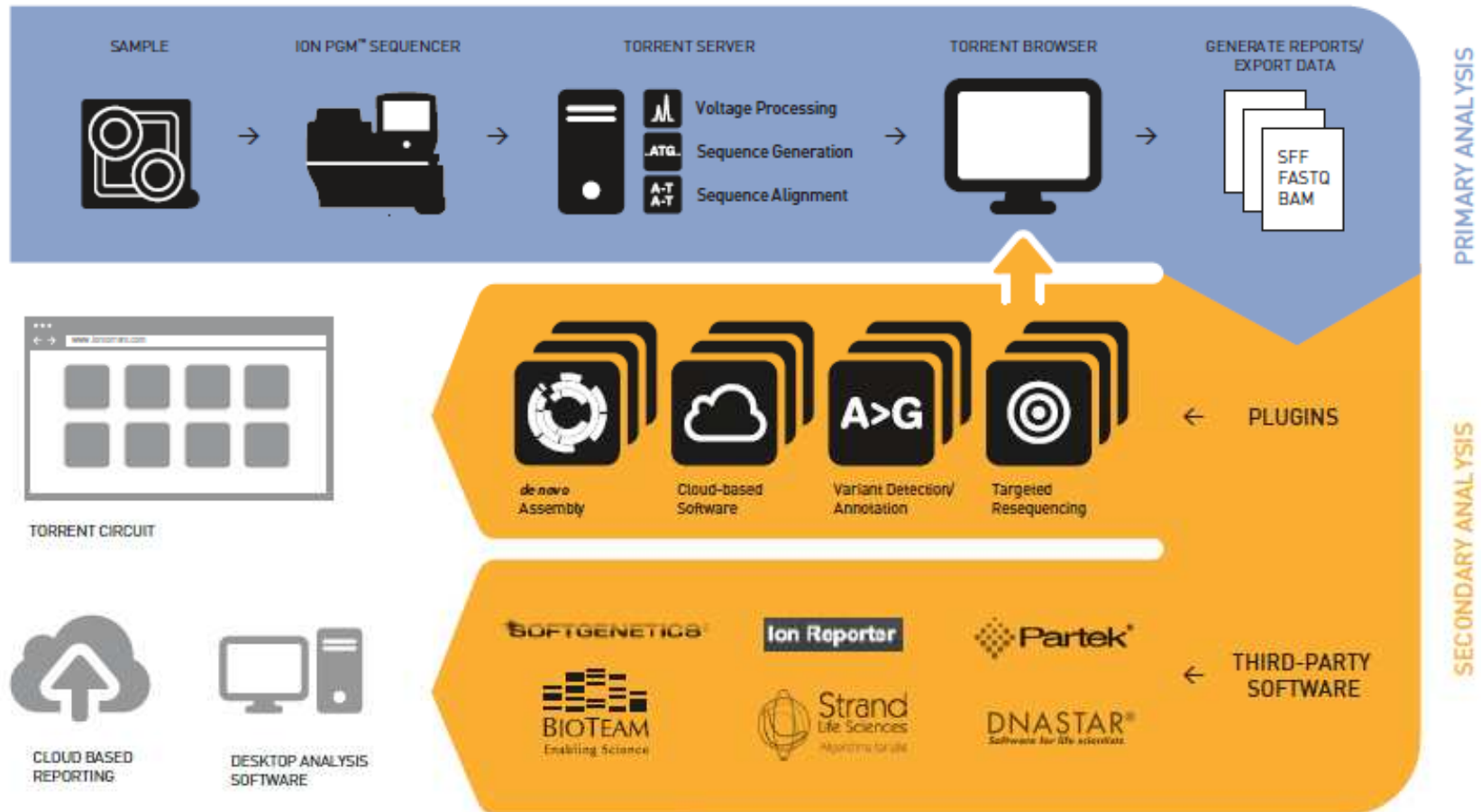**Thermo Fisher**
SCIENTIFIC

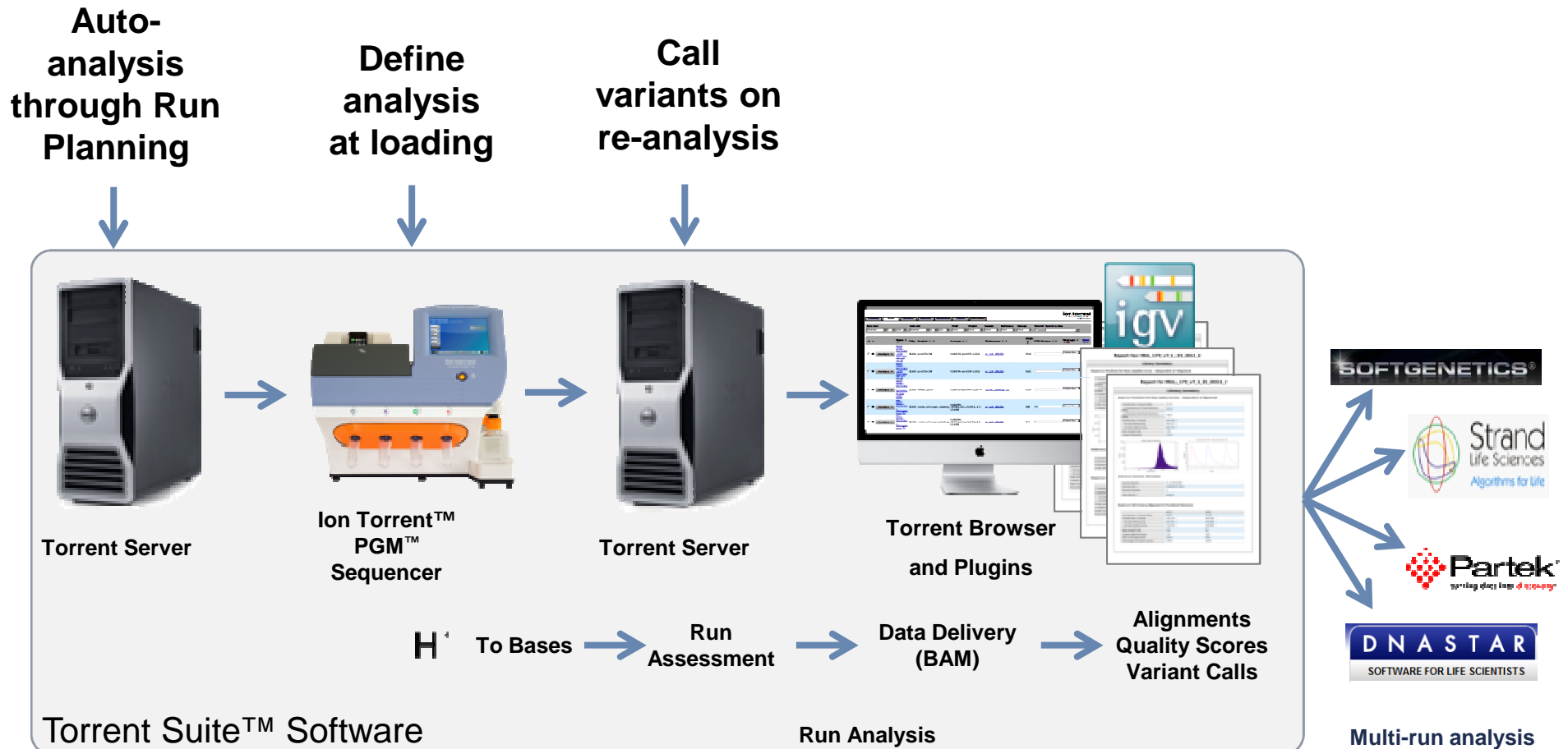# Ion University: Data Analysis

The world leader in serving science

Torrent Server and Torrent Suite™ Software

Web-based data delivery with integrated alignment, variant calling, and data analysis plugins

Thermo Fisher
S C I E N T I F I C

Example Workflow: Variant Calling

Auto-analysis through Run Planning

Define analysis at loading

Call variants on re-analysis

Torrent Server

Ion Torrent™ PGM™ Sequencer

Torrent Server

Torrent Browser and Plugins

H   To Bases   Run Assessment   Data Delivery (BAM)   Alignments Quality Scores Variant Calls

