

# **Torrent Suite™ Analysis Report Guide**

for use with Torrent Suite™ Software v5.0

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# Torrent Browser Analysis Report Guide

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## 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 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
- **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

## Report header

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

- **Run Summary** – Jumps to the Run Summary area
- **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)

**Run Summary: B32-299**

**Notes** Lib6457

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 (see One-Click Transfer 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 change 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:

Upload to IR ▾	Report Actions ▾	Reports ▾
Darwin	Review Plan	Summary PDF
IRProduction	Edit Run Plan	Plugins PDF
Configure IR accounts	Copy Plan	Classic Report
	Select plugins to run	
	Reanalyze	
	Data Management	

## Compare run reports

From a project listing page, you can compare report metrics for multiple runs side-by-side. See [Compare Multiple Run Reports](#).

## Old report analysis

The Torrent Browser cannot display run reports from 2.x versions of the Torrent Suite™ Software in the current run report format.

To work with results generated with a 2.x version of Torrent Suite™ Software, you have these options:

- **Re-analyze the run to generate a new report** – Opens the Data > Completed Runs & Reports > Reanalyze page. Typically you click **Start Analysis** button to re-analyze with the same settings. The **Advanced** page is also available to change the analysis settings. After you re-analyze the run, you then have all 3.x features available in the new run report.
  - **View the pre-3.0 report** – Open the run report in the old pre-3.0 style. Many new features are not available in the old style run report.
  - **View this report log** – Open the text log for this run.
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## Run Report Metrics

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#### Run Report Metrics

This section provides background information on run metrics and detailed descriptions of a run report. See [Run Metrics Overview](#) for explanations of quality metrics, read length calculations, and alignment.

[Run Metrics Overview](#)

[Run Report Metrics Before Alignment](#)

[Run Report Metrics on Aligned Reads](#)

For analyses that are members of a project, you can download a CSV file of run metrics. See [Project Result Sets Page](#) and [CSV Metrics File Format](#).

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## 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		<a href="#">BAM</a> <a href="#">BAI</a>
IonXpress_001	E2575-p7	18.7M	11.1M	235382	79 bp		<a href="#">BAM</a> <a href="#">BAI</a>
IonXpress_002	E2575-p7	24.7M	15.2M	312251	79 bp		<a href="#">BAM</a> <a href="#">BAI</a>
IonXpress_003	E2575-p7	29.2M	18.1M	366997	79 bp		<a href="#">BAM</a> <a href="#">BAI</a>
Q20							<a href="#">1</a> <a href="#">2</a>

Column	Description
<b>Barcode Name</b>	The individual barcode in the barcode set.  The row labeled as <b>No barcode</b> reports on unclassified barcodes, which are reads that could not be classified as matching one of the expected barcodes in the barcode set.
<b>Sample</b>	Name of the sample that was sequenced on instrument.
<b>Output</b>	Total number of reads.
<b>% &gt;= Q20</b>	The percentage of reads that have a predicted quality score of Q20 or better.  A Q20 score is the predicted quality of a Phred-like score of 20 or better, or one error in 100 bp.
<b>Reads</b>	Total number of filtered and trimmed library reads (independent of length). This number is reported in the barcode BAM file.
<b>Mean Read Length</b>	The average read length, in bp, of all filtered and trimmed library reads reported in the barcode BAM file.
<b>Read Length Histogram</b>	A thumbnail histogram of the read lengths for this barcode. Click on the thumbnail histogram to open a larger image.

<b>BAM</b>	Buttons to download the BAM and BAM index file (BAI) for this barcode. The BAM file contains aligned reads sorted by reference location.  See also <a href="#">Run Metrics Overview</a> for a description of alignment data.
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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.

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## 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.



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)

Quality Following Alignment (AQ20)

## 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  $-10 \times \log_{10}$  (error probability). Q20, therefore, corresponds to a predicted error rate of one percent.



Refer to [http://en.wikipedia.org/wiki/Phred\\_quality\\_score](http://en.wikipedia.org/wiki/Phred_quality_score) for a more complete description of Phred values.

## Quality Following Alignment (AQ20)

Alignment of reads can be a useful process 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

### How Is Aligned Read Length Calculated?

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.

### How Is Alignment Performed?

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.

[Technical Note - Analysis Pipeline](#)  
[Technical Note - TMAP Alignment](#)

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: 19907642  
 PLoS 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. For more detail on how to enable and specify this reference-specific limit see the Adding a Reference Sequence section of [Working with Reference Sequences](#).

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.

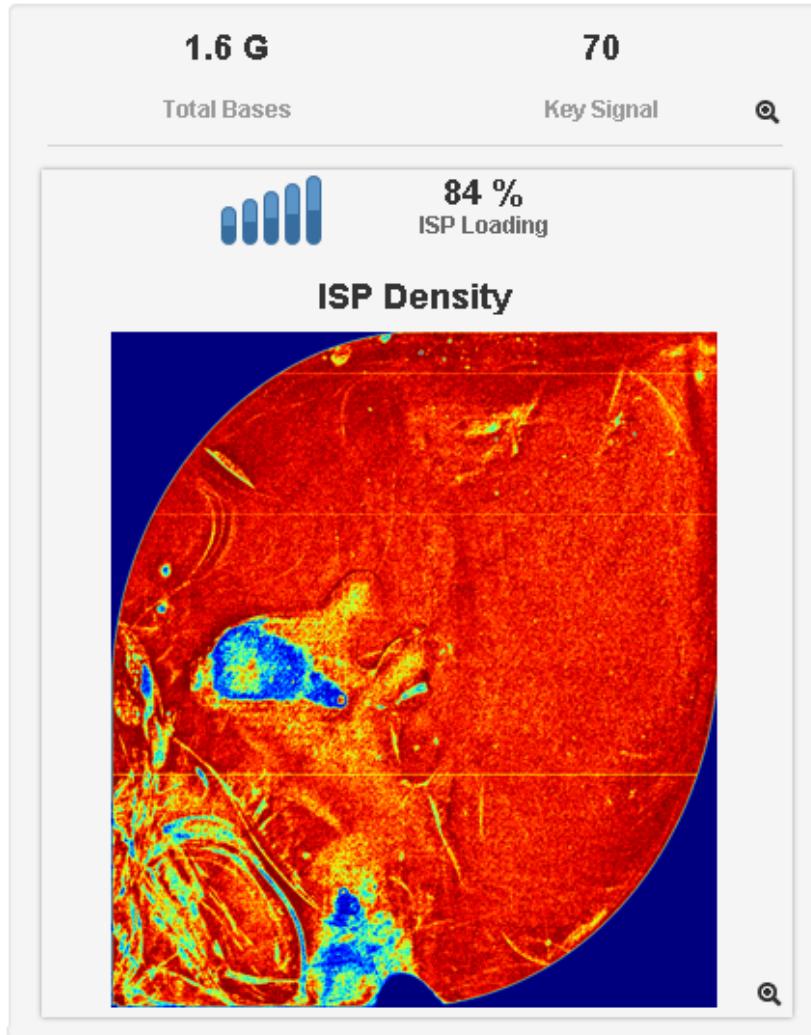
## Run Report Metrics Before Alignment

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



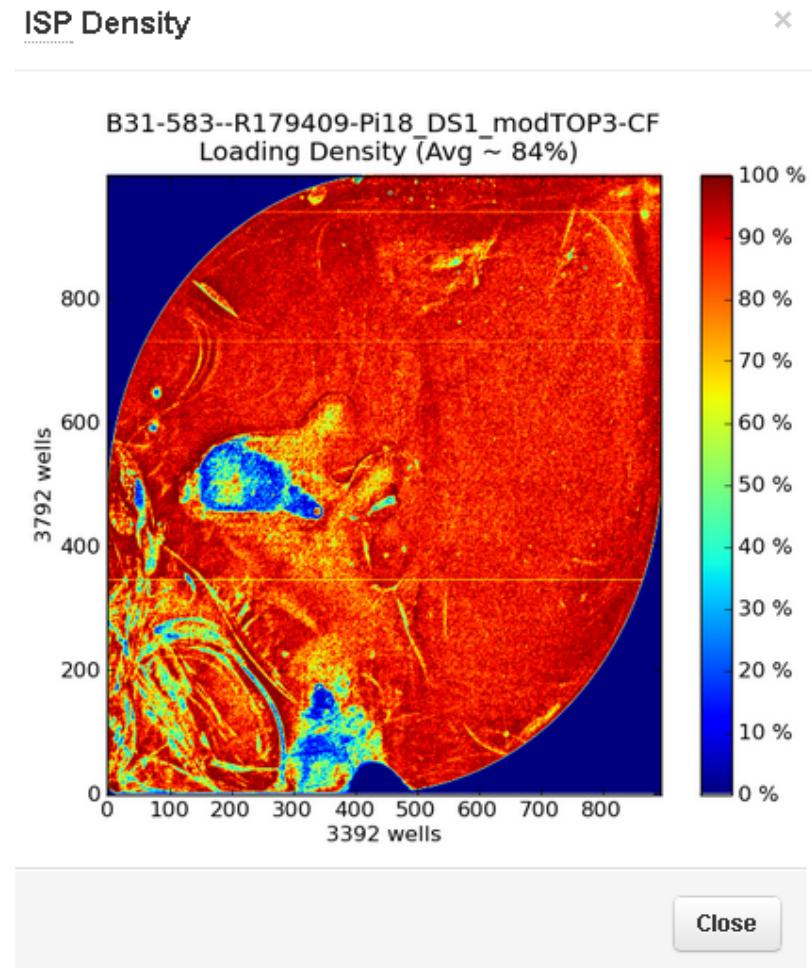
This table describes the Ion Sphere™ Particle (ISP) density metrics:

Metric	Description
Total Bases	Number of filtered and trimmed base pairs reported in the output BAM file.

<b>Key Signal</b>	Percentage of Live ISPs with a key signal that is identical to the library key signal.
<b>Bead Loading</b>	Percentage of chip wells that contain a live ISP. (The percentage value considers only potentially addressable wells.)

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

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

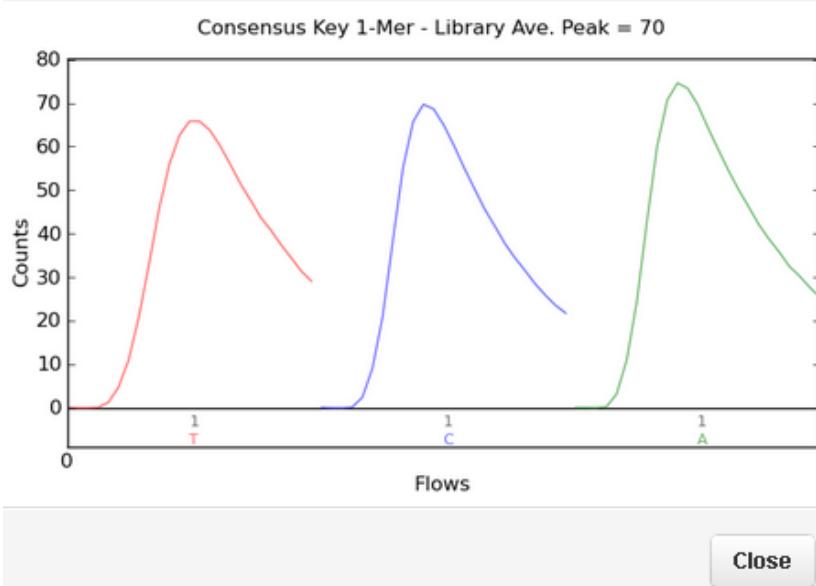


## Key signal



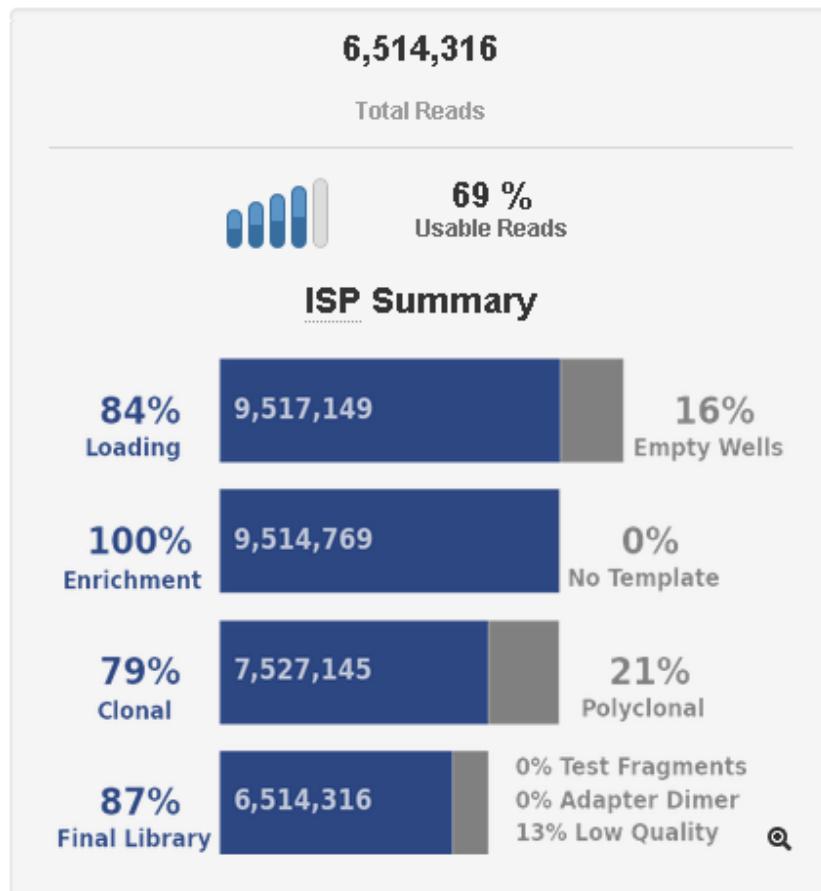
Click the magnify icon in the Key Signal area to open the key incorporation graphs:

## Key Incorporation Traces



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
<b>Total Reads</b>	Total number of filtered and trimmed reads independent of length reported in the output BAM file.	(Not calculated)
<b>Usable Sequence</b>	The percentage of library ISPs that pass the polyclonal, low quality, and primer dimer filters.	Final Library ISPs / Library ISPs
<b>Loading</b>	Percentage of chip wells that contain a live ISP. (The percentage value considers only potentially addressable wells.)	No. of Loaded ISPs / No. of potentially addressable wells
<b>Empty Wells</b>	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
<b>Enrichment</b>	Predicted number of Live ISPs that have a key signal identical to the library key signal. The Percent Enrichment value reported is the number of loaded ISPs that are Library ISPs, after taking out Test Fragment ISPs.	Library ISPs / (No. of Loaded ISPs minus TF ISPs)
<b>No Template</b>	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)
<b>Clonal</b>	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
<b>Polyclonal</b>	Percentage of polyclonal ISPs (ISPs carrying clones from two or more templates).	Polyclonal ISPs / Live ISPs

<b>Final Library</b>	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
<b>% Test Fragments</b>	Percentage of Live ISPs with a key signal that is identical to the test fragment key signal.	Test Fragment ISPs / Clonal ISPs
<b>% Adapter Dimer</b>	Percentage of ISPs with an insert length of less than 8 bp.	Primer dimer ISPs / Clonal ISPs
<b>% Low Quality</b>	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%
<b>Final Library ISPs</b>	<b>6,514,316</b>	<b>68.6%</b>

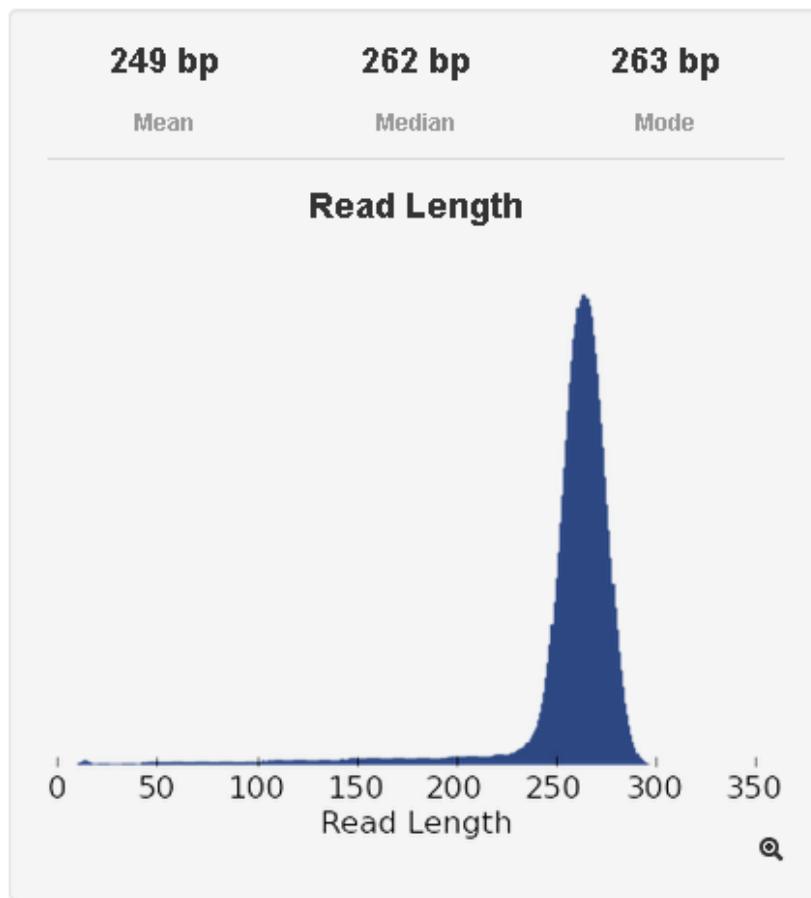
These metrics are described in this table:

Metric	Description	Calculation
<b>Addressable Wells</b>	Total number of addressable wells.	(Not calculated)

<b>With ISPs</b>	<p>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.</p>	Wells with ISPs / Total Addressable Wells
<b>Live</b>	<p>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:</p> <ul style="list-style-type: none"> <li>• Test Fragment</li> <li>• Library</li> </ul>	Live ISPs / Wells with ISPs
<b>Test Fragment</b>	<p>Number (and percentage of Live ISPs) of Live ISPs with a key signal that was identical to the Test Fragment key signal.</p>	Test Fragment ISPs / Live ISPs
<b>Library</b>	<p>Number (and percentage of Live ISPs) of Live ISPs with a key signal that was identical to the library key signal.</p>	Library ISPs / Live ISPs
<b>Library ISPs</b>	<p>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).</p>	Library ISPs
<b>Filtered: Polyclonal</b>	ISPs carrying clones from two or more templates.	Polyclonal ISPs / Library ISPs
<b>Filtered: Low quality</b>	Low or unrecognizable signal.	Low quality ISPs / Library ISPs
<b>Filtered: Primer dimer</b>	Insert length of less than 8 bp.	Primer dimer ISPs / Library ISPs

<b>Final Library ISPs</b>	<p>Number (and percentage of Library ISPs) of reads passing all filters, which are recorded in the output BAM file.</p> <p>This value may be different from the <b>Total number of reads</b> located in the Library Summary Section due to technicalities associated with read trimming beyond a minimal requirement resulting in <b>Total number of reads</b> being slightly less than <b>Final Library Reads</b>.</p>	Final Library / Library ISPs
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## Read Length

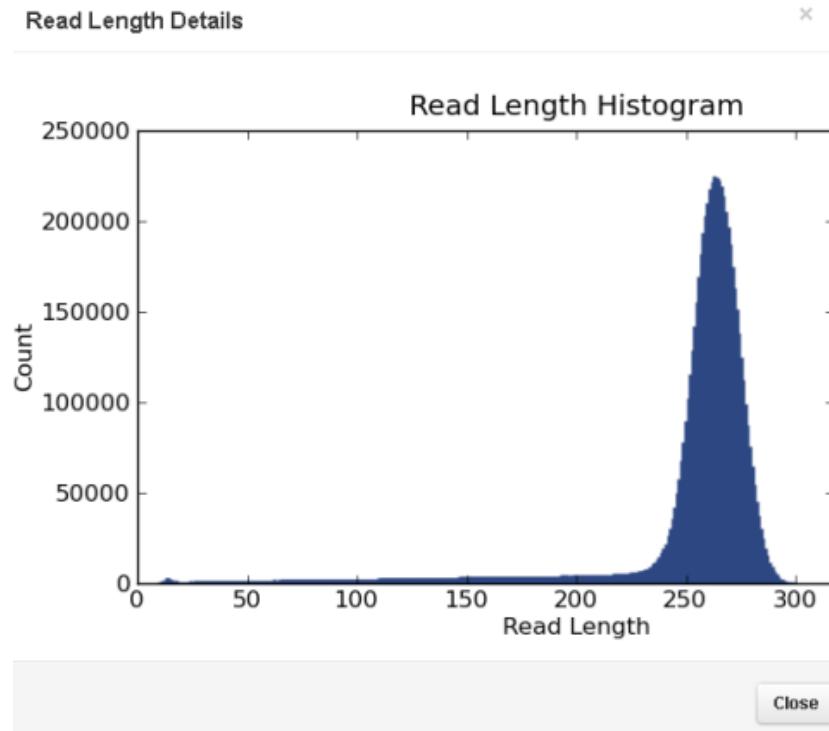


This table describes the read length metrics:

Metric	Description
<b>Mean Read Length</b>	Average length, in base pairs, of called reads.
<b>Median Read Length</b>	Median length of called reads.
<b>Mode Read Length</b>	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:

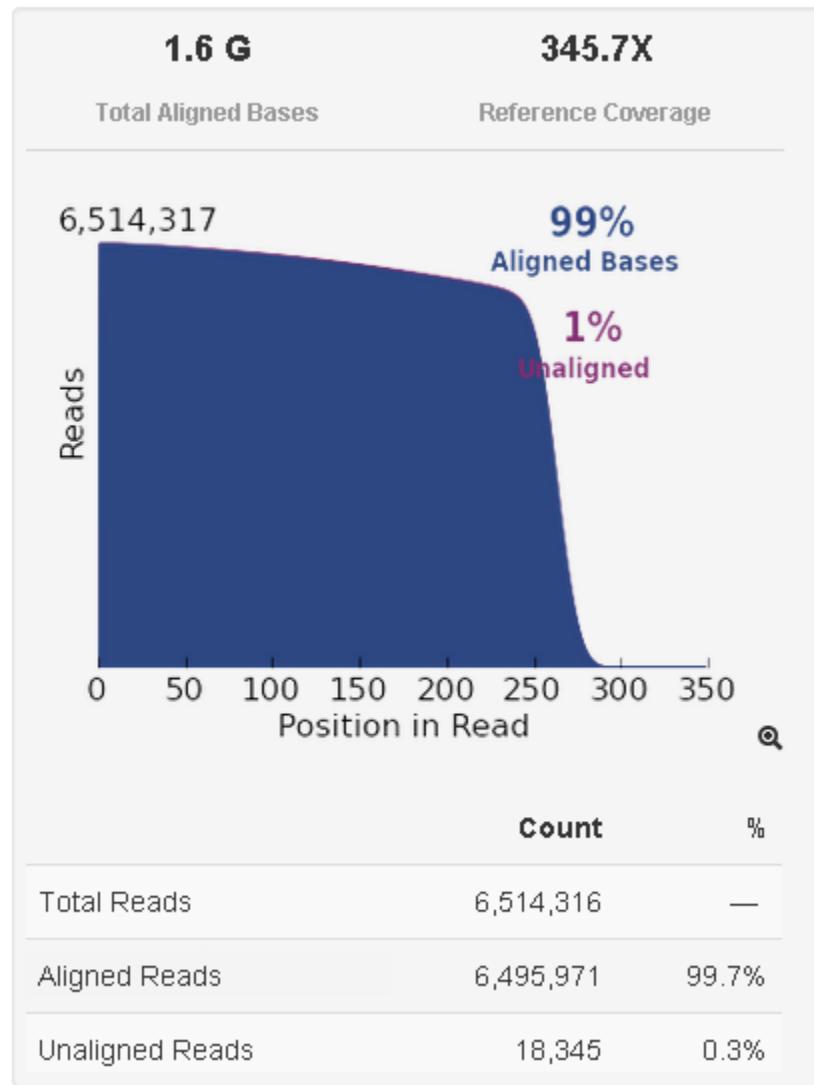


For more information on filtering and trimming, please see [Technical Note - Filtering and Trimming](#).

## Run Report Metrics on Aligned Reads

This section of a run report provides metrics on aligned reads.

### The Total Aligned Bases



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

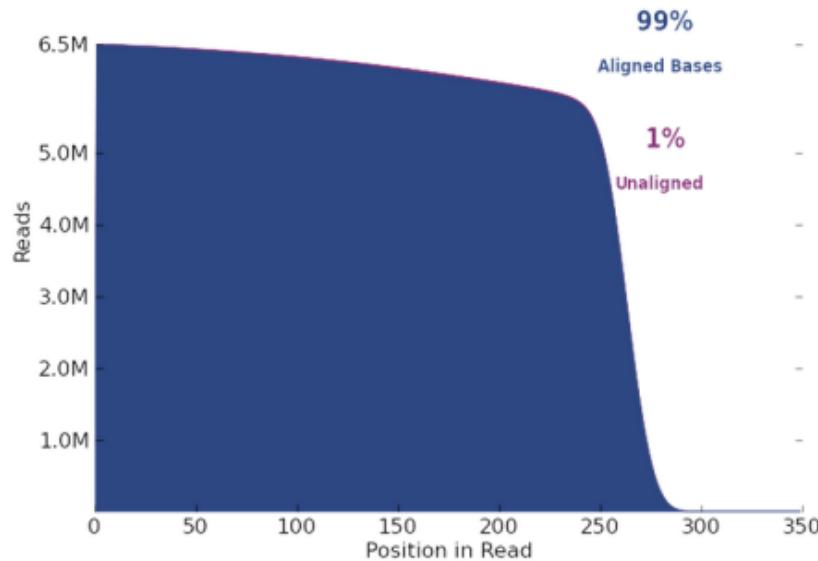
Metric	Description
<b>Total Aligned Bases</b>	Number of filtered and trimmed aligned base pairs reported in the output BAM file.  Total number of bases aligned to the reference sequence. Excludes the library key, barcodes, and 3' adapter sequences.

<b>Reference Coverage</b>	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.
<b>% Aligned Bases</b>	Percentage of Total Aligned Bases out of all reads.
<b>% Unaligned</b>	Percentage of bases not aligned to references.
<b>Total Reads</b>	Number of reads generated during basecalling.
<b>Aligned Reads</b>	Number of reads that aligned to the reference genome.
<b>Unaligned Reads</b>	Number of reads that did not align to the reference genome.

 For more information on filtering and trimming, please see [Technical Note - Filtering and Trimming](#).

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

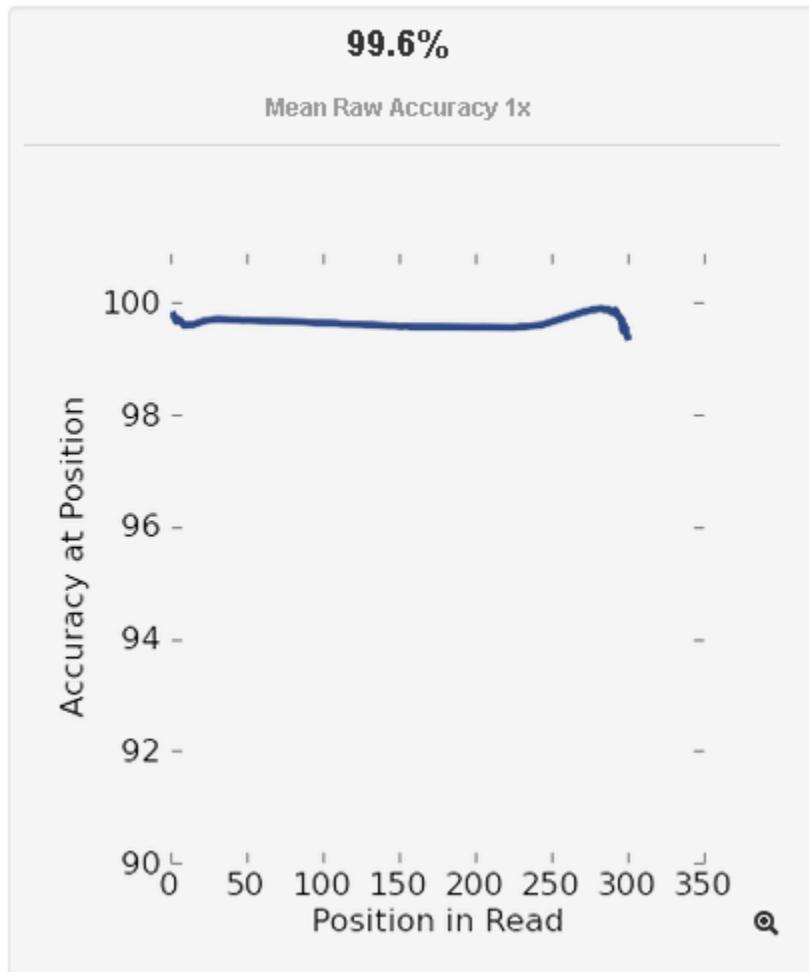
#### Alignment summary



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

See also [Run Metrics Overview](#) for information on alignment quality calculations.



