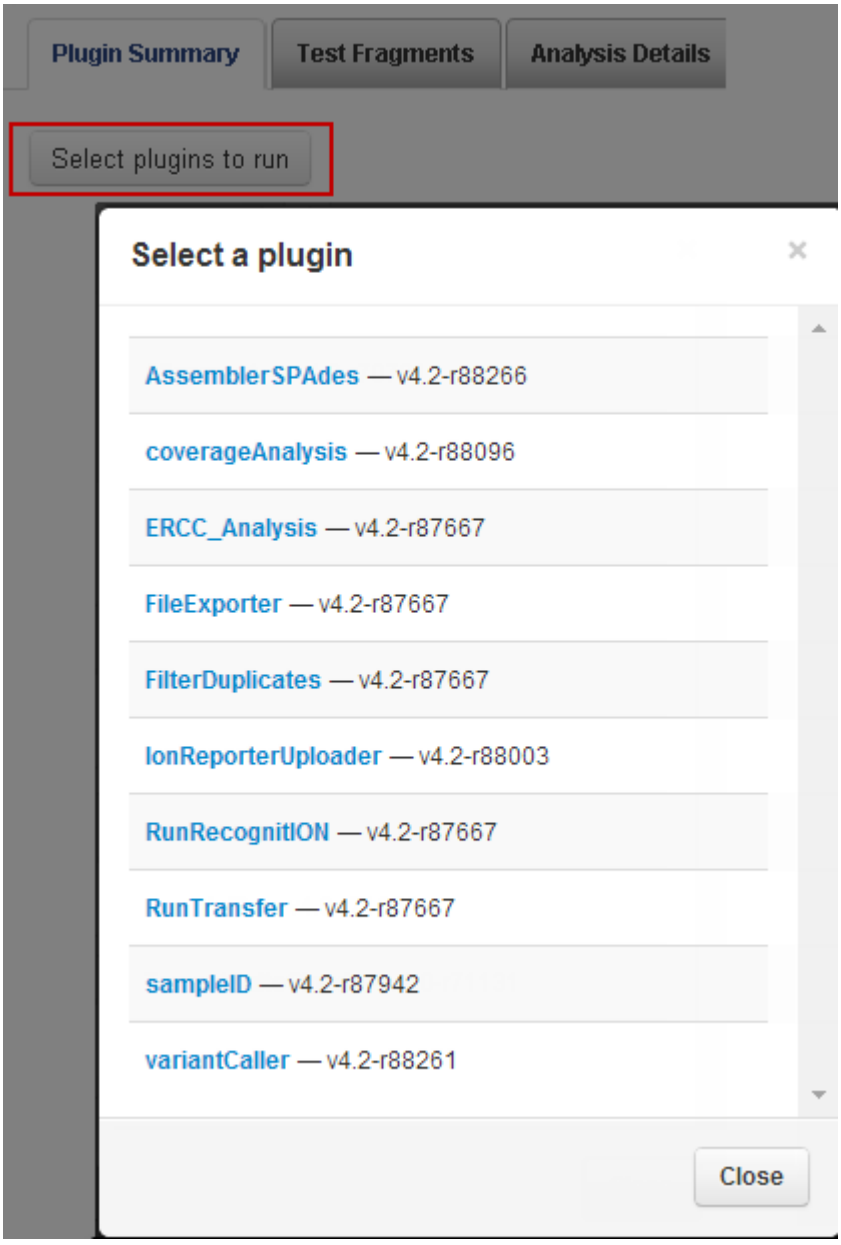


Your Torrent Suite™ software must be at least version 1.5.1 to use this plugin.

Run the RunRecognitION Plugin

Follow these steps to run to the RunRecognitION plugin:

1. Go to the report page for your run. Scroll down to the Plugin Summary section, and click **Select plugins to run**.
2. In **Select a plugin**, click **RunRecognitION**:

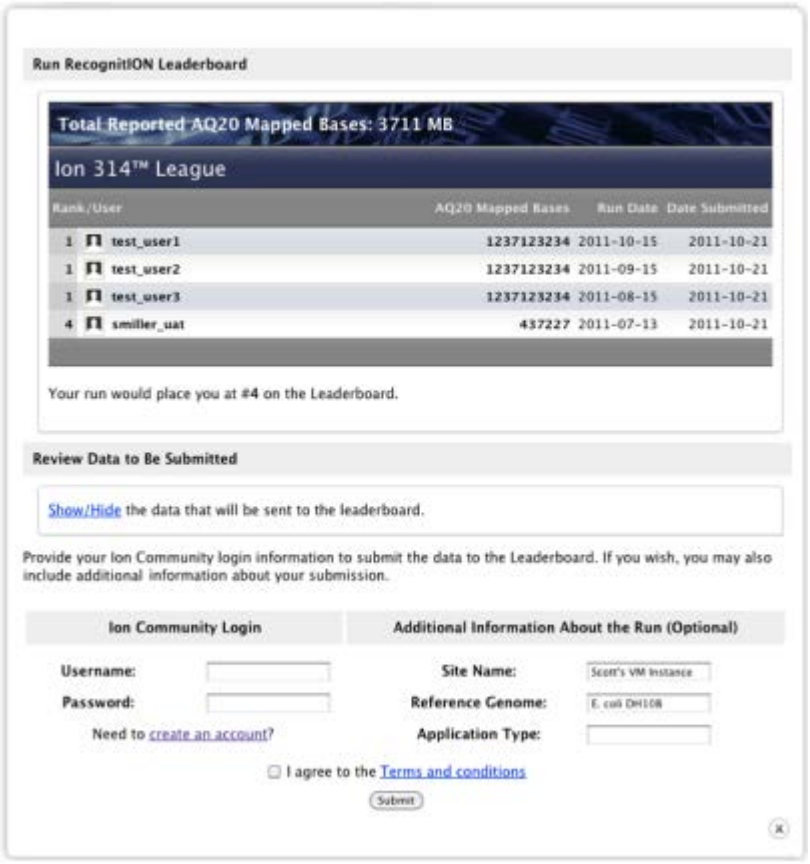


The leaderboard for your chip type is shown, with a message indicating where you rank among the leaderboard runs.

Submit your run to the RunRecognitION leaderboard

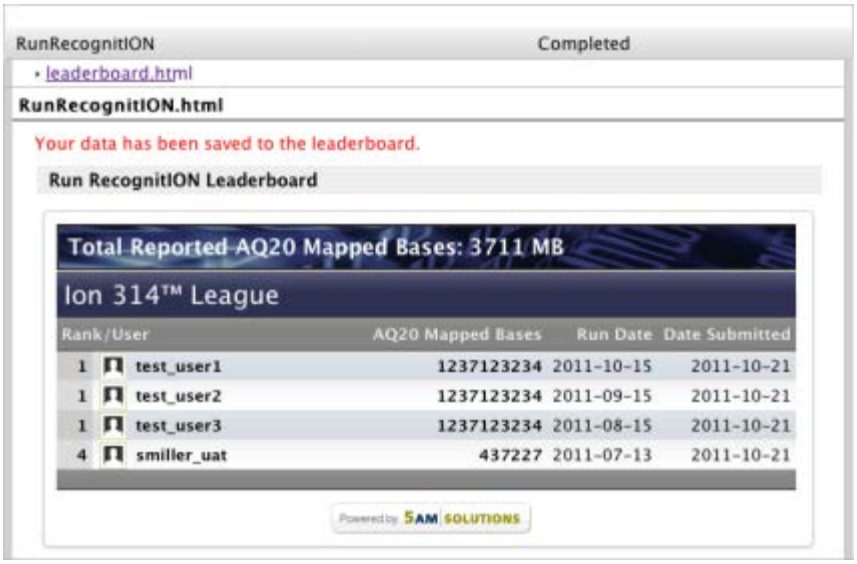
Follow these steps to submit a candidate run to the leaderboard:

1. Go to the report page for your run. Run the RunRecognitION plugin. The Run RecognitION leaderboard is displayed for your chip type.



2. Scroll to the Ion Community Login section below the leaderboard. If you do not have an Ion Community account, click **create an account** to register. Enter your community user name and password.

3. (*Optional*) Enter the any of the following information about your run:
   - Your site name
   - The reference genome used in this run
   - The application type for this run
4. Below the information fields, click **Terms and Conditions** and carefully read that information. Click the checkbox " **I agree to the Terms and Conditions** ".
5. Click **Submit** at the bottom of the page. If your run qualifies, it is added to the leaderboard:



What Information About Me Does RunRecognitION Make Public

If your run is published to the leaderboard, your Ion Community user name and avatar are visible to other members of the community.

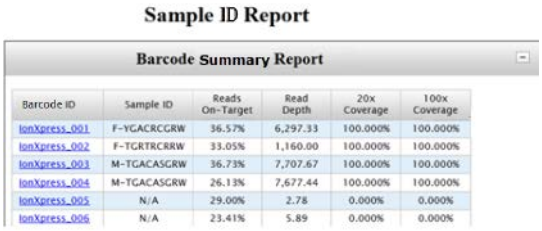
The SampleID Plugin

Ion AmpliSeq™ Sample ID Panel is a human SNP genotyping panel enabling accurate sample verification for increased confidence in sample data management. The plugin is comprised of nine primer pairs that can be combined with any Ion AmpliSeq™ Ready-to-Use or Custom Panel for the generation of a unique ID during post-sequencing analysis of research samples.

The Ion AmpliSeq™ Sample ID Panel can be used in combination with any Ion AmpliSeq™ Ready-to-Use or Custom Panel using the Ion AmpliSeq™ Designer 1.2 or greater. This plugin is compatible with the Ion Xpress barcodes set.

Plugin output

Example plugin output is shown below:



Sample ID Report

Barcode Summary Report

Barcode ID	Sample ID	Reads On-Target	Read Depth	20x Coverage	100x Coverage
IonXpress_001	F-YGACRCGRW	36.57%	6,297.33	100.000%	100.000%
IonXpress_002	F-TGRTRCRRW	33.05%	1,160.00	100.000%	100.000%
IonXpress_003	M-TGACASGRW	36.73%	7,707.67	100.000%	100.000%
IonXpress_004	M-TGACASGRW	26.13%	7,677.44	100.000%	100.000%
IonXpress_005	N/A	29.00%	2.78	0.000%	0.000%
IonXpress_006	N/A	23.41%	5.89	0.000%	0.000%

Use the Sample ID column to verify sample fingerprints.

Click on a barcode ID to open the detail report.

With the detail report, you can review the IUPAC SNP calls. The contents of the TaqMan® Assay ID column are links to order the corresponding TaqMan® Assay.

Plugin configuration

The sampleID plugin does not take any options or configuration.

Run the SampleID plugin automatically

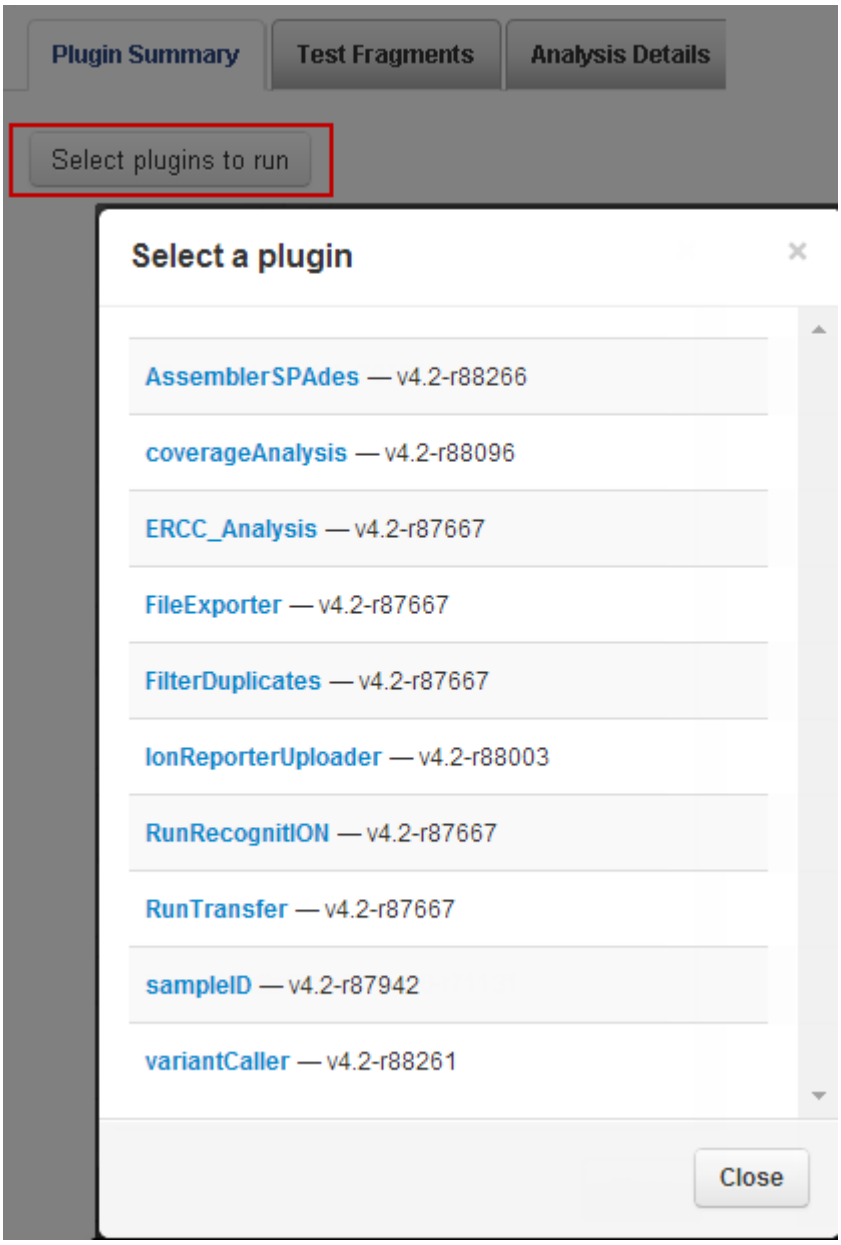
You set up the plugin to run automatically when you configure your template. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template.

Run the SampleID plugin manually

You can launch the plugin manually from a completed run report.

Follow these steps to run the plugin manually:

1. Open the run report and scroll down to the Plugin Summary button. Click **Select plugins to run**.



2. In the **Select a plugin** list, click **sampleID**. The sampleID plugin does not take user input. The plugin executes immediately (depending on server load) when you click it in the **Select a plugin** list.

On-target metrics

When you use the SampleID Panel, lower-than-expected number of on-target reads may occur. To recover the correct on-target reads metrics, add back the On-Target reads from the Sample ID Panel into the Ion AmpliSeq™ Ready-to-Use or Custom Panel Coverage Analysis plugin data.

The RunTransfer Plugin

With the RunTransfer plugin, you transfer the signal processing output files of a completed Torrent Suite™ Software analysis to a different Torrent Server and also launches a re-analysis of those file on the new server. On the receiving Torrent Server, after the re-analysis completes, the transferred run appears in the Torrent Browser as if it had been generated on that Torrent Server.

The files that are transferred are the BaseCaller Input category of files (as defined in the Data Management file categories), including the 1.wells file.

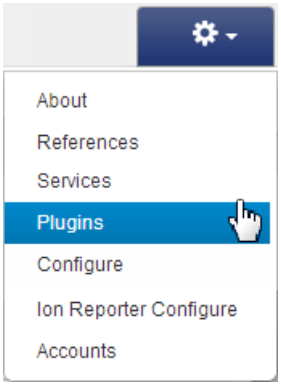
For Ion Proton™ analyses, you have the option of transferring only thumbnail files or transferring the 96 block files.

Pre-configure the plugin

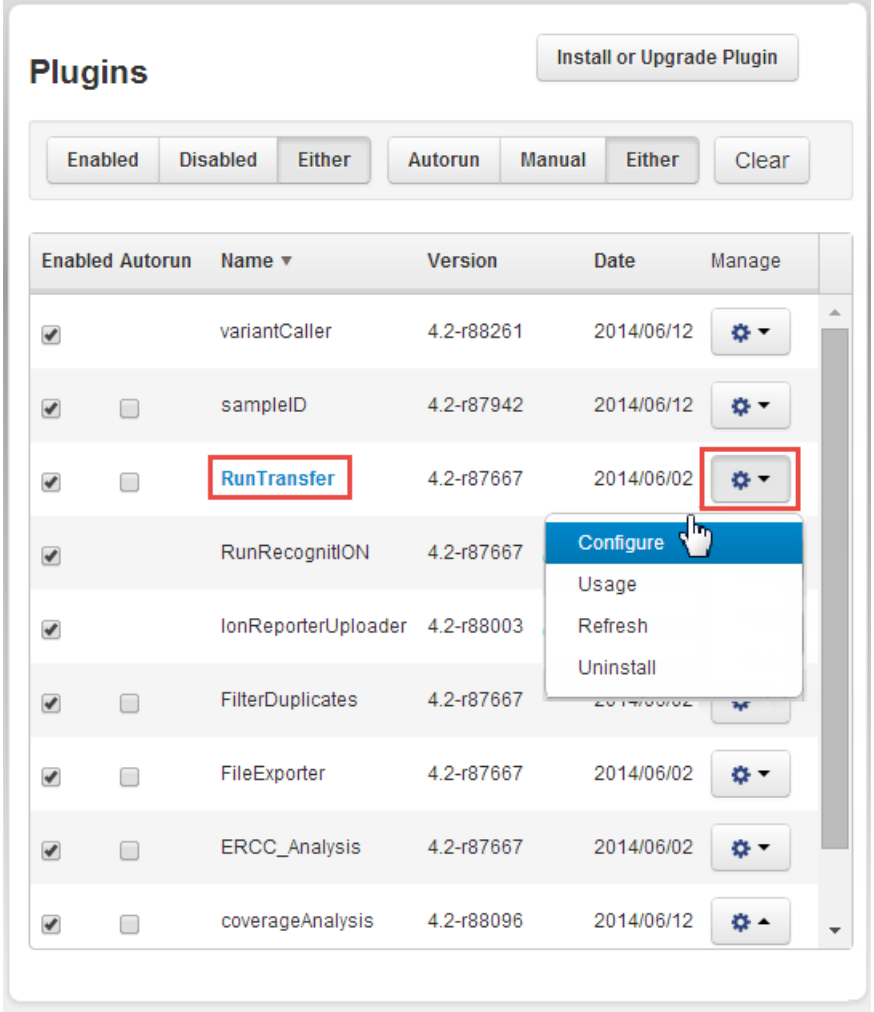
You can optionally configure the plugin ahead of time in the **Admin** ▸ **Plugins** tab. This configuration is known as "Global Settings". The Global Settings configuration is used whenever the plugin is automatically run and also is the default for manual launches of the plugin.

Follow these steps to create a global configuration for the RunTransfer plugin:

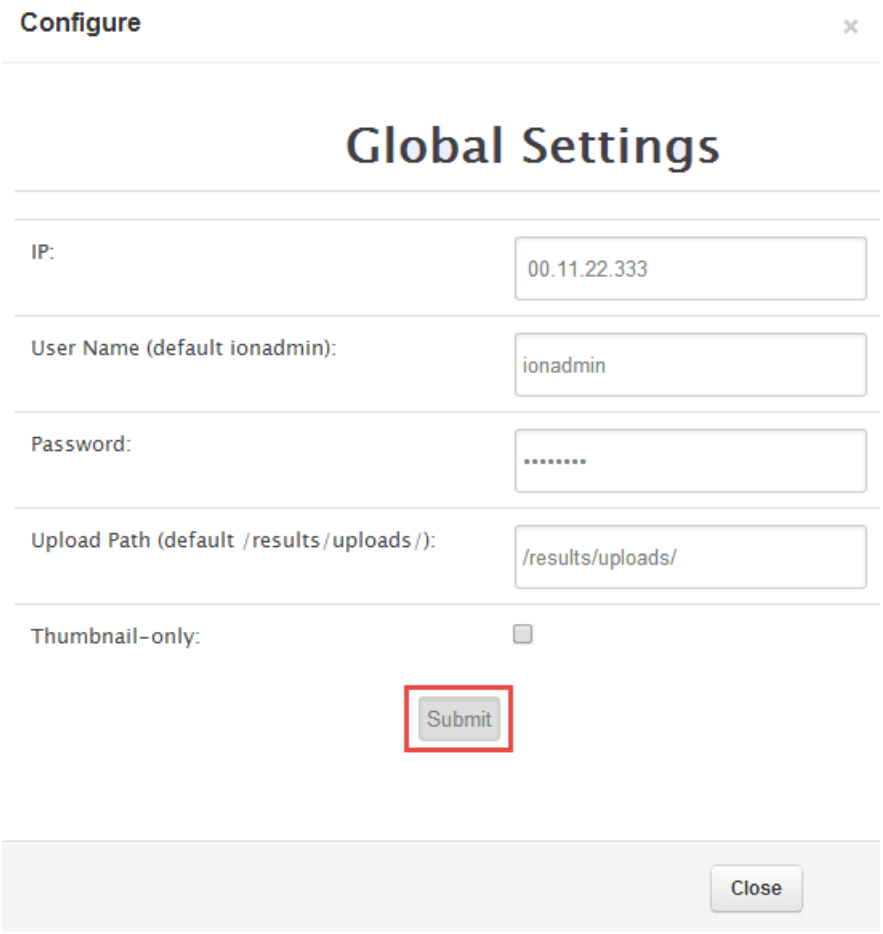
1. Go to the **Admin** > **Plugins** tab:



2. Find the entry for the RunTransfer plugin (you might have to scroll or re-order the Name column). With the gear menu for the RunTransfer plugin, select the **Configure** option:



3. Fill out the Global Settings page with information for the receiving Torrent Server (where the run is transferred to):



Field	Comments
IP	The IP address
User Name	The login name on the receiving Torrent Server
Password	Password for that login
Upload Path	The analysis directory path
Thumbnail-only	Enable to transfer only thumbnail data. Leave blank to transfer full data. Applies only to Ion Proton™ analyses.

4. Click **Submit**. (Your changes are lost if your click Close.)

Note: In this release, there is no way to remove a global configuration for the RunTransfer plugin.

Run the plugin automatically

You set up the plugin to run automatically when you configure your template or run plan. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template.

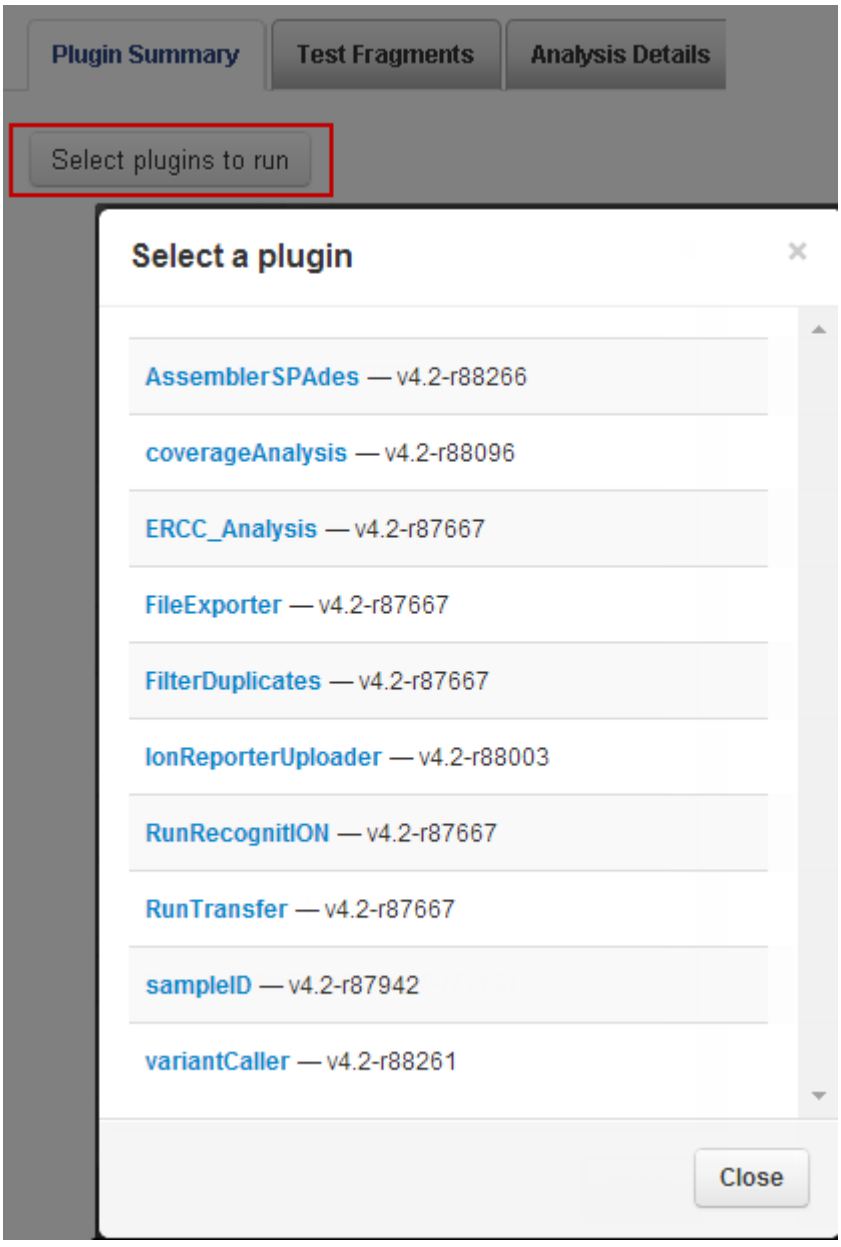
Create a plugin configuration in the Admin tab before running a plugin automatically.

Run the plugin manually

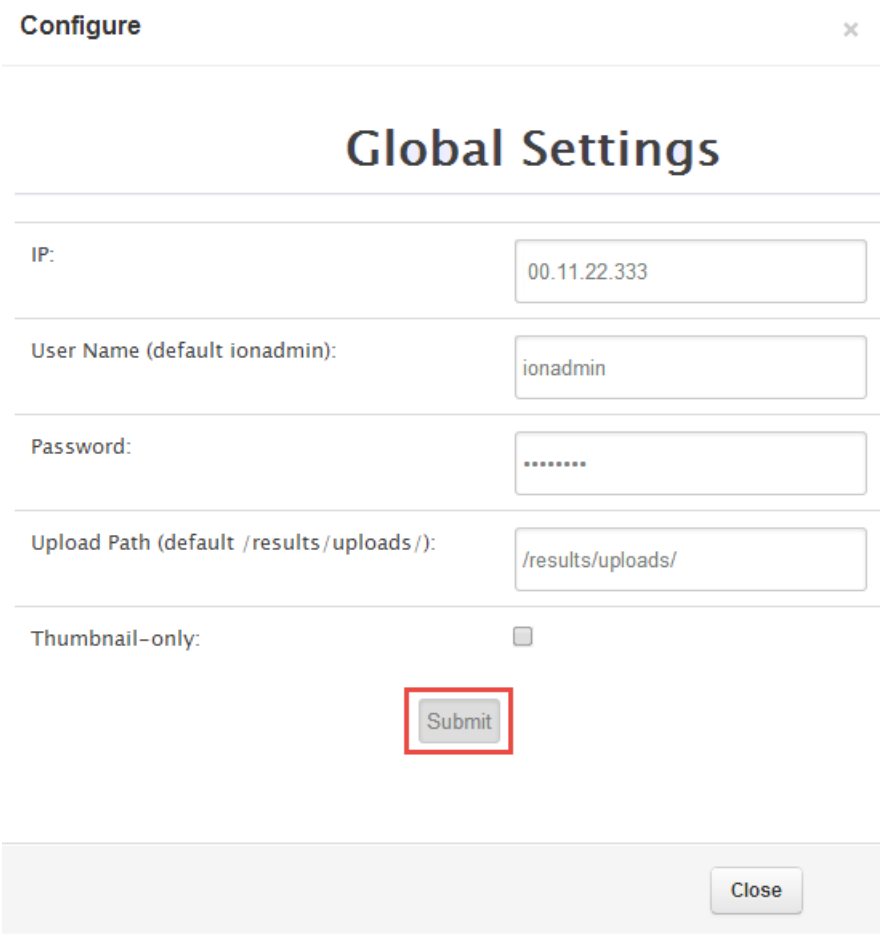
You can launch the plugin manually from a completed run report.

Follow these steps to run the plugin manually:

1. Open the run report and scroll down to the Plugin Summary button. Click **Select plugins to run**.



2. In the **Select a plugin** list, click **RunTransfer**.
3. The Configure page opens. If you made a global configuration for the plugin, the fields are filled in with that information. You can change any of the fields.



The Partek Flow Uploader Plugin

This plugin requires a separate purchase of Partek® Flow® Software, which is a third party software that is not supported by Ion Torrent™.

Run the plugin automatically

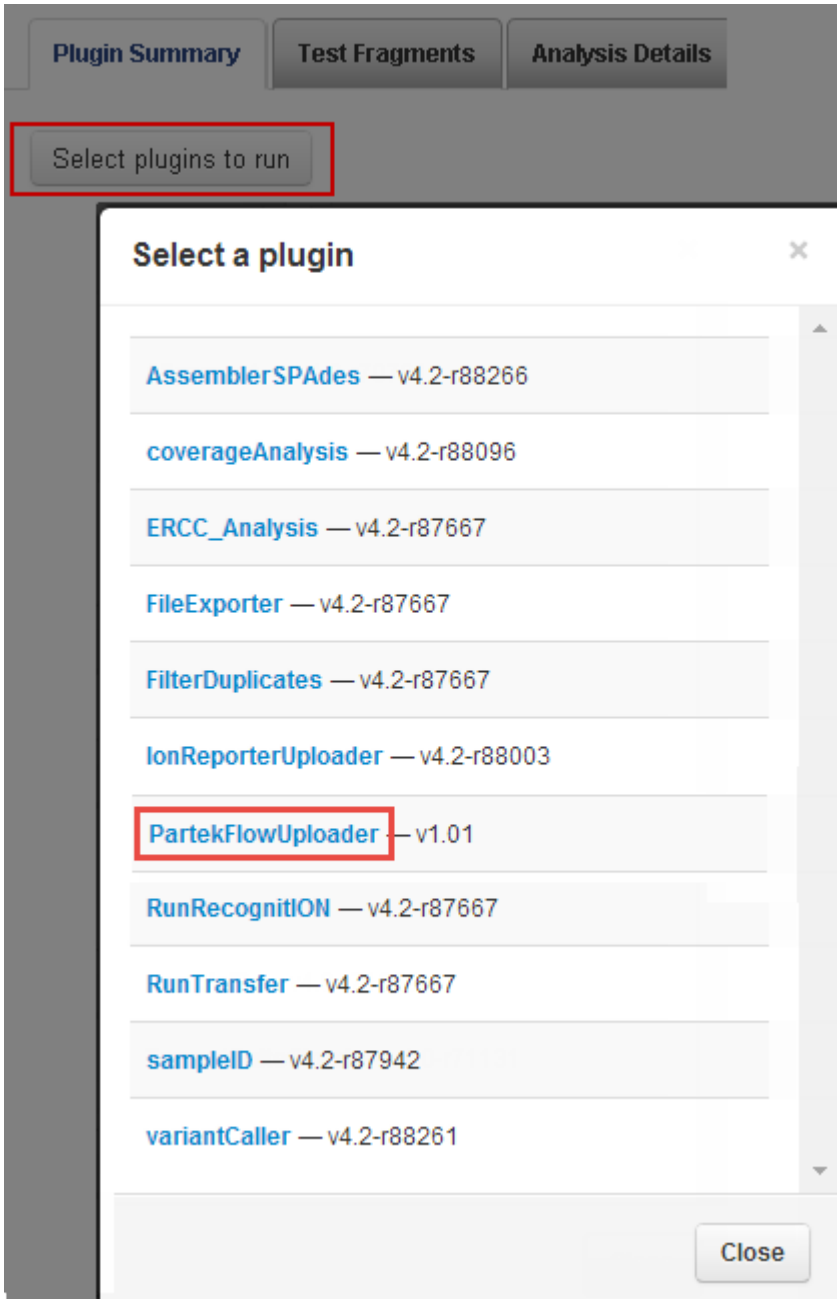
You set up the plugin to run automatically when you configure your template or run plan. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template.

Run the plugin manually

You can launch the plugin manually from a completed run report.

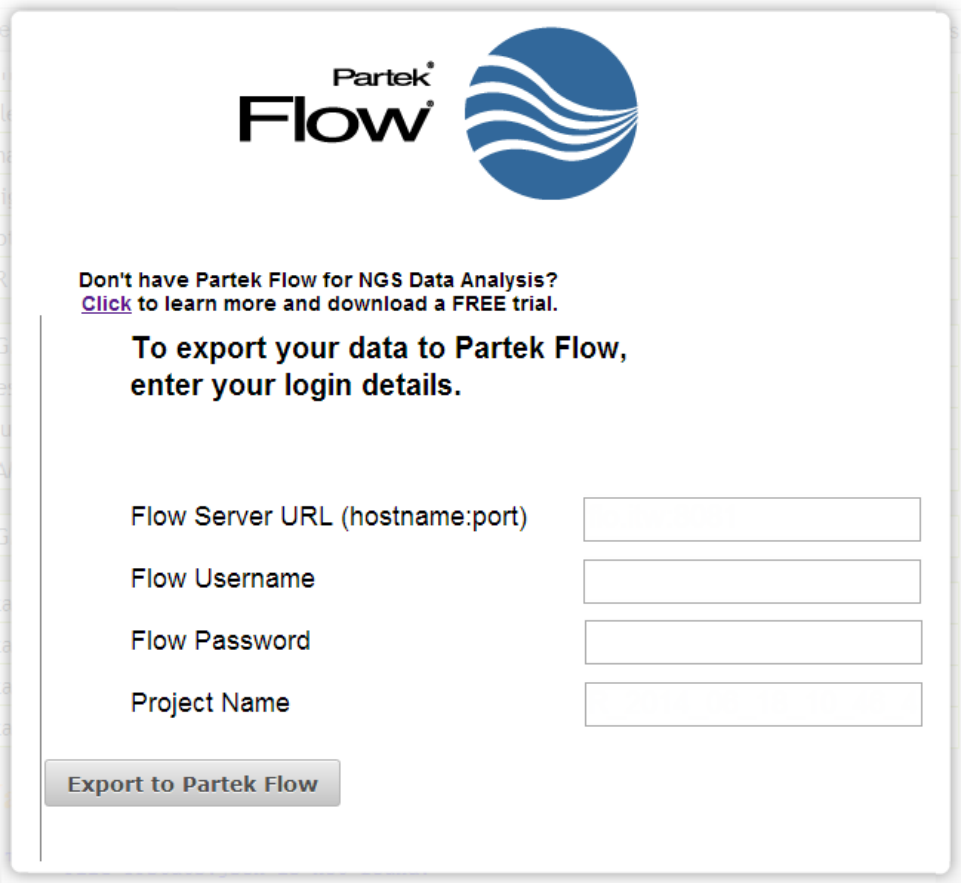
Follow these steps to run the plugin manually:

1. Open the run report and scroll down to the Plugin Summary button. Click **Select plugins to run**.



2. In the **Select a plugin** list, click **PartekFlowUplpoader**.

3. In the plugin export page, enter your Partek® Flow® Software information and click **Export to Partek Flow**:



Torrent Variant Calling

Introduction

The Torrent Variant Caller (TVC) plugin calls single-nucleotide polymorphisms (SNPs), multi-nucleotide polymorphisms (MNPs), insertions, and deletions 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 but is also very customizable. 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 over and over in your research. This is supported on both manual launches of the plugin and in automatic launches through the run plan template wizard.

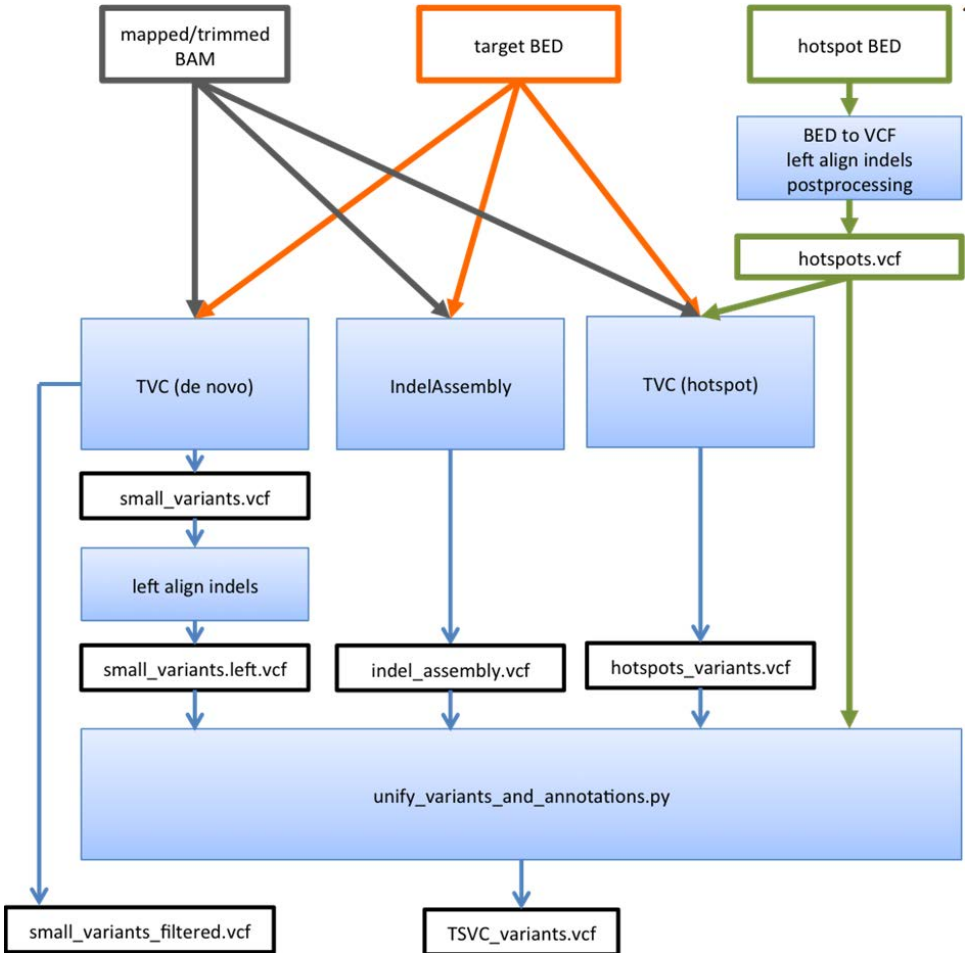
TVC Pipeline Introduction

Torrent Variant Caller (TVC) is a genetic variant caller for Ion Torrent™ Sequencing platforms, and is specially optimized to exploit the underlying flow signal information in the statistical model to evaluate variants. Torrent Variant Caller is designed to call single-nucleotide polymorphisms (SNPs), multi-nucleotide polymorphisms (MNPs), insertions, deletions, and block substitutions.

The basic operation of the TVC plugin is as follows:

1. Find all positions with any evidence for a variant.
2. Focus on these positions and evaluates if there is enough evidence for a SNP or indel call.
3. Filter the candidates based on key parameters (strand bias, enough coverage, known error prone position).

The image below gives a more detailed view of the variant calling process:



According to this diagram, in order to generate as final output the TSVC_variants.vcf file in Variant Call Format (VCF) 4.1, the plugin combines output from up to three calling modules:

1. The TVC (de novo) module uses freebayes to discover candidate variant locations, which are subsequently scored using adaptive signal model and filtered.
2. The second module performs assembly of reads to detect long indels.
3. If hotspots are provided, a separate TVC call scores hotspot locations and alleles using a model.

The resulting intermediate files are merged and overlapping records are reconciled to produce the final file. If a hotspot file is provided, the TVC_variants.vcf file, as well as the variant tables in the plugin report, will contain a record for every hotspot (with some exceptions*) including variant calls, reference calls, and no-calls. For no-calls, the filtering reason listed on the Variant Calls table in the UI and in the VCF tags.

For loci that are not present in the hotspots file, it is still possible to establish whether a lack of variant call is due to reference call or filtering. Candidate variants generated by de-novo TVC module that were either declared absent (reference calls) or were discarded by a filter (no calls) are saved to a separate file, small_variants_filtered.vcf.

Input

Torrent Variant Caller operates on input BAM files generated using Torrent Suite™ Software version 3.4.2 or higher and requires the presence of flow signal specific tags in the BAM file.

You optionally also supply TVC with target regions files and hotspot files:

- Target regions files — Sequencing is restricted to specified chromosome regions that appear in the regions of interest file.
- Hotspot files — TVC evaluates each listed position on the genome, and reports the filtering metrics for each position, including positions that are not called as a variant. When a hotspot position receives a NOCALL rather than a reference call or a variant call, the filtering reasons in the VCF output file explain the reasons for the NOCALL.

In the 4.x and 3.6.x releases, a hotspots file can be either BED format or VCF 4.1 format. The BED format might be deprecated in a future release. Both hotspots files and targeted regions of interest files are uploaded in the Torrent Browser References page and are associated with a specific reference.

Variant candidates

Candidate variants are generated if they pass a minimal set of requirements set in FreeBayes (such as base quality, apparent frequency, etc.). Candidate positions are then evaluated by a statistical model which matches basecalling predictions under each sequence alternative specified, and finally are filtered by a number of orthogonal criteria to determine if the assumptions of the model do not fit the data. Each of these processes are controlled by parameters supplied to the program. These parameters determine the effectiveness of generating, testing, and filtering variants.

Torrent Variant Caller uses a two-staged approach to calling variants in any given sample. Step 1 is to identify candidate positions where alternate allele frequency is above the minimum allele frequency threshold setting. Step 2 is to further evaluate the candidate variant identified in step 1 to assign the quality of the variant and also to assign the genotype.

A modified version of Freebayes software is used in step 1 to identify the candidate variants in a given sample. The minor modifications to FreeBayes are to model Ion Torrent™ Sequencing platforms specific error modes. Torrent Variant Caller supports Freebayes haplotype-based allele detection, where alleles can extend across several bases across a repeat sequence. This support enables Torrent Variant Caller to evaluate multiple haplotype-based alleles simultaneously (such as a SNP and an indel adjacent to each other).

Output

Variants that pass all the set filters are reported to a single output VCF file and all variants 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.

These reports are also available in tab-separated format:

- The Variant Calls table
- Hotspot Alleles table
- The Classic output table

The output page called "Beta Features" in 3.6.2 is the main output page in 4.x. The 3.6.2 main page layout is available through the Deprecated Features Classic button.

Standalone TVC

TVC can be run in a standalone mode as a part of user's analysis pipeline outside of the Torrent Suite plugin framework. It has been tested to work on Ubuntu 10.04, 11.10, 14.04, 15.04,

CentOS 6, 7, Debian Wheezy andJessie operating systems.

Parameters

TVC provides several ways of handling its parameter options:

- You can select one of TVC default pre-set parameter groups. TVC provides these defaults that are optimized for several experiment types.
- You can start with one of TVC default pre-set parameter groups and then make your own customizations in the TVC UI.
- You can import parameter settings that are optimized for fixed panels and community panels in ampliseq.com. (Optimized parameter sets for custom designs are not supported in this release.)
- You can download the parameters used in a TVC run and then either customize those parameters or reuse them in future TVC runs.

TVC's default parameters setting groups are organized according to these attributes:

- **Variant frequency** Somatic settings are optimized to detect low frequency variants. Germ-line settings are optimized for high frequency settings.
- **Sequencing instrument** The Ion PGM™ or the Ion Proton™ sequencer. Parameter defaults are different for Ion Proton™ data than for Ion PGM™ data.
- **Stringency** High stringency settings are optimized to minimize false positives. Low stringency settings minimize false negatives.
- **TargetSeq** Two sets of defaults are optimized for TargetSeq™ data.

Reference

The Reference Genome field names the reference used in the original Torrent Suite™ Software. The reference cannot be changed.

Library Type

The Library Type selection does not change or customize TVC's parameter settings. When the Library Type is set to AmpliSeq, the Trim Reads option is available. Trimming is recommended for Ion AmpliSeq™ data to remove the adapters from the reads.

Targeted Regions and HotSpot Regions menus

If you select Targeted Regions or HotSpot Regions files from their drop-down menus, and they are applied to your analysis:

- **Targeted 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.

Templates

Be aware that the TVC plugin is not selected in the templates shipped with Torrent Suite™ Software. Select the plugin when you create a run plan or a copy of a shipped template.

Templates that you download from ampliseq.com do have the TVC plugin selected.

Supported Ion AmpliSeq™ panels

The TVC plugin supports the various panels in the Ion AmpliSeq™ family of sequencing kits, including the following:

- Ion AmpliSeq™ BRCA1 and BRCA2 Panel
- Ion AmpliSeq™ Colon and Lung Cancer Panel
- Ion AmpliSeq™ CFTR Panel

The following table lists the TVC parameter options that are pre-defined and optimized for ampliseq.com panels or TargetSeq™ data:

Panel or application	TVC Pre-set parameter defaults
Ion AmpliSeq™ Exome	Germline - Proton - Low Stringency
CCP PGM	Somatic - PGM - Low Stringency
CCP Proton	Somatic - Proton - Low Stringency
CHP2 (HSM2)	Somatic - PGM - Low Stringency
CHv1	Somatic - PGM - Low Stringency
IDP	Germline - PGM - Low Stringency
TargetSeq™ data	Germline - Proton TargetSeq - Low Stringency

Parameter Settings defaults

The Torrent Variant Caller parameter settings change according to your Variant Caller configuration radio button selection. Data from Ion PGM™ and Ion Proton™ Sequencers require different default settings. Select settings that are appropriate to both your sequencing instrument and your experiment:

- **Germ-Line - Low Stringency** Optimized for high frequency variants and minimal false negative calls.
- **Somatic - Low Stringency** Optimized for low frequency variant detection with minimal false negative calls.
- **Germ-Line - TargetSeq Low Stringency** Optimized for high frequency variants and minimal false negative calls. (Ion Proton™ data only)
- **Germ-Line - TargetSeq High Stringency** Optimized for high frequency variants and minimal false positive calls. (Ion Proton™ data only)
- **Custom** Settings that you customize. (You cannot select this radio button. This button is enabled if you change a parameter value.)

Ion AmpliSeq™ and TargetSeq™ experiments

IMPORTANT! These two settings are optimized for IonTargetSeq experiments:

- Germ Line - Proton TargetSeq -Low Stringency
- Germ Line - Proton TargetSeq -High Stringency

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.

About the use of Variant Caller Parameter Settings radio buttons

First select the appropriate **Variant Caller Parameter Settings** radio button. Your radio button selection loads the correct set of default parameters for that type of run. If you want to customize parameters further, change parameter values in the main settings area. Advanced users can also click the **Show Advanced Settings** button to change values in the advanced settings.

These notes apply to the **Variant Caller Parameter Settings** and advanced settings selections:

- If you do customize settings in the advanced settings area, your changes are overwritten if you select a different **Variant Caller Parameter Settings** radio button (or again click on the same radio button).
- If you make changes in the advanced settings and later want to reset these parameters to their default values, again click your **Variant Caller Parameter Settings** radio button selection.

Variant Caller parameters

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.

Parameters are categorized as main settings, which are intended for general use, and advanced settings, which allow additional customization of the variant calling algorithm but are intended for advanced users only.

Upload your custom parameter values

Use the Upload Custom Settings Choose File button to upload your set of custom parameter settings:

You can use this mechanism for the following:

- To quickly apply your own settings to all your TVC plugin runs
- To know that your parameters are consistent (for instance, that a parameter change is not inadvertently forgotten in the UI)
- To apply a file of settings shared by others

The parameters file must be in JSON format.

After upload, the UI reflects the parameter values from your uploaded file. You can still make additional changes in the UI.

Follow these steps to upload a parameters file for your TVC plugin run:

1. Have the JSON file to be uploaded on your local machine. You can optionally edit values in the file before uploading.
2. In the TVC plugin launch page, click the **Choose File** button under Upload Custom Parameter Settings:

3. Browse to your parameters file and click **OK**.

   The optimized parameters are imported into your run and are reflected in the parameter table on the launch page.

Input files

This section describes input files that you provide for the TVC plugin.

Both a target regions file and a hotspots file must be associated with a reference before you use them with the TVC plugin. You upload these files to a specific reference, such as hg19, in the admin References page.

Target regions file

A target regions file controls the sequencing and downstream analysis of a targeted resequencing run in this way: sequencing is restricted to specified chromosome regions that appear in the regions of interest file. (In contrast, a whole genome analysis sequences every position that corresponds to the reference genome.)

The regions of interest file must be a Browser Extensible Data (BED) file, which is a tab-separated file format.

Hotspots file

A hotspots file contains a list of positions on the genome and when configured in a workflow affects the analysis results. For each position during variant calling:

1. Evidence for a variant is examined at that position (without regard to the hotspots positions) and a call is made.
2. Then the hotspots positions are examined. At each position listed in the file, if a variant is not already called, then one of the following variant calls is added:
   - **REF** Homozygous reference
   - **NO_CALL** A variant is not called at this position (for instance, because of lack of coverage)
3. The filtering metrics for each position are reported in the output VCF file, including for NO_CALLs.

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.

Run the Torrent Variant Caller plugin

There are two ways to run the Torrent Variant Caller plugin: automatically, by preconfiguring the plugin to run as soon as primary analysis has completed, or manually, allowing you to run the plugin at any time from a completed run report.

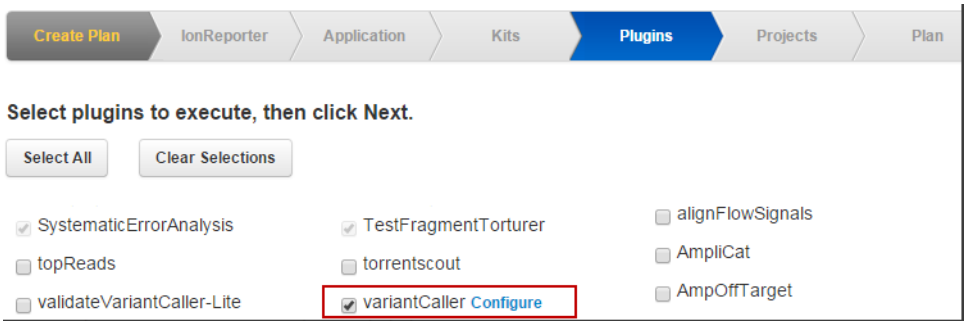
Note: The Torrent Variant Caller takes a significant amount of time to complete. Setting it up to run automatically saves time compared to running it manually.

Configure the Torrent Variant Caller in a template or run plan

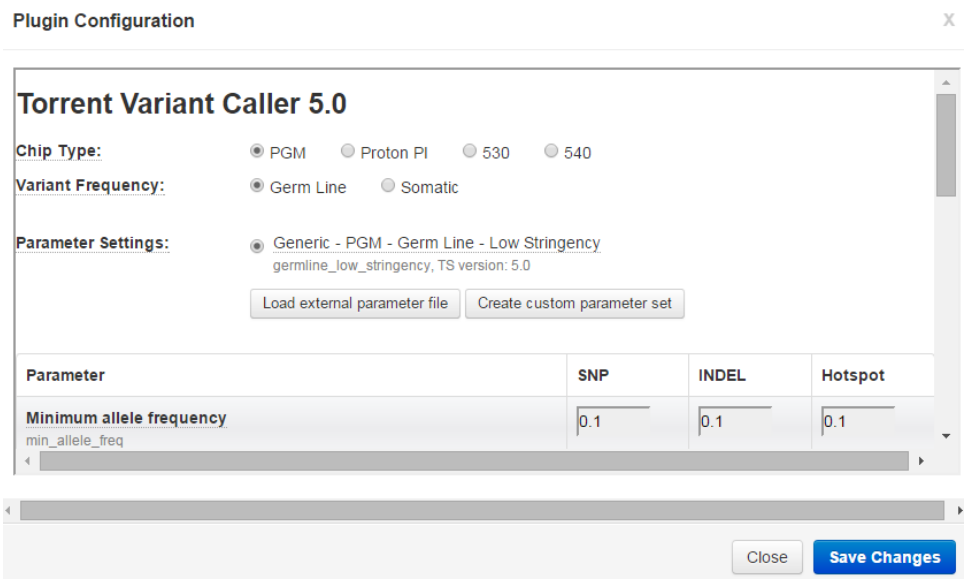
Use the run plan template wizard to have the TVC plugin run automatically after the Torrent Suite™ analysis completes.

Note: The TVC plugin run uses the same target regions file and hotspots file as the main Torrent Suite™ Software analysis (if those files are present in the main analysis). Through the wizard there is no facility in the TVC configuration to change the target regions file or hotspots file. You can use a different target regions file and hotspots file with a manual TVC launch from a completed run report.

1. When you select the plugin chevron in the template or run plan wizard and enable the variantCaller checkbox, a Configuration link appears next to thevariantCaller listing:



2. Link the **Configure** link to open the Variant Caller configuration popup:



3. Make your changes to the parameter values.
4. Advanced users can also click the Show Advanced Settings button and customize those parameters.
5. Click **Save Plugin Settings**.

   You can later return to the Variant Caller configuration page by clicking the **Configure** button next to variantCaller in the Plugin chevron.

   Note: Changes to parameters can dramatically affect the behavior and sensitivity of the Variant Caller. Parameter changes are not recommended if you are new to the Variant Caller plugin.

   IMPORTANT! The Variant Caller parameter settings are saved in templates but *are not saved* in run plans. Parameter changes that you make in a run plan affect only that specific run.

   When you change Variant Caller parameter settings in a template, your changes affect all users who create run plans from that template.

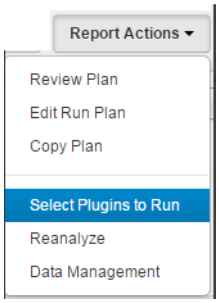
   IMPORTANT! The Torrent Variant Caller plugin is not run if you select Generic Sequencing as the sequencing run type.

Run the Torrent Variant Caller manually

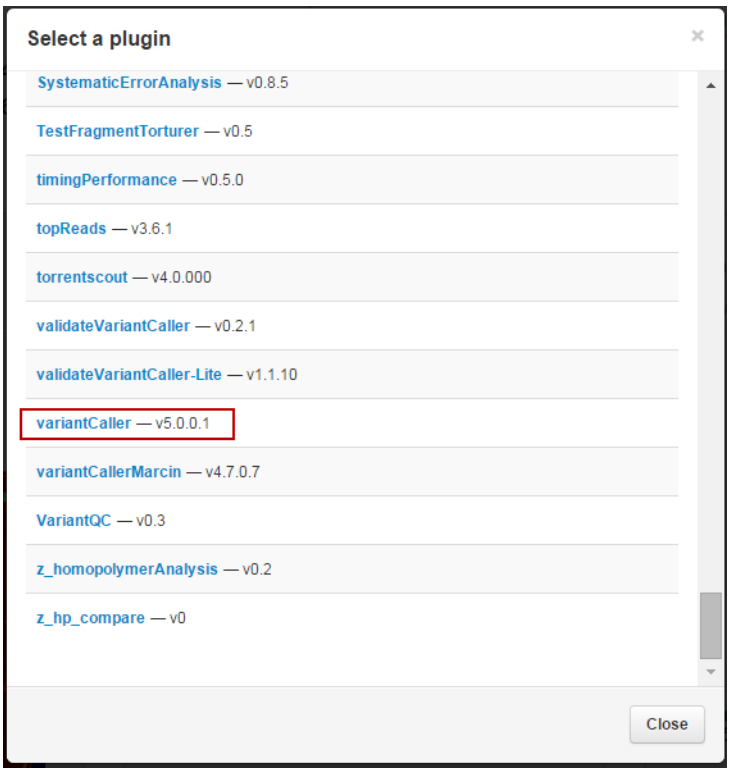
The TVC plugin supports multiple run analysis. The plugin can analyze a BAM file generated from Combine Alignment on multiple reports in a project.Combine Alignment creates a new run report (in the same project). You can open the new combined run report and use the **Select plugins to run** button to launch the TVC plugin.

To run the Torrent Variant Caller plugin manually, perform the following steps:

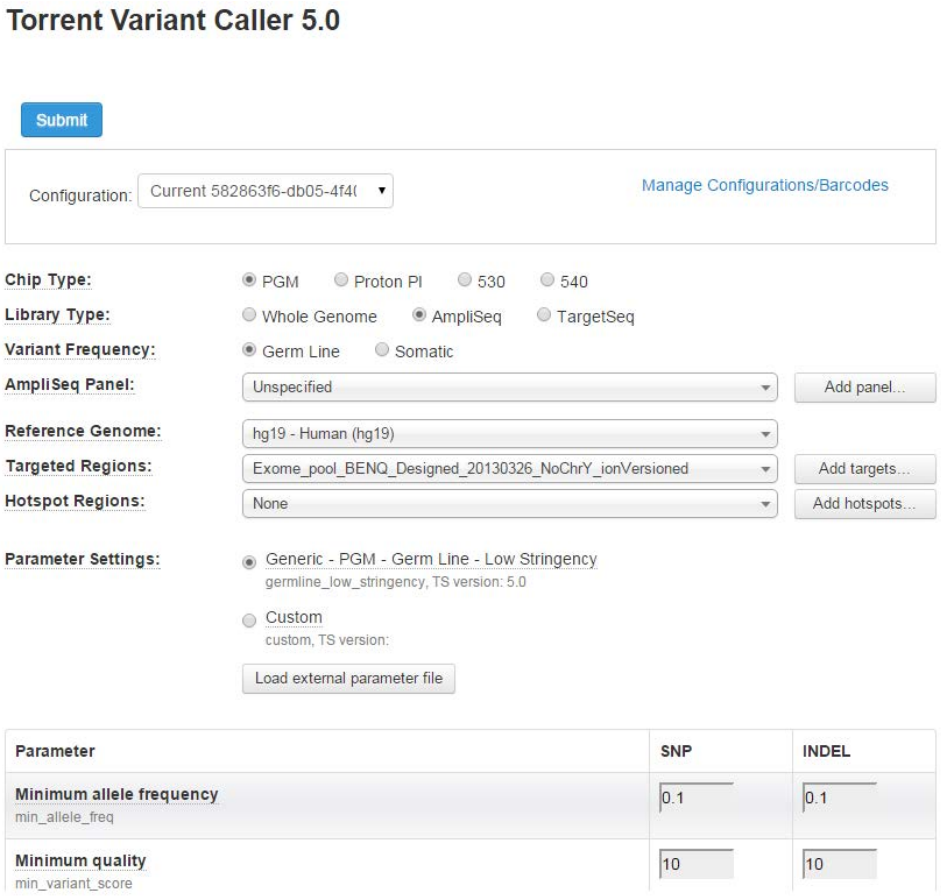
1. In the Torrent Browser, select a run report on the **Data > Completed Runs & Reports** page or on a **Data > Projects > projectname** page.
2. Click **Report Actions ▸ Select plugins to run**.



3. In the list of plugins, click on **variantCaller**.

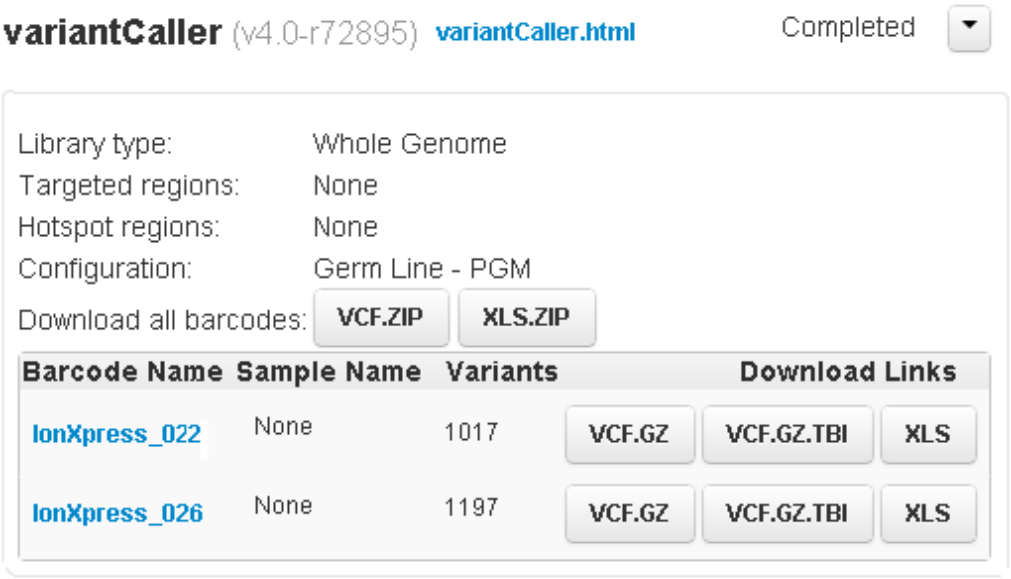


4. The Torrent Variant Caller Plugin interface appears:



5. When you are satisfied with your selections, click **Submit**. Your variant caller analysis is queued for execution.

Barcoded variantCaller summary area

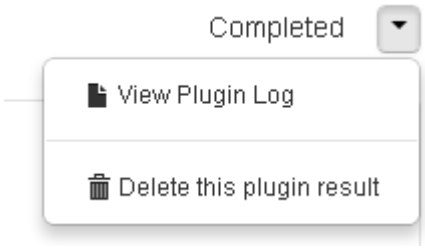


For a barcoded run:

- When the run contains multiple barcodes, the **variantCaller.html** link opens a listing of the barcodes.
- Links to a separate results page for each barcode.
- A link to download all results in one zipped file.

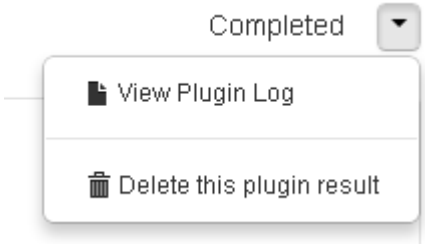
View plugin log file

To view the log file for a plugin run, in the plugin summary area, click the menu arrow next to the plugin status:



Delete plugin results

To delete the results for a plugin run, in the plugin summary area, click the menu arrow next to the plugin status:



This action removes the plugin's output files from the file system and removes the Torrent Browser plugin results page.

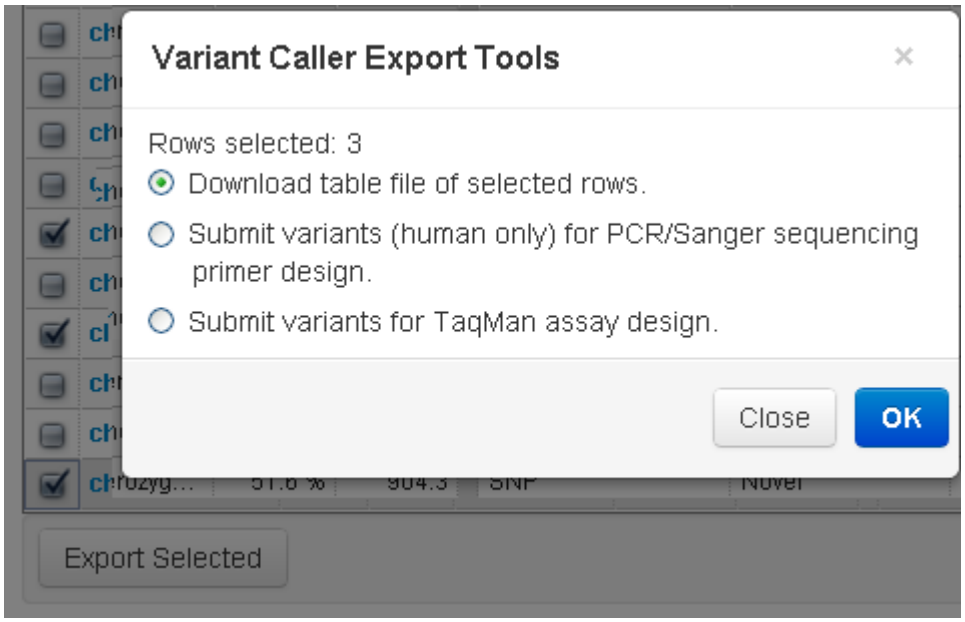
Download files and other actions

Field	Button	Description
Targeted regions	BED	Downloads the input targeted regions BED file (if any).
Hotspot regions	BED	Downloads the input hotspots BED file (if any).
Parameters Settings	Parameters File	Downloads a JSON text file of the TVC parameter values used on this run. **Note:** You can edit this file and later upload it to set your custom parameters in subsequent runs.
Mapped Reads	BAM, BAI	Downloads the BAM file (and its index) of mapped reads. This file is input to TVC.
Variant Calls	VCF.GZ,VCF.GZ.TBI,XLS	Downloads files of the variants calls: VCF.GZ,VCF.GZ.TBI: Zipped VCF file and its tabix index fileXLS: Tab-separated values file
Open Variants Calls in IGV	IGV	Link to open the results variants in the Integrated Genomic Browser (IGV).
Deprecated Features	Classic	Opens the plugin results page in the previous format.
Ion Community	Torrent Variant Caller documentation	Opens to theTorrent Variant Caller documentation page on the Ion Community (login is required).

Export to file

This option exports your variant calls to a tab-separated file. The exported file is named `subtable.xls` and has the same columns as 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:



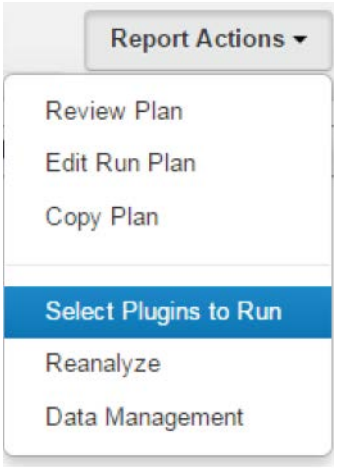
Barcode-aware Torrent Variant Calling

TVC manual launch now allows configuring and analyzing barcodes individually and in a customized manner. Every barcode can be associated with its own "configuration" that consists of Reference Genome, Target regions file, Hotspot file, TVC parameters JSON file, and TMAP parameters (remap if different from mapped BAM). In Torrent Suite™ Software v5.0, this feature is available today only via manual launch of TVC - not from run planning. On a completed run, select the TVC plugin to rerun. Then, select the Setup tab and customize parameters.

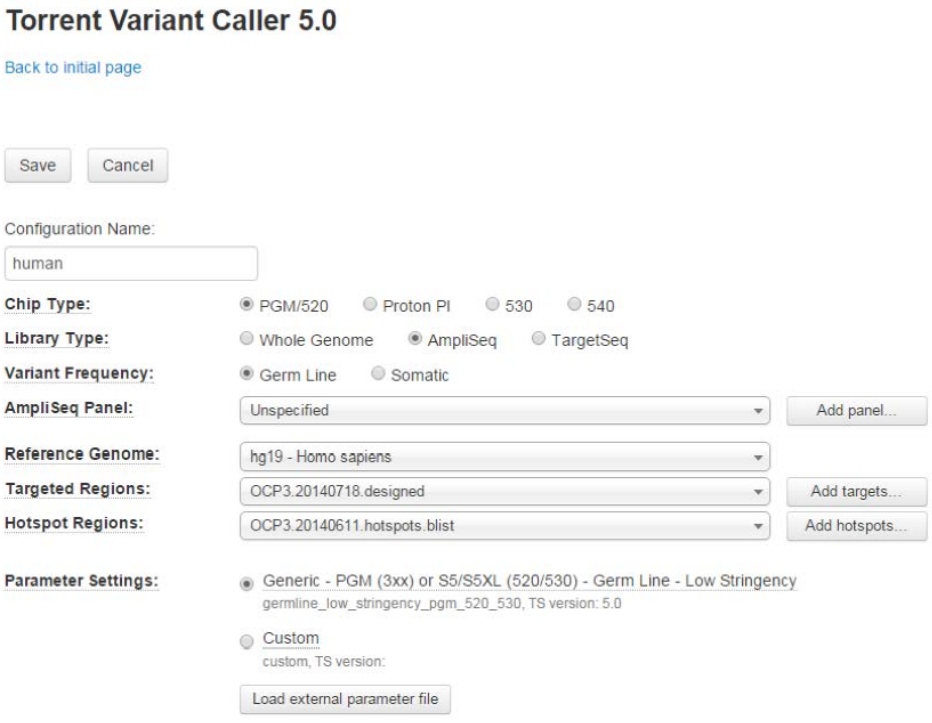
TVC manual launch for custom configuration per barcode

In Torrent Suite™ Software v5.0 and higher, you can configure individual barcodes in a run to be processed with their own reference genome, target regions file, hotspots file and TVC parameters. Please note, that this functionality is only available via manual launch of TVC after the run and not available from the run planning stage.

1. Select a completed run that you would like to reanalyze with TVC.
2. Click **Report Actions ▸ Select Plugins to Run**.