Torrent Suite™ Software

Run Analysis

Multi-run analysis

Thermo Fisher
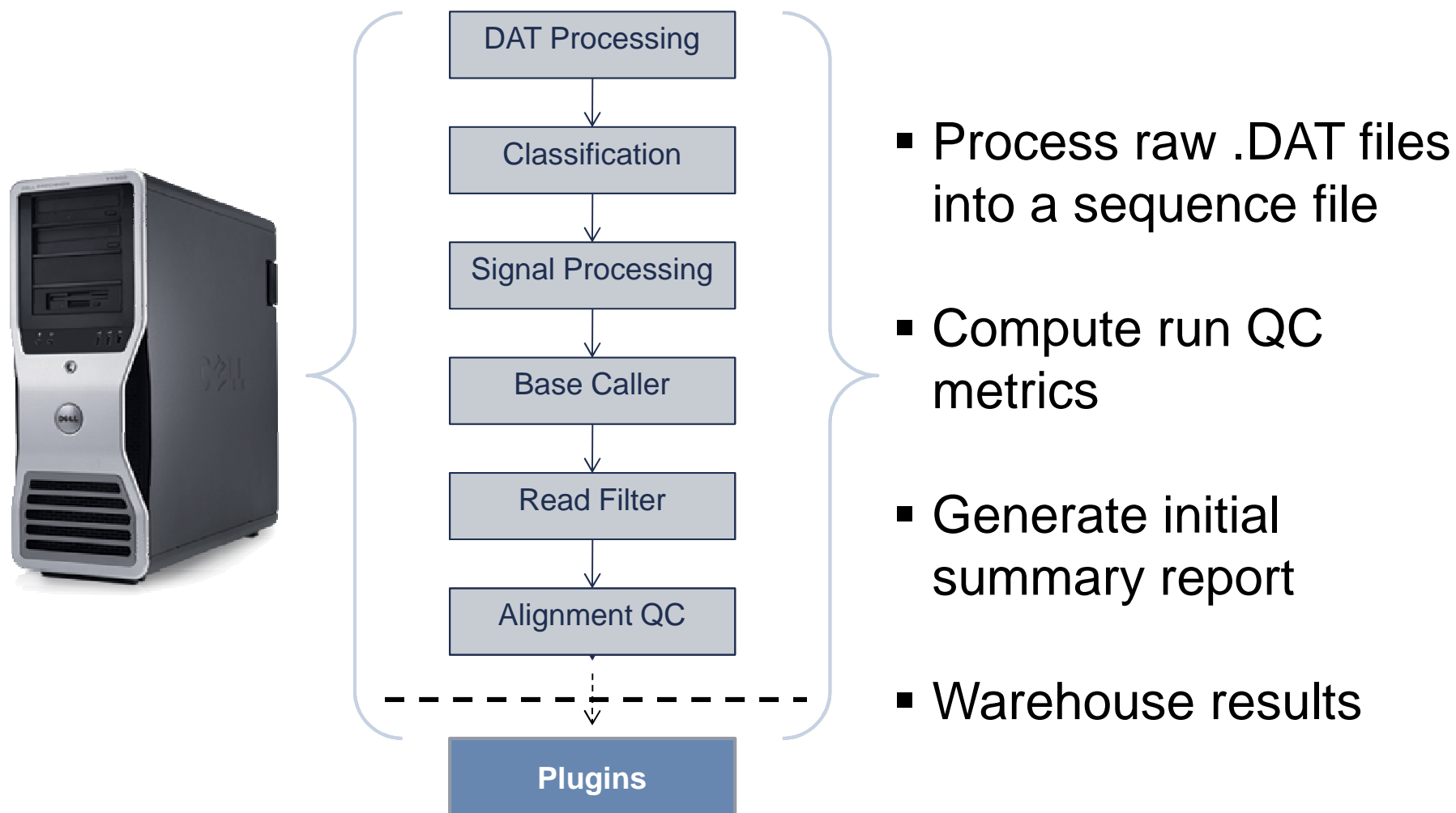S C I E N T I F I C

Preconfigured Torrent Server

- Preloaded with Torrent Suite™ Software

- Converts raw signal to base calls

- Performs preliminary alignment to a reference genome allowing run quality assessment

- Torrent Browser plugins

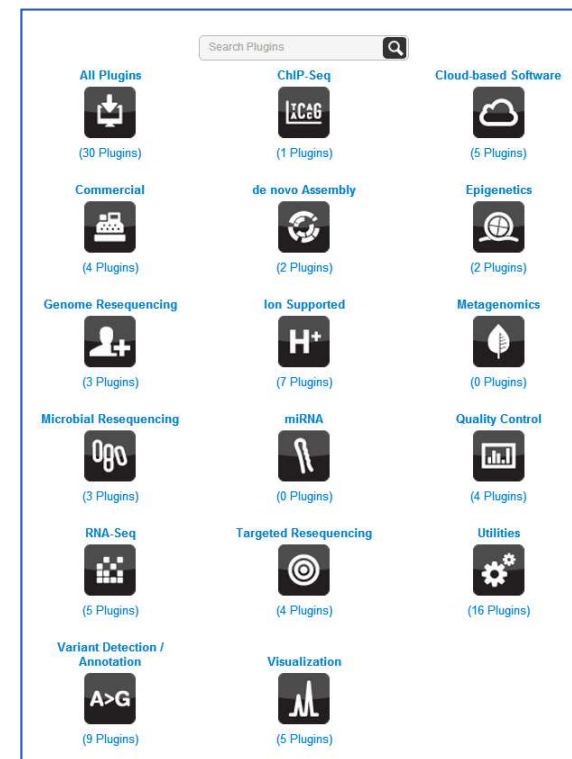- Hosts web portal for data review and distribution to collaborators

** Torrent Server is not designed to be a shared computing platform. It was engineered to be a *dedicated server* focused on the primary analysis of Ion Torrent™ data.

Thermo Fisher
S C I E N T I F I C

Torrent Server Analysis Pipeline

DAT Processing

↓

Classification

↓

Signal Processing

↓

Base Caller

↓

Read Filter

↓

Alignment QC

- - - - - - - - - - - - - - - -

Plugins

- Process raw .DAT files into a sequence file

- Compute run QC metrics

- Generate initial summary report

- Warehouse results

Torrent Browser Plugin Store & Torrent Suite™ Plugins

Your application. Your data. Your Plugin.

The App Store for Torrent Suite™ software

http://ioncommunity.lifetechnologies.com/community/products/torrent_browser_plugin_store

Thermo Fisher
SCIENTIFIC

Torrent Pipeline – Approximate Data Sizes*

Process Description	File Types	Ion 314™ chip	Ion 316™ chip	Ion 318™ chip	Ion PI™ chip
Raw Voltage Data	DAT	28 GB	129 GB	242 GB	2 TB
Signal Processing	WELLS	1 GB	7-9 GB	12-15 GB	80-100 GB
Base Calls - Aligned	BAM	0.1 GB	1.7-2.0 GB	2.7-3.0 GB	40-60 GB
Base Calls - Flow	SFF	1 GB/	4.5-6.0 GB/	8-10 GB/	100-120GB
Base Calls - Base	FASTQ	0.2 GB	1-1.25 GB	1.8-2.25 GB	20-30 GB

*v3.0 250 bp run (500 flows) Sept2012. The information presented here is an approximation.
User experiences may vary.