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

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## 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 (see [Barcode Reports](#)).

## Output Files

File Type	Reads	Aligned Reads
Library	<a href="#">BAM</a>	<a href="#">BAM</a> <a href="#">BAI</a>
Barcodes	<a href="#">BAM</a>	<a href="#">BAM</a> <a href="#">BAI</a>

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.  See <a href="#">Run Metrics Overview</a> for a description of the alignment data included in this BAM file.
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.



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
<b>SFF</b>	<p>Compressed (.zip) Standard Flowgram Format (SFF) -formatted file that contains "flow space" data.</p> <p>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.</p> <p>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. (See <a href="#">Technical Note - Filtering and Trimming</a>.)</p> <p><b>Note:</b> The SFF file format is deprecated and not produced by the default pipeline.</p>
<b>FASTQ</b>	<p>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. (See <a href="#">Technical Note - Filtering and Trimming</a>.)</p> <p><b>Note:</b> The FASTQ file format is deprecated and not produced by the default pipeline.</p>

## 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.)

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## 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](#)

[The Plugin Summary list](#)

[Plugin reports](#)

[Plugin log files](#)

## Run a plugin



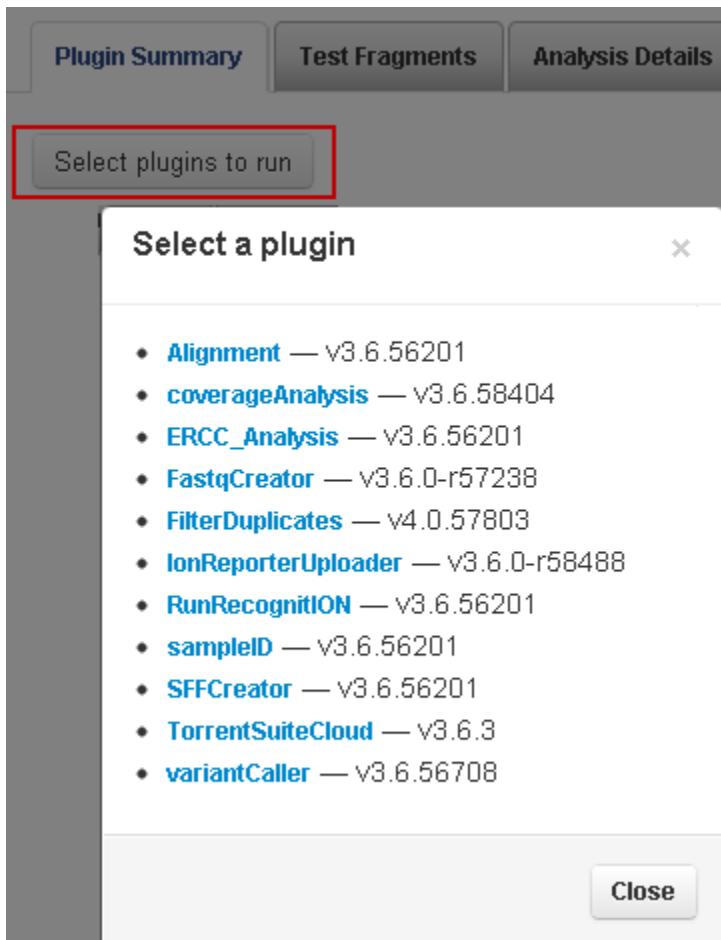
The following plugins are pre-installed. See [Run the Installed Plugins](#) for a description of these plugins.

- Alignment
- Coverage Analysis
- ERCC\_Analysis
- FileExporter
- FilterDuplicates
- IonReporterUploader
- Run RecognitION
- sampleID
- Torrent Suite Cloud

- Torrent Variant Caller

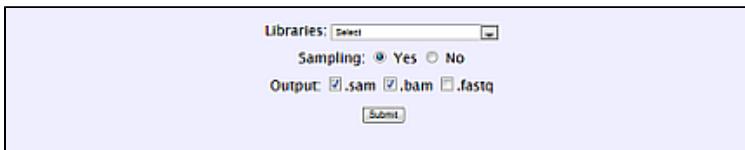
To manually run a post-analysis plugin on the report data:

1. Click **Select Plugins To Run**.
2. The Plugin List pops up and displays a list of available plugins:



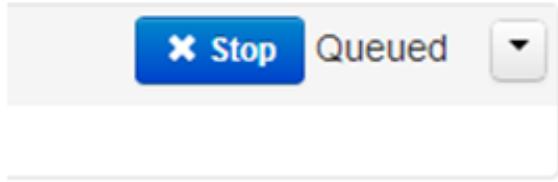
3. Click the plugin you want to run.

If the plugin does not require user input, it is immediately queued for execution. Other plugins displays their user interface. In this example, the **Alignment** plug in is selected, displaying the **Alignment** plugin dialog (see [Run the Installed Plugins](#) for more information about plugin options):



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:

Read Length [bp]	Reads	Unmapped	Excluded	Clipped	Perfect	1 mismatch	$\geq 2$ mismatches
50	102	46	2	0	16	13	25
100	47	20	1	14	0	0	12
150	18	8	0	8	0	0	2

+ Alignment.html

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. See [Run the Installed Plugins](#) for a description of report pages for the installed plugins.

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

Plugin Summary Test Fragments Analysis Details Support Software Version

Select plugins to run + Expand All - Collapse All Refresh plugin status

- coverageAnalysis — v3.6.58404 26.1 MB **Completed**
- coverageAnalysis.html
- FilterDuplicates — v4.0.57803 12.2 MB **Completed**
- sampleID — v3.4.50000 51.5 kB **Completed**
- sampleID.html
- variantCaller — v3.6.56316 704 kB **Completed**
- variantCaller.html

## Plugin log files

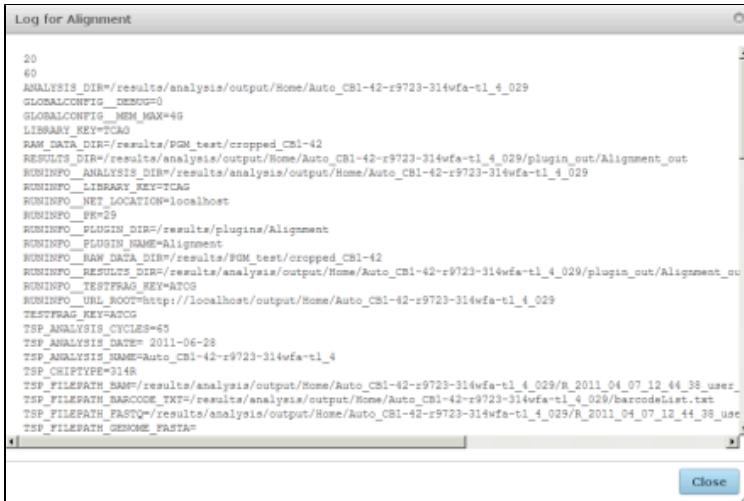
1. Hover your mouse pointer over the log icon to the right of your plugin to display the plugin log file:

Plugin Summary Test Fragments Analysis Details Support Software Version

Select plugins to run + Expand All - Collapse All Refresh plugin status

- coverageAnalysis — v3.6.58404 26.1 MB **Completed**
- coverageAnalysis.html
- FilterDuplicates — v4.0.57803 12.2 MB **Completed**
- sampleID — v3.4.50000 51.5 kB **Completed**
- sampleID.html
- variantCaller — v3.6.56316 704 kB **Completed**
- variantCaller.html

2. Click the icon to display the log:



```

20
60
ANALYSIS_DIR=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029
GLOBALCOMPTG_MAX=0
GLOBALCOMPTG_MM=MAX=40
LIBRARY_KEY=TCAG
RAW_DATA_DIR=/results/PGM_test/cropped_CBL-42
RESULTS_DIR=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029/plugin_out/Alignment_out
RUNINFO_ANALYSIS_DIR=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029
RUNINFO_LIBRARY_KEY=TCAG
RUNINFO_NET_LOCATION=localhost
RUNINFO_PGM=0
RUNINFO_PLUGIN_DIR=/results/plugins/Alignment
RUNINFO_PLUGIN_NAME=Alignment
RUNINFO_RAW_DATA_DIR=/results/PGM_test/cropped_CBL-42
RUNINFO_RESULTS_DIR=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029/plugin_out/Alignment_out
RUNINFO_TESTPAG_KEY=TCAG
RUNINFO_URL_ROOT=http://localhost/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029
TESTPAG_KEY=TCAG
TSP_ANALYSIS_CYCLE=65
TSP_ANALYSIS_DATE=2011-06-28
TSP_ANALYSIS_NAME=Auto_CBL-42-r9723-314wfa-tl_4
TSP_SCRIPTTYPE=J14R
TSP_FILEPATH_SAMP=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029/B_2011_04_07_12_44_38_user_
TSP_FILEPATH_BARCODE_TXT=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029/barcodeList.txt
TSP_FILEPATH_FASTQ=/results/analysis/output/Home/Auto_CBL-42-r9723-314wfa-tl_4_029/B_2011_04_07_12_44_38_use_
TSP_FILEPATH_GENOME_FASTA=

```

3. Click **Close** to exit the display.
-

## 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
- Plugin configuration
  - Admin plugin configuration area
- Manually run a plugin
- Automatically run plugins
  - The old method to autorun a plugin

## Available plugins

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

Plugin	Description
<a href="#">AssemblerSPAdes Plugin</a>	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.
<a href="#">Coverage Analysis Plugin</a>	Provides statistics and graphs describing the level of sequence coverage produced for targeted genomic regions.
<a href="#">ERCC Analysis Plugin</a>	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.
<a href="#">FileExporter Plugin</a>	Customizes the output file names of an analysis run. This plugin allows you to rename output files. The plugin also offers these options: <ul style="list-style-type: none"> <li>• Generates a Fastq format file of the analysis output</li> <li>• Generates an SFF format file of the analysis output</li> <li>• Renames Variant Caller plugin output files (when available)</li> <li>• Zips output files</li> </ul>
<a href="#">FilterDuplicates</a>	Removes duplicate reads. BAM files with duplicate reads removed are saved in the FilterDuplicates plugin directory.
<a href="#">IonReporterUploader</a>	Transfers run results files to your organization in Ion Reporter™ Software (available under a separate license). <p>Please read the Ion Reporter™ Software release notes for instructions about the Uploader plugin.</p>

<b>PartekFileUploader Plugin</b>	Transfers results of a completed Torrent Suite™ Software run to the Partek® Flow™ server running on the Ion Reporter™ Server.
<b>Pharmacogenomics</b>	Used with Ion AmpliSeq™ Pharmacogenomics Research Panels.
<b>RNASeqAnalysis</b>	Analyzes cDNA reads as produced by the RNA Seq planned run.
<b>Run Recognition Plugin</b>	Considers candidate runs for inclusion in Ion Community leaderboards. Within each chip type (Ion 314™ chip, Ion 316™ chip, and Ion 318™ chip), top runs are ranked according the number of AQ20 bases mapped.
<b>RunTransfer Plugin</b>	Transfers the signal processing output of a completed Torrent 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.
<b>sampleID Plugin</b>	Uses sample fingerprinting to identify any cross-contamination between samples or between barcodes in a run.
TorrentSuiteCloud	Not supported. See the RunTransfer plugin.
<b>Torrent Variant Caller Plugin</b>	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 (in 3.x releases) and that now is available in other areas of the Torrent Browser.

Previous plugin	Description and new location
<b>combineAlignment</b>	Combines reads aligned to the specified reference from multiple run reports. Intended for use when multiple runs analyze the same tissue sample, for example when a tissue sample is run on more than one chip.  You invoke combineAlignment from the project page (see <a href="#">Projects</a> ). The runs you combined must be members of the same project.

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

Previous plugin	Description

<b>Alignment</b>	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
<b>TorrentSuiteCloud</b>	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
<b>FastqCreator</b>	Creates a FASTQ format file from the BAM results file of a completed analysis run.
<b>SFFCreator</b>	Creates an SFF format file from the BAM results file of a completed analysis run.

## Plugin configuration

This section describes the various ways plugins are configured.

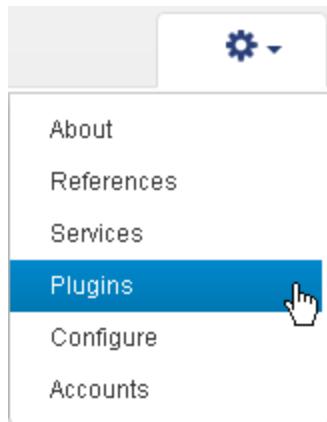
- **No configuration** – These plugins (sample ID and FilterDuplicates) do not take an user options. These plugins are ready to use as-is on new systems. When selected for manual launch on a completed run report, these plugins launch immediately.
- **The admin Plugins tab** – Several plugins can be configured 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 and variantCaller , 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
Assembler SPAdes	Yes	No	Yes	Yes
Coverage Analysis	Yes	No	Yes	Yes

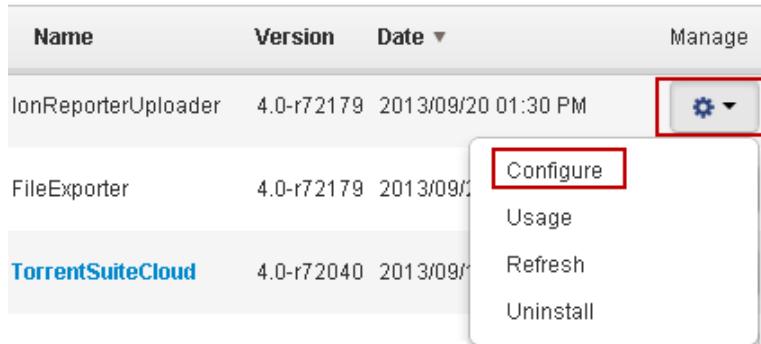
ERCC Analysis	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>
FileExporter	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>
FilterDuplicates	No	No	<b>Yes</b>	No
IonReporterUploader	<b>Yes</b>	<b>Yes</b>	<b>Yes</b> (IonReporter chevron)	<b>Yes</b>
RunRecognition	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>
RunTransfer	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>
SampleID	No	No	<b>Yes</b>	No
Torrent Variant Caller	<b>Yes</b>	No	<b>Yes</b>	<b>Yes</b>

## 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 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:

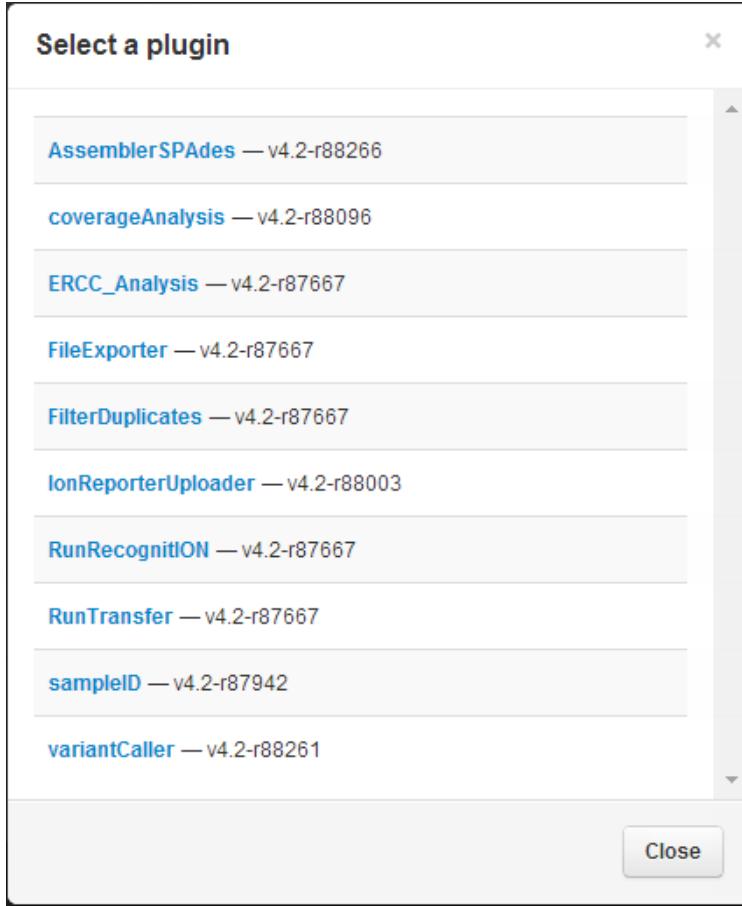
1. Go to the **Data > Completed Runs & Reports** tab, then click the link for your

- 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. See [Available Plugins](#) for the pre-installed plugins. Your server may have additional plugins or other versions installed.



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.

See the following for more information on specifying plugins in your templates and planned runs:

- Plan Tab
- Templates
- Planned Runs
- Template and Planned Run Wizard

## The old method to autorun a plugin

Your Torrent Suite™ Software administrator can set plugins to be executed automatically on every run; however, the preinstalled plugins are not good candidates to be run automatically.



The Alignment and RunRecognitION plugins cannot be be executed automatically because they require manual user input before every run.

For Ion Reporter™ Software users, enabling Autorun with the IonReporterUploader plugin could cause you extra expense, by transferring unnecessary results to Ion Reporter™ Software. (The Plan tab allows you to turn off the plugin for a template and for a planned run.)

# Assembler SPAdes Plugin

## Torrent Browser Analysis Report Guide

Torrent Suite™ Software space on Ion Community

[Analysis Report Guide TOC](#)

### 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.

- Run the AssemblerSPAdes plugin on a completed run
- Advanced settings
  - Custom assembly settings
    - The K field
    - The Mode menu
- Output
  - Downloads
  - Assembly Statistics
    - Parameter section
    - Metric section

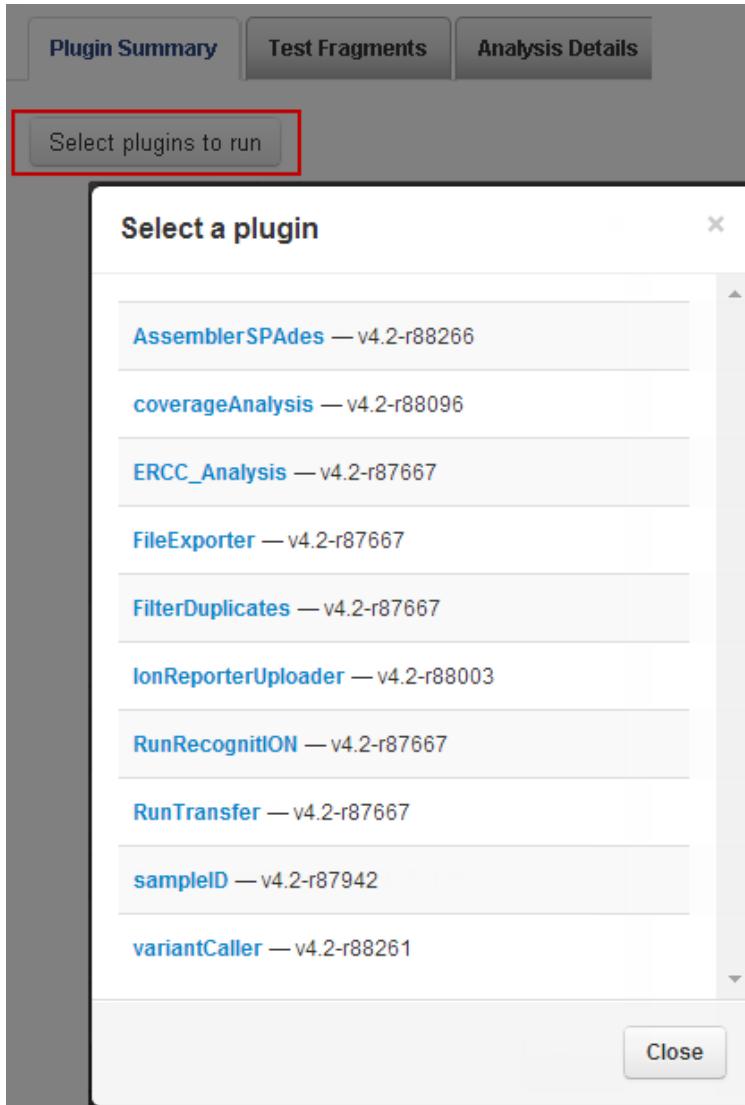


For *de novo* assembly, use a Generic Sequencing template for the Torrent Suite™ Software analysis.

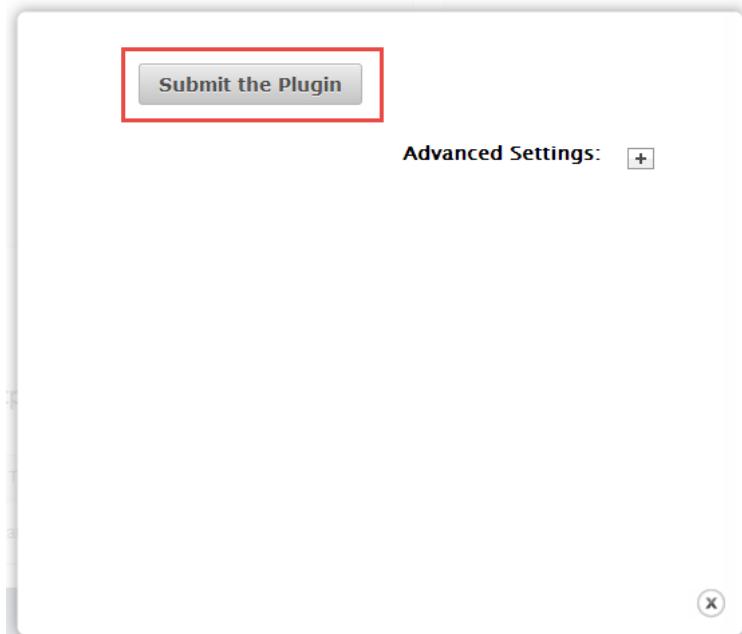
### Run the AssemblerSPAdes plugin on a completed run

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:

The "Advanced Settings" dialog box contains the following configuration options:

- Fraction of reads to use:** 100% (dropdown menu)
- Only process barcodes:** (text input field)
- Skip barcodes with fewer than**: 500 reads (text input field)
- RAM to allocate:** 32Gb (dropdown menu)
- SPAdes Version:** 3.1.0 (dropdown menu)
- Assembly settings:** Uniform coverage (dropdown menu)
- Run read correction before doing assembly**: checked (checkbox)
- Skip assembly if previous results exist**: unchecked (checkbox)

Field	Description
-------	-------------

<b>Fraction of reads to use</b>	If less than 100%, reads are randomly sub-sampled.  100% is recommended – the plugin automatically handles most changes in coverage.
<b>Only process barcodes</b>	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:
<b>Skip barcodes with fewer than</b>	Ignores barcodes whose number of reads do not meet this threshold. Intended to filter out barcode classification problems with noisy data.
<b>RAM to allocate</b>	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.  <b>Note:</b> The plugin crashes if the memory allocation fails.
<b>SPAdes version</b>	Select the version that you prefer. Select the default if you do not know.
<b>Assembly settings</b>	Set this menu as follows: <ul style="list-style-type: none"> <li>• <b>Uniform coverage</b> – Choose this setting for data with average or low GC content. This setting uses the default kmers.</li> <li>• <b>Non-uniform coverage</b> – Choose this setting for data with high GC content. This setting uses the default kmers.</li> <li>• <b>Highly non-uniform coverage</b> – Choose this setting for data with high GC content. This setting uses a different set of kmers.</li> <li>• <b>Custom</b> – See <a href="#">Custom Assembly Settings</a>.</li> </ul>
<b>Run read correction before doing assembly</b>	Recommended.
<b>Skip assembly if previous results exist</b>	Recommended.

## Custom assembly settings

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

Assembly settings:	<input style="border: 1px solid #ccc; padding: 2px; width: 150px; height: 20px;" type="button" value="Custom..."/> K: <input style="width: 150px;" type="text" value="21,33,55,77,99"/> (comma-delimited, no spaces)
	Mode: <input style="border: 1px solid #ccc; padding: 2px; width: 150px; height: 20px;" type="button" value="Multi-cell"/>

### ***The K field***

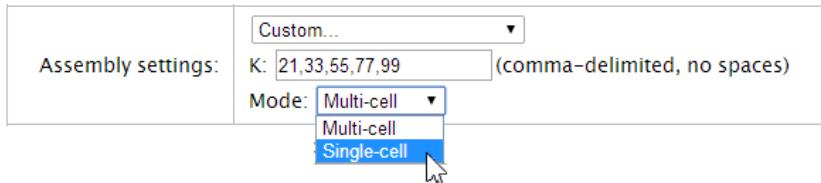
The SPAdes plugin is a De-Brujin graph assembler. The plugin breaks reads in kmers, makes a connected graph, and traverses through that graph to produce contigs.

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 contains 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

Download all your assembly result files.

[① Assembled Contigs \(FASTA\)](#) | [② SPAdes Log \(TXT\)](#) | [③ QUAST report \(HTML\)](#)

#### Assembly Statistics

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

Metric	Large Contigs ( $\geq$ 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

## 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](http://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 ( $\geq$ 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
<b>Largest Contig</b>	Length of the longest assembled contig
<b>Total Length</b>	Total number of base pairs contained in all assembled contigs that are at least 500 bp in length.
<b>Number of Contigs</b>	Number of assembled contigs that are at least 500 bp in length.

<b>N50</b>	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.
<b>N75</b>	The contig length such that using longer or equal length contigs produces 75% of the bases of the assembly.
<b>N90</b>	The contig length such that using longer or equal length contigs produces 90% of the bases of the assembly.
<b>N95</b>	The contig length such that using longer or equal length contigs produces 95% of the bases of the assembly.

 SPAdes only reports contigs that are 500 bp or longer.

# Coverage Analysis Plugin

## Torrent Browser Analysis Report Guide

Torrent Suite™ Software space on Ion Community

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### Coverage Analysis Plugin

The Coverage Analysis plugin provides statistics and graphs describing the level of sequence coverage produced for targeted genomic regions.

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:



### Run the Coverage Analysis plugin

You can run the Coverage Analysis plugin automatically or manually.