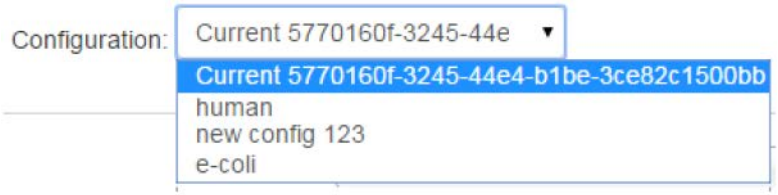


3. Select variantCaller. The Torrent Variant Caller 5.0 plugin configuration screen appears.



Apply TVC Settings to all barcodes

Use the Configuration drop-down menu to apply the same or different settings to all barcodes.

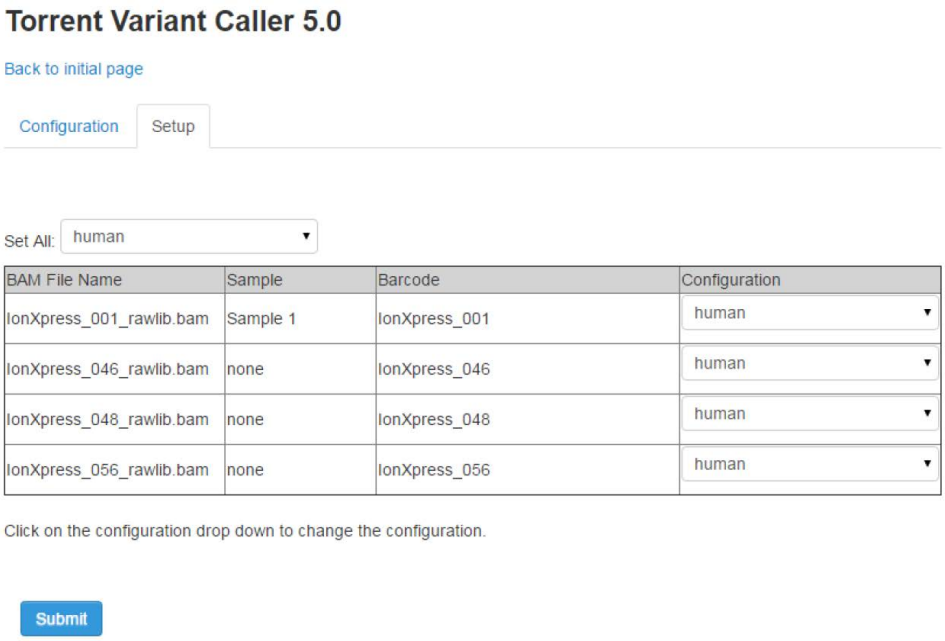


Modify and apply TVC settings for all or select barcodes

To customize your TVC settings, click the **Manage Configurations/Barcodes** link. The Configuration tab allows you to **Edit**, **Delete** or **Add** a configuration. The Setup tab allows you to apply settings to individual barcodes.

To apply TVC changes per barcode:

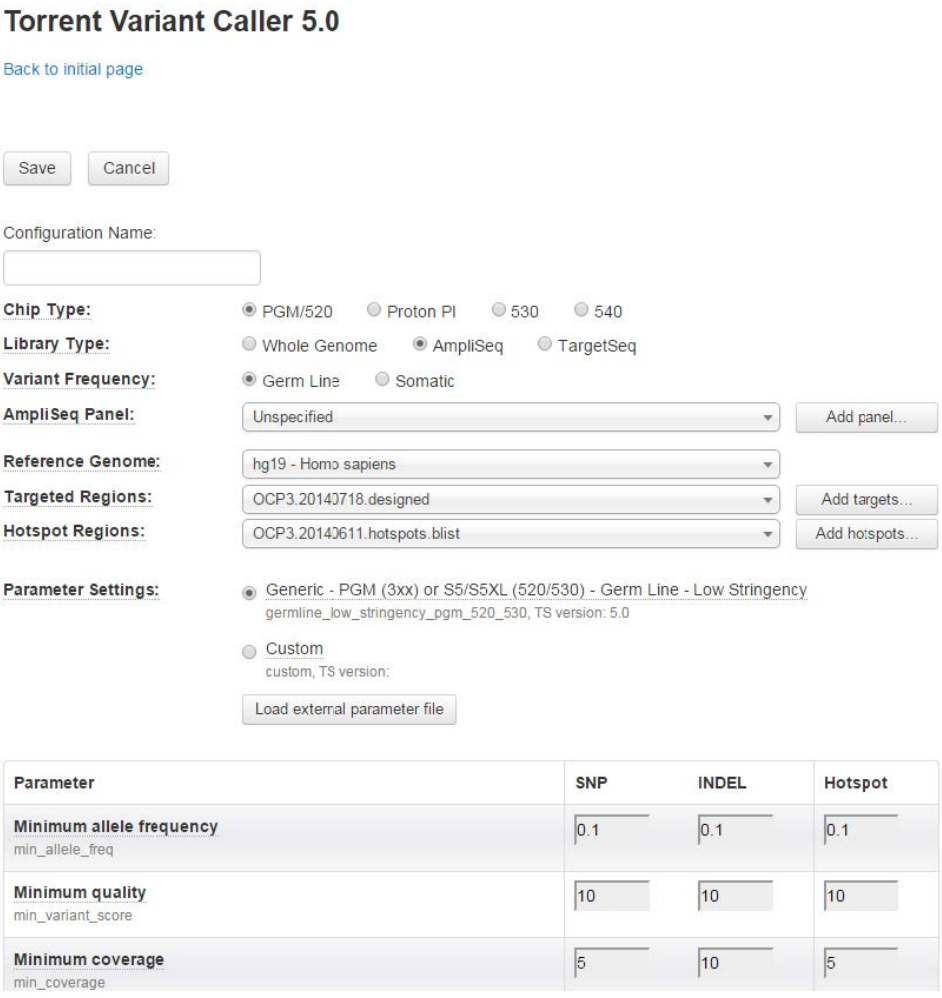
1. Click the **Setup** tab and modify settings per individual barcodes.



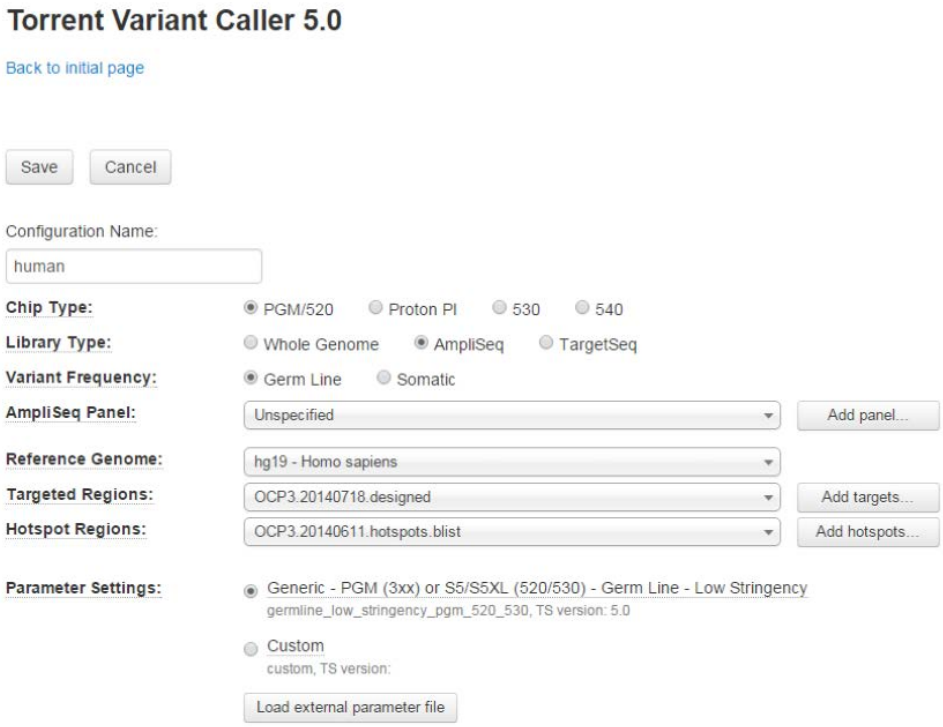
2. When finished making changes, click **Submit**. The TVC plugin reruns and applies the changes you made.

Add, edit and delete configurations

1. On the **Configuration** tab, you can Add new or Edit/Delete existing configurations.
2. Click the **Add** button to add new. Name the configuration and select your settings.



3. On the Edit screen, you can modify Chip and Library types, variant frequency, reference genome, targeted and hotspots regions and parameter settings.



4. At the bottom of the screen, you can click the **Show Advanced Settings** button and further adjust variant detection and alignment parameters.

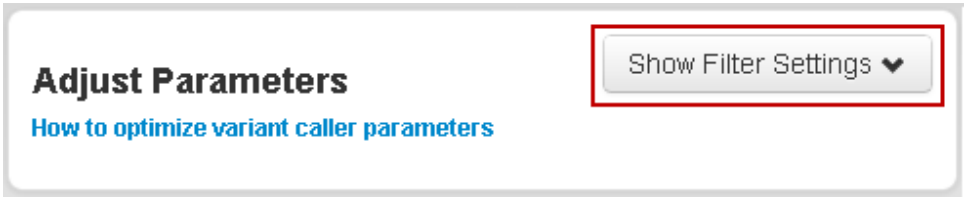
5. **Save** your new or modified configurations and then apply them to all or select barcodes.

6. Click **Submit** to rerun TVC.

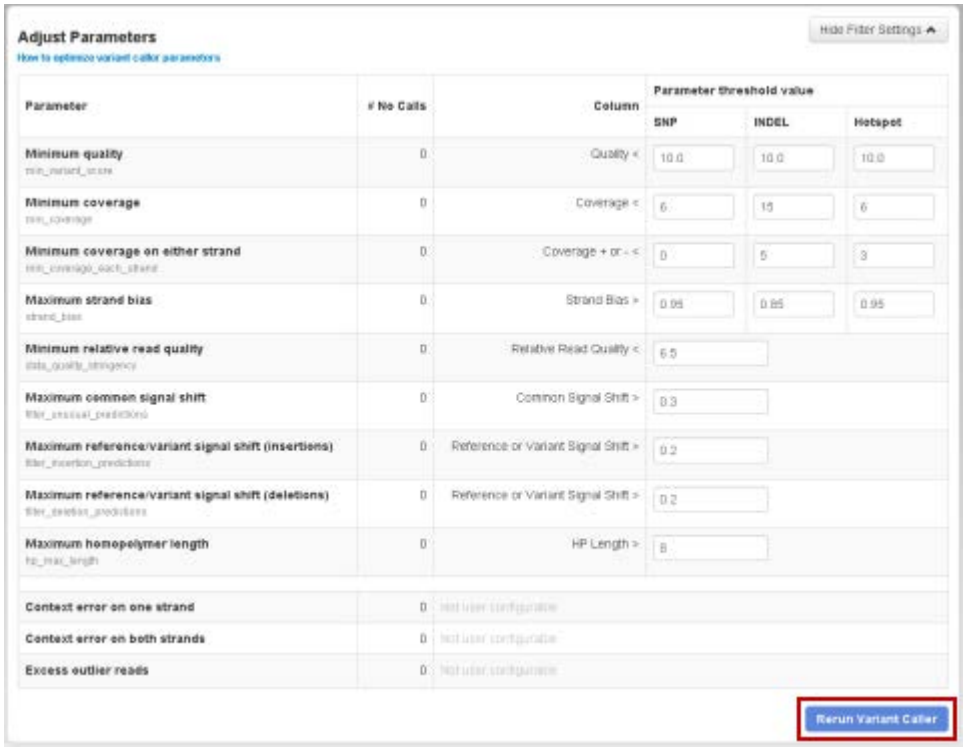
Rerun the variantCaller plugin

You can rerun the variantCaller plugin from the results page:

1. Scroll to the Adjust Parameters area at bottom of the results page, and click **Show Filter Settings**:



2. In the parameter listings, make your changes to the parameter settings (only main parameters are available):



3. Click **Rerun Variant Caller**. The plugin is submitted for execution.

Troubleshoot TVC results

Find False Negatives

In the case of missing variants (not called by TVC), an alignment viewer, such as Integrative Genomics Viewer (IGV) or IGV Light in Ion Reporter™ software, is a valuable tool to verify the presence of the variant in the sample at the position where it is expected.

- IGV may reveal problems that are not imputable to the TVC - for example, problems in mapping 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, TVC's built-in tools for displaying call details can be used.

If a hotspots 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. Run the variant caller again.

If no hotspots file was used:

1. Navigate to the variantCaller results directory on the Torrent Server and open the file small_variants_filtered.vcf. On Linux, the TS variantCaller 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 Browser, you can access the variantCaller results directory by opening the variantCaller report page for the sample or barcode of interest, removing the final 'variantCaller.html' from the URL, and hit **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 TVC v4.x.
4. Adjust parameters.
5. Run the variant caller again.

If the location of the SNP is NOT found in the filtered.vcf file create a hotspots file including 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 in dealing with homopolymer regions.

◦These can be resolved by:

1. Adjusting parameters that control the homopolymer calls, but this can increase the report of false negatives.
2. Or, when repeatedly running a panel, “blacklist” certain sites (positions) since the FP tend to happen in the same positions.

Ion Reporter™ Software Features Related to Variant Calling

Both Ion Reporter™ Software and Torrent Suite™ Software offer the Torrent Variant Caller and both provide a list of the variant positions called. This page describes additional features that are available in Ion Reporter™ Software.

In particular, the automated annotations, links to public databases, and near real-time filtering in the Ion Reporter™ ser interface provide time savings for researchers.

Annotations

In addition to the listing of the variant calls and positions, Ion Reporter™ software also annotates your called variants with information from both publicly and private databases. Except where noted, these annotations are pre-built within Ion Reporter™ software. You can select which of these annotation sources to include in your analysis workflows.

The following annotation sources are packaged with Ion Reporter™:

- **dbSNP** The Single Nucleotide Polymorphism Database, a free public-domain archive for simple genetic polymorphisms, at the following site:
  www.ncbi.nlm.nih.gov/projects/SNP
- **COSMIC** The Catalogue of Somatic Mutations in Cancer, which contains information about somatic mutations in cancer, with more than 100,000 somatic mutations from approximately 400,000 tumors. From their websites:
  http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/about.html
  http://www.sanger.ac.uk/resources/databases/cosmic.html
- **OMIM** From the website **http://www.ncbi.nlm.nih.gov/omim:**
  "Online Mendelian Inheritance in Man®. OMIM is a comprehensive, authoritative, and timely compendium of human genes and genetic phenotypes. The full-text, referenced overviews in OMIM contain information on all known Mendelian disorders and over 12,000 genes. OMIM focuses on the relationship between phenotype and genotype."
- **GeneModel** Ensembl or RefGene sources.
  - **GenePanel** Genomic regions panels that you download from AmpliSeq.com or your own custom panels.
  - **SIFT scores** A SIFT score predicts whether an amino acid substitution affects protein function.
- **PolyPhen-2 scores** The PolyPhen-2 score predicts the possible impact of an amino acid substitution on the structure and function of a human protein.
  - **Grantham scores** The Grantham score attempts to predict the distance between two amino acids, in an evolutionary sense.
- **PhyloP** PhyloP scores measure evolutionary conservation at individual alignment sites and report either slower evolution than expected or faster evolution than expected. For more information, visit this site and click the PhyloP link on the site's left navigation panel:
  compgen.bscb.cornell.edu/phast/background.php

- **Gene Ontology** The Gene Ontology project aims to standardize the representation of gene and gene product attributes across species and databases by providing a controlled vocabulary of terms for describing gene product characteristics and gene product annotation data. The Gene Ontology Consortium is supported by a grant from the National Human Genome Research Institute (NHGRI). See the following sites for more information:
  - **http://geneontology.org**
  - **http://amigo.geneontology.org**
- **Pfam** From their website at **http://pfam.sanger.ac.uk:** "The Pfam database is a large collection of protein families, each represented by multiple sequence alignments and hidden Markov models".
- **Ingenuity Variant Analysis** Software This is not a pre-built annotation source. From IR, you export your IR variants to this site to take advantage of the insight and filtering that Variant Analysis offers. If you have a large number of new variants in an analysis, Variant Analysis Software can help you filter the large set down to the most interesting variants, based to pathways, literature citations, and other categories. You can then import your variant set back into IR.
- **Local annotation sources** These are specific to your IR organization and are not pre-built annotation sources. You can configure annotation sources for the following:
  - Maintain a list of significant variants seen in your research
  - Flag variants seen previously in your research or of particular interest to your organization
  - Import positions in ampliseq.com panels
  - Contain your custom free-form annotations for specific positions
  - Limit variant calls to your list of preferred transcripts
  - Filter out known false positives

Notes on IR annotations:

- The use of Oncomine® annotations and the Ingenuity® Variant Analysis Software site involve additional charges.
- IR typically adds more annotation sources with each new release.
- Annotation is a separate module in IR workflows, called after the variant caller module. IR also has a workflow that only does annotations. This workflow takes a VCF input file of variant calls and adds annotations to those variants.

Multiple-sample workflows

Ion Reporter™ offers pre-built workflows for paired or trio related samples. During variant review, you can compare the incidence of variants in related samples. Variant calls in Torrent Suite™ software are on one sample at a time.

Ion Reporter™ also offers the pre-built Tumor/Normal workflow for a pair of input samples where one sample is a tumor sample and the other is a normal sample form the same individual. Tumor/Normal workflow provides more than a comparison of the presence of variants in each sample. For candidate variant positions seen in the tumor sample, the Tumor/Normal workflow also checks the background levels of those variants in the normal sample, providing an analysis of statistical significance of candidate variants in the tumor sample.

Additional variant types

Ion Reporter™ workflows support additional variant types that Torrent Suite™ software does not call. These include copy number variation and genetic disease variants such as compound heterozygous, trans-phase compound heterozygous, and male maternal X.

The Ion Reporter™ Exome Trio workflow also contains a pedigree check, which finds mis-labeling of the samples or mis-identification of the parent samples.

Variant filters

After your Ion Reporter™ analysis completes, you can filter your called variants directly in the Ion Reporter™ results review screen. For example, you can use pre-built filters to display only novel variants, or only variants that have an impact on the coded protein.

The filtering feature greatly reduces the effort required for you to review the results of large datasets. You can filter variants based on experimental or functional evidence. You can use Ion Reporter™ filters on the fly in the Ion Reporter™ user interface, or automate and reuse your filters.

Also on the Ion Reporter™ results review screen, variant details include links to variant information in public databases such as dbSNP or COSMIC (when appropriate).

Ion Reporter™ also offers a connection to the TaqMan® Assays site during variant review of a completed analysis. This site returns the TaqMan® SNP Genotyping Assay products related to your variants.

Output files

In Ion Reporter™ software you can download your variants in VCF format or as a text file.

Elasticity of the cloud

With larger datasets and in high-throughput labs, the use of Ion Reporter™ takes some compute load off your Torrent Server and relieves strain on your Torrent Server compute resources.

The Command-Line Torrent Variant Caller

This version of the Torrent Variant Caller is run on the Linux™ command-line and does not have a UI interface.

The command-line TVC is supported only on Torrent Server (release 4.2 or higher) and requires an input BAM file of Ion Torrent™ data.

Supported options

Many options have two equivalent versions. Choose the version that you prefer but use only one of the versions. Example:

- `-b BEDFILE`
- `--region-bed=BEDFILE`

Option	Description
-h --help	Show options in a help message and exit
-b BEDFILE --region-bed=BEDFILE	Limit variant calling to regions in this BED file. Optional.
-s HOTSPOT_VCF --hotspot-vcf=HOTSPOT_VCF	VCF.gz (+.tbi) file specifying exact hotspot positions.Optional.
-i BAMFILE --input-bam=BAMFILE	BAM file containing aligned reads
-r REFERENCE --reference-fasta=REFERENCE	FASTA file containing reference genome
-o OUTDIR --output-dir=OUTDIR	Output directory. Optional. Uses the current directory if this option is missing.
-p PARAMFILE --parameters-file=PARAMFILE	JSON file containing variant calling parameters. Optional butrecommended.
-B RUNDIR --bin-dir=RUNDIR	Directory path to location of variant caller programs. Optional. Usesthe directory where the `variant_caller_pipeline.py` script is located, if this option is missing.

Option	Description
-n NUMTHREADS --num-threads=NUMTHREADS	Sets the number of threads used by the TVC process. Optional. Defaults to 12,if this option is missing.
--postprocessed-bam=PTRIM_BAM	Perform primer trimming, storing the results in provided BAM file name. Optional. Requires the --primer-trim-bed option.
--primer-trim-bed=PTRIM_BED	BED file used for primer trimming. Required if--primer-trim-bam is used.

About target regions BED files

When a target regions BED file is uploaded to torrent servers, it is converted into 4 different versions: merged and unmerged, plain and detailed.

The merged plain version has the duplicate regions merged or removed. Use the merged plain BED file with the TVC `--region-bed` option to avoid redundant variant calls being made.

Use the unmerged detail version of the BED file with the TVC `--primer-trim-bed` option.

You can find the converted bed files under `/results/uploads/BED/`. The directory structure is shown in this example:

```
./22/hg19/merged/plain/ColonLung.20131001.designed.bed ./22/
hg19/merged/detail/ColonLung.20131001.designed.bed ./22/hg19/
unmerged/plain/ColonLung.20131001.designed.bed ./22/hg19/
unmerged/detail/ColonLung.20131001.designed.bed
```

Torrent Variant Caller Parameters

This page describes Torrent Variant Caller parameters.

Parameter customization

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.

The first group of parameters are intended for general use.

Main settings

The first five parameters support different thresholds for SNP, indel, and hotspot variants. The others use the same thresholds for all variant types.

Parameter	Comments
Minimum allele frequency `min_allele_freq`	Minimum observed allele frequency required for a non-reference variant call. Lowering this value improves sensitivity and decreases specificity (and increases the ratio of false positives to true positives). **Allowed values**: Floats 0.0 - 1.0 **Recommended values for SNPs**: Between 0.01 - 0.2 **Recommended values for indels**: Between 0.05 - 0.2
Minimum quality `min_variant_score`	Do not call variants if the phred-scaled call quality is below this value. Lowering this value improves sensitivity and decreases specificity. **Allowed values**: Integers >= 0 **Recommended values**: >= 10
Minimum coverage `min_coverage`	Do not call variants if the total coverage on both strands is below this value. For germ line workflows, lowering coverage improves sensitivity. Lowering this value is dangerous for homopolymer indels this decreases specificity drastically. **Allowed values**: Integers >= 0 **Recommended values for SNPs**: Between 5 - 20 **Recommended values for indels**: Between 15 - 30 **Recommended values for hotspots**: Between 5 - 20

Parameter	Comments
Minimum coverage on either strand `min_cov_each_strand`	Do not call variants if coverage on either strand is below this value. For indel calling, reducing this value improves sensitivity but at a high cost of specificity. **Allowed values**: Integers >= 0 **Recommended values**: >= 3
Maximum strand bias `strand_bias`	Do not call variants if the proportion of variant alleles comes overwhelmingly from one strand. **Allowed values**: Floats 0.5 - 1.0 **Recommended values for SNPs**: 0.95 **Recommended values for indels**: 0.85 **Recommended values for hotspots**: 0.95 Increasing strand bias increases sensitivity. SNP calling tolerates this adjustment better than indel calling. Related to VCF field STB.
Minimum relative read quality `data_quality_stringency`	Do not call variants if Relative Read Quality is below this threshold. A phred-scaled minimum average evidence per read or no-call. **Allowed values:** Floats >= 0 **Recommended values:** >= 6.5 Impact of changing this value: Lowering this value improves sensitivity and decreases specificity. Related to VCF field MLLD.
Maximum common signal shift `filter_unusual_predictions`	Do not call variants if Common Signal Shift exceeds this threshold. If the predictions are distorted to fit the data more than this distance (relative to the size of the variant), filter this candidate position out. **Allowed values:** Floats >= 0 **Recommended:** 0.3 = 30% of variant change size. Related to VCF field RBI.

Parameter	Comments
Maximum reference/variant signal shift (insertions) `filter_insertion_predictions`	Do not call insertions if Reference or Variant Signal Shift exceeds this threshold.Filter observed clusters that deviate from predictions by more than this amount (relative to the size of the variant). **Allowed values:** Floats >= 0 **Recommended:** 0.2 (which is 20% of variant change size). Related to VCF fields VARB and REFB.
Maximum reference/variant signal shift (deletions) `filter_deletion_predictions`	Do not call deletions if Reference or Variant Signal Shift exceeds this threshold. Filter observed clusters that deviate from predictions by more than this amount (relative to the size of the variant). **Allowed values:** Floats >= 0 **Recommended:** 0.2 (which is 20% of variant change size). Related to VCF fields VARB and REFB.

Torrent Variant Caller advanced parameters

Parameter	Comments	
hp_max_length	Maximum homopolymer length for calling indels. **Allowed values**: Integers >= 1 **Recommended value:** 8 Related to VCF field HRUN.	
downsample_to_coverage	Reduce coverage in over-sampled locations to this value. **Allowed values:** Integers>= 1 **Recommended values**: 400 (germline), 2000 (somatic)	
outlier_probability	Prior probability that a read comes from some other distribution. Lower numbers reduce the influence of outlier observations. Higher numbers increase the influence of outliers. Empirical adjustment indicates that increasing the influence of outliers leads to more false-positives and slightly more true positives, but at a poor tradeoff. **Allowed values:** Floats 0.0 - 1.0 **Recommended values**: Between 0.005 - 0.01	

Parameter	Comments	
do_snp_realignment	Realign reads in the vicinity of SNP candidates. **Allowed values**: • 0: Do no realign. Recommended for germline. • 1: Realign. Recommended for somatic.	
prediction_precision	Number of pseudo-data-points suggesting our predictions match the measurements without bias. **Allowed values:** Floats >= 0.0 **Recommended value:** 1.0 **Impact of changing this value:** Lowering this value increases specificity and decreases sensitivity.	
heavy_tailed	How heavy the T-distribution tails are to allow for unusual spread in the data. This value represents the prior probability that a given read comes from some distribution other than the possibilities being evaluated. Lower values mean that more reads are forced to be assigned to one of the tested alleles, even at very poor data fit (fewer reads are thrown out, with the likely tradeoff of more false positive calls). Higher values mean that reads that are merely slightly noisy are thrown away, resulting in poorer sensitivity. The proportion of reads that are discarded as outliers is shown in the FXX info tag in the output VCF file.	
suppress_recalibration	Ignore the base recalibration values from pipeline in TVC. (Changes the way signal is predicted.) **Allowed values:** • 0: Use base calibration values in TVC. Recommended for Ion Proton™ data. • 1: Ignore base calibration values in TVC.	

Parameter	Comments	
hotspot_strand_bias_pval indel_strand_bias_pval mnp_strand_bias_pval snp_strand_bias_pval	First we calculate a bootstrap estimate of the pval to test significance of observed strand bias against the null hypothesis of no strand bias. If this pval estimate is lower than the parameter value (hotspot, indel, mnp or snp_strand_bias_pval) and the observed strand bias is higher than the hotspot, indel, mnp or snp_strand_bias then this variant will be filtered. Lowering this value makes filtering less stringent. Allowed values: • 0-1 Default value: • 1	
mnp_min_allele_freq	Minimum observed allele frequency required for a non-reference variant call. Lowering this value improves sensitivity and decreases specificity (and increases the ratio of false positives to true positives). Allowed values: • 0.0-1.0 Default value: • 0.01-0.2	
mnp_min_variant_score	Do not call variants if the phred-scaled call quality is below this value.Lowering this value improves sensitivity anddecreases specificity. Allowed values: • integers >=0 Default value: • >=10	
mnp_min_cov_each_strand	Do not call variants if coverage on either strand is below this value. For indel calling, reducing this value improves sensitivity but at a high cost of specificity. Allowed values: • integers >=0	

Parameter	Comments	
mnp_min_coverage	Do not call variants if the total coverage on both strands is below this value.For germ line workflows, lowering coverage improves sensitivity. Lowering this value is dangerous for homopolymer indels - this decreases specificity drastically. Allowed values: • integers >=0 Default value: • 5-20	
mnp_strand_bias	Do not call variants if the proportion of variant allelesfrom one strand if higher than this ratio. Allowed values: • 0.5-1.0 Default value: • 0.95	
do_mnp_realignment	Realign reads in the vicinity of candidate mnp variants. Allowed values: • 0 = off • 1 = on	
realignment_threshold	Maximum allowed fraction of reads where realignment causes an alignment change Allowed values: • 0-1 Default value: • 1	
use_position_bias	Enables the position bias filter. Allowed values: • 0-1 Default value: • 0	

Parameter	Comments	
position_bias	Filter out variants with position bias relative to soft clip ends in reads > position-bias given pval < postion-bias-pval. Allowed values: • 0-1 Default value: – 0.75	
position_bias_pval	Filter out if position bias > position-bias given pval < position-bias-pval. Position_bias_pval is threshold for filtering if the pval for testing whether the position-bias is statistically significant against the null hypothesis of no position bias is lower than the threshold and the position_bias is higher than the position_bias threshold. Note that filtering only occurs if both the pval and the position bias is higher than the position_bias_pval and the position bias is higher than position_bias. Lowering this value makes filtering less stringent. Allowed values: • 0-1 Default value: • 0.05	
position_bias_ref_fraction	Skips position bias filtering if (reference read count)/ (reference + alt allele read count) <= to this value. Lowering this value makes filtering more stringent. Allowed values: • 0-1 Default value: – 0.05	
sse_prob_threshold*	Filters out variants in motifs with error rates above this. Advanced filtering: read the docs before changing. Allowed values: • 0-1 Default value: • 0.2	

Parameter	Comments	
indel_as_hpindel	Apply indel filters to non HP indels. Allowed values: • 0 = on • 1 = off Default value: • 0	

Advanced settings

These parameters allow additional customization of the variant calling algorithm but are intended for advanced users only.

Long indel assembly advanced settings

These parameters control the behavior of the long indel assembler (which is a module within TVC). Again, these parameters are recommended for advanced users only.

Both the FreeBayes module and the long indel assembler generate lists of variant candidates (other modules in TVC then 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 FreeBase module.

Parameter	Comments
kmer_len	Sets the length of the minimum suffix/prefix overlap (perfect match) of any two reads to be considered for assembly. Increasing this value requires longer overlaps, reducing the chances of finding matching pairs and therefore reducing the chances of calling false positives. (Increasing values make indel calls less sensitive but more specific.) Reducing the value has the opposite effect. **Allowed values**: Integers > 5 **Recommended values**: Between 11 - 30
min_var_count	Sets the number of times a variant appears in the assembled contigs in order to be considered for evaluation. Increasing this value requires more coverage of the candidate indel to be taken in consideration reducing the chances of false positive calls.(Increasing values make indel calls less sensitive but more specific.) **Allowed values:** Integers > 1 **Recommended values**: Between 3 - 30

Parameter	Comments
short_suffix_match	In order for a contig to be considered for the coverage of an indel, both sides of the variant have to match perfectly the reference sequence. Increasing the size of the matching sequence sets more stringent conditions, reducing the chances of a contig to be picked as containing an indel. (Increasing values make indel calls less sensitive but more specific.) **Allowed values:** Integers > 2 **Recommended values**: Between 4 and kmer_len
min_indel_size	Sets the minimum size of an indel (from assembled reads) to be reported. Increasing this size reduces the number (and increases the size) of the reported indels. Increasing values make indel calls less sensitive but more specific. **Allowed values:** Integers > 0 **Recommended values:** Between 2 - 30
max_hp_length	Sets the maximum length of the homopolymer to be reported. The default value has been optimized according to the physics of semiconductor sequencing. Increasing values make indel calls less sensitive but more specific. **Allowed values**: Integers > 1 **Recommended values:** Between 2 - 11
min_var_freq	Sets the minimum value of the frequency of an indel to be reported. **Allowed values**: Floats 0.0 - 1.0 **Recommended values**: Between 0.1 - 0.4 Increasing this value requires a variant to be highly present in the sample in order to be called. Increasing values make indel calls less sensitive but more specific.

Parameter	Comments
relative_strand_bias	Indels appearing in the sample more frequently in one strand than in the other one have an increased strand bias value. Assembled indels for which their bias value exceeds the value of this parameter are not called. **Allowed values**: Floats 0.0 - 1.0 **Recommended values:** Between 0.6 and 1.0 Increasing this value makes indel calls more sensitive but less specific.
output_mnv	Whether or not to include MNV variants in TVC output. **Allowed values:** • 0: Do not include MNVs. • 1: Also include MNVs.

FreeBayes advanced settings

These parameters control the behavior of the FreeBayes module, which generates a list of variant candidates.

Again, these parameters are recommended for advanced users only.

Parameter	Comments
allow_indels	Enable indels in FreeBayes hypothesis generator. When set to 0, indels are not called. **Allowed values:** • 0 = Do not generate indel hypotheses • 1 = Generate indel hypotheses(default)
allow_snps	Enable SNPs in FreeBayes hypothesis generator. When set to 0,SNPs are not called. **Allowed values:** • 0 = Do not generate SNP hypotheses • 1 = Generate SNP hypotheses (default)
allow_mnps	Enable MNPs, including equal-length block substitutions, in the FreeBayes hypothesis generator. When set to 0,MNPs are not called. **Allowed values**: • 0 = Do not generate MNPhypotheses • 1 = Generate MNP hypotheses(default)

Parameter	Comments
allow_complex	Enable the generation of block substitution variants candidate in FreeBayes hypothesis generator. When set to 0, block substitution variants are not called. **Allowed values:** • 0 = Do not generate block substitution hypotheses (default) • 1 = Generate block substitution hypotheses Notes about setting allow_complex to 1: • When on, allow_complex results in the call of more true positives, but also increases the false positive rate in germ line analyses on Ion AmpliSeq™ exome data. • When on, allow_complex overrides the settings of allow_mnps
min_mapping_qv	Minimum mapping QV value required for reads to be allowed into the pileup. If a read has a mapping QV lower than this value, filter the position out. **Allowed values:** Integers >= 0 **Recommended value:** 4 Impact of changing this value: Increasing this value decreases sensitivity and improves specificity.
read_mismatch_limit	The number of mismatches allowed. If a read has more mismatches than this value, filter the read out. **Allowed values**: Integers>= 0 **Recommended value:** 10
read_max_mismatch_fraction	Maximum fraction of mismatches allowed in the length of read. Filters out potentially mis-mapped reads. **Allowed values:** Floats 0.0 - 1.0 **Recommended value**: 1.0 Decreasing this value decreases sensitivity and improves specificity (fewer but more accurate reads).
gen_min_alt_allele_freq	An early-on filter for allele frequency. Filter out variant candidates that do not have at least this frequency in the pileup. **Allowed values:** Floats 0.0 - 1.0 **Recommended value:** 0.02 - 0.15

Parameter	Comments
gen_min_indel_alt_allele_freq	An early-on filter for allele frequency for indel callings. Filter out indel candidates that do not have at least this frequency in the pileup. **Allowed values**: Floats 0.0 - 1.0 **Recommended value:** 0.02 - 0.15
gen_min_coverage	An early-on filter for minimum coverage. Filter out variant candidates that do not have at least this depth of coverage. **Allowed values**: Integers>= 0 **Recommended value**: 6

Torrent Variant Caller arguments

Parameter	Comments
Torrent Variant Caller arguments	This field is for internal use. **Recommended value:** "tvc"

IMPORTANT! The Variant Caller parameter settings are saved in templates but *are not saved* in run plans. Parameter changes that you make in a run plan affect only that specific run.

When you change Variant Caller parameter settings in a template, your changes affect all users who create run plans from that template.

Torrent Variant Caller Output

Introduction

This page describes the Torrent Variant Caller output, which includes the following reports and sections.

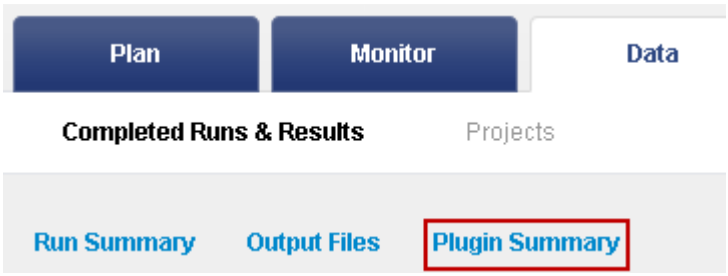
- Run report plugin summary
- Variant Caller Report
- Variant Caller Report summary section
- Variant Caller Report Variant Calls table

Run report plugin summary

The run report contains a short summary of plugin output. These summaries appear below the run report metrics and above the Output Files section.

To jump to the plugin results:

1. Click the Plugin Summary jump near the top of the run report:



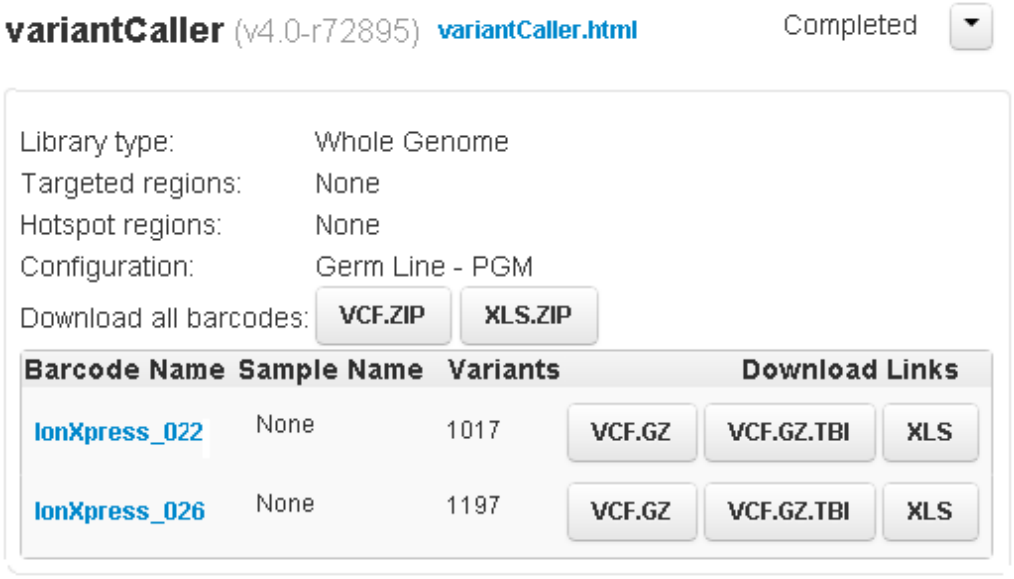
2. Click the link to go to the TVC results summary:

3. The browser jumps to the variantCaller summary.

The variantCaller summary area is slightly different for barcoded and non-barcoded runs. In both cases, the summary section includes the following:

- Information about the analysis type, targeted regions and hotspot files, and variantCaller parameter settings.
- The total number of variants called.
- The **variantCaller.html** link to the results page.
- Download links:
  - The zipped VCF file of variant calls.
  - The Zipped VCF index file (required for IGV).
  - The results in a tab-separated file.

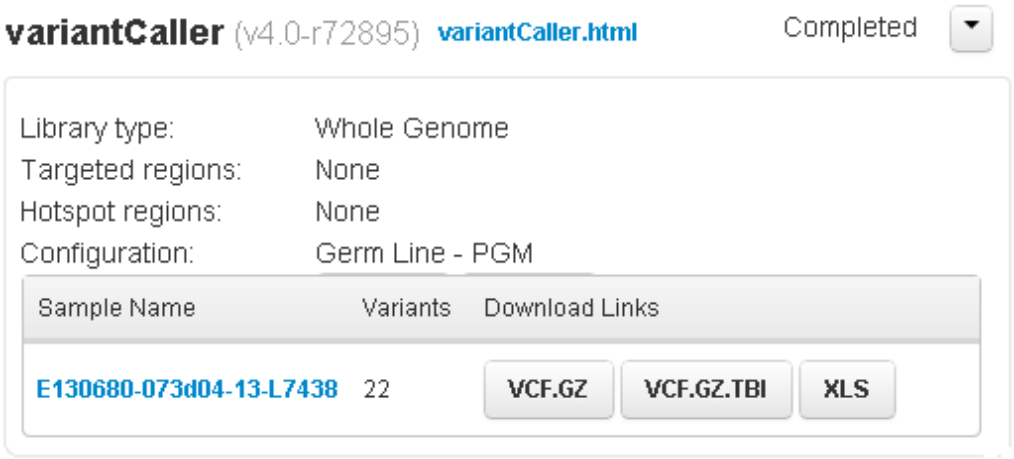
Barcoded variantCaller summary area



For a barcoded run:

- When the run contains multiple barcodes, the **variantCaller.html** link opens a listing of the barcodes.
- Links to a separate results page for each barcode.
- A link to download all results in one zipped file.

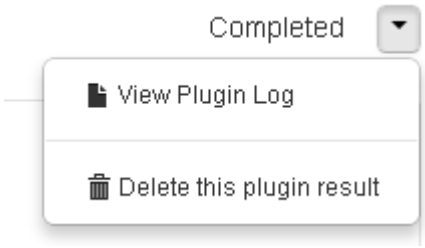
Non-barcoded variantCaller summary area



For a non-barcoded run, the sample name is listed. This link and the **variantCaller.html** link open the same results page.

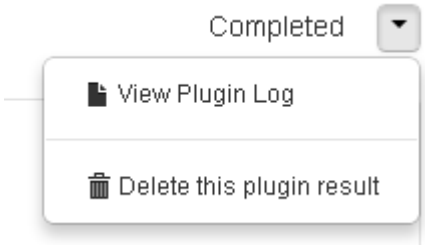
View plugin log file

To view the log file for a plugin run, in the plugin summary area, click the menu arrow next to the plugin status:



Delete plugin results

To delete the results for a plugin run, in the plugin summary area, click the menu arrow next to the plugin status:



This action removes the plugin's output files from the file system and removes the Torrent Browser plugin results page.

Variant Caller Report

The open the plugin report, click the `variantCaller.html` link in the run report Plugin Summary area:

The plugin report begins with a listing of information and download links, as described in the following tables:

Buttons for downloads and other actions

Field	Button	Description
Targeted regions	BED	Downloads the input targeted regions BED file (if any).
Hotspot regions	BED	Downloads the input hotspots BED file (if any).

Field	Button	Description
Parameters Settings	Parameters File	Downloads a JSON text file of the TVC parameter values used on this run. **Note:** You can edit this file and later upload it to set your custom parameters in subsequent runs.
Mapped Reads	BAM, BAI	Downloads the BAM file (and its index) of mapped reads. This file is input to TVC.
Variant Calls	VCF.GZ,VCF.GZ.TBI,XLS	Downloads files of the variants calls: VCF.GZ,VCF.GZ.TBI: Zipped VCF file and its tabix index fileXLS: Tab-separated values file
Open Variants Calls in IGV	IGV	Link to open the results variants in the Integrated Genomic Browser (IGV).
Deprecated Features	Classic	Opens the plugin results page in the previous format.
Ion Community	Torrent Variant Caller documentation	Opens to the Torrent Variant Caller documentation page on the Ion Community (login is required).

Variant Calls by Allele table

The following list summarizes the features of the Variant Calls table:

- Each position is a link to open the variant in 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.
- You can export selected variants to a table file or to the Life Technologies PCR and Sanger Sequencing For TaqMan® Assay Design web sites.
- 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 View tabs on the right of the table 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. Larger values mean more certainty in the call. Typically very large for reads strongly distinguishing variants (SNPs) 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.

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 COMPLEX Complex block substitution
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 target regions file
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

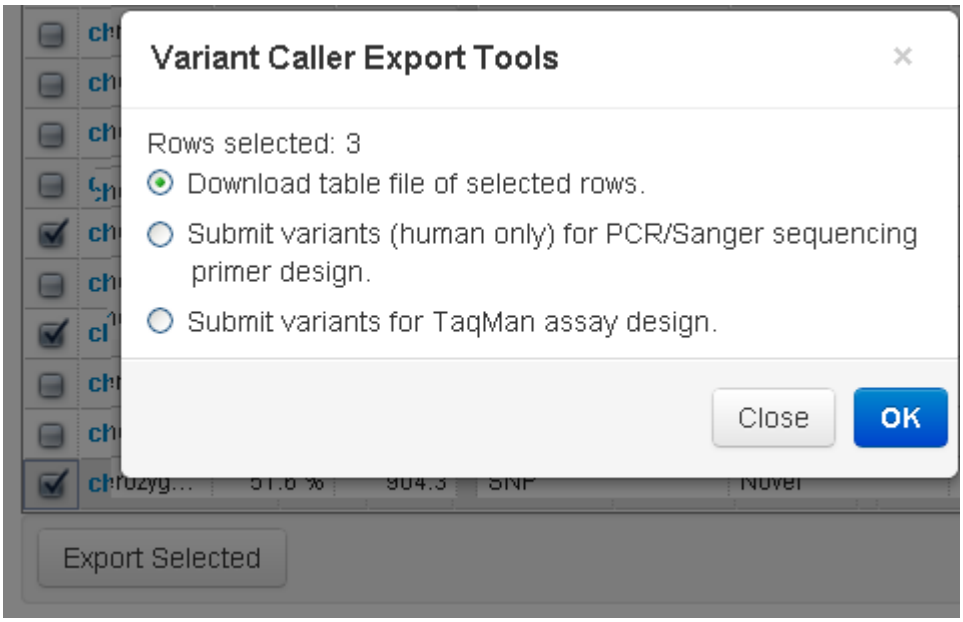
These columns are displayed in the run report in the View Coverage Metrics tab:

Column	Description
Coverage	Total coverage at this position, after downsampling
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

Export to file

This option exports your variant calls to a tab-separated file. The exported file is named `subtable.xls` and has the same columns as 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:



View Quality Metrics

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

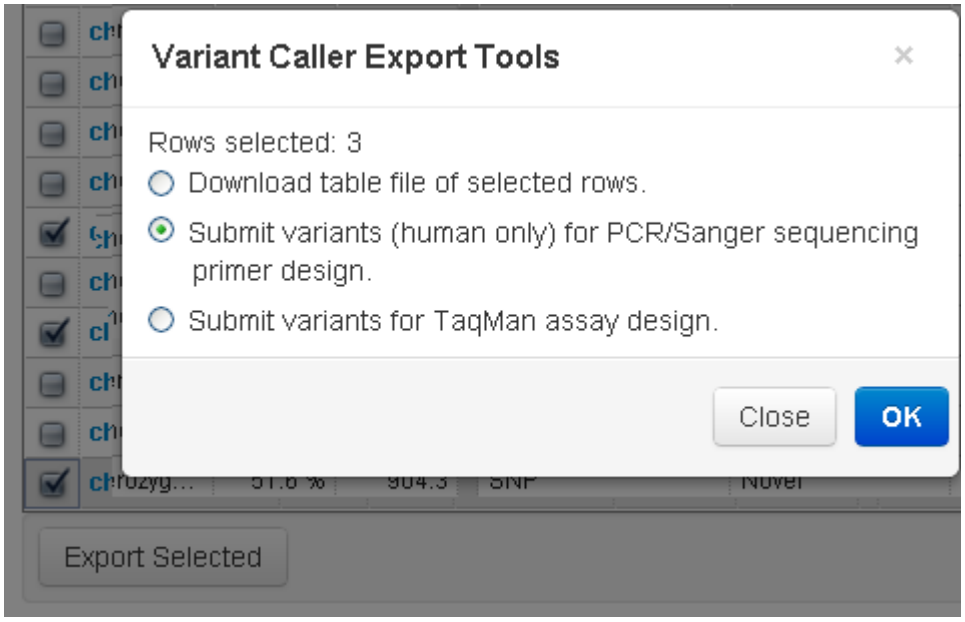
Column	Description
Common Signal Shift	Distance between predicted and observed signal at the allele locus. [RBI]
Reference Signal Shift	Distance between predicted and observed signal in the reference allele. [REFB]
Variant Signal Shift	Distance between predicted and observed signal in the variant allele. [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).

Column	Description
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 to Pre-Designed Primers for PCR and Sanger Sequencing site

With this export option, you select variants to search for pre-designed primers for PCR and Sanger sequencing.

To initiate a search, use the checkboxes column (on the left) to select variants from the TVC plugin report and click on the **Export Selected** button. A pop-up window allows you to submit variants for the PCR and Sanger sequencing pre-designed primers site:

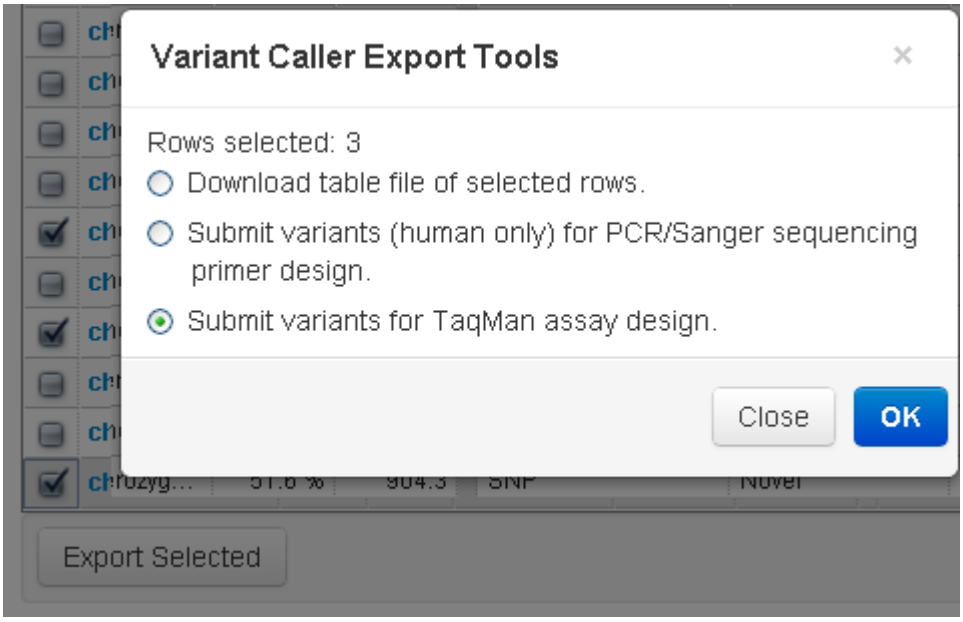


A new browser window opens to this site:
Pre-Designed Primers for PCR and Sanger Sequencing. You must be connected to the internet to access this feature.

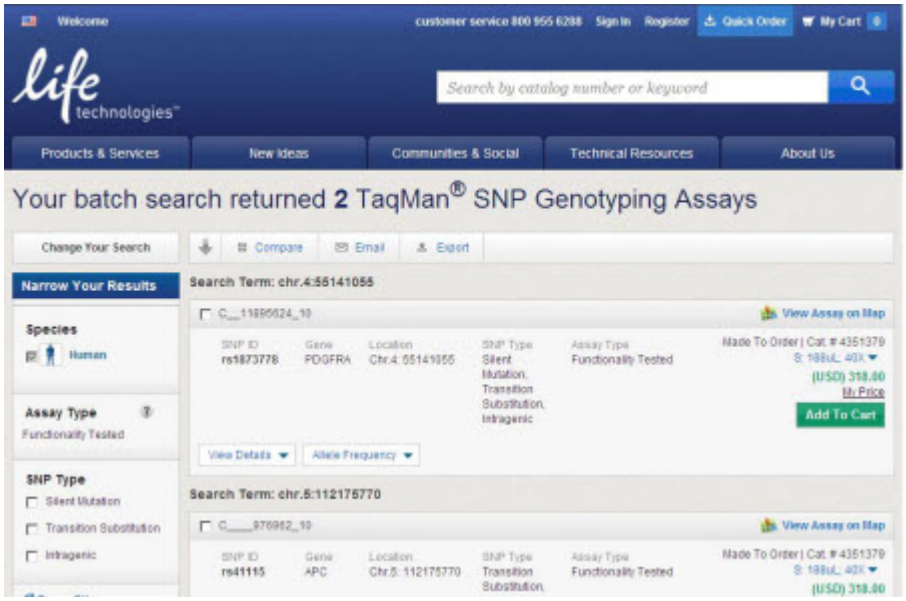
TaqMan® Assay Search

After running the TVC plugin, you can select variants from a report table and submit a search against the TaqMan® Assay Search webpage. When the search is submitted, you can choose which TaqMan® Assay database to search. A new browser window appears with the search results. If TaqMan® assays are available for the selected variant, you can immediately order the assay directly from the landing page. You must be connected to the internet to access this feature.

To initiate a search, use the checkboxes column (on the left) to select variants from the TVC plugin report and click the **Export Selected** button. A pop-up window allows you to submit variants for TaqMan® assay design.



You are then presented with a list of verification assays that you can order from Thermo Fisher.



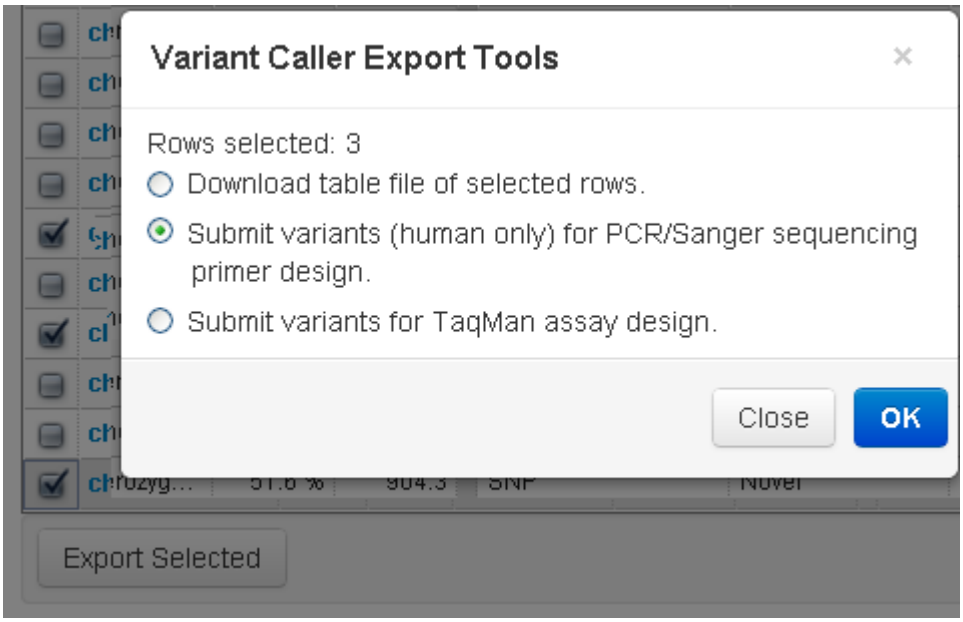
Torrent Variant Caller Integration with TaqMan® and PCR

This page describes how to use your Torrent Variant Caller (TVC) results forpre-designed primers for PCR and Sanger sequencing or with the TaqMan® Assay Search web page.

Export to Pre-Designed Primers for PCR and Sanger Sequencing site

With this export option, you select variants to search for pre-designed primers for PCR and Sanger sequencing.

To initiate a search, use the checkboxes column (on the left) to select variants from the TVC plugin report and click on the **Export Selected** button. A pop-up window allows you to submit variants for the PCR and Sanger sequencing pre-designed primers site:

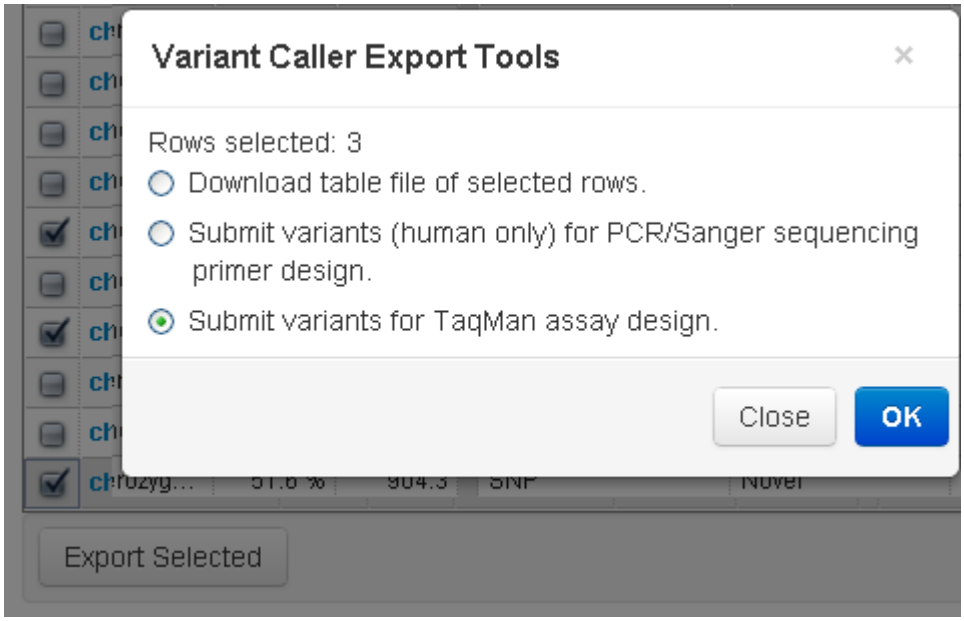


A new browser window opens to this site:
Pre-Designed Primers for PCR and Sanger Sequencing. You must be connected to the internet to access this feature.

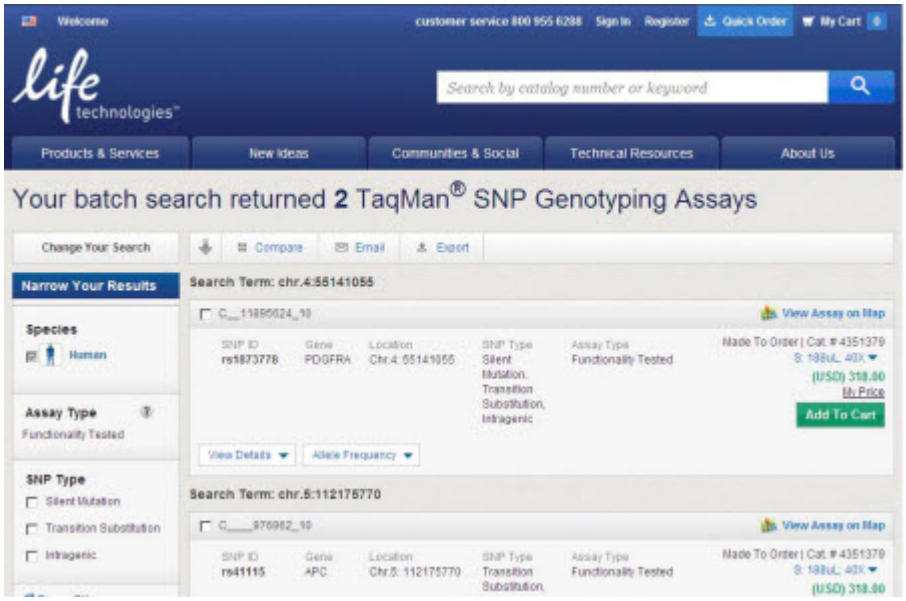
TaqMan® Assay Search

After running the TVC plugin, you can select variants from a report table and submit a search against the TaqMan® Assay Search webpage. When the search is submitted, you can choose which TaqMan® Assay database to search. A new browser window appears with the search results. If TaqMan® assays are available for the selected variant, you can immediately order the assay directly from the landing page. You must be connected to the internet to access this feature.

To initiate a search, use the checkboxes column (on the left) to select variants from the TVC plugin report and click the **Export Selected** button. A pop-up window allows you to submit variants for TaqMan® assay design.



You are then presented with a list of verification assays that you can order from Thermo Fisher.



Integration with Ion Reporter™ Software

The IonReporterUploader plugin

Overview

The Ion Reporter Uploader plugin transfers results files directly from a Torrent Suite™ Software analysis to your Ion Reporter™ Software organization (available under separate license).

You include the Uploader plugin settings in the run plan that you use for your sequencing run. When your Torrent Suite™ Software analysis completes, the run plan automatically launches the plugin and begins your file transfer. After the transfer, your Ion Reporter™ Software is automatically launched.

One-time manual launch from a completed run report (with the **Select plugins to run** button) is also supported. Manual plugin launch does not support automatic Ion Reporter™ Software workflow launch.

Configure your Ion Reporter™ Uploader Plugin Access Credentials

This page describes how to configure your Torrent Browser to transfer files to your organization on Ion Reporter™ Software.

The IonReporterUploader plugin supports multiple configurations, each with a different login and different display name (that you assign). With these multiple configurations, the IonReporterUploader plugin supports the transfer of results from one Torrent Server to different Ion Reporter™ Software organizations, without the need to change the plugin configuration.

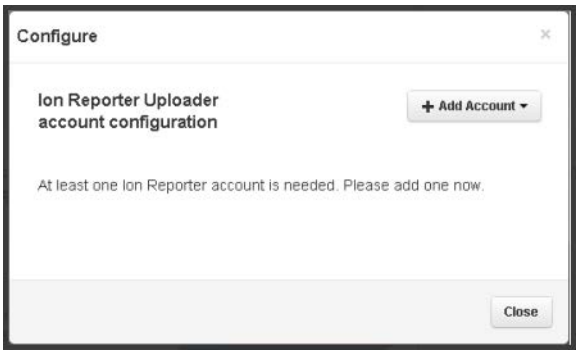
When multiple users (with different Torrent Suite™ Software logins) configure the IonReporterUploader plugin, each user can only see (and use) the IonReporterUploader plugin configuration that they created themselves. When multiple users share one Torrent Suite™ Software login, they can see and use all

IonReporterUploader plugin configurations created with that Torrent Suite™ Software account.

IMPORTANT! When you upgrade your Ion Reporter™ software, you must configure your IonReporterUploader plugin to access the new version of the software. Repeat the configuration steps each time you upgrade.

---

1. Log into the Torrent Browser (as either an ionadmin or a regular user).
2. Click the Admin gear menu (in the upper right) and select Ion Reporter™ **Configure**.
   The Ion Reporter™ Uploader Configuration page opens. If the plugin is not configured yet, this page opens:



3. Click the **+ Add Account** menu. For the hosted cloud Ion Reporter™ Software solution, click **Ion Reporter™ Cloud**. For your own Ion Reporter™ Server System, click Ion Reporter™.
4. In the Configure popup, make these entries the hosted cloud Ion Reporter™ Software solution:

Field	Directions
Server Type	Enable HTTPS
Display Name	Enter a meaningful name of your choice. This name is used in the run plan template wizard and is seen by other Torrent Browser users. Use only alphanumeric characters, spaces, and underscores.
Server	Enter: 40.dataloader.ionreporter.iontorrent.com
Port	Enter: 443
Username	Enter your Ion Reporter™ Software login (your email address).
Password	Enter your Ion Reporter™ Software login password

   For your own Ion Reporter™ Server System, these entries depend on your location system configuration: Server Type (HTTP or HTTPS), Server, and Port. Ask your local Ion Reporter™ Server System administrator for these values.
5. The "Default Account" is the account that is configured by default in run templates and run plans. If this is the main account to be used for file transfers, enable the Default Account checkbox. (You can always change this selection in the run plan template wizard and in the Upload to Ion Reporter™ quick link.)

6. Click the **Get Versions** button to see what versions are available on your server. Select the desired version.

7. Click the **Add** button in the bottom right.

   As soon as at least one account is successfully configured, the Uploader plugin is ready to transfer files and also launch Ion Reporter™ Software analyses. If you configure more than one account, you have a choice of accounts to use for the transfers, in the run plan template wizard, the Uploader plugin manual launch page, and the

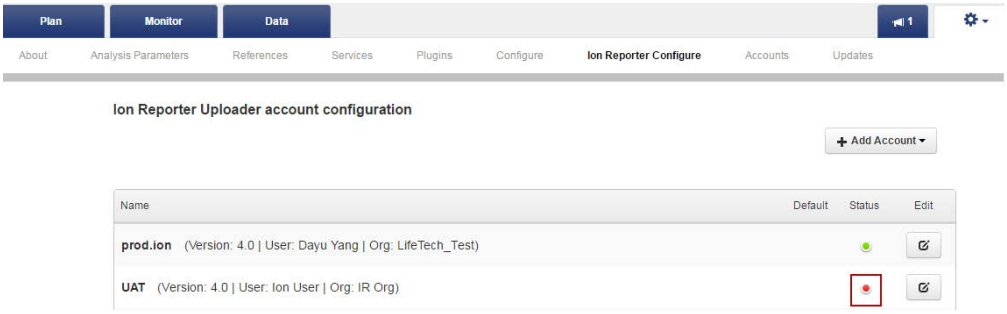
   Upload to Ion Reporter™ quick link. Each Torrent Browser user can only see the configurations they created, but if several people share a Torrent Browser login account, those people can see and potentially use each others configurations.

   The more common causes of a configuration error are:

   - HTTP is selected instead of HTTPS.
   - The server name is incorrect.
   - The port number is incorrect.
   - The login or password is incorrect or deactivated.
   - There are spaces before or after the server name, port number, username, or password.

Red status on Ion Reporter account configuration page

When you change your Ion Reporter account password or upgrade the account, the status column on the Ion Reporter Uploader account configuration page shows a red radio button.



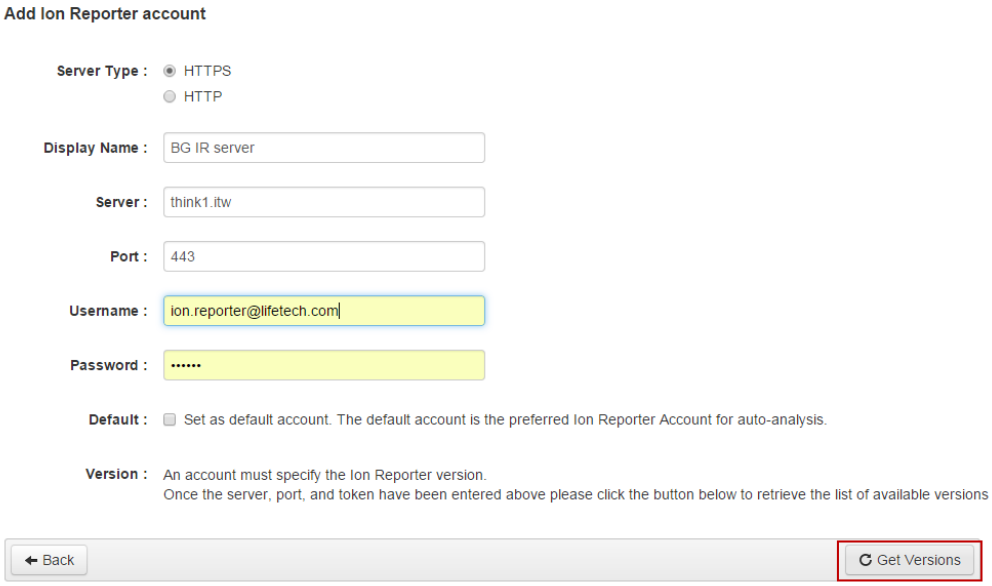
If you changed your password, click the Edit icon and enter your new password.

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

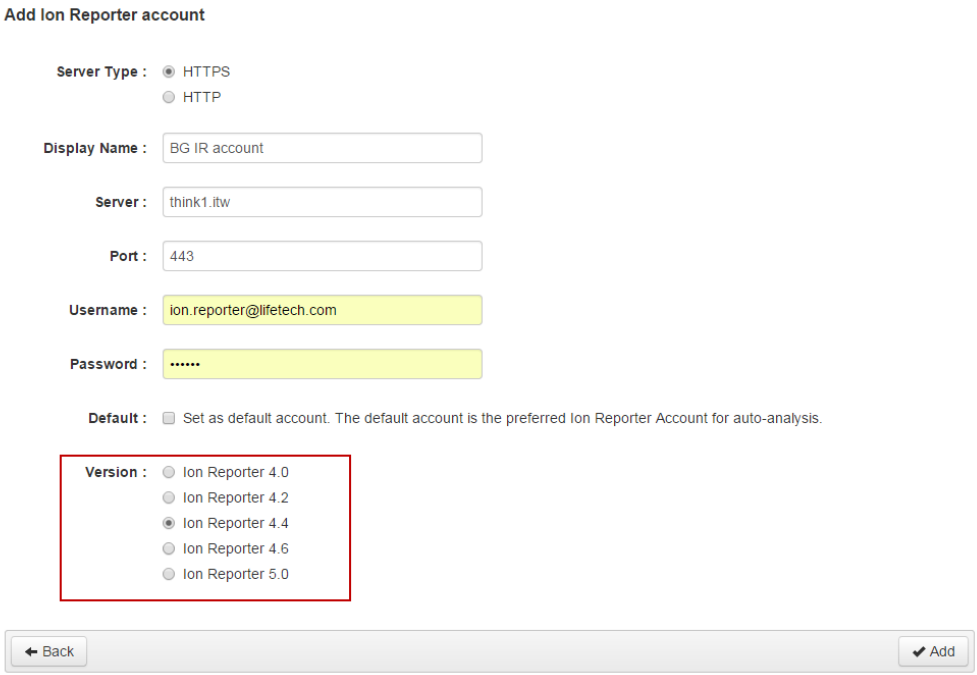
Manage multiple configurations

With the release of Ion Reporter™ Software 4.0, the Uploader plugin can manage multiple configurations. Repeat the process above to add another configuration.