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How many runs can be stored?

Process	Equipment	Ion 314™ chip	Ion 316™ chip	Ion 318™ chip
Data Acquisition	PGM™ Sequencer	40 runs	8 runs	5 runs
Raw Data	Torrent Server	300 runs	65 runs	40 runs
Archived Data	Torrent Server	4100 runs	880 runs	530 runs

File sizes for a typical run of 520 flows that enables 200 base pair read length.
The Torrent Server has 12 TB of space with n=2 redundancy.

The information presented here is an approximation. User experiences may vary.

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Analyze twice the data in half the time

TSS 3.4

Total runtime ~ 21 hours with TSS 3.4

Ion Proton™ Run (3.3 hrs)

(7G total throughput, 168 bp reads)

Data Processing (7.0 hrs) on Proton

Data Analysis (18 hrs) on Torrent Server

TSS 4.0

Total runtime ~ 8 hours with TSS 4.0

Ion Proton™ Run (2.4 hrs)

(14.5G total throughput, 200 bp reads)

Data Processing (6.4 hrs) on Proton

Data Analysis (5.6 hrs) on Torrent Server

440 flow TargetSeq exome run
Start: Ion Proton™ instrument run starts
End: Availability of complete sequence and mapping data

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Torrent Browser
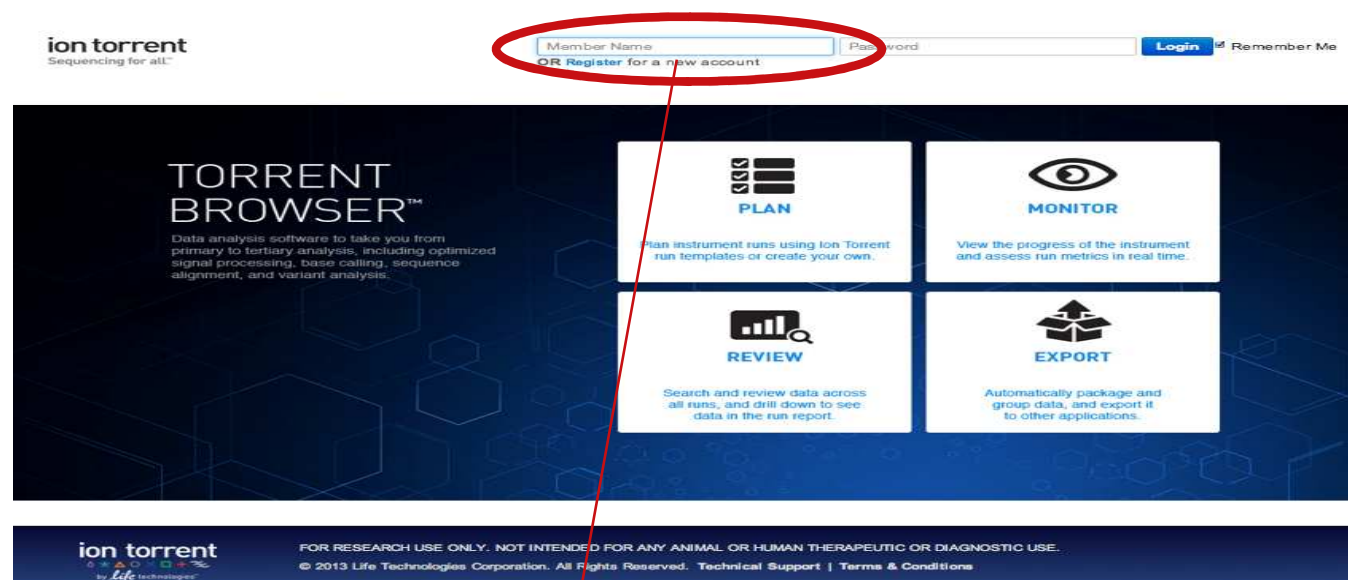
- Plan run

- Monitor run

- Access data

- Export data

Support for all Ion instruments

Planning a Run by Sample or Template

Planning a Run by Sample

Planning a Template Run

Create new run templates and plan instrument runs. You can use Ion Torrent templates or create new ones that match your own protocols.

Whole-Genome Seq | Add New Template | Plan New Run

RNA Seq | Add New Template | Plan New Run

Ampliseq DNA | AmpliSeq.com Import | Add New Template | Plan New Run

AmpliSeq Exome | AmpliSeq.com Import | Add New Template | Plan New Run

AmpliSeq RNA | AmpliSeq.com Import | Add New Template | Plan New Run

TargetSeq | Add New Template | Plan New Run

16S Target Sequencing | Add New Template | Plan New Run

Generic Sequencing (Other) | Add New Template | Plan New Run
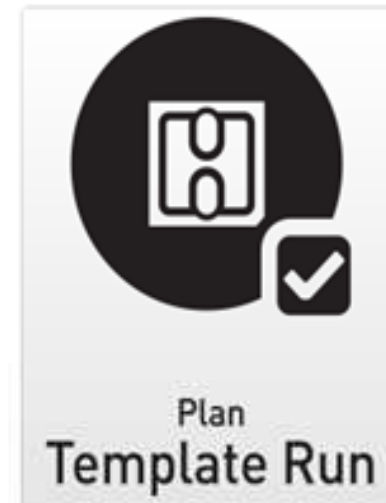
Plan
Template Run