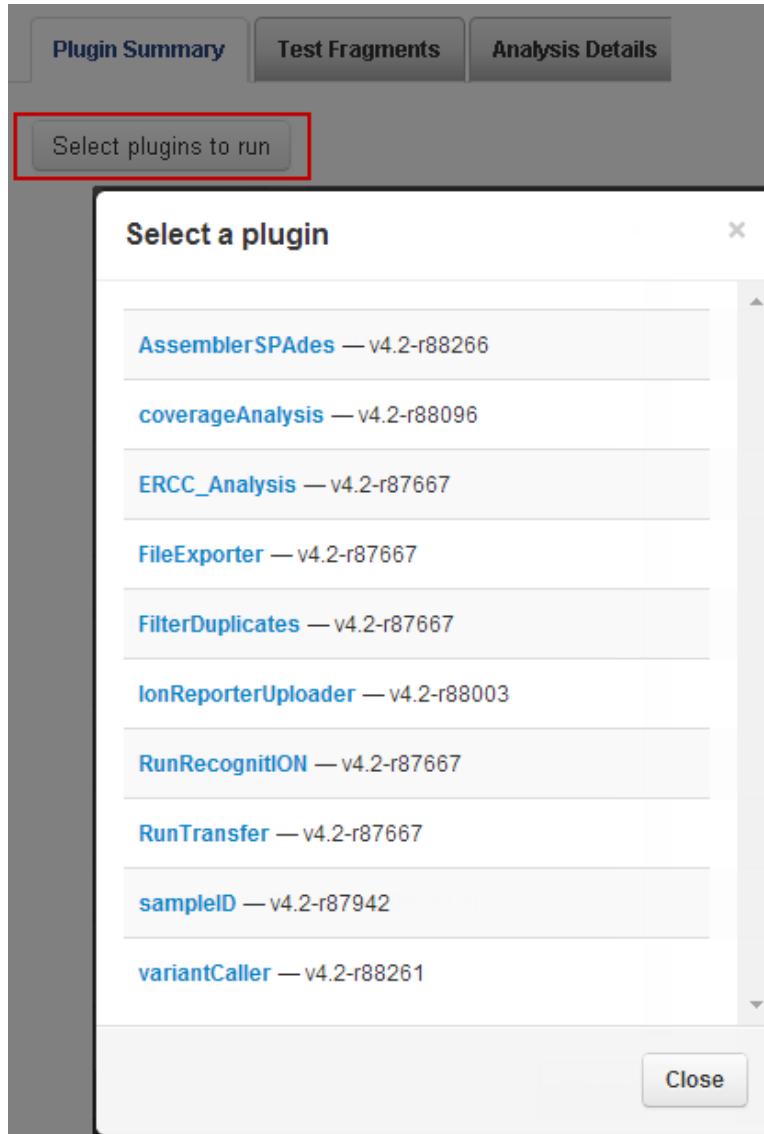
#### Include the Coverage Analysis plugin in a run plan

To run the Coverage Analysis plugin automatically, you select Coverage Analysis plugin during template setup. Refer to the [Plan Tab](#) and [Templates](#) pages section of the [Torrent Browser User Interface Guide](#) 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 dropdown 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.

**Torrent Coverage Analysis Plugin**

**Genome and Targeted Re-sequencing Coverage Analysis**

Reference Genome: `e_coli_dh10b`

Library Type: `Whole Genome`

Targeted Regions: `None`

Use Only Uniquely Mapped Reads:

**Description and Usage Notes**

This plugin generates statistics, downloadable data files and interactive visualization of coverage over targeted regions of the reference genome.

The Library Type should be set to the type of enriched fragment library used for the run (aka 'Run Type') and the Targeted Regions selected should correspond to the enriched regions of the reference (if any). Some options are only presented as available for the Library Type selected, and coverage will only be reported over the specified target regions. Typically a Targeted Regions selection is required for enriched library types and set to 'None' for Whole Genome runs. (The default selections will match those defined by the Run Plan for this report.)

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**.

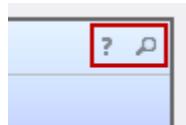
The analysis runs and a group of output reports is created.

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.

Most Coverage Analysis chart have help and options icons in the top right corner:



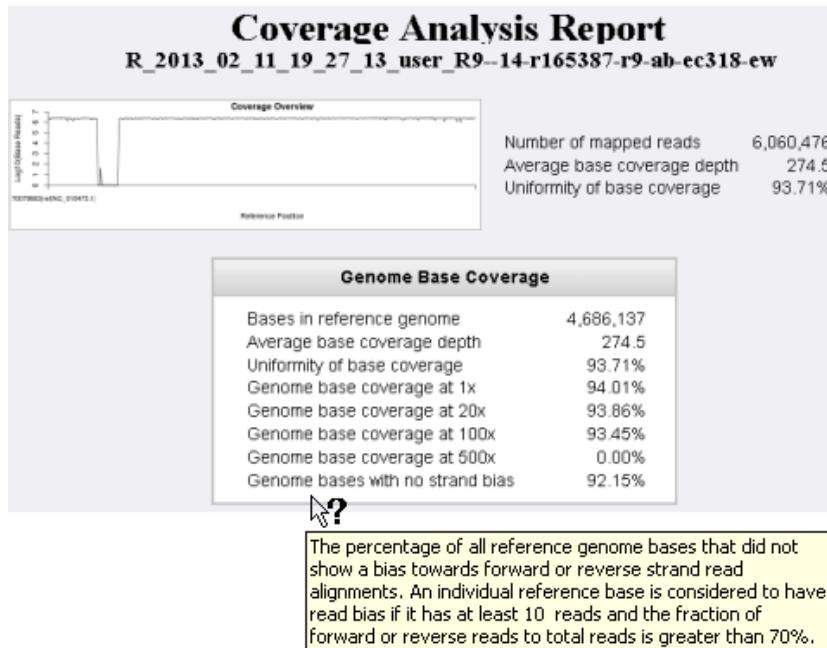
Click a chart's help icon ? to open a description of the chart.

Click a chart's options icon ☰ to open a panel of options for the chart.

Most fields in the report offer hover help.

### Example statistics

The following is an example of the plugin statistics for a whole genome run. Most fields names offer hover help.



Click on the Coverage Overview graph to see a larger image (then click Back in your browser to return to the report).

## Reads statistics

The library type determines which statistics are presented.

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

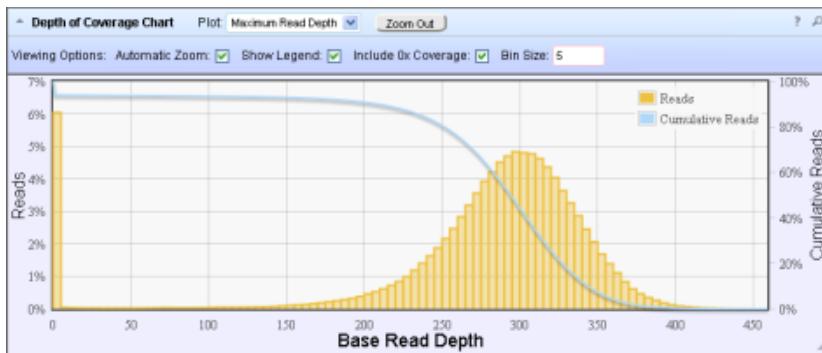
<b>Bases in targeted reference</b>	The total number of bases in all target regions of the reference.
<b>Bases covered (at least 1x)</b>	The total number of target bases that had at least one read aligned over the proximal sequence. Only the aligned parts of each read are considered. For example, unaligned (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.
<b>Average base coverage depth</b>	The average number of reads of all targeted reference bases.
<b>Uniformity of base coverage</b>	The percentage of bases in all targeted regions (or whole genome) covered by at least 0.2x the average base coverage depth.
<b>Maximum base read depth</b>	The maximum number of times any single target base was read.
<b>Average base read depth</b>	The average number of reads of all targeted reference bases that were read at least once.
<b>Std.Dev base read depth</b>	The standard deviation (root variance) of the read depths of all targeted reference bases that were read at least once.
<b>Genome Base Coverage</b>	Summary statistics for base reads of the reference genome.
<b>Genome base coverage at Nx</b>	The percentage of reference genome bases covered by at least $N$ reads.
<b>Target coverage at Nx</b>	The percentage of target bases covered by at least $N$ reads.
<b>Targets with no strand bias</b>	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%.
<b>Amplicon Read Coverage</b>	Summary statistics for reads assigned to specific amplicons. Each sequence read will be assigned to exactly one of the amplicons specified by the targets file. Reads are assigned to particular amplicon targets based if their (5') mapping location being sufficiently close to the end of the amplicon region, taking the read direction (mapping strand) in to account.
<b>Number of amplicons</b>	The number of amplicons specified in the target regions file.

<b>Percent assigned amplicon reads</b>	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.
<b>Average reads per amplicon</b>	The average number of reads assigned to amplicons.
<b>Uniformity of amplicon coverage</b>	The percentage of bases in all targeted regions (or whole genome) covered by at least 0.2x the average base read depth.
<b>Amplicons with at least <math>N</math> reads</b>	The percentage of all amplicons that had at least $N$ reads.
<b>Amplicons with no strand bias</b>	The percentage of all amplicons that did not show a bias towards forward or reverse strand read alignments. An individual amplicon is considered to have read bias if it has at least 10 reads and the fraction of forward or reverse reads to total reads is greater than 70%.
<b>Amplicons reading end-to-end</b>	The percentage of all amplicons that were considered to have a sufficient proportion of assigned reads (70%) that covered the whole amplicon target from 'end-to-end'. To allow for error the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.

## Example charts

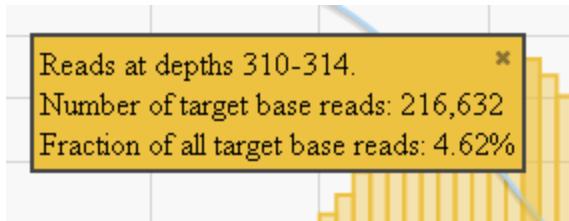
This section shows a couple example charts. Many charts have a Plot menu that allows you to change characteristics of the chart, for instance, to show both strands.

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

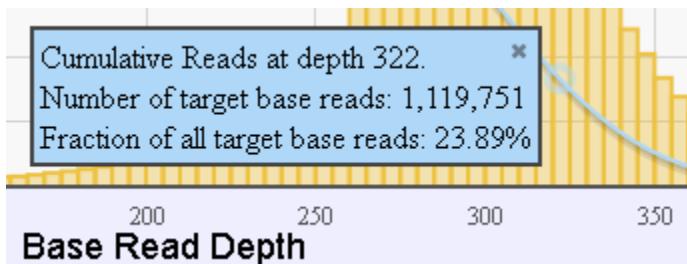


In the Depth of Coverage chart above, the left Y-axis (% reads) is the number of reads at a particular read depth (or bin of read depths) as a percentage of the total number of (base) reads. The right Y-axis (% cumulative reads) is the cumulative count of the number of reads at a given read depth *or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

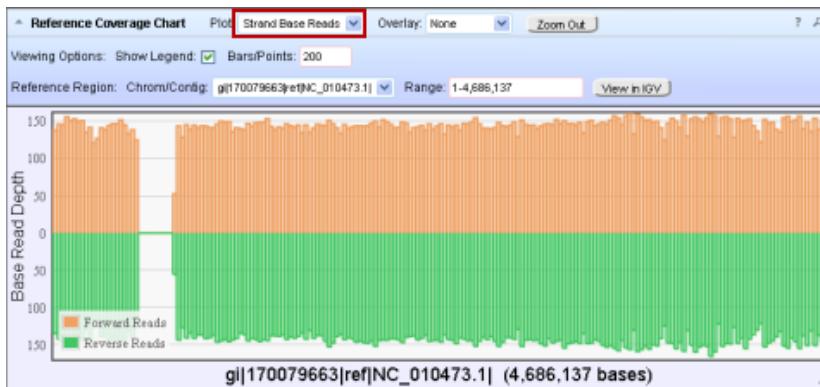
In most charts you click on a data point to open a detail panel for that data:



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:



**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

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

File Links	
<a href="#">Download the coverage statistics summary file.</a>	?
<a href="#">Download the base depth of coverage file.</a>	?
<a href="#">Download the amplicon coverage summary file.</a>	?
<a href="#">Download the chromosome base coverage summary file.</a>	?
<a href="#">Download the aligned reads BAM file.</a>	?
<a href="#">Download the aligned reads BAI file.</a>	?
<a href="#">Link to targets (BED) file upload page.</a>	?
<a href="#">Download the download ZIP report.</a>	?

Ion 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:

<b>Base depth of coverage file</b> This is a tab-separated-values text file with a .xls filename extension. It has 5 named fields: <b>read_depth</b> : The depth at which a (targeted) reference base has been read. <b>base_cov</b> : The number of times any base was read (covered) at this depth. <b>base_cum_cov</b> : The cumulative number of reads (coverage) at this read depth or greater. <b>norm_read_depth</b> : The normalized read depth (depth divided by average base read depth). <b>pc_base_cum_cov</b> : As <b>base_cum_cov</b> but represented as a percentage of the total base reads.	<a href="#">Download the aligned reads BAI file.</a> ?
---	--

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. See also <a href="#">Example Coverage Analysis Report</a> .
Base depth of coverage	Coverage summary data used to create the Depth of Coverage Chart. This file contains these fields: <ul style="list-style-type: none"> <li><b>read_depth</b> – The depth at which a (targeted) reference base has been read.</li> <li><b>base_cov</b> – The number of times any base was read (covered) at this depth.</li> <li><b>base_cum_cov</b> – The cumulative number of reads (coverage) at this read depth or greater.</li> <li><b>norm_read_depth</b> – The normalized read depth (depth divided by average base read depth).</li> <li><b>pc_base_cum_cov</b> – As <b>base_cum_cov</b> but represented as a percentage of the total base reads.</li> </ul>

Amplicon coverage summary	<p>Coverage summary data used to create the Amplicon Coverage Chart. This file contains these fields:</p> <ul style="list-style-type: none"> <li>• <b>contig_id</b> – The name of the chromosome or contig of the reference for this amplicon.</li> <li>• <b>contig_srt</b> – The start location of the amplicon target region. <b>Note:</b> This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file.</li> <li>• <b>contig_end</b> – The last base coordinate of this amplicon target region. <b>Note:</b> The length of the amplicon target is given as tlen = (contig_end - contig_srt + 1).</li> <li>• <b>region_id</b> – The ID for this amplicon as given as the 4th column of the targets BED file.</li> <li>• <b>gene_id</b> – The gene symbol as given as the last field of the targets BED file.</li> <li>• <b>gc</b> – The number of G and C bases in the target region. Hence, %GC = 100% * gc / tlen.</li> <li>• <b>overlaps</b> – The number of times this target was overlapped by any read by at least one base. <b>Note:</b> Individual reads might overlap multiple amplicons where the amplicon regions themselves overlap.</li> <li>• <b>fwd_e2e</b> – The number of assigned forward strand reads that read from one end of the amplicon region to the other end.</li> <li>• <b>rev_e2e</b> – The number of assigned reverse strand reads that read from one end of the amplicon region to the other end.</li> <li>• <b>total_reads</b> – 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).</li> <li>• <b>fwd_reads</b> – The number of forward strand reads assigned to this amplicon.</li> <li>• <b>rev_reads</b> – The number of reverse strand reads assigned to this amplicon.</li> </ul>
Target coverage summary	<p>Coverage summary data used to create the Target Coverage Chart. This file contains fields:</p> <ul style="list-style-type: none"> <li>• <b>contig_id</b> – The name of the chromosome or contig of the reference for this target.</li> </ul>

- **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 =  $100\% * \text{gc} / \text{tlen}$ .
- **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 =  $100\% * \text{covered} / \text{tlen}$ .  
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 Ion 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.

	<ul style="list-style-type: none"> <li><b>depth</b> – 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, sort and end).</li> <li><b>fwd_reads</b> – The number of forward strand reads assigned to this target.</li> <li><b>rev_reads</b> – The number of reverse strand reads assigned to this target.</li> </ul>
Chromosome base coverage summary	<p>Base reads per chromosome summary data used to create the default view of the Reference Coverage Chart. This file contains these fields:</p> <ul style="list-style-type: none"> <li><b>chrom</b> – The name of the chromosome or contig of the reference.</li> <li><b>start</b> – Coordinate of the first base in this chromosome. This is always 1.</li> <li><b>end</b> – Coordinate of the last base of this chromosome. Also its length in bases.</li> <li><b>fwd_reads</b> – Total number of forward strand base reads for the chromosome.</li> <li><b>rev_reads</b> – Total number reverse strand base reads for the chromosome.</li> <li><b>fwd_ontrg</b> (if present) – Total number of forward strand base reads that were in at least one target region.</li> <li><b>rev_ontrg</b> (if present) – Total number and reverse strand base reads that were in at least one target region.</li> <li><b>seq_reads</b> – Total sequencing (whole) reads that are mapped to individual contigs.</li> </ul>
Aligned reads BAM file	Contains all aligned reads 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.

### **Example Coverage Analysis Report**

#### Coverage Analysis Report

Reference (File): e\_coli\_dh10b  
Alignments: R\_2013\_02\_11\_19\_27\_13\_user\_R9--14-r16538  
Using: Uniquely Mapped Non-duplicate Reads

Number of mapped reads: 6060476

Total base reads on target: 1286521202  
Bases in reference genome: 4686137  
Average base coverage depth: 274.5  
Uniformity of base coverage: 93.71%  
Genome base coverage at 1x: 94.01%  
Genome base coverage at 20x: 93.86%  
Genome base coverage at 100x: 93.45%  
Genome base coverage at 500x: 0.00%  
Genome bases with no strand bias: 92.15%

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# ERCC Analysis Plugin

## Torrent Browser Analysis Report Guide

[Torrent Suite™ Software space on Ion Community](#)

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### 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.

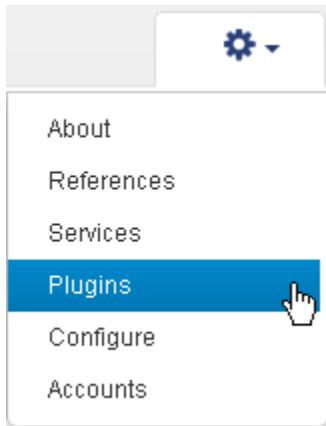
- Enable the ERCC Analysis plugin
- Manually run the ERCC Analysis plugin
- Configure a template to run the ERCC plugin
- Plugin run times
- View analysis results
- Interpret the data
- View transcript details
- Definitions
- (Optional) Configure the ERCC Analysis plugin
- View plugin error information
- ERCC resources

#### 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:

## Plugins

Enabled   Disabled   Either   Autorun   Manual   Either

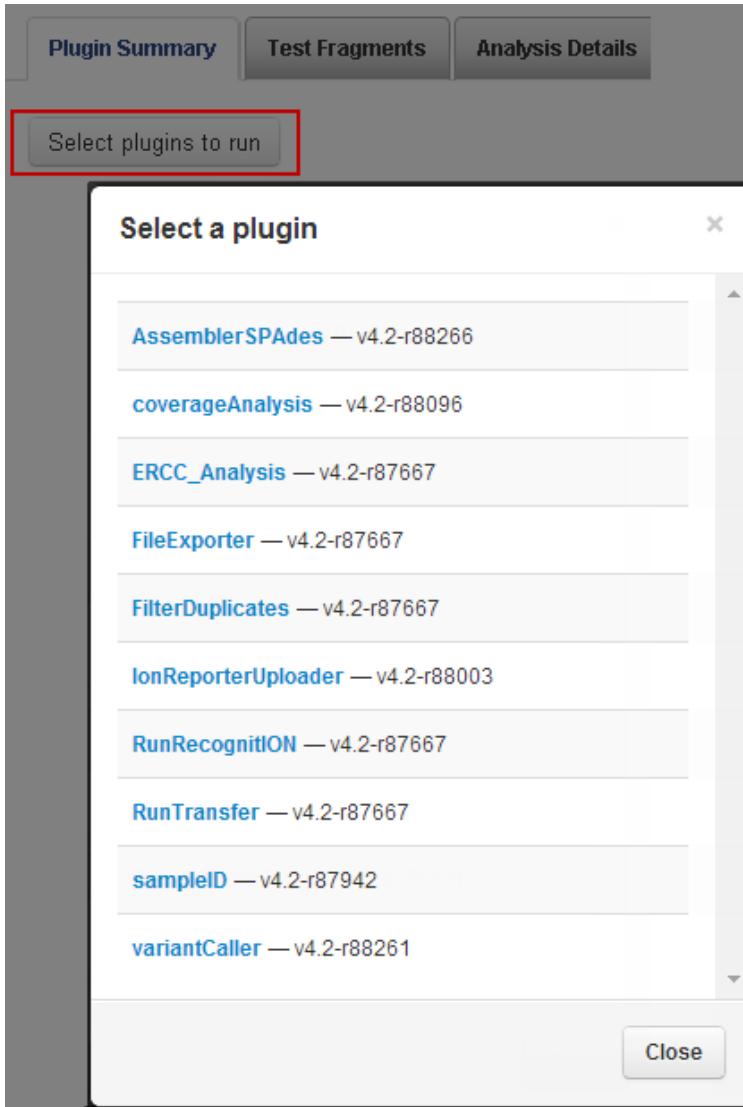
Enabled	Autorun	Name	Version	Date	Manage
<input checked="" type="checkbox"/>	<input type="checkbox"/>	ERCC_Analysis	2.2.11	2012/08/19	 
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	variantCaller	3.0.41	2012/07/29	 

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

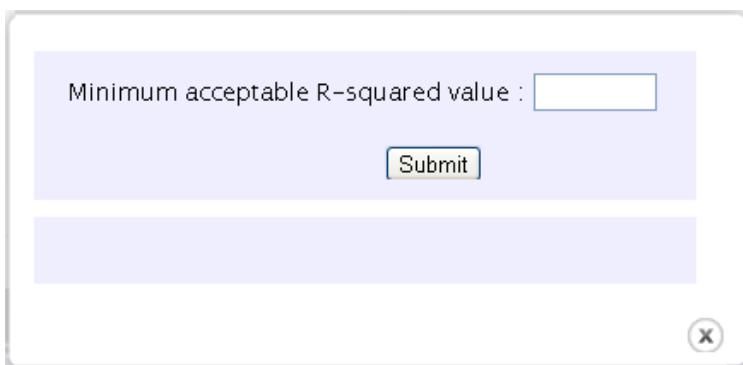
### Manually run the ERCC Analysis plugin

Follow these steps to manually launch the ERCC\_Analysis plugin. You can use the default minimum R-squared value or enter your 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.



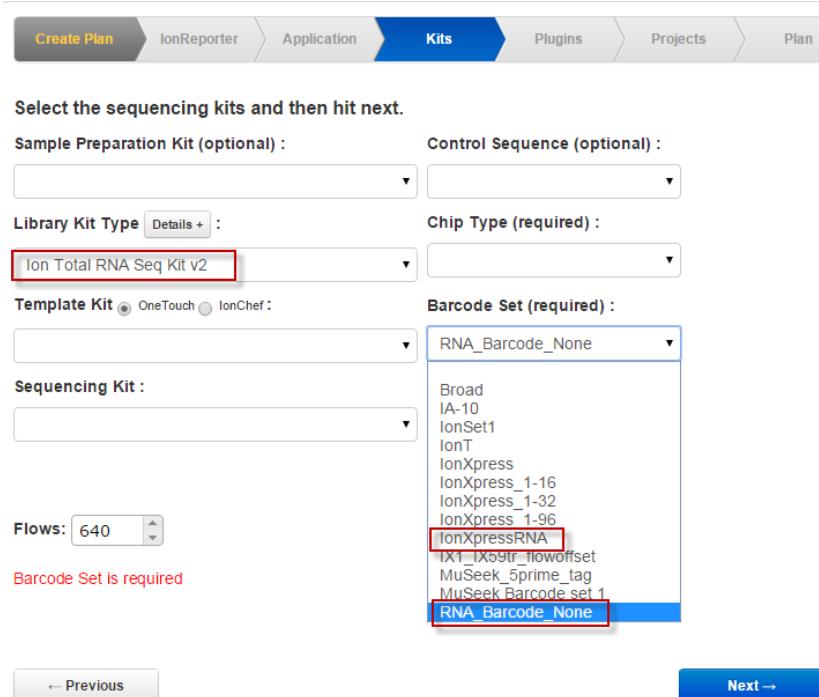
5. Click **Submit** to start the plugin.

## 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\_BarcodNone** – 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.

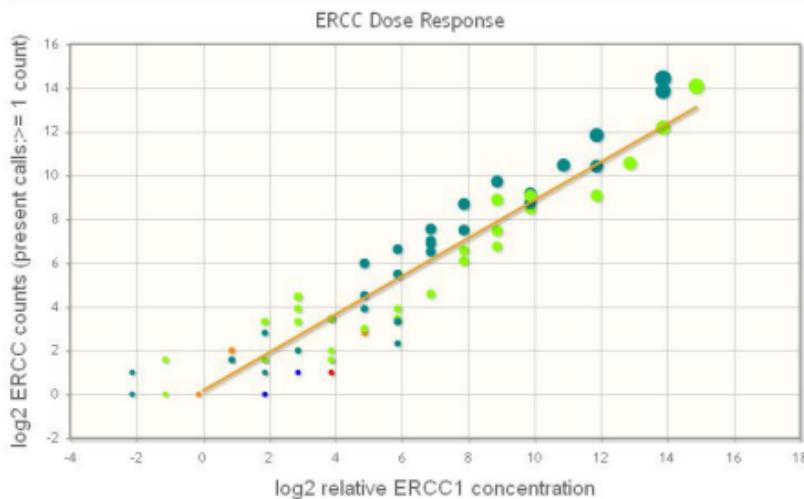
## View analysis results

Plugin analysis results appear in the plugin summary area:

ERCC_Analysis — v2.2.11	Completed
<ul style="list-style-type: none"> <li><a href="#">ERCC_Analysis.html</a></li> </ul>	

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:

## ERCC Report



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

### 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

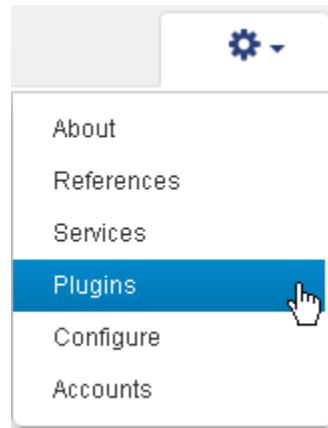
This section defines terms used in the plugin output.

- **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:

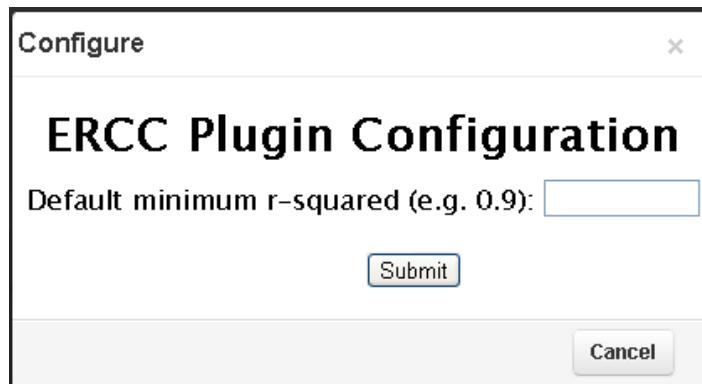
1. If the window showing the minimum acceptable R-squared value is open, close it. Then scroll to the top of the Torrent Browser and click **Plugins** in the gear menu on the top right:



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\_Analysis row, click the Manage column gear menu, then select **Configure**.

The screenshot shows a 'Plugins' management interface. At the top, there are filters for 'Enabled', 'Disabled', 'Either', 'Autorun', 'Manual', and 'Either'. Below is a table with columns: Enabled, Autorun, Name, Version, Date, and Manage. Two rows are listed: 'ERCC\_Analysis' (version 2.2.11, 2012/08/19) and 'variantCaller' (version 3.0.41, 2012/08/19). The 'variantCaller' row has a context menu open with 'Configure' and 'Uninstall' options.

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:

The screenshot shows the 'ERCC\_Analysis — v2.2.11' summary. The status is 'Error' (with a red box around the error icon). Below it is a link to 'detailedReport.html'.

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

```

Log for detailedReport

Plugin: detailedReport - 1.0
Host: gogots
Running under SGE: /var/lib/gridengine/iontorrent
Job: 1808 - ion_plugin_detailedReport_launch.sh
Home:
Submit User:
Submit Host: gogots
Work Dir: '/'
pk 800
version 0
plugin detailedReport
Plugin Update returns: 'True'
Plugin 'detailedReport' Successfully Updated
Set the Status of the plugin 'detailedReport' on report '80
version=1.0
start time=Sat Jul 21 05:39:41 CDT 2012
command line=
detailedReport: starting execution of plugin code
start time=2012-07-21 5:39:41.654257759
=====
Traceback (most recent call last):
File "/results/plugins/detailedReport/detailedReport.py", l
from ion.results import parseCafie, parseCafieRegions, tfg;
ImportError: cannot import name parseCafie
ERROR: Plugin exited unexpectedly with error: 1 - Plugin E>
=====
```

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.