The **Get Versions** button allows you to see all the Ion Reporter™ versions available on the Cloud or your Ion Reporter™ local server.



In the Version section, select the Ion Reporter™ version that you wish to access workflows from.



Notes about multiple Uploader configurations:

- When you use the Uploader plugin in a Torrent Browser run template or manual launch, you select the correct configuration based on the display name that you entered on the plugin configuration Add Ion Reporter Account form.
- Each Torrent Suite™ Software user can only see and use configurations that are created with that Torrent Suite™ Software user login.

Integration with Ion Reporter™ Software

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

In a run plan template wizard, you can set up the IonReporterUploader plugin to automatically transfer the output files to Ion Reporter™ Software, after each completion of your Torrent Suite™ Software analyses.

Command-line version of the Ion Reporter™ Uploader

A UNIX® command-line version of the Uploader is available. The command-line Uploader runs on any standard UNIX® box (and is not limited to Torrent Servers).

Download the command-line Uploader from your Ion Reporter™ Software profile. Instructions for the command-line Uploader available in the command-line version and are included with the Ion Reporter™ Software documentation (which is also downloaded from your Ion Reporter™ Software profile and available in the Ion Reporter™ Software help).

Integration with Ion Reporter™ Software

In your analysis template, you can specify that your Torrent Suite™ Software results files are automatically transferred to your organization in Ion Reporter™ Software. Your results transfer, including sample definition and workflow launch in Ion Reporter™ Software, is handled by the IonReporterUploader plugin.

You include the Uploader plugin settings in the run plan that you use for your sequencing run. When your Torrent Suite™ Software analysis completes, the run plan automatically launches the plugin and begins your file transfer. After the transfer, your Ion Reporter™ Software is automatically launched.

You can run the IonReporterUploader in these three ways to transfer your Ion Reporter™ Software results files to Ion Reporter™ Software:

- **The analysis template wizard** In the wizard you use configure your analysis template to use Ion Reporter™ Software. Then every Torrent Suite™ Software analysis that is based on your analysis template automatically transfers results files to your Ion Reporter™ Software organization and defines Ion Reporter™ Software samples from your newly transferred files. You have the option to configure automatic Ion Reporter™ Software launch on the new samples.
- **On-demand manual plugin launch** A one-time manual launch from a completed run report (with the **Select plugins to run** button) transfers your results files and defines samples in Ion Reporter™ Software. Manual plugin launch does not support automatic Ion Reporter™ Software workflow launch.
- **On-demand one-click transfer to Ion Reporter™ Software** A one-time manual launch from a completed run report (with the **Upload to IR** menu) transfers your results files and defines samples in Ion Reporter™ Software. This transfer does not support automatic Ion Reporter™ Software workflow launch.

Each of these requires that you first configure the IonReporterUploader plugin with your Ion Reporter™ Software login ID and password.

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

What Ion Reporter Software does

Ion Reporter™ Software comprises a suite of bioinformatics tools that streamlines and simplifies the data analysis, annotation, and reporting of Ion Torrent™ semiconductor sequencing data. Designed for both exploratory and production use in performing sequencing assays, Ion Reporter™ Software helps you overcome the bioinformatics barriers to interpret DNA variants faster and more consistently. For researchers needing a simple, reproducible bioinformatics tool that helps ensure consistency in their results, Ion Reporter™ Software is an ideal solution.

Integrate your variants with comprehensive public and curated annotations, along with your own lab-specific content. Ion Reporter™ Software is designed to reduce the time-to-results. Configurable analysis modules and parameters allow customization of workflows to fit your research needs.

All the steps, from data import to annotating variants, are automated in Ion Reporter™ Software workflows. Key features include the following:

- Detection of SNPs, indels, and CNVs in one easy to use workflow
- Automated analysis workflows for 1, 2, or 3 samples
- Hosted and local deployment options to suit your lab's needs
- Simple interface to drive intuitive user experience
- Role-based logins and full auditability so you can tell who did what and when
- Extensive annotations to guide understanding of your sample's variants
- Flexible batch export for integration into laboratory systems and APIs in support of third-party tools for external analysis

Integration with Ion Reporter™ Software

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 Torrent Variant Caller plugin. Use the IonReporterUploader plugin to transfer these output files to Ion Reporter™ Software.

In a run plan template wizard, you can set up the IonReporterUploader plugin to automatically transfer the output files to Ion Reporter™ Software, after each completion of your Torrent Suite™ Software analyses.

Add the Ion Reporter™ Software to a run plan

To set a run plan to automatically transfer files (after the completion of the Torrent Suite™ Software analysis), you define a run plan in the Torrent Browser Plan tab before your sequencing run. Your results files are transferred to Ion Reporter™ Software and also defined as samples in Ion Reporter™ Software, and your selection of Ion Reporter™ Software workflow is automatically launched on your newly-transferred samples.

Manual launch of the Ion Reporter™ Uploader plugin

When you run the Uploader plugin from a completed run report, your results files are transferred to Ion Reporter™ Software and also defined as samples in Ion Reporter™ Software. An Ion Reporter™ Software workflow is not automatically launched.

Email notifications

The Uploader plugin sends two email notifications for each plugin run:

- When the plugin begins 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.

Prerequisites

- There is a one-time configuration of the plugin in both the Ion Reporter™ Software UI and the Torrent Browser, before the plugin can be used.
- Because this one-time plugin configuration requires an authentication token from Ion Reporter™ Software, you (or an administrator) must already have an Ion Reporter™ Software user account).
- The plugin has these requirements:
  - The plugin only works on completed Torrent Suite™ Software analyses that have a run plan with the following:
    - IonReporterUploader selected in the run plan wizard Export chevron,
    - The Ion Reporter™ Software sample name(s), workflow(s), and sample relationships properly defined in the Plan chevron,
    - And, for barcoded runs, the correct barcode kit selected ion the Kits chevron.
  - Manual launches of the plugin (on a completed run report) also depend on the run plan.

Transfer limitations

The following limitations apply to the IonReporterUploader plugin:

- The Uploader plugin transfers results files for a completed run plan that executed on the Torrent Server where the plugin is configured. (In addition, each run must have the IonReporterUploader plugin enabled and configured in its run plan.)
- You cannot copy results file from a different Torrent Server and have the plugin transfer those files.
- Only files appearing in the File Links section of a run report are transferred.
- You cannot add supplemental files to the results files of a run, in order to have the plugin transfer those files.
- For barcoded runs:
  - For automatic runs with barcoded data, the Uploader plugin only transfers samples if the barcode kit selection in the run plan wizard is correct. If you correct or add the barcode kit selection on the sequencing instrument, the Uploader plugin still uses the old run plan information and the results file transfer fails.
  - For manual launches of the plugin on barcoded data, the Uploader plugin uses the barcode kit selection that you make on the sequencing instrument.

Command-line version of the Ion Reporter™ Uploader

A UNIX® command-line version of the Uploader is available. The command-line Uploader runs on any standard UNIX® box (and is not limited to Torrent Servers).

Download the command-line Uploader from your Ion Reporter™ Software profile. Instructions for the command-line Uploader available in the command-line version and are included with the Ion Reporter™ Software documentation (which is also downloaded from your Ion Reporter™ Software profile and available in the Ion Reporter™ Software help).

Torrent Suite™ Software output

Typically the BAM file output of your Torrent Suite™ Software analysis is uploaded to Ion Reporter™ Software and then Ion Reporter™ Software runs through its analysis phases of mapping, variant calling, and 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 (TVC) plugin	Annotation only

The Ion Reporter™ Uploader plugin by default uploads both the BAM file and the VCF file from your Torrent Server to Ion Reporter™ Software.

Ion Reporter™ Software analysis files

Ion Reporter™ Software analysis involves the following major analysis phases:

1. Mapping
2. Variant calling
3. Annotation

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 Torrent Suite™ Software Torrent Variant Caller (TVC) plugin analysis, or a different source.

File Transfer

You can configure the Ion Reporter™ Uploader plugin to transfer your files automatically and you can also transfer files on a completed Torrent Suite™ Software analysis.

Set an Analysis Template to Transfer to Ion Reporter™ Software

Overview

The Ion Reporter Uploader plugin transfer results files directly from a Torrent Suite™ Software analysis to your Ion Reporter™ Software organization (available under separate license).

You include the Uploader plugin settings in the run plan that you use for your sequencing run. When your Torrent Suite™ Software analysis completes, the run plan automatically launches the plugin, begins your file transfer, and eventually defines samples and launch the analysis in Ion Reporter™ Software.

These instructions apply to Ion Reporter™ Software users.

Add Ion Reporter™ Software to your analysis template

To set an analysis to automatically transfer files (after the completion of your Torrent Suite™ Software analysis), use the IonReporter and Plan chevrons in the Torrent Browser analysis template wizard. (The Ion Reporter Uploader does not use the wizard's plugin chevron.)

For barcoded data, also select the correct barcode kit in the Kits chevron. When you select a barcode kit, the Plan chevron creates a sample name field for each barcode.

We recommend that you use the plan-by-sample feature. Plan-by-sample helps fill out the run plan wizard with your sample information.

Here is a summary of what takes place. After your run plan is created, saved, and then started on the sequencing instrument, each step is automated until you review your results in the Ion Reporter™ Software UI:

1. **Analysis template** Make sure that your Torrent Suite™ Software analysis template is ready. For multi-sample analyses in Ion Reporter™ Software, create a multi-sample version of your run template in your Torrent Browser.

2. **Samples and sample set** You define your samples and sample set in the Torrent Browser **Plan ▸ Samples** tab. After you save your sample set, click the **Plan Run** option in the sample sets' gear menu. The Torrent Browser prompts you to select your run template.

3. **Run plan wizard** You follow the prompts to define a run plan based on your run template. In the run plan, you select the Ion Reporter™ Software workflow and barcoding kit (if required). The Torrent Browser fills out the sample information in the wizard, based on your sample set.

4. **Plan code** When you save your plan, it is assigned a plan code.

5. **Sequencing** You enter the plan code on the Ion PGM™ or Ion Proton™ sequencing instrument to begin the sequencing run.

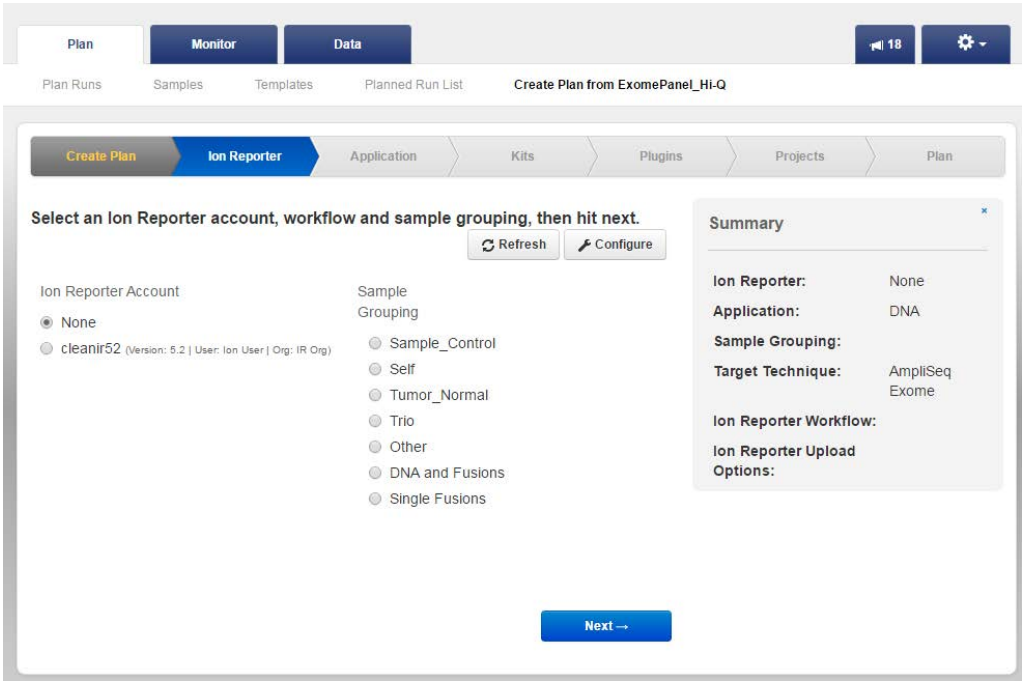
6. **Torrent Suite™ Software analysis** After sequencing, the results are transferred to your Torrent Suite™ Software for analysis.

7. **Ion Reporter™** Software analysis When your Torrent Suite™ Software analysis is complete, your results are transferred to Ion Reporter™ Software:
   a. Your samples are defined in Ion Reporter™ Software.
   b. Your choice of workflow is launched in Ion Reporter™ Software.
   c. Ion Reporter™ Software sends you an email to notify you that your results are ready for review.

8. **Ion Reporter™** Software results You review your results in the Ion Reporter™ Software UI.

The Ion Reporter chevron

Click the Ion Reporter chevron:



Sample Grouping

Sample Grouping corresponds to the sample relationship within Ion Reporter™ Software. When you select a Sample Grouping, the workflow menu displays only workflows that match that number of samples.

Existing Workflow

Use this menu to select your Ion Reporter™ Software analysis type. When you select a workflow from this menu, the Sample Grouping radio buttons show the sample relationship required by that workflow.

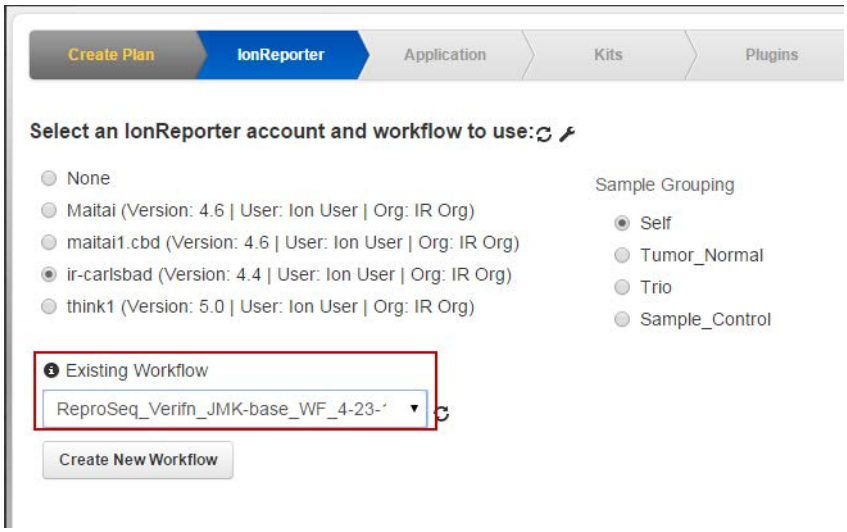
Create New Workflow

Click this button to open Ion Reporter™ Software in a new browser window. In Ion Reporter™ Software, create your new workflow and save it.

When you return to your Torrent Browser, refresh your browser. Then the newly-created workflow appears in the Existing Workflow menu.

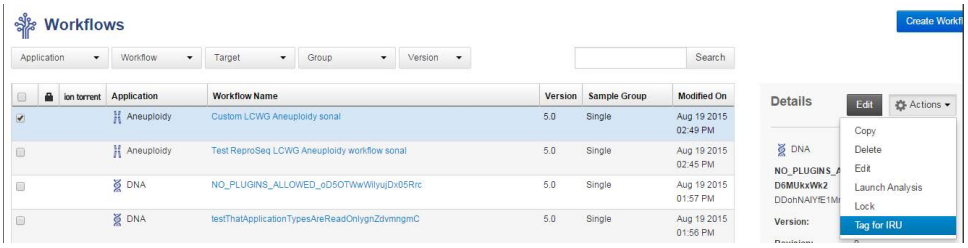
Manage Ion Reporter™ Workflow List

To reduce the number of Ion Reporter™ workflows that appear on the drop-down menu on the Ion Reporter™ chevron during planned run creation in Torrent Suite™ Software.



This is achieved by applying "Tag for IRU" in Ion Reporter™ Software. "IRU" stands for Ion Reporter™ Uploader and it is the plugin that transfers Torrent Suite™ run data to Ion Reporter™ software, but it can also tag the Ion Reporter™ workflows that you want to see in Torrent Suite™ software.

1. Log into Ion Reporter™ software.
2. Go to **Workflows** ▸ **Overview**.
3. Select a workflow, then click **Actions** ▸ **Tag for IRU**. Repeat for each workflow of interest.



Only the 'tagged for IRU' workflows will now show up when planning your runs in Torrent Suite™ Software.

4. To undo, select **Untag for IRU**.

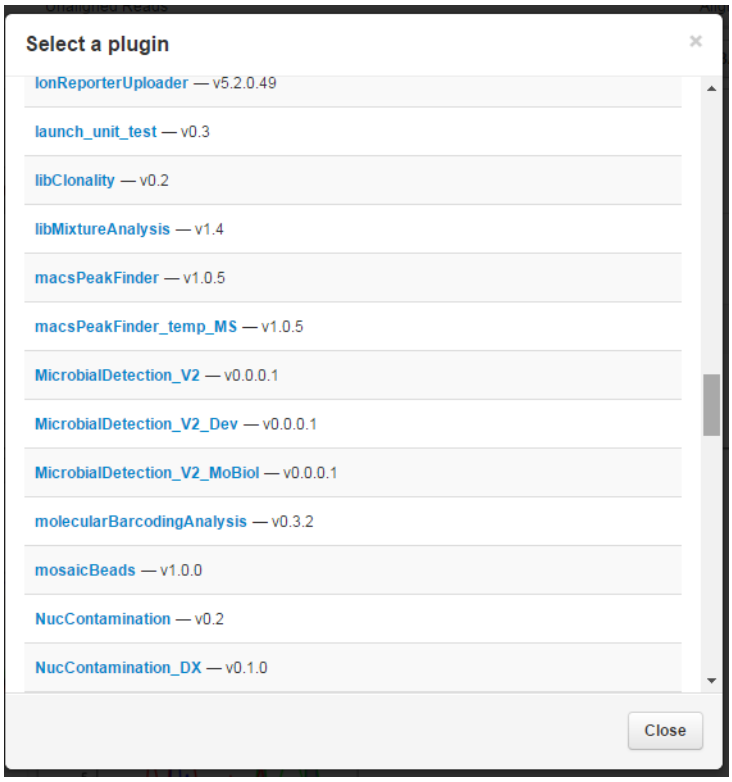
Advanced settings

The Ion Reporter Uploader plugin now allows you to tune upload speeds and set file segment size on the run report.

Tune Ion Reporter™ Uploader speed parameters

New in Ion Reporter™ Uploader v5.2, you can adjust upload speed parameters.

1. In Torrent Suite™ software, go to **Data** ▸ **Completed Runs** and select a run you wish to upload into Ion Reporter™ software.
2. Scroll down to Output files and click **Select Plugins to Run**, then select **IonReporterUploader**.



3. Click **Advanced Settings**.
   a. Set the Number of Parallel Streams to **Default** (recommended optimal speed) or choose **1-5** to slow down upload.

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

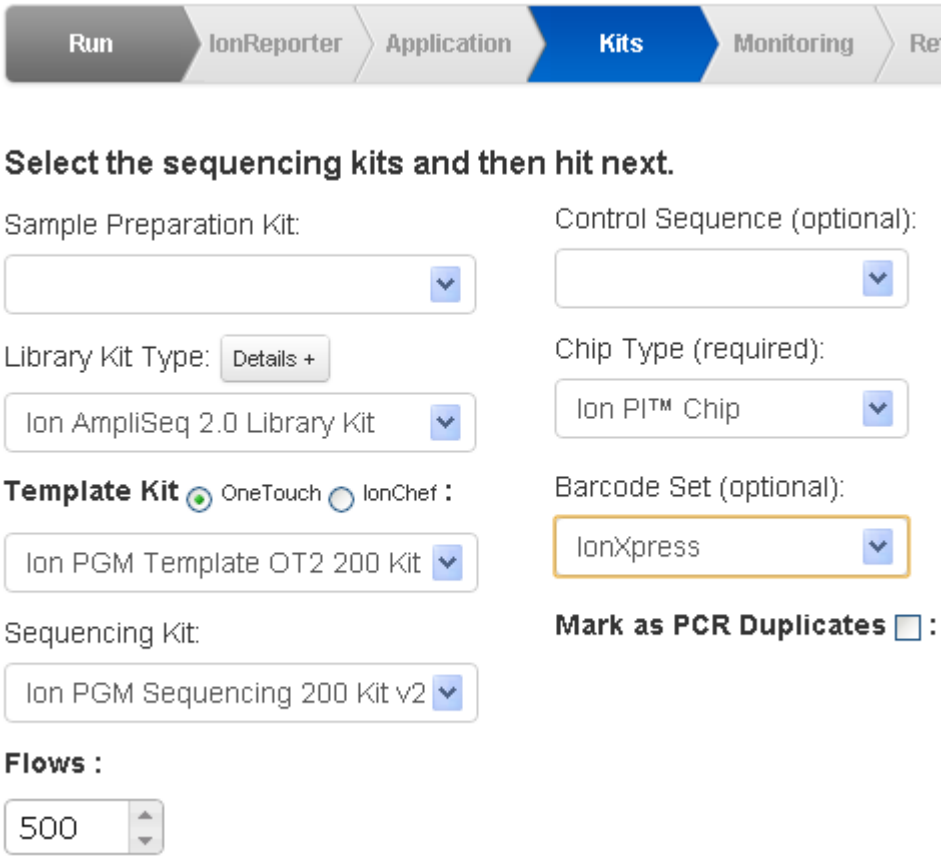
   Note: Only change the settings if you have a slow network and have had trouble with the default settings in the past.

The Kits chevron

The Chip Type is required. If the template contains the chip type, that information is silently entered into the Chip Type field here. Otherwise, select the chip from the Chip Type menu in this chevron.

If your sequencing run uses a barcode kit, select that kit.

Important: If your analysis will use an Ion Reporter™ Software multi-sample workflow, such as a paired or trio workflow, you must use barcoding to sequence your samples together on one chip. Samples that are not sequenced on the same chip cannot be automatically analyzed in Ion Reporter™ Software.



Based on your barcode kit selection, the Plan chevron pre-populates a sample field for each barcode.

Sample gender

Whenever the gender of a sample is known, enter the gender in the plan (the gender column is not shown in the example images). Several workflows, especially copy number variation detection and Genetic Disease Screening (GDS), are limited when gender is not known and also return unexpected results when gender is incorrectly specified for a sample.

For example, with the (GDS) workflow, when the gender of the proband is not known, variants cannot be assigned the categories HasMaleMaternalX and HasUnknownX.

If you transfer a research sample without specifying the gender, follow this step as a workaround: after the files are transferred, go to the Sample > Sample Management screen in Ion Reporter™ Software and edit the GDS sample to specify the gender attribute.

Note: You cannot edit samples that have been launched in an analysis. Instead, define new samples from the raw data files, and add the correct gender metadata to the new samples.

Note about sample gender for Ion Reporter™ Software users:

- IGV assumes that a sample's gender is female, if the sample's gender is not specified or specified as "Unknown".

Save the run plan or template

The title for last chevron of the wizard is different for templates, run plans, and run plans that are planned by sample set:

- **Templates** The chevron title is Save. Here you enter the new template name and optionally mark it as a favorite.
- **Run plans** The chevron title is Plan. Here you enter the new run plan name and sample information.
- **Plan by sample set** The chevron title is Save & Finish.Here you enter the new run plan name. (Sample information is automatically entered into the Barcoding chevron.)

Note: Templates that are marked as favorites are listed in their own section at the top of the Templates tab.

Results files for barcoded sequencer runs

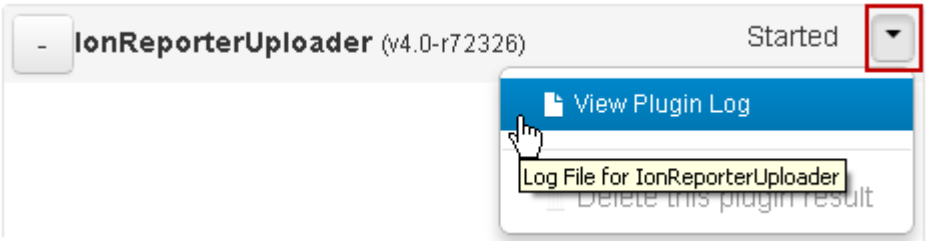
For barcoded runs, all barcoded results files are transferred, except for results files with a file size of zero.

The plugin logs warnings for these files:

- Files with a file size of zero
- Missing files

Note: Results files for unused barcodes are transferred, if the results file size is not zero.rc_Plugin log files

The plugin log files are found on the Torrent Browser Completed Runs & Reports tab Plugin Summary section, in the run report for which the plugin was run. The icon opens the system log for the plugin run. The status arrow opens the Uploader plugin log:

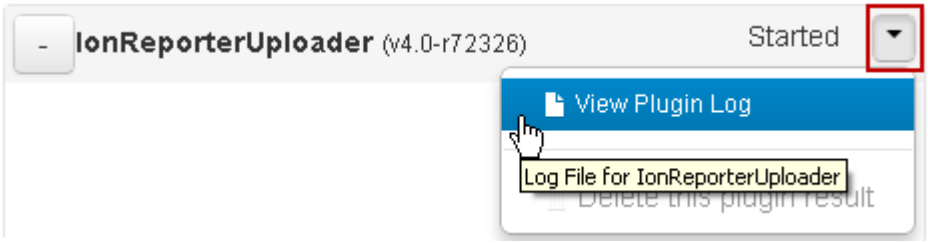


While the transfer is in progress, the plugin has the following message. Click the here link to open the Uploader's progress table:

Upload to Ion Reporter is in progress. Please click here for the upload status.

Plugin log files

The plugin log files are found on the Torrent Browser Completed Runs & Reports tab Plugin Summary section, in the run report for which the plugin was run. The menu next to the plugin status opens the system log for the plugin run:



Manual launch of the IonReporterUploader plugin

Unlike a run that is launched through a planned run, a manual launch of the Uploader plugin does not automatically launch the Ion Reporter™ Software workflow for you. With a manual launch of the Uploader plugin, your Torrent Suite™ Software results files are transferred to Ion Reporter™ Software and also defined as samples in Ion Reporter™ Software.

Display names

The plugin launch uses display names that the Uploader plugin uses to transfer files to the correct version of Ion Reporter™ Software and to the correct Ion Reporter™ Software organization.The display names are defined in the admin Plugin tab, in the IonReporterUploader Configure option.

Files transferred

BAM files are the output of the mainTorrent Suite™ Software analysis pipeline. VCF files are the output of the Torrent Variant Caller plugin. The default action is to upload both BAM and VCF files, if available.

A VCF file is only available if the Torrent Variant Caller plugin already completed for this run report.

Information from the run plan

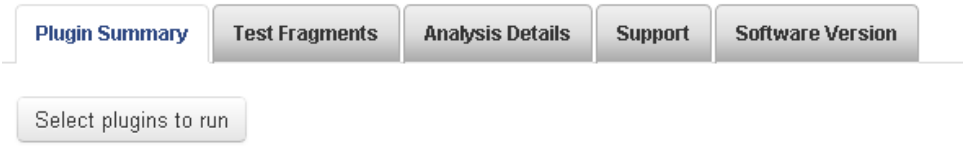
The Uploader's behavior is different if the original Torrent Suite™ Software analysis was executed because of a run plan than if the analysis did not have a run plan:

- **Part of a planned run** The Uploader reads the run plan and transfers files based on the run plan.
- **Not part of a planned run** The Uploader checks for BAM and VCF files and uploads all eligible files.

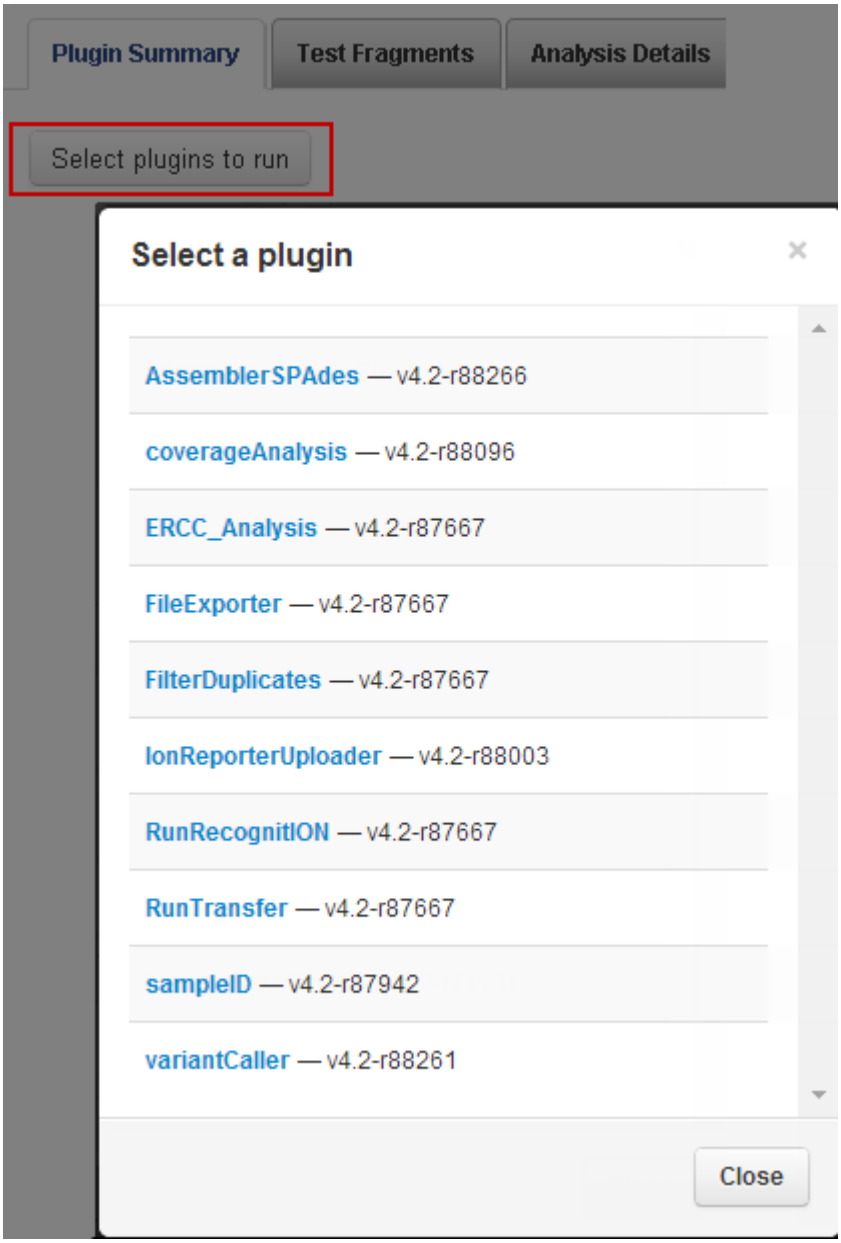
Launch

Follow these steps to manually run the Uploader plugin:

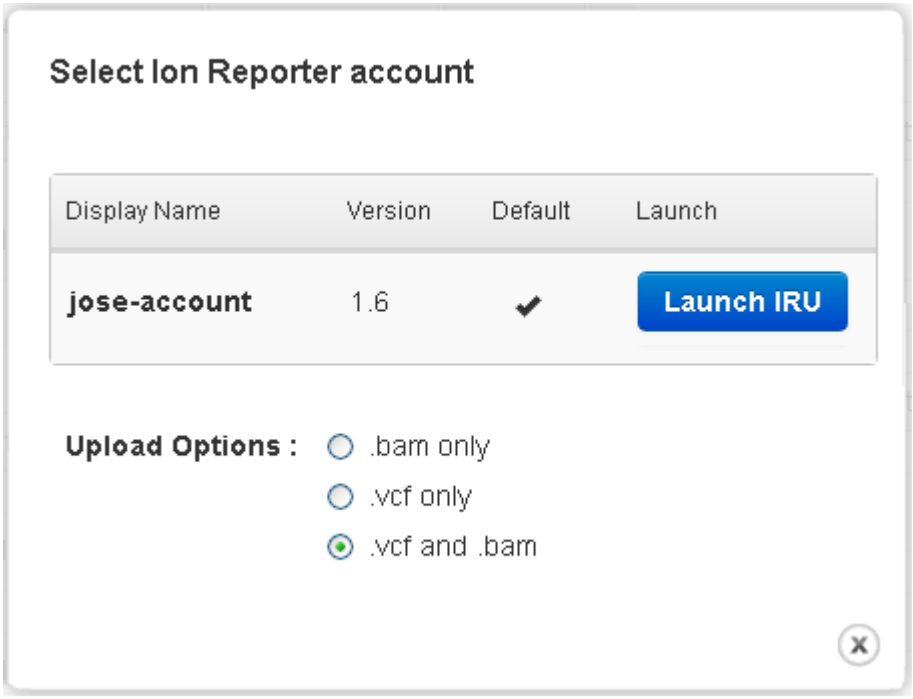
1. Go to the **Data > Completed Runs & Reports** tab, then click the link for your completed analysis run.
2. In the run report, scroll down to Plugin Summary tab.



3. Click **Select plugins to run** button, then click **IonReporterUploader**:



4. The Select Ion Report Account page opens:



5. First select the correct Upload Option for this run:
   a. **BAM files** The output of the main Torrent Suite™ Software analysis pipeline.
   b. **VCF files** The output of the Torrent Variant Caller plugin (if already run and completed for this run report).
6. When multiple Ion Reporter™ Software user account are supported, your selection on this page determines which Ion Reporter™ Software organization receives the transferred files. Click the **Launch IRU** button for the correct set of credentials and to the correct version of Ion Reporter™ Software
7. If you are sure, click **Yes** on the confirmation popup.
8. Please read the submission notice and do not relaunch the Uploader on this run report without allowing time for this Uploader launch to finish transferring its files. Click **OK**.
9. Click the **Refresh Plugin Status** button to monitor the Uploader's progress. The Uploader section is easier to find if you click the **Collapse All** button.

Monitor the upload progress

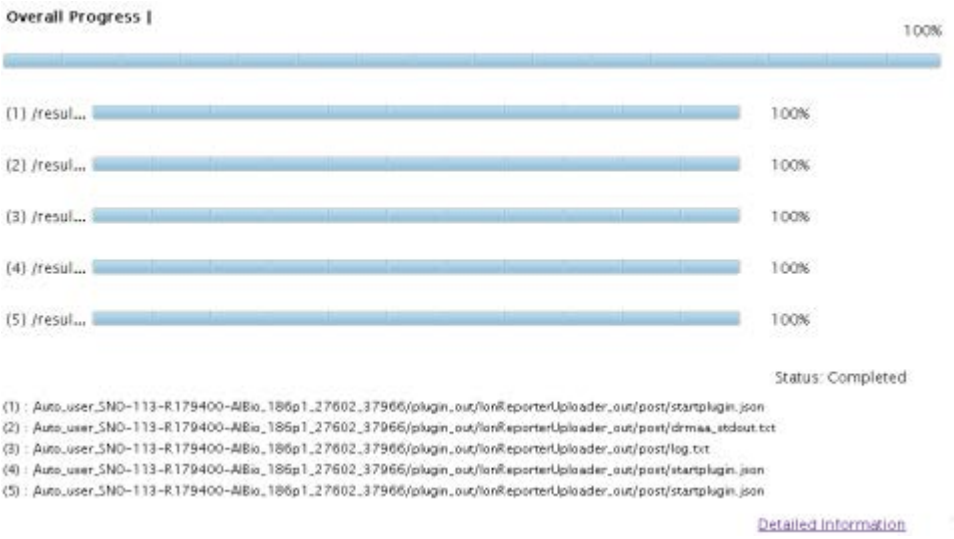
Use the plugin's progress table (in Plugin Summary section for IonReporterUploader) to track the progress of the file transfer. While the transfer is in progress, the plugin has the following message.

Upload to Ion Reporter is in progress. Please click here for the upload status.

Click the **here** link to open the Uploader's progress table in the plugin summary section. The **status.html** link opens the same progress table, but as the only content in a new browser tab. An example progress table is shown below:

The underlined fields are links to additional information. the **progress** link is best for seeing how much of the transfer is complete. the **log** link is the best source for troubleshooting a failure.

- **stdout** The standard output messages for the plugin run.
- **log** Plugin log messages, including errors.
- **input** The input file of plugin parameters and options, in JSON format.
- **progress** Opens a page of progress bars that track the transfer:



Notes about the Uploader plugin

- Use only IonReporterUploader. Other versions of IonReporterUploader plugins are not compatible with Ion Reporter™ Software 1.6.x.
- If IonReporterUploader does not appear in the Select Plugin to Run list, it is either not enabled or not configured on your Torrent Server.
- The plugin log file (in the Torrent Browser Plugin Summary area) contains information about the location the data files are transferred to in Ion Reporter™ Software.
- For Torrent Suite™ Software analyses that do not have the Ion Reporter™ Software configured in a run plan (in the IonReporter chevron), sample names in Ion Reporter™ Software are created using the Torrent Suite™ Software analysis name. With barcoded runs, the barcode name is also added to the sample name.

Double submission warning message

This warning is shown when there is the possibility that an upload of the current results is already in progress:

If the Uploader plugin has Error status, you can safely click **Yes** and retry the upload.

If the Uploader plugin status is Queued or Started, click **No**. A second submission of the plugin on the same run report causes both submissions to fail.

One-Click Transfer to Ion Reporter™ Software

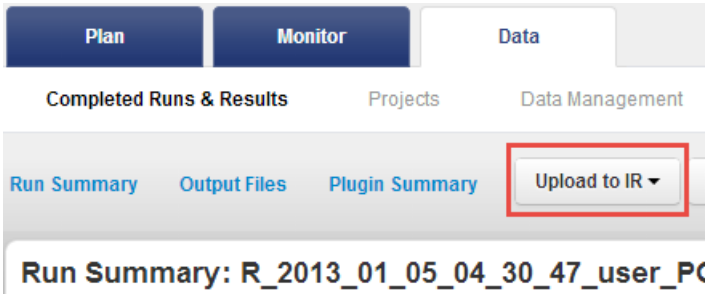
In the report header for a completed analysis, you can use the **Upload to IR** button to transfer the output of that analysis to Ion Reporter™ Software. You choose whether to transfer the BAM or VCF output files, or both file types. This button transfers the data but does not define a sample in Ion Reporter™ Software or launch an analysis in Ion Reporter™ Software.

The Ion Reporter™ Uploader plugin must be configured before you can use the **Upload to IR** button.

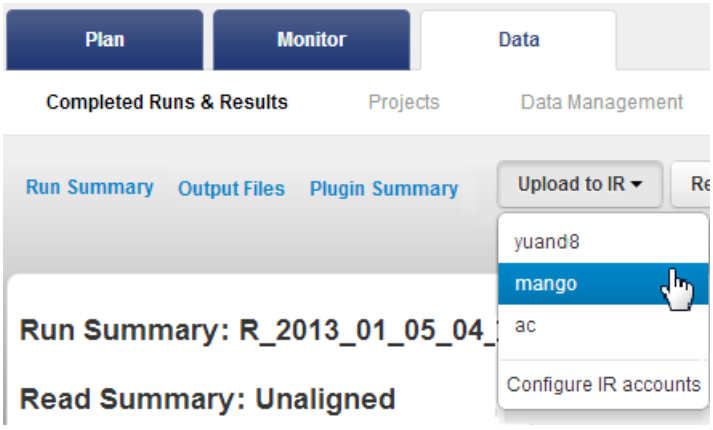
Transfer to Ion Reporter™ Software

Follow these steps to transfer the run report's output files to Ion Reporter™ Software with the **Upload to IR** button:

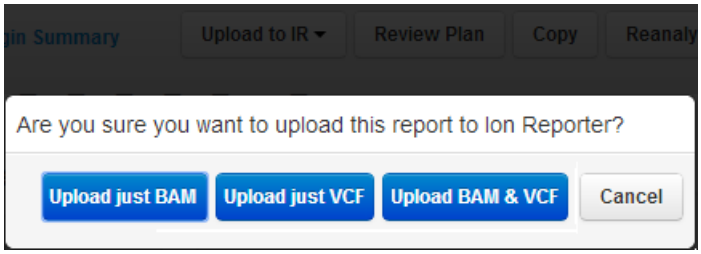
1. Open the run report in the Torrent Browser. The analysis must be complete.
2. Click the **Upload to IR** button in the run report header area:



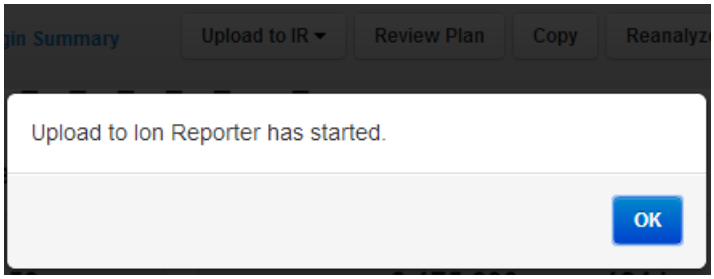
3. Select your IonReporterUploader configuration:



4. In the popup, select which type of output file you want to transfer:

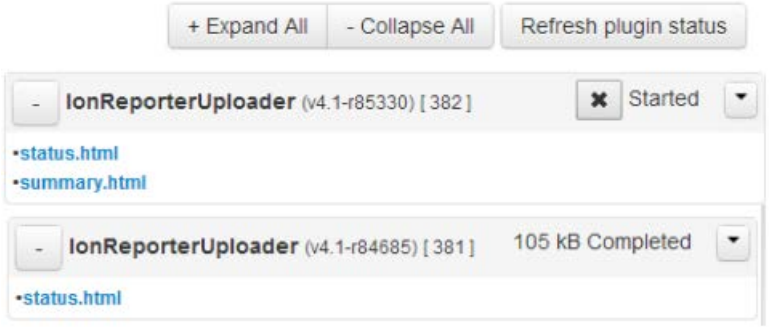


5. You see a message confirming that the transfer has started:

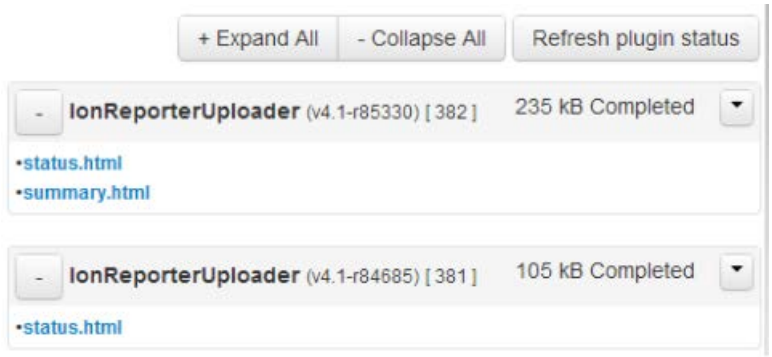


Monitor the transfer

A new IonReporterUploader instance opens in the run report's Plugin Summary area. The summary of any previous IonReporterUploader run is not overwritten.



When transfer is finished, the plugin shows a status of Completed:



Nuances of the Ion Reporter Uploader plugin

- You can configure the Ion Reporter™ Uploader plugin to transfer your files automatically (in your run plan template).
- You can also transfer files on a completed Torrent Suite™ Software analysis, by launching the plugin on a completed run report.
- The Ion Reporter™ Uploader plugin defines samples in Ion Reporter™ Software for your newly- transferred files. The plugin also defines sample relationships for paired and trio samples and defines sample attributes.
- You can configure the Ion Reporter™ Uploader plugin to automatically start an Ion Reporter™ Software analysis after your files are transferred and samples are defined.

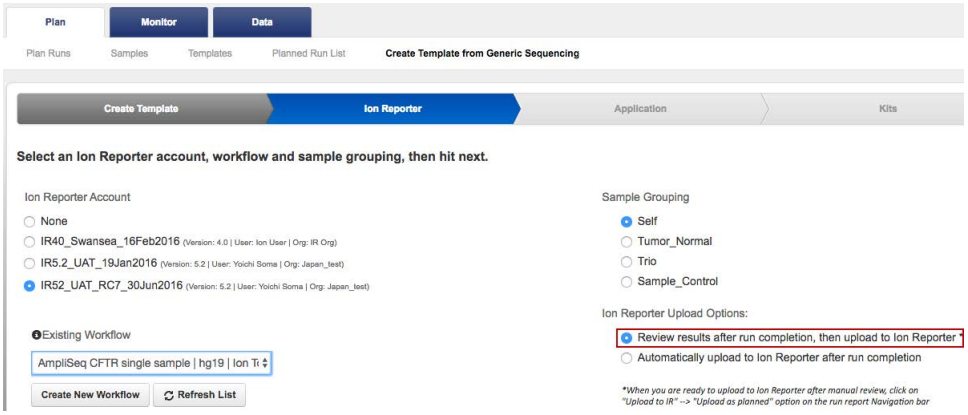
A manual launch of the IonReporterUploader plugin in a run report's Plugin Summary area supports defining the sample in Ion Reporter™ Software and launching anIon Reporter™ Software analysis on your newly-defined sample.

Review Torrent Suite™ run results before auto upload to Ion Reporter™ software

New in Torrent Suite™ software v5.2, you can review run results and decide whether or not to upload them to Ion Reporter™ software for further analysis. You can set this quality check pause when setting up your run plan template or when editing a planned run.

Set Run Plan template for QC pause

1. Go to **Plan** ▸ **Templates** and click template of interest.
2. In the Ion Reporter tab, select your Ion Reporter server or cloud account.
3. Select an existing workflow and then select **Review results after run completion, then upload to Ion Reporter**.

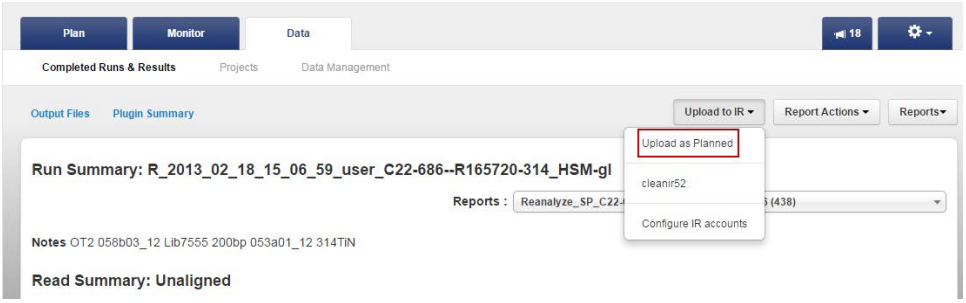


4. Make any other required changes, name your template, and click **Save** and **Finish**.
5. Go back to **Plan** ▸ **Templates**, locate your new template, click the gear button in its row and select **Plan Run**.

6. Execute the run on the sequencer.

   After run is completed, the Plugin Summary reports the IonReporterUploader status as completed. In order to proceed with the IRU upload or auto-launch, you must do the next step.

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



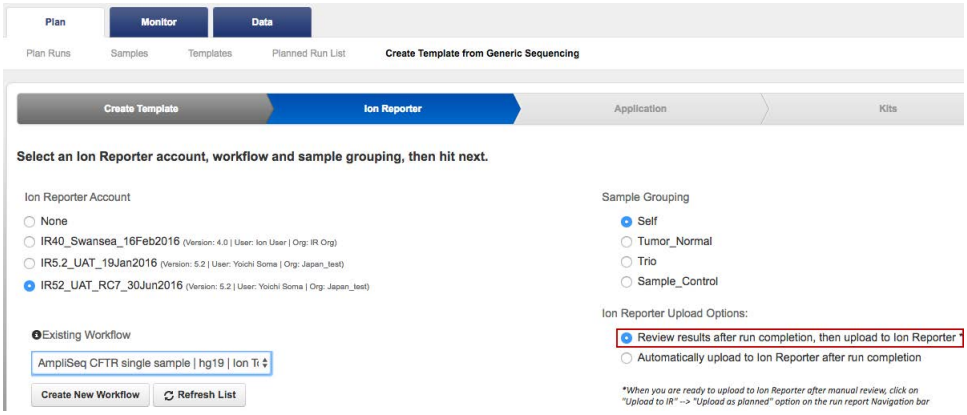
   A confirmation window appears.

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

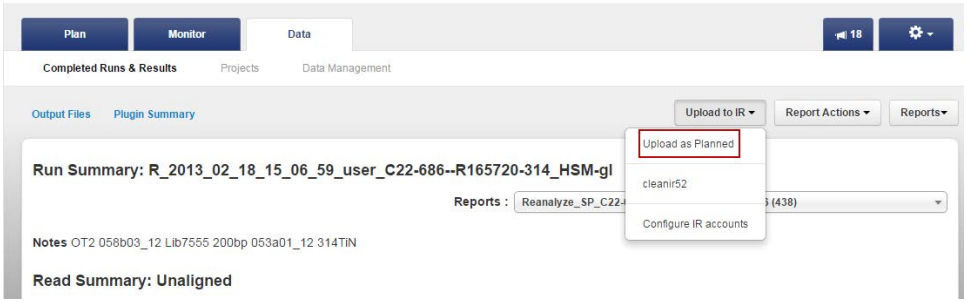
8. Review the Ion Reporter Uploader results in the status.html or summary.html files.

Edit Run Plan for QC pause

1. In the **Data** ▸ **Completed Runs & Results** page, select your run and review the results.
2. Click **Report Actions** ▸ **Edit Run Plan**.
3. In the Ion Reporter screen, select your Ion Reporter server or cloud account.
4. Select an existing workflow and then select **Review results after run completion, then upload to Ion Reporter**.



5. Make any other necessary changes and click **Update Run & Reanalyze**.
6. Review the Run Results. If results are acceptable, click **Upload to IR** ▸ **Upload as Planned**.



A confirmation window appears.

- Select **Yes** to upload as planned.
- Select **No** to cancel the Ion Reporter workflow upload or workflow auto launch.

- Select **Review-Plan** to look at the run results.

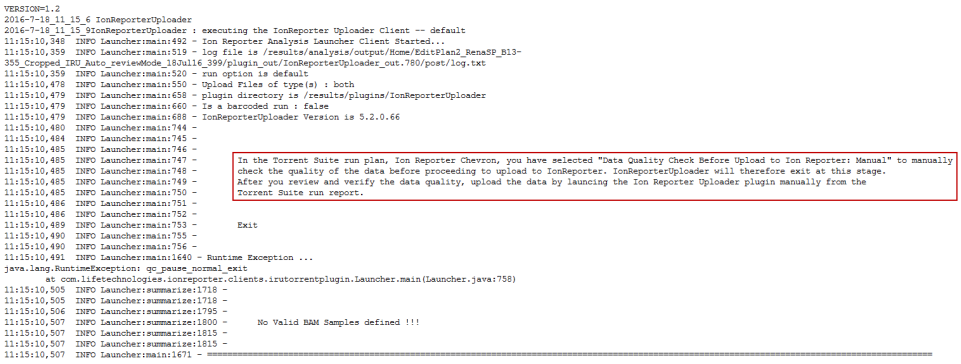
7. Review the Ion Reporter Uploader results in the status.html or summary.html files.

Check IRU status in log file

If there is no status.html file, you can check the status of the IonReporterUploader in the log file:

1. After the report is complete, click H+ to launch Metal.

2. Go to `(your server name)/report/399/metal/plugin_out/IonReporterUploader_out.(plugin number)/post/log.txt` to see the status:



```
VERSION=1.2
2016-7-18_11_15_6 IonReporterUploader
2016-7-18_11_15_9IonReporterUploader : executing the IonReporter Uploader Client -- default
11:15:10,348  INFO Launcher:main:492 - Ion Reporter Analysis Launcher Client Started...
11:15:10,359  INFO Launcher:main:519 - log file is /results/analysis/output/Home/EditPlan2_RenaSP_B13-
355_Cropped_IRU_Auto_reviewMode_18Jul16_399/plugin_out/IonReporterUploader_out.780/post/log.txt
11:15:10,359  INFO Launcher:main:520 - run option is default
11:15:10,478  INFO Launcher:main:550 - Upload Files of type(s) : both
11:15:10,479  INFO Launcher:main:658 - plugin directory is /results/plugins/IonReporterUploader
11:15:10,479  INFO Launcher:main:660 - Is a barcoded run : false
11:15:10,479  INFO Launcher:main:688 - IonReporterUploader Version is 5.2.0.66
11:15:10,480  INFO Launcher:main:744 -
11:15:10,484  INFO Launcher:main:745 -
11:15:10,485  INFO Launcher:main:746 -
11:15:10,485  INFO Launcher:main:747 -
11:15:10,485  INFO Launcher:main:748 -
11:15:10,485  INFO Launcher:main:749 -
11:15:10,485  INFO Launcher:main:750 -
11:15:10,486  INFO Launcher:main:751 -
11:15:10,486  INFO Launcher:main:752 -
11:15:10,489  INFO Launcher:main:753 -
11:15:10,490  INFO Launcher:main:755 -
11:15:10,490  INFO Launcher:main:756 -
11:15:10,491  INFO Launcher:main:1640 - Runtime Exception ...
java.lang.RuntimeException: qc_pause_normal_exit
        at com.lifetechnologies.ionreporter.clients.irutorrentplugin.Launcher.main(Launcher.java:758)
11:15:10,505  INFO Launcher:summarize:1718 -
11:15:10,505  INFO Launcher:summarize:1718 -
11:15:10,506  INFO Launcher:summarize:1795 -
11:15:10,507  INFO Launcher:summarize:1800 -       No Valid BAM Samples defined !!!
11:15:10,507  INFO Launcher:summarize:1815 -
11:15:10,507  INFO Launcher:summarize:1815 -
11:15:10,507  INFO Launcher:main:1671 - ================================================================================================================================================
```

In the Torrent Suite run plan, Ion Reporter Chevron, you have selected "Data Quality Check Before Upload to Ion Reporter: Manual" to manually check the quality of the data before proceeding to upload to IonReporter. IonReporterUploader will therefore exit at this stage. After you review and verify the data quality, upload the data by launcing the Ion Reporter Uploader plugin manually from the Torrent Suite run report.

Exit

Check the Progress of your File Transfer

Email notifications

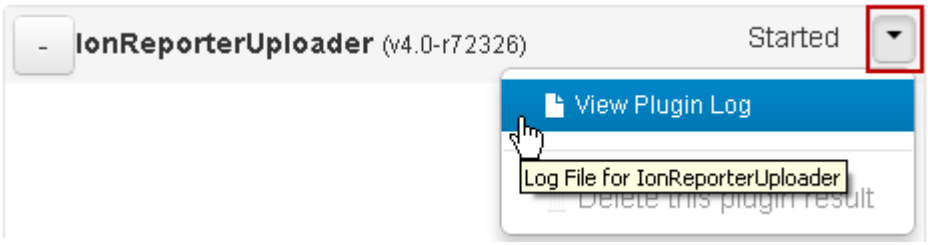
The Uploader plugin sends two email notifications for each plugin run:

- When the plugin begins 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.

Plugin log files

The plugin log files are found on the Torrent Browser Completed Runs & Reports tab Plugin Summary section, in the run report for which the plugin was run. The menu next to the plugin status opens the system log for the plugin run:



Plugin progress table

The plugin progress table is in Plugin Summary section for IonReporterUploader and is useful to monitor the transfer of large files. While the transfer is in progress, the plugin has the following message.

Upload to Ion Reporter is in progress. Please click here for the upload status.

Click the **here** link to open the Uploader's progress table in the plugin summary section. An example progress table (empty):

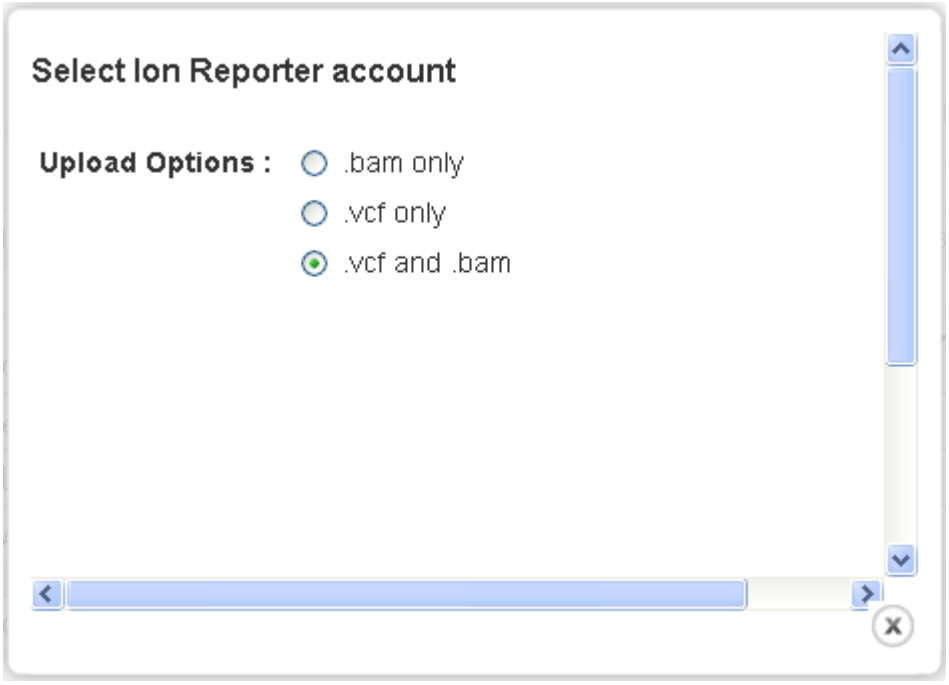
The status.html link opens the same progress table, but as the only content in the browser tab.

When the IonReporterUploader Plugin is Not Configured

This pages shows how various IonReporterUploader-related pages appear when your IonReporterUploader plugin is not yet configured to transfer to your Ion Reporter™ Software organization.

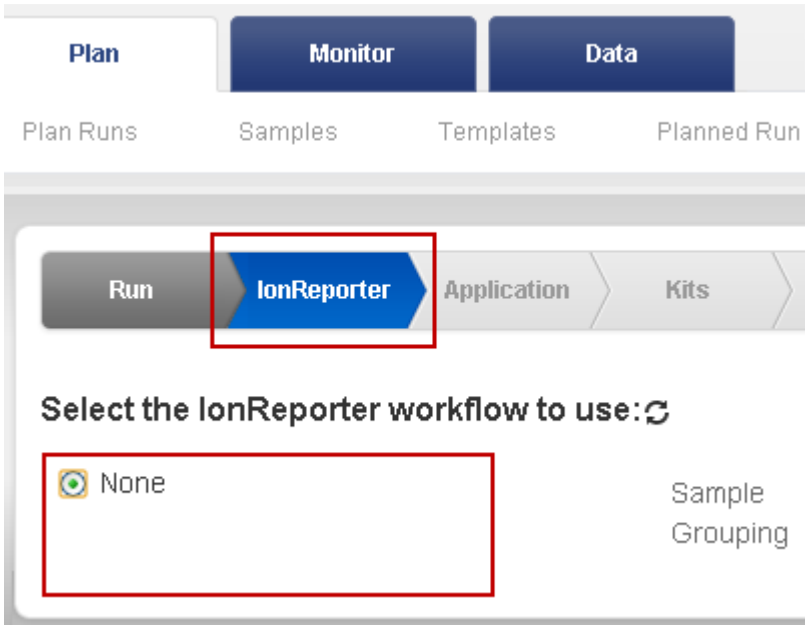
In the plugin manual launch page

Before the plugin is configured, if you launch the IonReporterUploader plugin on a completed run report (with the **Select plugins to run** button), the following page opens with an empty "Select Ion Reporter™ account" area. When the IonReporterUploader plugin is properly configured, theIon Reporter™ Software account or accounts appear in this area. (Multiple accounts are not supported when transferring to Ion Reporter™ Software1.6.x.)



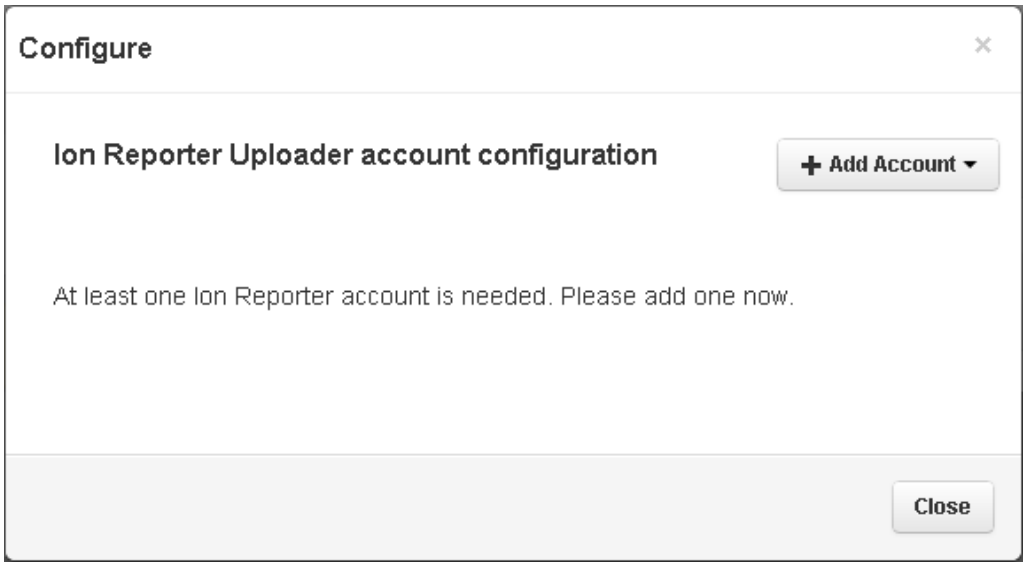
In the run plan template wizard

Before the plugin is configured, if you click the Ion Reporter™ chevron in the run plan template wizard, the Ion Reporter™ Software workflow selection menu does not appear. Also, IonReporterUploader credential choices do not appear (these normally are listed below the None radio button.



In the plugin configuration page

When the plugin is not configured, the admin **Plugins** configuration page contains this:



Click the **Add Account** button to begin the configuration.

IonReporterUploader Plugin Usage Page

The Uploader plugin Usage page lists the run reports on which the plugin has been run, the Uploader's completion status, and the sizes of the plugin output folders. You can check for errors and delete large plugin output folders.

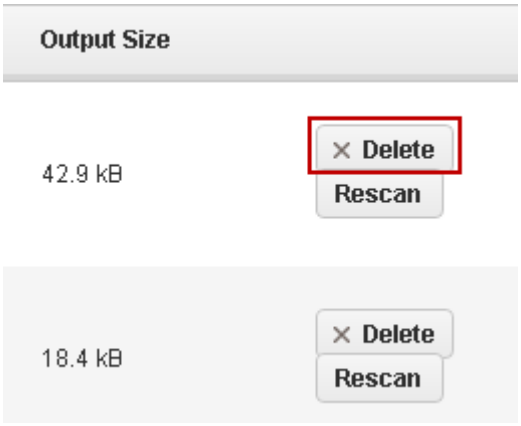
Open the Uploader plugin Usage page

Open the Usage page from the plugin table in the Services tab. Click the Usage option in the gear menu for the IonReporterUploader entry:

Delete plugin report files

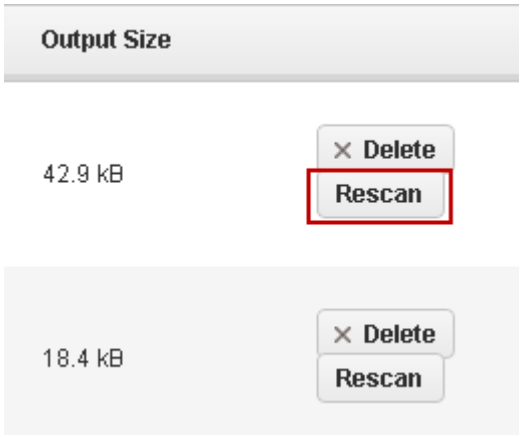
The **Delete** button permanently deletes the Uploader plugin report for a run. This action is the same as the trash can icon on the run report page plugin summary.

Use the **Delete** button to permanently delete the plugin output files for a specific Uploader run:



Rescan plugin output files for a specific run

Click the **Rescan** button to recalculate the size of plugin's output files for a specific run:



Configure Your IonReporterUploader Plugin Access Credentials

Overview

This page describes how to configure your Torrent Browser to transfer files to your organization on Ion Reporter™ Software. (Beginning with the Ion Reporter™ Software 4.0 release, you configure the IonReporterUploader plugin with yourIon Reporter™ Software login ID and password.)

Multiple configurations

The IonReporterUploader plugin now supports multiple configurations, each with a different login and different display name (that you assign).With these multiple configurations, the IonReporterUploader plugin supports the transfer of results from one Torrent Server to different Ion Reporter™ Software organizations, without the need to change the plugin configuration.

When multiple users (with different Torrent Suite™ Software logins) configure the IonReporterUploader plugin, each user can only see (and use) the IonReporterUploader plugin configuration that they created themselves. When multiple users share one Torrent Suite™ Software login, they can see and use all IonReporterUploader plugin configurations created with that Torrent Suite™ Software account.

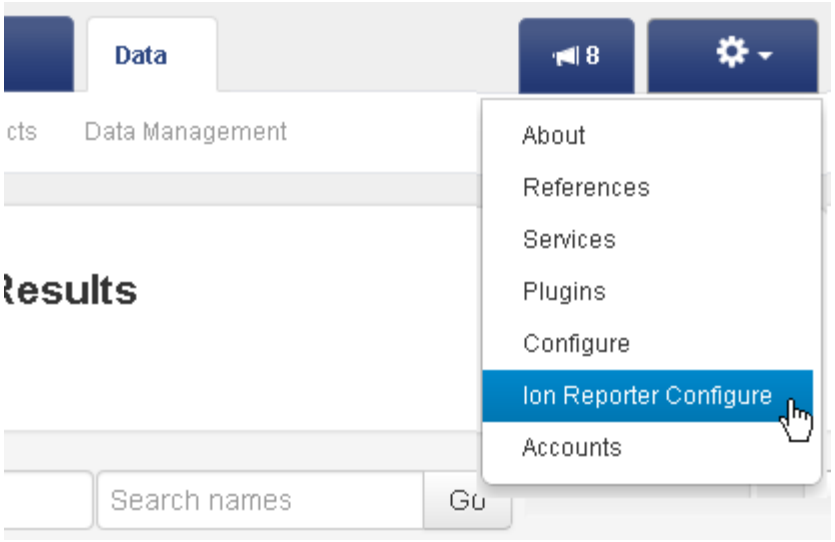
Configure the Uploader plugin in the Torrent Browser

This sections describes the last step in configuring the plugin. You configure the plugin in the Torrent Browser with the Ion Reporter™ Software authentication token.

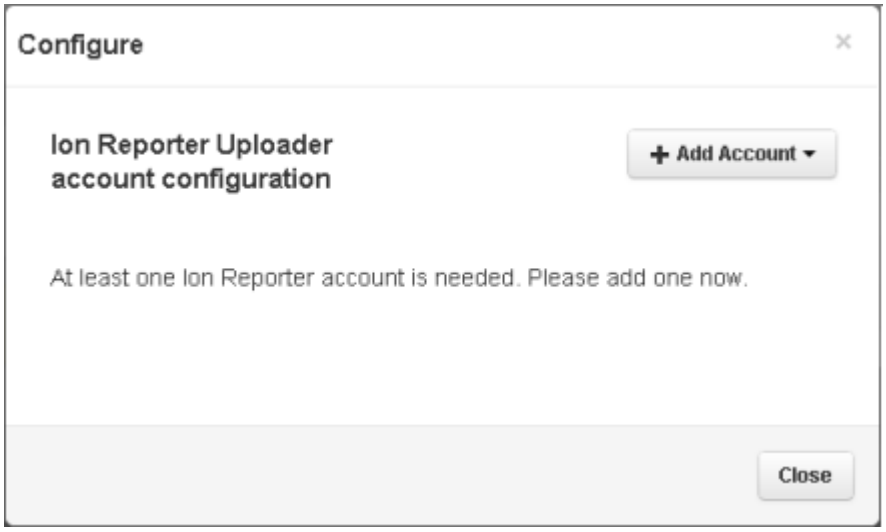
IMPORTANT! See the Ion Reporter™ Software documentation for the latest configuration information. Both Ion Reporter™ Software and the Uploader plugin may be updated on a different schedule than Torrent Suite™ Software (and these documentation pages).