Set up a run
- Use pre-installed template (application/product-based) or Create your own
- Start run on PGM™ sequencer using run code of planned run

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Plan Tab

Sample or Template Run Planning

- Reusable experimental designs,

  - Run Parameters

  - Ion Reporter (Integrate IR workflow & sample grouping selection)

  - Application (Whole genome, Ion Ampliseq™, Targeted Seq, RNA Seq, Generic sequencing)

  - Kits (Ion kit, Barcode kit…)

  - Monitor thresholds (Bead Loading, Usable Sequence, Key Signal)

  - Reference (Fasta and BED files)

  - Plugins

  - Output

  - Plan

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Monitoring a Run
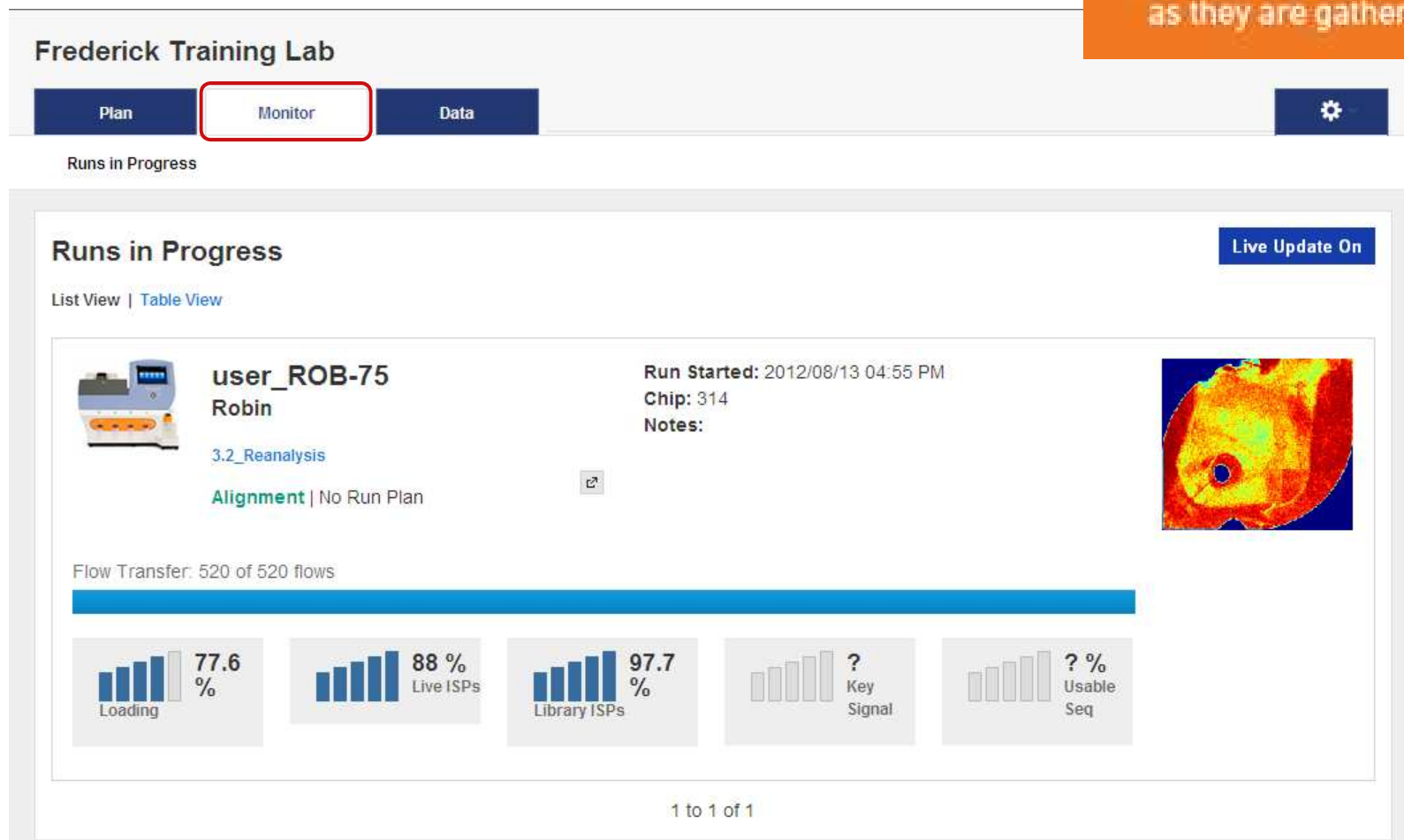
Track your run in progress

Reviewing Data - Run Reports

Search and review across all of your runs and drill down to see your data in the run report. View your data grouped into projects.