# FileExporter Plugin

## Torrent Browser Analysis Report Guide

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### 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
- Plugin configuration
  - Configuration scope
  - Manual launch
  - Admin Plugin tab
  - Output file options
  - Plugin naming pattern (required)
- Run the plugin automatically
- Run the plugin manually

### Plugin output

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

The screenshot shows the FileExporter plugin interface. At the top, it displays "FileExporter (v4.0-r70587)" and "25.5 KB Completed". Below this, there are several configuration settings listed:

- Create SFF? True
- Create FASTQ? True
- Link Variants? True
- Create ZIP? False
- DELIMITER: ":"
- SELECTIONS:**
- Auto\_user\_B22-884--26953
- E129294--L\_
- 2013-09-17

Below these settings, under the heading "Files created.", are links to the generated files:

- [VCF files](#)
- [Auto\\_user\\_B22-884--26953.E129294--L\\_.2013-09-17.bam](#)
- [Auto\\_user\\_B22-884--26953.E129294--L\\_.2013-09-17.bam.bai](#)
- [Auto\\_user\\_B22-884--26953.E129294--L\\_.2013-09-17.fastq](#)
- [Auto\\_user\\_B22-884--26953.E129294--L\\_.2013-09-17.sff](#)

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

### Plugin configuration

#### 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.

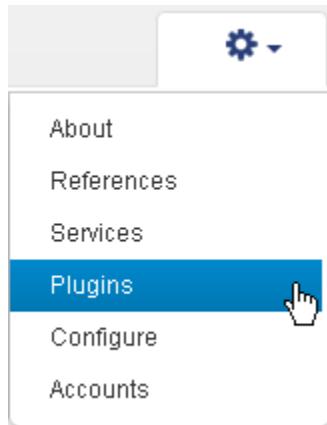
## Manual launch

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

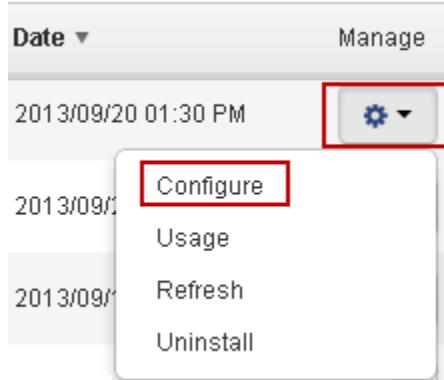
## Admin Plugin tab

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:

## Output File Options

Create SFF?

Create FASTQ?

Include Variant Caller Files (if available)?

Zip results?

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 not 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: <b>Files created.</b> <a href="#">Link</a>

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



For more information on SFF-format and FASTQ-format files, see [Technical Note - Transition from SFF to BAM format](#).

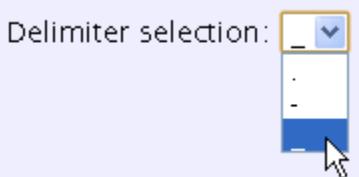
### Plugin naming pattern (required)

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 one delimiter):



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

## Customize file names

Delimiter selection:

Field 1:

## Example name string

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:

## Customize file names

Delimiter selection:

Field 1:

**Example name string**

The dropdown menu for Field 1 shows the following options:

- Select option
- Run Name
- Report Name** (highlighted)
- Report Date
- Chip Type
- Sequencer Name
- Sample ID
- Barcode Info. (if applicable)

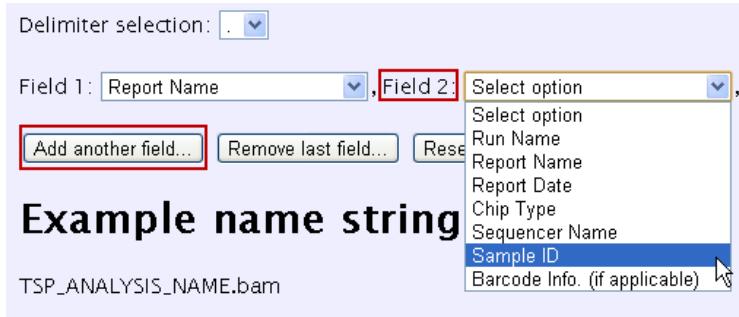
Both Field 1 and the example name string show Report Name (TSP\_ANALYSIS\_NAME.bam is an internal name for Report Name):

Delimiter selection:

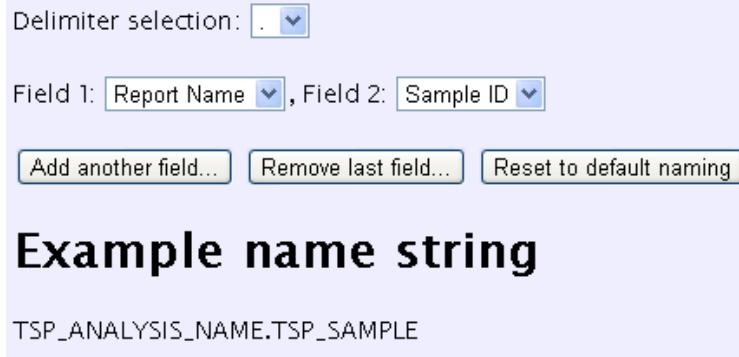
Field 1:  ,

## Example name string

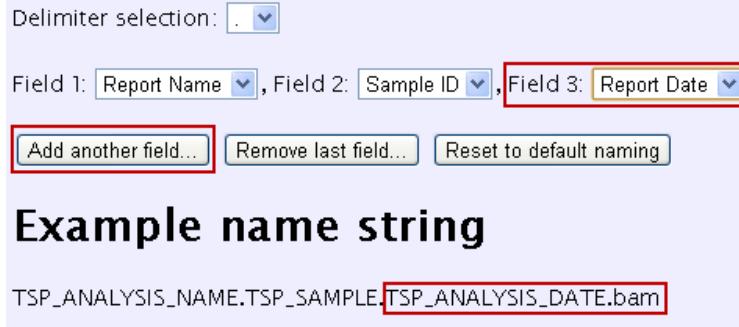
3. Click the **Add another field** button. In the Field 2 menu, select **Sample ID**:



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



- 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 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. See the [Templates](#) page in the [Torrent Browser User Interface Guide](#).

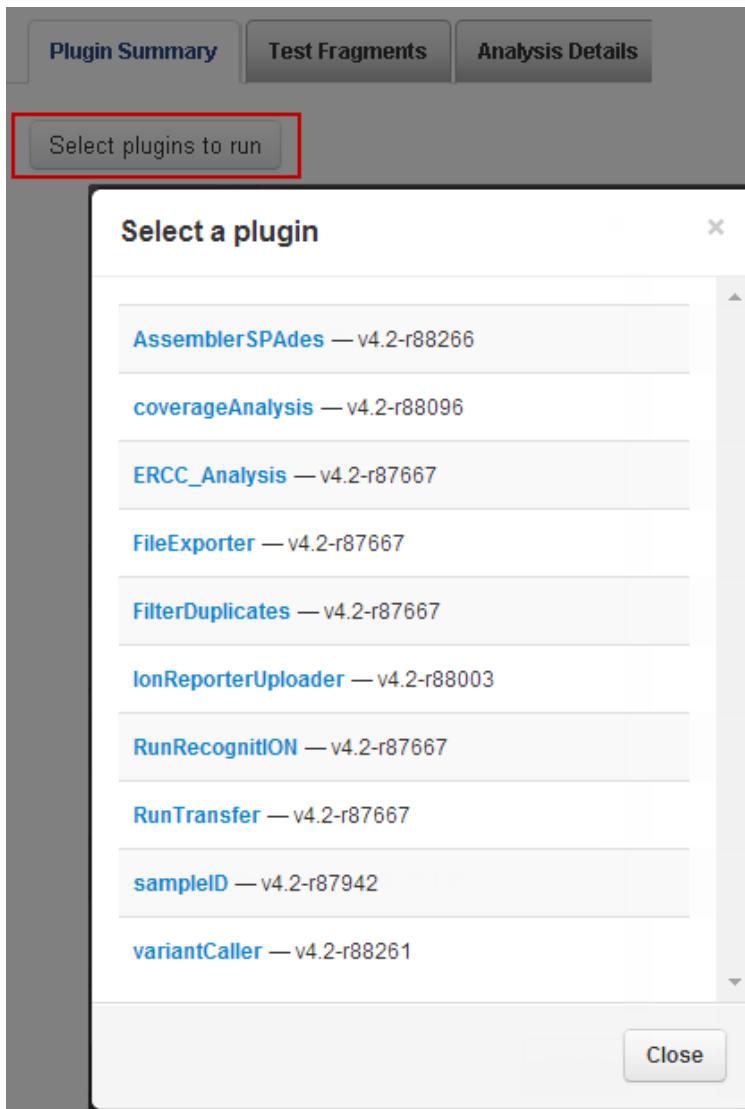
**Important:** Before you use the plugin in a planned run, configure the plugin in the admin Plugins page. A run template and planned run accept the plugin when it is not configured, but your plugin fails at run time.

## 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 **FileExporter**. Select your output file options and configure the file naming pattern.

**Note:** In this release, you must configure a file naming pattern.

3. Click **Submit**.

## Notes

- If you run the plugin multiple times on the same run report, the zip includes all output files from the previous FileExporter runs.
- VCF files are not included in the zipped file.
- In this release, if launched with no file options selected, the plugin creates FASTQ and SFF files if those files have not already been created.

# SFFCreator and FastqCreator Plugins

## Torrent Browser Analysis Report Guide

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### The SFFCreator and FastqCreator Plugins

These plugins are deprecated in the 4.0 release. The [FileExporter](#) plugin creates SFF and Fastq format output files (and more).

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# Torrent Browser Analysis Report Guide

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## 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.

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

- Plugin output
- Barcoded runs
- Plugin configuration
- Run the plugin automatically
- Run the plugin manually
- ZC tag

## 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
<a href="#">rawlib.bam</a>	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.

## Plugin configuration

The sampleID plugin does not take any options or configuration.

## Run the plugin automatically

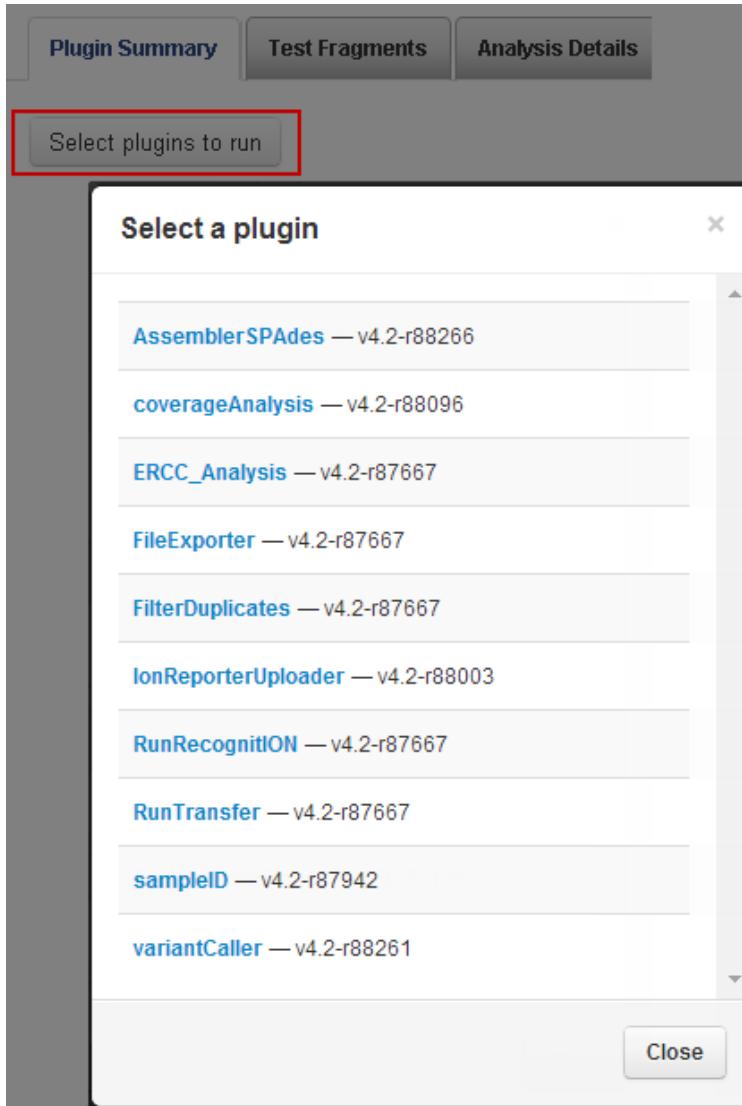
You set up the plugin to run automatically when you configure your template or run plan. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template. See the [Templates](#) page in the [Torrent Browser User Interface Guide](#).

## 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. The plugin is *submitted immediately* when you click it in the **Select a plugin** list.

## 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.

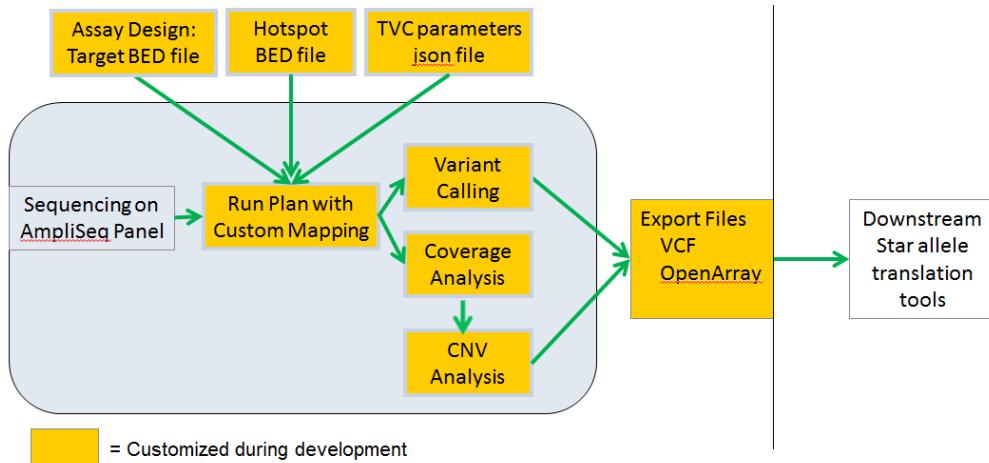
The FilterDuplicates plugin groups reads with matching start coordinate, strand, and ZC tag value, and selects one read from each group to not be a duplicate read:

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.)
-

## Pharmacogenomics Analysis Plugin

The Pharmacogenomics (PGx) Analysis Plugin is designed to be used with the Ion AmpliSeq™ Pharmacogenomics panel, which is a hotspot panel that allows the interrogation of Pharmacogenomics variants in samples using the Torrent Suite™ variantCaller plugin for genotyping and CoverageAnalysis Plugin for CYP2D6 copy number detection.



The plugin reports SNVs, Indels, and CNVs in VCF format or AlleleTyper format. For detailed instructions on the plugin, see the standard and custom panel instructions.

[Create a Planned Run Using the Ion AmpliSeq Pharmacogenomics Research Panel Plugin.pdf](#)

[Customization Guidelines for Ion AmpliSeq Pharmacogenomics Research Panels.pdf](#)

## Run Recognition Plugin

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## 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:

The screenshot displays three separate league tables for different chip types, each showing the total reported AQ20 Mapped Bases and a list of users ranked by their submission date.

Ion 314™ League			
Rank/User	AQ20 Mapped Bases	Run Date	Date Submitted
1	test_user1	1237123234	2011-10-15
1	test_user2	1237123234	2011-09-15
1	test_user3	1237123234	2011-08-15
4	smiller_uat	437227	2011-07-13

Ion 316™ League			
Rank/User	AQ20 Mapped Bases	Run Date	Date Submitted
1	test_user3	1123456	2011-10-24
2	test_user2	649214	2011-10-24
3	test_user1	456413	2011-10-24

Ion 318™ League			
Rank/User	AQ20 Mapped Bases	Run Date	Date Submitted
1	test_user1	762345	2011-10-24
2	test_user2	403567	2011-10-24

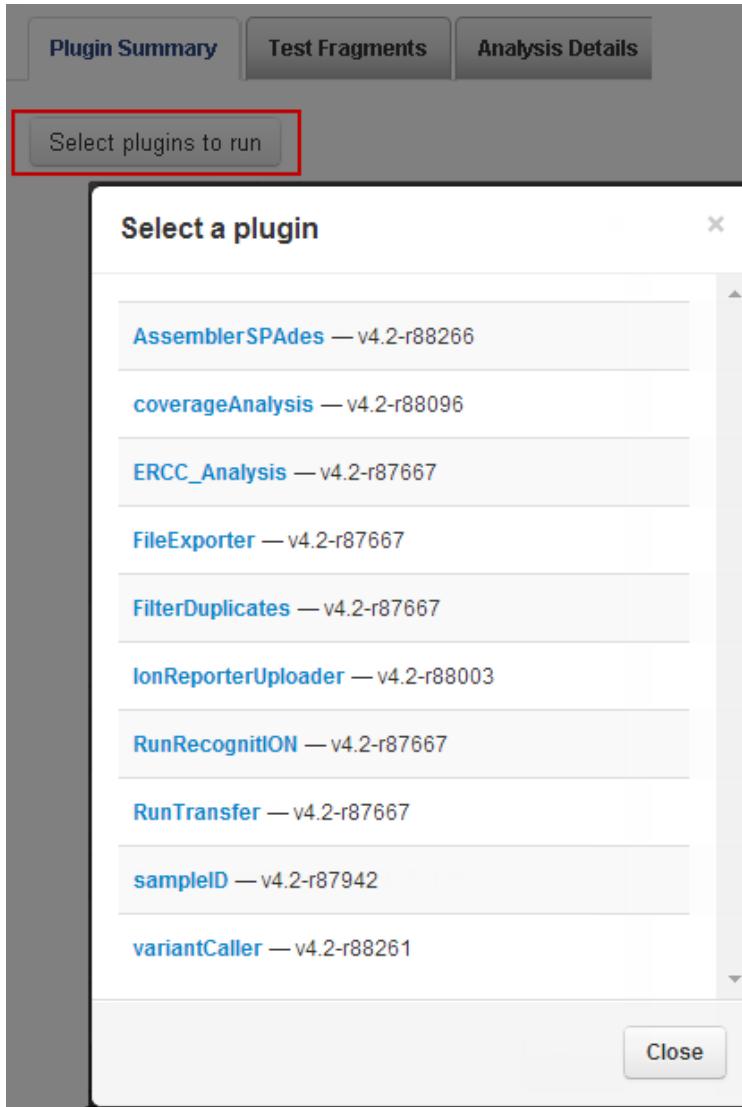
Powered by **SAM SOLUTIONS**

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

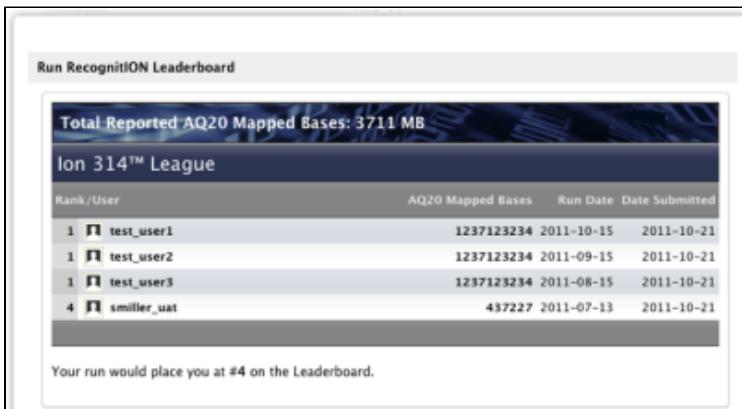
## Run the RunRecognitON Plugin

Follow these steps to run to the RunRecognitON 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 **RunRecognitON**:



The leaderboard for your chip type is shown, with a message indicating where your ranks 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, as described in [Run the RunRecognition Plugin](#).

The Run RecognitionON leaderboard is displayed for your chip type.

The screenshot shows a web-based submission form for the Run RecognitionON Leaderboard. At the top, it displays "Total Reported AQ20 Mapped Bases: 3711 MB". Below this is a table titled "Ion 314™ League" showing four entries:

Rank/User	AQ20 Mapped Bases	Run Date	Date Submitted
1 test_user1	1237123234	2011-10-15	2011-10-21
1 test_user2	1237123234	2011-09-15	2011-10-21
1 test_user3	1237123234	2011-08-15	2011-10-21
4 smiller_uat	437227	2011-07-13	2011-10-21

Below the table, a message says "Your run would place you at #4 on the Leaderboard." The next section, "Review Data to Be Submitted", contains a "Show/Hide" link to view the data sent to the leaderboard. The final section, "Ion Community Login", includes fields for "Username" (empty), "Site Name" (Scott's VM Instance), "Password" (empty), "Reference Genome" (E. coli OH108), and "Application Type" (empty). It also includes a checkbox for "I agree to the Terms and conditions" and a "Submit" button.

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. Optionally enter the any of this information about your run:

- Your site name
- The reference genome used in this run
- The application type for this run

1. Below the information fields, click **Terms and Conditions** and carefully read that information. Click the checkbox "**I agree to the Terms and Conditions**".
2. Click **Submit** at the bottom of the page. If your run qualifies, it is added to the leaderboard:

The screenshot shows the same web-based submission form after a run has been submitted. The "Completed" status is indicated at the top. The "Run RecognitionON.html" section shows the message "Your data has been saved to the leaderboard." The "Run RecognitionON Leaderboard" section shows the updated table:

Rank/User	AQ20 Mapped Bases	Run Date	Date Submitted
1 test_user1	1237123234	2011-10-15	2011-10-21
1 test_user2	1237123234	2011-09-15	2011-10-21
1 test_user3	1237123234	2011-08-15	2011-10-21
4 smiller_uat	437227	2011-07-13	2011-10-21

At the bottom, there is a "Powered by SAM SOLUTIONS" logo.

## **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.

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# Torrent Browser Analysis Report Guide

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## The IonReporterUploader plugin

See the [Ion Reporter Software Integration Guide](#) for complete information on the IonReporterUploader plugin. A brief description is included below.

### Overview

The IonReporterUploader 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.

## 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.

See [Integration with Ion Reporter™ Software](#) for an overview of Torrent Suite™ Software output files and how these files are used with Ion Reporter™ Software.

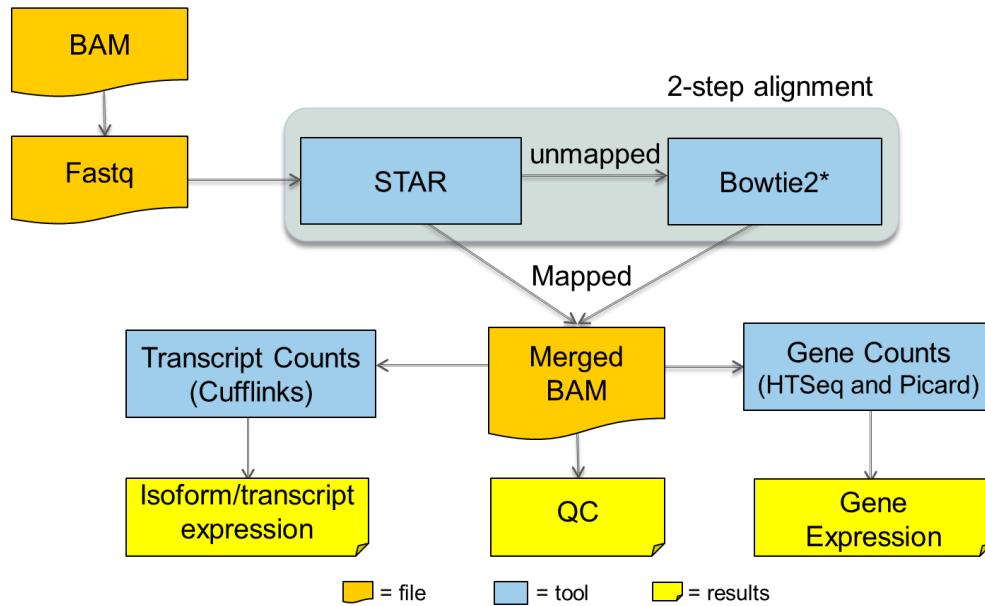
## Command-line version of the Uploader

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).

## RNASeq Analysis 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 Picard tools to collect 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.



\* A secondary alignment is performed against rRNA sequences for reporting the fraction of total reads represented by ribosomal RNA species. This serves as a useful QC metric to estimate effectiveness of rRNA depletion procedures and/or effects on detection sensitivity for mRNAs of interest.

For detailed instructions on setting up an RNA Seq Planned Run and reviewing results, see the [RNASeq Plugin User Bulletin.pdf](#)

# Torrent Browser Analysis Report Guide

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## 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

[Run the SampleID plugin automatically](#)

[Run the SampleID plugin manually](#)

[On-target metrics](#)

## 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
<a href="#">IonXpress_001</a>	F-YGACRCGRW	36.57%	6,297.33	100.000%	100.000%
<a href="#">IonXpress_002</a>	F-TGTRTRCRRW	33.05%	1,160.00	100.000%	100.000%
<a href="#">IonXpress_003</a>	M-TGACASGRW	36.73%	7,707.67	100.000%	100.000%
<a href="#">IonXpress_004</a>	M-TGACASGRW	26.13%	7,677.44	100.000%	100.000%
<a href="#">IonXpress_005</a>	N/A	29.00%	2.78	0.000%	0.000%
<a href="#">IonXpress_006</a>	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:

Chrom	Position	Target ID	TaqMan Assay ID	Call	Ref	r2P	Con
chr3	293202300	SHAPE1	G_21749022_10	Y	8	91.87%	3033
chr4	389903615	SHAPE1	G_11245602_10	S	T	87.94%	5828
chr5	179886725	SHAPE1	G_3163086_10	A	G	87.37%	42296
chr7	112320505	SHAPE1	G_3004175_10	C	T	90.16%	5219
chr9	272932465	SHAPE1	G_2622938	A	A	90.72%	5771
chr12	69445814	SHAPE1	G_2164724_1	C	C	90.69%	12668
chr16	97456723	SHAPE1	G_1171206_10	R	G	90.42%	9165
chr22	32058505	SHAPE1	G_11887118_1	W	Y	91.4%	5747

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

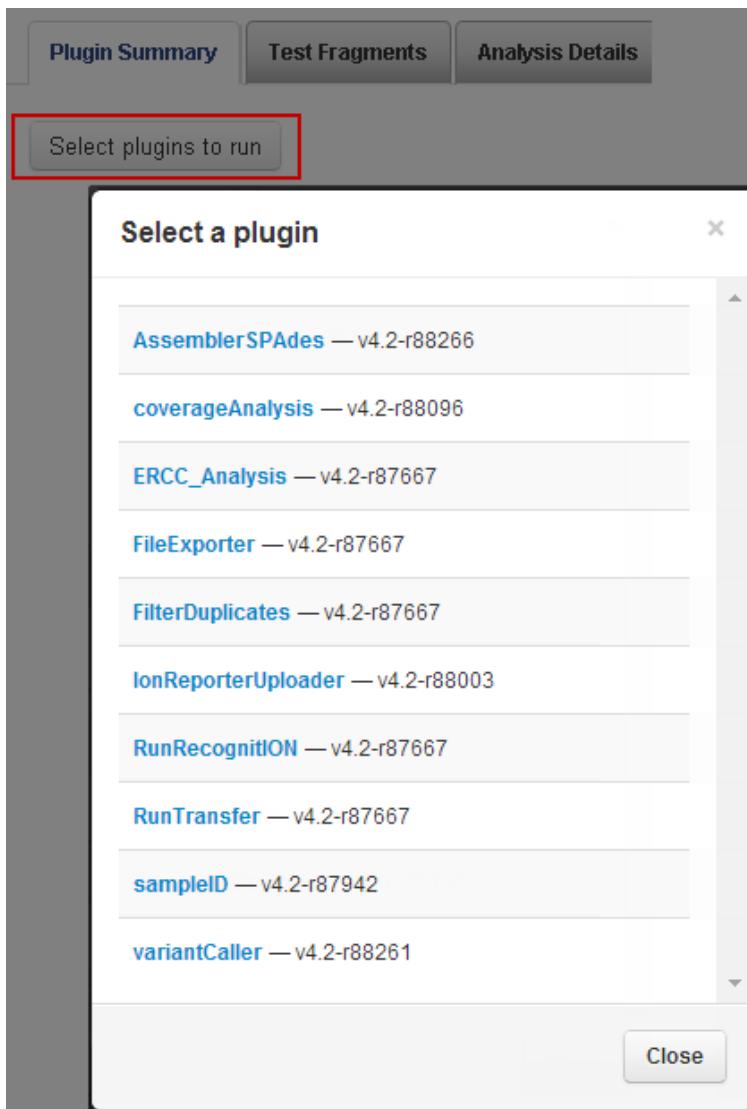
You set up the plugin to run automatically when you configure your template. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template. See the [Templates](#) page in the [Torrent Browser User Interface Guide](#).

## 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

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## 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.