Follow these steps to configure your Ion Reporter™ Uploader plugin in your Torrent Browser:

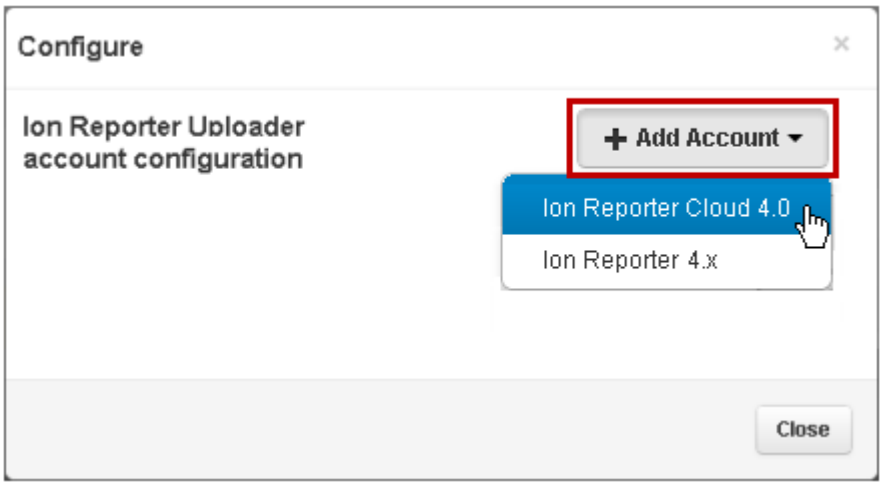
1. Log into the Torrent Browser (as either an `ionadmin` or a regular user).
2. Click the Admin gear menu (in the upper right) and select **Ion Reporter™ Configure**:



3. The Ion Reporter™ Uploader Configuration page opens. If the plugin is not configured yet, this page opens:



4. Click the **+ Add Account** menu. For the hosted cloud Ion Reporter™ Software solution, click **Ion Reporter™ Cloud 4.0**:



   For your own Ion Reporter™ Server System, click **Ion Reporter™ 4.x**.

5. In the Configure popup, make these entries the hosted cloudIon Reporter™ Software solution:

Field	Directions
Server Type	Enable HTTPS.
Display Name	Enter a meaningful name of your choice. This name is used in the run plan template wizard and is seen by other Torrent Browser users. Use only alphanumeric characters, spaces, and underscores.
Server	Enter: 40.dataloader.ionreporter.iontorrent.com
Port	Enter: 443
Username	Enter your Ion Reporter™ Software login (your email address)
Password	Enter your Ion Reporter™ Software login password

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

6. The "Default Account" is the account that is configured by default in run templates and run plans. If this is the main account to be used for file transfers, enable the Default Account checkbox. (You can always change this selection in the run plan template wizard and in the Upload to IR quick link.)

7. Click the **Add** button in the bottom right.

As soon as at least one account is successfully configured, the Uploader plugin is ready to transfer files and also launch Ion Reporter™ Software analyses. If you configure more than one account, you have a choice of accounts to use for the transfers, in the run plan template wizard, the Uploader plugin manual launch page, and the Upload to IR quick link. Each Torrent Browser user can only see the configurations they created, but if several people share a Torrent Browser login account, those people can see and potentially use each others configurations.

Configuration errors

The more common causes of a configuration error are:

- HTTP is selected instead of HTTPS.
- The server name is incorrect.
- The port number is incorrect.
- The login or password is incorrect or deactivated.
- There are spaces before or after the server name, port number, username, or password.

Reference Management

GRCh38 human reference

New in Torrent Suite™ Software v5.2, you can begin 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 and is the first major update since 2009. Highlights include: changes to chromosome coordinates, fixed errors in the former sequence, addition of Mitochondria, and multiple loci for some highly variable genes.

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

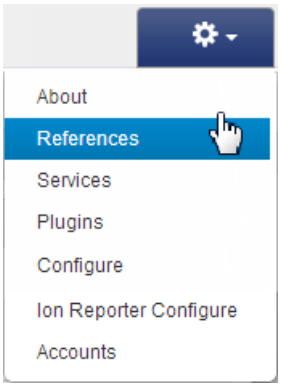
AmpliSeq™ Designer includes many preloaded reference genomes, including:

- 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)

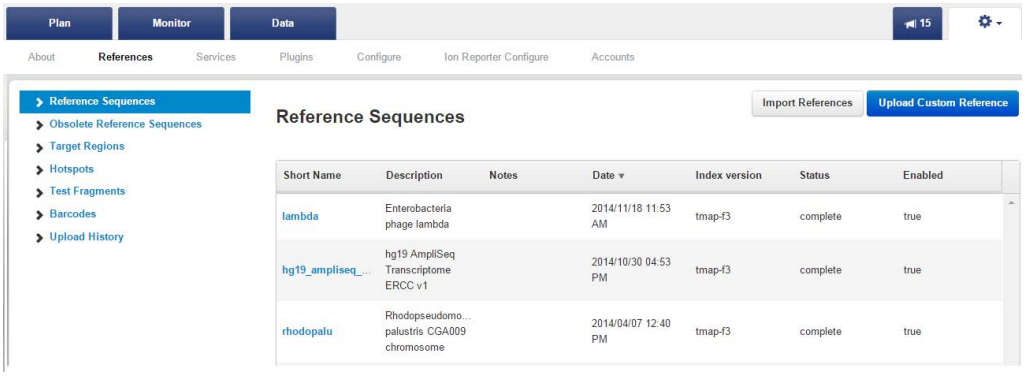
References Management Guide

Use the Admin **References** tab to enter nucleotide sequence **Test Fragments**, **Reference Genomes** for aligning reads, and **DNA Barcodes** for barcode set management.

Access the References tab from the Admin gear menu (near the top right of a Torrent Browser page), with the References option:



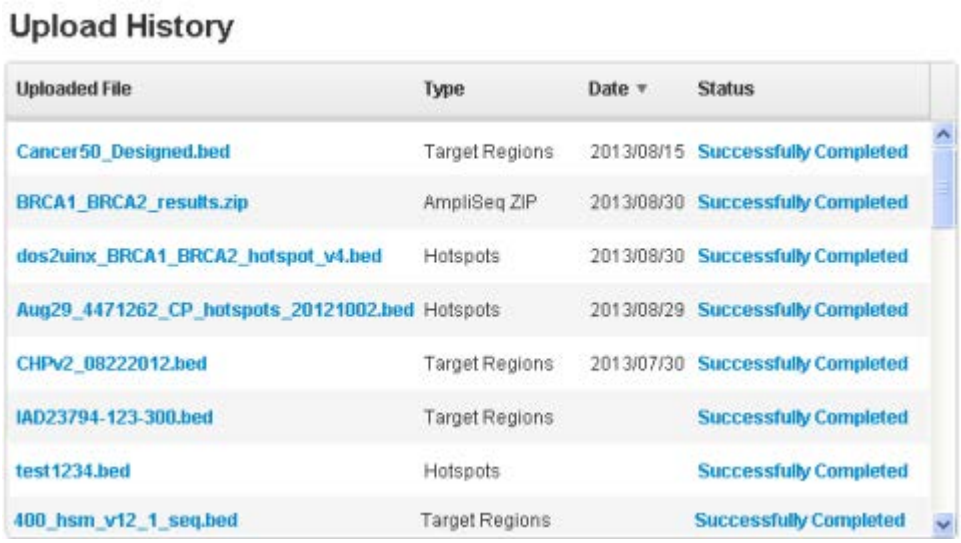
The main reference management page opens:



In this page you can click into 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 (shown above).
- 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.

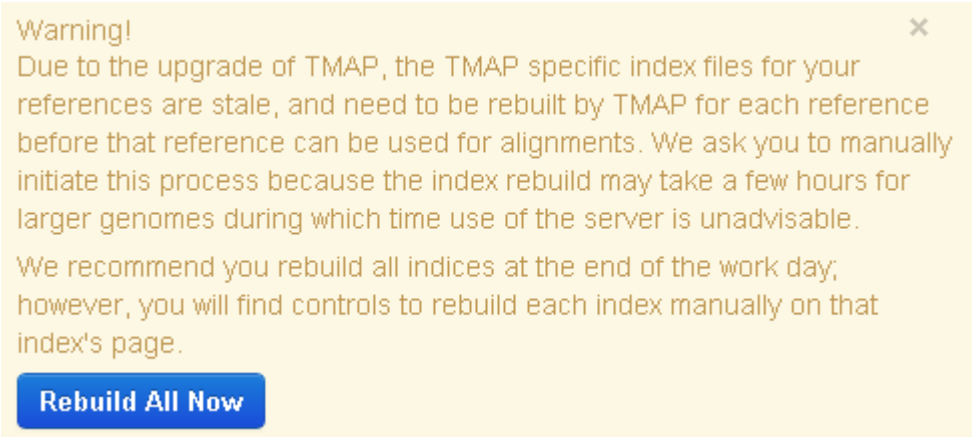
- Upload History. Shows the recent uploads of target regions, hotspots, and ampliseq.com zip files:



  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, TVC 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.

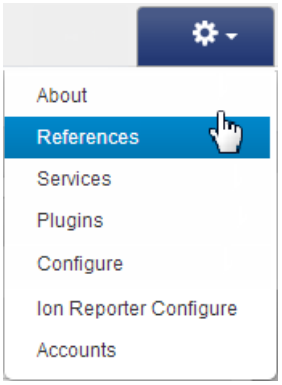
- Prepare a descriptive name for the genome.
- Prepare the short name for the genome.
- Prepare a version for the genome.
- Know the number of reads to randomly sample for alignment.
- Prepare a regions of interest file or hotspots file (on your client machine).

  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/**.

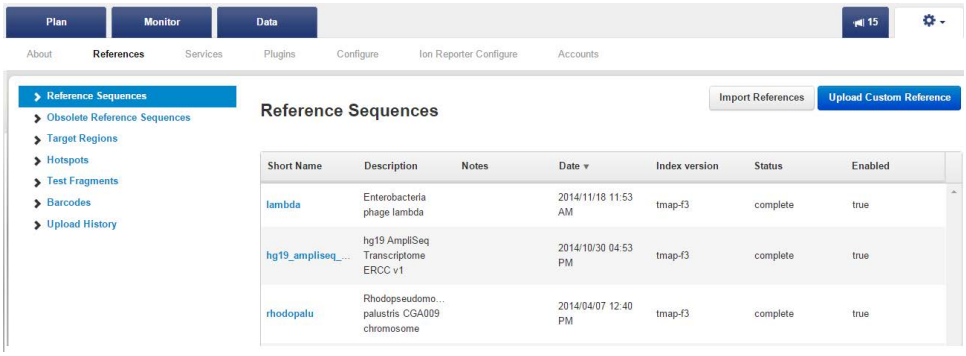
Upload the reference

You upload reference files with the Admin gear menu References tab:

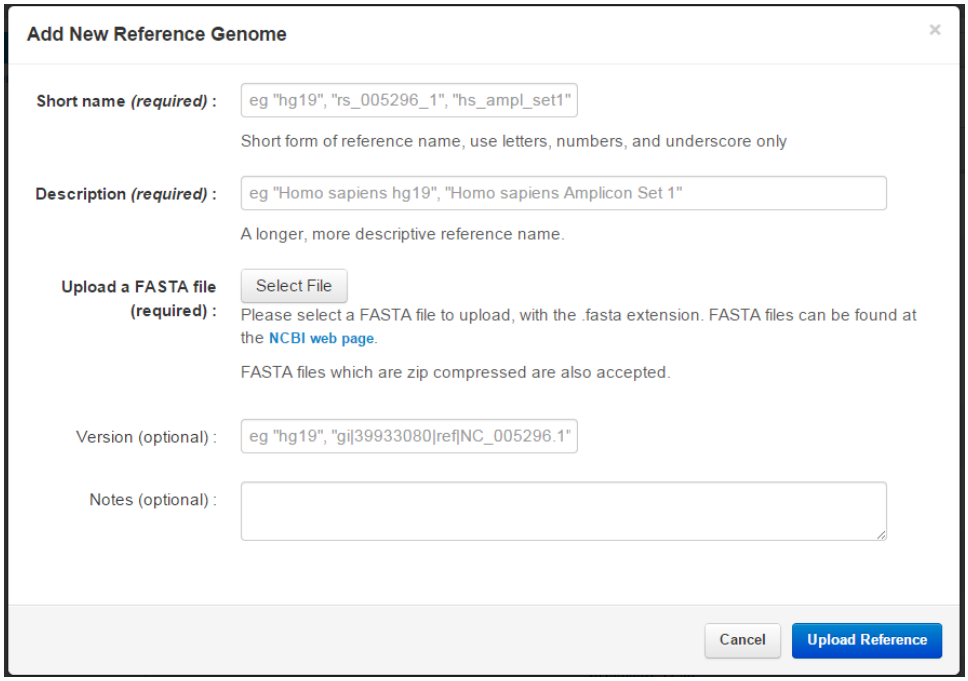


Follow these steps to upload a reference genome:

1. On the **Admin** ▸ **References** tab, in the References Sequences section, click the **Upload a Custom Reference** button:



2. Fill out the Add New Reference Genome page. Required fields are noted on the page.



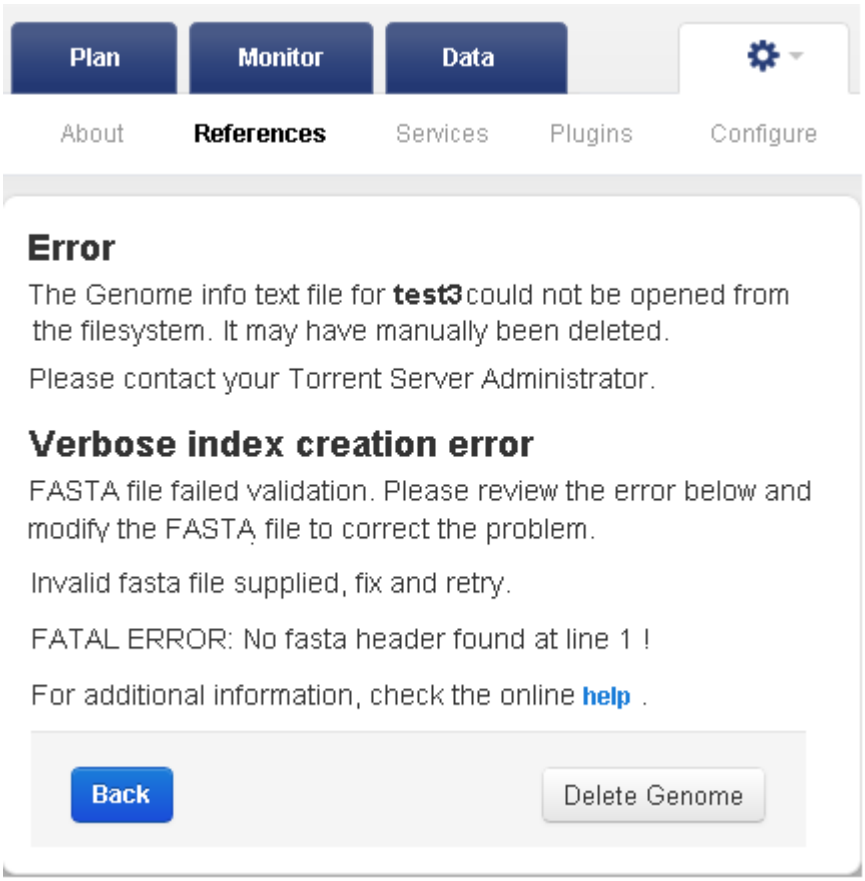
Field	Description
Descriptive name	[required] This entry may be any text string. The description usually includes the genus-species, version, and other identifying information. The description entered here is displayed in various report output, and is listed in the Reference Sequences section of the Admin > **References** tab.
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.
Genome 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

3. Click the **Select File** button and browse to the genome file (on your local machine).
4. Click the **Upload file and create reference** button.
5. Wait while the genome is uploaded.

   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.

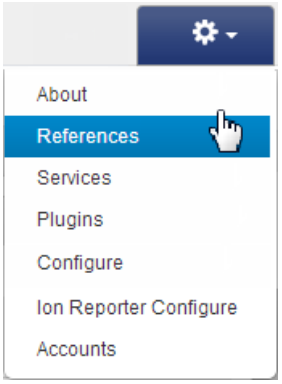
Target Regions Files and Hotspot Files

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)

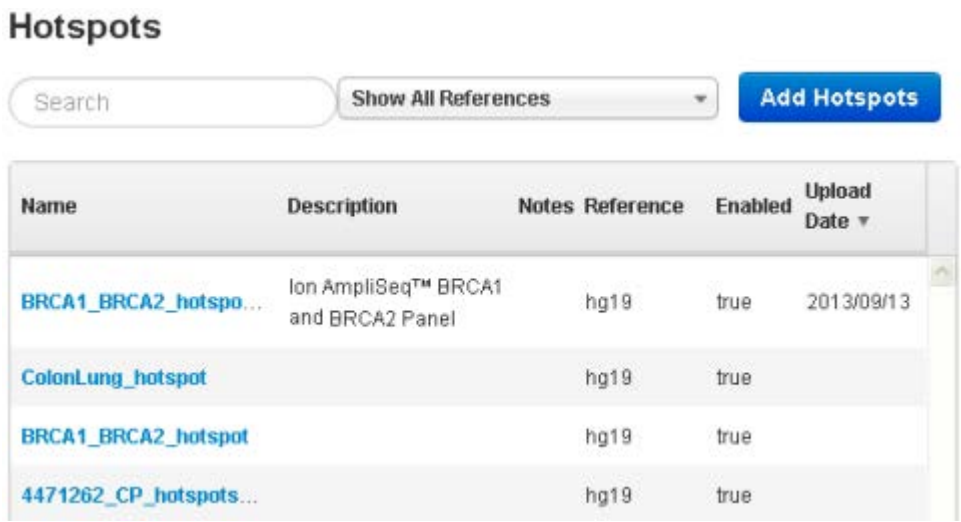
Target regions files and hotspot files are listed in the admin References tab. These files are uploaded to a specific reference and available for use only when that reference is used for an analysis.

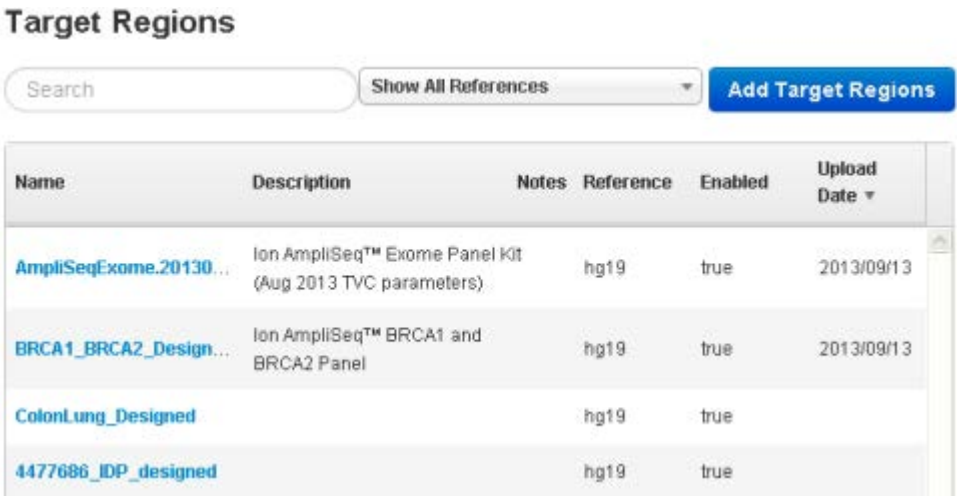
To view the target regions files and hotspot files on your system, click the admin gear menu **References** option:



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:





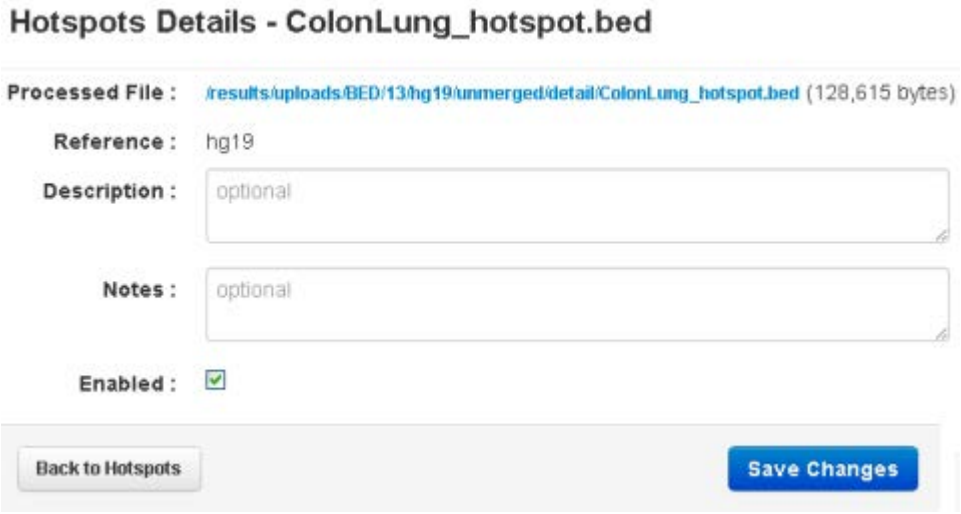
Both Hotspots andTarget Regions pages offer the following actions:

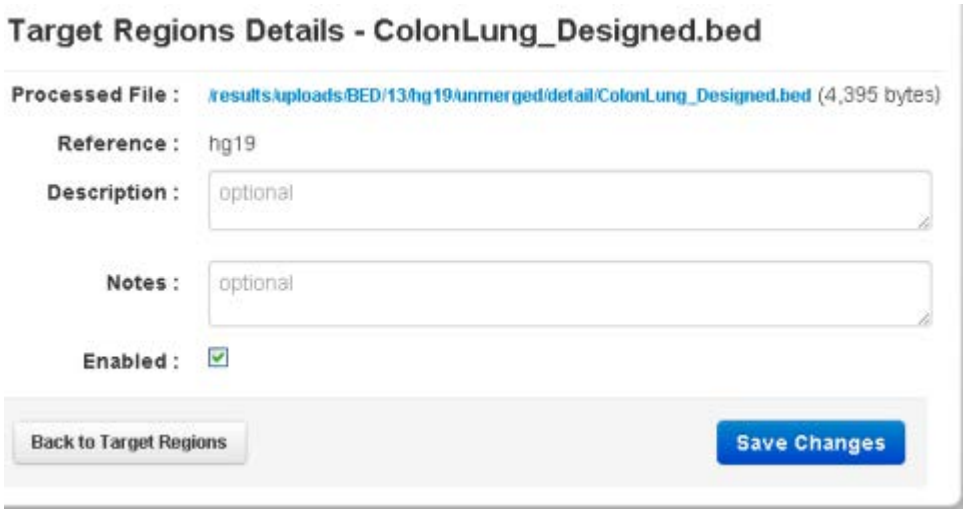
- Click the file name to open its details page.
- Use the references selection menu (default Show all References) to display only files of one reference.
- Click the **Add Hotspot** or **Add Target Regions** button to upload a new file (to associate with any reference).

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):





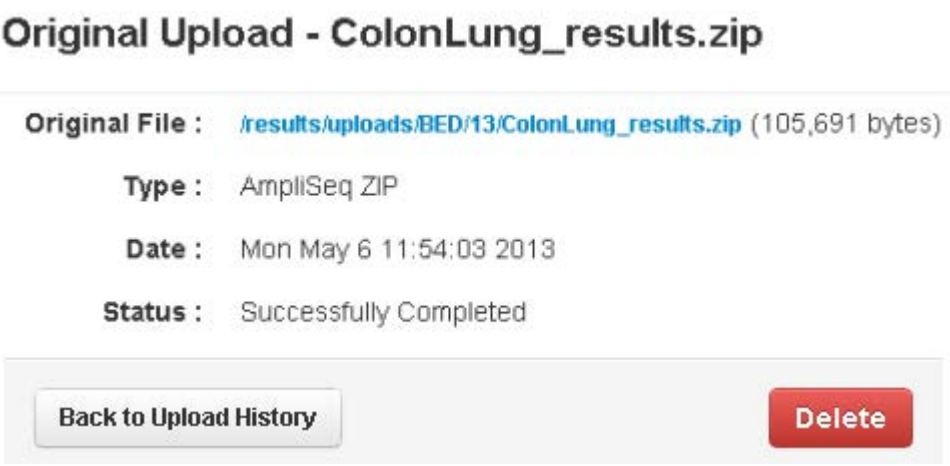
In these details sections, you can do the following:

- 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 333 (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 chevron.

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).

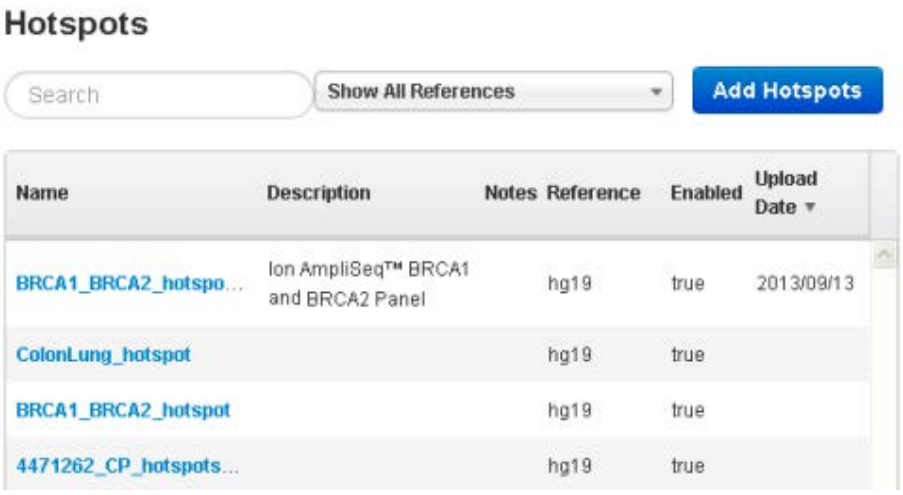
2. Upload a BED or VCF file for a different species.

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

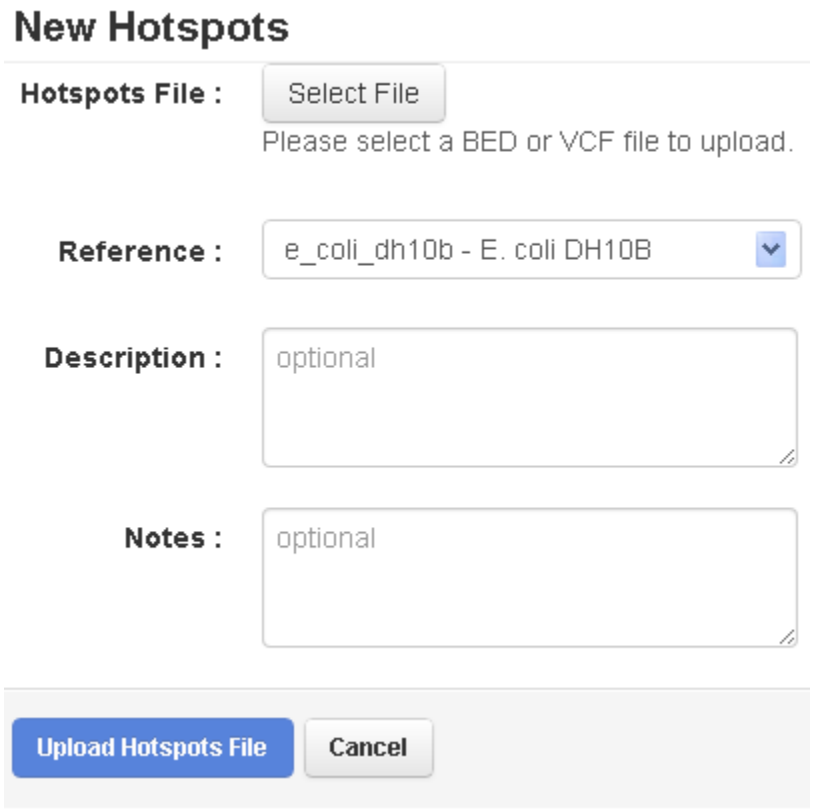
   Follow these steps to upload a target regions BED file or hotspots BED or VCF file to a reference:

   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:

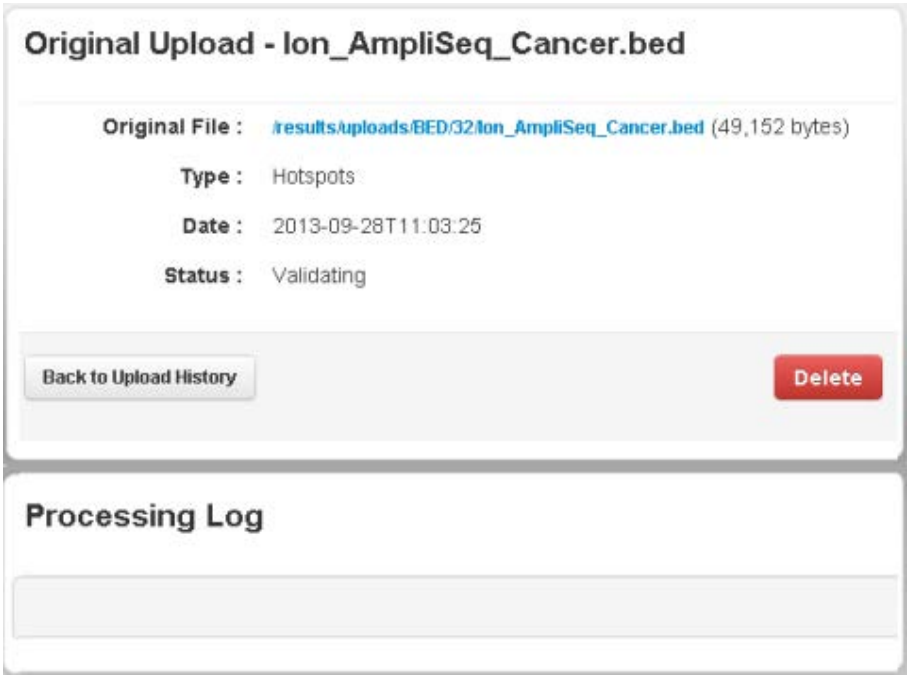


c. Click the **Select File** button and browse to the file to be uploaded.

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.

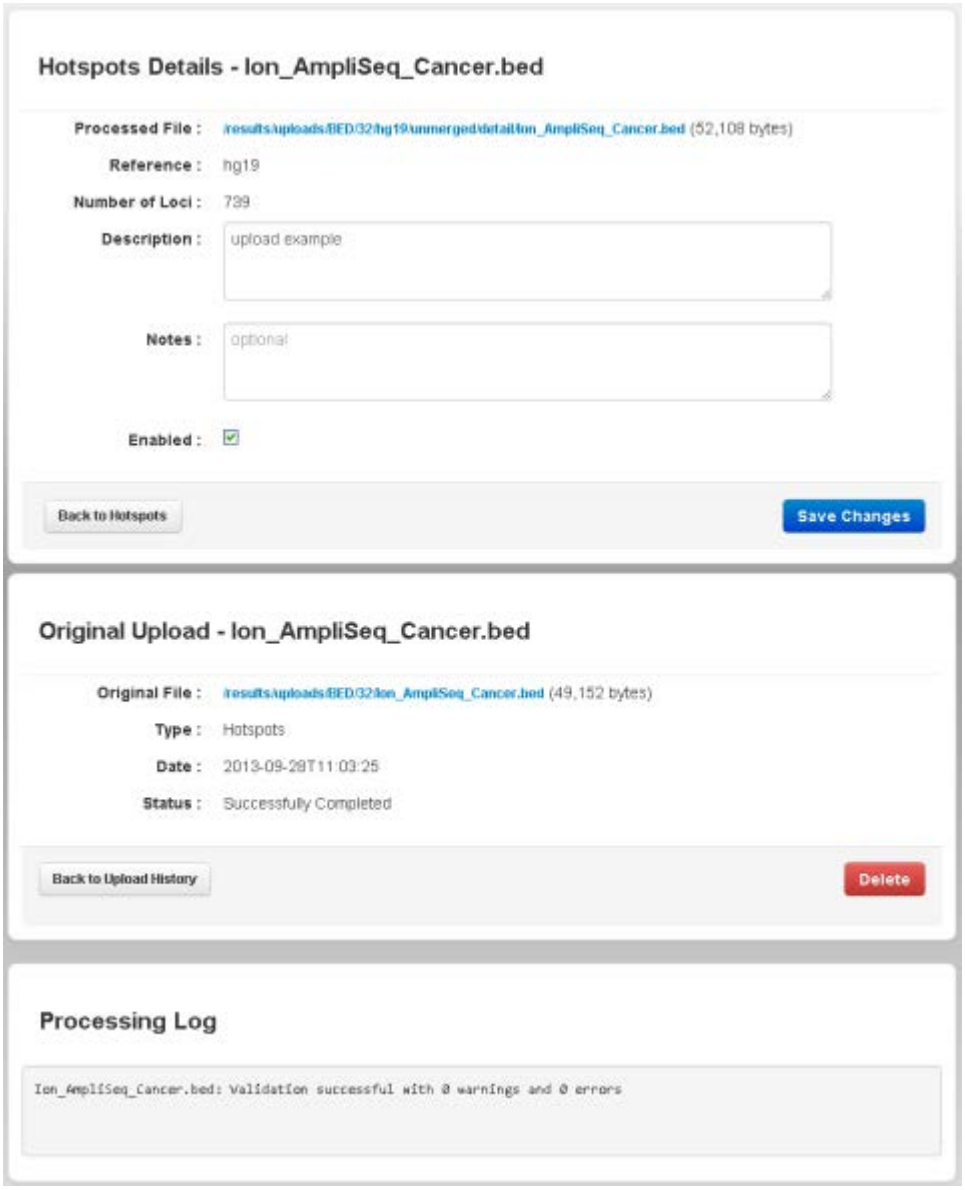
f. Click the **Upload Hotspots File** (or **Upload Target Regions File**) button. Wait while the file is validated:



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:

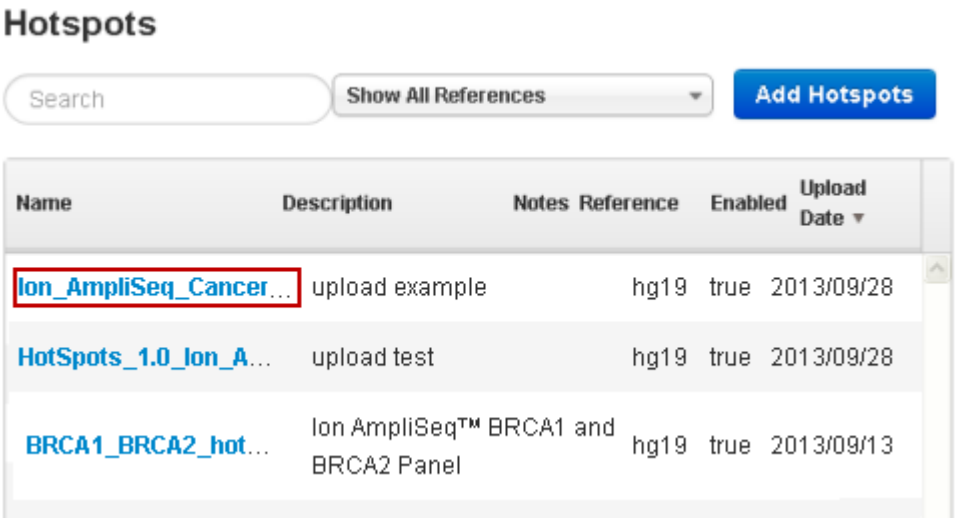
- Incorrect file format
- Incorrect file extension
- Zip contains 0 or multiple files
- A corrupted .zip .gz file

Download a hotspots or target regions file

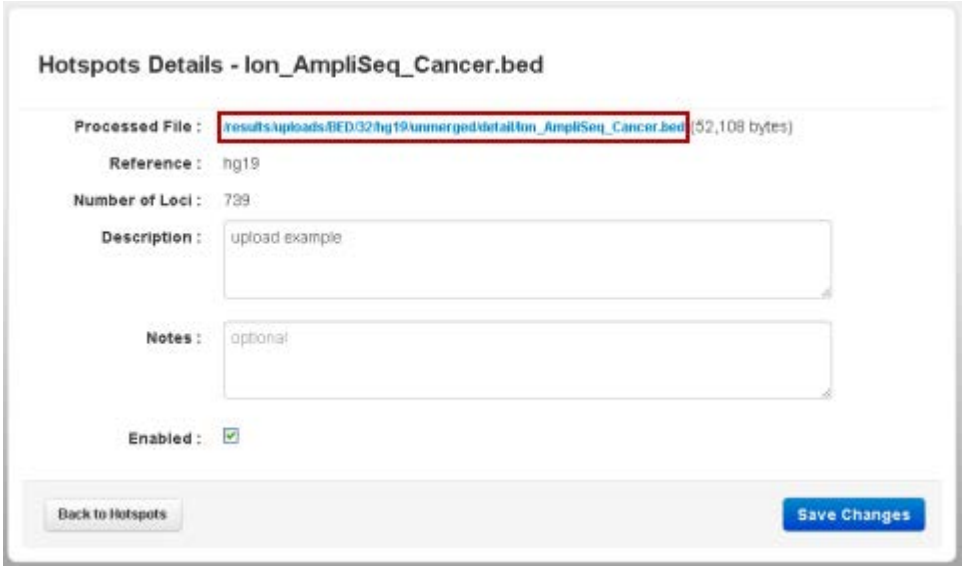
Follow these steps to download a hotspots BED or VCF file, or a target regions BED file:

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:



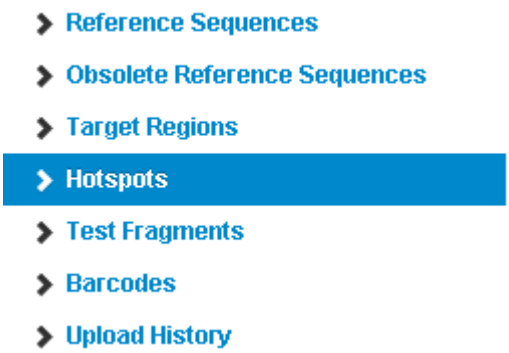
The Original File link in the Original Upload section also downloads the same file.

Delete a hotspots or target regions file

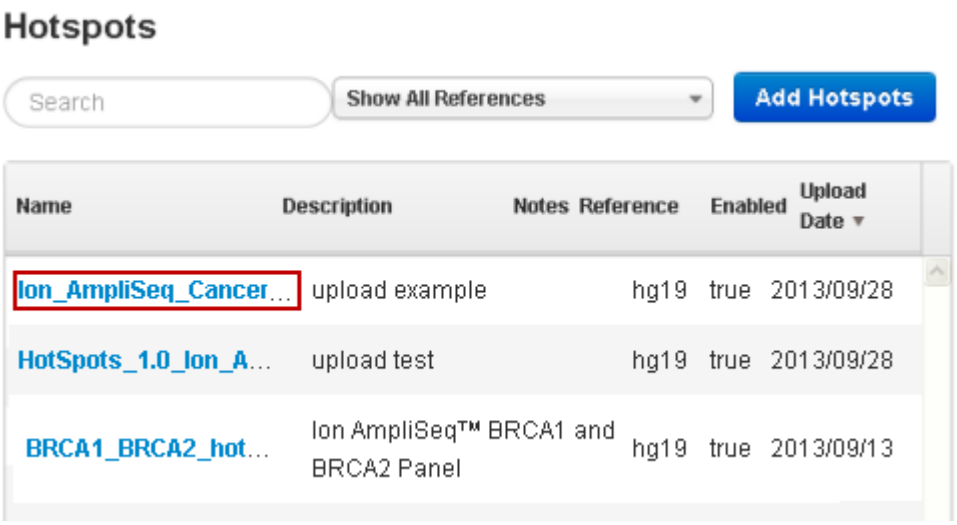
Note: This step removes the file from the system. There is no recovery or undo. Consider first downloading the file as a backup.

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.

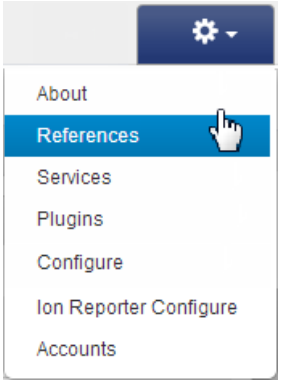
With the pre-installed Ion Torrent™ barcodes, you can view the barcode sets and the barcodes, incuding the barcode sequences.

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

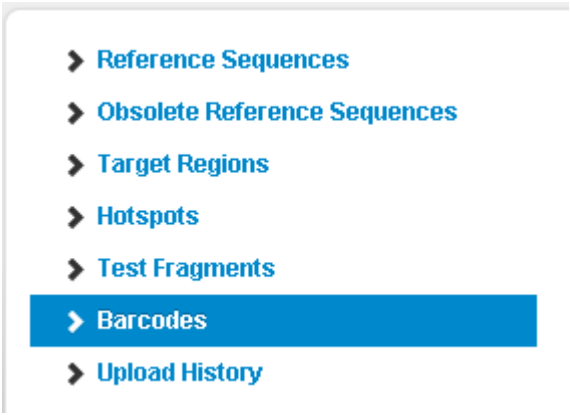
- View a DNA barcode or barcode set
- Add a custom DNA barcode set
- Delete a DNA barcode set
- Add a barcode to an existing DNA barcode
- Edit or delete an individual barcode

Access the DNA barcode set pages

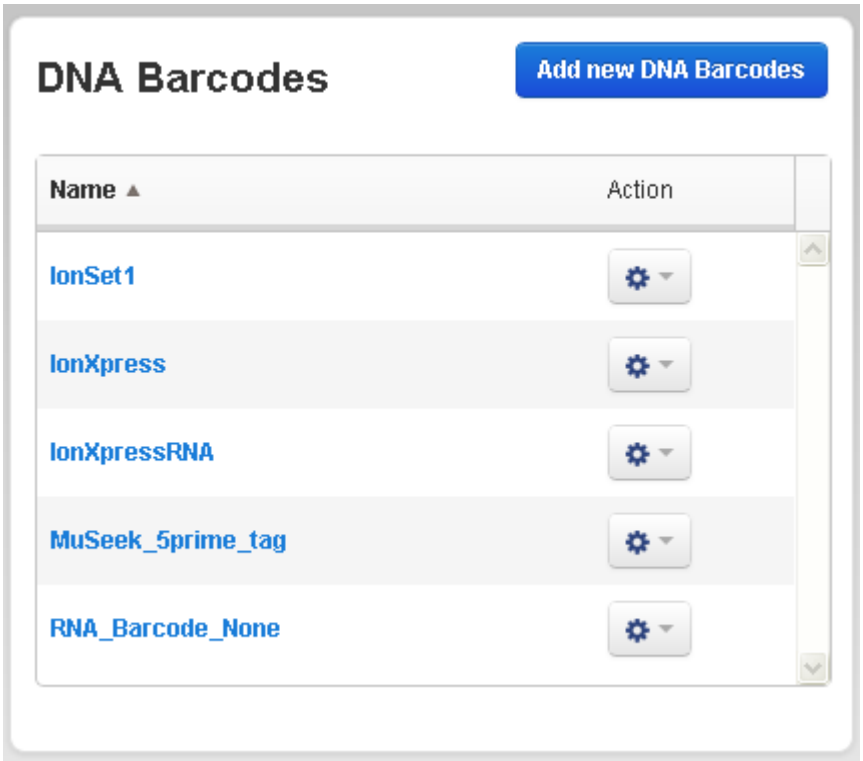
You access the barcode sets in the Torrent Browser by clicking the gear menu (near the top right) and clicking the References option:



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

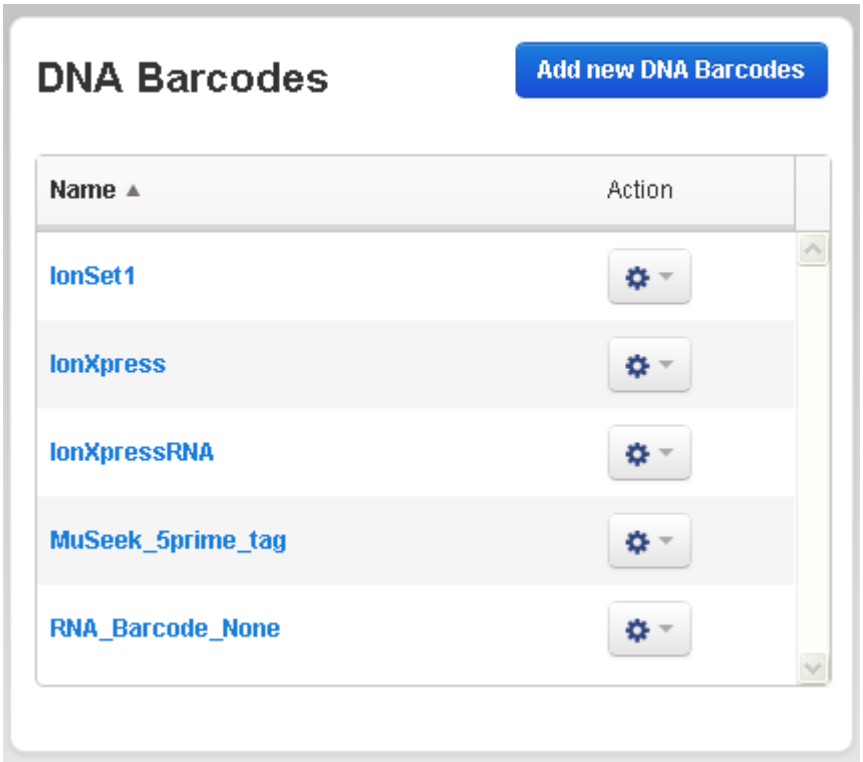


The DNA Barcodes page opens:



Pre-installed DNA barcode sets

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

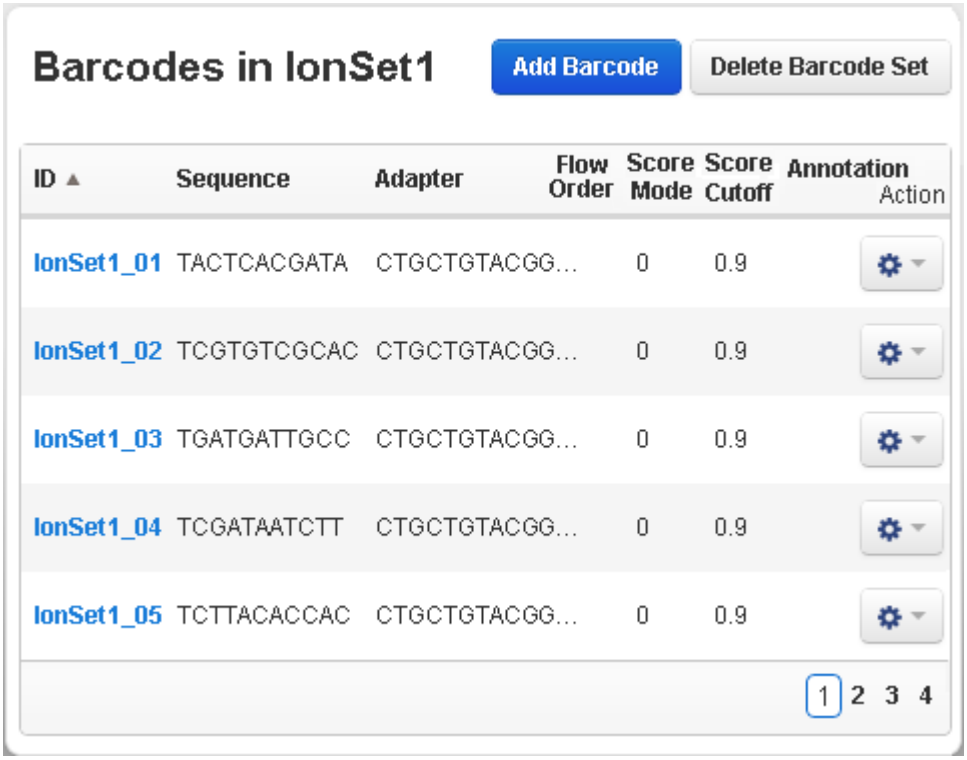


View a DNA barcode or barcode set

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

1. Click the gear menu, select **References**, and scroll down to the DNA Barcodes panel.
2. Either click the name of the barcode set to view or click the gear menu **Edit** option for that barcode.

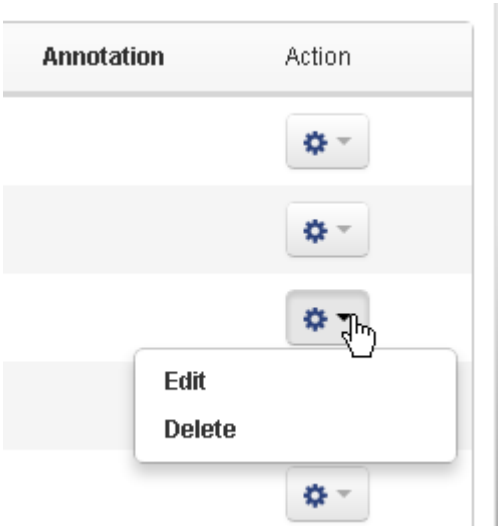
This displays the barcodes in the set:



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 Action column gear menu provides **Edit** and **Delete** options:



The gear 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:

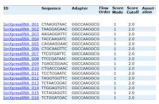
ID	Sequence	Adapter	Flow Order	Score Mode	Score Cutoff Annotation
IonSet1_01	TACTCACGATA	CTGCTGTACGGCCAAGGCGT		0	0.9
IonSet1_02	TCGTGTCGCAC	CTGCTGTACGGCCAAGGCGT		0	0.9
IonSet1_03	TGATGATTGCC	CTGCTGTACGGCCAAGGCGT		0	0.9
IonSet1_04	TCGATAATCTT	CTGCTGTACGGCCAAGGCGT		0	0.9
IonSet1_05	TCTTACACCAC	CTGCTGTACGGCCAAGGCGT		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:

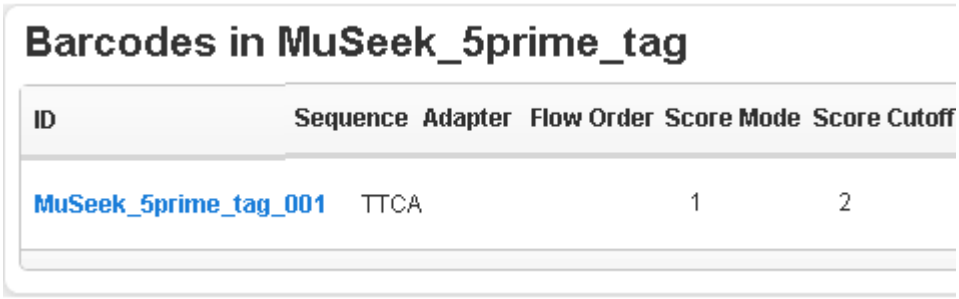


RNA_Barcodes_None barcode

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:

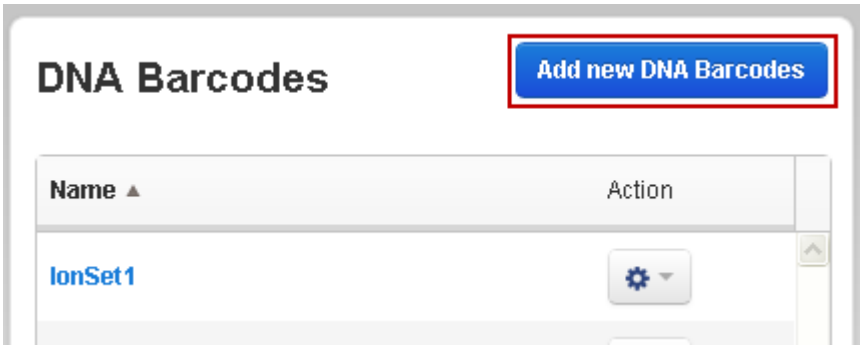
- View a DNA barcode or barcode set.
- Add a custom DNA barcode set.
- Delete a DNA barcode set.
- Add a barcode to an existing DNA barcode.
- Edit or delete an individual barcode.

Add a custom DNA barcode set

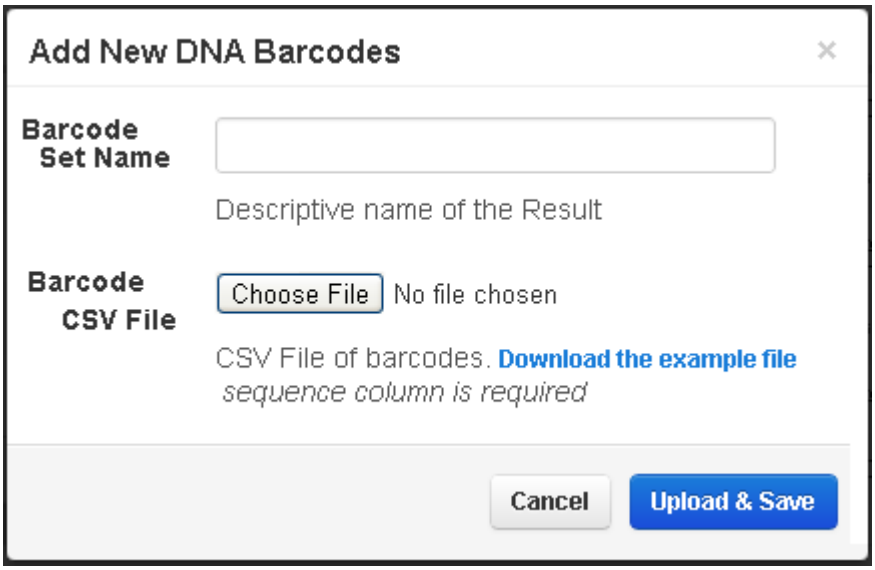
To add a barcode set, packaged as a list of barcodes in a Comma-separated Variable (CSV) text file, create the CSV file then select the file to add it to the barcode set list.

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 the Torrent Browser gear menu (near the top right), select the **References** option, and scroll down to the DNA Barcodes panel. Click **Add new DNA Barcodes** on the right side of the **DNA Barcodes** panel:



3. 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**:



4. 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.

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

6. 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:

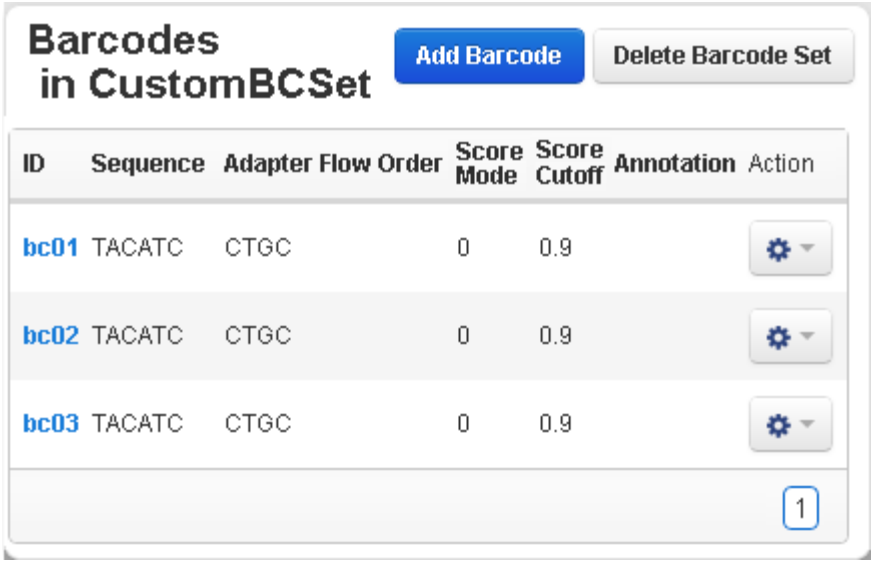
2. Click **Yes, Delete!** to delete the entire barcode set. Click **Cancel** to keep the displayed barcodes.

Add a barcode to an existing DNA barcode set

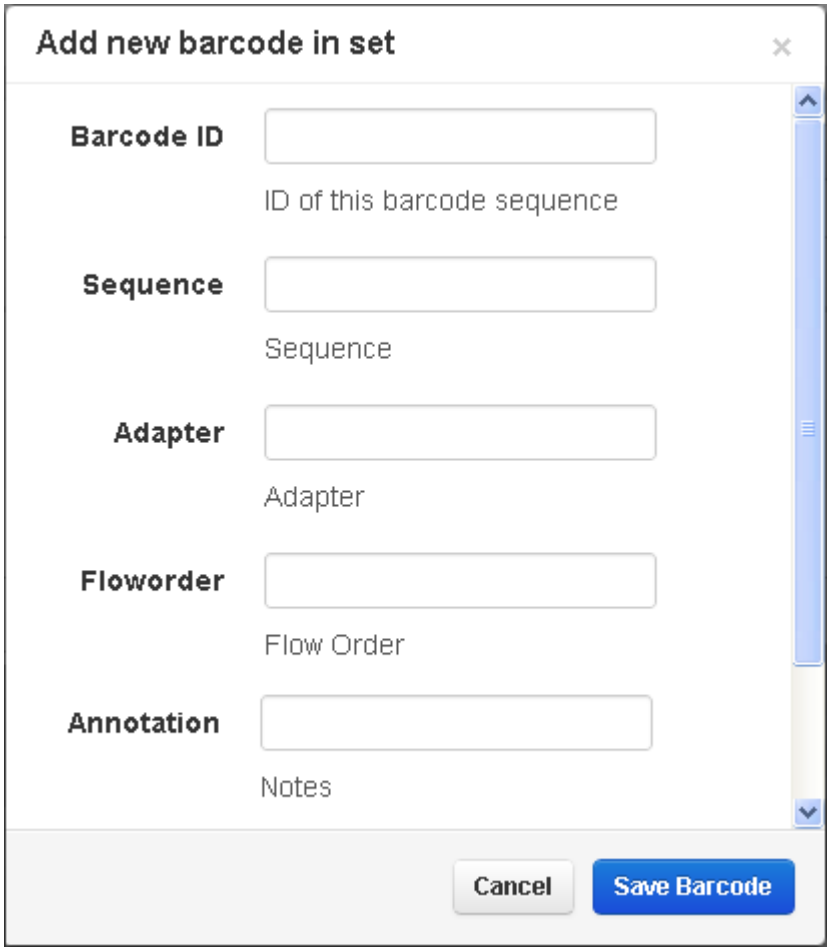
This feature is only for custom barcode sets that you install.

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.

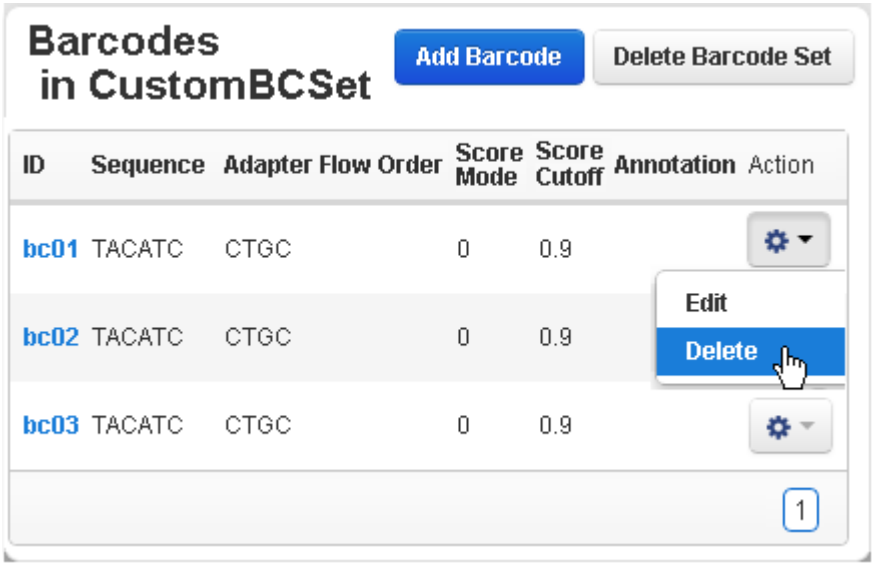
Delete an individual barcode

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 on the gear menu for that barcode and select **Delete**:



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

The barcode is removed for the barcode set.

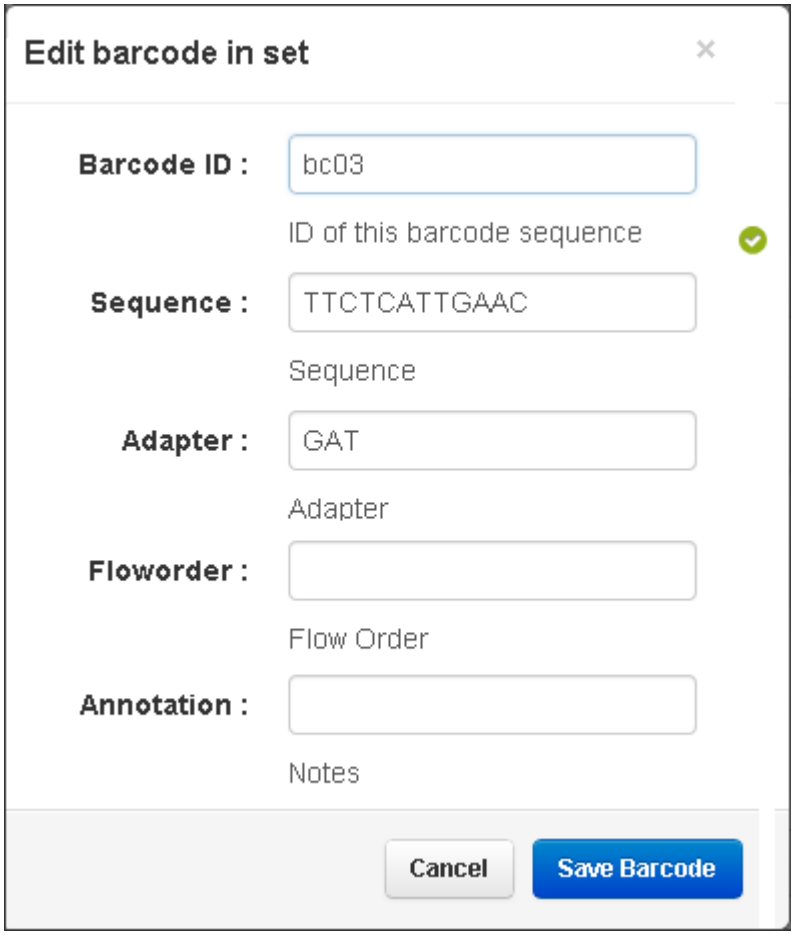
Edit an individual barcode

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:



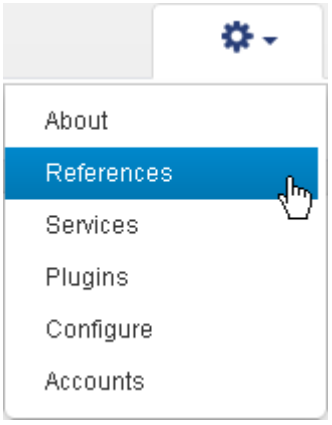
2. To edit the barcode details, make your changes and click **Save Barcode**.

Update Reference Library Indices

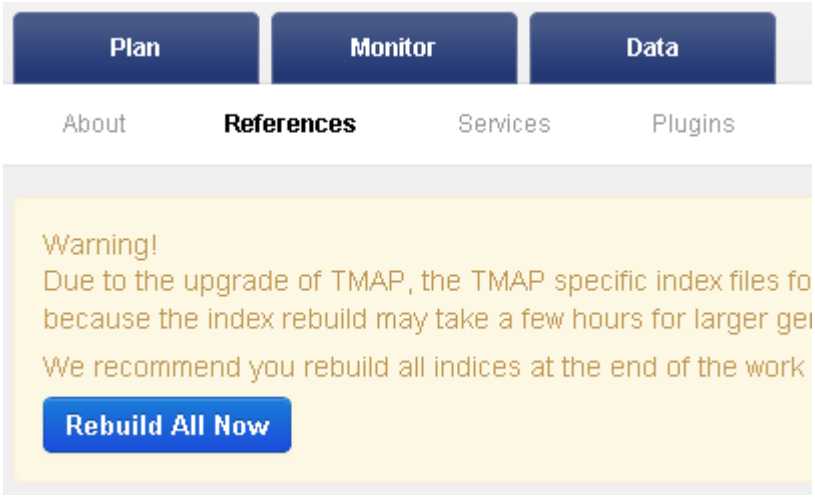
Note: When you upgrade your Torrent Suite™ Software from a version earlier than 3.0, you must rebuild your reference indices. This process can take a few hours for larger reference genomes. Your users should not submit data analysis jobs while the reference indices are being rebuilt.

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 the Admin gear menu **References** option:



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



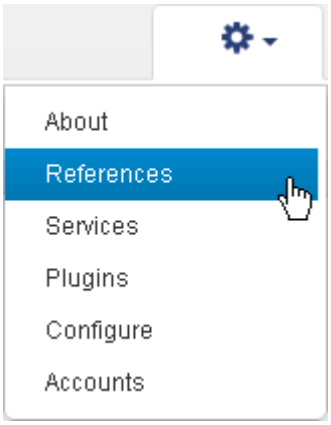
The TMAP index version used in 3.x and 4.x releases is tmap-f3.

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. Access the Test Fragments section by clicking on the Admin gear menu (near the top right of a Torrent Browser page) and clicking the **References** option:



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.

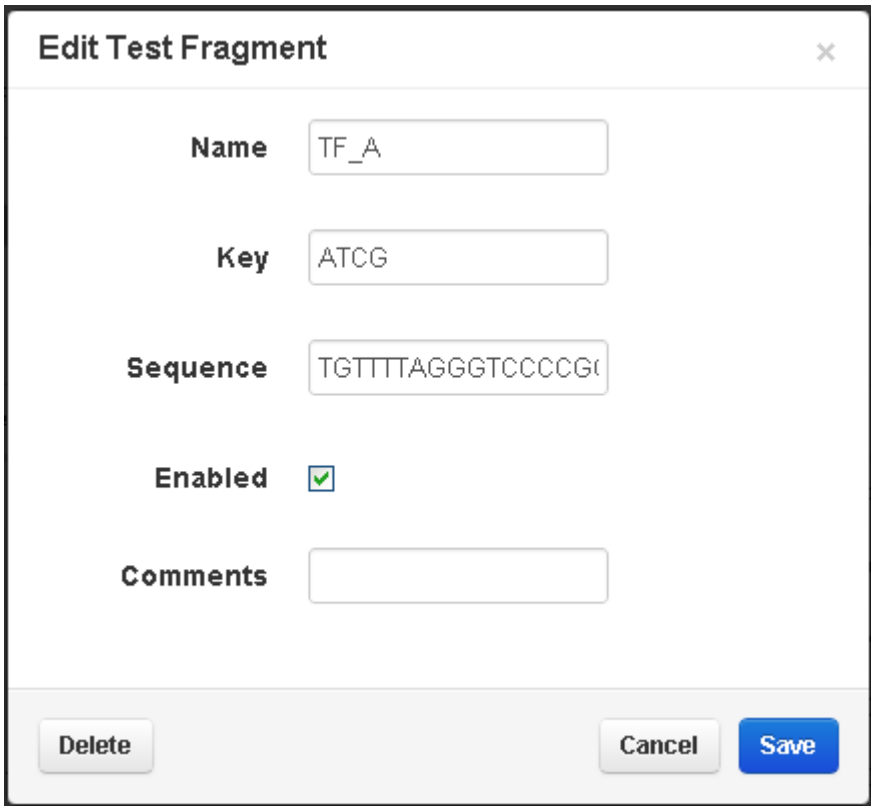
Contact your Ion Torrent™ representative if you have questions about the test fragment templates installed in your Torrent Browser.