Filter Data

List of runs

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Run Report – Summary of Unaligned reads

New Median and Mode metrics added
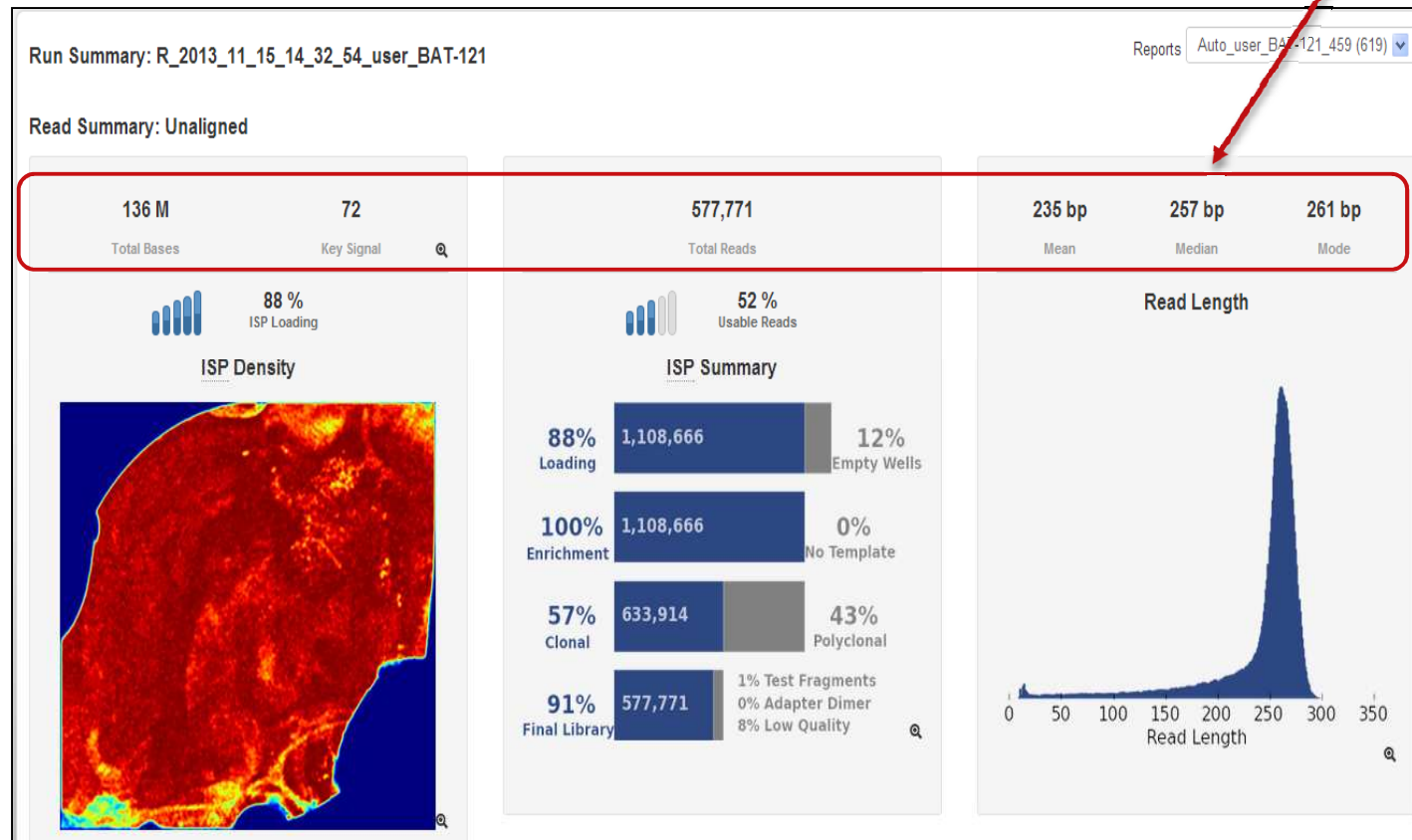
Click for details of each section