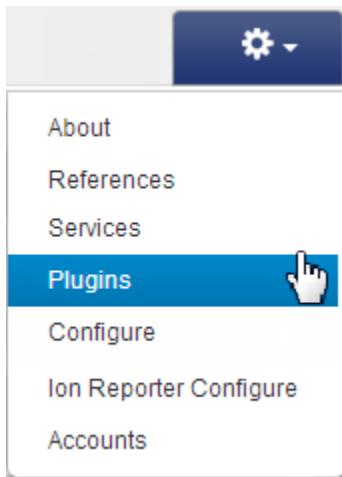
- Pre-configure the plugin
- Run the plugin automatically
- Run the plugin manually

### 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:

The screenshot shows the 'Plugins' section of a software interface. At the top, there are tabs for 'Enabled', 'Disabled', 'Either', 'Autorun', 'Manual', 'Either', and 'Clear'. Below this is a table with columns: Enabled, Autorun, Name, Version, Date, and Manage. The 'RunTransfer' plugin is highlighted with a red box around its name. A context menu is open over the 'RunTransfer' row, with the 'Configure' option highlighted and also surrounded by a red box. Other options in the menu include 'Usage', 'Refresh', and 'Uninstall'. The table rows list other plugins like 'variantCaller', 'sampleID', 'RunRecognition', etc.

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

### Configure

**Global Settings**

IP:	00.11.22.333
User Name (default ionadmin):	ionadmin
Password:	*****
Upload Path (default /results/uploads/):	/results/uploads/
Thumbnail-only:	<input type="checkbox"/>
<input style="border: 2px solid red; padding: 5px; width: fit-content; margin: auto;" type="button" value="Submit"/>	

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 you click Close.)



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. See the [Templates](#) page in the [Torrent Browser User Interface Guide](#).

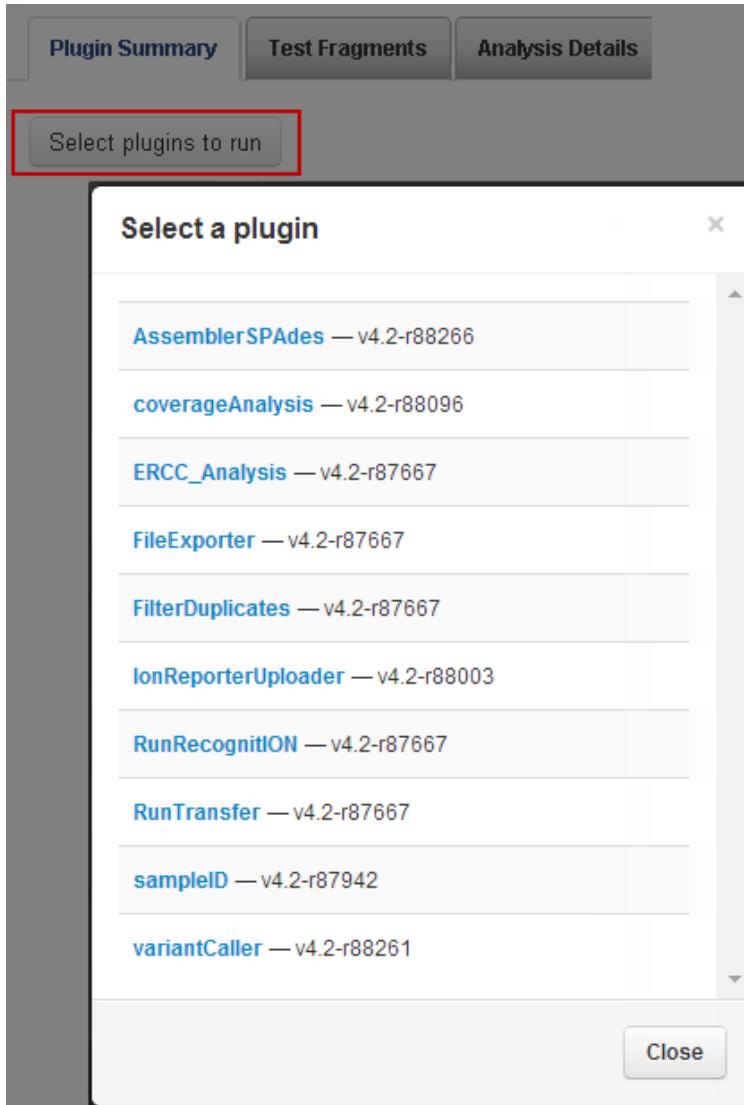
Create a plugin configuration in the Admin tab before running a plugin automatically. See [Pre-configure the plugin](#).

## 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.

Configure ×

## Global Settings

IP:

User Name (default ionadmin):

Password:

Upload Path (default /results/uploads/):

Thumbnail-only:

Submit Close

See [Pre-configure the plugin](#) for details.

# Torrent Browser Analysis Report Guide

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Torrent Suite™ Software space on Ion Community

[Analysis Report Guide TOC](#)

# Torrent Variant Caller Plugin

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## 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.

## 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 S5™, Ion PGM™, or the Ion Proton™ sequencer. Parameter defaults are different for Ion Proton™ data than for Ion PGM™ and Ion S5™ 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 Ion TargetSeq™ data.

See [Parameter Settings defaults](#) and [Variant Caller parameters](#) for more information about TVC's parameters.

## 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 dropdown 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.

See also [Input files](#).

 If you do not select a Target Regions file or a HotSpot Regions file, the Torrent Variant Caller plugin assumes there are no targeted regions when it runs the analysis. (See [Manage Target Regions Files](#) and [Hotspot Files](#) for information about file formats and about uploading these files in the Torrent Browser.)

## 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 research panel
- Ion AmpliSeq™ Colon and Lung Cancer research panel
- Ion AmpliSeq™ CFTR research panel

See [Create a Template with Ion AmpliSeq.com Import](#) for steps to import settings optimized for the above panels into your TVC run.

The following table lists the TVC parameter options that are pre-defined and optimized for ampliseq.com panels or Ion 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
Ion 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™ Sequencer's require different default settings. Select settings that are appropriate to both your sequencing instrument and your experiment:

### Variant Caller Parameter Settings:

- Germ Line - PGM - Low Stringency
- Germ Line - PGM - High Stringency
- Somatic - PGM - Low Stringency
- Somatic - PGM - High Stringency
- Germ Line - Proton - Low Stringency
- Germ Line - Proton - High Stringency
- Germ Line - Proton TargetSeq - Low Stringency
- Germ Line - Proton TargetSeq - High Stringency
- Somatic - Proton - Low Stringency
- Somatic - Proton - High Stringency
- Custom

- **Germ-Line - Low Stringency** — Optimized for high frequency variants and minimal false negative calls.
- **Germ-Line - High Stringency** — Optimized for high frequency variants and minimal false positive calls.
- **Somatic - Low Stringency** — Optimized for low frequency variant detection with minimal false negative calls.
- **Somatic - High Stringency** — Optimized for low frequency variant detection with minimal false positive 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 Ion TargetSeq™ experiments**

These two settings are optimized for Ion TargetSeq™ 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. See [Create a Template with Ion AmpliSeq.com Import](#).

## 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

Individual TVC parameters are described in [Torrent 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.

**Note:** To download the parameters used in a completed TVC run, see [Parameters File](#) in [Buttons for downloads and other actions](#).

## Upload your custom parameter values

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

**Upload Custom Settings:**

No file chosen

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. For an example of this type of file, see [Example Torrent Variant Caller Parameter File](#).

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:

**Upload Custom Settings:**

No file chosen

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. See [Manage Target Regions Files](#) and [Hotspot Files](#).

### 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. For format information, see [Manage Target Regions Files](#) and [Hotspot Files](#).

### 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.

The hotspots file must be in BED or VCF format. For format information, see [Manage Target Regions Files and 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.

## 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.

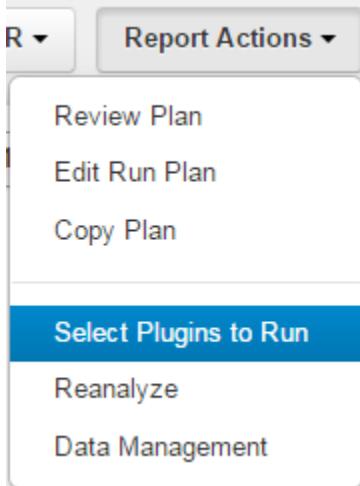
 The Torrent Variant Caller takes a significant amount of time to complete. Setting it up to run automatically saves time compared to running it manually.

See also [Variant Caller parameters](#).

## Torrent Variant Caller plugin manual launch for custom configuration per barcode

New in Torrent Suite™ Software v5.0, 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** and **Select Plugins to Run**.



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

## Torrent Variant Caller 5.0

[Submit](#)

Configuration:	Current 5770160f-3245-44e ▾	Manage Configurations/Barcodes												
<p><b>Chip Type:</b> <input checked="" type="radio"/> PGM/520 <input type="radio"/> Proton PI <input type="radio"/> 530 <input type="radio"/> 540</p> <p><b>Library Type:</b> <input type="radio"/> Whole Genome <input checked="" type="radio"/> AmpliSeq <input type="radio"/> TargetSeq</p> <p><b>Variant Frequency:</b> <input checked="" type="radio"/> Germ Line <input type="radio"/> Somatic</p> <p><b>AmpliSeq Panel:</b> Unspecified <a href="#" style="float: right;">Add panel...</a></p> <p><b>Reference Genome:</b> hg19 - Homo sapiens <a href="#" style="float: right;">▼</a></p> <p><b>Targeted Regions:</b> OCP3.20140718.designed <a href="#" style="float: right;">Add targets...</a></p> <p><b>Hotspot Regions:</b> OCP3.20140611.hotspots.blist <a href="#" style="float: right;">Add hotspots...</a></p> <p><b>Parameter Settings:</b></p> <ul style="list-style-type: none"> <li><input checked="" type="radio"/> Generic - PGM (3xx) or S5/S5XL (520/530) - Germ Line - Low Stringency germline_low_stringency_pgm_520_530_TS version: 5.0</li> <li><input type="radio"/> Custom custom, TS version: <input type="button" value="Load external parameter file"/></li> </ul> <table border="1" style="margin-top: 10px; width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="padding: 5px;">Parameter</th> <th style="padding: 5px;">SNP</th> <th style="padding: 5px;">INDEL</th> <th style="padding: 5px;">Hotspot</th> </tr> </thead> <tbody> <tr> <td style="padding: 5px;">Minimum allele frequency min_allele_freq</td> <td style="padding: 5px; text-align: center;">0.1</td> <td style="padding: 5px; text-align: center;">0.1</td> <td style="padding: 5px; text-align: center;">0.1</td> </tr> <tr> <td style="padding: 5px;">Minimum quality</td> <td style="padding: 5px; text-align: center;">10</td> <td style="padding: 5px; text-align: center;">10</td> <td style="padding: 5px; text-align: center;">10</td> </tr> </tbody> </table>			Parameter	SNP	INDEL	Hotspot	Minimum allele frequency min_allele_freq	0.1	0.1	0.1	Minimum quality	10	10	10
Parameter	SNP	INDEL	Hotspot											
Minimum allele frequency min_allele_freq	0.1	0.1	0.1											
Minimum quality	10	10	10											

## Apply TVC Settings to all Barcodes

To apply the same or different settings, select from the Configuration drop-down menu.

Configuration: [Current 5770160f-3245-44e](#) ▾

[Current 5770160f-3245-44e4-b1be-3ce82c1500bb](#)  
[human](#)  
[new config 123](#)  
[e-coli](#)

## 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.

### Apply TVC changes per barcode

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

**Torrent Variant Caller 5.0**[Back to initial page](#)[Configuration](#)[Setup](#)

Set All: **human** ▾

BAM File Name	Sample	Barcode	Configuration
IonXpress_001_rawlib.bam	Sample 1	IonXpress_001	human ▾
IonXpress_046_rawlib.bam	none	IonXpress_046	human ▾
IonXpress_048_rawlib.bam	none	IonXpress_048	human ▾
IonXpress_056_rawlib.bam	none	IonXpress_056	human ▾

Click on the configuration drop down to change the configuration.

[Submit](#)

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

**Add, Edit, and Delete Configurations**

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

**Torrent Variant Caller 5.0**[Back to initial page](#)[Save](#)[Cancel](#)

Configuration Name:

Chip Type:  PGM/520  Proton PI  530  540Library Type:  Whole Genome  AmpliSeq  TargetSeqVariant Frequency:  Germ Line  SomaticAmpliSeq Panel:  [Add panel...](#)Reference Genome: [Add targets...](#)Targeted Regions: [Add targets...](#)Hotspot Regions: [Add hotspots...](#)Parameter Settings:  Generic - PGM (3xx) or S5/S5XL (520/530) - Germ Line - Low Stringency  
germline\_low\_stringency\_pgm\_520\_530\_TS version: 5.0 Custom  
custom, TS version:[Load external parameter file](#)

Parameter	SNP	INDEL	Hotspot
Minimum allele frequency min_allele_freq	0.1	0.1	0.1
Minimum quality min_variant_score	10	10	10
Minimum coverage min_coverage	5	10	5

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

## Torrent Variant Caller 5.0

[Back to initial page](#)

[Save](#) [Cancel](#)

Configuration Name:

Chip Type:  PGM/520  Proton PI  530  540

Library Type:  Whole Genome  AmpliSeq  TargetSeq

Variant Frequency:  Germ Line  Somatic

AmpliSeq Panel:  [Add panel...](#)

Reference Genome:  [Add targets...](#)

Targeted Regions:  [Add hotspots...](#)

Hotspot Regions:

Parameter Settings:  Generic - PGM (3xx) or S5/S5XL (520/530) - Germ Line - Low Stringency  
germline\_low\_stringency\_pgm\_520\_530\_TS version: 5.0  
 Custom  
custom\_TS version:  
[Load external parameter file](#)

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.

## 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.

Before following these steps, read the [Introduction](#), [Supported panels](#), [Parameter Settings defaults](#), and [Variant Caller parameters](#) sections for information about the options and customizations for the plugin.

**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.

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



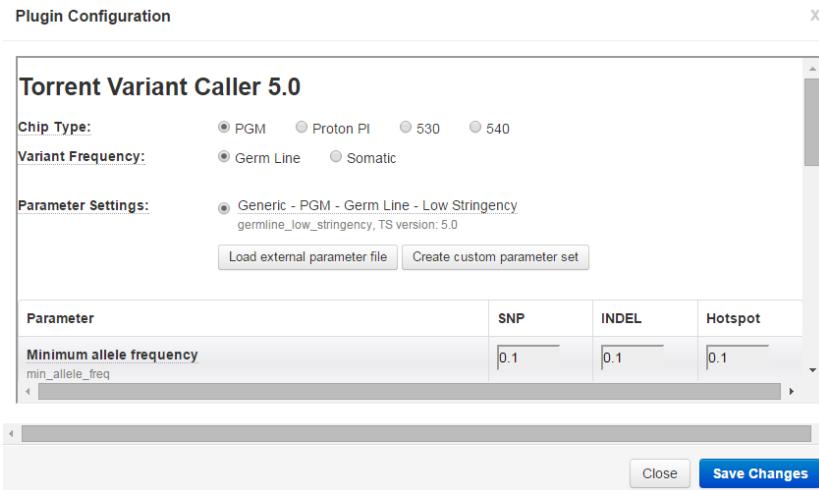
Select plugins to execute, then click Next.

[Select All](#)

[Clear Selections](#)

- |   |   |   |
|---|---|---|
| <input checked="" type="checkbox"/> SystematicErrorAnalysis | <input checked="" type="checkbox"/> TestFragmentTorturer                    | <input type="checkbox"/> alignFlowSignals |
| <input type="checkbox"/> topReads                           | <input type="checkbox"/> torrentscount                                      | <input type="checkbox"/> AmpliCat         |
| <input type="checkbox"/> validateVariantCaller-Lite         | <input checked="" type="checkbox"/> variantCaller <a href="#">Configure</a> | <input type="checkbox"/> AmpOffTarget     |

Link the **Configure** link to open the variantCaller configuration popup:



1. Make your changes to the parameter values.
2. Advanced users can also click the Show Advanced Settings button and customize those parameters.
3. Be sure to click the **Save Changes** button before leaving this page.

Be sure to click the **Save Plugin Settings** button before you click **Close**. Your changes are lost if you do not use the **Save Changes** button.

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.



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.



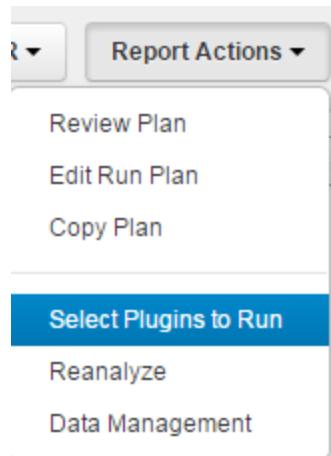
The Torrent Variant Caller plugin is not run if you select Generic Sequencing as the sequencing run type.

## Run the Torrent Variant Caller manually

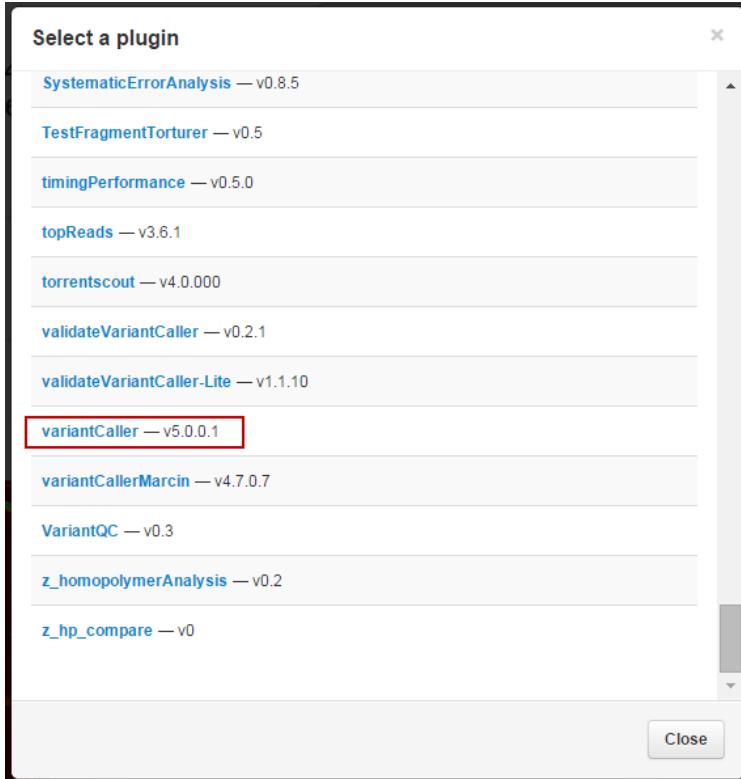
The TVC plugin supports multiple run analysis. The plugin can analyze a BAM file generated from Combine Alignment on multiple reports in a project. Combine Alignment creates a new run report (in the same project). You can open the new combined run report and use the **Select plugins to run** button to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



The Torrent Variant Caller Plugin interface appears:

## Torrent Variant Caller 5.0

**Submit**

Configuration: Current 582863f6-db05-4f41 ▾ Manage Configurations/Barcodes

**Chip Type:**  PGM  Proton PI  530  540

**Library Type:**  Whole Genome  AmplicSeq  TargetSeq

**Variant Frequency:**  Germ Line  Somatic

**AmplicSeq Panel:** Unspecified

**Reference Genome:** hg19 - Human (hg19)

**Targeted Regions:** Exome\_pool\_BENQ\_Designed\_20130326\_NoChrY\_ionVersioned

**Hotspot Regions:** None

**Parameter Settings:**

- Generic - PGM - Germ Line - Low Stringency  
germline\_low\_stringency, TS version: 5.0
- Custom  
custom, TS version:

Parameter	SNP	INDEL
Minimum allele frequency min_allele_freq	0.1	0.1
Minimum quality min_variant_score	10	10

These options are described in the [Introduction](#), [Input files](#), [Supported panels](#), [Parameter Settings defaults](#), and [Variant Caller parameters VCopts](#) sections.

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

## Torrent Variant Caller plugin output

The Torrent Variant Caller plugin output includes the following reports and sections:

[Run report plugin summary](#)

[Variant Caller Report](#)

[Variant Caller Report summary section](#)

[Variant Caller Report Variant Calls table](#)

See [Torrent Variant Caller Output](#) for descriptions of the reports and tables.

### 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:

Click the [Plugin Summary](#) jump near the top of the run report:

The screenshot shows the BioNumerics software interface. At the top, there are three tabs: 'Plan', 'Monitor', and 'Data'. The 'Monitor' tab is currently selected. Below the tabs, there are two main sections: 'Completed Runs & Results' and 'Projects'. Under 'Completed Runs & Results', there are three tabs: 'Run Summary', 'Output Files', and 'Plugin Summary'. The 'Plugin Summary' tab is highlighted with a red border.

Click the link to go to the TVC results summary:

The screenshot shows a run summary card for a 'variantCaller (v4.0-r72895)' run. The card includes the run name, version, size (8.1 MB), completion status (Completed), and a dropdown menu. A link labeled 'See plugin results above' is highlighted with a red border.

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.
- The genome VCF file

#### Barcoded variantCaller summary area

The screenshot shows the 'Variant Caller' summary for a barcoded run ('Run: R\_231b\_UV\_22'). The left side displays a list of barcodes with their corresponding sample names and sequencing parameters. The right side contains sections for 'Targeted Genes', 'Hotspot Regions', and 'Variant Statistics', each with various links and download options.

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

The screenshot shows the variantCaller plugin summary page. At the top, it displays the plugin name "variantCaller (v4.0-r72895)" and a link to "variantCaller.html". To the right is a status indicator "Completed" with a dropdown arrow. Below this, there's a table with the following configuration details:

Library type:	Whole Genome
Targeted regions:	None
Hotspot regions:	None
Configuration:	Germ Line - PGM

Below the configuration table is a navigation bar with three tabs: "Sample Name", "Variants", and "Download Links". The "Sample Name" tab is selected, showing the sample ID "E130680-073d04-13-L7438" and the count "22". To the right of the sample ID are three download buttons: "VCF.GZ", "VCF.GZ.TBI", and "XLS".

For a non-barcoded run, the sample name is listed. This link and the **variantCaller.htm** 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:

The screenshot shows a dropdown menu for a completed plugin run. The menu header says "Completed" with a dropdown arrow. Inside the menu, there are two items: "View Plugin Log" and "Delete this plugin result".

## 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:

The screenshot shows a dropdown menu for a completed plugin run. The menu header says "Completed" with a dropdown arrow. Inside the menu, there are two items: "View Plugin Log" and "Delete this plugin result".

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

## Download files, other actions

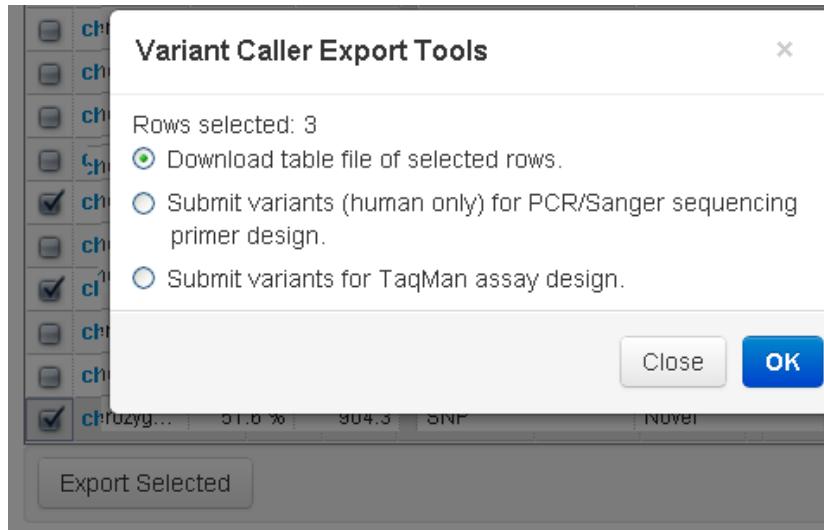
Field	Button	Description
Targeted regions	BED	Downloads the input targeted regions BED file (if any).
Hotspot regions	BED	Downloads the input hotspots BED file (if any).

Parameters Settings	Parameters File	<p>Downloads a JSON text file of the TVC parameter values used on this run.</p> <p><b>Note:</b> You can edit this file and later upload it to set your custom parameters in subsequent runs. For an example of this type of file, see <a href="#">Example Torrent Variant Caller Parameter File</a>.</p>
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, gVCF.GZ	<p>Downloads files of the variants calls:</p> <p>VCF.GZ, VCF.GZ.TBI: Zipped VCF file and its tabix index file            XLS: Tab-separated values file            gVCF.GZ: Reports positions on the genome where no variants were found.</p>
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).

## 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).

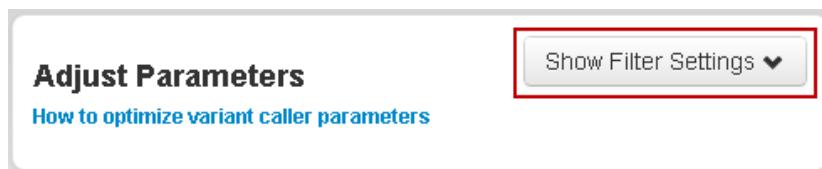
Click the left column checkboxes to select your variants, then click the **Export Selected** button:



## Rerun the variantCaller plugin

You can rerun the variantCaller plugin from the results page:

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



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

Parameter	# No Calls	Column	Parameter threshold value		
			SNP	INDEL	Hotspot
Minimum quality min_variant_score	0	Quality <	10.0	10.0	10.0
Minimum coverage min_coverage	0	Coverage <	6	15	6
Minimum coverage on either strand min_coverage_either_strand	0	Coverage + or - <	0	5	3
Maximum strand bias strand_bias	0	Strand Bias >	0.95	0.85	0.95
Minimum relative read quality min_quality_threshold	0	Relative Read Quality <	6.5		
Maximum common signal shift thr_common_predictions	0	Common Signal Shift >	0.3		
Maximum reference/variant signal shift (insertions) thr_insertion_predictions	0	Reference or Variant Signal Shift >	0.2		
Maximum reference/variant signal shift (deletions) thr_deletion_predictions	0	Reference or Variant Signal Shift >	0.2		
Maximum homopolymer length hp_max_length	0	HP Length >	8		
Context error on one strand	0	Not user configurable			
Context error on both strands	0	Not user configurable			
Excess outlier reads	0	Not user configurable			

Rerun Variant Caller

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

## Integration with other Thermo Fisher sites

See [Torrent Variant Caller Integration with TaqMan and PCR](#) for the following:

To select variants to search for pre-designed primers for PCR and Sanger sequencing

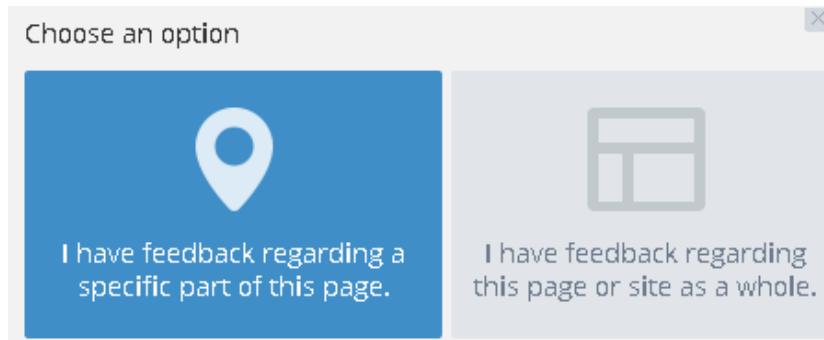
To select variants to submit a search on the TaqMan® Assay Search webpage

## Send feedback

Use the Send Feedback button, in the bottom right of the browser, to send your comments about the Torrent Variant Caller plugin:



The feedback app asks if your comments are about a specific part of the current page or if your comments are more general:



If you select the specific part of the current page option, you next click on that feature in the page and then fill out a text form with your comments.

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## Example Torrent Variant Caller Parameter File

### Torrent Browser Analysis Report Guide

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## Example Torrent Variant Caller Parameter File

This page contains an example JSON-format parameters file.