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

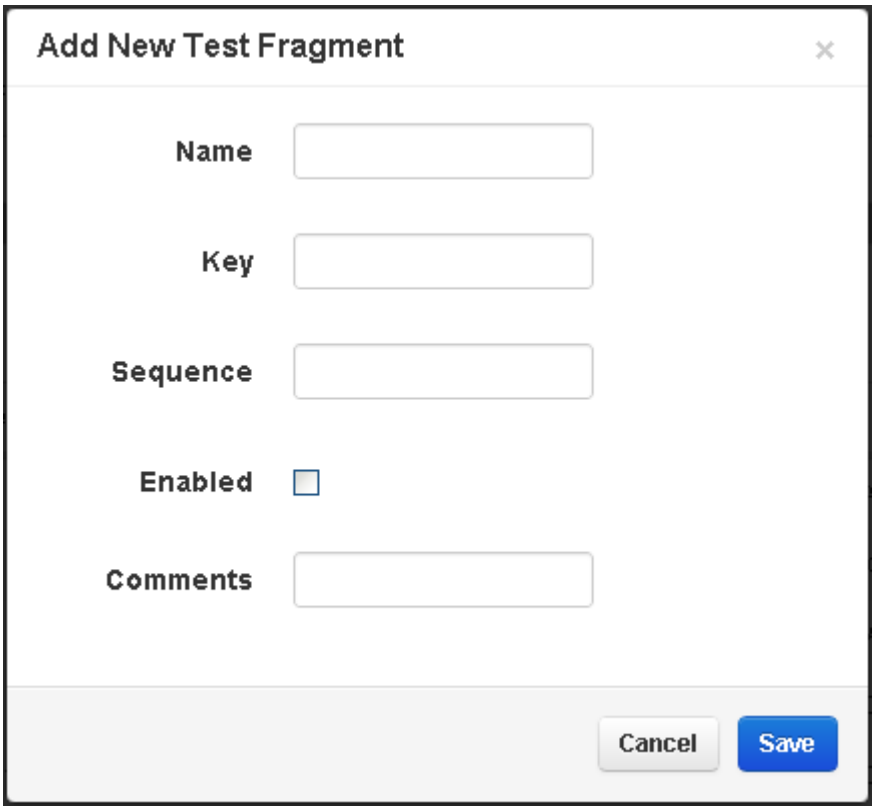
1. Click the **Name** column label to display test fragment details. This example showstest fragment **TF-C** selected for editing:



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

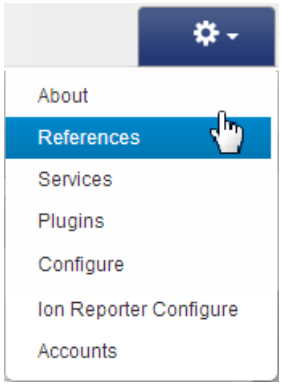
In the admin References tab, you can download a GHRC38, MM10, hg19, or *E. coli* reference file.

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

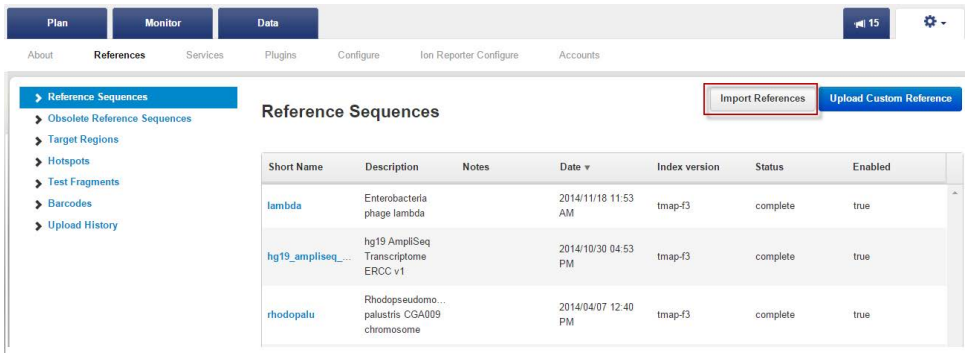
t_Steps to download a reference file

Follow these steps to download a reference file:

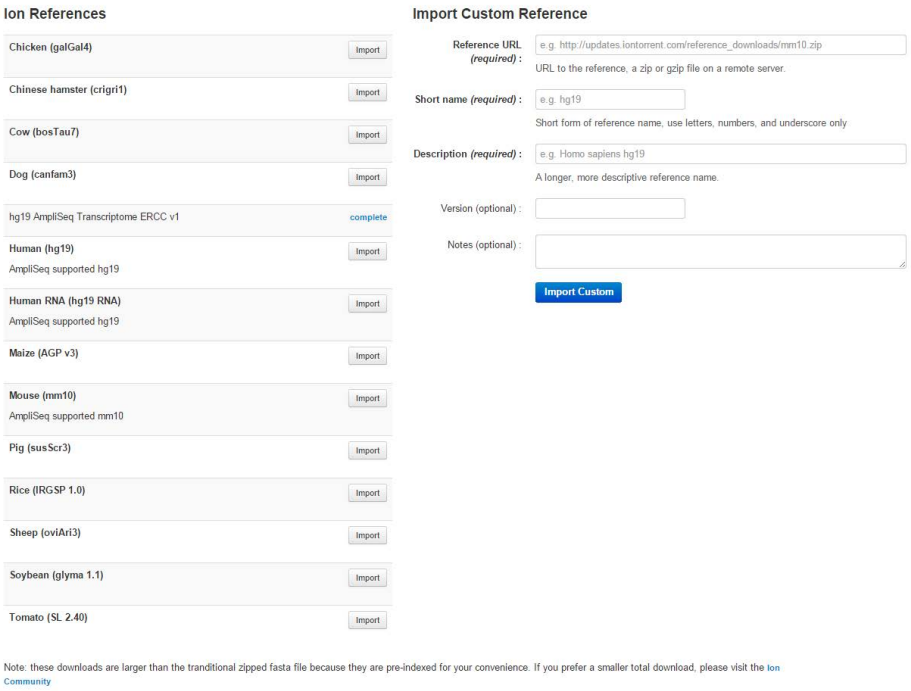
1. Click the Admin gear menu **References** option:



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

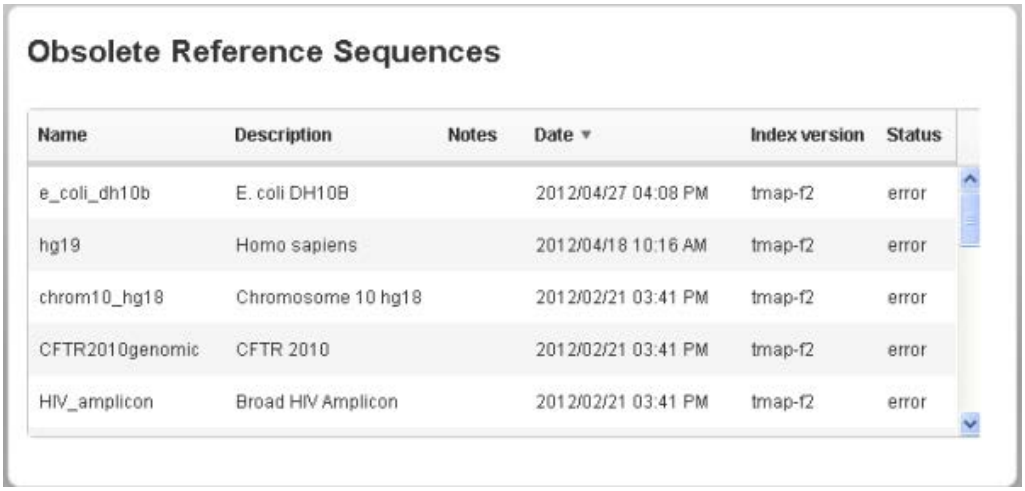
We use the Cambridge Reference Sequence (rCRS) for chromosome M with the GenBank accession number NC_012920. UCSC has announced that they also are using this version in the next human assembly release.

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.

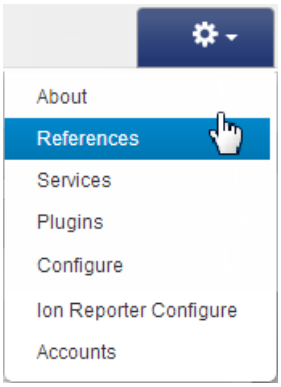
Delete a Reference Sequence

The section provides instructions to delete a reference sequence.

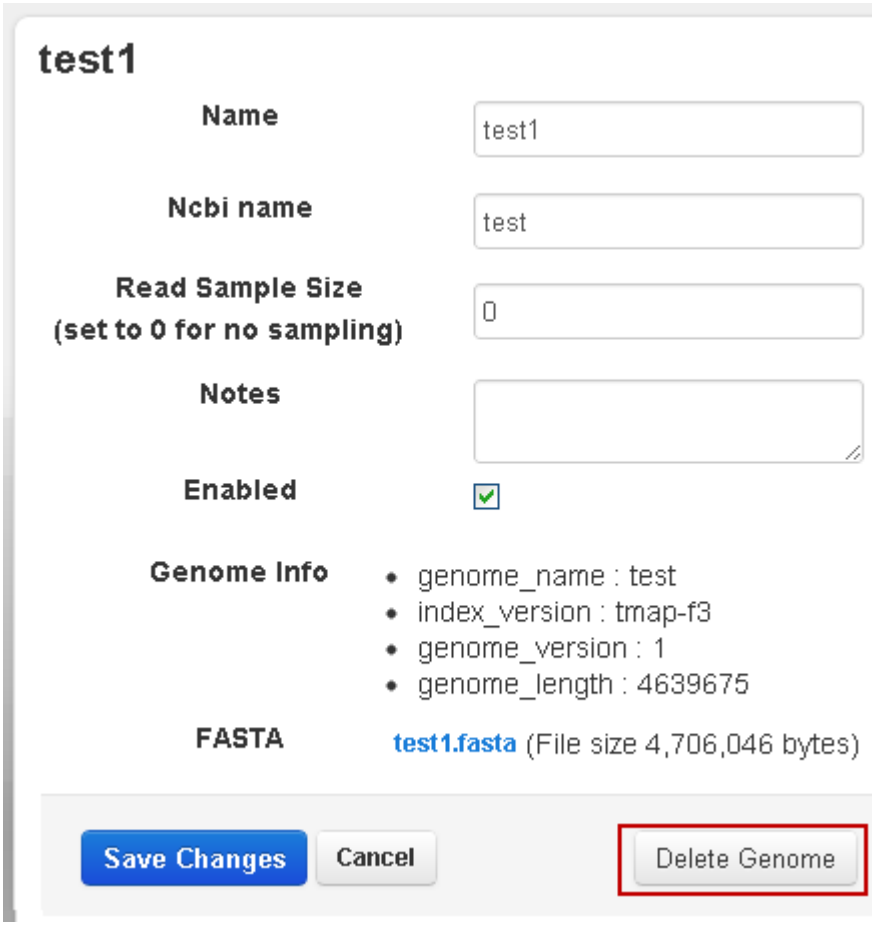
Recovery of a deleted reference sequence is not supported.

Delete a Reference Sequence

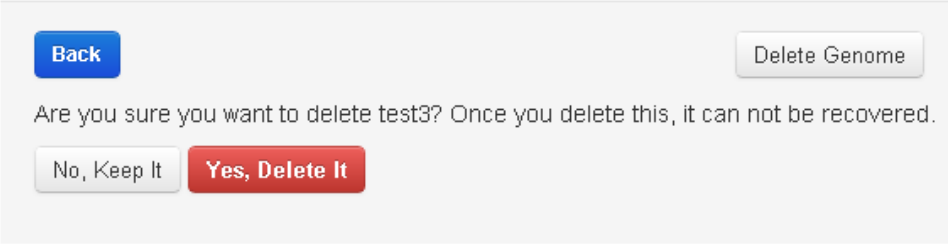
1. Go to the Admin gear menu and select the Reference option:



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:



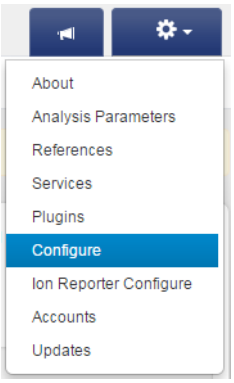
3. Click **Yes, Delete It** only if you are sure this genome should be deleted. Click **No, Keep It** to exit the dialog without deleting the reference sequence: The deleted reference sequence is removed from the **Reference Sequences** list.

In this release you cannot delete a reference from the Obsolete Reference Sequences section.

Software Administration and Data Management

Administration

To perform these administrative tasks, in the Torrent Browser user interface, click the Admin gear menu (near the top right) and select the **Configure** option:



The Admin Configure tab opens. Here you can perform these tasks:

- Add customer support contacts
- Add IT Contact
- Customize site name
- Change Time zone
- Enable nightly email notifications
- Administer database
- Configure Torrent Storage
- Configure Torrent Mesh

WARNING! You must use your `ionadmin` account for steps described in the Torrent Suite™ Software Administration Guide. (Do not use your `ionuser` account.)

Change Your Torrent Browser Password

It is possible for a user to change the UI password and lock themselves out of the Torrent Browser Admin screen, or locked out of the Torrent Browser UI altogether.

The UI password is stored in a database field. When you access to the database, you can change the password.

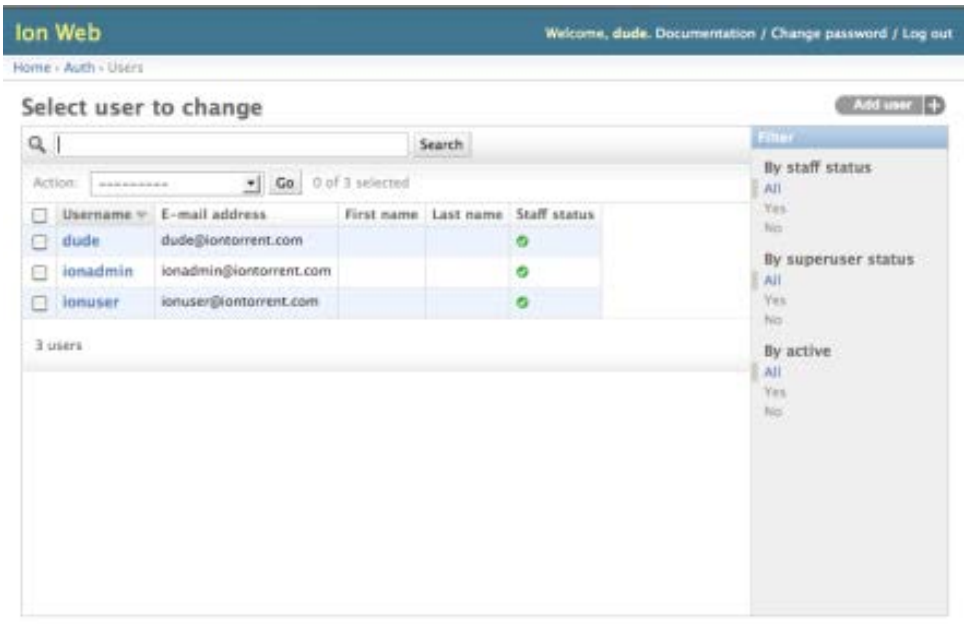
Method one

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. Once the new superuser account has been created, login to the admin page with the newly created username and password.
3. Select the user section under Auth:

Note: The Auth section does not appear if you login with an `ionuser` account.

4. Select the account you want to change the password for:



5. Click **Change password form**:

6. Enter the new desired password and click **Change Password**:

Method two

IMPORTANT! This process updates the database directly, and has no undo or recover in case of error. Do not proceed with this process if you are not comfortable with SQL commands.

1. Login to the database

   Log 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

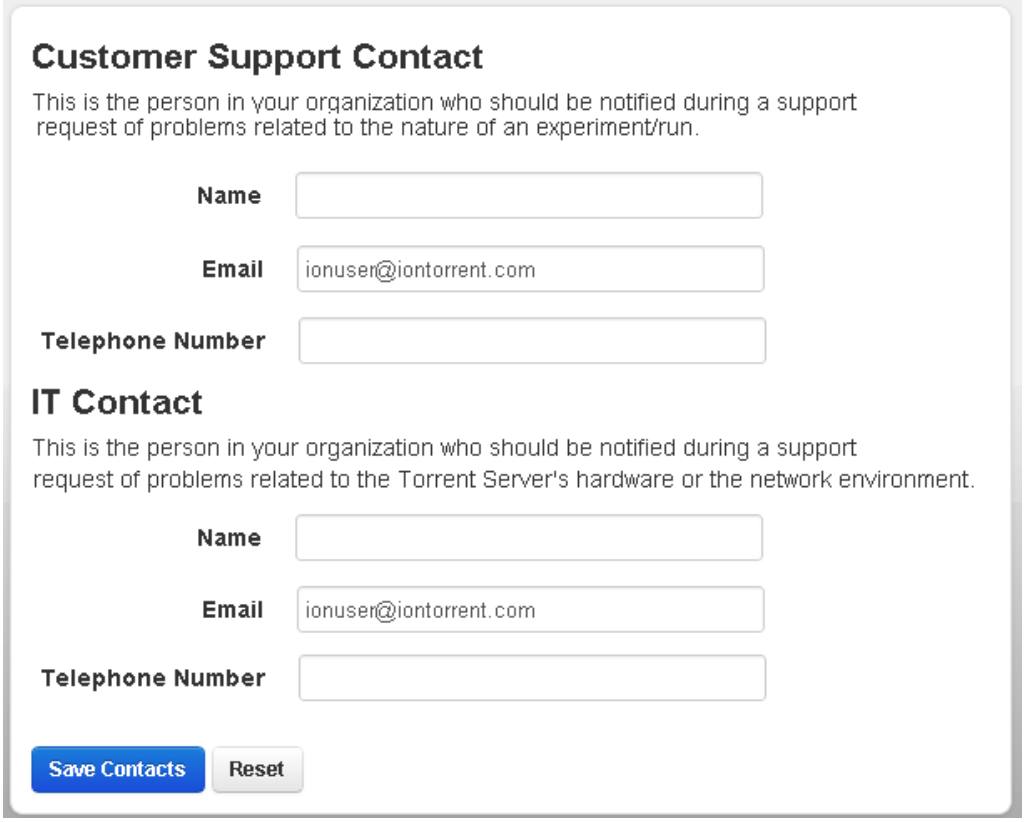
```
iondb=> SELECT username, password from auth_user; username
|
                          password ----------
+-------------------------------------------------
                          ionuser |
sha1$7e254$476582a5fa365cdd6081a80ac161c1904cc9c374
ionadmin |

sha1$7e254$476582a5fa365cdd6081a80ac161c1904cc9c374 ion |
                          sha1$7798b
$c025c463682f84b66cf3b5168356a04e3ce3b899 (3 rows)
```

5. Reset the password

   Now you can log in via the UI as `ionadmin`, and reset the password. Remember to change the password via the **Change password** form.

Add Customer Support Contacts

Fill in the information for a customer support contact and an IT contact at your organization, and click the **Save Contacts** button.



Change the displayed server name

This section describes how to change the server name displayed in the Torrent Browser. The default name is `Torrent Server`. This change only affects the server name shown in the Torrent Browser, and the default bookmark entry if you create a browser bookmark for the server.

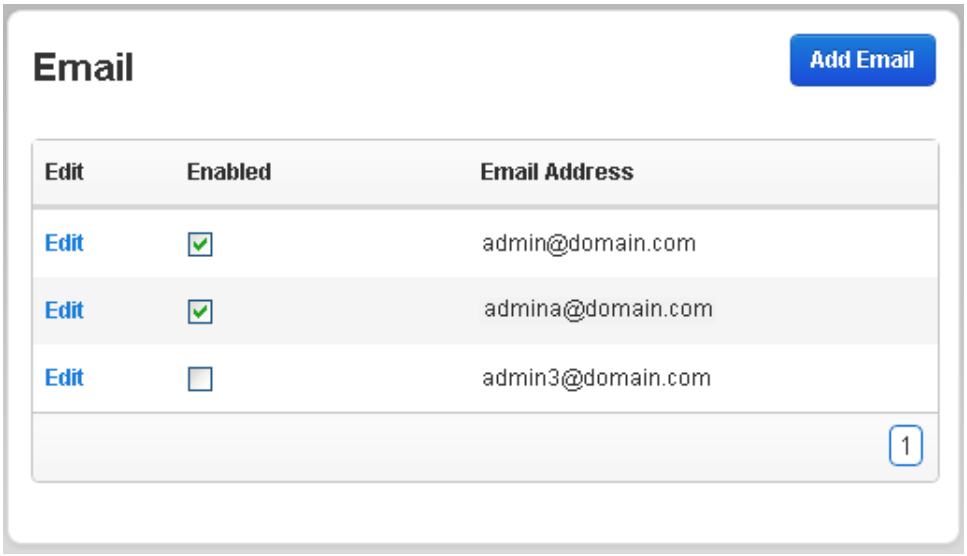
1. Click the Admin gear menu (near the top right) and select the **Configure** option:
2. Scroll down to the Customize Site Name section.

3. Enter the name of your choice and click **Save Name**. The server display name is now changed.

Nightly email configuration

The Torrent Suite™ Software Data Management system attempts to send email notifications of archive space problems through Postfix.

1. In the email address configuration panel, click **Add Email** to add an email address. This displays the **Add Email** dialog.
2. Enter an email address. Click the **Selected** checkbox to enable this email address for sending email. Click **Save**.
3. The new email address is displayed in the **Email** panel:



Use the Enable column checkboxes to enable or disable email to specific email addresses.

Database Administration

In the **Database Administration** panel, click **Admin Interface** to access the administrative configuration dialog, which displays in a new window:

Where configuration options are directly available through an interface tab, such as **Templates**, the interface tab mechanism should be used to configure the item.

Configure Chips

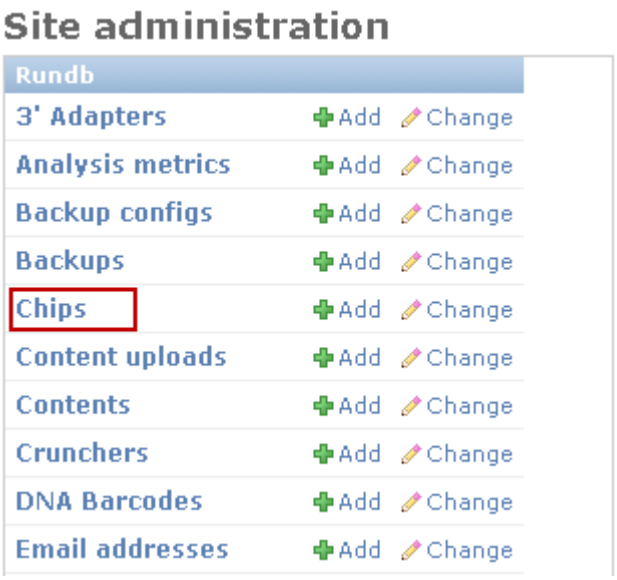
The **Chips** dialog controls default analysis processing for a particular Ion Torrent™ chip.

IMPORTANT! Please check with Ion Torrent™ before modifying this information.

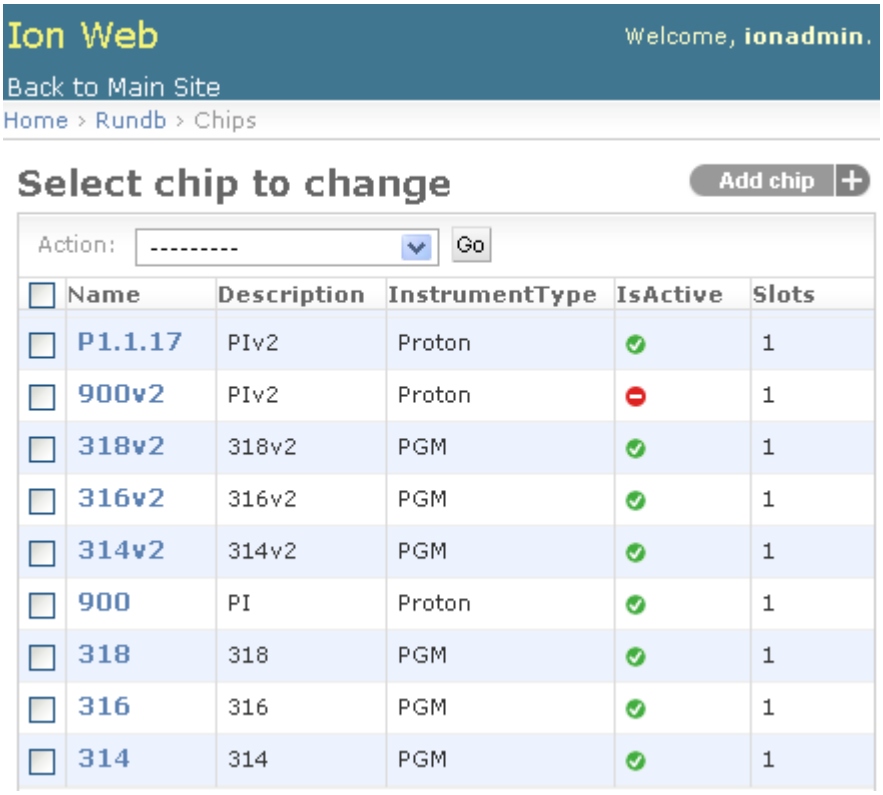
When an analysis job is started, the Torrent Browser looks up the chip type in the database Analysis Args table for default analysis settings.

To modify the default behavior:

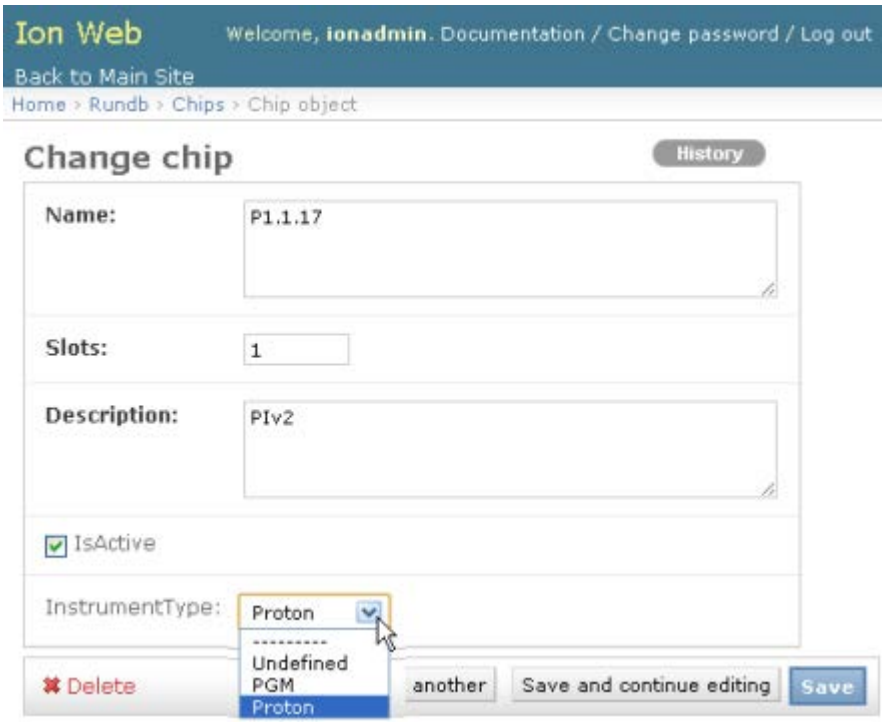
1. Click **Chips**, or **Change**, on the **Chips** line:



2. In the **Name** column, click the chip you want to change:



3. Modify the chip fields, including the Instrument Type menu, as needed:

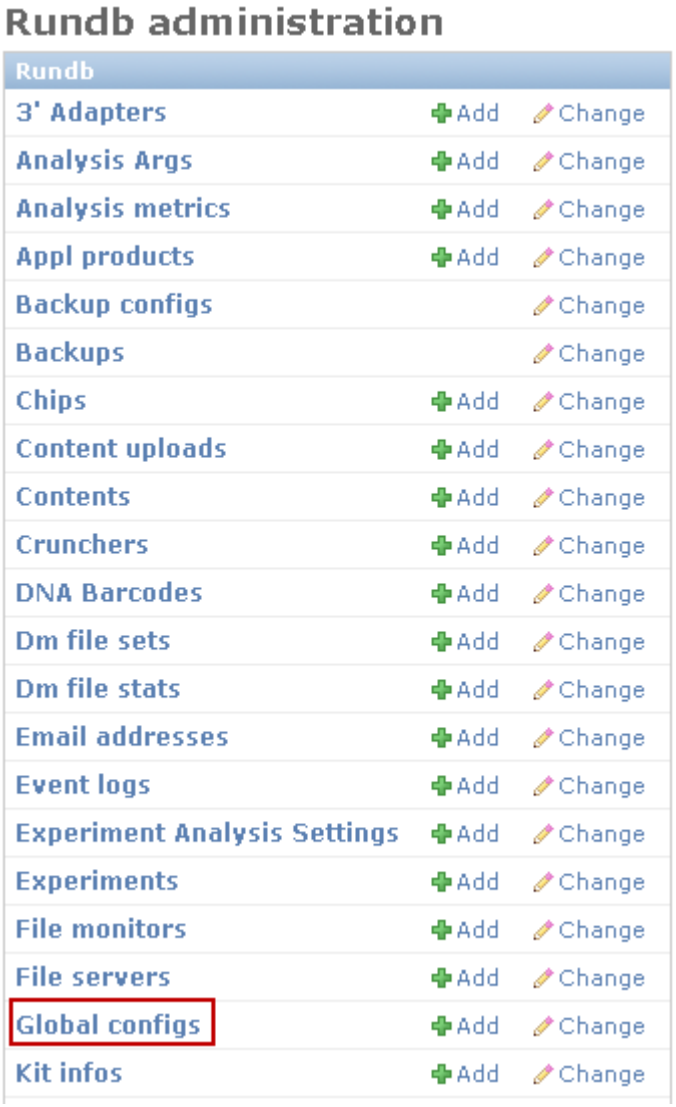


4. Click **Save** to save your change.

Configure Global Configs

The **Configure Global Configs** dialog permits you to specify run parameters.

This page describes advanced configuration options. Check with Ion Torrent™ if you are unsure of the effect of modifying this information.



Add a configuration

IMPORTANT! There should only be one Global Config record. Do not create multiple Global Configs!

1. Click **Add** on the **Global configs** line to set the values of the configuration parameters:
   - Plugin folder
   - Fasta path
   - Reference path
   - Records to display
   - Default test fragment key
   - Default library key
   - Default flow order
   - Plugin output folder
   - Default plugin script
   - Web root
   - Site name
   - Default storage options
   - Auto-Acknowledge Delete
   - Enable Auto Actions
   - Barcode args
   - Enable Package Auto Download
   - Enable Ts version lock
   - Ts update status
   - Base recalibrate
   - Mark duplicates
   - Realign
   - Check for news posts
   - Enable Security Updates
   - Sec update status
   - Enable Oncomine
   - Enable Support Upload
   - Enable Nightly Email
   - Notifications
   - Automatically disable SGE
   - Queue on node errors
2. Click the **Save** button when you are done making changes.

Change an existing configuration

1. Click **Change**, to the right of **Global configs**, to modify an existing configuration.
2. Click the **Name** of the configuration you want to change.

3. Enter the values for the items you want to change.
4. Click the **Save** button to save your change(s).

Configure Experiments

Users typically do not make any changes in the Experiments table. Run plans and other tables depend on the integrity of the Experiments table. Bad things can happen.

IMPORTANT! Please check with Ion Torrent™ before modifying this information.

IMPORTANT! Runs are automatically added to the database by the Crawler process, so you should not add a run to the database using this dialog.

Modify an experiment

1. Click **Experiments**, or **Change** on the **Experiments** line, to modify an existing experiment description:

2. Click the **ExpName** of the run you want to change:

3. Edit the experiment description fields as necessary. Typically, you only need to modify items that were not entered correctly at the time the information was captured on the Ion PGM™ or Ion Proton™ Sequencer.
4. Click **Save** options to save your changes.

Delete an experiment

These procedures delete the experiment and any child records generated, such as reports.

If the raw data are still present in the file system, the Crawler re-discovers the data and adds it to the database. To completely remove the run, you must delete the raw data before deleting the experiment from the database.

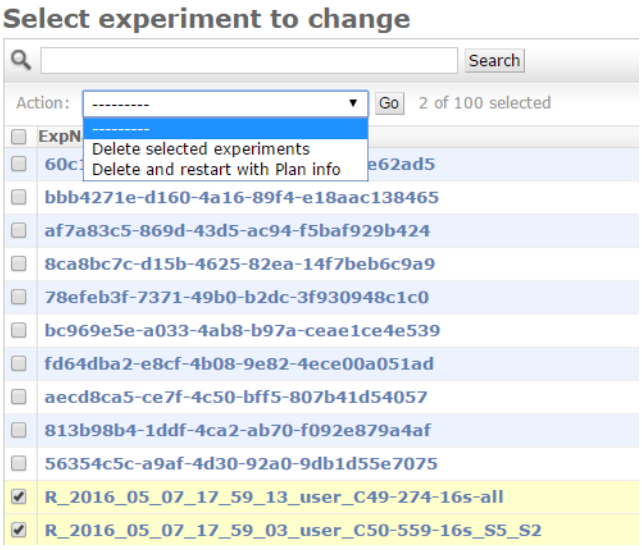
Delete a single experiment

1. Click the **ExpName** you want to delete.
2. Click **Delete**, on the lower left
3. Confirm the deletion.

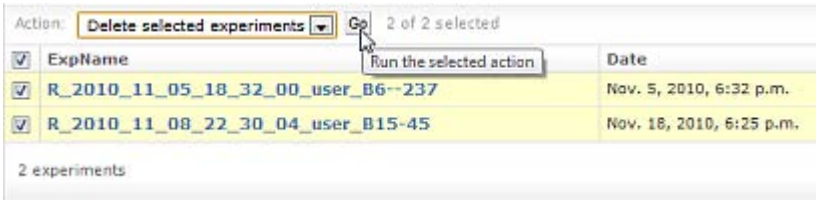
Delete multiple experiments

1. Check the checkbox of the experiment(s) you want to delete, or check the top checkbox to select all experiments for deletion:

2. In the drop-down menu, select **Delete selected experiments**:



3. Click the **Go** button to perform the requested **Action**:

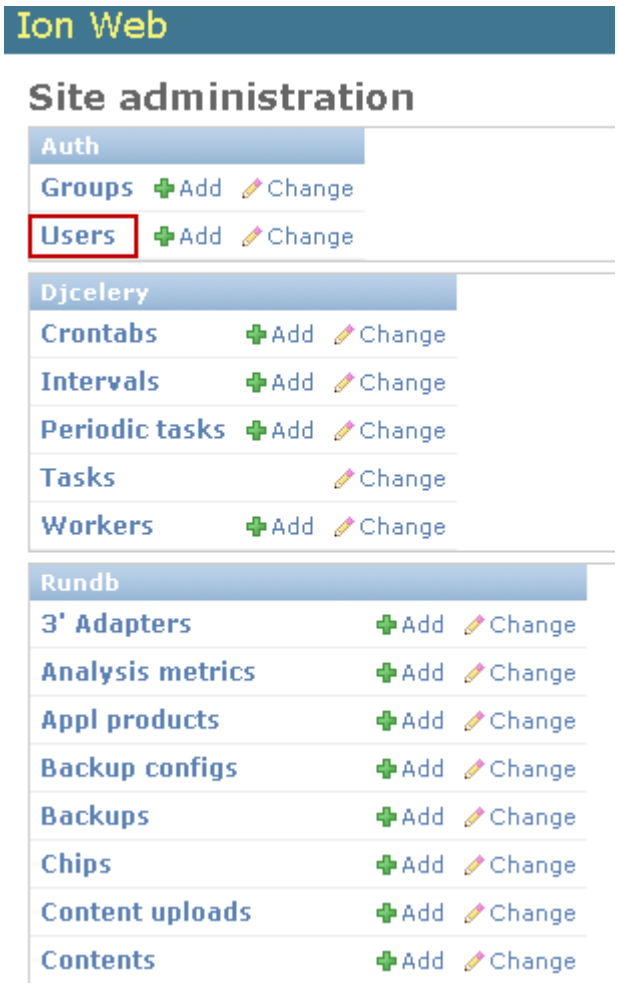


4. When prompted to confirm the deletion, click **Yes, I'm sure**. The list of experiments confirms your deletion.

Configure Users

IMPORTANT! The recommended mechanism to create user accounts is to add them through the login page Register link.

The **Users** dialog provides for creating or modifying user accounts to access Torrent Suite™ Software using the Torrent Browser:

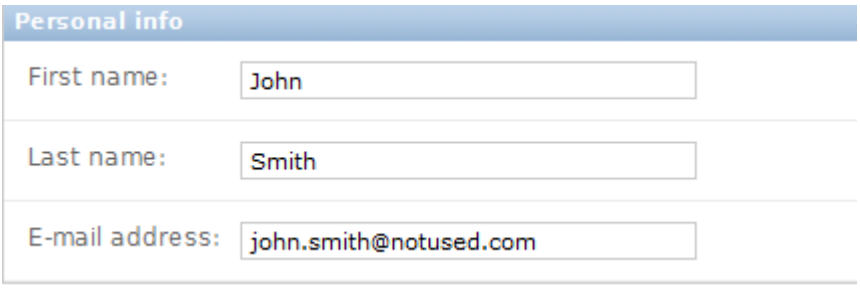


Add a User

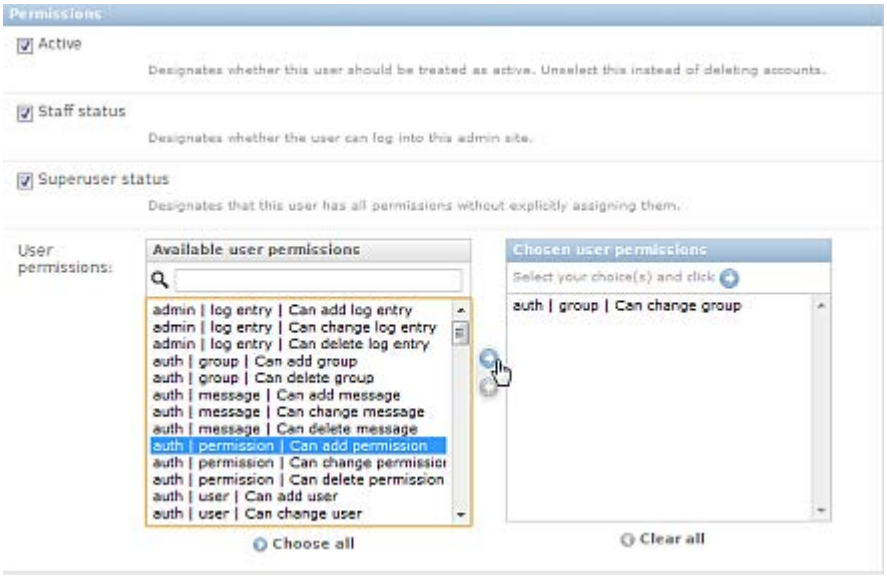
1. Click **Add** on the **Site administration** menu for **Users**.
2. Enter a **Username** and **Password** ; enter the password twice to confirm:

   Note: If the user already exists or the password is invalid, you will be prompted to enter the correct information before continuing.

3. Click the wanted save option. Clicking **Save** permits you to provide additional user information using the **Change user** dialog, as follows.
4. In the Personal info dialog, optionally, enter a **First name**, **Last name** and **E-mail address**:



5. (Optional) In the **Permissions** dialog, check the **Active**, **Staff status** and **Superuser status** checkbox(es), as needed, and select the wanted **User permissions**:

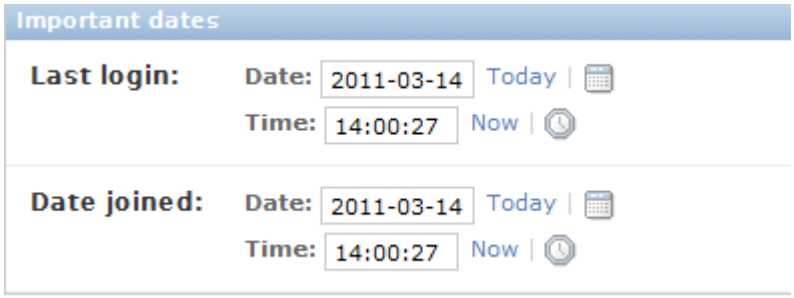


Checkbox	Description
Active	Designates whether or not this user is treated as active. The recommended method is to uncheck this item rather than delete this account.
Staff status	Designates whether or not this user can login to this administration site.
Superuser status	Designates that this user has all permissions without explicitly assigning them.

Select User permissionsThere are three ways to specify **User permissions**:

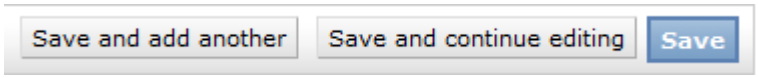
a. Enter a string in the search window. All permissions matching the string are displayed, from which you may select permissions by highlighting the permission(s) and clicking the right arrow, in the center.
b. Scroll through the permissions list. Highlight the wanted permission and click the right arrow to select the highlighted permission. Also hold down the control key to select more than one permission.
c. Click **Choose all** at the bottom of the dialog, to highlight all available permissions, and click the right arrow to select all permissions. To deselect any permission, highlight selected permission(s), in the right window, or click **Clear all** followed by clicking the left arrow.

6. (Optional) Set the **Last login** and **Date joined** times, manually or using the calendar and clock icons. Click **Today** and **Now** to set the values to the current date and time:



7. (Optional) Click the plus sign to display the **Add group** dialog.

8. (Optional) Currently, the **Add group** dialog is the same as the **User permissions** dialog in step 5, above. Add the user to the group in the same way as described in step 5.
9. Choose one of the following **Save** options to complete adding the new user.

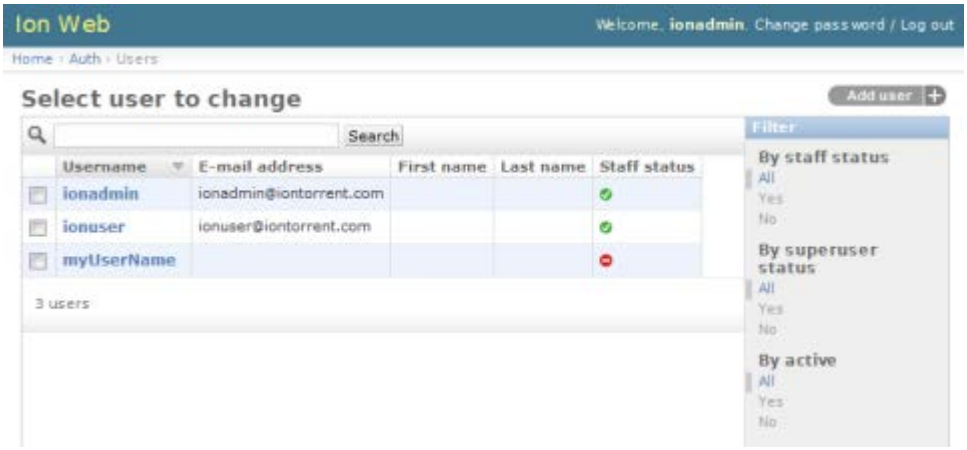


- – **Save and add another** Choose this option to complete adding the new user, and return to the **Add user** page to another user.
  – **Save and continue editing** Choose this option to complete adding the new user, and return to the **Change user** page.
  – **Save** Choose this option to complete adding the new user. Clicking Save takes you to the Select user to change page.

Modify a User Entry

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** page, 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 in the same way as described for adding a user, beginning in step 4 above.
   To be able to login to the server, it is important that you remember to check the **Staff status** checkbox in the **Permissions** dialog, shown in the following figure.

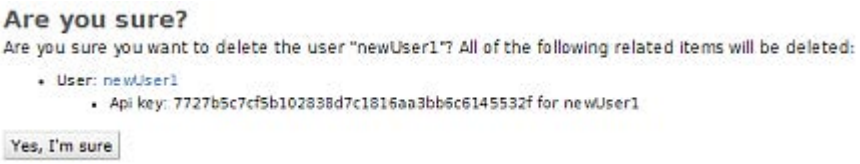
4. Choose one of the **Save** options at the bottom of the page to save your changes.

Delete a User Entry

Use one of the following procedures to delete an existing user.

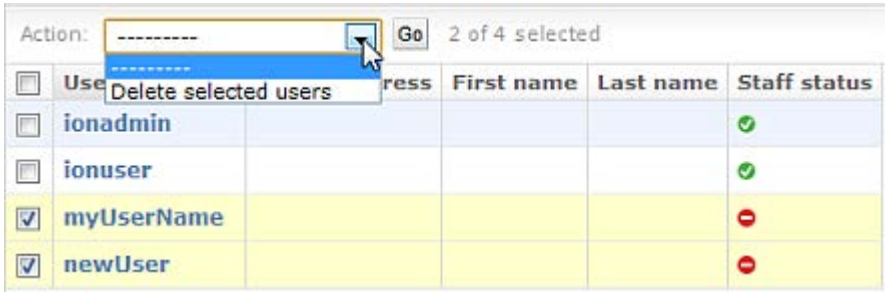
Delete a Single User

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 user information page, click **Delete**.
4. Confirm that you want to delete the user by clicking **Yes, I'm sure**:

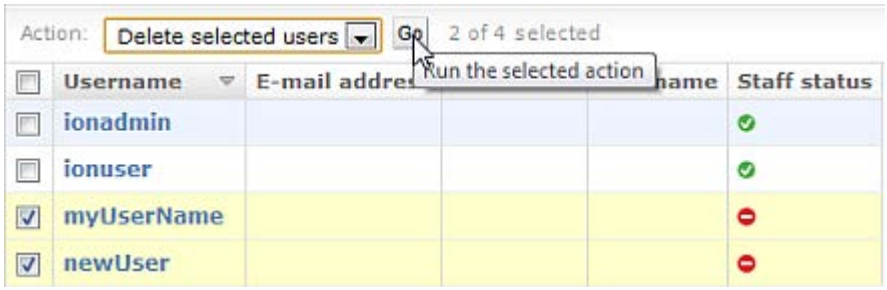


Delete Multiple Users

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 of the users you want to delete.
3. Click the drop-down menu and select **Delete selected users**:



4. Click **Go**:



5. Confirm the list of users you want to delete by clicking **Yes, I'm sure**.
6. On the **Select user to change** page, the list of users confirms your deletion(s).

Configure Basecaller Default Parameters

This section describes how to use the Admin Interface to change the basecaller default parameters, which include the quality trimming parameters.

Each chip type has a separate basecaller parameter field. The basecaller parameters and quality trimming parameters can be customized for each chip type.

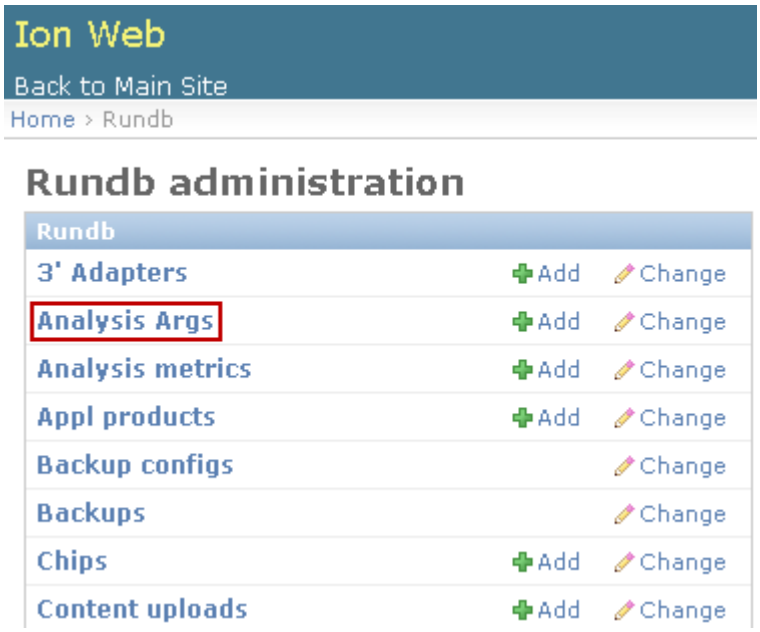
The quality and trimming parameters are described in this table:

Parameter	Description	
Qual cutoff	Sets the threshold for the average quality values within the qual window. A read is trimmed when the average of quality values measured over the (sliding) window of size `Qual window` drops below the `Qual cutoff` value. Basecaller parameter: `--trim-qual-cutoff`	
Qual window	Sets the size of a sliding window inside of which quality values are measured and averaged. Basecaller parameter: `--trim-qual-window-size`	
Adapter cutoff	Controls how accurate a match is required between the called sequence and adapter sequence this field sets the cutoff required to actually perform the adapter trimming. The lower the value, the more accurate match between the called sequence and adapter sequence is required to trim. (The value also depends on adapter length. Typically a value of half the adapter length [0.5 * (adapter length)] is reasonable.) Basecaller parameter: `--trim-adapter-cutoff`	

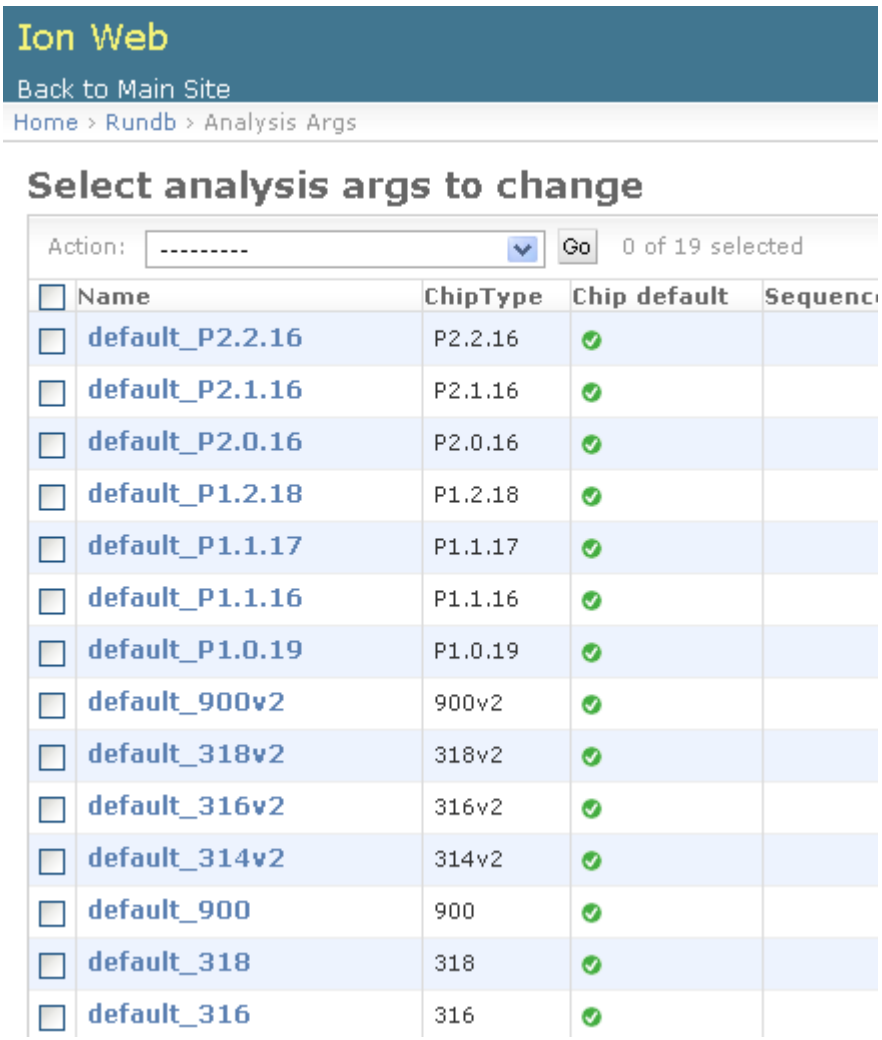
Note: Basecaller arguments by default are different for sequencer type, regular analyses, thumbnail analysis, and recalibration. This example shows the Basecaller arguments for regular analyses on Ion PGM™ data.

Follow these steps to change or customize basecaller parameters:

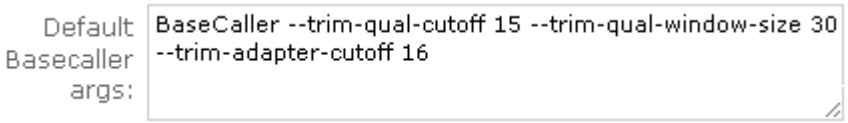
1. In the Rundb administration page, select **Analysis Args**:



2. In the Name column, click on the name for your chip type:



3. Make your basecaller parameter and quality parameter changes to the **Default Basecaller args:** field:



   Note: Enter the basecaller arguments in one line. The example here is wrapped.)

4. Click **Save**.

5. Repeat your changes to other chip types also, if required.

Note: Take care not to unintentionally have different quality and trimming settings for different chip types. Differences in these settings can lead to differences in results.

Adapter trimming is described in the section Removal of Adapter Sequence.

Quality trimming is described in the section Removal of lower-quality 3' Ends with Low Quality Scores.

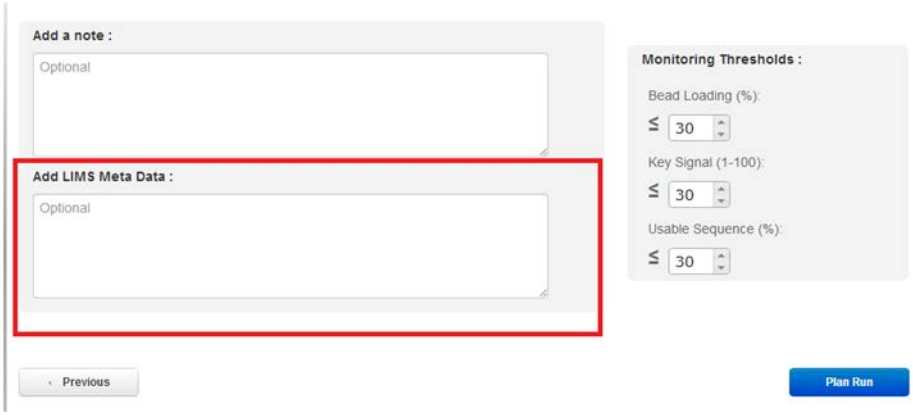
Configure LIMS Metadata export from Planned Runs

Users can add text in the Add LIMS Metadata text-entry box on the Plan page when setting up a planned run. The entered text is associated with the Torrent Suite planned run and can be extracted using APIs for Laboratory Information Management System (LIMS) consumption.

The purpose of the metadata field is to allow users to insert arbitrary strings during Torrent Suite™ driven planning that a LIMS system can use to identify runs during post-processing (via a plugin or querying the REST API).

If the LIMS system is driving planning through the API, then it fills in the metadata field directly. However, during manual plan creation, the user can also use this field to provide hints to the LIMS system to link with external data later.

Specifics of content should be determined by the users' LIMS system. This text field is essentially a hook for external systems, but is likewise user driven and user visible. Use your laboratory protocols to determine what types of data to retrieve from this field.



1. Enter text in the text entry box is at the bottom of the Plan page in the planned run setup wizard.
2. View the text on the Review Plan screen (**Data ▸ Completed Runs & Results ▸ Review Plan**.)

Approve User Account Requests

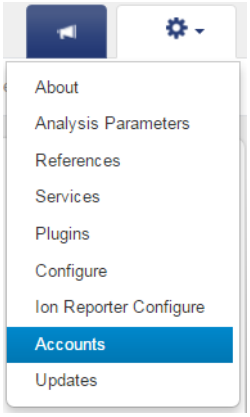
New users can request accounts on the Torrent Browser login page. An admin must approve each request in the admin Account tab before the new account is active.

An `ionadmin` account is required to approve a user account request.

Approved accounts are created with `ionuser` permissons.

Notification

1. Open the admin Accounts tab with the gear menu (near the top right of the Torrent Browser). Click the **Accounts** option:



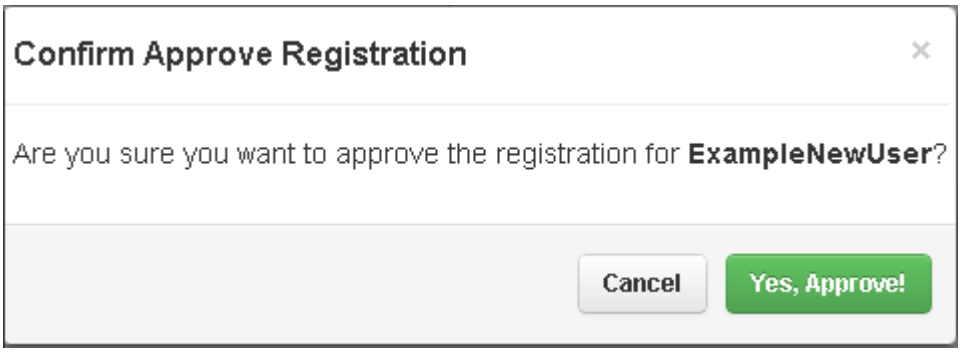
2. When user account requests are pending, the Accounts tab contains notifications such as the following:

   New pending user registration for 'ExampleNewUser'. Please visit Account Management (as an admin user) to review.

Approval and rejection

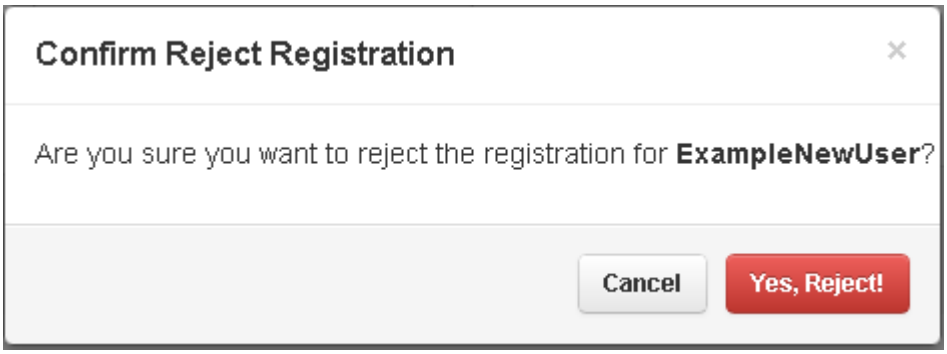
The User Registration section of the Admin Accounts tab shows the pending requests for new user accounts:

To approve a new account, click the green **Approve** button in the User Registration section, and in the popup, click the **Yes, Approve!** button:



When you approve an account request, the account status is changed to Active in the user database and the user can log into the Torrent Browser.

To reject a new account request, click the red **Reject** button in the User Registration section, and in the popup, click the **Yes, Reject!** button:



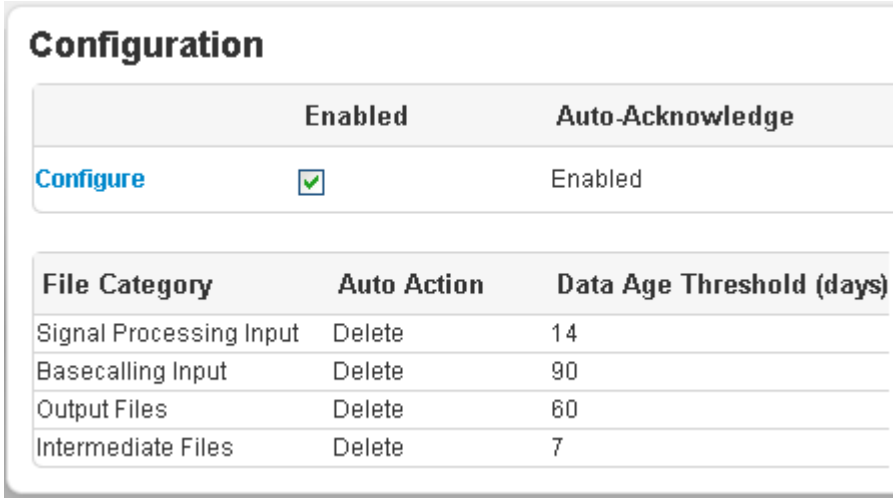
Data Management tab

In the **Data > Data Management** tab, you perform the following data management tasks:

- Configure data management rules for archival, deletion, export, or protecting results sets and file categories
- Import files from removable media
- Monitor disk usage and free disk space
- View active data management jobs
- View data management statistics
- Manually change data management settings for specific analyses

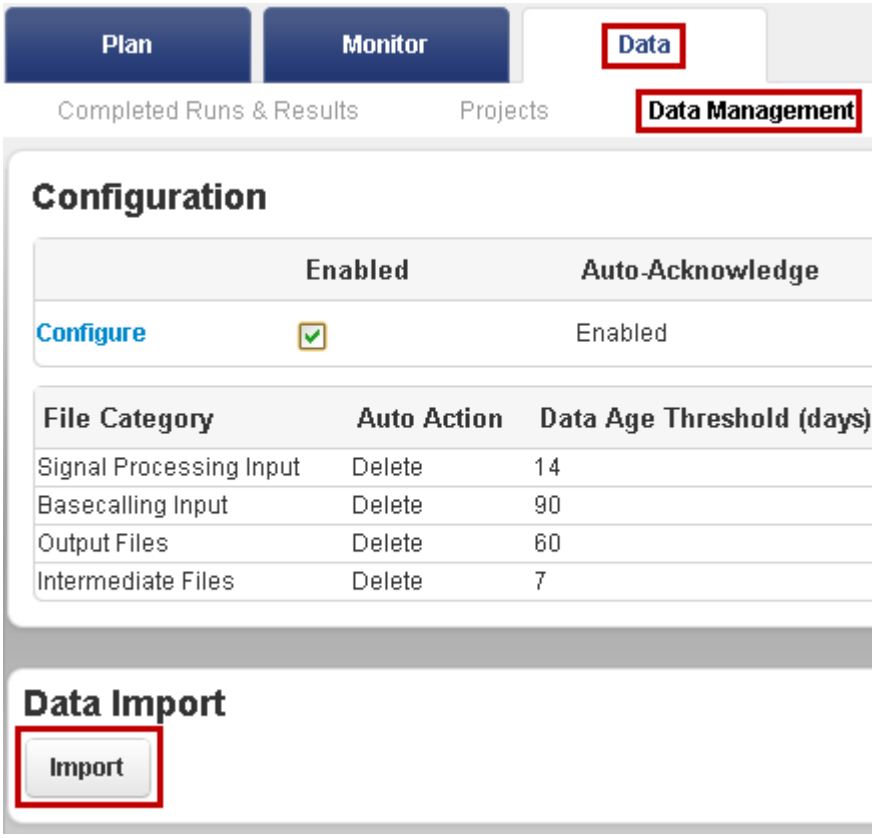
Configuration

The Configuration link opens the Data Management Configuration tool, where you set data management rules for archival, deletion, export, or protecting results sets and file categories. You define automatic data management action based on file categories (such as output files and intermediate files), run report age rules, and other factors.



Data Import

Use the **Import** button to import files from external media:



Import brings in your selected files categories of previously exported or archived runs.

Notes about the **Import** button:

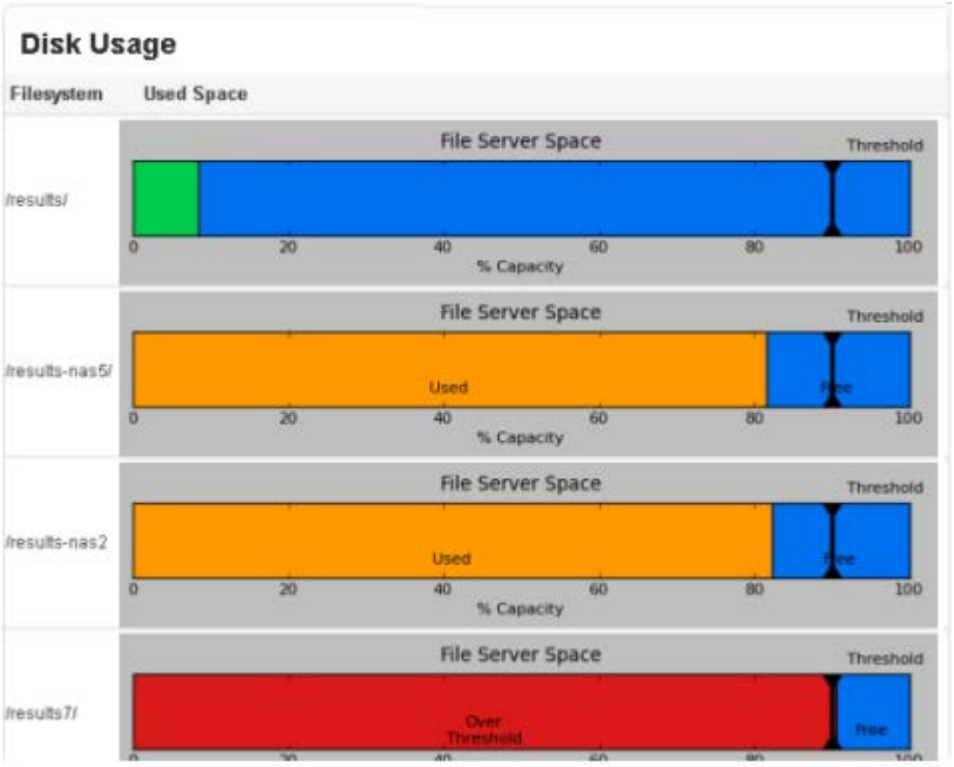
- After the import, you can use these files just as if they are normal analysis files.
- Import cannot retrieve file categories that were notpreviously exported or archived. For instance, if you import files from an archive that does not include the Signal Processing Input or Basecalling Input categories, the Import action cannotretrieve these files for you.
- If exported or archived files are still on your local Torrent Server, you do not have to import them. The Torrent Browser can use these files directly in their exported or archived location. This is also true if the exported or archived location is on media that is currently mounted.

Disk Usage

It is critical that enough disk space is available on the server to avoid data loss. It is important to have a strategy that periodically monitors disk space and archives or deletes data as needed.

Graph usage indicators

The **Data > Data Management** tab Disk Usage section reports disk space usage for both server file systems and archive locations:



The color keys are explained in this table. The descriptions are based on the default disk usage threshold of 90%. (The threshold for the Signal Processing Input file category is used if your file categories have different thresholds.)

Color	Meaning
Green	Your Torrent Server hard drive is less than 70% full. The height of the green bar corresponds to the percentage of disk space in use.
Orange	Your Torrent Server hard drive is between 70% and 90% full.
Red	Your Torrent Server hard drive is more than 90% full.
Blue	Free space.

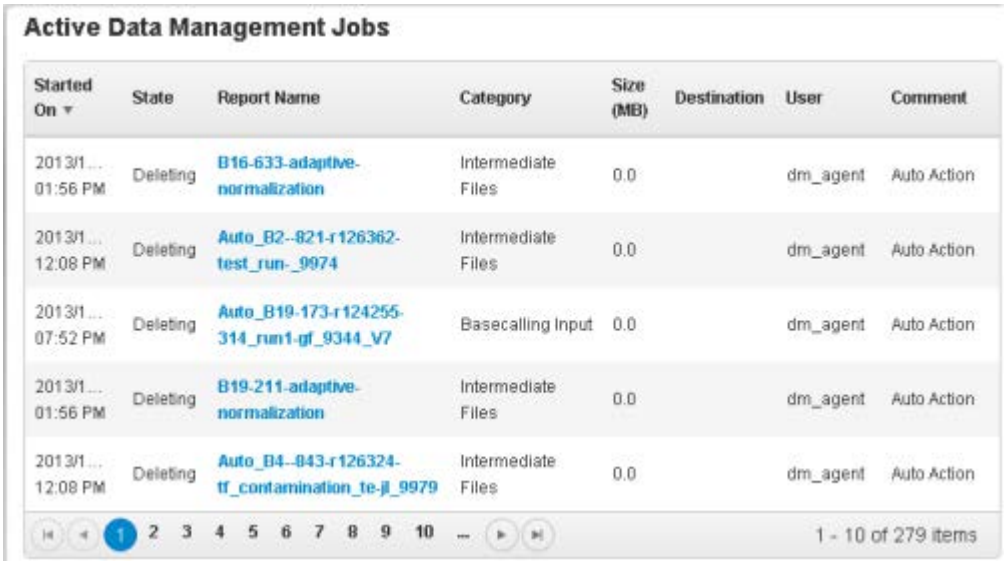
Error messages

Monitor the Disk Space Management section for messages that require administrator 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.

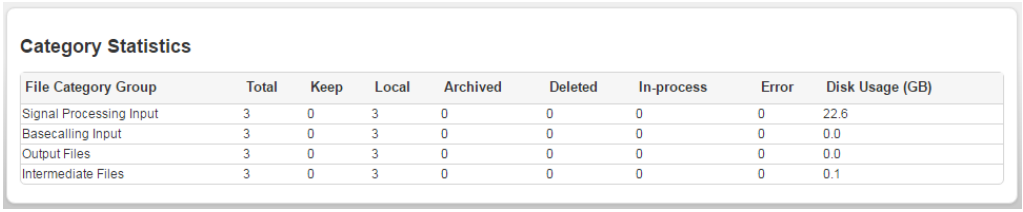
Active Data Management Jobs

This section lists processes that are carrying out either automatic data management rules or manual data management actions. Each entry lists the report name, the file category involved, the size of those files, and whether the job is initiated by an automatic rule or manual action. For archival and export jobs, the destination media is also listed.