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Run Report – Chip Loading & Key Signal
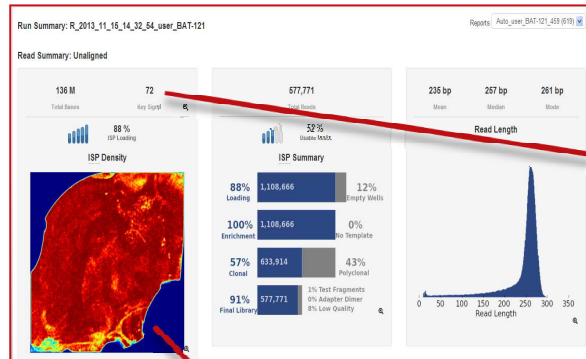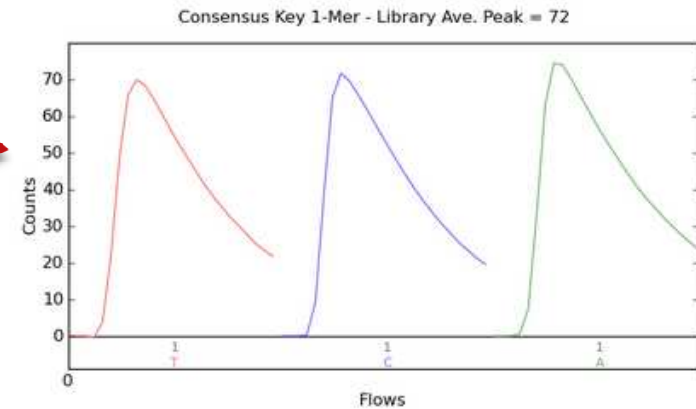
Consensus Key 1-mer Graph
Signal strength of the first three bases of the key
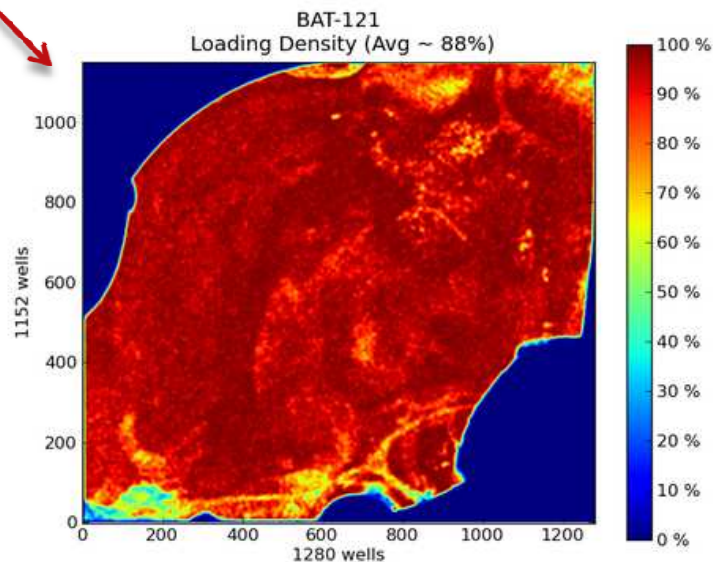
Visualization of loading density

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Run Report – Chip Well Details
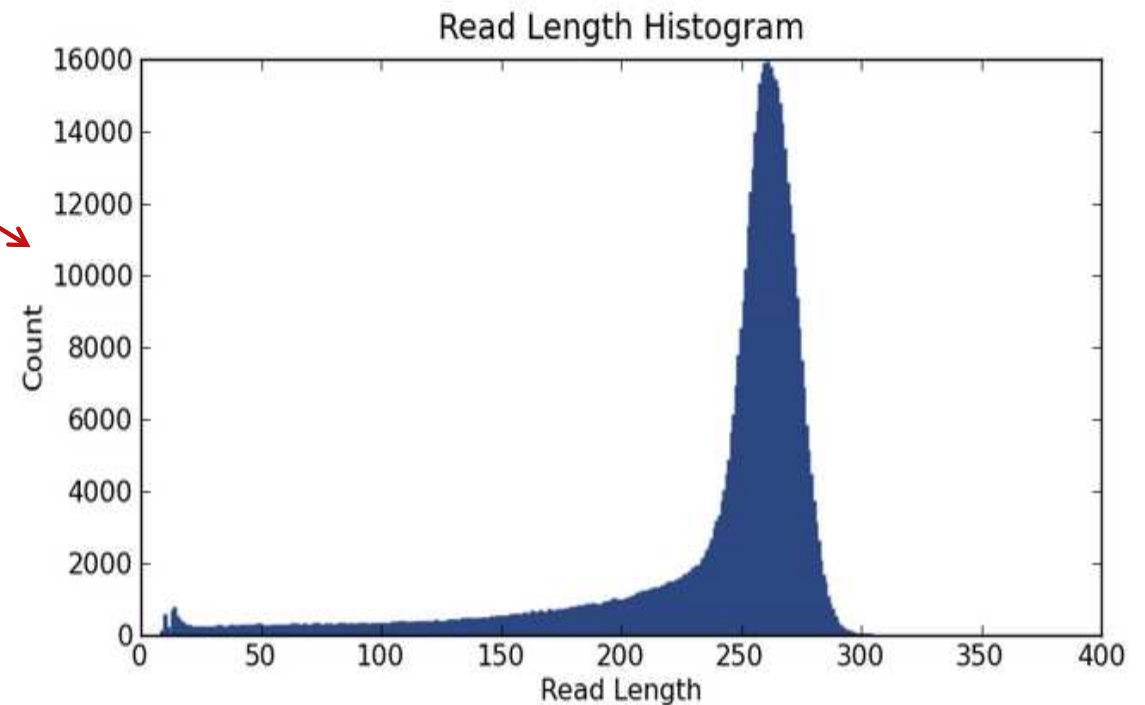

- Measure loading performance and enrichment efficiency
- Quantify read filter metrics: assess polyclonality and low quality reads