A file of this type is used to upload custom parameter settings to the Torrent Variant Caller (TVC) plugin (see [Upload a JSON file of parameter values](#)). You can download a similar file with the settings used in your TVC run, with the plugin report **Settings File** button (see [Variant Caller Report](#)).

```
{
    "torrent_variant_caller": {
        "snp_min_allele_freq": "0.15",
        "snp_strand_bias": "0.95",
        "hotspot_min_coverage": "20",
        "hotspot_min_cov_each_strand": "3",
        "hotspot_min_allele_freq": "0.15",
        "snp_min_variant_score": "10",
        "snp_beta_bias": "8",
        "hotspot_strand_bias": "0.95",
        "hp_max_length": "8",
        "filter_insertion_predictions": "0.2",
        "indel_min_variant_score": "10",
        "indel_beta_bias": "8",
        "indel_min_coverage": "20",
        "heavy_tailed": "3",
        "outlier_probability": "0.01",
        "data_quality_stringency": "8.5",
        "snp_min_cov_each_strand": "3",
        "hotspot_min_variant_score": "10",
        "indel_strand_bias": "0.85",
        "downsample_to_coverage": "400",
        "filter_unusual_predictions": "0.25",
        "indel_min_allele_freq": "0.15",
        "prediction_precision": "1",
        "indel_min_cov_each_strand": "3",
        "filter_deletion_predictions": "0.2",
        "snp_min_coverage": "20",
        "hotspot_beta_bias": "8"
    },
    "meta": {
        "tvcargs": "tvc"
    },
    "long_indel_assembler": {
        "short_suffix_match": "5",
        "min_indel_size": "4",
        "min_var_count": "5",
        "min_var_freq": "0.15",
        "kmer_len": "19",
        "max_hp_length": "8",
        "relative_strand_bias": "0.8"
    },
    "freebayes": {
        "gen_min_coverage": "6",
        "allow_mnps": "0",
        "read_max_mismatch_fraction": "1",
        "allow_snps": "1",
        "allow_indels": "1",
        "min_mapping_qv": "4",
        "gen_min_alt_allele_freq": "0.15",
        "read_mismatch_limit": "10",
        "min_base_qv": "4",
        "gen_min_indel_alt_allele_freq": "0.15"
    }
}
```

# Ion Reporter Software Features Related to Variant Calling

## Torrent Browser Analysis Report Guide

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## Ion Reporter™ Software Features Related to Variant Calling

Both Ion Reporter™ Software (IR) and Torrent Suite™ Software (TSS) 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 IR UI provide time savings for researchers.

- Annotations
- Multiple-sample workflows
- Additional variant types
- Variant filters, links, and export
- Output files
- Elasticity of the cloud
- Cost
- IR space on the Ion Community

### Annotations

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

The following annotation sources are packaged with IR:

- **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](http://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 PolyPhen2 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](http://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

IR 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 TSS are on one sample at a time.

IR 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 from 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

IR workflows support additional variant types that TSS 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 IR Genetic Disease Screening workflow also contains a pedigree check, which finds mis-labeling of the samples or mis-identification of the parent samples.

## Variant filters, links, and export

After your IR analysis completes, you can filter your called variants directly in the IR 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 IR's filters on the fly in the IR UI, or automate and reuse your filters.

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

IR 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 IR 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 IR takes some compute load off your Torrent Server and relieves strain on your Torrent Server compute resources.

## Cost

Beginning with the IR 4.0 release, IR usage is connected to Thermo Fisher eCommerce. IR charges are based on the amount of storage used (with lower storage usage being free). Use of the Ingenuity® Variant Analysis™ Software website involves a separate purchase.

## IR space on the Ion Community

For more information about Ion Reporter™ Software, see the [Ion Reporter™ Software space](#) on the Ion Community.

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# The Command-Line Torrent Variant Caller

## Torrent Browser Analysis Report Guide

[Torrent Suite™ Software space on Ion Community](#)

[Analysis Report Guide TOC](#)

### The Command-Line Torrent Variant Caller

This version of the Torrent Variant Caller (TVC) 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.

- [Example command](#)
- [Supported options](#)
- [About target regions BED files](#)

#### Example command

The following is an example TVC command. The options are described in the table after the example.

```
### Put your own values in these variables
BEDS=/results/uploads/BED/22/hg19
DIR=your_directory
INPUT=$DIR/yourBAM.bam
PARAMFILE=somatic_lowstringency_pgm_parameters.j
son
###

TVCDIR=/results/plugins/variantCaller
OUT=$DIR/out
BAM=`echo $(basename $INPUT) | sed -e
's/.bam$//'`^
echo $BAM

$TVCDIR/variant_caller_pipeline.py \
--input-bam $BAM \
--reference-fasta
/results/referenceLibrary/tmap-f3/hg19/hg19.fasta \
--output-dir $OUT/$BAM \
--parameters-file
$TVCDIR/pluginMedia/parameter_sets/$PARAMFILE \
--input-bam $BAM \
--postprocessed-bam $OUT/$BAM/PTRIM.bam \
--primer-trim-bed
$BEDS/unmerged/detail/designed.bed \
--bin-dir /results/plugins/variantCaller \
--hotspot-vcf $DIR/hotspots.vcf.gz \
--region-bed $BEDS/merged/plain/designed.bed
> $OUT/$BAM/log.txt 2>&1
```

## 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 but recommended.
-B RUNDIR --bin-dir=RUNDIR	Directory path to location of variant caller programs. Optional. Uses the directory where the <code>variant_caller_pipeline.py</code> script is located, if this option is missing.
-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.desig  
ed.bed  
./22/hg19/merged/detail/ColonLung.20131001.desig  
ned.bed  
./22/hg19/unmerged/plain/ColonLung.20131001.desi  
gned.bed  
./22/hg19/unmerged/detail/ColonLung.20131001.desi  
gned.bed
```

# Torrent Variant Caller Parameters

This page describes Torrent Variant Caller (TVC) parameters.

- A note about parameter customizations
- Main settings
- Advanced settings
  - Torrent Variant Caller advanced parameters
  - Long indel assembly advanced settings
  - FreeBayes advanced settings
  - Advanced Settings

## A note about parameter customizations

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
<b>Minimum allele frequency*</b>  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).  <b>Allowed values:</b> Floats 0.0 - 1.0 <b>Recommended values for SNPs:</b> Between 0.01 - 0.2 <b>Recommended values for indels:</b> Between 0.05 - 0.2
<b>Minimum quality*</b>  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.  <b>Allowed values:</b> Integers >= 0 <b>Recommended values:</b> >= 10

<b>Minimum coverage*</b>	<p>Do not call variants if the total coverage on both strands is below this value.</p> <p>For germ line workflows, lowering coverage improves sensitivity.</p> <p>Lowering this value is dangerous for homopolymer indels – this decreases specificity drastically.</p> <p><b>Allowed values:</b> Integers <math>\geq 0</math>  <b>Recommended values for SNPs:</b> Between 5 - 20  <b>Recommended values for indels:</b> Between 15 - 30  <b>Recommended values for hotspots:</b> Between 5 - 20</p>
<b>Minimum coverage on either strand*</b>	<p>Do not call variants if coverage on either strand is below this value.</p> <p>For indel calling, reducing this value improves sensitivity but at a high cost of specificity.</p> <p><b>Allowed values:</b> Integers <math>\geq 0</math>  <b>Recommended values:</b> <math>\geq 3</math></p>
<b>Maximum strand bias*</b>	<p>Do not call variants if the proportion of variant alleles comes overwhelmingly from one strand.</p> <p><b>Allowed values:</b> Floats 0.5 - 1.0  <b>Recommended values for SNPs:</b> 0.95  <b>Recommended values for indels:</b> 0.85  <b>Recommended values for hotspots:</b> 0.95</p> <p>Increasing strand bias increases sensitivity. SNP calling tolerates this adjustment better than indel calling.</p>
<b>Minimum relative read quality*</b>	<p>Do not call variants if Relative Read Quality is below this threshold. A phred-scaled minimum average evidence per read or no-call.</p> <p><b>Allowed values:</b> Floats <math>\geq 0</math>  <b>Recommended values:</b> <math>\geq 6.5</math></p> <p>Impact of changing this value: Lowering this value improves sensitivity and decreases specificity.</p>
<b>Maximum common signal shift*</b>	<p>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.</p> <p><b>Allowed values:</b> Floats <math>\geq 0</math>  <b>Recommended:</b> 0.3 = 30% of variant change size</p>

<b>Maximum reference/variant signal shift (insertions)*</b>  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).  <b>Allowed values:</b> Floats $\geq 0$ <b>Recommended:</b> 0.2 (which is 20% of variant change size)
<b>Maximum reference/variant signal shift (deletions)*</b>  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).  <b>Allowed values:</b> Floats $\geq 0$ <b>Recommended:</b> 0.2 (which is 20% of variant change size)

\*Override parameters that can also be used to customize hotspot calling. See note below.

**Note:** Torrent Variant Caller v4.6 allows particular hotspots to be customized with overrides that trigger filtering. Once TVC completes, you can rerun the following parameters to override the original hotspot calls. These limits are applied per variant, not per position. Use a 6-tab delimited fields format such as: chr1 43814978 43814979 . REF=G;OBS=A;strand\_bias=1; NONE. The Override Parameters are flagged with an asterisk \* in the table above.

## Advanced settings

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

### Torrent Variant Caller advanced parameters

Parameter	Comments
<b>hp_max_length</b>	Maximum homopolymer length for calling indels.  <b>Allowed values:</b> Integers $\geq 1$ <b>Recommended value:</b> 8
<b>downsample_to_coverage</b>	Reduce coverage in over-sampled locations to this value.  <b>Allowed values:</b> Integers $\geq 1$ <b>Recommended values:</b> 400 (germline), 2000 (somatic)

<b>outlier_probability</b>	<p>Prior probability that a read comes from some other distribution.</p> <p>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.</p> <p><b>Allowed values:</b> Floats 0.0 - 1.0  <b>Recommended values:</b> Between 0.005 - 0.01</p>
<b>do_snp_realignment</b>	<p>Realign reads in the vicinity of SNP candidates.</p> <p><b>Allowed values:</b></p> <ul style="list-style-type: none"> <li>• 0: Do no realign. Recommended for germline.</li> <li>• 1: Realign. Recommended for somatic.</li> </ul>
<b>prediction_precision</b>	<p>Number of pseudo-data-points suggesting our predictions match the measurements without bias.</p> <p><b>Allowed values:</b> Floats &gt;= 0.0  <b>Recommended value:</b> 1.0</p> <p><b>Impact of changing this value:</b> Lowering this value increases specificity and decreases sensitivity.</p>
<b>heavy_tailed</b>	<p>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.</p> <p>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.</p> <p>The proportion of reads that are discarded as outliers is shown in the FXX info tag in the output VCF file.</p>
<b>suppress_recalibration</b>	<p>Ignore the base recalibration values from pipeline in TVC. (Changes the way signal is predicted.)</p> <p><b>Allowed values:</b></p> <ul style="list-style-type: none"> <li>• 0: Use base calibration values in TVC. Recommended for Ion Proton™ data.</li> <li>• 1: Ignore base calibration values in TVC.</li> </ul>

## 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
<b>kmer_len</b>	<p>Sets the length of the minimum suffix/prefix overlap (perfect match) of any two reads to be considered for assembly.</p> <p>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.</p> <p><b>Allowed values:</b> Integers &gt; 5  <b>Recommended values:</b> Between 11 - 30</p>
<b>min_var_count</b>	<p>Sets the number of times a variant appears in the assembled contigs in order to be considered for evaluation.</p> <p>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.)</p> <p><b>Allowed values:</b> Integers &gt; 1  <b>Recommended values:</b> Between 3 - 30</p>
<b>short_suffix_match</b>	<p>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.</p> <p>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.)</p> <p><b>Allowed values:</b> Integers &gt; 2  <b>Recommended values:</b> Between 4 and kmer_len</p>
<b>min_indel_size</b>	<p>Sets the minimum size of an indel (from assembled reads) to be reported.</p> <p>Increasing this size reduces the number (and increases the size) of the reported indels. Increasing values make indel calls less sensitive but more specific.</p> <p><b>Allowed values:</b> Integers &gt; 0  <b>Recommended values:</b> Between 2 - 30</p>

<b>max_hp_length</b>	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.  <b>Allowed values:</b> Integers > 1 <b>Recommended values:</b> Between 2 - 11
<b>min_var_freq</b>	Sets the minimum value of the frequency of an indel to be reported.  <b>Allowed values:</b> Floats 0.0 - 1.0 <b>Recommended values:</b> 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.
<b>relative_strand_bias</b>	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.  <b>Allowed values:</b> Floats 0.0 - 1.0 <b>Recommended values:</b> Between 0.6 and 1.0  Increasing this value makes indel calls more sensitive but less specific.
<b>output_mnv</b>	Whether or not to include MNV variants in TVC output.  <b>Allowed values:</b> <ul style="list-style-type: none"> <li>• 0: Do not include MNVs.</li> <li>• 1: Also include MNVs.</li> </ul>

## 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
<b>allow_indels</b>	Enable indels in FreeBayes hypothesis generator. When set to 0, indels are not called.  <b>Allowed values:</b> <ul style="list-style-type: none"> <li>• 0 = Do not generate indel hypotheses</li> <li>• 1 = Generate indel hypotheses (default)</li> </ul>

<b>allow_snps</b>	Enable SNPs in FreeBayes hypothesis generator. When set to 0, SNPs are not called. <b>Allowed values:</b> <ul style="list-style-type: none"> <li>• 0 = Do not generate SNP hypotheses</li> <li>• 1 = Generate SNP hypotheses (default)</li> </ul>
<b>allow_mnps</b>	Enable MNPs, including equal-length block substitutions, in the FreeBayes hypothesis generator. When set to 0, MNPs are not called. <b>Allowed values:</b> <ul style="list-style-type: none"> <li>• 0 = Do not generate MNP hypotheses</li> <li>• 1 = Generate MNP hypotheses (default)</li> </ul>
<b>allow_complex</b>	Enable the generation of block substitution variants candidate in FreeBayes hypothesis generator. When set to 0, block substitution variants are not called. <b>Allowed values:</b> <ul style="list-style-type: none"> <li>• 0 = Do not generate block substitution hypotheses (default)</li> <li>• 1 = Generate block substitution hypotheses</li> </ul> <p>Notes about setting allow_complex to 1:</p> <ul style="list-style-type: none"> <li>• 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.</li> <li>• When on, allow_complex overrides the settings of allow_mnps</li> </ul>
<b>min_mapping_qv</b>	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. <b>Allowed values:</b> Integers >= 0 <b>Recommended value:</b> 4  Impact of changing this value: Increasing this value decreases sensitivity and improves specificity.
<b>read_mismatch_limit</b>	The number of mismatches allowed. If a read has more mismatches than this value, filter the read out. <b>Allowed values:</b> Integers >= 0 <b>Recommended value:</b> 10
<b>read_max_mismatch_fraction</b>	Maximum fraction of mismatches allowed in the length of read. Filters out potentially mis-mapped reads. <b>Allowed values:</b> Floats 0.0 - 1.0  <b>Recommended value:</b> 1.0  Decreasing this value decreases sensitivity and improves specificity (fewer but more accurate reads).

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

## Advanced Settings

At the bottom of the variantCaller configuration page are two Advanced Settings text entry boxes.

### Advanced Settings

#### Torrent Variant Caller arguments