Category Statistics

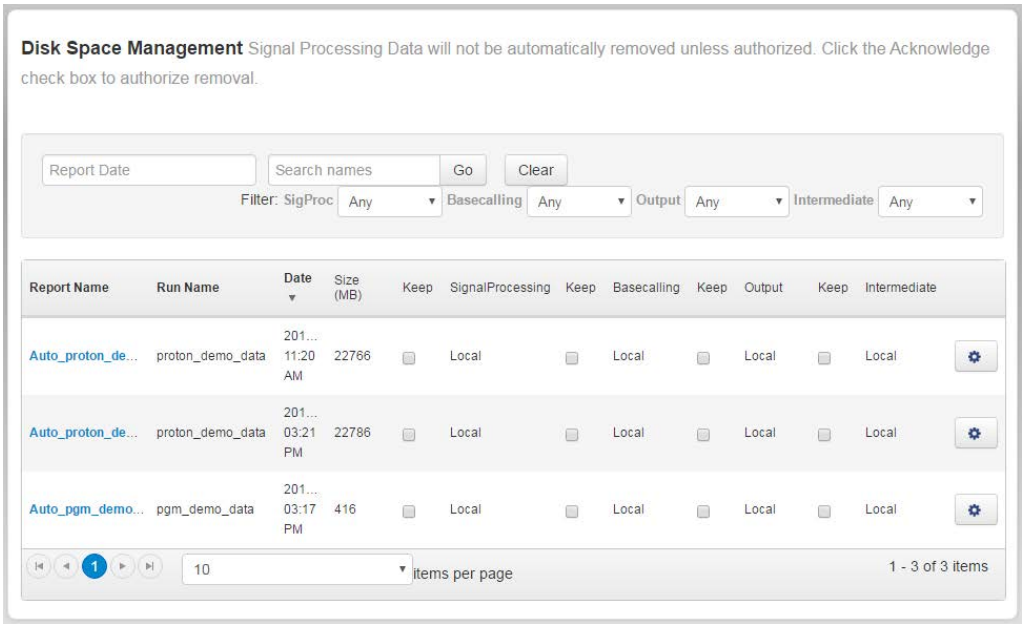
This table tracks the size of the file categories currently on your system (in the Local column), and shows the totals of file categories that have been removed form your system by data management archival and deletion. Error totals for data management jobs are also given.



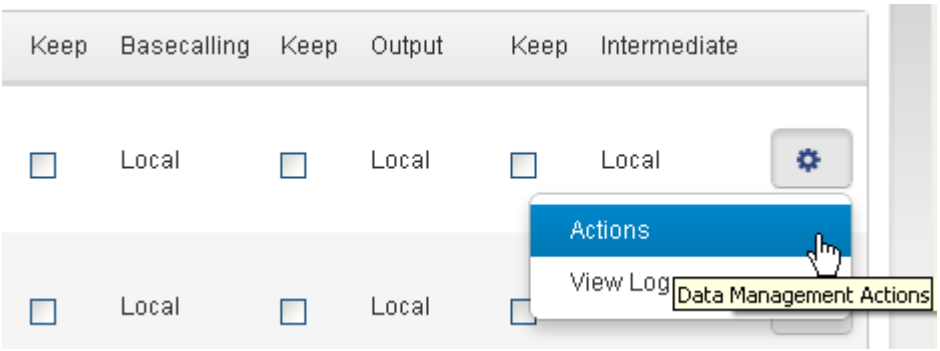
Note: Error column displays the count of file categories that are currently in an error state. If a data management action is re-run on one of these file categories and completes successfully, then that file category no longer appears in the error count.

Disk Space Management

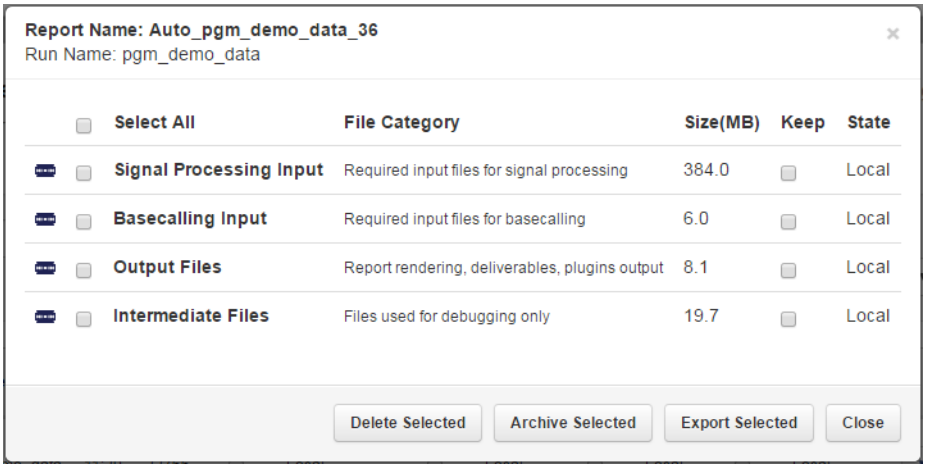
This section lists each run report in your file system partitions:



Click the **Keep** checkboxes for any file categories you want to save. To change the other settings for any report, click the gear menu to the right and click **Actions**:



This opens the Data Management popup, which you use to reset the data management actions for this report or to initiate deletion, archival, or export on-demand.



Back Up and Restore Data

The Torrent Server maintains the following types of data in separate locations:

- Ion PGM™ and Ion S5™ sequencer data are stored in the `/results/<PGM_Name/S5 Name>` directory, by default.
- Ion Proton™ Sequencer data are stored in the `/rawdata/<Proton_Name>` directory, by default.
- Report data are stored in the `/results/analysis/output/Home` directory, by default.
- Database records are stored in the PostgreSQL database.

The nightly backup of the database is created and 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-8.4 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:

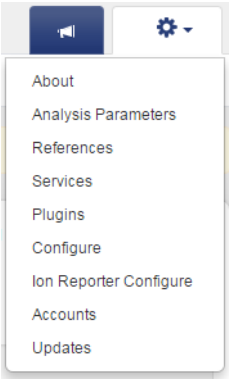
a. Drop database.

b. Create database.

c. Import data.

The Admin Menu

The Torrent Browser Admin pages are accessed through the gear symbol menu near the top right of a Torrent Browser page:



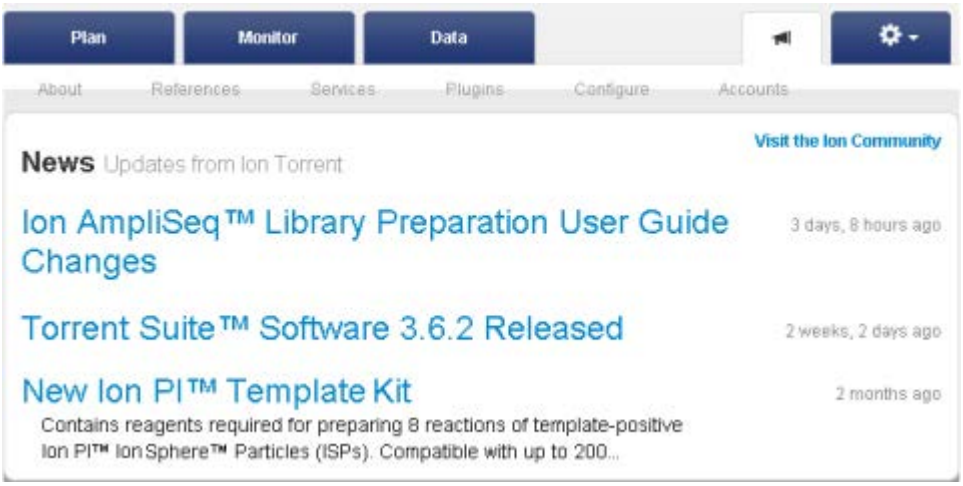
- **About** View information on installed packages and versions, and access links to community, support, documentation, and diagnostic resources.
- **Analysis Parameters** View or copy and customize analysis parameters by chip type.
- **References** Manage your references, regions of interest files, hotspot files, barcode sets, and test fragments.
- **Services** Monitor your system and perform Data Management tasks:
  - View the status of Torrent Suite™ Software modules, running analyses, file system space, and RAID drives.
  - View Data Management logs, and view or configure Data Management settings.
  - Initiate archive, export, or import actions.
- **Plugins** Install, configure, and manage plugins.
- **Configure** Access the Admin Interface, and configure contact information, your Torrent Server name, and email addresses.
- **Ion Reporter Configure** Configure one or many Ion Reporter accounts.
- **Accounts** Configure your user profile, and respond to requests for new user accounts.
- **Updates** Select and install product and plugin updates that occur between software launches.

Announcements

The Torrent Browser provides announcements of product updates and releases from Ion Torrent™.

The announcement flag next to the admin gear menu shows the number of new Ion Torrent™ announcements, if any:

The following is an example announcements listing:



Click on a headline to see a brief text summary of the announcement.

Introduction

This document supports the deployment and maintenance of the Ion Torrent™ Server.

The Ion Torrent™ Server is a dedicated server that performs DNA sequencing analysis of data received from the Ion Proton™ and Ion Personal Genome Machine™ (Ion PGM™) Sequencers.

Audience

This document is intended for system and network administrators responsible for setting up and maintaining server hardware, software and network connectivity. By presenting typical use cases, we identify best-practice operational scenarios to ensure that your system installs correctly and continues to run smoothly.

Server configuration

Dell® T630 for Ion S5™ Sequencer

Feature	Ion Torrent™ Server
CPU	Two Intel® Xeon® CPU E5=2680 v3, 12 cores @ 2.50GHz
GPU	NVIDIA®Tesla K40 GPU
RAM	128 GB
OS	Ubuntu®14.04 Server
Network controllers	Two Intel® Built-in Ports Broadcom Quad-Port NIC
Hard Drives	10 4-TB hard drives in RAID6 25 TB usable space
Optical Drive	None
Keyboard, Mouse	Included

Dell® T620 for Ion Proton™ Sequencer, Rev 2

Feature	Ion Torrent™ Server
CPU	Two Intel® Xeon® CPU E5=2690 , 8 cores @ 2.60GHz
GPU	NVIDIA®Tesla K20 GPU
RAM	128 GB
OS	Ubuntu® 10.04 and 14.04 Server
Network controllers	Two Intel® Built-in Ports Broadcom Quad-Port NIC
Hard Drives	12 3-TB hard drives in RAID5 25 TB usable space
Optical Drive	None
Keyboard, Mouse	Included

Dell® T620 for Ion PGM™ Sequencer

Feature	Ion Torrent™ Server
CPU	Two Intel® Xeon® CPU E5=2670 , 8 cores @ 2.60GHz
GPU	NVIDIA®Tesla K20 GPU
RAM	64 GB
OS	Ubuntu® 10.04 and 14.04 Server
Network controllers	Two Intel® Built-in Ports Broadcom Quad-Port NIC
Hard Drives	6 3-TB hard drives in RAID5 12 TB usable space
Optical Drive	None
Keyboard, Mouse	Included

Dell® T7500 PGM Sequencer

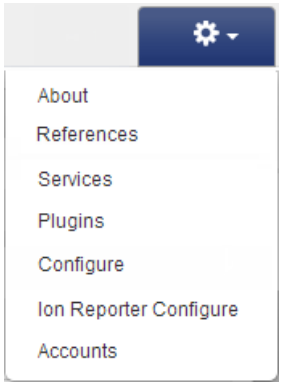
Feature	Ion Torrent™ Server
CPU	Two Intel® Xeon® 5650 6-core processors @ 2.67 GHz
GPU	NVIDIA® Tesla C2075 GPU
RAM	48 GB
OS	Ubuntu® 10.04 and 14.04 Server

Feature	Ion Torrent™ Server
Network controllers	Broadcom Quad-Port NIC
Hard Drives	8 2-TB hard drives in RAID 5 12 TB of usable storage
Optical Drive	Included
Keyboard, Mouse	None

For more information on Ubuntu® technical specs, including version numbers of other associated software packages like Apache™ Software and PostgreSQL, please visit **https://help.Ubuntu.com/community/Server/TechSpecs/1004LTS**.

Admin tabs

Use the admin gear menu to access the admin areas of the Torrent Browser:



- **About** View information on installed packages and versions, and access links to community, support, documentation, and diagnostic resources.
- **References** Manage your references, regions of interest files, hotspot files, barcode sets, and test fragments.
- **Services** Monitor your system and perform Data Management tasks:
  - View the status of Torrent Suite™ Software modules, running analyses, file system space, and RAID drives.
  - View Data Management logs, and view or configure Data Management settings.
  - Initiate archive, export, or import actions.
- **Plugins** Install, configure, and manage plugins, including the Ion Reporter™ Uploader plugin.
- **Configure** Access the Admin Interface and Report Data Management settings, and configure contact information, your Torrent Server name, and email addresses.
- **Accounts** Configure your user profile, and respond to requests for new user accounts.

Deploy Your System

This section covers initial Torrent Server installation procedures, from the time when your system is delivered to when you are able to run your first analysis.

IMPORTANT! You must use your `ionadmin` account for steps described in the Torrent Suite™ Software Administration Guide. (Do not use your `ionuser` account.)

Prepare Your Site

The Torrent Server requires internet connectivity to install software updates and patches from Ion Torrent™. To facilitate deployment, it is important that you prepare your site network infrastructure in advance.

IMPORTANT! On the scheduled deployment date, IT support personnel should be scheduled to be available to ensure that the Torrent Server is properly connected to the network and, if needed, assist the FAS in troubleshooting connectivity issues.

Review the following topics to prepare your site for server installation:

Network connectivity preparation

Network provisioning

Network provisioning should be completed before the scheduled installation:

- A network access jack should be identified and enabled in the area where the Torrent Server will be deployed. An Ethernet cable is provided with the server. The cable connects to the port labeled **LAN** on your Torrent Server; the remaining ports are used to connect to the Ion Proton™ and Ion PGM™ Sequencers.
- DNS should be configured in advance of the scheduled installation.

Port assignment

To fully support the 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 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.

Name resolution

A DHCP-assigned address is recommended. (The Torrent Server can be reconfigured to use a static IP address, if necessary.)

Server run, report and configuration data are accessed using your workstation Web browser. You must be able to access the server UI by entering the server hostname in your browser URL address field.

Install the Server

IMPORTANT! Server installation should only be done by a Thermo Fisher Field Service Engineer (FSE).

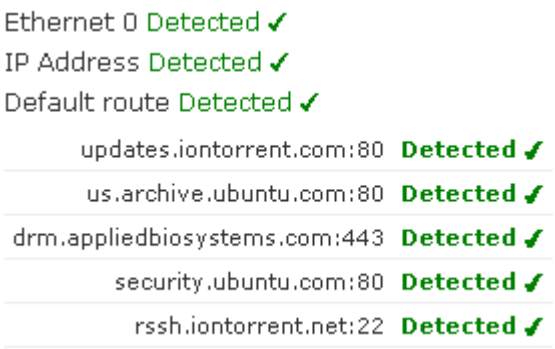
Server installation includes the following activities.

Network Connection

Use the following procedures to verify correct network configuration.

Test network connectivity (preferred)

The automated network tests (requires an `ionadmin` account).



Alternate network connection tests

The server is configured with DHCP. If DHCP is configured in the site network, the server should have already acquired an IP address. Verify that the server has acquired an IP address using the following command.

```
ifconfig eth0
```

To test connectivity for updates, type the following command:

```
links -dump http://updates.iontorrent.com/
```

This should return:

```
Welcome to the Ion Torrent™
 Updates Server. ----------------------------------------------
[ICO] Name Last modified Size Description
------------------------------------------
```

followed by a few [DIR] lines.

If not, check that the light is on, for the port connected to the network. Try to ping other on-site servers by name and IP address.

Access the web browser

Use the following procedure to test web browser access.

IMPORTANT! To access the web browser, the site IT administrator may need to configure the hostname to point to the IP address of this server. Some networks may handle this automatically.

---

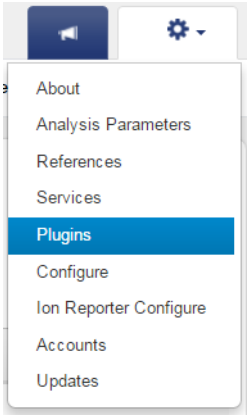
1. Go to another desktop or laptop on the network.
2. Open a browser and enter either **http://ion-torrent-server** or the new host name, as applicable.
3. If the server does not respond, try using `http://<<ip-address>>`. If you are able to open the browser, it indicates there is a name resolution issue on the network, which must be fixed.
4. If there is still no response, `ping` the hostname and the IP address of the server.

Update Torrent Suite™ Software

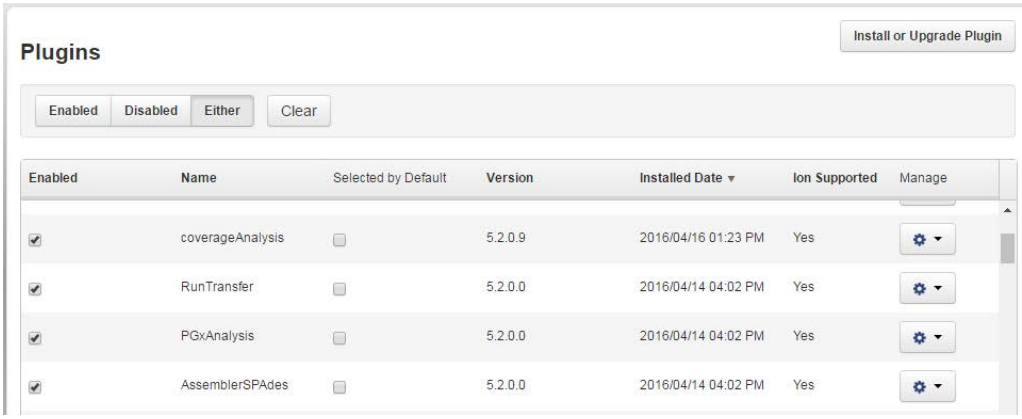
These instructions describe how to update your Torrent Suite™ Software to a new version. Visit the Management Actions page for instructions to update Torrent Suite™ Software.

Next steps

After upgrading Torrent Suite™ Software, click the Admin gear menu and select **Plugins**:



Check the list of enabled plugins:



Manually enable plugins of interest by checking the checkboxes in the Enabled column.

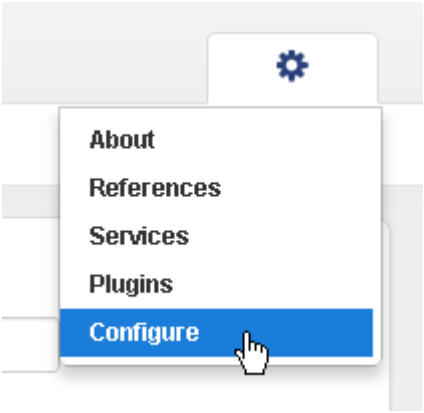
Configure Torrent Suite™ Software

IMPORTANT! Follow these configuration steps only after updating the Torrent Suite™ Software version.

Torrent Suite™ Software works out-of-the-box.

Configuration tab

Some configuration tasks use the Linux™ command line and some use the Torrent Browser configuration tab. To access the Torrent Browser configuration tab, use the gear menu near the top right of a Torrent Browser page and select **Configure**:

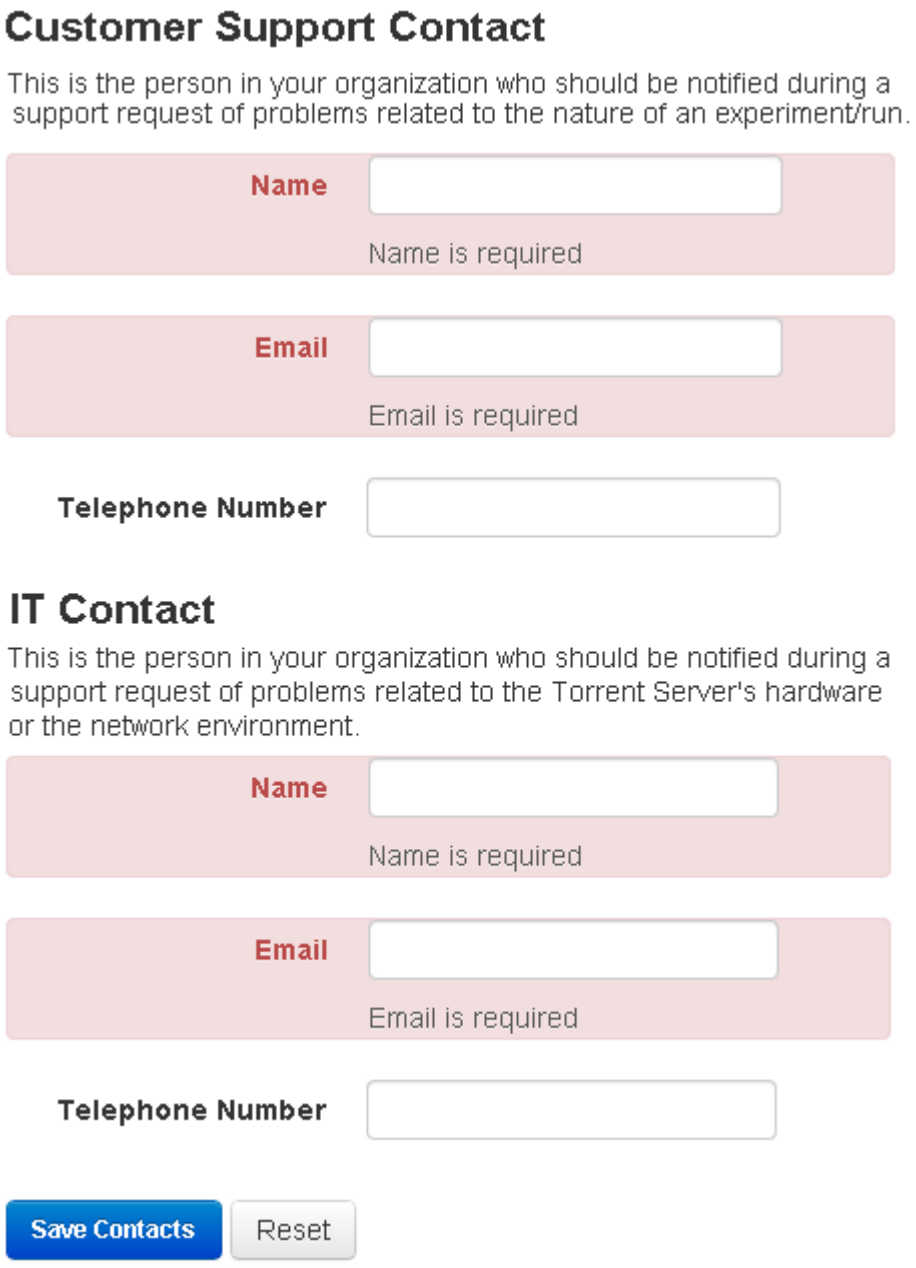


Configure disk space notification email address

The Data Management system can automatically notify you when available disk space on a Torrent Server disk partition becomes low. However, in order to receive these notifications, you must configure an email address in the Data Management system.

Configure contact information

Add the name, email address, and phone number of the person responsible for the server and of an I.T. contact person, and click the **Save Contacts** button:



Change the hostname

Use the following command to change the hostname:

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

The server must reboot after the hostname is changed. The above command automatically reboots the server.

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
```

Configure HTTPS

The Torrent Server is shipped with a self-signed SSL certificate that allows you to enable HTTPS quickly but provides a limited degree of security. Torrent Suite™ Software administrators who are interested in securing the connection further should read the Ubuntu® Server Guide on Certificates and Security, and investigate getting either an SSL certificate signed by their organization or a trusted Root Certificate Authority.

For more information on the level of security provided by Ubuntu®, see the following site for 10.04: **https://help.Ubuntu.com/10.04/serverguide/certificates-and-security.html**, or for 14.04: **https://help.ubuntu.com/14.04/serverguide/certificates-and-security.html**.

With a self-signed SSL certificate, each user sees an error message on his or her first visit to the site and is asked whether or not to trust the certificate.

See also the following Apache™ file for information. This file is installed on your Torrent Server as `/etc/ Apache™ 2/sites-available/torrent-server-ssl.example`:

```
# So, you want to configure HTTPS...

# [Optional] consider creating and signing your own ssl-cert,

# and replace the 'snakeoil' cert below.

?

# Copy this file to torrent-server-ssl, edit as needed? [[ NB:
Step missing in current file... ]]

cd /etc/ Apache™ 2/sites-available

sudo cp torrent-server-ssl.example torrent-server-ssl

?

# To activate this vhost:

?

sudo apt-get install lib Apache™ 2-mod-gnutls ssl-cert

sudo a2enmod ssl

sudo a2dissite default-ssl? ? ## should already be disabled,
but just in case

sudo a2ensite torrent-server-ssl

sudo service  Apache™ 2 restart

?

# [Optional] If you want to block access by plain http,

# you can disable the default site.

# This may block Ion PGM™ Sequencer access to the Torrent
Server.

# Disable at your own risk.

# echo "I'm sure I want to do this." && sudo a2dissite torrent-
```

```
server
```

```
?
```

```
# [Optional] Enable http site which redirects to https,
forcing https access
```

```
# a2ensite force-torrent-server-ssl
```

```
?
```

IMPORTANT! The Ion PGM™ and Ion Proton™ sequencing instruments do not support HTTPS. If you configure HTTPS on the Torrent Server, ensure that Apache™ is running both HTTPS and HTTP concurrently, so that the sequencing instruments can connect to the Torrent Server over HTTP. If you disable HTTP, the sequencing instruments are not be able to connect to the Torrent Server.

Change the timezone

Use the following command to change the timezone:

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

Configure Postfix for nightly email

Postfix is a Linux™ mail server ("post office") program. It is open-source software and was not designed by Ion Torrent™. It is more similar to Exchange than Outlook® software in that it is an e-mail server, not an e-mail client. Postfix is designed to work on various infrastructures, so there are many configuration options for IT administrators to adjust mail routing parameters.

You can find the official Postfix documentation here: **http://www.postfix.org/documentation.html**.

Prepare site IT

It is useful to confirm the following with the site IT administrator:

- **Outbound email from the server is allowed** Many sites block the ability for machines to send an e-mail message directly to a mail server because this is a common avenue for virus propagation. If the site has such a restriction in place, the IT administrator may need to make an exception for this server.
- **Ideally, the domain is configured with a default "MX record"** The "MX record" is the mail exchange information that automatically knows how to handle any email generated from the Torrent Server. If this is not in place, the nightly email might not work.

Add email recipients

We recommend that you add only two recipients while you are testing.

Follow these steps to enter the destination e-mail addresses.

Add Web Root and Site Name to Admin and Global Config

Make certain these fields are configured in the database so they will show up correctly on the e-mail message:

1. Click the Torrent Browser gear menu and select **Configure**.
2. Scroll down to the Database Administration section and click **Admin Interface**. If prompted to log in, use your `ionadmin` account.
3. Click on **Global configs**:

4. Make certain a value is entered in the **Web root** field and the **Site name** field, as shown in the example:

The value for **Web root** should be the main URL that people use to access this Torrent Browser from the desktop.

Configure Postfix for nightly email

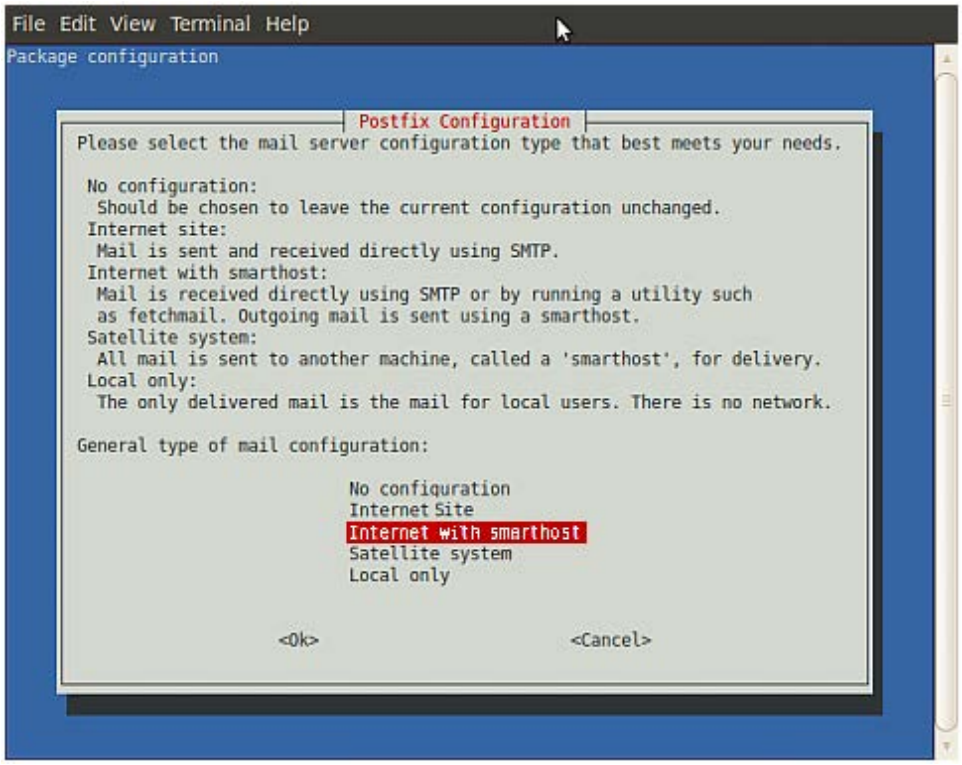
There is no way to know it advance what Postfix configuration settings work for a particular site. The following example procedure shows basic configuration settings that have worked for the servers configured to date. There is no guarantee that these steps work for every situation. If email is still not appearing, you almost certainly require assistance from the site IT administrator, who may or may not have experience with Postfix.

This configuration can be re-executed at anytime:

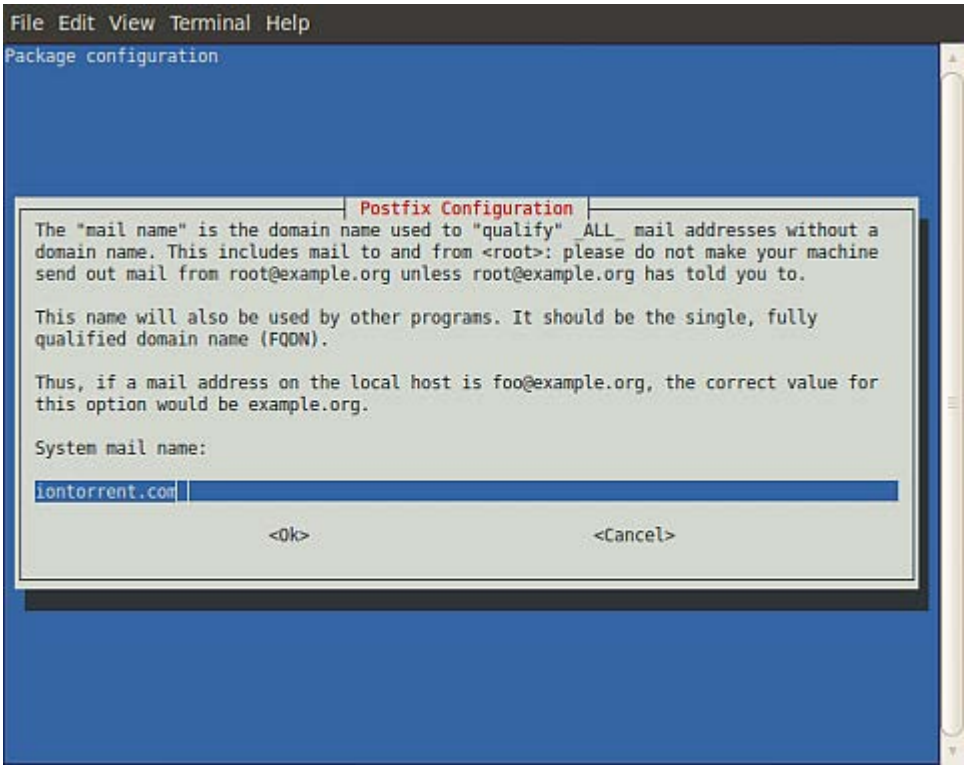
1. Go to the Linux™ command line and start the Postfix configuration utility:

```
sudo TSconfig --configure-postfix
```

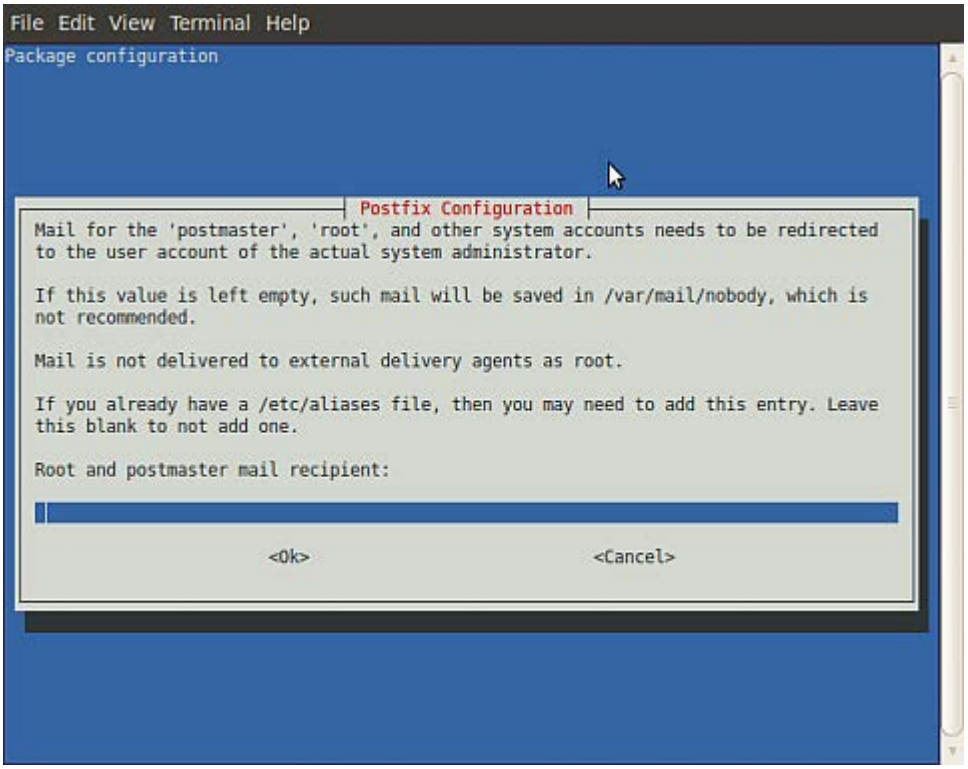
2. Select the type of mail configuration. Typically, **Internet with smarthost**.



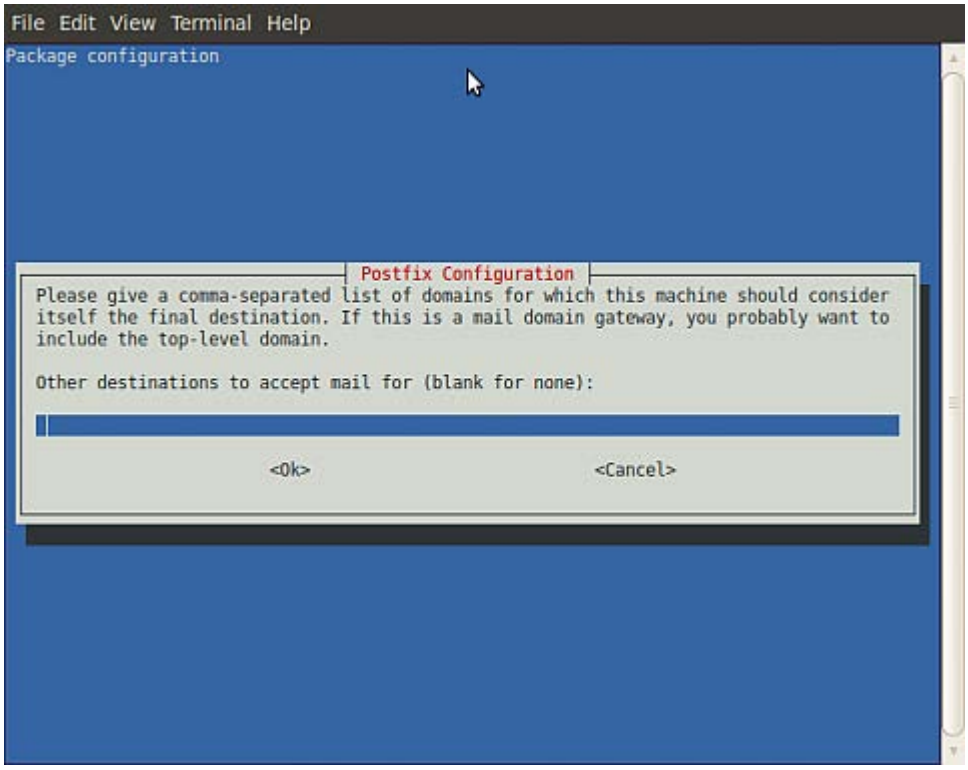
3. For **System mail name**, enter the site domain name. This domain needs to be configured with a default "MX" (mail exchange) record.



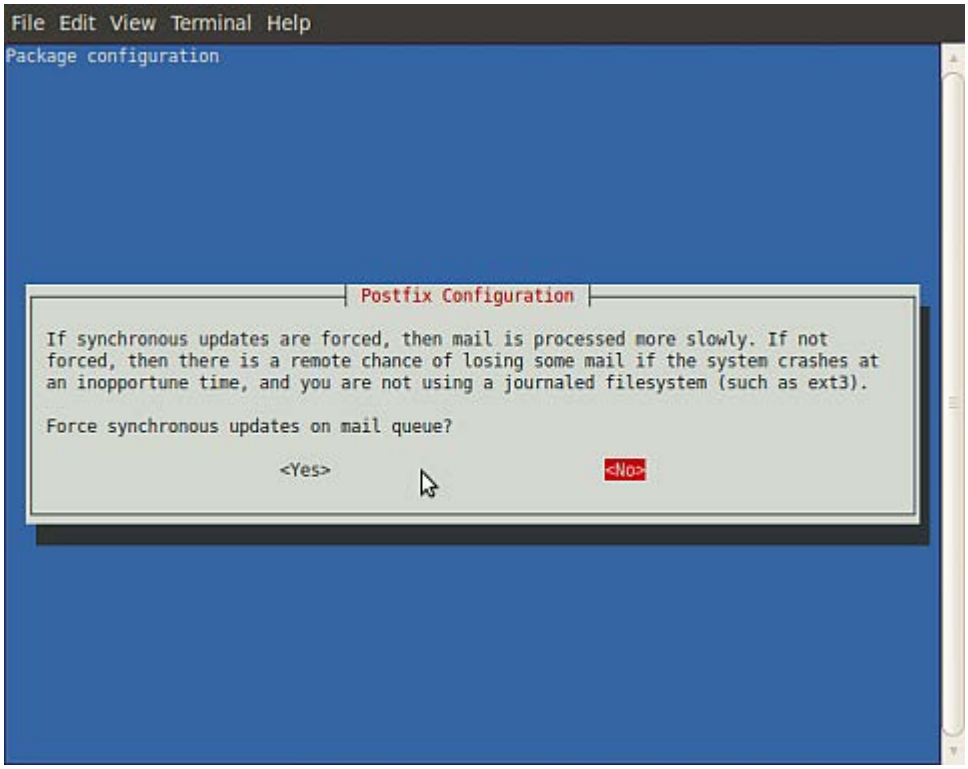
4. Leave **mail recipient** blank.



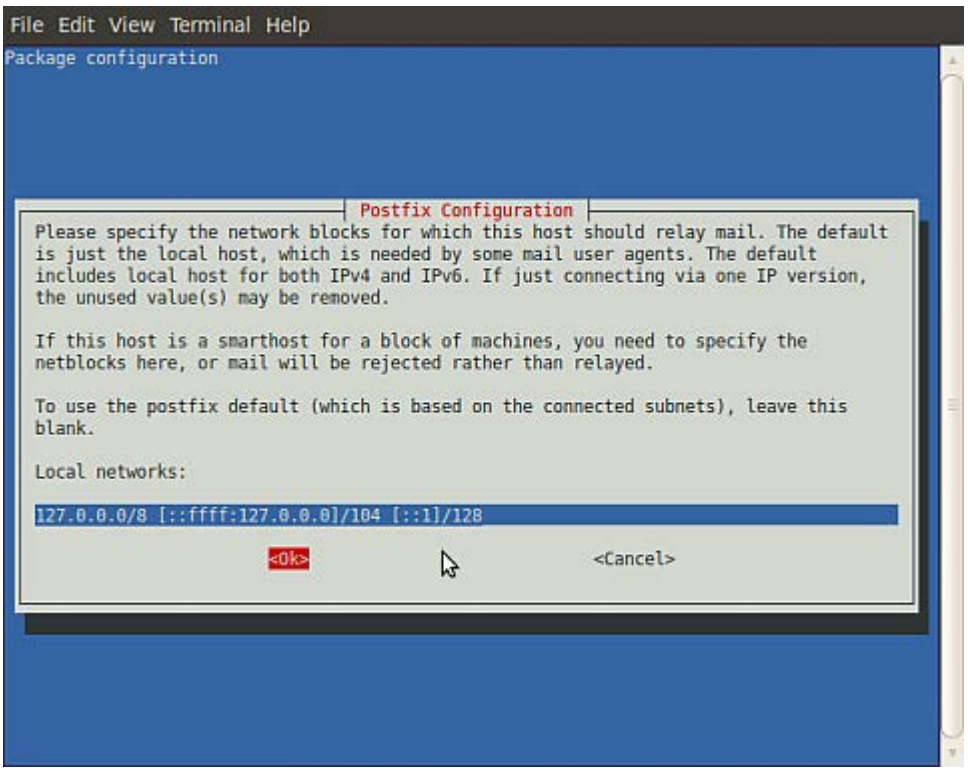
5. Leave **other destinations** blank.



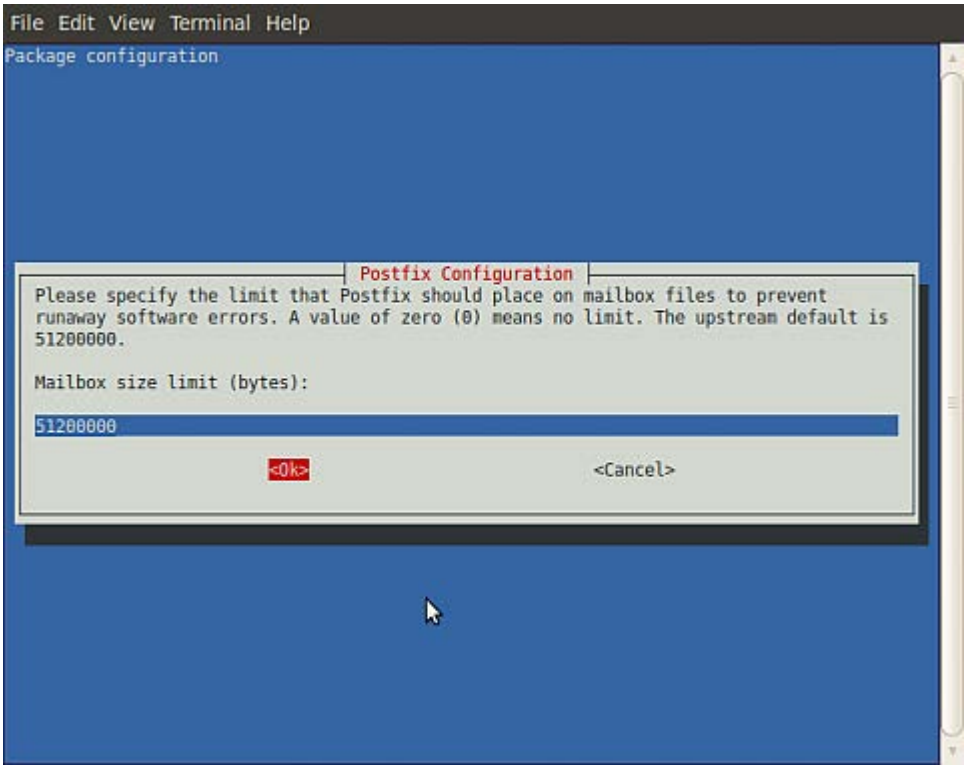
6. Set **Force synchronous updates on mail queue** to **<No>**.



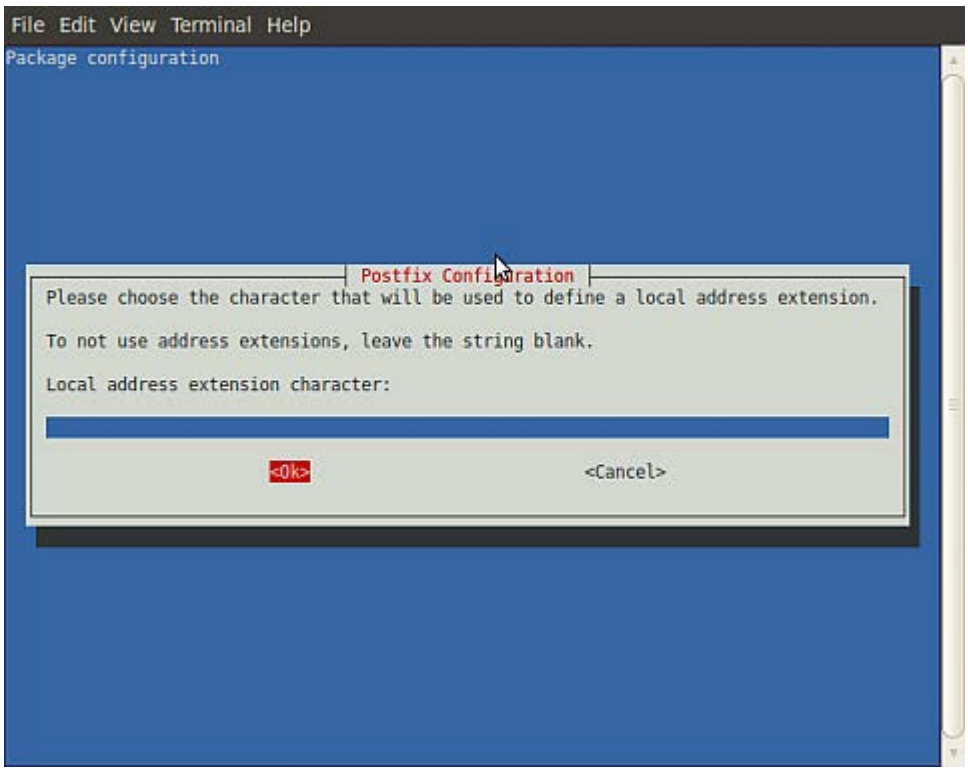
7. For **Local networks**, accept the default, and select **<OK>**.



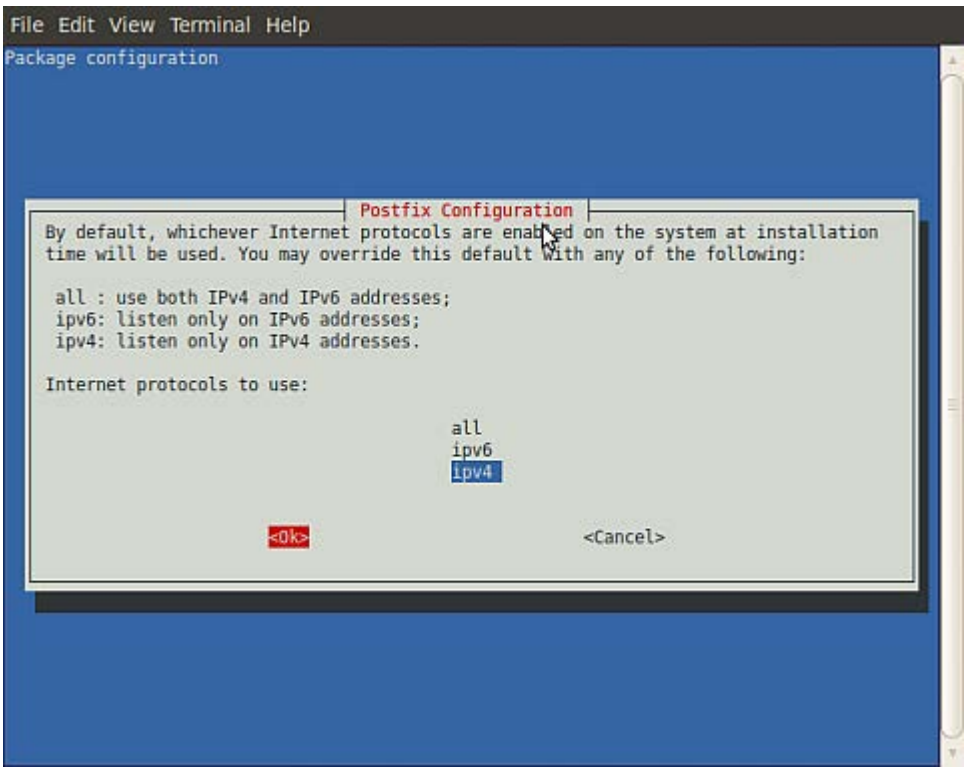
8. For **Mailbox size limit**, it is recommended that you not have no limit, so enter "51200000" to not consume all disk space.



9. Leave **Local address extension character** blank.



10. For **Internet protocols to use**, accept the default and select **<OK>**.



11. After the last screen, the console returns to text mode and displays the status of the Postfix configuration files update.

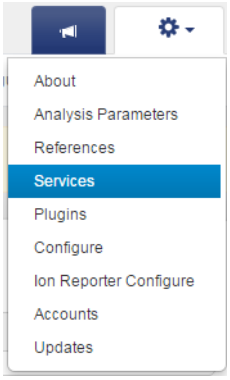
```
Running newaliases * Stopping Postfix Mail Transport Agent
postfix [ OK ] *
                Starting Postfix Mail Transport Agent postfix
[ OK ] root@kauai:~# sudo
                dpkg-reconfigure postfix * Stopping Postfix
Mail Transport Agent postfix [ OK ]
                setting synchronous mail queue updates: false
setting myorigin setting destinations:
                setting relayhost: setting mynetworks:
127.0.0.0/8 []/104 []/128 setting
                mailbox_size_limit: 51200000 setting
recipient_delimiter: setting inet_interfaces:
                all setting inet_protocols: ipv4 WARNING: /etc/
aliases exists, but does not have a
                root alias. Postfix is now set up with the
changes above. If you need to make
                changes, edit /etc/postfix/main.cf (and
others) as needed. To view Postfix
                configuration values, see postconf(1). After
modifying main.cf, be sure to run
                '/etc/init.d/postfix reload'. Running
newaliases * Stopping Postfix Mail
                Transport Agent postfix [ OK ] * Starting
Postfix Mail Transport Agent postfix [ OK
                ]
```

This completes Postfix configuration.

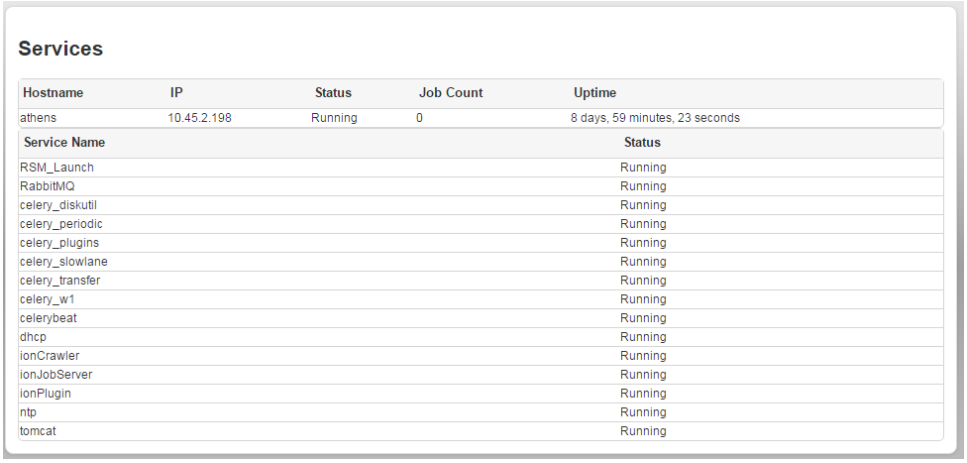
Verify Functionality

This procedure verifies that the server installation and the Torrent Suite™ Software configuration are complete, and that the Torrent Suite™ Software is ready to run analysis programs:

1. Go to the admin Services tab, and view the Service Name table (in the Jobs Server section at the top of the page):



2. All services should have status Running:



Services

Hostname	IP	Status	Job Count	Uptime
athens	10.45.2.198	Running	0	8 days, 59 minutes, 23 seconds

Service Name	Status
RSM_Launch	Running
RabbitMQ	Running
celery_diskutil	Running
celery_periodic	Running
celery_plugins	Running
celery_slowlane	Running
celery_transfer	Running
celery_w1	Running
celerybeat	Running
dhcp	Running
ionCrawler	Running
ionJobServer	Running
ionPlugin	Running
ntp	Running
tomcat	Running

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 Instrument Settings from the Torrent Browser

From your Torrent Browser, you can do the following remotely:

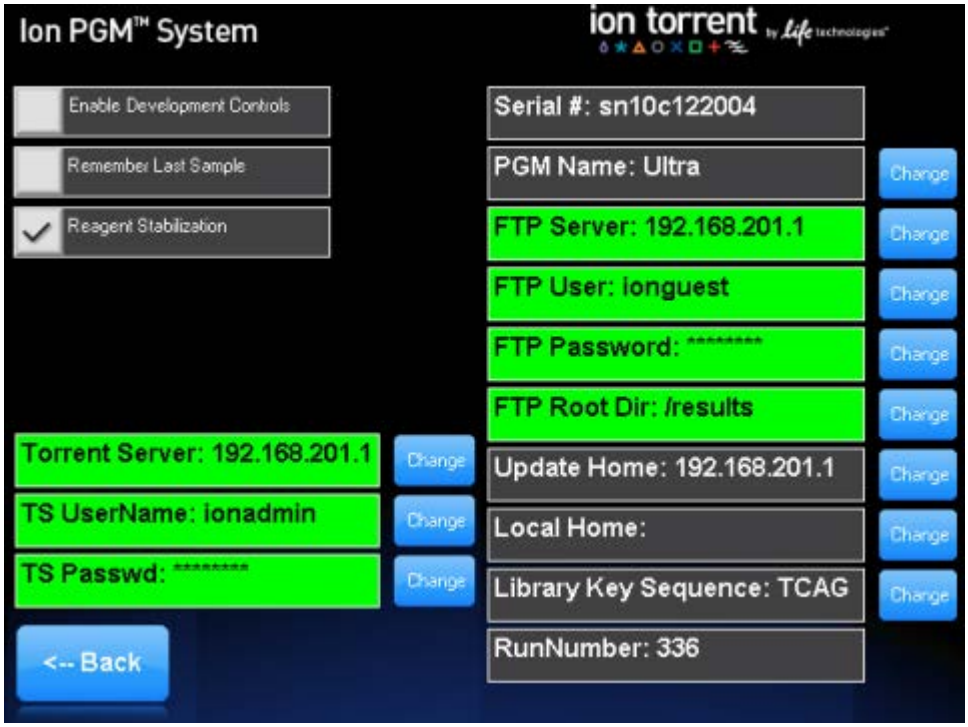
- Set FTP information
- Set Update server address
- Monitor the state of the Ion PGM™ or Ion Proton™ sequencing instrument, such as Idle or In experiment
- View the most recent alarms
- Get the software versions

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, e.g. 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! T he 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.



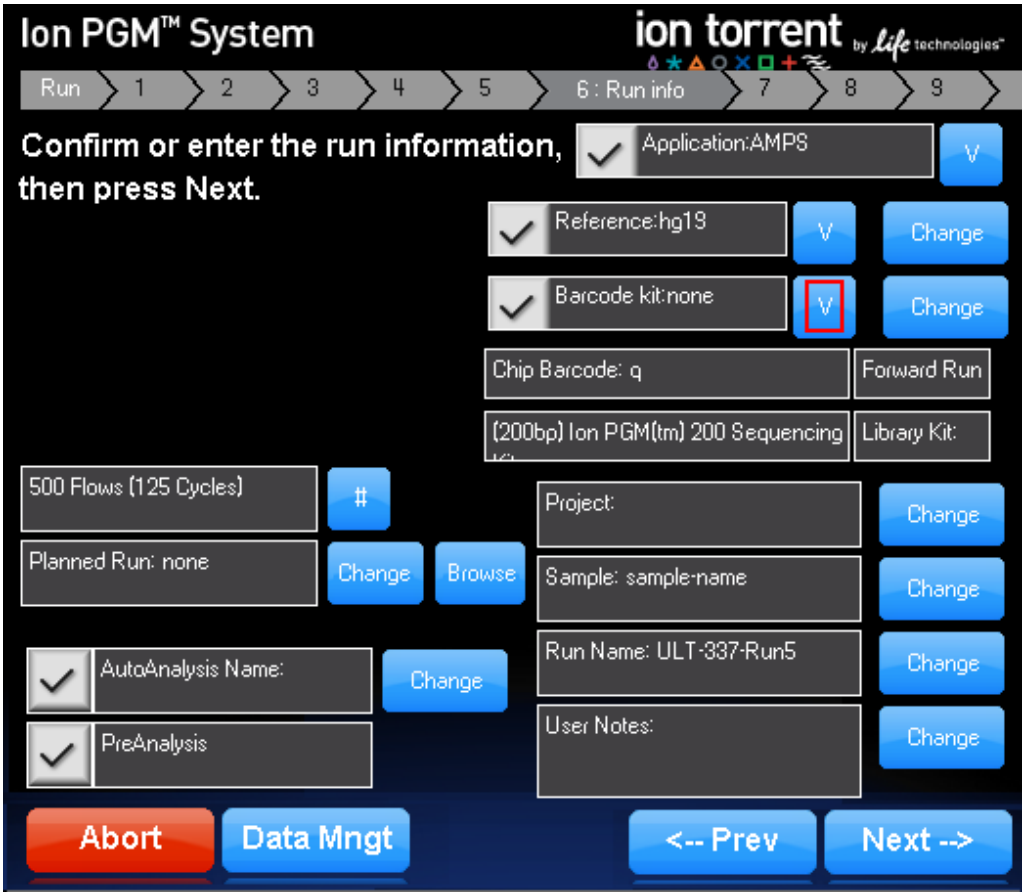
Update information on an Ion sequencer via the Torrent Browser

Ion PGM™ and Ion Proton™ instrument information is stored inside **Rigs** under Site administration. First click on the **Config** tab; under **Database Administration** section, click on **Admin Interface**. It prompts for user name and password. The user needs to have staff status and the permission to update **Rigs** (`rundb | rigs`). By default, `ionadmin` has this permission.

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™ orIon 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. You can go to **Reference** tab (with the Admin gear menu **References** option) on Torrent Browser 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):



View Ion sequencer status and information

You can view the status of Ion PGM™ or Ion Proton™ instrument in the **State** field. It can be *Idle*, *Initing*, or *In Experiment*. You also can view the recent alarm such "No connectivity to ftp server". You can find out the software versions in the **Version** field.

Maintain Your System

Perform the following tasks as needed to maintain your system in good working order.

- Disk space, archival, data:
  1. “Monitor Free Disk Space“ on page 436.
  2. “Back Up and Restore Data“ on page 409.

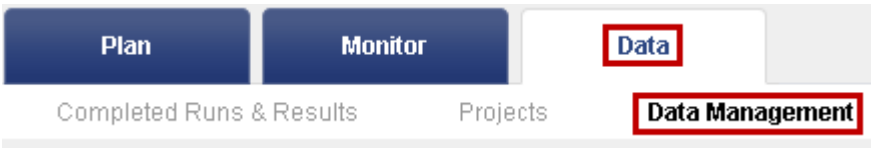
IMPORTANT! You must use your `ionadmin` account.

Monitor Free Disk Space

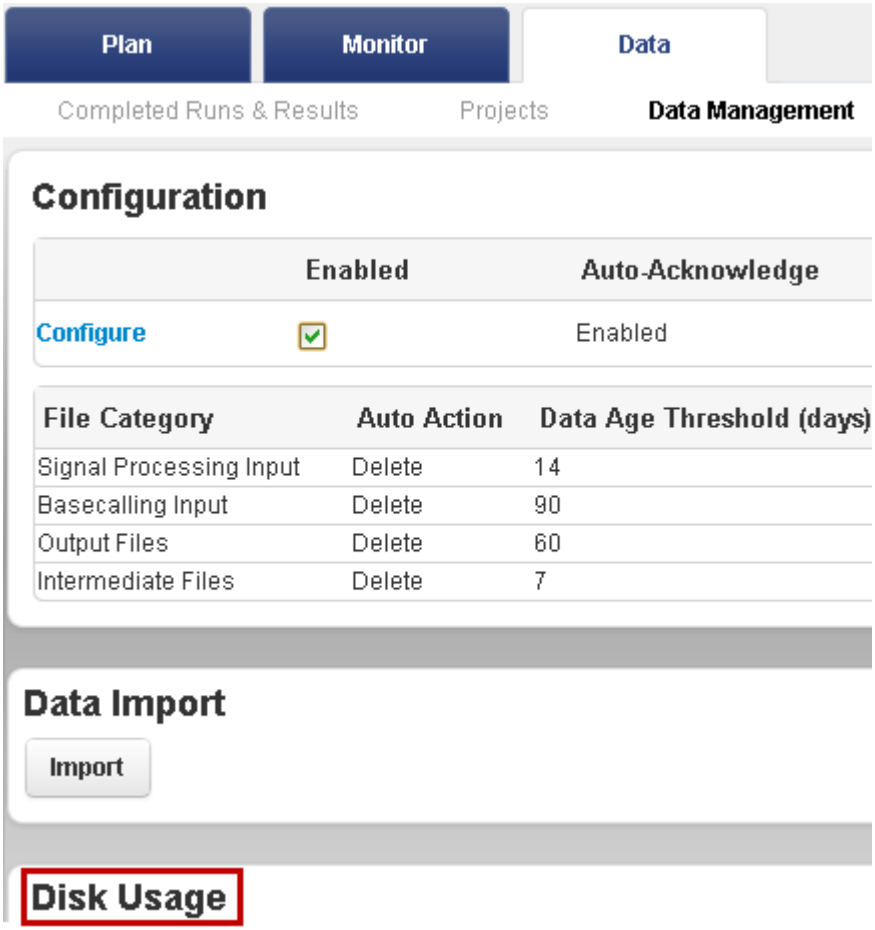
It is critical that enough disk space is available on the server to avoid data loss so it is important to have a strategy that periodically monitors disk space and archives or deletes data as needed.

Disk Usage section

To access the Disk Usage section, click the **Data** tab and the **Disk Management** subtab:

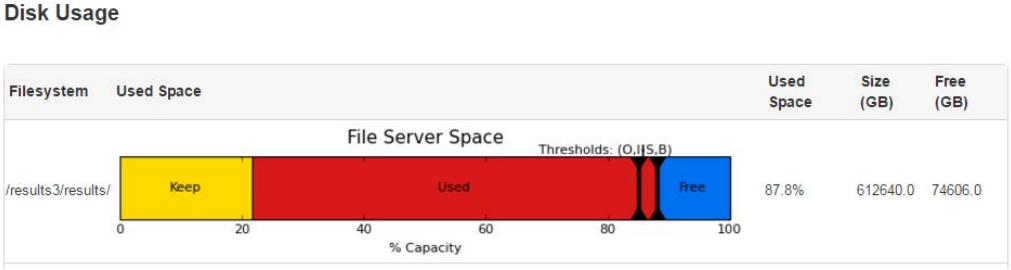


Scroll down to the Disk Usage section:



Usage totals

The Disk Usage section also reports space and usage totals for each file system. These totals appear to the right of the File server Space graphs in the Disk Usage section. (Totals for only one file system are shown here.)



Error messages

Monitor the Disk Space Management section for messages that require administrator 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 messages

When any storage device reaches 95% full (and again at 99%), a warning banner is displayed across the top of Torrent Browser pages:

***** CRITICAL! /results/: Partition is getting very full - 95% *****

IMPORTANT! Torrent Suite™ Software performance is impacted when a disk partition is more than 95% full.

When the Torrent Browser UI is not available

Use the following procedure to monitor disk space when the Torrent Browser UI is not available:

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.

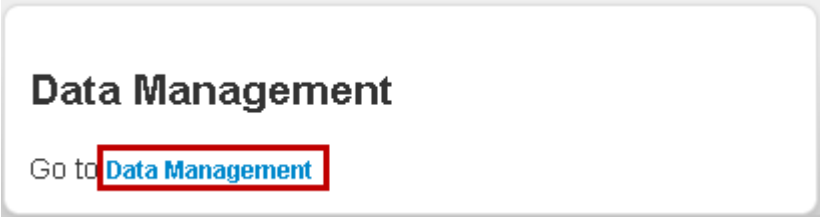
The `Use%` column indicates how much space is being used.

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.

Torrent Browser Data Management features

A disk space monitoring policy can be implemented using the **Data** tab's **Disk Management** subtab. Additionally, your I.T. department may choose to deploy a monitoring tool such as Ganglia.

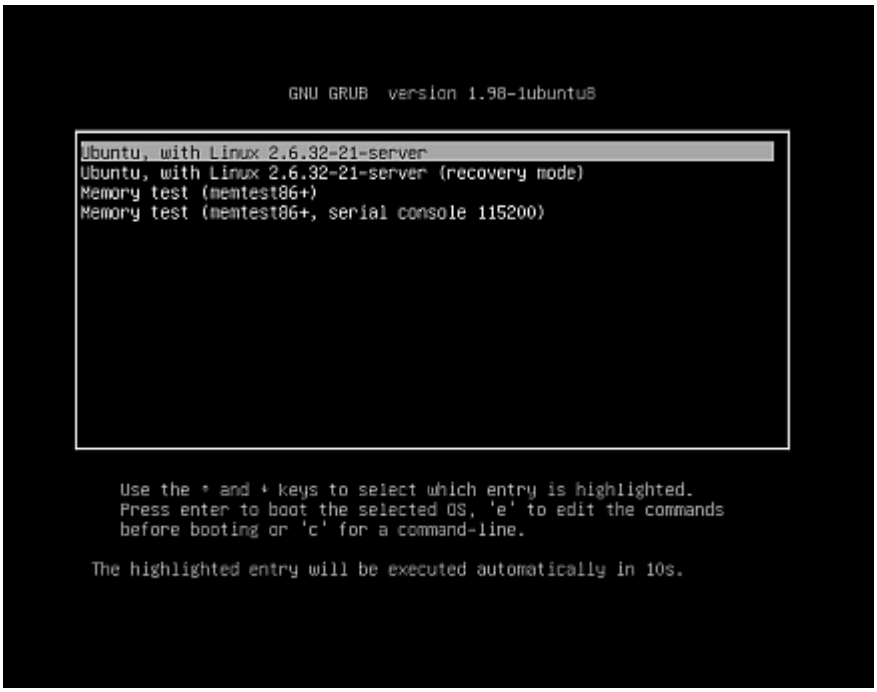
The admin menu Services tab also has a link to the **Data** tab's **Disk Management** subtab (scroll to the bottom of the Services tab for this link):



Boot into Single-User Mode

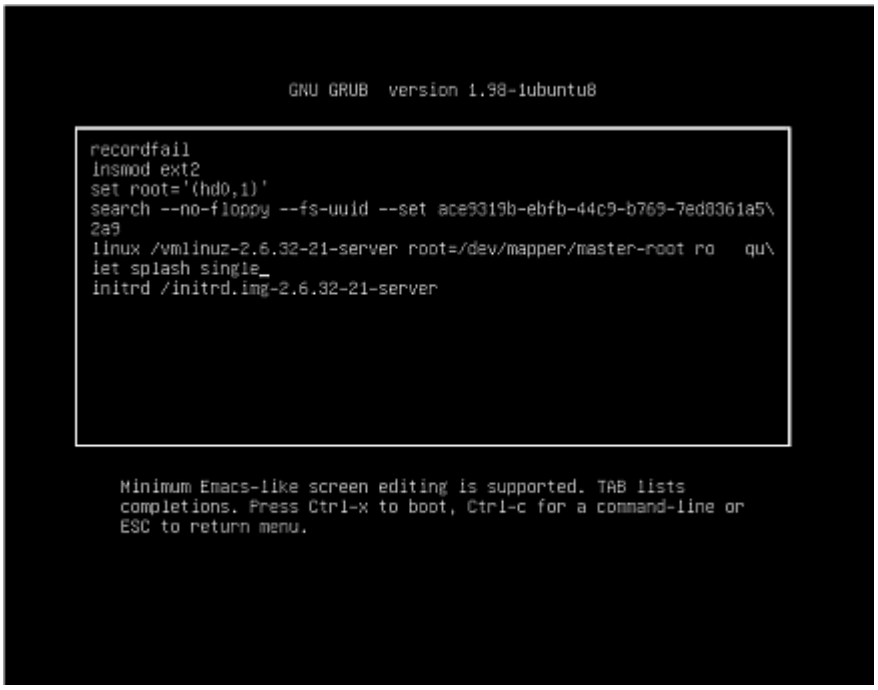
You may sometimes need to boot the Torrent Server host into single user mode. One reason would be if you forgot and need to reset your password.

1. Reboot the server. A hard reboot may be needed if there is no way to login to the system.
2. Immediately after the BIOS completes, press the **Esc** key to display the grub menu:



3. Press the **E** key to edit the boot command line.
4. Scroll down to the **linux ...** command and scroll to the end of the line.

5. Add the word **single** to the end of the line:



6. Press **Ctrl-X** to boot.

7. At the next window, scroll down to the **netroot** option and press Enter:

You are now logged into the system with root privileges.

Mount a USB Drive

This describes the procedure for manually mounting and unmounting an external USB drive. To follow these steps, you should feel comfortable using the Linux™ command line and have a basic understanding of disk drives and partitions.

By default, Ubuntu® Desktop automatically mounts an external USB drive when the drive is attached to the machine, similar to Macintosh™ or Windows™ operating systems.

The Ubuntu® Server, however, does not mount external hard drives automatically, so the `ion-usbmount` utility is included with the Torrent Server Software, which automatically mounts attached USB drives in the `/media` directory. If a particular USB drive is not being automatically mounted by `ion-usbmount`, you may need to mount the drive manually.

Note: These instructions only provide an overview of the required steps, and may be a helpful reminder if you are new to the Linux™ operating system. We recommend a system administrator perform the Linux™ mount and unmount procedures.

For more detailed instructions and background information, refer to the Ubuntu® documentation: **https://help.ubuntu.com/community/Mount**

Mount a USB Drive

To see a list of the drives in the system, type the following command before connecting the USB drive:

```
sudo fdisk \-l
```

Make a note of the drives that are present, so you can be sure which drives are in the server. 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
```

To see a list of drives, including the new drive:

1. Connect the USB drive.

2. Wait about ten seconds and re-type: `sudo fdisk -l`. You should see a new drive. You need to know the device name of your USB drive, which is usually called `/dev/sdb` or `/dev/sdc`, depending on the number of drives installed. The partition is a number appended 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 2GB partition (1953512001 blocks) attached to the system and named `/dev/sdb1`. It is configured with a Linux™ partition. (If the drive was formatted on Windows™, it is either a FAT or 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
```

If the drive is a Windows™ FAT or NTFS partition, reformat the drive as an ext3 partition to preserve the Linux™ file information.

IMPORTANT! Be careful that you are formatting the correct hard-drive!

To reformat the drive as ext3 partition, type `sudo mkfs.ext3 <your_device>`. For example:

```
sudo mkfs.ext3 /dev/sde5
```

Label the partition on external USB drive. To label the partition, type

```
sudo e2label <your_device_place> <partition_label>.?
```

For example, external drive connected in /dev/sdbl will be 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 TS at the same time.

Test to see if external USB drive mounts automatically. Disconnect the external USB drive and connect it back. Wait ~10 seconds. The external USB drive should now appear under the Services tab in Torrent Browser.

Unmount a USB Drive

Before disconnecting a drive, it is recommended that you unmount it first, to ensure that all data has been completely written to disk. If you pull out the USB cable, there is a real risk of data loss.

The command to unmount the drive is almost identical to the command to mount the drive:

```
sudo umount /dev/sdb1 /media/external
```

TorrentNAS storage device

The TorrentNAS is a convenient option for backing up or archiving your run data. This storage device is supported in Torrent Suite Software v5.2 and beyond.

Network Attached Storage (NAS), which supports NFS protocol, can be used as an archive or export directory.

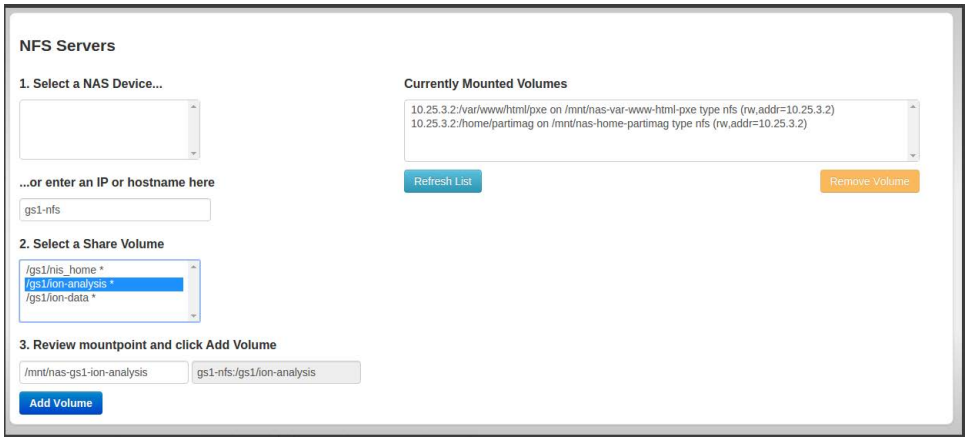
Torrent Suite™ Software will detect any storage mounted over NFS to the server as external storage.

Your Torrent Server administrator (or local I.T. person) might need to use the "sudo mount" command to mount an NAS drive. After the drive is mounted, it appears in the Archive Directory drop down menus. You may need to consult with your Linux® system administrator.

Set up TorrentNAS storage device

The TorrentNAS can be plugged in directly to your Torrent Server or it can be implemented on a network and shared among users.

1. If you are directly connecting TorrentNAS to your Torrent Server, just plug the TorrentNAS into your server.
   The TorrentNAS IP address self populates in box 1 on the interface.
2. If your TorrentNAS resides on a shared network, enter it's IP address or host name in the box below box 1.
3. Select a Share Volume (directory of folders) where you plan to store your run data.
   The network path to your storage location appears in box 3.
4. Review the mountpoint path, make any changes necessary, and click **Add Volume**.



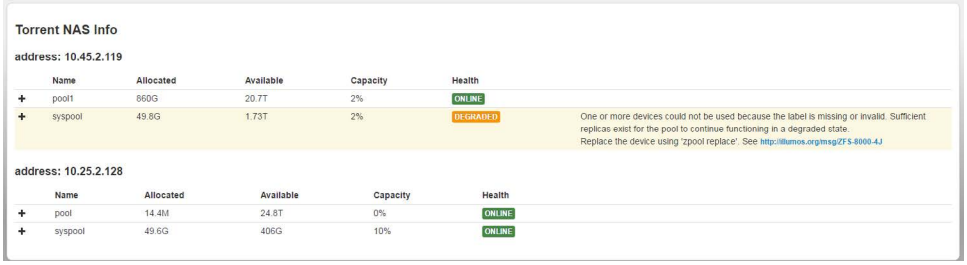
The new storage location appears in the Currently Mounted Volumes list on the right.

To remove a storage location, select the volume and click **Remove Volume**.

Monitor TorrentNAS storage device

You can check the status of your TorrentNAS devices.

1. Log in as administrator.
2. Click the gear button, then **Services**.
3. Scroll down to the TorrentNAS Info section and view all the TorrentNAS devices networked to your server.
   This section lists storage pool names, allocated and available storage capacity, usage, and health of the device.



Archive files on TorrentNAS storage device

See “Data Management tab“ on page 404 for details on storing, archiving and deleting data from your TorrentNAS device.

Axeda Remote System Monitoring (RSM)

Overview

The Axeda® RSM (Remote System Monitoring) agent is a software component installed automatically on the Torrent Server and Ion S5™, Ion PGM™, and Ion Proton™ Sequencers via the software update process.

Approximately every sixty 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 collected by the agents. Since the heartbeat message is sent many times an hour, Tech Support can quickly see if a machine is online, the software versions, and some technical details about the instrument such as temperature and hard drive status.

The agent also allows Ion Torrent™ to remotely log into the Ion S5™, Ion PGM™, andIon Proton™ systems and the Torrent Server, which is required for system support. Without remote access, Thermo Fisher Field Application Scientists cannot access, view, and troubleshoot issues regarding machine performance.

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	
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	

Event Name	Type	Sample Value	
TS.Nexenta<n>_vol<v>	String	pool1 size=32.5T allocated=860G free=31.7T capacity=2% health=ONLINE	
TS.Nexenta<n>_vol<v>_d<d>	String	c0t5d1 health=ONLINE vendor=SEAGATE product=ST6000NM0034 serial=Z4D1XT26 size=6TB	
TS.Server.celerybeat	String	ok/offline/error	
TS.Server.celery_diskutil	String	ok/offline/error	
TS.Server.celery_periodic	String	ok/offline/error	
TS.Server.celery_plugins	String	ok/offline/error	
TS.Server.celery_slowlane	String	ok/offline/error	
TS.Server.celery_transfer	String	ok/offline/error	
TS.Server.celery_w1	String	ok/offline/error	
TS.Server.dhcp	String	ok/offline/error	
TS.Server.ionCrawler	String	ok/offline/error	
TS.Server.ionJobServer	String	ok/offline/error	
TS.Server.ionPlugin	String	ok/offline/error	
TS.Server.ntp	String	ok/offline/error	
TS.Server.RabbitMQ	String	ok/offline/error	
TS.Server.RSM_Launch	String	ok/offline/error	
TS.Server.tomcat	String	ok/offline/error	
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	

Event Name	Type	Sample Value	
TS.Version.tmap	String	0.0.19-1	
TS.Version.tsconfig	String	1.3-9	

Ion PGM™ data

Event Name	Type	Sample Value
Instrument.Event.LastExperiment	String	R_2011_04_22_15_34_58_usr_S-1
Instrument.Event.Pressure	Analog	0 (chart)
Instrument.Event.ValveBoard	String	Valve Board not accessible Valve Board Down Stream Errors Valve Board Up Stream Errors
Instrument.Event.RunAborted	String	Run aborted
Instrument.Event.LostChipCon	String	Lost chip connection, run aborted
Instrument.Event.UBoot	String	U-boots don't match
Instrument.Event.Kernel	String	Kernels don't match
Instrument.Event.ResultsDrive	String	Results drive not accessible
Instrument.Event.BootDrive	String	Bad boot drive detected
Instrument.Event.DataDrive	String	Bad data drive detected
Instrument.HW.HD1	Analog	34.001 (chart)
Instrument.InstrumentName	String	Stork
Instrument.Pressure	Analog	10.2 (chart)
Instrument.Temperature	Analog	27.06 (chart)
Instrument.TYPE	String	PGM1
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

Event Name	Type	Sample Value
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
Event.DatacollectStarted	String	Datacollect Started
Event.InstrumentMustBeInitialized	String	Instrument must be initialized
Event.PostRunCleanHasNotBeenRun	String	Post Run Clean has not been run
InstrumentState	String	Idle
RunData.a1a2	String	R_2016_02_17_13_01_08_user_F4--145 W1.dat dffffe cntArry 9 0 0 9
RunData.AutoPhFinal	Analog	7.660635
RunData.AutoPhInitial	Analog	6.321023
RunData.AutoPhIterations	Analog	4
RunData.AutoPhResult	String	Pass

Data Item Name	Type	Sample value
RunData.AutoPhTotalW1Volume	Analog	1.0
RunData.ChipGain	Analog	1.066389
RunData.ChipPixelAverage	Analog	8241
RunData.ChipPixelsInRange	Analog	164698460
RunData.ChipPixelsPinnedHigh	Analog	0
RunData.ChipPixelsPinnedLow	Analog	676
RunData.ChipTemp	Analog	81.826172
RunData.CpuTemp0	Analog	53
RunData.CpuTemp1	Analog	74
RunData.efuse	String	**********L:Q6C841,W:4,J:WC2012C00086-C00272,P:16,C:PT4,F:F6,Y:4,X:0,B:3,SB:31,B:1P,N:343**********
RunData.FpgaMasterTemp	Analog	113
RunData.FpgaSlaveTemp	Analog	118.4
RunData.GpuTempC	Analog	82
RunData.LastAutoPhRealPh	Analog	766
RunData.LastAutoPhRef	Analog	745
RunData.LastAutoPhTarget	Analog	770
RunData.R1pH	Analog	7.00
RunData.R2pH	Analog	7.00
RunData.R3pH	Analog	7.00
RunData.R4pH	Analog	7.00
RunData.W1pH	Analog	8762
RunData.W2pH	Analog	7619
RunData.W3RefpH	Analog	7.45
Status.HDPctFull	Analog	0.823612
Status.SsdPctFull	Analog	6.220454
System.CpuUsagePct	Analog	7

Data Item Name	Type	Sample value
System.Date	String	2013-01-0
System.FreeMemoryKB	Analog	129951948
System.Hostname	String	d1.ite
System.IpAddress	String	10.25.3.150
System.PhysMemTotalGB	Analog	128
System.Time	String	03:42:58 PM GMT
TYPE	String	Proton1
Version.Datacollect	String	3371
Version.DiskImage	String	2015_06_04
Version.Graphics	String	80
Version.KernelRelease	String	3.13.9-ionrt1
Version.LiveView	String	2166
Version.OIA	String	5203
Version.OS	String	17
Version.Reader FPGA	String	3d400109
Version.Reader FPGA1	Analog	33400109
Version.Reader Woddr FPGA	String	3400043
Version.Reader Woddr FPGA1	String	340004b
Version.RSM	String	24
Version.Scripts	String	2.0.63
Version.S5 Release	Analog	5.2
Version.S5 Script	String	0.1.13
Version.TSLink	String	1.0.2r5
Version.Valve FPGA	String	c010

Remote access for troubleshooting

In the event that there is a problem with the Ion sequencer or Torrent Server, this agent will allow Thermo Fisher support personnel to remotely:

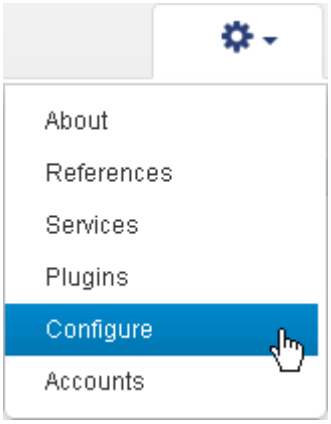
- Collect log files from the systems for review
- Restart the device
- Upgrade software
- Provide a remote login connection to the device for further diagnostic work

These types of activities would only be initiated at the request of the administrative contact, to ensure compliance with site security policies.

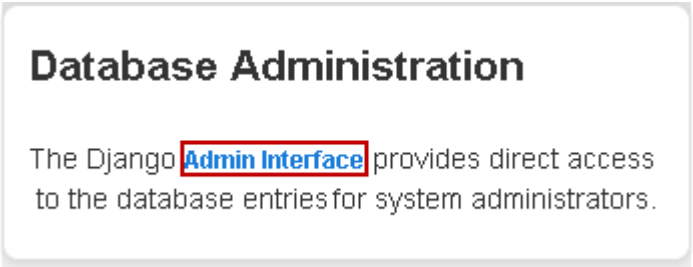
When a issue with the Ion S5™, Ion PGM™ system, Ion Proton™ system, or Torrent Suite™ Software is reported, we make every effort to solve the issue by telephone or email. If remote access is required for additional troubleshooting, a member of Thermo Fisher support staff requires authorization from the technical contact to initial remote connection. Only after getting authorization does anyone from Thermo Fisher proceed with remote troubleshooting. Once the issue is resolved you are be notified. Additional authorization is required before initiating any further remote assistance.

Management Actions

Enter the Management Actions section with the admin gear menu **Configure** selection:



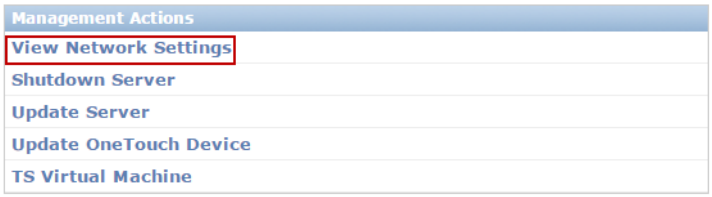
Scroll down to the Database Administration section, and click the **Admin Interface** link:



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

IMPORTANT! These procedures require your `ionadmin` account. (Do not use your `ionuser` account.)

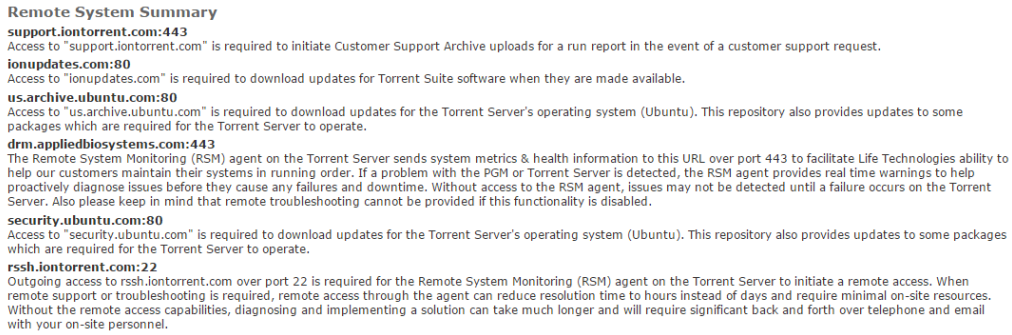
View Network Settings



Click the **View Network Settings** link to see the following information about the Torrent Server:

The Network Settings page also describes the following ports and remote sites in its Remote system Summary section:

- `support.iontorrent.com:443`
- `ionupdates.com:80`
- `us.archive.ubuntu.com:80`
- `drm.appliedbiosystems.com:443`
- `security.Ubuntu.com:80`
- `rssh.iontorrent.com:22`

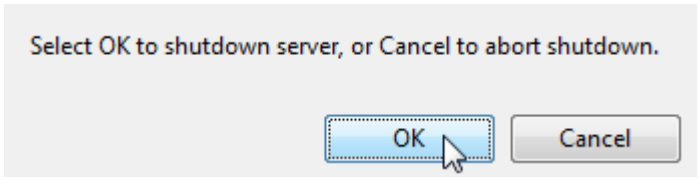


Shutdown Server

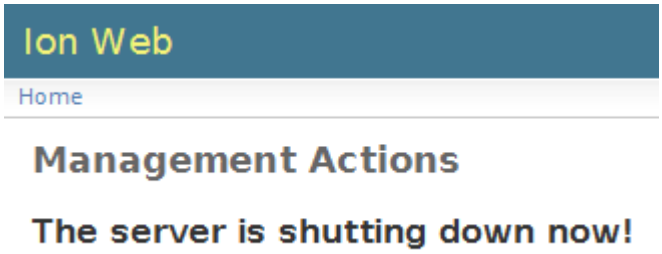
The Management Actions panel displays the **Shutdown Server** dialog:

1. In the **Management Actions** panel, click **Shutdown Server** to start the shutdown dialog.:

2. Click **OK** to confirm your shutdown request. Click **Cancel** to exit without shutting down the server.



You will see a confirmation message indicating the server is shutting down:



Torrent Virtual Machine BIOS Instructions

The evaluation virtual machine (TS-VM) requires virtualization to be supported on your machine. These instructions require a reboot.

IMPORTANT! Incorrect BIOS changes can cause unexpected results including non-operation of your server. If you are not comfortable making these changes, contact your local Life Technologies FSE.

BIOS instructions

This section describes BIOS changes required to enable virtualization support on your Torrent Server. These steps are different for T7500 servers and T620 servers.

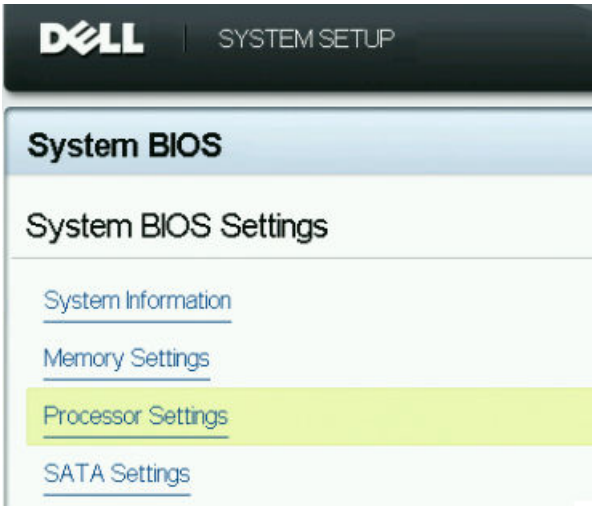
Steps for T7500 servers

1. During system boot, click **F12** for boot options.
2. Click **System Setup**.
3. In the left navigation panel, click the **Virtualization** option under Virtualization Support.
4. In the Virtualization panel on the right, click the checkbox **Enable Intel™ Virtualization Technology**.
5. Back in the left navigation panel, click the **VT for Direct I/O** option under Virtualization Support.
6. In the Virtualization panel on the right, click the checkbox **Enable Intel™ VT for Direct I/O**.
7. Click **Apply** and click **Exit** to leave BIOS.
8. Restart your system.

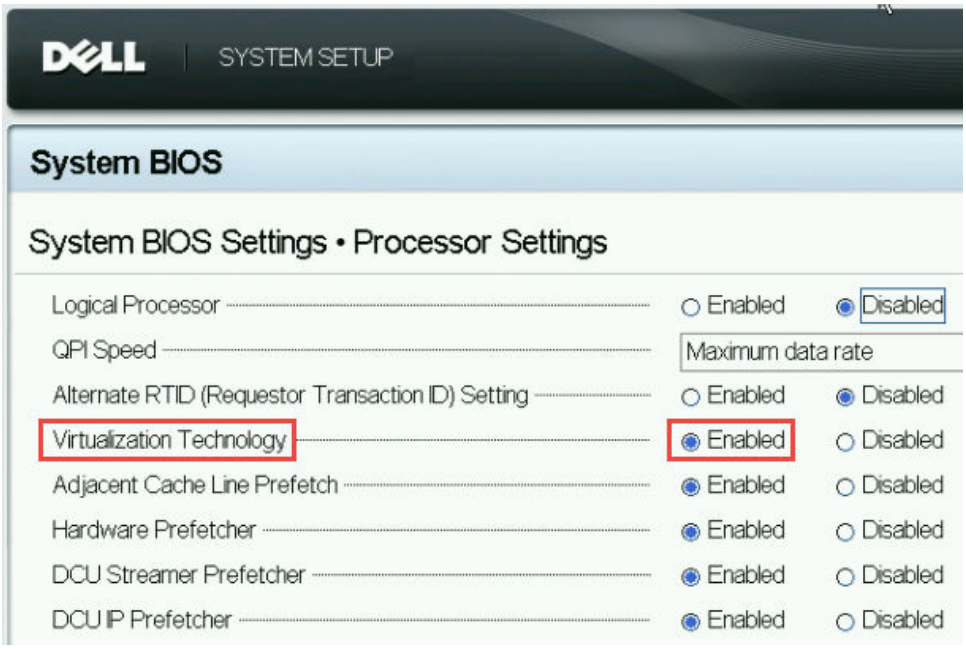
Steps for T620 servers

1. During system boot, click **F2** to select the System Menu.
2. Select **System Bios**.

3. Select **Processor Settings**.



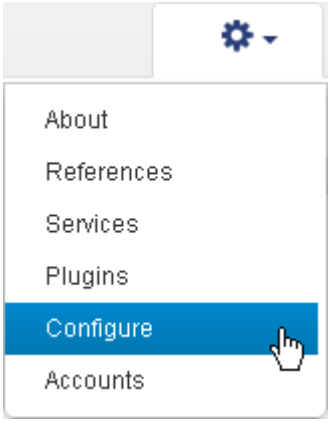
4. Click **Enabled** on the Virtualization Technology row.



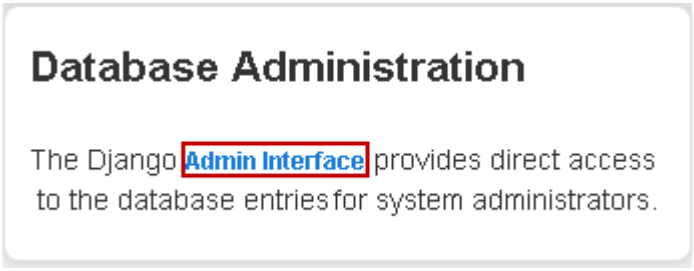
5. Save your settings and reboot your server.

Update the Ion Ion OneTouch™ Device

Enter the Management Actions section with the admin gear menu **Configure** selection:



Scroll down to the Database Administration section, and click the **Admin Interface** link:



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

IMPORTANT! These procedures require your `ionadmin` account. (Do not use your `ionuser` account.)

This procedure requires actions on both the Ion OneTouch™ device and in the Torrent Browser Management Actions section.

Follow these steps to update the Ion OneTouch™ device software:

1. Connect the Ion OneTouch™ device and the Torrent Server with an Ethernet connection.
2. 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.

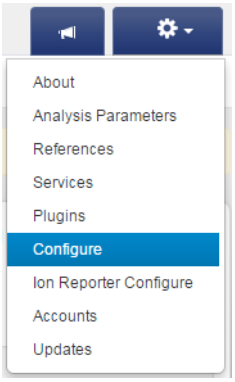
   More than one update may appear for the optional download.
3. Click **Update**.
   On the Ion OneTouch™ device, a splash screen appears with update progress.
4. After update is complete, the Ion OneTouch™ device reboots itself.

Update Server

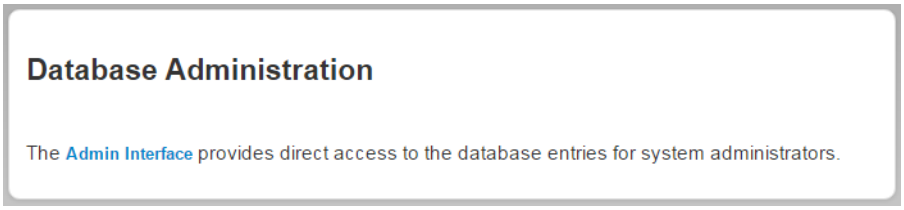
1. Update your Torrent Suite™ Software.
2. Disable updates to your Torrent Suite™ Software.

   IMPORTANT! After the Torrent Suite™ Software upgrade is completed, also upgrade your instruments to the same software level to ensure that they are compatible.

   Enter the Management Actions section with the admin gear menu **Configure** selection:



Scroll down to the Database Administration section, and click the **Admin Interface** link:



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

IMPORTANT! These procedures require your `ionadmin` account. (Do not use your `ionuser` account.)

Update your Torrent Suite™ Software

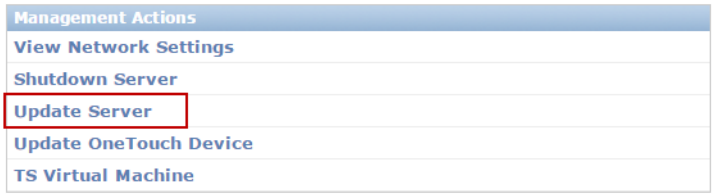
Update to your Torrent Suite™ Software cause the Torrent web services to restart. Make sure no analysis jobs are running on the server or are queued to run.

If you have old references present in the Obsolete References section, this triggers a warning to rebuild your references. Rebuild is not required if your references are already using the index `tmap-f3`. The upgradeto a3.x release does require rebuilding your reference indices *only if* you upgrade from a release earlier than 2.2.

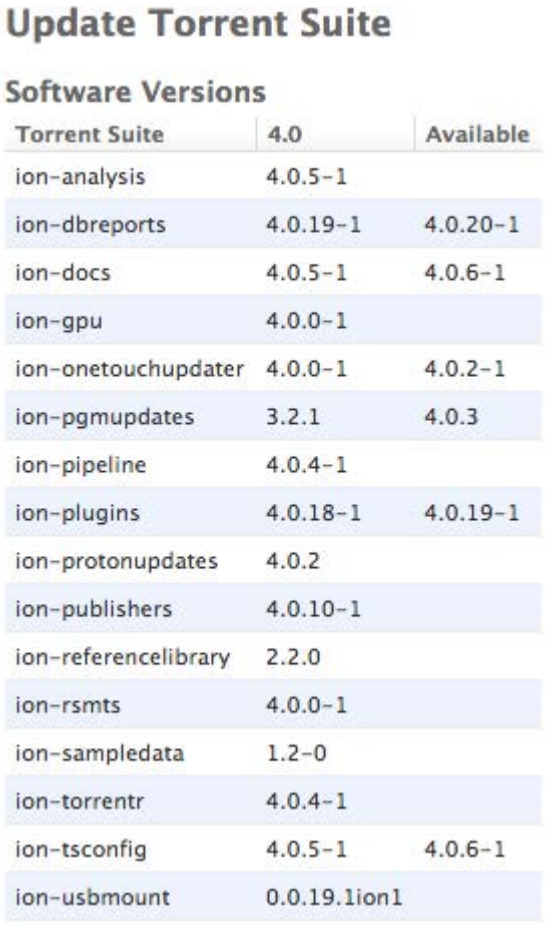
IMPORTANT! To get the full instructions for updating your Torrent Suite™ Software, please refer to the latest Release Notes on the Ion Community. There may be additional steps and procedures required, depending on the type of software upgrade.

Follow these step to update your Torrent Suite™ Software:

1. Log in with your `ionadmin` account.
2. Select the Admin gear menu **Configure** option, click the **Admin Interface** link in the Database Administration section, and scroll down on the Management Actions section.
3. Click the **Update Server** link:



The Software Versions table, in the second column header, lists the current version of Torrent Suite™ Software ("4.0" in this example. The Available column shows which packages are available for update and their versions:

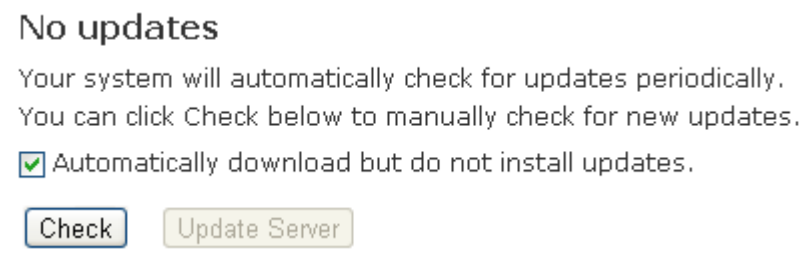


Update Torrent Suite

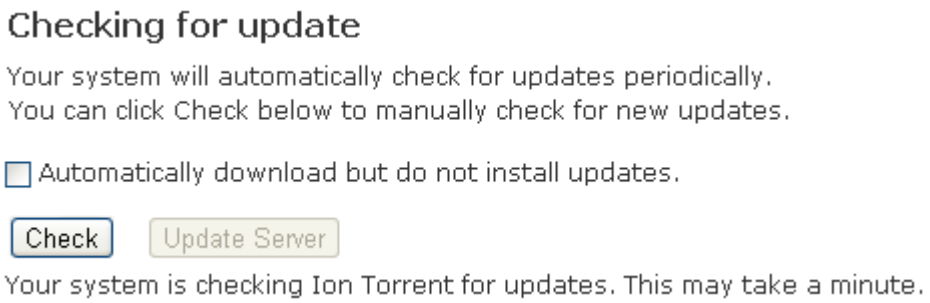
Software Versions

Torrent Suite	4.0	Available
ion-analysis	4.0.5-1	
ion-dbreports	4.0.19-1	4.0.20-1
ion-docs	4.0.5-1	4.0.6-1
ion-gpu	4.0.0-1	
ion-onetouchupdater	4.0.0-1	4.0.2-1
ion-pgmupdates	3.2.1	4.0.3
ion-pipeline	4.0.4-1	
ion-plugins	4.0.18-1	4.0.19-1
ion-protonupdates	4.0.2	
ion-publishers	4.0.10-1	
ion-referencelibrary	2.2.0	
ion-rsmts	4.0.0-1	
ion-sampledata	1.2-0	
ion-torrentr	4.0.4-1	
ion-tsconfig	4.0.5-1	4.0.6-1
ion-usbmount	0.0.19.1ion1	

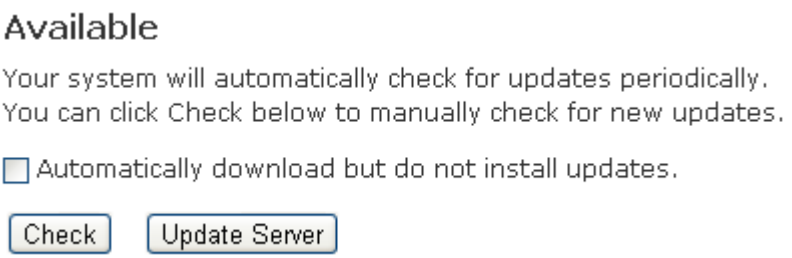
4. Below the Software Versions list is the update area. If no updates are available, this section is titled "No updates":



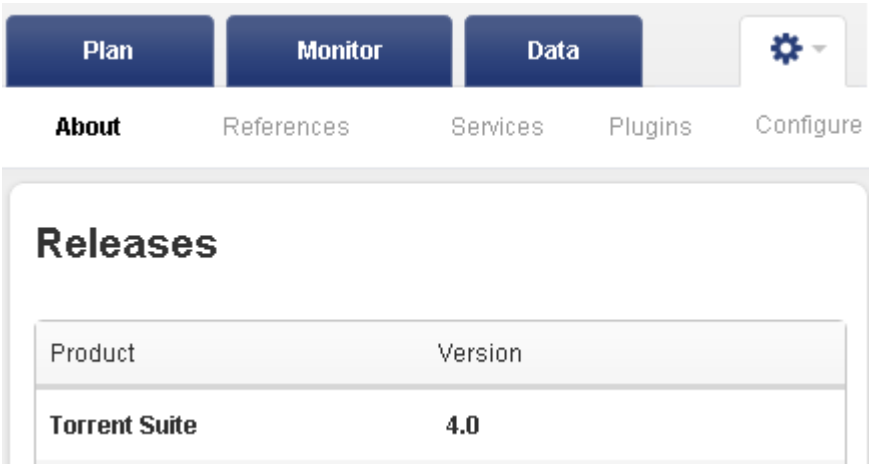
If you click the **Check** button, the following appears:



If updates are available, this section is titled "Available":

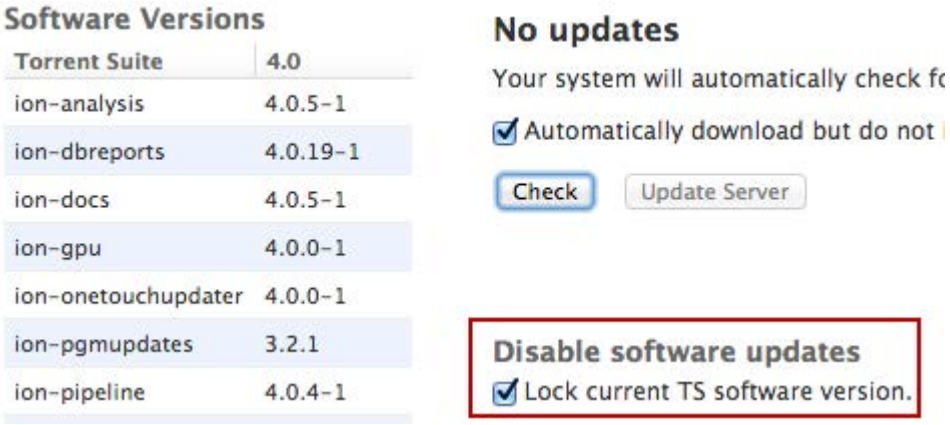


5. After the Torrent Suite™ Software update is done, please verify the version number in the Admin gear menu **About** tab. The version number should now reflect the current release:



Disable updates to your Torrent Suite™ Software

Click the **Lock current TS software version** checkbox to prevent accidental updates to your software:



Use a Torrent Virtual Machine to Evaluate a New Release of Torrent Suite™ Software

With some new releases ofTorrent Suite™ Software, we provide a virtual machine (TS-VM) of the new release, for evaluation purposes only.

You use TS-VM to reanalyze (in the new release version) an analysis that you already ran in your current version of Torrent Suite™ Software. You can then compare analysis results in your Torrent Browser. You can also run a plugin from the new release on an analysis that you already ran in your current version of Torrent Suite™ Software. Your Torrent Server remains on its current release during your evaluation.

After you install and initialize TS-VM on your Torrent Server, use the TS-VM Torrent Browser's Data Management features to import the analyses and raw data from your native Torrent Suite™ Software. With TS-VM, you do not have to copy your analysis results or other files from your native Torrent Suite™ Software.

The output of TS-VM analyses is in a separate directory (`/results/analysis/output/TSVM5.2` for the 5.2 TS-VM) and is not in the same location as your native Torrent Suite™ Software analyses.

This page describes how to download, initialize, and use TS-VM. This page uses the term *native* to refer to your Torrent Server or Torrent Suite™ Software where you install TS-VM.

IMPORTANT! While TS-VM is a fully-functioning instance of Torrent Suite™ Software, TS-VM is for evaluation purposes only and is not supported for production use. Concurrent use of TS-VM and your native Torrent Suite™ Software is not supported (due to resource contention, processing delays, and probable system hangs).

Evaluation overview

This list provides an overview how to use TS-VM to evaluate a new release:

1. Install and initialize TS-VM. TS-VM is configured with the time zone, rigs, and references from your native Torrent Suite™ Software.
2. Open a Torrent Browser pointing to TS-VM.
3. Import the data set for the run you want to reanalyze in TS-VM.
4. From the completed run report (for a previously analyzed 5.0 run), click the **Reanalysis** button.
5. Wait for the reanalysis to complete (analysis times are longer in TS-VM than in the native Torrent Suite™ Software).
6. Import the 5.0 report files.
7. Go to the Projects tab to compare the 5.0 and 5.2 results side-by-side. Results to be compared must be added to the same project in TS-VM.

Prerequisites

- TS-VM runs only on Torrent Servers.
- TS-VM requires that virtualization be enabled in BIOS (these changes require a reboot).
- TS-VM installation requires internet access.

Restrictions

- TS-VM cannot utilize the GPU.
- TS-VM has degraded disk access. Expect analysis times to be several times longer than in your native Torrent Suite™ Software.
- TS-VM does not have Torrent Suite™ Software's resource optimizations.

These restrictions are in keeping with the TS-VM's purpose of evaluating a new release before your upgrade your server. However, as a result, analysis jobs take considerably longer than they do on your native Torrent Suite™ Software.

System resource information

- All but 1 of your Torrent Server's CPU and all but 4G of its RAM are allocated to TS-VM.
- TS-VM uses ~9 GB of disk space in your `/results` partition. (The VM results files are not deleted automatically when you remove your VM.

IMPORTANT! Do not run analyses in both TS-VM and your native Torrent Suite™ Software. When TS-VM is running, system resources are allocated to TS-VM's jobs, and analyses on yournative Torrent Suite™ Software cannot complete.

Install TS-VM on your Torrent Server and initialize TS-VM

Steps

1. From the gear menu, select **Configure**.
2. In the Management Actions section, click on the TS Virtual Machine link.
3. If your Torrent Server's BIOS is not set to support virtualization, follow the instructions in Torrent VM BIOS Instructions. A reboot is required.
4. Click the **Start TS-VM Setup** button. This starts the installation and configuration.

5. If there is a newer ion-tsconfig package available, click the Update button to install first.

   Note: The Update button is disabled if there is a TS-VM currently installed. Stop the current TS-VM and delete it to enable the Update button.

   Your TS-VM is now ready to use.

Use the Torrent Browser with TS-VM

To access TS-VM, point your browser to either of these URLs:

- http://ts-vm-< *nativeTS*>
- http://< *nativeTS*>:8082

Import analysis files and resources from your native Torrent Suite™ Software

Use the Torrent Browser's Data Management Import feature. You do not have to copy any of your native Torrent Suite™ Software files.

You import analysis files based on Data Management file categories. The file categories that you import determine what type of analysis you can do in TS-VM:

File category imported	What you can do in TS-VM
Report Files	Rerun plugins
Basecaller Input	Reanalyze from Base Calling
Signal Processing Input	Reanalyze from Signal Processing

Import raw data sets from your native Torrent Suite™ Software

The TS-VM does not include the ionCrawler. Your import the native Torrent Suite™ Software analysis files that you want to analyze in TS-VM. (You do not have to copy your native Torrent Suite™ Software files.)

Follow these steps to import data files from your native Torrent Suite™ Software to TS-VM (in order to reanalyze them in TS-VM):

1. In your TS-VM Torrent Browser, go to the **Data** -> **Data Management** tab -> **Data Import** section and click the **Import** button.

2. In the **Import Data** popup, click the **Browse** button and go to **More**

Reference genomes from your native Torrent Suite™ Software

TS-VM as installed includes the e-coli genome. Other references in your native Torrent Suite™ Software are automatically imported into TS-VM.

Analyses in the TV-SM

- Auto-analysis is turned off. You manually launch analyses in TS-VM from raw data.
- See “Import raw data sets from your native Torrent Suite™ Software“ on page 465 for steps to analyze with the `From wells` option.

TS-VM shell commands

Run these commands in a terminal session or a Linux™ command prompt window on your Torrent Server.

IMPORTANT! These procedures require your `ionadmin` account. (Do not log in with your `ionuser` account.)

Start TS-VM

```
cd /results/tsvm
```

```
./tsvm-ctrl start
```

Stop TS-VM

```
cd /results/tsvm
```

```
./tsvm-ctrl stop
```

Suspend TS-VM

```
cd /results/tsvm
```

```
./tsvm-ctrl suspend
```

The suspend command saves the current state of TS-VM. The next time you start TS-VM, this state is restored.

Remove TS-VM

This command deletes the files that comprise the TS-VM virtual machine. To use TS-VM after running this command, you must re-create the virtual machine with the TS-VM initialize step (see the initialization step in Install TS-VM on your Torrent Server and initialize TS-VM).

This command does not delete any analysis files generated by your TS-VM analyses.

```
cd /results/tsvm
```

```
./tsvm-ctrl destroy
```

Purge TS-VM and associated VM files

This command both deletes the files that comprise the TS-VM virtual machine and uninstalls the `ion-tsvm` package. To use TS-VM after running this command, you must follow all the steps in “Steps“ on page 464

This command does not delete any analysis files generated by your TS-VM analyses.

```
sudo apt-get purge ion-tsvm
```

Evaluation alternative

This table compares pros and cons of using TS-VM or a separate Torrent Server to evaluate a new release ofTorrent Suite™ Software.

Issue	TS-VM	Separate Torrent Server
Requires a server reboot	Yes	No
Requires data transfer	No	Yes
Must stop analyses (and possibly stop sequencing)	Yes	No

Note:

- TS-VM requires a reboot if BIOS settings need to be changed to support virtualization.
- Data transfer to a separate Torrent Server can be done with the TorrentSuiteTransfer plugin (formerly named the TorrentSuiteCloud plugin) or with the Data Management feature ofexporting to a removable drive.

Enable Off-cycle Product Updates

Beginning in Torrent Suite™ Software v5.2, you can add new kits, chips, templates, plugins and Ion Chef scripts that are launched outside of the software's full launches.

When you learn of a new product that you would like to use, check to see if a software update is available.

1. Log in as administrator.
2. Click the Gear menu and select **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 you selected.

Update off-cycle release plugins

Beginning in Torrent Suite™ Software v5.2, you can add new plugins that are launched outside of the software's full launches.

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

1. Log in as administrator.
2. Click the Gear menu and select **Updates**.
3. Scroll down to the Update Plugins section at the bottom of the screen.
4. Select the desired new plugin and click **Update**.
   Your installed version of Torrent Suite™ Software is updated to include the new plugin you selected.

Update Ion Chef™ scripts

New in Torrent Suite™ Software v5.2, 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.

Troubleshooting

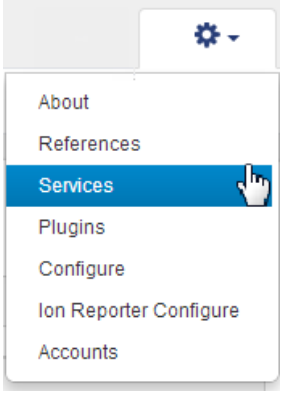
These troubleshooting suggestions apply to system level issues such as networking, disk space, and system load.

For investigations of an individual failed analysis run, see instead "Handle a failed analysis run" on page 552.

Check Crawler and Job Server status

Access the Crawler and Jobs Server page:

Click the Admin gear menu (near of top right of the Torrent Browser) and select **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.

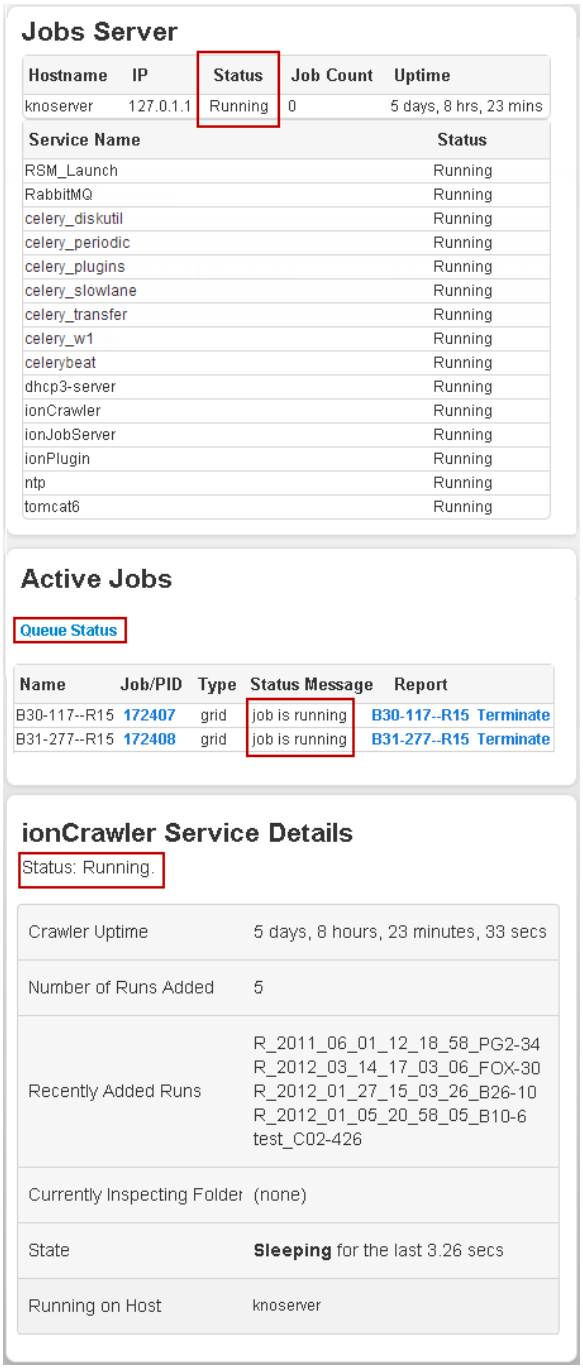
Note: Startup scripts for each process can be found in the `/etc/init.d` directory.

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

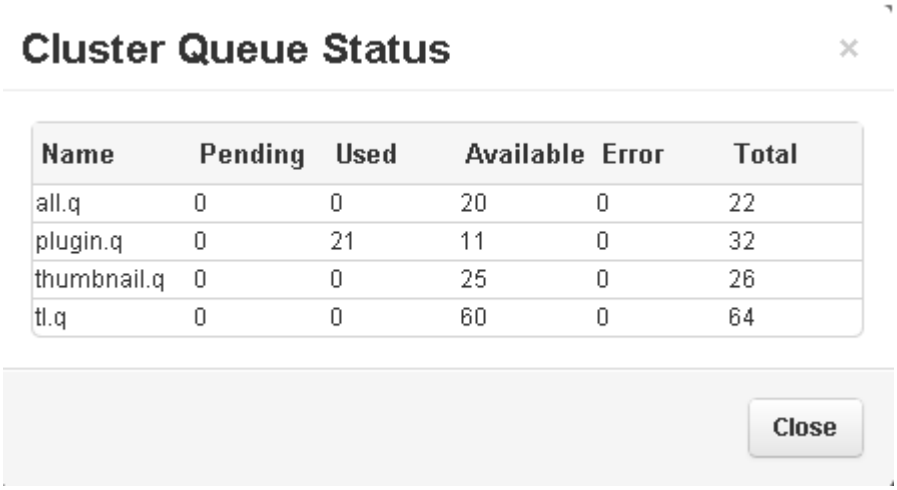
Status: Running.

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".

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 UI. The easiest approach 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.

You can also 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
```

Use the `ps ax | grep py` command or the Torrent Browser UI to verify that the processes are running.

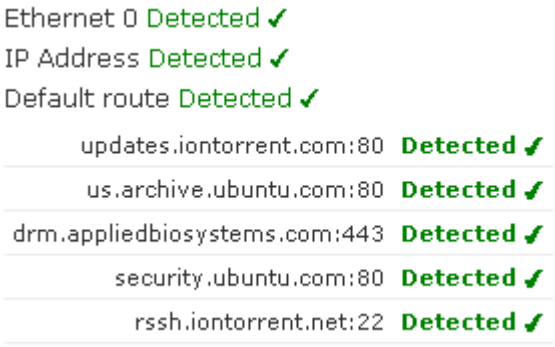
After restarting a process, it continues from the point where it was interrupted, and no more user interaction is needed.

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:

1. Go to the admin interface Management Actions area and click **View Network Settings**. The Torrent Browser performs several network checks:



2. You can also 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 472.

If you cannot reach the Torrent Server an IP address, you are likely to need help from the site I.T. administrator who understands how the local network is configured.

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 I.T. 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 siteI.T. 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 I.T. 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

Verify that all files successfully transferred from the Ion PGM™ and Ion Proton™ Sequencers to the Torrent Server. Manually transfer files, if needed, by going to the Data Management screen of the sequencer and select the Re-transfer option for any of the runs in question. You can safely re-transfer data.

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.

Use QMON to manage the SGE cluster

`QMON` is a graphical tool to manage the SGE Cluster. With `QMON` you can do tasks such as the following:

- See the status of the servers in the cluster
- Check server CPU load and memory utilization
- See the status of jobs, are they running, completed, or still waiting in the queue
- Monitor these job queues:
  - `tl.q` Top level control jobs
  - `all.q` Analysis jobs
  - `plugin.q` Plugin jobs
  - `thumbnail.q` Ion Proton™ thumbnail analysis jobs
- Delete jobs that are currently processing or waiting in queue
- Disable servers from accepting new jobs, and re-enable them later
- And many other advanced configuration options

System diagnostics

System diagnostics information can help diagnose network, disk space, and system status issues.

Instrument diagnostics

Use instrument diagnostics to investigate chip and sequencing instrument issues, such as pH levels.

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

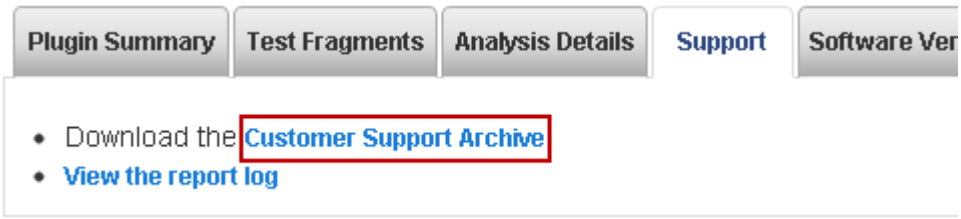
Please contact your Ion Torrent™ representative for assistance before you attempt any of these steps.

Customer Support Archive

This page describes the contents of a Customer Support Archive and how you can generate one. The Customer Support Archive is a ZIP file of log files and other technical data about your Torrent Suite™ Software and analysis runs. This data is useful for our support team to diagnose issues with your Torrent Suite™ Software. A Customer Support Archive can only be generated by a request in your Torrent Browser an archive is not generated automatically or forwarded automatically to Ion Torrent™.

Generate a Customer Support Archive

You can generate a Customer Support Archive from a run report in the Torrent Browser Data > Completed Runs & Reports tab. The Support tab appears in this tab ribbon (after the run metrics and before the plugin detailed output):



Click the **Support** tab and then the **Customer Support Archive** download link. Wait while the file is downloaded. The zipped archive file can be 15 or 20 MB or more.

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
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

File	Description
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
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

File	Description
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)
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.

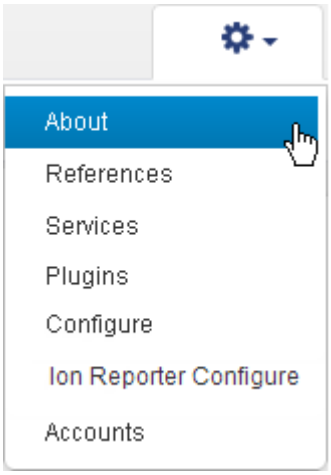
File
NucStep_frametime.txt
NucStep_inlet_head.txt
NucStep_inlet_empty.txt
NucStep_inlet_empty_sd.txt
NucStep_inlet_step.txt
NucStep_middle_head.txt
NucStep_ middle_empty.txt
NucStep_ middle_empty_sd.txt
NucStep_ middle_step.txt
NucStep_outlet_head.txt
NucStep_ outlet_empty.txt

File
NucStep_outlet_empty_sd.txt
NucStep_outlet_step.txt

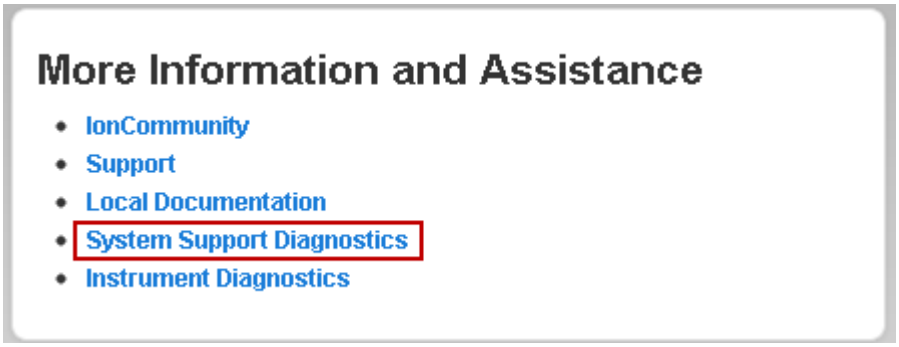
View System Support Diagnostics

System diagnostics information can help in troubleshooting network, disk space, and system status issues.

To access system diagnostics information, click the Admin gear menu (near of top right of the Torrent Browser) and select **About**:



Scroll down to the More Information and Assistance section and click the **System Support Diagnostics** link:



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/T
|/ Err?=(none)/Reinst-required (Status,Err: uppercase=bad)
||/ Name                                Version
+++-=====================================-============================
ii  ion-alignment                        3.0.2-1
```

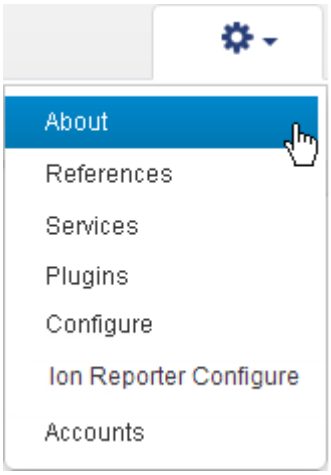
Data

```
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
```

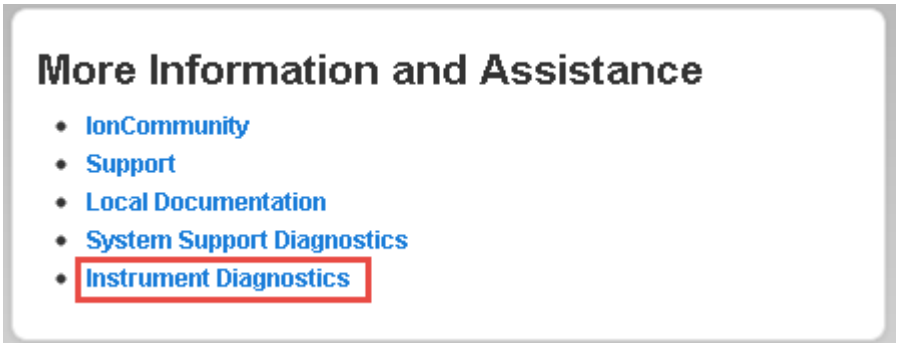
View Instrument Diagnostics

Use instrument diagnostics to investigate chip and sequencing instrument issues, such as pH levels

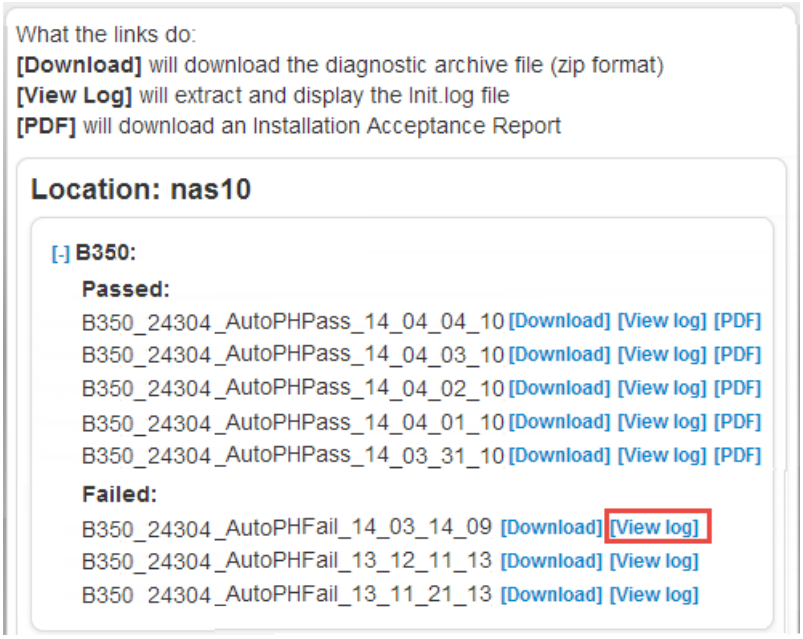
To access the instrument diagnostics information, click the Admin gear menu (near of top right of the Torrent Browser) and select **About**:



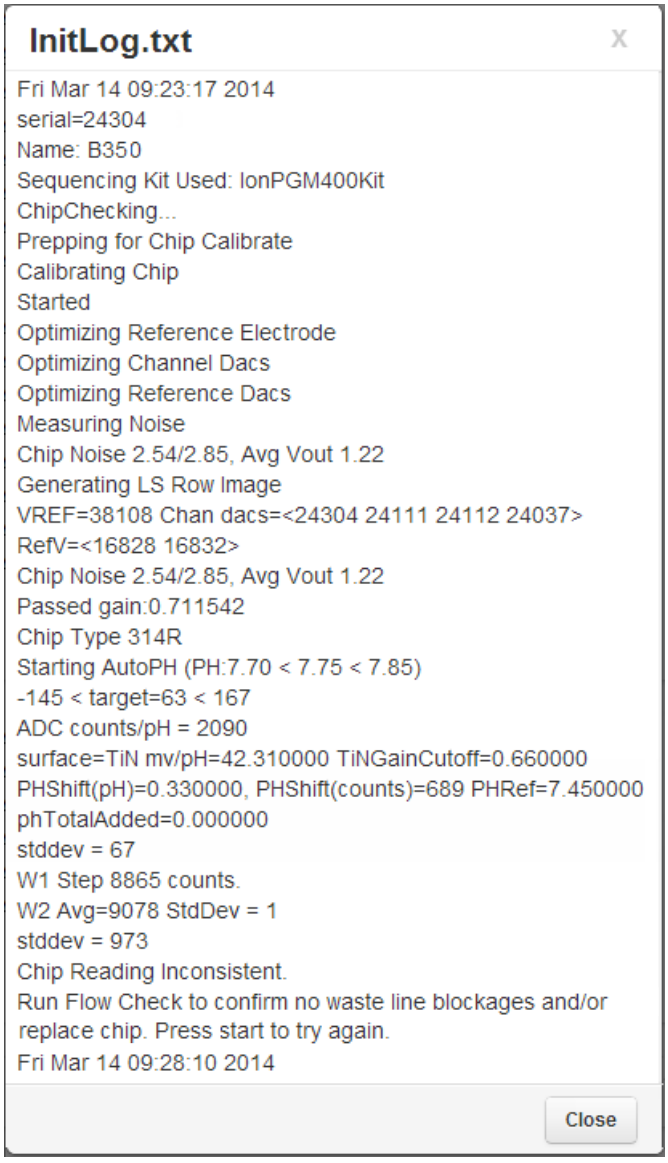
Scroll down to the More Information and Assistance section and click the **Instrument Diagnostics** link:



The instrument diagnostics page lists the sequencing instruments associated with each of your results partitions. Passed and failed analysis runs are shown for each instrument. To investigate a failed run, click the **View log** link for that run:



The InitLog.txt file opens for that run on the instrument:



The InitLog.txt file includes diagnostic measurements and if possible presents a probable cause and suggests next steps.

Manage your SGE Cluster with QMON

QMON is a graphical tool to monitor and manage the SGE Cluster. With QMON you can do tasks such as the following:

- See the status of the servers in the cluster
- Check server CPU load and memory utilization
- See the status of jobs, are they running, completed, or still waiting in the queue
- Disable servers from accepting new jobs, and re-enable them later
- Delete jobs that are currently processing or waiting in queue
- And many other advanced configuration options

SGE is typically used in a cluster of several servers to distribute data processing jobs. With the Torrent Server, SGE is used in the cluster configuration and also in the single-server configuration. This design makes it it easy for users to start with one server and add more "compute nodes," as needed, to ramp up the data processing capacity.

Client OS support

A GUI is not installed on Torrent Server. Use `ssh` to start `QMON` remotely,

To display `QMON` on your desktop, you need a client that supports the X Windows protocol; a Linux™ client automatically includes X Windows support. There are numerous software packages that add X Windows functionality to Windows™, but the easiest and most economical solution is to run Linux™ in a virtual machine on a Windows™ system.

Start QMON

To start `QMON`, login to the server with `ssh`, and include the `-X` option, this tells the server that you are going to be using an application that uses the X Windows display.

```
ssh -X ionadmin@ion-torrent-server
```

After you are logged into the server, just type `QMON` from the command line to launch the tool.