```
tvc
```

#### Alignment arguments

```
tmap mapall ... --context -J 25 --end-repair 15 --do-repeat-clip stage1 map4
```

Parameter	Comments
tvc	Torrent Variant Caller arguments
tmap mapall	Overrides alignment arguments if arguments entered on rerun of TVC plugin are different from arguments set in the original run. If this setting is not change, TVC does not rerun alignment arguments.



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
  - Run report plugin summary
    - Barcoded variantCaller summary area
    - Non-barcoded variantCaller summary area
  - View plugin log file
  - Delete plugin results
  - Variant Caller Report
    - Information fields
    - Buttons for downloads and other actions
    - VCF File
    - Variant Calls by Allele table
      - View Allele Annotations
      - View Coverage Metrics
      - View Quality Metrics
    - Filtering codes
  - Export to file
  - Export to Pre-Designed Primers for PCR and Sanger Sequencing site
  - TaqMan® Assay Search

## Introduction

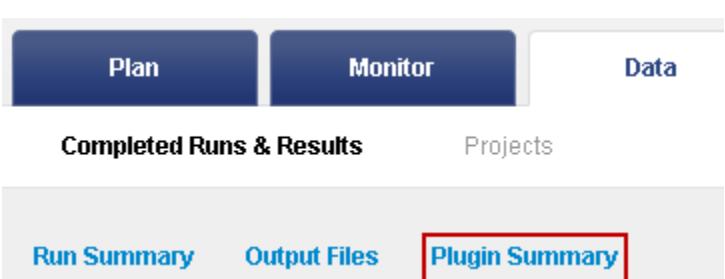
This page describes the Torrent Variant Caller (TVC) output, which includes the following reports and sections.

- 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.

#### Barcode variantCaller summary area

**variantCaller** (v4.0-r72895) [variantCaller.html](#) Completed ▾

Library type:	Whole Genome				
Targeted regions:	None				
Hotspot regions:	None				
Configuration:	Germ Line - PGM				
Download all barcodes:	<a href="#">VCF.ZIP</a> <a href="#">XLS.ZIP</a>				
Barcode Name	Sample Name	Variants	Download Links		
<a href="#">IonXpress_022</a>	None	1017	<a href="#">VCF.GZ</a>	<a href="#">VCF.GZ.TBI</a>	<a href="#">XLS</a>
<a href="#">IonXpress_026</a>	None	1197	<a href="#">VCF.GZ</a>	<a href="#">VCF.GZ.TBI</a>	<a href="#">XLS</a>

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

**variantCaller** (v4.0-r72895) [variantCaller.html](#) Completed ▾

Library type:	Whole Genome			
Targeted regions:	None			
Hotspot regions:	None			
Configuration:	Germ Line - PGM			
Sample Name	Variants	Download Links		
<a href="#">E130680-073d04-13-L7438</a>	22	<a href="#">VCF.GZ</a>	<a href="#">VCF.GZ.TBI</a>	<a href="#">XLS</a>

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

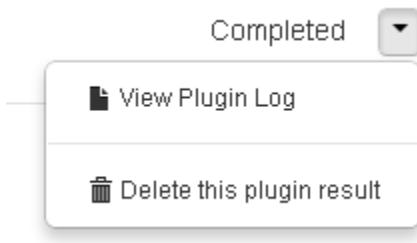
To view the log file for a plugin run, in the plugin summary area, click the menu arrow next to the plugin status:

Completed ▾

-  [View Plugin Log](#)
-  [Delete this plugin result](#)

## 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

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

**variantCaller** (v4.0-r72895) [variantCaller.html](#) Completed ▾

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

### Information fields

Field	Description
Run	The run report name
Barcode	Barcode name (only for barcoded runs)
Sample Name	Name of the sample
Reference	Name of the reference used in the analysis
Library Type	Library type
Variant Caller Version	Version of the plugin

### 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).

Parameters Settings	Parameters File	Downloads a JSON text file of the TVC parameter values used on this run.  <b>Note:</b> You can edit this file and later upload it to set your custom parameters in subsequent runs. For an example of this type of file, see <a href="#">Example Torrent Variant Caller Parameter File</a> .
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 file XLS: 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).

## VCF File

The VCF files contains all the information used by the other summary tables and all the variant calls. In its header it also contain a QC metric useful for FFPE samples:

### Deamination Metric

The deamination metric denotes the quality of an FFPE sample. The deamination score is the fraction of C>T plus G>A type even counts (at read level) within all substitution event counts. Currently, the following basic requirements apply for a substitution event to be counted:

1. The substitution position needs to be within the amplicon insert region for the amplicon that a read is assigned to.
2. The substitution position needs to have >30 coverage on both sides.
3. The ratio of a particular type of substitution at a given position needs to be >0.001 and <0.15 on both strands.

## 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 Thermo Fisher PCR and Sanger Sequencing Por TaqMan® Assay Design web sites. See [Export to File](#), [Export to Pre-Designed Primers for PCR and Sanger Sequencing](#), [Export to TaqMan® Assay Design](#).
- 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:

allele 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:

View Allele Annotations	View Coverage Metrics	View Quality Metrics
-------------------------	-----------------------	----------------------

Column	Description
<b>Position</b>	The chromosome (or contig) name in the reference genome, and the one-based position in the reference genome.
<b>Ref</b>	The reference base(s).
<b>Variant</b>	Variant allele base(s).
<b>Var Fred</b>	Frequency of the variant allele.
<b>Quality</b>	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 region name 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 the reverse strand, after downsampling
Strand bias	Discrepancy between allele frequencies on the forward and reverse strands

### **View Quality Metrics**

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

Column	Description
Common Signal Shift	Distance between predicted and observed signal at the allele locus. [RBI]

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

## Filtering codes

This table lists TVC filtering codes, which describe why a candidate is filtered out and not considered a variant.

Filtering reason	Description
COVERAGE	Corresponds to the various counting VCF tags on each strand for alleles: FDP, FSAF, FSAR, FSRF, FSRR. Set by the TVC parameter <code>min-cov-per-strand</code> for the appropriate variant type, as well as parameter <code>min-coverage</code> .
FAIL	The filtering is based on the quality score (probability that the variant at the first location has an allele-frequency above the specified location, or for a reference call, that we can exclude the variant having an allele-frequency above the specified frequency), controlled by <code>min-allele-frequency</code> and <code>min-variant-score</code> .  <b>Note:</b> <code>min-allele-frequency</code> is the frequency at which the cutoff happens due to sampling variability, and the quality value for an observation at <i>exactly</i> this frequency is quite low.
HPLEN	Corresponds to the VCF tag HRUN. It is set by TVC parameter <code>hp-max-length</code> . This tag filters Insertion and deletion variants that happen in long runs. Defaults to 8.

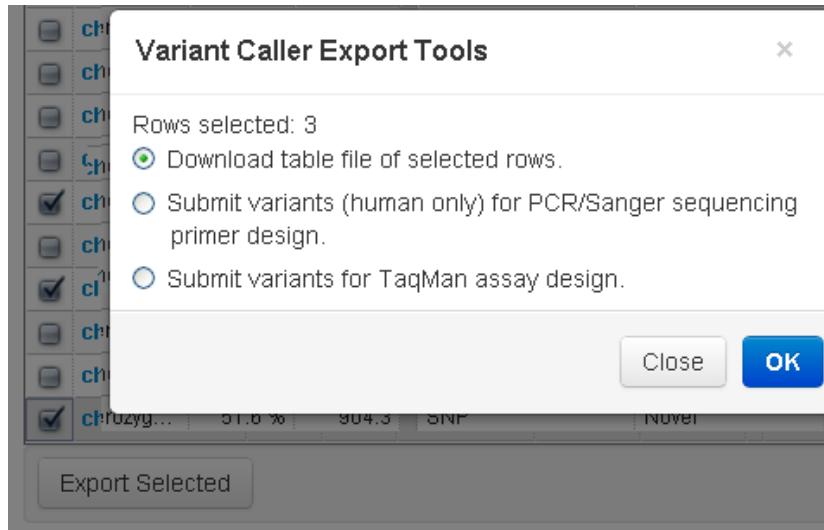
PREDICTIONSHIFT	Corresponds to the VCF tag RBI, which measures the amount by which the predictions had to be distorted to match the measurements. A value such as 0.3=30% of the difference the variant would make to the read (e.g., a shift of 0.3 for a 1 base deletion, a shift of 0.6 for a 2-base deletion). Set by TVC parameter <code>filter-unusual-predictions</code> . Usual range is 0.2 - 0.3. Smaller values mean predictions are required to be more precise. Larger values mean that more shift is allowed.
REFERENCE	We generate a candidate variant at this location, but after considering the evidence, generate a 0/0 genotype call at the location. Because this clutters the VCF file with entries at locations that are not variants, there is a flag <code>suppress-reference-calls</code> that filters out candidates where we evaluate the genotype to be 0/0.
SSE	Corresponds to the VCF tags SSEN and SSEP, and means that at this location we predict a potential error due to the local sequence context, on either the positive or negative strand. Set by the two TVC internal filter controls <code>sse-prob-threshold</code> (0.2), <code>min-ratio-reads-non-sse-strand</code> (0.2). We filter on either of these scenarios: <ul style="list-style-type: none"> <li>If both SSEN and SSEP are larger than the probability threshold set, <b>or</b> ...</li> <li>If only one is set and the other strand appears to be untrusted.</li> </ul>
STDBIAS	Corresponds to the VCF tag STB. Filters out variants if the stand bias for the variant is too large. Set by TVC parameter <code>strandbias</code> (and the corresponding internal parameters <code>snp-strand-bias</code> , <code>indel-strand-bias</code> , and <code>hotspot-strand-bias</code> ).
STRINGENCY	Corresponds to the VCF tag MLLD, which is mean-log-likelihood difference per read for the variant. This is approximately phred-scaled, so that MLLD=10 = 10% error rate per read, MLLD=20 = 1% error rate per read. Variants are filtered by the TVC parameter <code>data-quality-stringency</code> . Smaller values imply worse performance per read.

## 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**

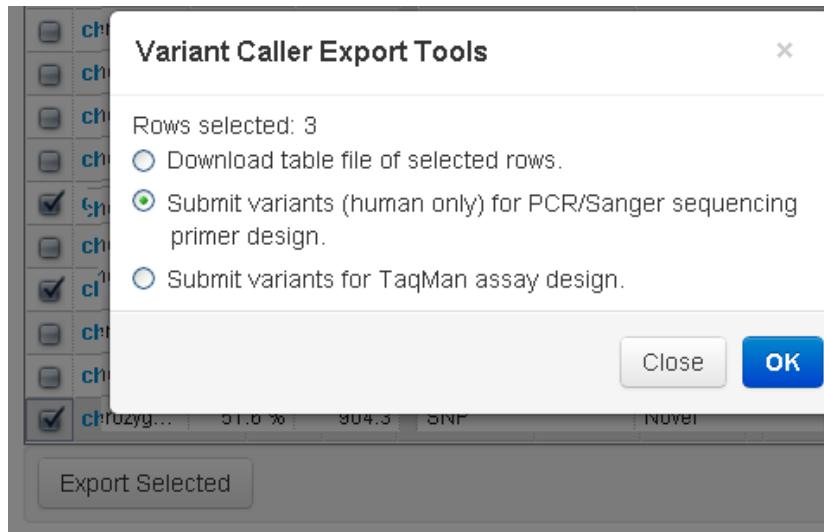
**Selected button:**



## 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:



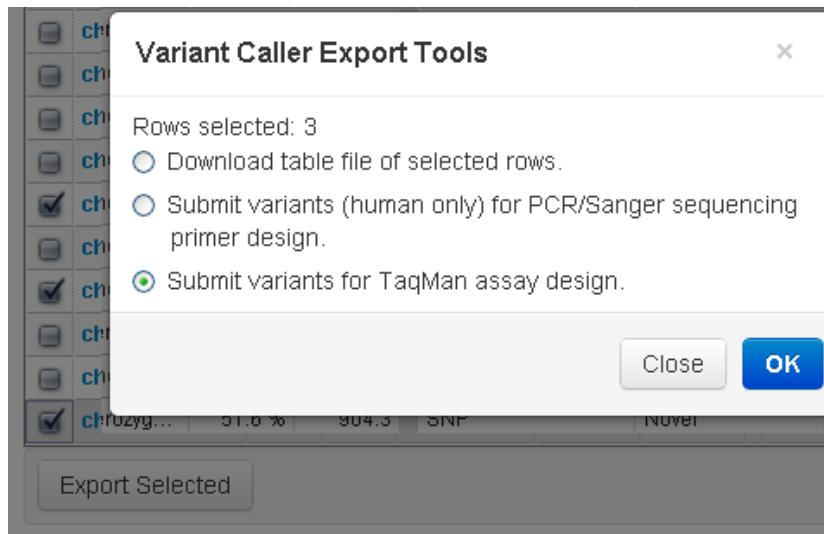
A new browser window opens to this site: [Pre-Designed Primers for PCR and Sanger Sequencing](#). You must be connected to the internet to access this feature.

## 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

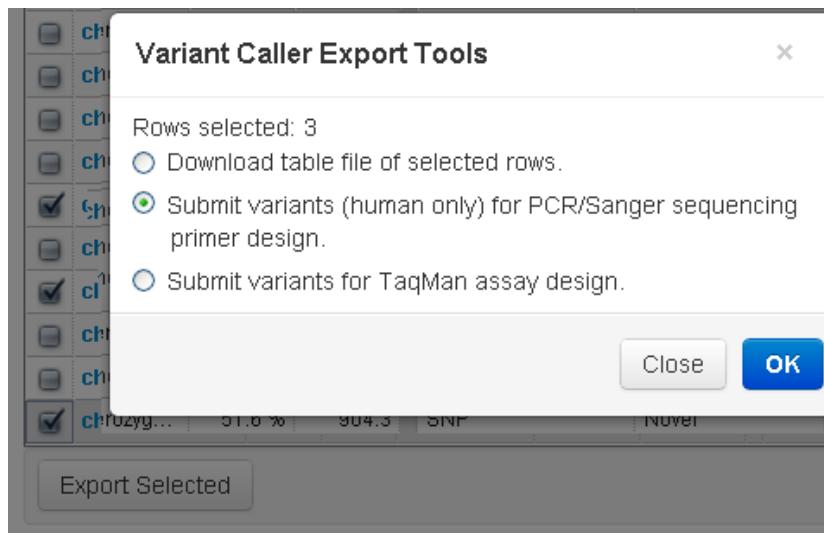
This page describes how to use your Torrent Variant Caller (TVC) results for pre-designed primers for PCR and Sanger sequencing or with the TaqMan® Assay Search webpage.

- Export to Pre-Designed Primers for PCR and Sanger Sequencing site
- TaqMan® Assay Search

### 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:

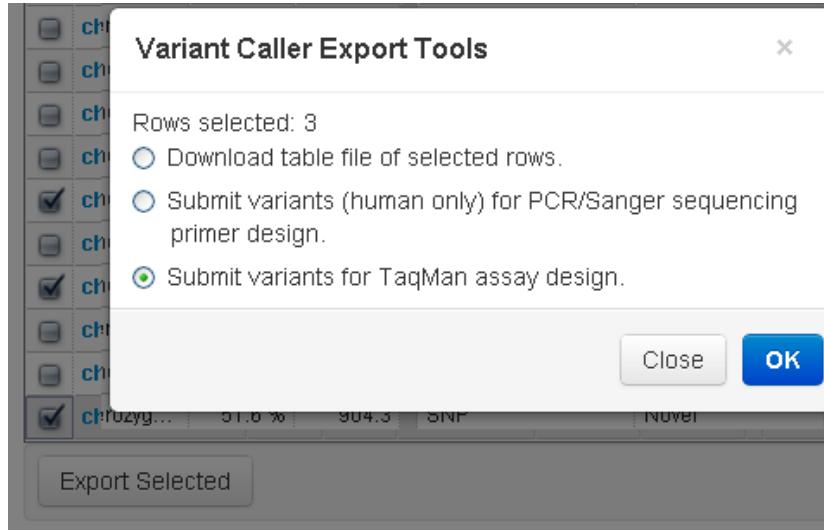


A new browser window opens to this site: [Pre-Designed Primers for PCR and Sanger Sequencing](#). You must be connected to the internet to access this feature.

### 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 Life Technologies.

Welcome    customer service 800 955 6288    Sign In    Register    Quick Order    My Cart

life technologies™

Search by catalog number or keyword

Products & Services    New Ideas    Communities & Social    Technical Resources    About Us

Your batch search returned **2** TaqMan® SNP Genotyping Assays

Change Your Search    Compare    Email    Export

Narrow Your Results

Search Term: chr.4:55141055

Species	SNP ID	Gene	Location	SNP Type	Assay Type	Made To Order   Cat. #	Unit	Price
Human	rs1873778	POGFRA	Chr.4: 55141055	Silent Mutation, Transition Substitution, Intron/exon	Functionality Tested	4351379	198UL, 40V	\$318.00 (USD)

Add To Cart

View Details    Allele Frequency

Search Term: chr.5:112175770

SNP ID	Gene	Location	SNP Type	Assay Type	Made To Order   Cat. #	Unit	Price
rs41115	APC	Chr.5: 112175770	Transition Substitution	Functionality Tested	4351379	198UL, 40V	\$318.00 (USD)

View Assay on Map

# Torrent Browser Analysis Report Guide

Torrent Suite™ Software space on Ion Community

[Analysis Report Guide TOC](#)

## Test Fragment Report

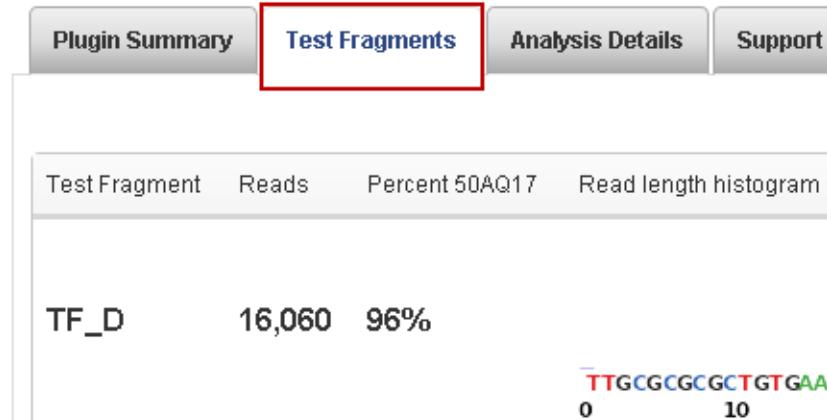
The **Test Fragment Summary** section of the Analysis Report provides information about the performance of each Test Fragment included in the experiment.

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.

 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
<b>Test Fragment</b>	Test fragment name (defined in the Admin > References tab of Torrent Browser).
<b>Reads</b>	Number of filtered & trimmed reads identified for this test fragment.

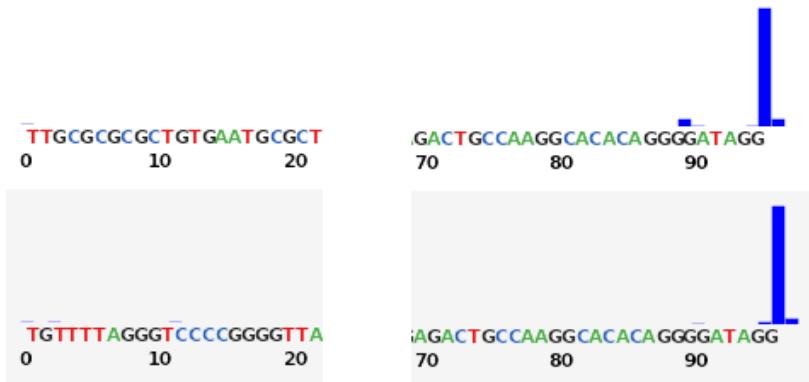
**Percentage 50AQ17**

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 histogram**

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.

# Torrent Browser Analysis Report Guide

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## Report Information

This section describes the following run report buttons:

- [Analysis Details](#)
- [Software Version](#)
- [Support](#)

## Analysis Details

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

Plugin Summary	Test Fragments	<b>Analysis Details</b>	Support	Software Version
<b>Run Name</b>	test_GOG-428			
<b>Run Date</b>	2012-07-09 13:14:07.357181			
<b>Run Cycles</b>	16			
<b>Run Flows</b>	520			
<b>Project</b>	BLACK			
<b>Sample</b>	2126-21418-MCB-AG			
<b>Library</b>	hsa_ERCC92			
<b>PGM</b>	2-4-RegressionTests			
<b>Flow Order</b>	TACGTACGTCTGAGCATCGATCGATGTACAGC			
<b>Library Key</b>	TCAG			
<b>TF Key</b>	ATCG			
<b>Chip Check</b>	Passed			
<b>Chip Type</b>	318B			
<b>Chip Data</b>	single			
<b>Notes</b>				
<b>Barcode Set</b>	IA-10			
<b>Analysis Name</b>	Batch_GOG-428_Build_201208171640			
<b>Analysis Date</b>	2012-08-17			
<b>Analysis Flows</b>	0			
<b>runID</b>	5Y5AE			

Parameter	Description
<b>Run Name</b>	Name of the run.
<b>Run Date</b>	Date and time the Ion S5™, Ion PGM™, or Ion Proton™ run was started.
<b>Run Cycles</b>	Number of Ion S5™, Ion PGM™, or Ion Proton™ cycles analyzed for this report. Note that this number can differ from the total number of cycles run on the sequencer.

<b>Run Flows</b>	Number of Ion S5™, Ion PGM™, or Ion Proton™ nucleotide flows analyzed for this report. Note that this number can differ from the total number of flows occurring on the sequencer.
<b>Project</b>	Names of the projects the result set is a member of.
<b>Sample</b>	Name of the sample assigned to the run used to generate this analysis. This is assigned on the Ion S5™, Ion PGM™, or Ion Proton™ Sequencer.
<b>Library</b>	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.
<b>PGM</b>	Name of the Ion S5™, Ion PGM™, or Ion Proton™ Sequencer on which the run was performed. This value is typically entered on the sequencer.
<b>Flow Order</b>	<p>Flow order selected on Ion S5™, Ion PGM™, or Ion Proton™ Sequencer:</p> <p>Samba = TACGTACGTCTGAGCATCGATC GATGTACAGC [Default] Regular = TACG</p> <p>The "regular" flow order adds bases most rapidly to sequenced molecules but is vulnerable to phase errors. The Samba flow order consists of a 32-base sequence, repeated. This flow order resists phase errors by providing opportunities for out-of-phase molecules to catch up and is designed to sample all dimer (nucleotide pair) sequences, efficiently. Samba is the default flow order because it improves sequencing accuracy for longer reads by resisting phase errors.</p>
<b>Library Key</b>	A short known sequence of bases used to distinguish the library fragment from the test fragment. Example: "TCAG"
<b>TF Key</b>	A short known sequence of bases used to distinguish the test fragment.
<b>Chip Check</b>	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 <b>Passed</b> or <b>Failed</b> .
<b>Chip Type</b>	Type of chip used on the Ion PGM™ Sequencer. Usually, 314, 316, or 318 (for the Ion 314™ chip, Ion 316™ chip, and Ion 318™ chip.) A letter follows the numbers, indicating the chip version.
<b>Chip Data</b>	In this release, the value is <b>single</b> , for a forward run.
<b>Notes</b>	A space for text notes entered during the Ion S5™, Ion PGM™, or Ion Proton™ Sequencer run.

<b>Barcode Set</b>	The name of the barcode set assigned to the run. Blank for non-barcode libraries.
<b>Analysis Name</b>	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.
<b>Analysis Date</b>	Date the analysis was performed.
<b>Analysis Flows</b>	Number of Ion S5™, Ion PGM™, or Ion Proton™ nucleotide flows analyzed for this report. Note that this number can differ from the total number of flows occurring on the Ion PGM™ or Ion Proton™ Sequencer.
<b>runID</b>	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 Torrent Suite™ Software and its modules installed on your Torrent Server.

 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™ Software Release Notes for the package versions in a specific release.



<b>Torrent_Suite</b>	<b>3.6</b>
<b>Datacollect</b>	
<b>Graphics</b>	
<b>LiveView</b>	
<b>OS</b>	
<b>Script</b>	
<b>host</b>	
<b>ion-alignment</b>	
<b>ion-analysis</b>	
<b>ion-gpu</b>	
<b>ion-pipeline</b>	

Parameter	Description
<b>Torrent Suite</b>	Version of Torrent Suite™ Software used to generate the analysis.
<b>Datacollect</b>	Version of the Datacollect package.
<b>Graphics</b>	Version of the Graphics package

<b>LiveView</b>	Version of the LiveView package.
<b>Script</b>	Version of the Script package.
<b>host</b>	Host name where the Torrent Server is installed.
<b>ion-alignment</b>	Version of the Torrent Suite™ Software alignment module used for this analysis.
<b>ion-analysis</b>	Version of the Analysis Pipeline used to generate the analysis.
<b>ion-gpu</b>	Version of the NVIDIA Tesla GPU driver.
<b>ion-pipeline</b>	Version of the analysis pipeline.

## 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.



- Download the [Customer Support Archive](#)
- [Download the New Customer Support Archive](#)
- [View the report log](#)

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

**Report Error Log** Refresh the page to see updates

# Torrent Browser Analysis Report Guide

## Classic Run Reports

This section describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features.

To view the classic-style report for a run, open the current run report in your Torrent Browser and then click the **Classic Report** button (near the top right of the page):



To view analysis runs generated with earlier version of the Torrent Suite™ Software, you must either re-analyze the run to create a 3.x-style run report or use the older report format.

Some features that previously were available in run reports are moved in 3.x releases. For example, in 3.x, to combine alignments you create a project, use a template that creates run results in that project, and use the **Data > Projects > projectname > Combine Selected...** button. This button replaces the combinAlignments plugin that was available in previous releases.

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	<a href="#">Classic Report Information</a>
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## Classic TBAR Table Of Contents

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## Pre-3.0 Library Summary

### Torrent Browser Analysis Report Guide

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#### Pre-3.0 Library Summary

This section provides **Library Summary** background information and a detailed description of the report.

[Library Summary Overview](#)  
[Library Summary Report](#)

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	<a href="#">Run Report Metrics on Aligned Reads</a>
	<a href="#">Compare Multiple Run Reports</a>
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	<a href="#">FilterDuplicates Plugin</a>
	<a href="#">IonReporterUploader Plugin</a>
	<a href="#">See  The Ion Reporter™ Software Integration Guide</a>
	<a href="#">Run Recognition Plugin</a>
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	<a href="#">Torrent Variant Caller Plugin</a>
	<a href="#">Torrent Variant Caller Parameters</a>
	<a href="#">Example Torrent Variant Caller Parameter File</a>
	<a href="#">Torrent Variant Caller Output</a>
	<a href="#">The Command-Line Torrent Variant Caller</a>
	<a href="#">Ion Reporter™ Software Features Related to Variant Calling</a>
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## Classic Library Summary Overview

### Torrent Browser Analysis Report Guide

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#### Classic Library Summary Overview

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



This **Library Summary** section of the Torrent Browser Analysis Report gives performance metrics for reads whose initial bases match the library key.

 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.

[Using Predicted Quality \(Q17/Q20\)](#)  
[Using Quality Following Alignment \(AQ17/AQ20\)](#)

### Using Predicted Quality (Q17/Q20)

The number of called bases with a predicted quality of Q17 or Q20 is reported. The predicted quality values are reported on the Phred scale, defined as  $-10 \times \log_{10}$  (error probability). Q20, therefore, corresponds to a predicted error rate of one percent and Q17 corresponds to a predicted error rate of two percent.

 Refer to [http://en.wikipedia.org/wiki/Phred\\_quality\\_score](http://en.wikipedia.org/wiki/Phred_quality_score) for a more complete description of Phred values.

### Using Quality Following Alignment (AQ17/AQ20)

Alignment of reads can be a useful process 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 three quality levels:

- AQ17
- AQ20
- Perfect

#### How Is Aligned Read Length Calculated?

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 AQ17 length of a read is the greatest length at which the read error rate is 2% or less, and 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 AQ17 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 AQ17 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 and AQ17 lengths are 80 and 100 bases, respectively.

#### **How Is Alignment Performed?**

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 BFEST algorithm. Please, contact Ion Torrent™ Technical Support for more information about TMAP.

[Technical Note - Analysis Pipeline](#)  
[Technical Note - TMAP Alignment](#)

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

Homer N, Merriman B, Nelson SF.  
BFEST: An alignment tool for large scale genome resequencing.  
PMID: 19907642  
PLoS 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. For more detail on how to enable and specify this reference-specific limit see the Adding a Reference Sequence section of [Working with Reference Sequences](#).

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.

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## Classic Library Summary Report

### Torrent Browser Analysis Report Guide

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#### Classic Library Summary Report

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



Performance, based on either predicted quality or quality as measured following alignment, is provided in the **Library Summary** section of the Detailed Report. This section of the report contains the following information:

#### Based on Predicted Per-Base Quality Scores - Independent of Alignment

The **Based on Predicted Per-Base Quality Scores - Independent of Alignment** section gives performance measurements based on predicted quality:

Total Number of Bases [Mbp]	44.02
Number of Q20 Bases [Mbp]	24.69
Total Number of Reads	438,836
Mean Length [bp]	100
Longest Read [bp]	201

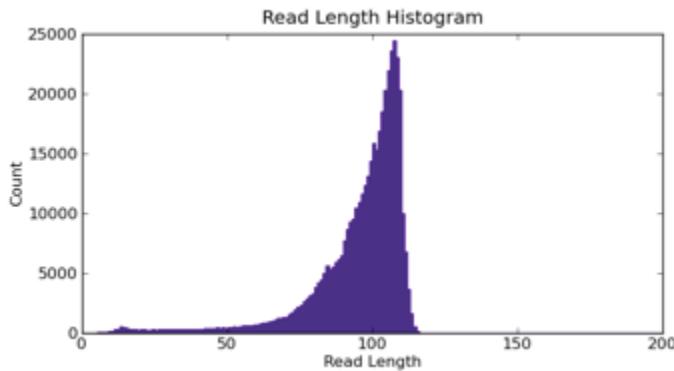
Parameter	Description
<b>Total Number of Bases [Mbp]</b>	Number of filtered and trimmed million base pairs reported in the output files.
<b>Number of Q20 Bases [Mbp]</b>	Number of bases with predicted quality of Q20 or greater.
<b>Total Number of Reads</b>	Total number of filtered and trimmed reads independent of length reported in the output files.

<b>Mean Length [bp]</b>	Average length, in base pairs, of all filtered and trimmed library reads reported in the output files.
<b>Longest Read [bp]</b>	Maximum length, in base pairs, of all filtered and trimmed library reads reported in the file.

 For more information on filtering and trimming, please see [Technical Note - Filtering and Trimming](#).

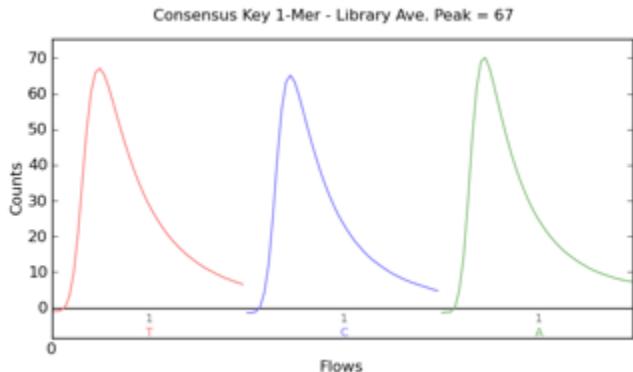
### Read Length Histogram

The **Read Length Histogram** is a histogram of the trimmed lengths of all reads present in the output BAM file. The following figure illustrates an example graph:



### Consensus Key 1-Mer

The **Consensus Key 1-Mer** graph shows the strength of the signal from the first three one-mer bases of the library key. This graph represents the consensus signal measurement of release of H<sup>+</sup> during nucleotide incorporation.



The y-axis shows signal strength, measured in **Counts**, which is an arbitrary but consistent unit of measure. The x-axis shows time as nucleotide **Flows** over the chip.

There is a known key at the beginning of every library read. Typically, three bases are shown of the 4-mer key. Note that the graph is displayed in "flow order" rather than "base order." For example, the four-base library key is typically TCAG for nucleotides one through four and is graphed as TCA, representing the nucleotide flow order. Negative flows are not displayed.

Q: If the library key is 4 bases, why are only three bases displayed in the key one-mer graph?

A: The next base after the last key base is the first library base. This base varies depending on the library fragment. If the last key base is a G and the first library base is a G, both of these are incorporated in the same flow resulting in a signal roughly 2X of a one-mer. Thus, the last base of the library read is not informative for quality purposes because that flow can contain library information in addition to key information. The one-mer key pass graph only contains n-1 flows for an n-mer library key.

## Reference Genome Information

The **Library Summary** includes the **Reference Genome Information**:

Genome Name	<i>E. coli DH10B</i>
Genome Size	4,686,137 bases
Genome Version	1
Index Version	tmap-f2

Parameter	Description
<b>Genome Name</b>	Name of reference genome.
<b>Genome Size</b>	Number of bases in the reference genome.
<b>Genome Version</b>	Version information for the genome used.
<b>Index Version</b>	Version information for the genome index used.

If an alignment error occurred, a message prompts you to view the report log for information about the error.

## Based on Full Library Alignment to Provided Reference

This section of the **Library Summary** report shows performance as measured following alignment.

	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	112.63	92.40	73.80
Mean Length [bp]	125	107	88
Longest Alignment [bp]	254	242	227
Mean Coverage Depth	24.00×	19.70×	15.70×
Percentage of Library Covered	100%	100%	100%

Parameter	Description
<b>Total Number of Bases [Mbp]</b>	Number of million of base pairs that have been aligned to the genome at the specified quality level.
<b>Mean Length [bp]</b>	Average length, in base pairs, of all library reads that aligned to the genome at <b>Q20</b> and <b>Perfect</b> (the longest perfectly aligned segment).
<b>Longest Alignment [bp]</b>	Maximum length, in base pairs, of all library reads that aligned to the genome at a specific quality level.

<b>Mean Coverage Depth</b>	Average number of times that a base was independently sequenced and aligned to the reference genome. 1X means that every base was sequenced and aligned, on average, once. 2X means that every base was sequenced and aligned, on average, twice.
<b>Percentage of Library Covered [bp]</b>	Percentage of the reference genome that is covered at a minimum of 1X by filtered library reads at a specific quality.

### Using the TMAP Alignment Algorithm

When the reference genome is a large genome, alignment is performed on a random subset of the reads in the unmapped BAM file. You can specify sampling and the number of reads to sample on a per-genome basis. Sampling only occurs if this number is set during reference upload. Otherwise, complete alignment is performed. The values in this table are extrapolated to the full number of reads.

### Read Alignment Distribution

Summarizing the alignments produces the following report format:

Read Length [bp]	Reads	Unmapped	Excluded	Clipped	Perfect	1 mismatch	≥2 mismatches
50	1,053,091	14,965	9	0	708,749	197,844	131,524
100	1,038,999	14,170	8	38,660	400,053	238,881	347,227
150	1,019,061	13,347	7	137,130	159,433	157,260	551,884
200	983,824	11,988	6	312,005	54,986	70,017	534,822
250	480,929	7,805	3	311,487	8,264	12,059	141,311
300	62,035	2,682	0	59,350	0	0	3

 The number of rows displayed varies depending on the read length.

Each column shows data based on alignment of the sample.

Column	Description
<b>Read Length [bp]</b>	The number of bases in each read considered for the row in the table.
<b>Reads</b>	Number of reads with at least <b>Read Length</b> bases.
<b>Unmapped</b>	Number of reads that TMAP could not map.
<b>Excluded</b>	Number of reads mapped but not having 90% accuracy in first 50 bases.
<b>Clipped</b>	Number of reads mapped and with accuracy of greater than 90% in first 50 bases, but with align length less than the <b>Read Length</b> threshold.
<b>Perfect</b>	Number of aligned reads with zero mismatches in the first <b>Read Length</b> bases.
<b>1 mismatch</b>	Number of aligned reads with one mismatch in the first <b>Read Length</b> bases.
<b>2 mismatches</b>	Number of aligned reads with two or more mismatches in the first <b>Read Length</b> bases.

 SAM/BAM files for reports with sampled data only include alignment information for the sampled subset.

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# PartekFlowUploader Plugin

# Torrent Browser Analysis Report Guide

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[Torrent Suite™ Software space on Ion Community](#)

[Analysis Report Guide TOC](#)

## 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](#)
- [Run the plugin manually](#)

### Run the plugin automatically

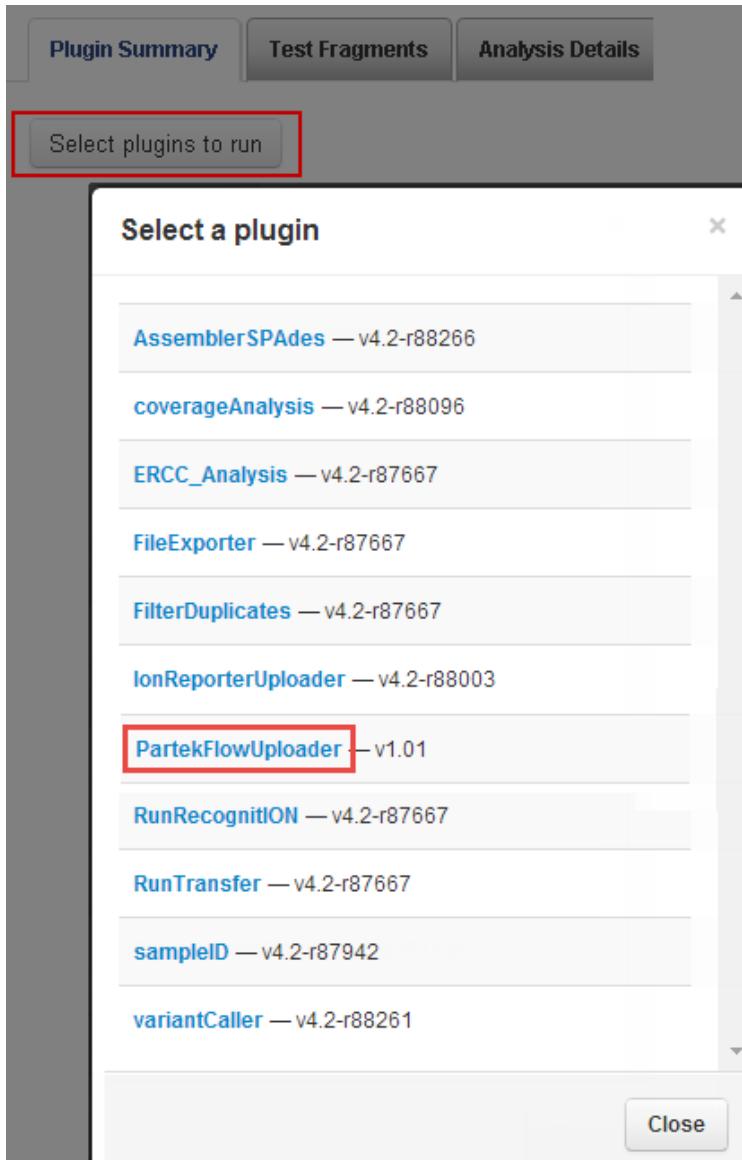
You set up the plugin to run automatically when you configure your template or run plan. In the Plugin chevron of the template wizard, you select which plugins run automatically on planned runs created from that template. See the [Templates](#) page in the [Torrent Browser User Interface Guide](#).

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

 Partek®  
**Flow**

Don't have Partek Flow for NGS Data Analysis?  
[Click](#) to learn more and download a FREE trial.

To export your data to Partek Flow,  
enter your login details.

Flow Server URL (hostname:port)

Flow Username

Flow Password

Project Name

**Export to Partek Flow**

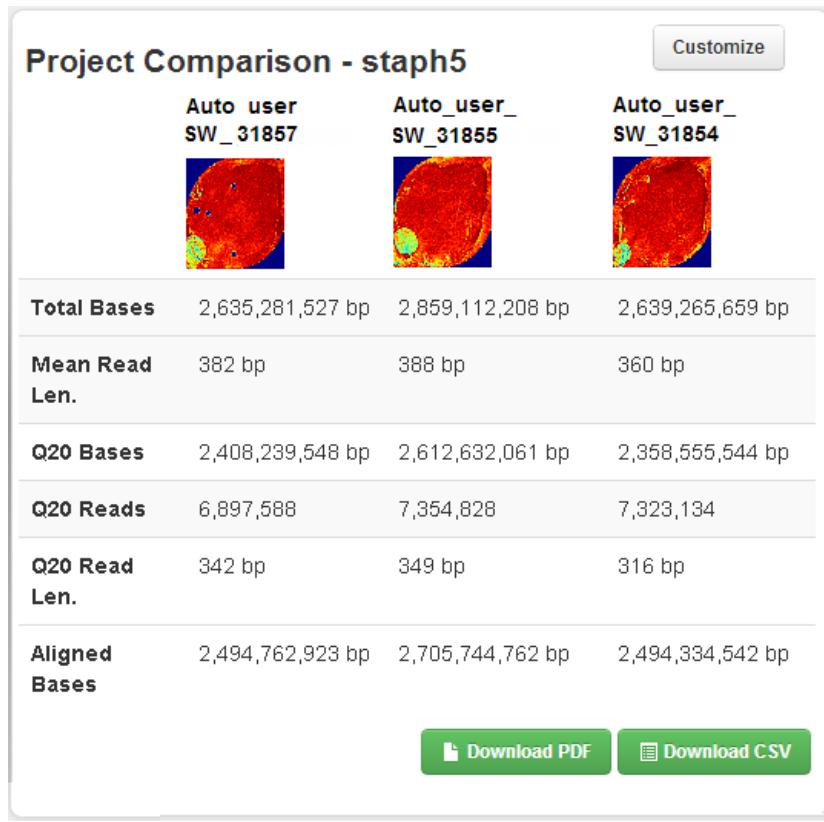
# Torrent Browser Analysis Report Guide

Torrent Suite™ Software space on Ion Community

## Compare Multiple Run Reports

- Display only reports of interest or metrics of interest
- Comparison of barcoded runs
- Download CVS or PDF output

This page describes how to compare multiple run reports in your Torrent Browser. An example comparison page is shown here:



Notes:

- The reports to be compared must all belong to the same project.
- The project should contain a reasonable number of reports (not hundreds or thousands), because the comparison page display metrics for all reports in the project.
- You then filter to display only your reports of interest and only your fields of interest.
- You can also download the comparison report as a PDF or CSV file.

## Steps to compare run reports

Follow these steps to compare multiple run reports:

1. Have the run reports be in one project or create a project and add the reports to that project.
2. Open the project listing and click the **Compare** button:

## Result Sets in staph5

Report Date    Search names    Go    Clear

<input type="checkbox"/>	Name	Status
<input type="checkbox"/>	Auto_user_SW_31854	Completed
<input type="checkbox"/>	Auto_user_SW_31857	Completed
<input type="checkbox"/>	Auto_user_SW_31855	Completed
<input type="checkbox"/>	Auto_user_SW_31856	Completed

1

Combine Selected ▾    Process Selected ▾    **Compare**

- The Project Comparison page opens and displays a columns of metrics for each report in the project:

Project Comparison - staph5			
Auto user SW_31857	Auto_user_SW_31856	Auto_user_SW_31855	Auto_user_SW_31854
			
Date	March 18, 5:56 a.m.	March 18, 4:44 a.m.	March 18, 5:55 a.m.
Chip	318C	318C	318C
Total Bases	2,635,281,527 bp	2,448,529,886 bp	2,859,112,208 bp
Total Reads	6,897,588	6,597,797	7,354,828
Mean Read Len.	382 bp	371 bp	388 bp
Q20 Bases	2,408,239,548 bp	2,218,102,188 bp	2,612,632,061 bp
Q20 Reads	6,897,588	6,597,797	7,354,828
Q20 Read Len.	342 bp	324 bp	349 bp
Reference	Staph5	Staph5	Staph5
Aligned Bases	2,494,762,923 bp	2,315,150,681 bp	2,705,744,762 bp
Aligned Reads	6,806,547	6,490,466	7,275,763
<a href="#">Download PDF</a>		<a href="#">Download CSV</a>	

**i** The checkboxes in the project listing page do not affect which reports are displayed in the comparison page.

## Display only reports of interest or metrics of interest

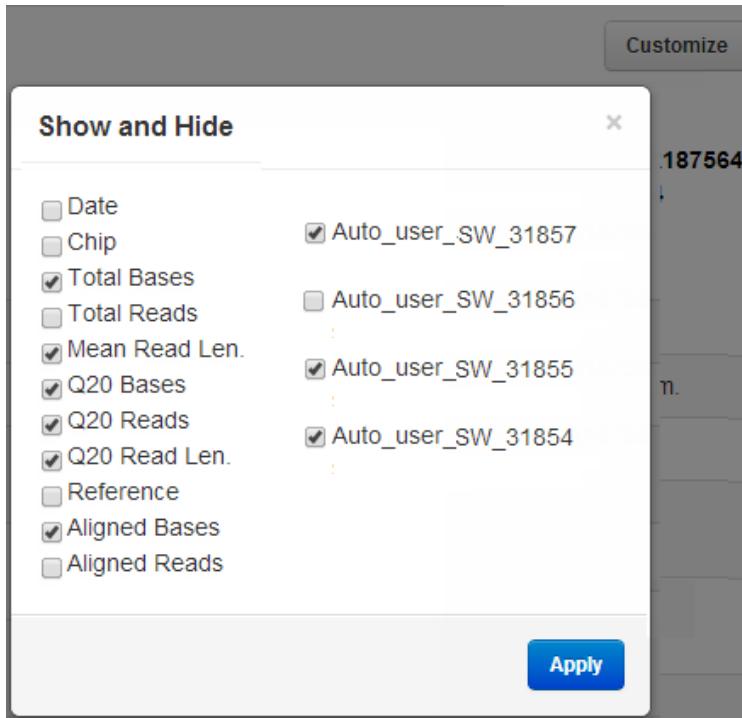
Follow these steps to filter the displayed reports:

1. In the Project Comparison page, click the **Customize** button in the top right corner:

Date	March 18, 5:56 a.m.	March 18, 4:44 a.m.	March 18, 5:55 a.m.	March 18, 8:53 a.m.
Chip	318C	318C	318C	318C
Total Bases	2,635,281,527 bp	2,448,529,886 bp	2,859,112,208 bp	2,639,265,659 bp

2. In the Show and Hide popup, in the left column, click the checkboxes for unwanted fields. Leave only the fields of interest enabled:

3. In the right column, click the checkboxes for unwanted reports. Leave only the reports of interest enabled:



4. Click **Apply**. The Project Comparison page changes to display on the fields of interest and the reports of interest:

**Project Comparison - staph5**

	Auto_user_SW_31857	Auto_user_SW_31855	Auto_user_SW_31854
Total Bases	2,635,281,527 bp	2,859,112,208 bp	2,639,265,659 bp
Mean Read Len.	382 bp	388 bp	360 bp
Q20 Bases	2,408,239,548 bp	2,612,632,061 bp	2,358,555,544 bp
Q20 Reads	6,897,588	7,354,828	7,323,134
Q20 Read Len.	342 bp	349 bp	316 bp
Aligned Bases	2,494,762,923 bp	2,705,744,762 bp	2,494,334,542 bp

**Customize**

**Download PDF** **Download CSV**

In this example, several metrics fields are not included in the display and the report Auto\_user\_SW\_31856 also is not displayed.

## Comparison of barcoded runs

In this release, when an analysis includes multiple barcodes, the comparison page shows one view of that analysis. This view is made of all barcodes in the analysis,

combined.

A separate comparison of individual barcodes currently is not supported.

## Download CVS or PDF output

Click [Download PDF](#) or [Download CSV](#) to download a PDF or comma-separated file of the comparison report.

---

# Torrent Browser Analysis Report Guide

## Classic Barcode Reports

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



The Barcode Reports section displays histograms for the following metrics per barcode:

Chart	Description
<b>Total number of reads</b>	Total number of filtered and trimmed reads independent of length. This number is reported in the barcode unmapped BAM file.
<b>AQ20 Bases</b>	Number of base pairs of sequence from reads with aligned quality score of AQ20 or better.
<b>Mean AQ20 read length</b>	An AQ20 read length is the length, in <i>bp</i> units, that after alignment has a Phred-like score of 20 or better, or one error in 100 bp. This histogram charts the mean of these AQ20 read lengths per barcode.
<b>AQ20 Reads</b>	The number of reads with an aligned quality score of AQ20 or better.



The number of barcodes shown in the reports 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 chart.

Each bar is labeled with the barcode ID. Data labeled as barcode ID X reports the number of unclassified barcodes: reads which could not be classified as matching one of the expected barcodes in the barcode set.

# Torrent Browser Analysis Report Guide

## Classic Test Fragment Report

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



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

The report has a heading for each Test Fragment, in the form **Test Fragment - <TestFragmentName>**.

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.



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

## Test Fragment Summary

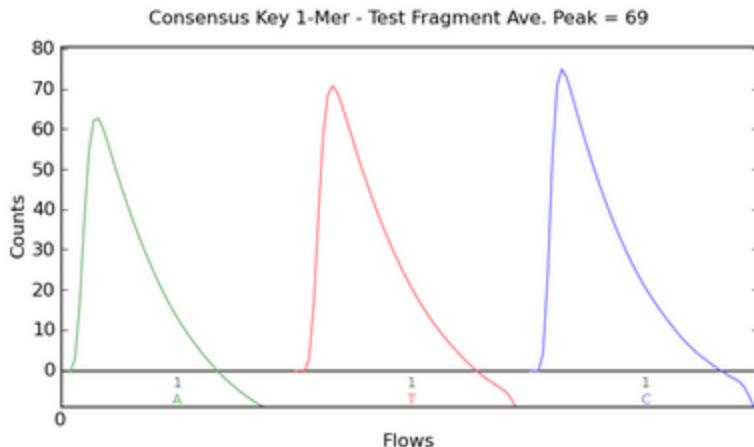
The **Test Fragment Summary** part of the **Test Fragment Report** displays the following information:

### Test Fragment List

Test Fragment	Percent (50AQ17 / Num)
TF_A	82%
TF_B	64%
TF_C	95%
TF_D	96%

### Consensus Key 1-Mer

The Consensus Key 1-Mer graph shows the strength of the signal from the first three one-mer bases of the Test Fragment key. This graph represents the consensus signal measurement of release of H<sup>+</sup> during nucleotide incorporation.



The y-axis shows signal strength, measured in Counts, which is an arbitrary but consistent unit of measure. The x-axis shows time as nucleotide Flows over the chip.

There is a known key at the beginning of every read. Typically, three bases are shown of the 4-mer key. Note that the graph is displayed in "flow order" rather than "base order." For example, the four-base Test Fragment key is typically ATCG for nucleotides one through four and is graphed as ATC, representing the nucleotide flow order. Negative flows are not displayed.

## Quality Metrics

The **Quality Metrics** part of the **Test Fragment Report** displays the following information:

TF Name	TF_A
TF Seq	TGTTTTAGGGTCCCCGGGTTAAAGGTTTCGAACCTAACAGCTGTCGG CAGCTCGCTACGATCTGAGACTGCCAGGCACACAGGGATAGG
Num	1,856
Avg Q17 read length	67
50AQ17	1,522

Parameter	Description
TF Name	Test Fragment name, as defined in the <b>Templates</b> tab of Torrent Browser.
TF Seq	Test Fragment sequence.
Num	Number of filtered & trimmed reads identified for this Test Fragment.
Avg Q17 read length	Average read length with Q17, or better, for this Test Fragment.
50AQ17	Number 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.

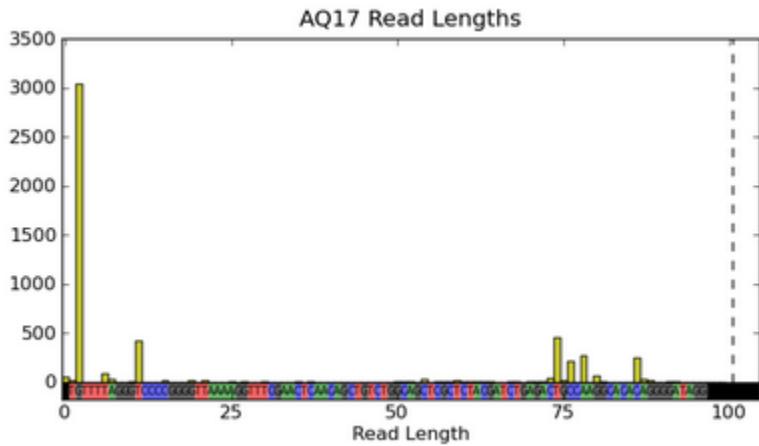
## Graphs

The **Graphs** part of the **Test Fragment Report** displays the following information:

### AQ17 Read Lengths

The **AQ17 Read Lengths** graph 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.

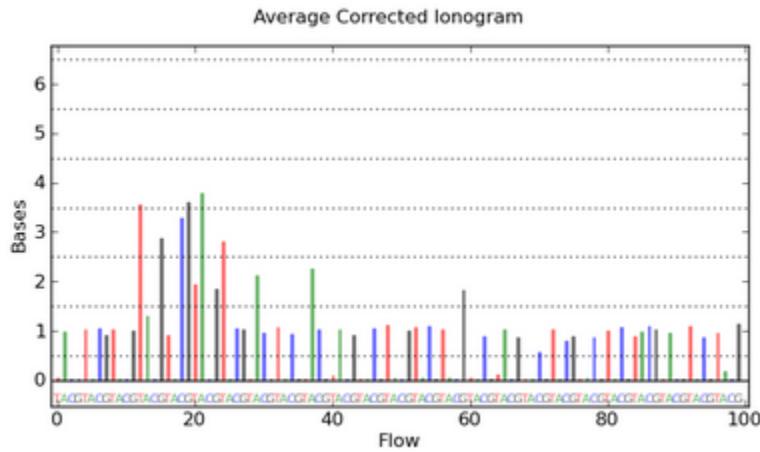
Distributions skewed to the right are ideal, showing longer read lengths (remembering that Test Fragments are a discrete length). It is likely that the sequence can extend all the way through the Test Fragment, if enough flows are run, so the histogram only displays a maximum size based on the length of the Test Fragment.



#### Average Corrected Ionogram

In the **Average Corrected Ionogram** graph, the x-axis is in flow space and the y-axis shows the signal intensity.

A unit of one indicates that there is only one base. A unit of two indicates that there are two bases of that specific nucleotide incorporated during the single flow event.

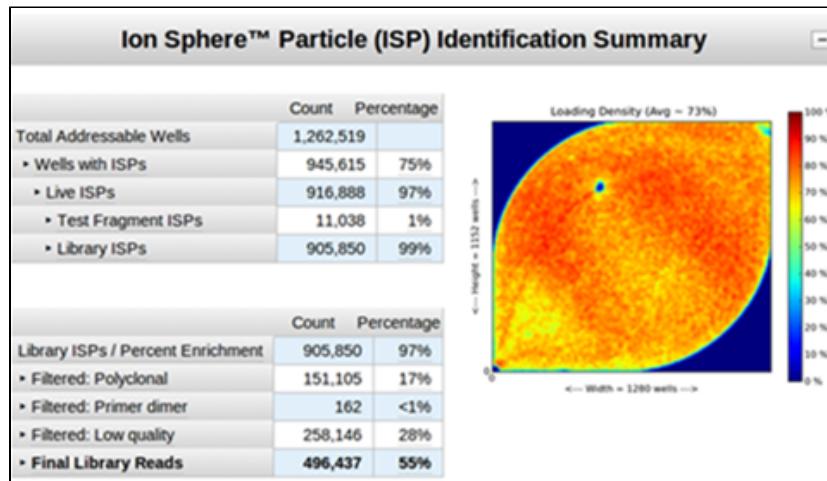


# Classic Ion Sphere™ Particle (ISP) Summary

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



The **Ion Sphere™ Particle (ISP) Identification Summary** section of the Analysis Report gives summary statistics of Ion Sphere™ Particle performance:



This section of the report is available before the **Library Summary** or **Test Fragment Summary** sections, providing a quick determination of whether or not the analysis should be permitted to continue.

The report displays:

## Well Information



The data generated in this section are created "early" in the analysis process, before base calling, and are intended to be a coarse, initial assessment of run performance. The well data are subject to more stringent filtering in later stages of the analysis.

	Count	Percentage
Total Addressable Wells	1,262,519	
• Wells with ISPs	945,615	75%
• Live ISPs	916,888	97%
• Test Fragment ISPs	11,038	1%
• Library ISPs	905,850	99%

Parameter	Description	Percentage

<b>Total Addressable Wells</b>	Total number of addressable wells.	Not calculated
<b>Wells with ISPs</b>	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
<b>Live ISPs</b>	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: <ul style="list-style-type: none"> <li>• Test Fragment</li> <li>• Library</li> </ul>	Live ISPs / Wells with ISPs
<b>Test Fragment ISPs</b>	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
<b>Library ISPs</b>	Number (and percentage of Live ISPs) of Live ISPs that have a key signal identical to the library key signal. These reads are input into the Library filtering process.	Library ISPs / Live ISPs

## Library ISP Details

The Library ISP Details table is available after basecalling and read filtering are complete. This table provides information on a collection of read filters, which are applied after basecalling to ensure only high-quality reads are written to the final results.

The Polyclonal filter removes ISPs carrying clones from two or more templates.

The Primer dimer filter removes reads carrying an insert of fewer than 8 bp.

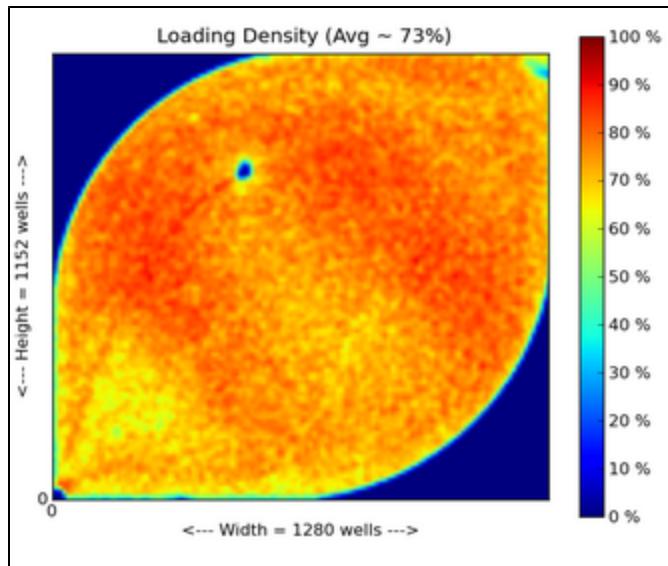
The Low quality filter removes reads that fail to attain a high level of accuracy.

	Count	Percentage
<b>Library ISPs / Percent Enrichment</b>	905,850	97%
▶ Filtered: Polyclonal	151,105	17%
▶ Filtered: Primer dimer	162	<1%
▶ Filtered: Low quality	258,146	28%
<b>Final Library Reads</b>	<b>496,437</b>	<b>55%</b>

Parameter	Description	Percentage
<b>Library ISPs/Percent Enrichment</b>	<p>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).</p> <p>The <b>Percent Enrichment</b> value reported is the number of loaded ISPs that are Library ISPs, after taking out Test Fragment ISPs.</p>	Library ISPs / (No. of Loaded ISPs minus TF ISPs)
<b>Filtered: Polyclonal</b>	ISPs carrying clones from two or more templates.	Polyclonal ISPs / Library ISPs
<b>Filtered: Primer dimer</b>	Insert length of less than 8 bp.	Primer dimer ISPs / Library ISPs
<b>Filtered: Low quality</b>	Low or unrecognizable signal.	Low quality ISPs / Library ISPs
<b>Final Library Reads</b>	<p>Number (and percentage of Library ISPs) of reads passing all filters, which are recorded in the unmapped BAM file.</p> <p>This value may be different from the <b>Total number of reads</b> located in the Library Summary Section due to technicalities associated with read trimming beyond a minimal requirement resulting in <b>Total number of reads</b> being slightly less than <b>Final Library Reads</b>.</p>	Final Library / Library ISPs

## Chip Loading Image

The **Ion Sphere™ Particle Identification Summary** section includes a Chip Loading Image, similar to the image shown in the following figure:



This is a pseudo-color image of the Ion CHIP™ wells showing percent loading across the physical surface.

The **Loading Density** percentage is displayed above the image, with the percentage value that considers only potentially addressable wells.

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## Classic Report Information

# Torrent Browser Analysis Report Guide

## Classic Report Information

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



### Analysis Info

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

#### Report Information

##### Analysis Info

Run Name	R_2012_01_04_12_34_00_user_COL-3
Run Date	2012-01-04 12:34:00
Analysis Name	Auto_COL-3_29
Analysis Date	2012-01-04
Analysis Cycles	16
Analysis Flows	520
Project	project.colossusnewpgm
Sample	sample.colssus
Library	e_coli_dh10b
PGM	COLOSSUS
Chip Check	Passed
Chip Type	316D
Chip Data	single
Notes	
Barcode Set	
Flow Order	TACGTACGTCTGAGCATCGATCGATGTACAGC
Library Key	TCAG

Parameter	Description
Run Name	Name of the sequencing run entered. This value is typically entered on the Ion S5™, Ion PGM™, or Ion Proton™ sequencer.
Run Date	Date and time the PGM™ or Proton™ run was started.

<b>Analysis Name</b>	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.
<b>Analysis Date</b>	Date the analysis was performed.
<b>Analysis Cycles</b>	Number of cycles analyzed for this report. Note that this number can differ from the total number of cycles run on the sequencer.
<b>Analysis Flows</b>	Number of nucleotide flows analyzed for this report. Note that this number can differ from the total number of flows occurring on the sequencer.
<b>Project</b>	Name of the project assigned to the run. This is typically assigned on the Ion S5™, Ion PGM™, or Ion Proton™ sequencer.
<b>Sample</b>	Name of the sample assigned to the run used to generate this analysis. This is assigned on the Ion S5™, Ion PGM™, or Ion Proton™ sequencer.
<b>Library</b>	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.
<b>PGM</b>	Name of the Ion S5™, Ion PGM™ or Ion Proton™ sequencer where the run was performed.
<b>Chip Check</b>	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 <b>Passed</b> or <b>Failed</b> .
<b>Chip Type</b>	Type of chip used on the 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.
<b>Barcode Set</b>	The name of the barcode set assigned to the run. Blank for non-barcode libraries.
<b>Notes</b>	A space for text notes entered during the Ion S5™, Ion PGM™, or Ion Proton™ sequencer run.

<b>Flow Order</b>	<p>Flow order selected on PGM™ or Proton™ sequencer:</p> <p>Samba = TACGTACGTCTGAGCATCGATC GATGTACAGC [Default] Regular = TACG</p> <p>The "regular" flow order adds bases most rapidly to sequenced molecules but is vulnerable to phase errors. The Samba flow order consists of a 32-base sequence, repeated. This flow order resists phase errors by providing opportunities for out-of-phase molecules to catch up and is designed to sample all dimer (nucleotide pair) sequences, efficiently. Samba is the default flow order because it improves sequencing accuracy for longer reads by resisting phase errors.</p>
<b>Library Key</b>	A short known sequence of bases used to distinguish the library fragment from the Test Fragment. Example: "TCAG"

## Software Version

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



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.

## Software Version

Torrent_Suite	5.0.0
Datacollect	5.0.0
LiveView	5.0.0
Script	5.0.0
host	5.0.0
ion-alignment	5.0.0=0
ion-analysis	5.0.0=0
ion-dbreports	5.0.0=0
ion-gpu	5.0.0=0
ion-plugins	5.0.0=0
ion-torrentR	5.0.0=0
tmap	5.0.0=0

Parameter	Description

<b>Torrent Suite</b>	Version of Torrent Suite™ Software software used to generate the analysis.
<b>Datacollect</b>	Version of the Datacollect package.
<b>LiveView</b>	Version of the LiveView package.
<b>Script</b>	Version of the Script package.
<b>ion-alignment</b>	Version of the Torrent Suite™ Software alignment module used for this analysis.
<b>ion-analysis</b>	Version of the Analysis Pipeline used to generate the analysis.
<b>ion-dbreports</b>	Version of the ion-dbreports package.
<b>ion-gpu</b>	Version of the NVIDIA® Tesla® GPU driver.
<b>ion-plugins</b>	Version of the pre-installed plugins.
<b>ion-torrentR</b>	Version of the TorrentR stats package.
<b>tmap</b>	Version of the TMAP alignment package.

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# Classic File Links

# Torrent Browser Analysis Report Guide

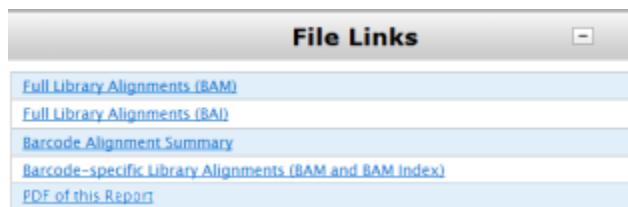
## Classic File Links

This page describes the old style run reports used before release 3.0. The old style run reports use an older layout and do not offer 4.x features. The Classic run report is viewed with the **Classic Report** button (near the top right of a run report page):



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.

Right-click the wanted file type and follow the **Save link as** dialog 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 links only appear for runs on barcoded data.



File Type	Description
<b>Library Alignments (BAM)</b>	Binary Sequence Alignment/Map (BAM), is a compressed, binary form of the SAM file. BAM files can be indexed, using the BAM Index file, for quick access to sequence alignment data. See <a href="http://samtools.sourceforge.net">http://samtools.sourceforge.net</a> for more a more detailed description of the SAM/BAM file format. Many tools are available for working with SAM files.  See <a href="#">Classic Library Summary Overview</a> for a description of the alignment data included in the BAM file. The reads in the file are sorted by reference location.
<b>Library Alignments (BAM Index)</b>	Binary Sequence Alignment/Map Index (BAI) -formatted file. A BAM index file speeds up the access time for a coordinate-sorted BAM file, enabling software to more quickly access random parts of the genomic information in a BAM file. Each BAM index file is associated with a BAM file so be careful not to confuse them. They share the same file name but the BAM index file has a .bai extension. To access information in a BAM file, a BAM index file is not required but does improve time-to-access.
<b>Barcode Alignments Summary</b>	A summary file of alignment metrics for barcodes. The metrics include the quality and read lengths at which each barcode aligns to reference. This link appears on barcode runs only.

<b>Barcode-specific Library Alignments (BAM and BAM Index)</b>	The same format and purpose as the Library Alignments (BAM) and Library Alignments (BAM Index) files, with content for specific barcodes. The BAM and BAM Index files for each barcode are zipped together. This link appears on barcode runs only.
<b>PDF of this Report</b>	Complete detailed <b>Analysis Report</b> in PDF format.

 The SFF and FASTQ files formats are deprecated. See [Technical Note - Transition from SFF to BAM format](#) and [FileExporter Plugin](#).

 The Customer Support Archive is available on the regular run report, not the Classic report. Next to the Plugin Summary area, click the **Support** tab. See [Customer Support Archive](#) for more information.

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## Pre-3.0 combineAlignments Plugin

# Torrent Browser Analysis Report Guide

## Pre-3.0 combineAlignments Plugin

The combineAlignment plugin combines reports from multiple runs that analyze the same tissue sample. The plugin is used, for example, when you run a tissue sample on more than one chip. The plugin combines the reports from those runs into one report. You run the plugin from any one of the related reports.



All runs must use the same reference.

Analyses using barcoded libraries are not supported in this release.

## Run the Plugin

You run the plugin from the Report tab run report of one of the runs to be combined:

Report	Status	Flows
<a href="#">Auto_B19-448--R145851-HSM-bl_15604</a>	Completed	260

That report appears in the selected reports section. Click search to find related reports:

Add	Report	Project	AQ17 Reads	TMAP #	Analysis Date
	B19-448	2012 val_chem2	230,970	0.3.7-1	May 10 2012

Selected 1 Reports      Total AQ17 Reads: 230970

Remove	Report	Project	AQ17 Reads	TMAP #	Analysis Date
Remove	<a href="#">B19-448</a>	2012 val_chem2	230,970	0.3.7-1	May 10 2012

Combined Alignments Name:

Related reports appear in the search section. Your original report has the Add button grayed out:

Add	Report	Project	AQ17 Reads	TMAP #	Analysis Date
<a href="#">Add</a>	B19-449	2012val_chem2	262,955	0.3.7-1	May 10 2012
<a href="#">Add</a>	B19-448	2012val_chem2	230,970	0.3.7-1	May 10 2012
<a href="#">Add</a>	B18-542	2012val_chem2	417,722	0.3.7-1	May 10 2012
<a href="#">Add</a>	B18-543	2012val_chem2	386,597	0.3.7-1	May 10 2012

Click **Add** for each run to be combined. Additional search results can be seen by clicking the **More** button on the lower right.

When you have all runs selected and appearing in the selected report section (the lower table), select a name for the combined alignments report, and click **Submit**.

## Plugin Notes

the plugin includes these notes in the submission page:

This plugin combines reads aligned to the specified reference from multiple run reports. The resulting combined alignment files may be downloaded from the plugin report page or used as input for other Torrent Browser plugins that support the use of these files, such as the Torrent Variant Caller.

Use the filters in the Report Locator to find reports then click the Add button to add them to the Selected Reports table. Initially the list of selected reports will contain the current report name. This report, or any selected report, may be removed from the list by clicking the Remove button. The alignment file from selected reports is combined by this plugin after clicking the Submit button. You may also modify the default name for the combined alignment files generated.

Note that the Report Locator will only include reports that are completed and does not support barcoded runs. Also, the Report Locator only lists reports for runs associated with the selected reference.

Unable to render {include}

The included page could not be found.

For support visit [thermofisher.com/techresources](http://thermofisher.com/techresources) or email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)  
lifetechnologies.com