```
ionadmin@ion-torrent-server:~$ qmon
```

The server should be preconfigured to support `QMON` so you should not get any error message.

The most common error is that you forgot the `-X` option, which causes the following error when you try to start `QMON`:

```
ionadmin@ion-torrent-server:~$ qmon
```

```
Error: Can't open display:
```

Shortly after entering the `QMON` command, the "Grid Engine" splash screen should appear, indicating your X Windows display is working. If you are on a slow connection, it may take a few seconds for the tool to launch. After a short time, the splashscreen automatically disappears.

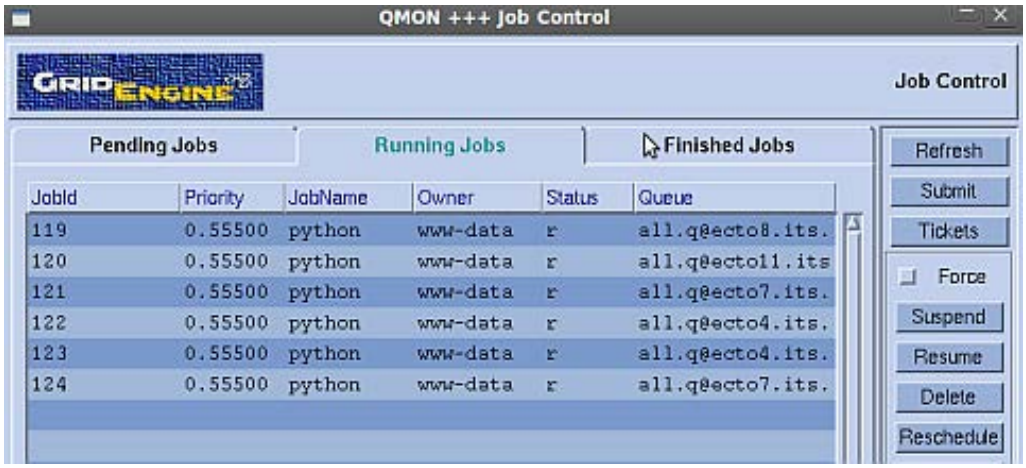
When you see the following toolbar, QMON is ready to use:

There are many buttons here to access advanced options. Typically, only use the first two buttons on the upper-left: Job Control and Queue Control.

Job Control

The Job Control screen shows you the job status.

IMPORTANT! The display does not automatically refresh, so remember to click **Refresh** to update the status.

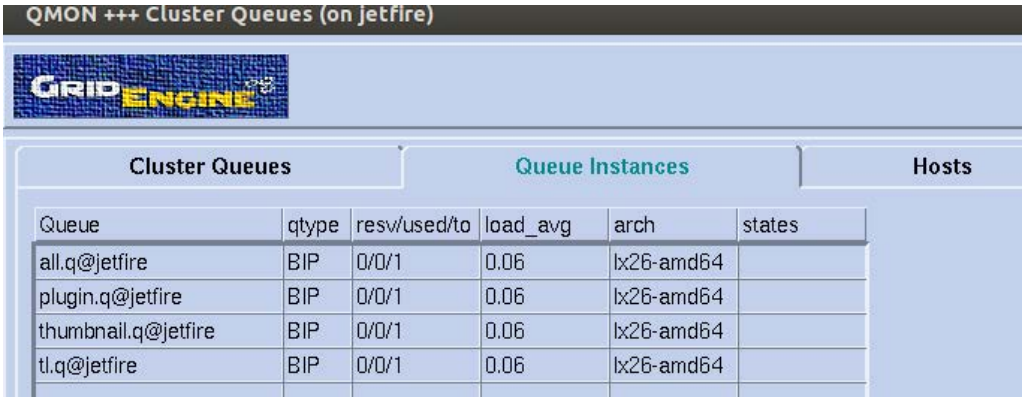


Tab	Description
Pending Jobs	Jobs that are in the queue waiting to be process.
Running Jobs	Jobs currently being processed.
Finished Jobs	Jobs that have completed processing.

You can delete jobs from this screen by highlighting the job then pressing the **Delete** button.

Queue Control

The Queue Control Screen shows the different servers that are available for processing jobs.



the **used/total** column shows the number of jobs currently running on the server (**used**), and the number of processing slots available on the server (**total**). This example shows seven processing slots available and six jobs currently being processed.

IMPORTANT! There are four queues:

- `tl.q` Top level control jobs
- `all.q` Analysis jobs
- `plugin.q` Plugin jobs
- `thumbnail.q` Ion Proton™ thumbnail analysis jobs

To show the status of all jobs, enter the command: `qstat -f`.

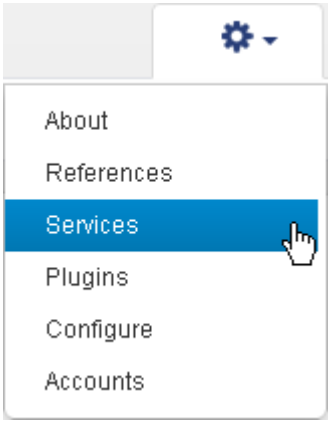
For jobs indicating an error, where an "E" is displayed in the last column of the job status, clear the error using one of the following methods:

- Enter the command `qmod -cq all.q` on the command line.
- Login to `QMON` and, for any queues are marked with "E," highlight the entry and click the **Clear Error** button.

Monitor Your Torrent Server and Torrent Suite™ Software

Use the admin **Services** tab to view the current status of the essential system services:

Access the Services tab from the admin gear menu and select **Services**:



IMPORTANT! The Job Server and ionCrawler services should always be active. The Archive service is only active if archiving has been configured.

Jobs Server service

The **Jobs Server** panel lists services used by Torrent Suite™ Software.

Jobs Server

Hostname	IP	Status	Job Count	Uptime
kingrider	127.0.1.1	Running	0	42 days, 12 hours, 13 minutes, 15 seconds

Service Name	Status
RSM_Launch	Running
RabbitMQ	Running
celery_periodic	Running
celery_plugins	Running
celery_w1	Running
celerybeat	Running
dhcp3-server	Running
ionCrawler	Running
ionJobServer	Running
ionPlugin	Running
ntp	Running
tomcat6	Running

During normal operation each service's status is "Running". A status of "Down" indicates the service should be restarted.

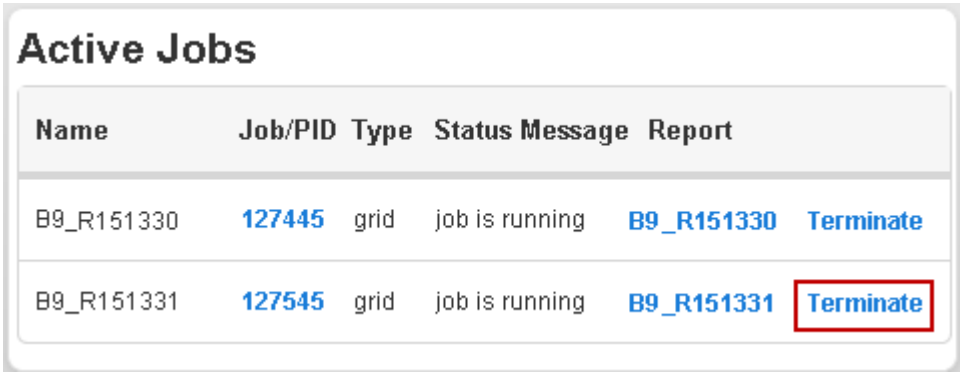
Start a job

Job requests are initiated by clicking the **Analyze** button for a given run or by specifying**auto-analysis** on the Ion PGM™ or Ion Proton™ Sequencer for the run. After data transfer following an Ion PGM™ or Ion Proton™ Sequencer auto-analysis completes, an analysis job starts automatically for that run.

Up to two jobs can be run concurrently, by default, using the Sun Grid Engine (SGE). Running jobs in parallel results in a longer run time for each job but gives a shorter overall processing time compared to running the jobs concurrently.

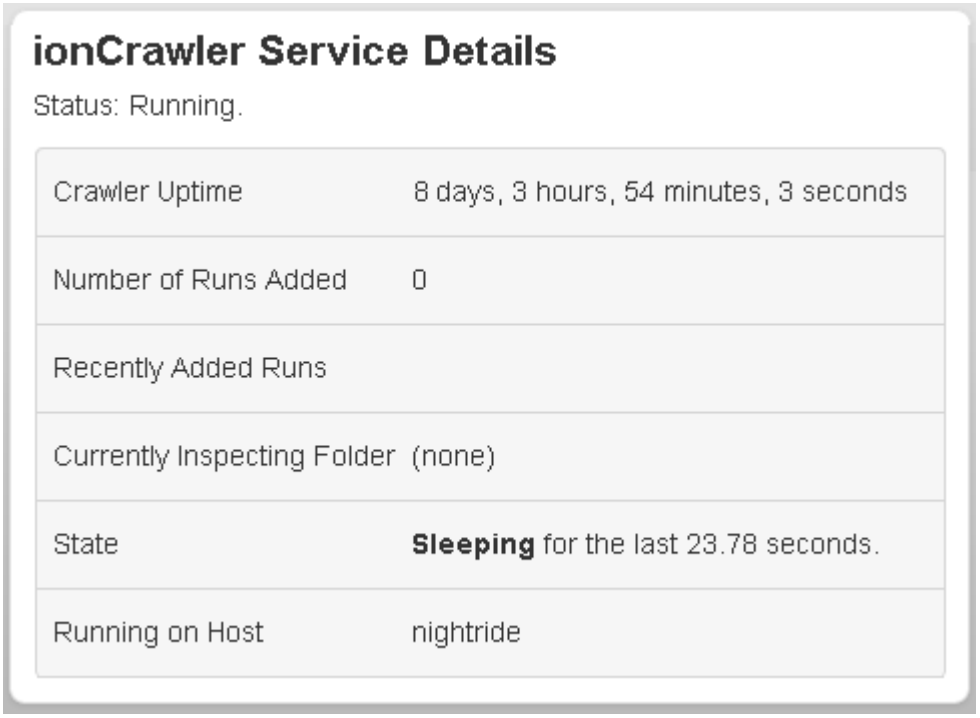
Stop a job

In the **Active Jobs** panel, you can stop a job by clicking **Terminate** on the line for the job:



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 presents the status of RAID drives on your Torrent Server:

The Show Details link opens a popup with details of the RAID drives (only one shown here):

Slot 0	
Media Error Count	0
Other Error Count	0
Predictive Failure Count	0
Firmware state	Online, Spun Up
Inquiry Data	SEAGATE ST32000444SS KS679WM0L47T
Needs EKM Attention	No
Foreign State	None
Port-0	
Port status	Active
Port-1	
Port status	Active
Drive has flagged a S.M.A.R.T alert	No
Drive Temperature	30C (86.00 F)

Refresh your browser to see changes in status. This information is not updated automatically.

Screen descriptions

Planned Runs screen

How to...	Learn more about...
"Create a planned run using AmpliSeq™ DNA template" on page 30	"Plan Tab" on page 508
"Plan by sample set" on page 36	"Templates " on page 505
"Create multiple planned runs via CSV upload" on page 40	"Wizard Plan or Save Chevron" on page 501
"Create a Planned Run using DNA and Fusions template" on page 30	
"Plan a run using Generic Sequencing template" on page 32	
"Plan a run using Ion 16S™ Target Sequencing template" on page 170	
"Copy a template" on page 32	
"Create a Template with Ion AmpliSeq.com Import" on page 34	

Plan > Samples screen

How to...	Learn more about...
"Enter new sample" on page 20	"Sample information" on page 25
"Create sample sets manually" on page 21	"Sample attributes" on page 23
"Import samples to create a sample set" on page 21	"CSV Metrics File Format" on page 516
"Create multiple planned runs via CSV upload" on page 40	
"Search samples" on page 26	
"Edit samples" on page 26	
"Delete samples" on page 27	
"Sort samples" on page 28	

Plan > Templates screen

How to...	Learn more about...
"Create a planned run using AmpliSeq™ DNA template" on page 30	"Plan Tab" on page 508
"Create a Planned Run using DNA and Fusions template" on page 30	"Templates " on page 505
"Plan a run using Generic Sequencing template" on page 32	"Wizard Plan or Save Chevron" on page 501
"Plan a run using Ion 16S™ Target Sequencing template" on page 170	
"Copy a template" on page 32	
"Create a Template with Ion AmpliSeq.com Import" on page 34	
"Plan by sample set" on page 36	

Plan > Planned Run List screen

How to...	Learn more about...
"Execute a run plan on your sequencer" on page 59	"Plan Tab" on page 508
"Start your planned run on the Ion S5™ or Ion S5™ XL sequencer" on page 98	"Customizing and editing templates" on page 29
	"Wizard Plan or Save Chevron" on page 501
	"Example Planned Runs page" on page 515

Monitor > Runs in Progress screen

How to...	Learn more about...
"Review the planned run settings" on page 63	"Monitor tab" on page 61
	"Example monitoring metrics" on page 61
	"Views" on page 62
	"Auto Update" on page 63

Monitor > Ion Chef screen

How to...	Learn more about...
"Analyze Ion AmpliSeq™ on Ion Chef™ samples" on page 42	

Plan > Planned Run List screen

How to...	Learn more about...
"Execute a run plan on your sequencer" on page 59	"Plan Tab" on page 508
"Start your planned run on the Ion S5™ or Ion S5™ XL sequencer" on page 98	"Customizing and editing templates" on page 29
	"Wizard Plan or Save Chevron" on page 501
	"Example Planned Runs page" on page 515

Data > Completed Runs & Results screen

How to...	Learn more about...
"Manage completed runs and results" on page 67	"Test fragment report" on page 137
"Search for a run" on page 68	"Analyze Ion AmpliSeq™ on Ion Chef™ samples" on page 42
"Add a run to a project" on page 72	"Plugin Summary" on page 175
"Terminate an analysis run" on page 73	
"Change the analysis reference" on page 74	
"Change run metadata" on page 74	
"Add barcoding to a completed run" on page 75	
"View the Data Management log" on page 69	
"Delete, archive, or export run data" on page 68	
"Reanalyze a run" on page 70	
"Edit a run plan" on page 70	

Run Report

How to...	Learn more about...
"Review pre-alignment metrics" on page 111	"Introduction" on page 110
"Review alignment metrics" on page 112	"ISP density" on page 121
"Download results set" on page 112	"ISP summary" on page 124
"Manually run a plugin on the run results" on page 112	"Read length" on page 128
"Review the planned run settings" on page 114	"Key signal" on page 122
"Review the test fragments and their quality metrics" on page 115	"Output files" on page 135 "Run metrics overview" on page 118
"Review Chef Summary" on page 115	"Predicted quality (Q20)" on page 119
"Review Calibration Report" on page 116	"Quality following alignment (AQ20)" on page 119
"Review analysis information" on page 116	
"Compare run reports" on page 118	
"Tune Ion Reporter™ Uploader speed parameters" on page 297	

Data > Projects screen

How to...	Learn more about...
"Add a run to a project" on page 72	"The Projects tab" on page 101
"Download a CSV file of metrics" on page 107	"The Projects Listing Page" on page 103
"Add to Project" on page 108	"Project menus and actions" on page 107
"Remove from Project" on page 108	"Actions on members of a project" on page 108
"Search" on page 108	"Mark as Duplicates Read" on page 108
"Filter by date" on page 109	

Data > Data Management screen

How to...	Learn more about...
"Access the database" on page 79	"Data Management tab" on page 404
"Set up TorrentNAS storage device" on page 445	"Configuration" on page 404
"Monitor TorrentNAS storage device" on page 446	"Data Import" on page 405
"Archive files on TorrentNAS storage device" on page 446	"Disk Usage" on page 406
	"Active Data Management Jobs" on page 407
	"Category Statistics" on page 407
	"Disk Space Management" on page 408
	"Dataflow file sizes" on page 524

Analysis Parameters screen

How to...	Learn more about...
"Configure Custom Analysis Parameters" on page 526	

References screen

How to...	Learn more about...
"Upload a new reference file" on page 323	"Prerequisites" on page 323
"Add the Ion GRCh38 Reference to Torrent Suite™ Software" on page 320	"GRCh38 human reference" on page 320
"Upload the reference" on page 324	"Error handling" on page 327
"Manage Target Regions Files and Hotspot Files" on page 331	"Target Regions Files and Hotspot Files" on page 327
"Modify a BED file" on page 333	"BED File Formats and Examples" on page 340
"Download a hotspots or target regions file" on page 338	"Target Regions File Formats" on page 340
"Delete a hotspots or target regions file" on page 339	"RNA Fusions BED File Formats and Examples" on page 350

Services screen

How to...	Learn more about...
"Verify Functionality" on page 433	"Alternate checks" on page 433
"Check Crawler and Job Server status" on page 468	
"Restart services" on page 471	
"Monitor Your Torrent Server and Torrent Suite™ Software" on page 490	

Plugins screen

How to...	Learn more about...
"Enable an installed plugin" on page 173	"Available plugins" on page 178
"Delete a plugin" on page 174	"Plugin configuration" on page 181
"Enable or disable automatic plugin execution" on page 174	"Admin plugin configuration area" on page 182
"Modify and apply TVC settings for all or select barcodes" on page 250	"ERCC Analysis Plugin" on page 204
"Add, edit and delete configurations" on page 251	"The FileExporter Plugin" on page 212
	"The FilterDuplicates Plugin" on page 218
	"Overview" on page 285
	"Pharmacogenomics Research Analysis Plugin" on page 143
	"Run RecognitION Plugin" on page 221
	"Ion RNASeq Plugin" on page 144
	"The SampleID Plugin" on page 224
	"The RunTransfer Plugin" on page 227
	"The Partek Flow Uploader Plugin" on page 232
	"Introduction" on page 235

Configure screen

How to...	Learn more about...
"Add Customer Support Contacts" on page 383	"Administration" on page 379
"Change the displayed server name" on page 384	"Nightly email configuration" on page 384
"Configure Chips" on page 385	"Database Administration" on page 385
"Configure Global Configs" on page 387	
"Add a configuration" on page 388	
"Change an existing configuration" on page 389	
"Configure Experiments" on page 389	
"Modify an experiment" on page 390	
"Delete an experiment" on page 391	
"Configure Users" on page 392	
"Delete Multiple Users" on page 397	
"Configure Basecaller Default Parameters" on page 398	
"Configure LIMS Metadata export from Planned Runs" on page 401	
"Approve User Account Requests" on page 402	

Ion Reporter configure screen

How to...	Learn more about...
"Configure your Ion Reporter™ Uploader Plugin Access Credentials" on page 285	"In the plugin configuration page" on page 314
"Configure the Uploader plugin in the Torrent Browser" on page 316	"Multiple configurations" on page 316
"Manual launch of the IonReporterUploader plugin" on page 300	"Configuration errors" on page 319
"Tune Ion Reporter™ Uploader speed parameters" on page 297	"Advanced settings" on page 296

Account Settings

How to...	Learn more about...
"Approve User Account Requests" on page 402	"Approval and rejection" on page 403

Product and Plugin updates

How to...	Learn more about...
"Enable Off-cycle Product Updates" on page 467	
"Update off-cycle release plugins" on page 467	

Reference

Wizard Plan or Save Chevron

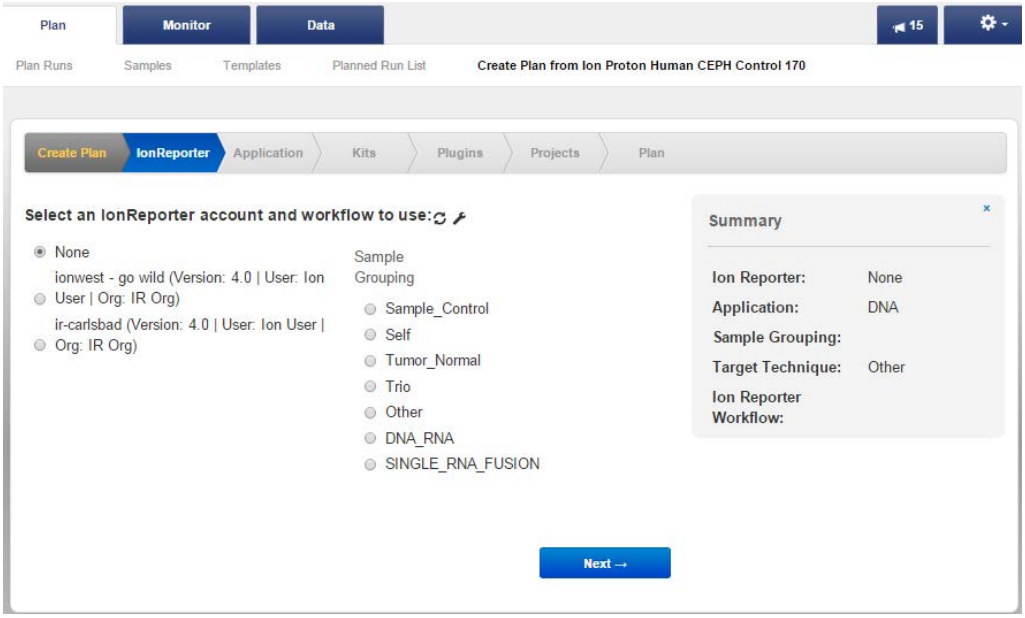
The title for last chevron of the wizard is different for templates, run plans, and run plans that are planned by sample set:

- **Templates** The chevron title is Save. Here you enter the new template name and optionally mark it as a favorite.
- **Run plans** The chevron title is Plan. Here you enter the new run plan name and sample information.
- **Plan by sample set** The chevron title is Save & Finish. Here you enter the new run plan name. (Sample information is automatically entered into the Barcoding chevron.)

Note: Templates that are marked as favorites are listed in their own section at the top of the Templates tab.

Wizard Ion Reporter Page

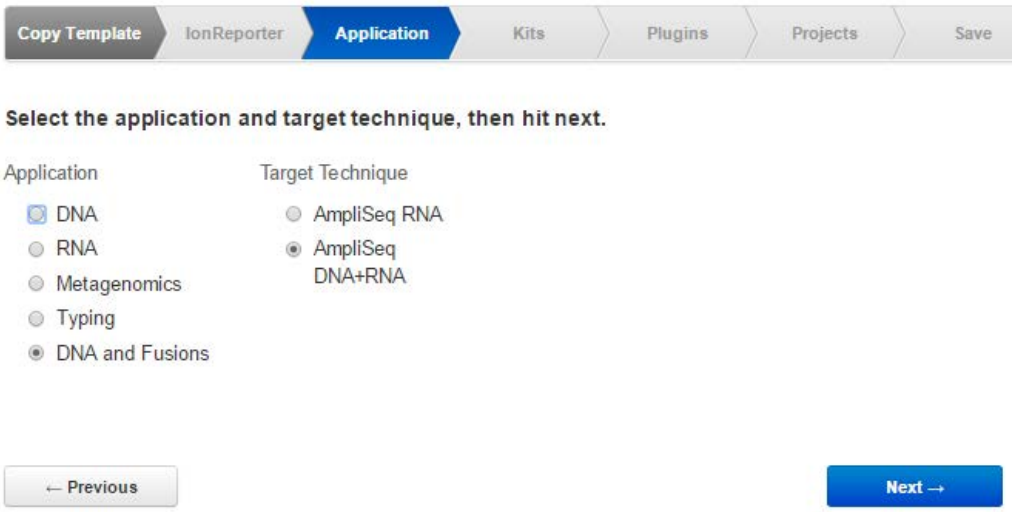
Ion Reporter is the first page in the Torrent Browser run template wizard. When you select an Ion Reporter account in this page, features related to Ion Reporter™ Software appear in the other wizard pages.



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

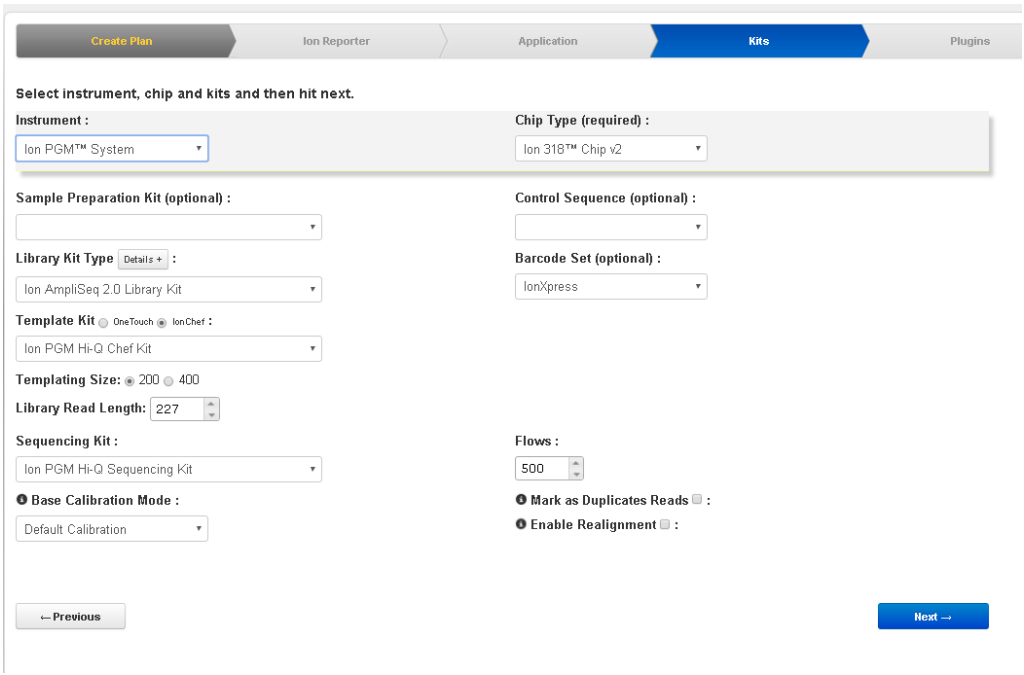
On the Kits wizard page, enter the following information about laboratory kits and other sequencing parameters:

- (*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™

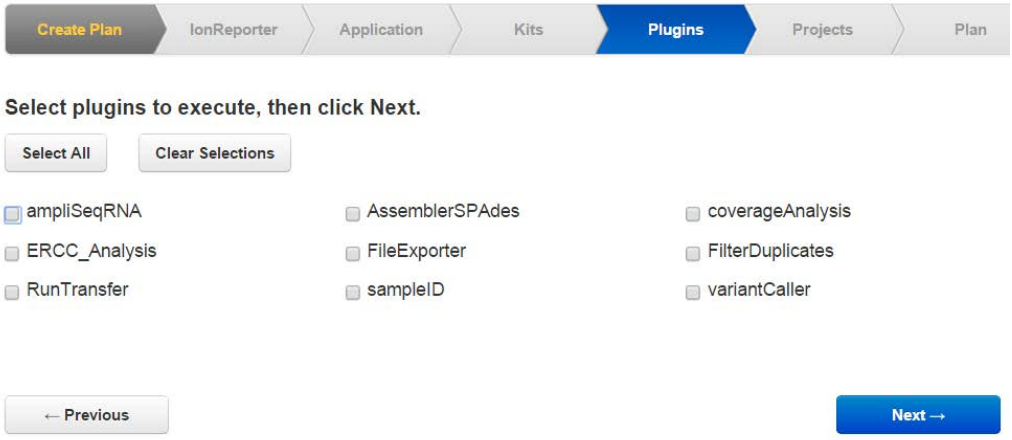
runs as a quality control for higher homopolymer performance and as a known reference for base recalibration.

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:

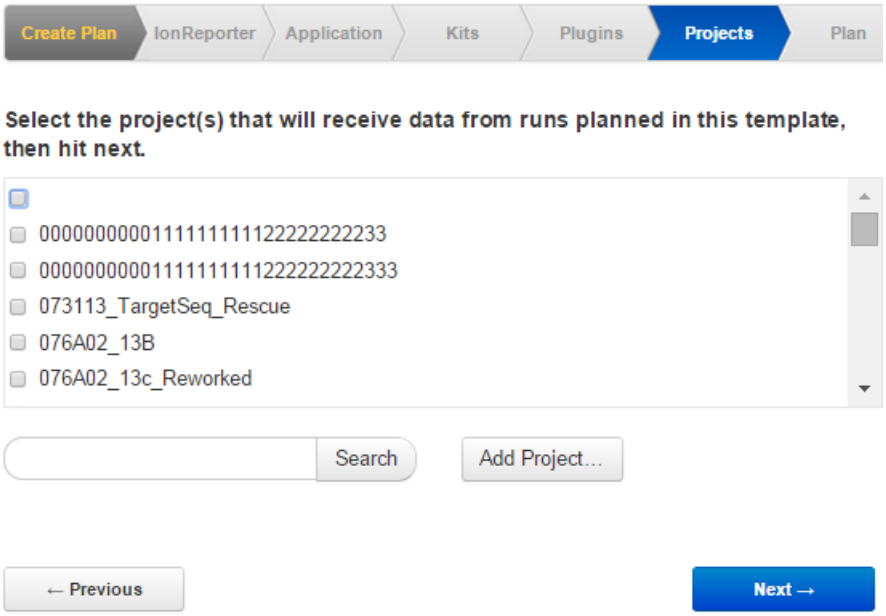


Note:

- 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 (TVC) 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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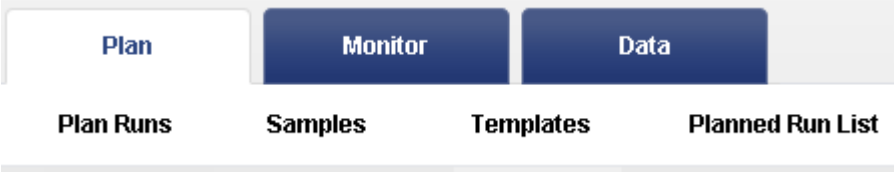
- **16S Target Sequencing** Ion AmpliSeq™ 16S metagenomics applications.

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 the gear button and selecting **Plan Run**.
- In **Plan ▸ Planned Run List** gear button, **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.

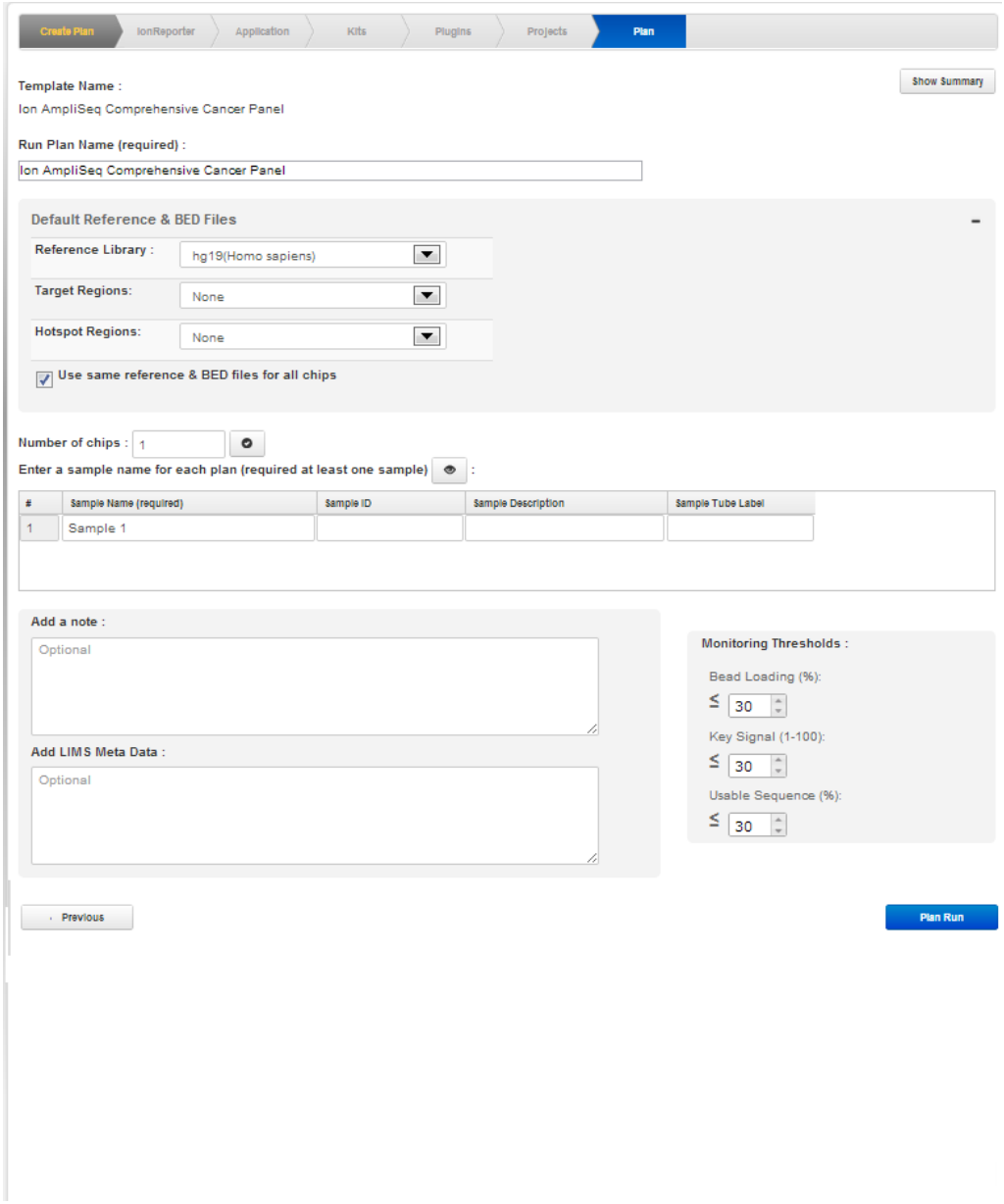
You can your run plans based on your sample sets or on run plan templates.

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.

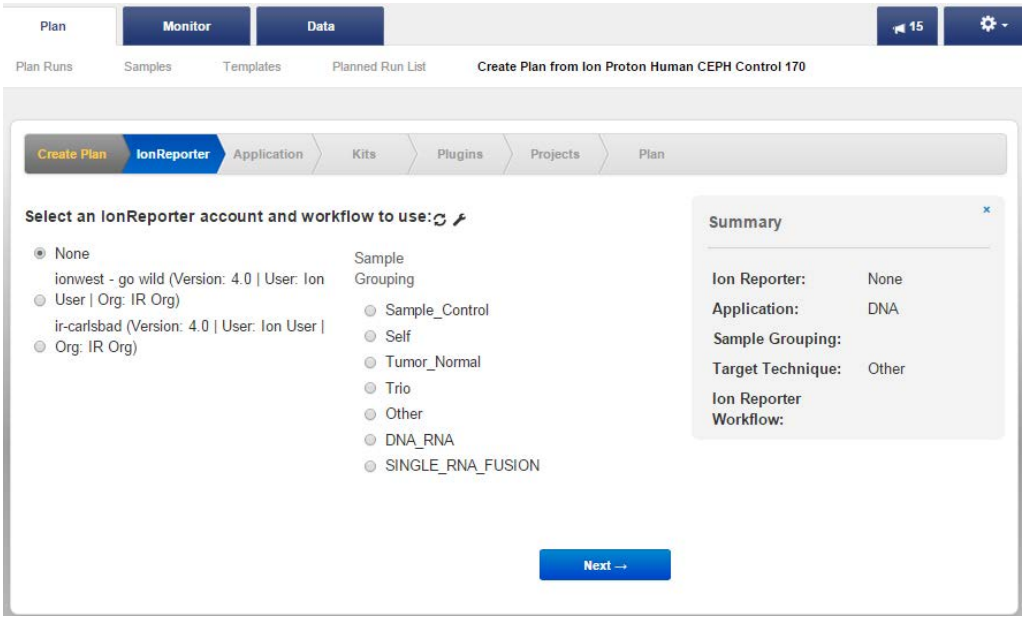
How you start the wizard is important, especially if your sequencing workflow uses common sequencing products. Pre-installed templates are available for these common sequencing products:

- 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

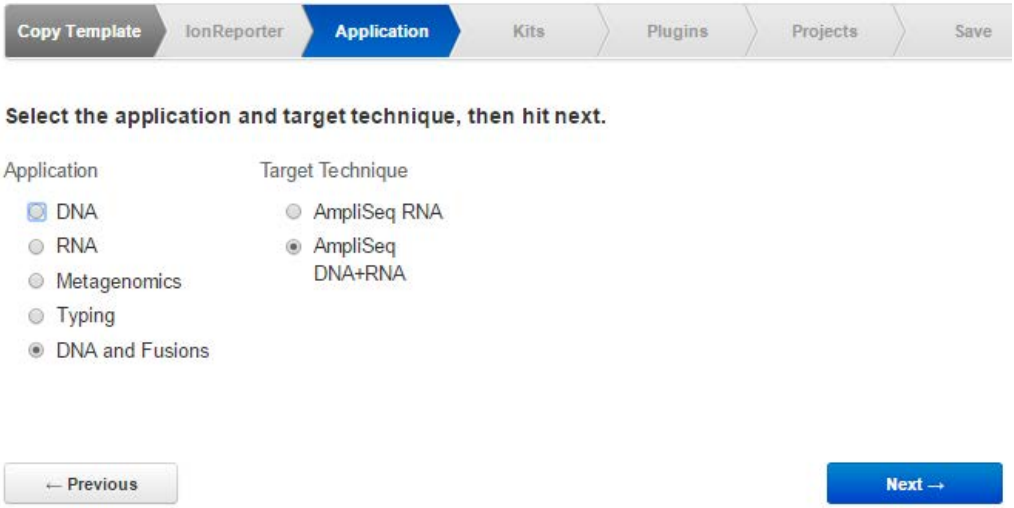
Ion Reporter is the first page in the Torrent Browser run template wizard. When you select an Ion Reporter account in this page, features related to Ion Reporter™ Software appear in the other wizard pages.



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

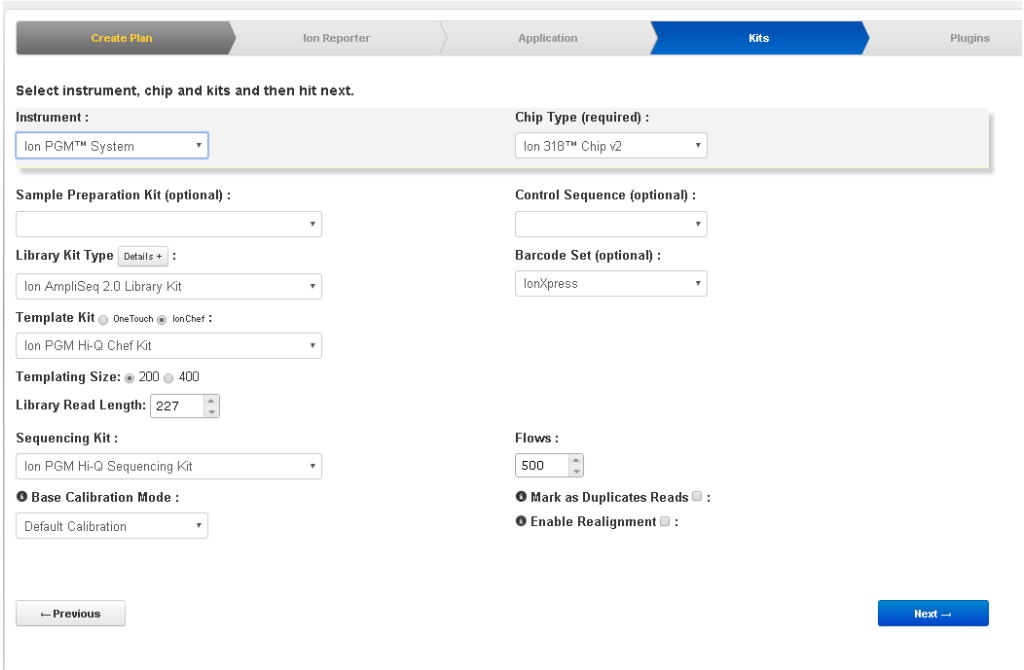
On the Kits wizard page, enter the following information about laboratory kits and other sequencing parameters:

- (*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.

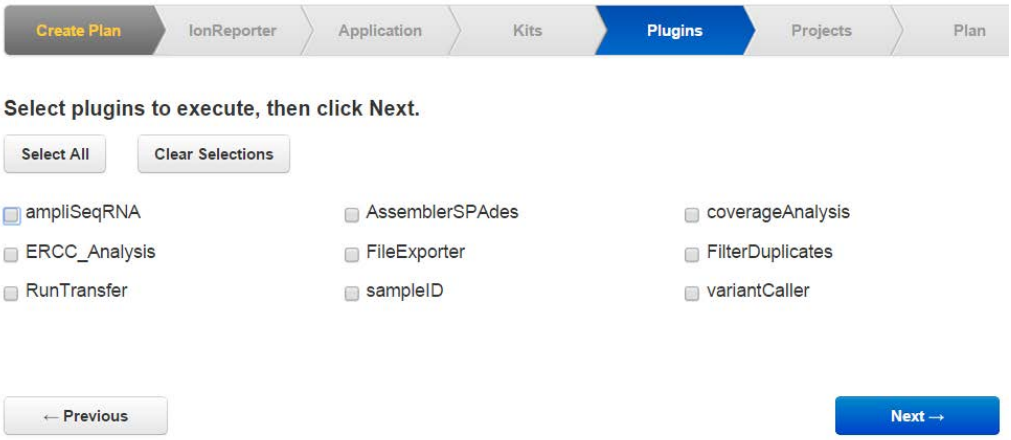
Example Kits page:



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:

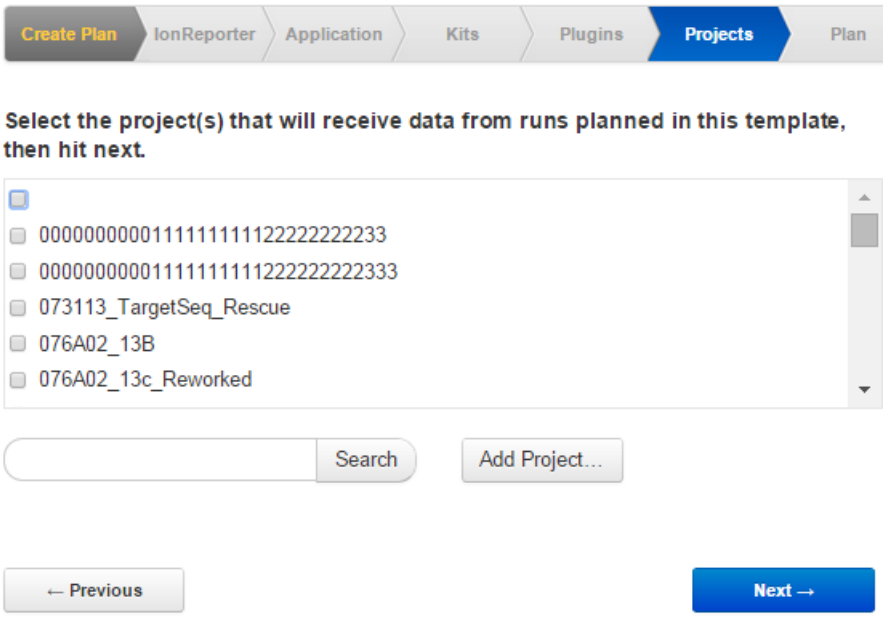


Note:

- 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 (TVC) 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 Chevron

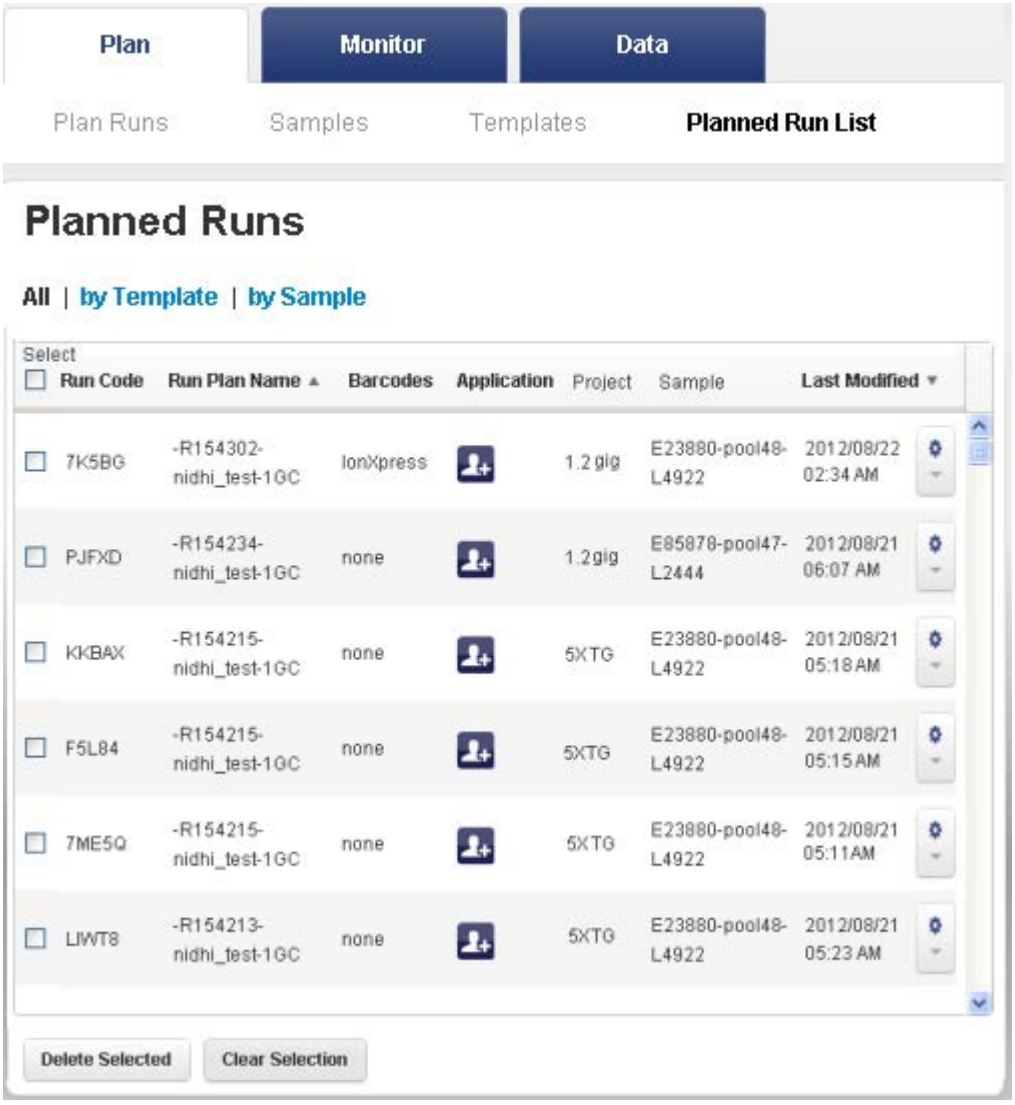
The title for last chevron of the wizard is different for templates, run plans, and run plans that are planned by sample set:

- **Templates** The chevron title is Save. Here you enter the new template name and optionally mark it as a favorite.
- **Run plans** The chevron title is Plan. Here you enter the new run plan name and sample information.
- **Plan by sample set** The chevron title is Save & Finish. Here you enter the new run plan name. (Sample information is automatically entered into the Barcoding chevron.)

Note: Templates that are marked as favorites are listed in their own section at the top of the Templates tab.

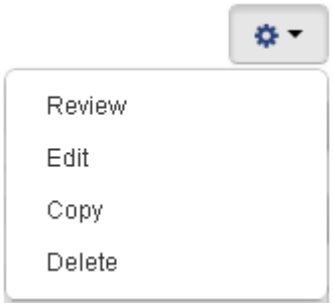
Example Planned Runs page

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.
Gear menu	The gear menu on the right side of a planned run allows you to review, edit, copy, or delete 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

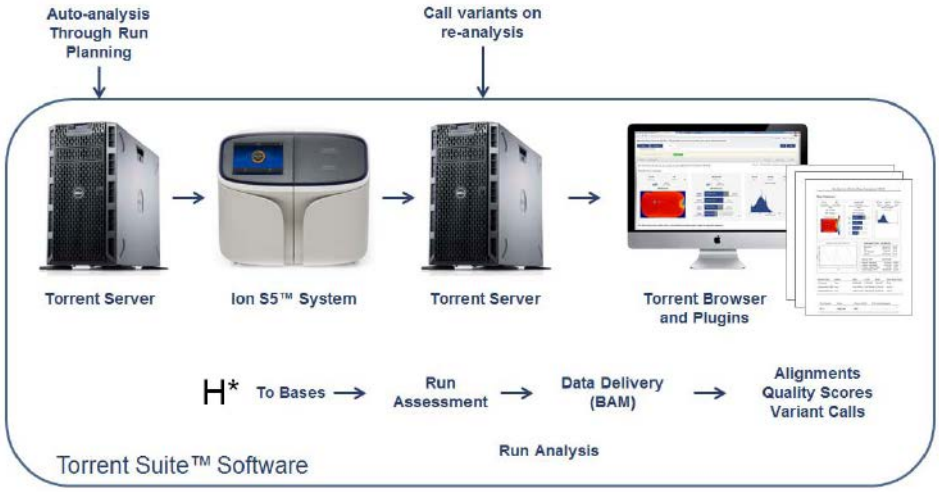
Ion Torrent uses a collection of custom tags to store sequencing and alignment information useful for downstream processing. In general, custom BAM tags starting with Z or Y are written by the BaseCaller and BAM tags starting with X stem from TMAP. As a consequence, tags starting with Z or Y are present 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.

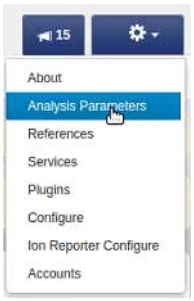
Step	Resulting file type	Ion Proton™ Chip	Ion 318™ Chip	Ion 316™ Chip	Ion 314™ Chip
Flows	--	500	500	520	520
Raw image acquisition	DAT	2.7 TB	225 GB	135 GB	30 GB
Image processing	WELLS	219 GB	16.4 GB	9.0 GB	2.0 GB
Signal processing and base calling	BAM	44 GB	4.2 GB	3.1 GB	0.5 GB

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 Custom Analysis Parameters

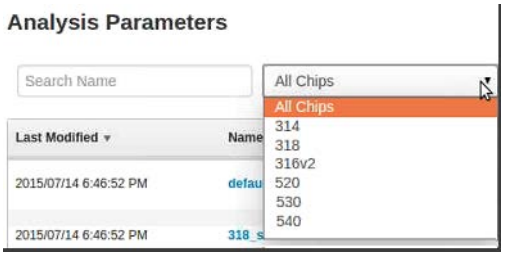
To create custom analysis parameters:

1. Log into Torrent Suite™ Software v5.0 or higher as Admin or User.
2. Click the **Gear** button and select **Analysis Parameters**.



The Analysis Parameters page appears. This page contains all the parameters that exist. Factory parameters are denoted by the Ion icon in the Source column.

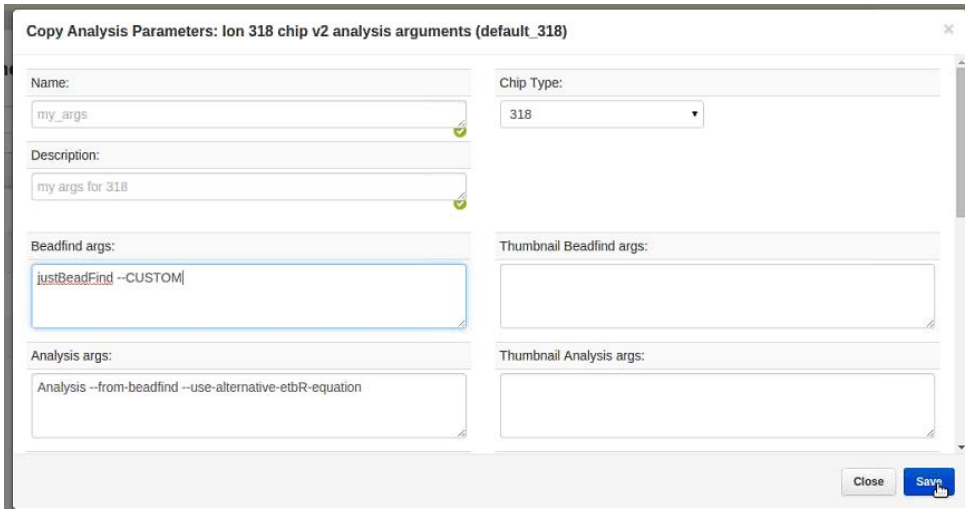
3. Select your chip type from the All Chips drop-down menu to see what parameter sets are available for it. In our example, we chose 318.



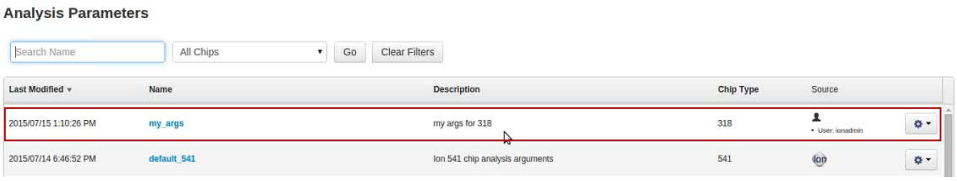
4. Pick a set to modify. Click the **Gear** button in the set's row and select **Copy**. For our example, we chose **default_318**.

The Copy Analysis Parameters screen appears.

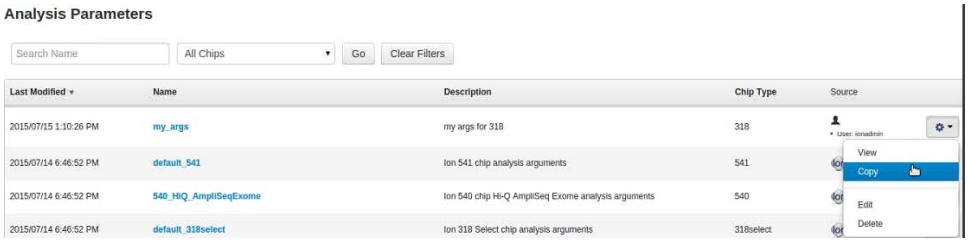
5. Enter a name, description, and make parameter changes. Click **Save**.



Your new analysis parameter set is now available on the Analysis Parameters table. Notice the Source column denotes that a user or admin created it.

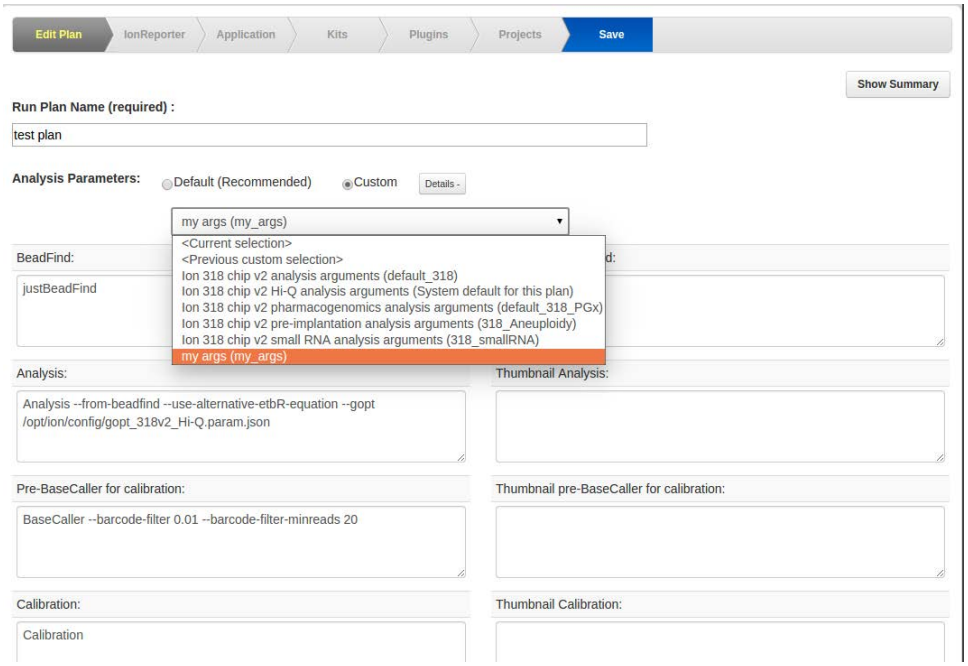


6. From the Gear button, you can View, Copy, Edit, or Delete this parameter set.

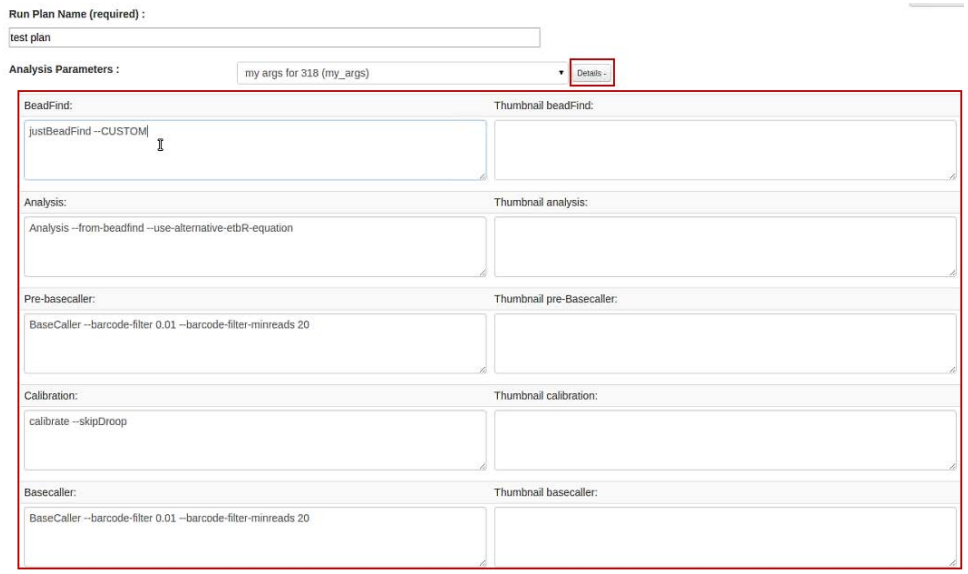


Your custom parameter set is now available for run planning.

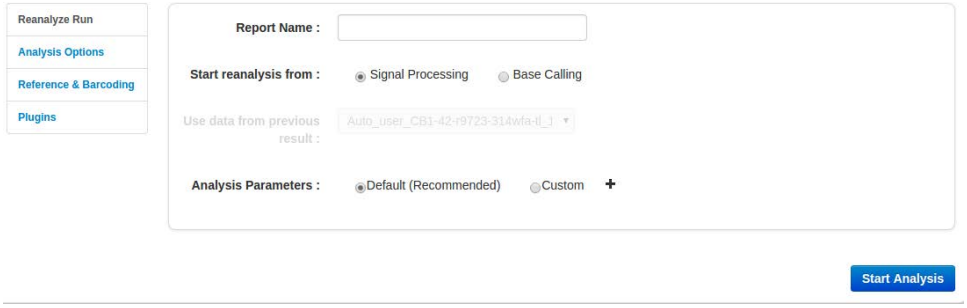
7. On the **Edit Plan** (or **Plan**) tab, Analysis Parameters section, select **Custom**. Then select your custom parameters from the drop-down menu.



8. Click **Details** to review the parameters.



9. You can also access the default and custom analysis parameters from the Reanalyze Run page:

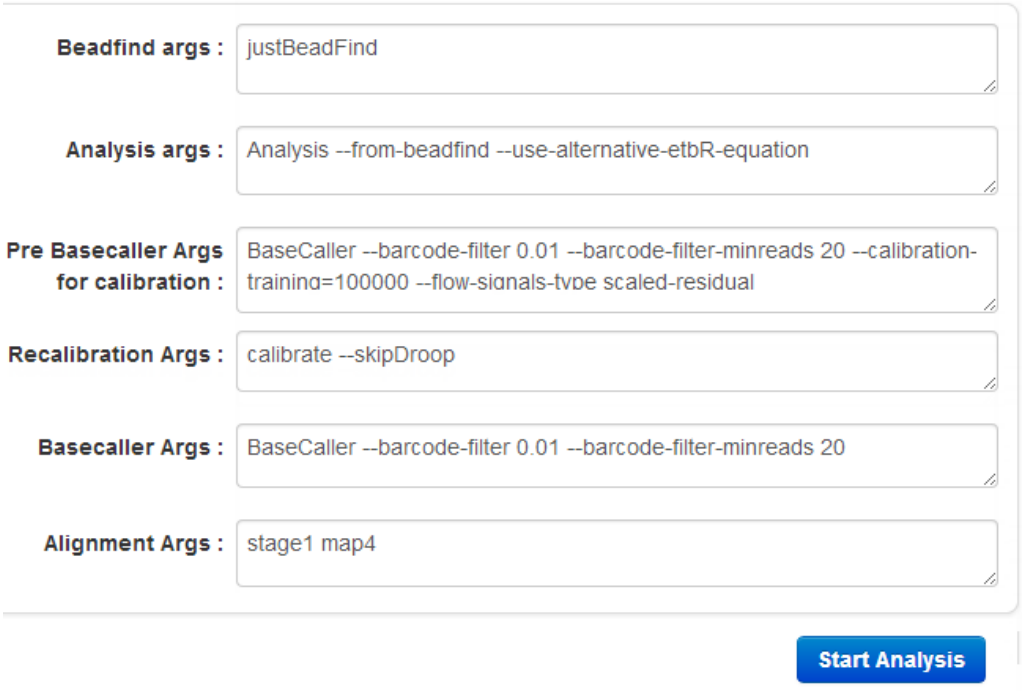


Find the TMAP command for a specific analysis

See "TMAP examples" on page 544 for steps to open the run report log and search for the TMAP command. (The analysis must be completed before you can find the command.)

The Command Line Args (Advanced) tab

An example Advanced Options page is shown here:



Setting	Description
Beadfind args	Beadfind module command line arguments. Should not be modified unless instructed by Ion Torrent™ Technical Support.
Analysis args	Analysis command line arguments. Should not be modified unless instructed by Ion Torrent™ Technical Support.
Pre Basecaller args for calibration	BaseCaller command line arguments. See Basecaller arguments for information on `--barcode-mode`, `--barcode-cutoff`, and `--barcode-filter`. Other Basecaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support. This field is used only if a Base Calibration Mode other than 'No Calibration' is used.
Recalibration Args	Recalibration command line arguments.
Basecaller args	BaseCaller command line arguments. See Basecaller arguments for information on `--barcode-mode`, `--barcode-cutoff`, and `--barcode-filter`. Other Basecaller arguments should not be modified unless instructed by Ion Torrent™ Technical Support.
Alignment Args	Arguments for the TMAP aligner. (Replaces the TMAP Args field that appears in previous releases.)

Overview of BaseCaller and Barcode Classification

This page discusses BaseCaller operations in general and issues around BaseCaller parameters, barcode classification, and filtering and trimming.

The settings of BaseCaller parameters control barcode classification as well as filtering and trimming.

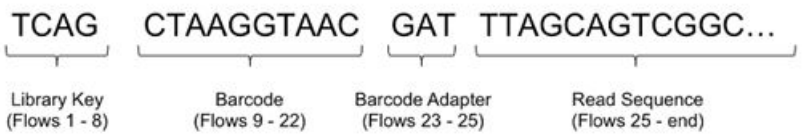
About barcodes

Barcodes are short base sequences that during library preparation are placed between the library key and the read. The barcode sequences provide a mechanism to distinguish and identify reads from different samplesduring data analysis.

The use of barcodes allows multiple samples to be sequenced together on one chip during a sequencing run, and still have the run's read data be analyzed separately afterward as distinct samples.

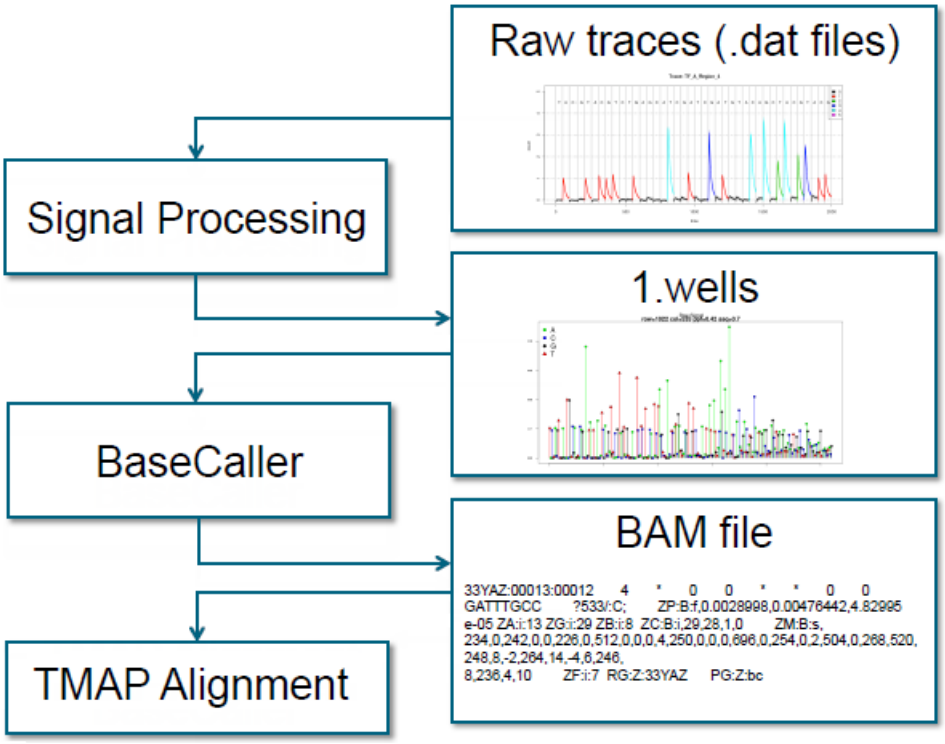
This diagram shows the placement of the barcode sequence, as well as the library key and adapters, with the read sequence (which is labeled "Template Bases"). The key is on the 5' end.

This example shows the location of the barcode sequence in both base space and flow space, using barcode IonPress_001 as an example:



Analysis pipeline overview

The beginning steps of the Torrent Suite™ Software analysis pipeline are shown below:



Steps:

1. The sequencing instrument generates DAT files of electrical signals' raw traces.
2. The signal processing step converts the raw traces into a single number per flow per well, in the 1.wells file.
3. The BaseCaller converts the 1.wells file information into a sequence of bases and writes the sequence into an unaligned BAM file.
4. The BAM file is passed to TMAP for alignment.

The signal processing step also marks several types of low-quality reads:

- Polyclonal reads (reads with two template beads instead of one)
- Reads with high signal processing residual (indicating an ambiguous signal value)
- Reads that do not contain a valid library key

Thesignal processing step marks these problematic reads but does not remove them.

Overview of BaseCaller functionality

In addition to creating a sequence of bases from the 1.wells file information, the BaseCaller module also performs read filtering and read trimming.

Notes on read filtering:

- Filters out low-quality reads that were marked during signal processing.
- Filters out reads that fail basecalling filters.
- Filtered out reads *do not*appear in the BAM file. The BaseCaller keeps counts of these reads but there is no record of specific reads that are filtered out.

Notes on read trimming:

- Removes certain bases from the read for quality reasons.
- The read appears in the BAM file.
- The removed bases do not appear in theBAM file.

These are the steps performed in the BaseCaller:

1. Remove low-quality reads that were marked during the signal processing step.
2. Do base calling:
    1. From the signal values, create the sequence of bases.
    2. Estimate the base quality value for each base.
3. Do barcode classification:
    1. Assign each read to a barcode.
    2. Trim the barcode sequence away if `--trim-barcodes=on` is specified. (The default is 'on').
4. Trim 5' unique molecular tag (only done if `--trim-barcodes=on`).
5. Trim extra bases at the 5' end. Controlled by `--extra-trim-left` (default is 0, meaning no extra trimming).
6. Filter out reads that are too short. Controlled by `--min-read-length` and `--trim-min-read-len`.
7. Filter out reads that do not have the correct library key. Can be turned off by `--keypass-filter`.
    1. Trim 3′ unique molecular tag (only done if P1 adapter was found).
    2. Trim extra bases on the 3′ end. Controlled by --extra-trim-right (default is 0, meaning no extra trimming. Only done if P1 adapter was found).
8. Trim the P1 adapter (at the 3' end).
9. Perform quality trimming. Affect ed by `--trim-qual-window-size` and `--trim-qual-cutoff`.

Notes about quality trimming:

- The purpose of quality trimming is to identify where quality issues begin at the end of a read. We try to identify when bases fall below a quality threshold and trim both those bases and a bit before those bases.
- The parameter `--trim-qual-window-size` sets the window size for quality trimming. The algorithm slides through the sequence of bases and, each time the window shifts, computes the mean Base QV value for all bases in the window.
- If the mean Base QV value for all bases in the window falls below a threshold (set the by parameter `--trim-qual-cutoff`, default 16), then we trim all bases from the center of the window at that time to the 5' end.

Notes about barcode classification and barcode filtering

Barcode classification determines which barcode group a read is assigned to. Barcode classification is done for each read immediately after base calling.

Barcode filtering determines if a specific barcode is included in the run report or is filtered out. Barcode filtering works on the barcode groups as a whole

Troubleshooting Barcode Classification Issues

Barcode classification metrics are available in the file `basecaller_results/datasets_basecaller.json` in the Torrent Suite™ Software analysis directory.

This file contains information about all barcodes, no matter whether they appear in the run report or are filtered out. This information describes the numbers of barcodes that would be included or discarded if you reanalyze with certain changed BaseCaller settings.

A sample of this file is shown here. Later examples in this page use this file:

```
"IEXL3.IonXpress_033": { "Q20_bases": 98859279,
"barcode_adapter": "GAT", "barcode_bias": [ 0.026, -0.028,
-0.034, 0.011, -0.019, -0.001, 0.072, -0.061, 0.103, -0.008,
-0.062, 0.110, -0.021, 0.001], "barcode_distance_hist":
[ 907546, 50122, 10793, 4498, 5342 ], "barcode_errors_hist":
[ 949782, 24584, 3935 ], "barcode_match_filtered": 162,
"barcode_name": "IonXpress_033", "barcode_sequence":
"TTCTCATTGAAC", "description": "1T 058a0112 Lib6457 0bp lr2
lr226b04", "filtered": false, "index": 33, "library": "hg19/
IonXpress_033", "platform_unit": "PGM/318/IonXpress_033",
"read_count": 978301, "recalibrate": true, "sample": "None",
"total_bases": 109292583 },
```

Explanation of fields in the BaseCaller JSON file

Read count

The read_count field shows how many reads were assigned to this barcode.

```
"read_count": 978301,
```

Filtered

The `filtered` field is `true` if this barcode is filtered out and `false` if the barcode appears on the run report.

```
? "filtered": false,
```

Barcode errors histogram

The barcode errors histogram shows the number of reads with difference levels of basecalling errors in this barcode:

- **First field**: The number of reads that have 0 basecalling errors (949782 in this example). This is the number of reads that perfectly match this barcode (in base space).
- **Second field**: The number ofreads that have one basecalling error(24584 in this example).
- **Third field**: The number of reads that have two basecalling errors(3935in this example).

From the 3935 value with 2 basecalling errors, we know that if we reanalyze with the number of allowed errors set to 1 instead of 2, then 3935 fewer reads will be assigned to this barcode.

```
? "barcode_errors_hist": [ 949782, 24584, 3935 ],
```

This histogram is typical of a real barcode. A large majority of reads are perfect matches, a few have one error, and a smaller number have two errors.

If the pattern is reversed (with very few perfect matches, some reads with one error, and many reads with 2 errors), we suspect that this is probably a fake barcode.

Barcode distance histogram

The barcode distance histogram shows, *in signal space*, the number of reads at various squared residual distances between the predictedsignal and the observed signal.

The distance fields are given in 0.2 increments:

- The first field gives the number of reads with a squared residual distance of between 0 and 0.2.
- The second field gives the number of reads with asquared residual distance of between 0.2 and 0.4.
- The third field gives the number of reads with a squared residual distance of between 0.4 and 0.6, etc.

Smaller distances reflect better matches of the read to barcode. Larger distances reflect poorer matches.

This example reflects the pattern that is typical of a real barcode:

- The most reads have shorted distance residuals.
- Fewer reads have larger distance residuals.
- The entry 5342 in the fifth field tells us that reducing `--barcode-cutoff` to 0.8 would cause those 5342 reads not to be assigned to a barcode.

```
? "barcode_distance_hist": [ 907546, 50122, 10793, 4498,
5342 ],
```

Barcode match filtered

The `barcode_match_filtered` field gives the number of reads that perfectly match the barcode *in base space* and also are filtered out because they do not meet the separation criteria *in signal space*. The signal for these reads are in-between two barcodes and are not close enough to either barcode to be assigned.

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

Barcode bias

The `barcode_bias` values show the mean signal deviation by flow: how much the observed signal is off from the expected signal. Low bias values, for example with the value shown here, are indications of good signal.

Bias values around 0.33 indicate a signal that is about a third of a base off. Values near 0.5 indicate a signal that is half a base off. Values in this range indicate a problem with the sequencing run or with the barcode classification.

```
? "barcode_bias": [ 0.026, -0.028, -0.034, 0.011, -0.019,
-0.001, 0.072,? -0.061, 0.103, -0.008, -0.062, 0.110, -0.021,
0.001],
```

BaseCaller arguments

This section describes select arguments used with the BaseCaller module.

BaseCaller Parameters

This page describes BaseCaller parameters that are available when you reanalyze a completed run.

Note: The default BaseCaller parameters are tuned for Ion Torrent™ data. In most cases, you do not need to modify these settings. Modifying these parameters is recommended for advanced users only.

However, if you use a custom barcode set, please see the cautions and requirements in Design Custom Barcodes. Correct parameter settings require knowledge of your barcode's distances in signal space. The BaseCaller defaults are optimized for the IonXpress barcode set, and likely are not correct for a custom barcode set.

When you reanalyze a run, other parameters are also listed in the BaseCaller arguments field. These parameters are for internal use please do not change or remove these fields.

Note: Barcode classification is the process by which reads are assigned to one of the barcodes present in one analysis run. Correct barcode classification is important because a classification error results in a read being assigned to the wrong barcode, which in turn leads to the read beinganalyzed as belonging to a wrong sample.

Barcode classification determines which barcode group a read is assigned to. Barcode classification is done for each read immediately after base calling.

Barcode filtering determines if a specific barcode is included in the run report or is filtered out. Barcode filtering works on the barcode groups as a whole.

Barcode classification parameters

This table lists the more common BaseCaller parameters relating to barcode classification. (All parameters listed in this table are barcode classification parameters.)

Parameter	Default	Description
--barcode-cutoff	1.0 (Float)	Maximum distance allowed in barcode matches. A threshold that sets the stringency for barcode matches. Lower values require more exact matches when assigning reads to barcodes. Higher values allow less exact matches. Reads that have a distance greater than this value are counted as barcode no-matches.
--barcode-mode	2 (Integer)	Allowed values: 1, 2 • **1**: A barcode is scored by comparing each read sequence to each barcode sequence in a flow space alignment. Errors in each flow are summed over the length of the barcode flows.Then any barcode with a number of errors equal to or less than the `--barcode-cutoff` value can be considered, and the barcode with the fewest errors with respect to the input sequence is the matching barcode. (The default in 4.0, known as hard decision classification.) **2**: Barcode classification is based on signal information,specifically on the squared distance between the measured signal and the predicted barcode signal. (The default in 4.4, known as soft decision classification.) **Note:** `--barcode-mode 0` is no longer supported.

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 544 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 | sff | 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)

Troubleshooting

Handle a failed analysis run

If an analysis run fails, you need to attempt to determine the cause of the failure an, 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 verify complete data transfer. You can re-transfer the data if you are not sure it was completely transmitted.

2. Go to the Torrent Browser **Data > Completed Runs & Reports** tab and see 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.

Glossary

Index