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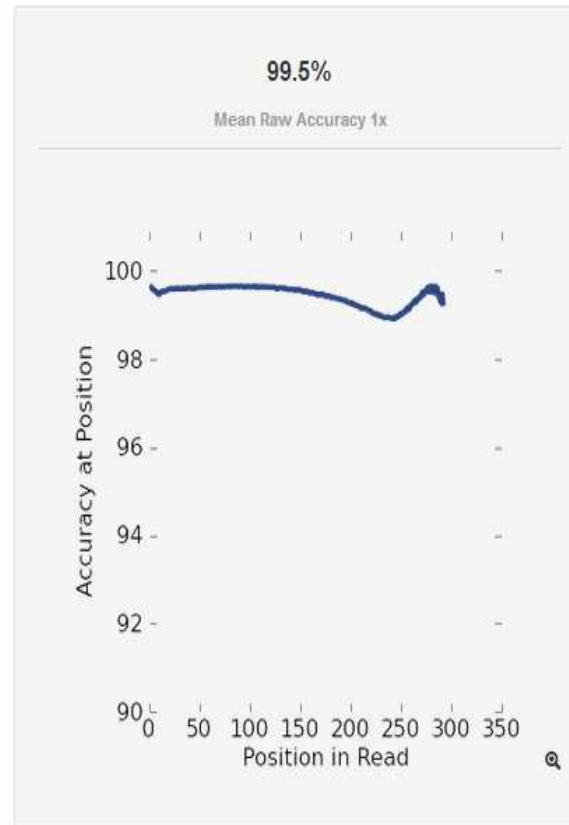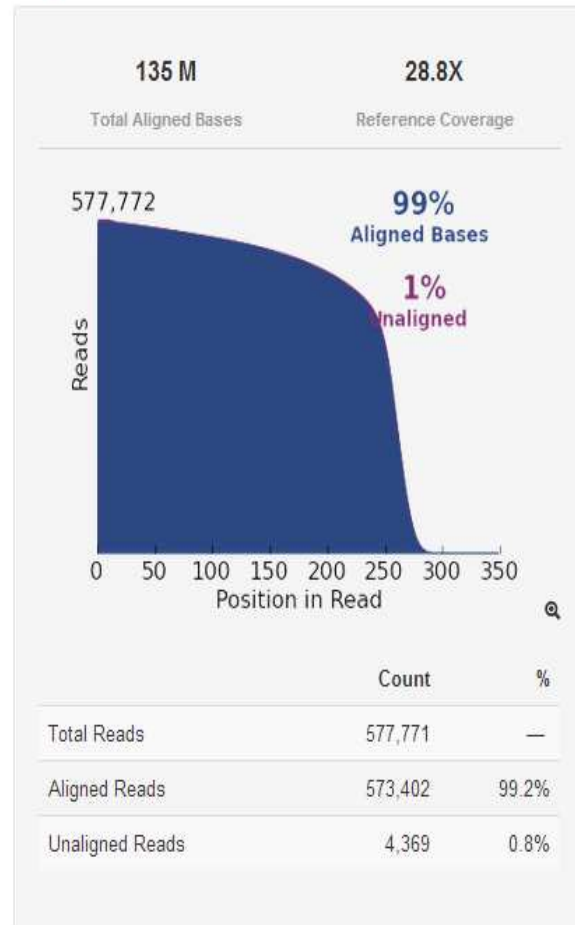# Run Report – Read Length Details
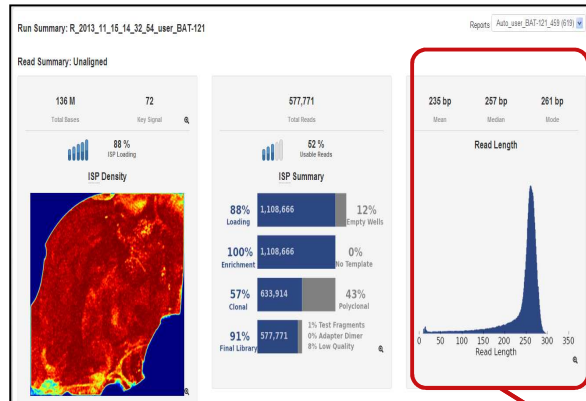
Histogram of read length for all reads in output files

Run Report – Reads Aligned to Reference

Aligned to E. coli DH10B

135 M	**28.8X**
Total Aligned Bases	Reference Coverage

577,772

99%
Aligned Bases

1%
Unaligned

Reads vs. Position in Read

	Count	%
Total Reads	577,771	—
Aligned Reads	573,402	99.2%
Unaligned Reads	4,369	0.8%

99.5%

Mean Raw Accuracy 1x

Accuracy at Position vs. Position in Read

131 M

AQ17 Total Bases

Alignment Quality

	AQ17	AQ20	Perfect
Total Number of Bases [bp]	131 M	126 M	103 M
Mean Length [bp]	237	231	195
Longest Alignment [bp]	325	322	320
Mean Coverage Depth [x]	28.1	27.0	22.1

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Run Report – Output Files

File format of older

File formats produced for extensive data analysis via third party software packages (offered on Webstore) or other bioinformatics tools.

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Plugins

Reviewing Data - Projects

Filtering – Which reads make it to alignment?

For a read to be represented, the following must be achieved:

- Well is "positive" for an Ion Sphere™ Particle (determined by the Ion Sphere™ Location Solution)
- Well produced strong signal across each of the first three key nucleotides
- Read contains a minimum of 8 bases
- The key of the read is an **exact** match to the defined library key
- Remove polyclonal reads with mixed read filter
- The read fits the "expected signal model"

http://ioncommunity.lifetechnologies.com/docs/DOC-7011 (p. 327)
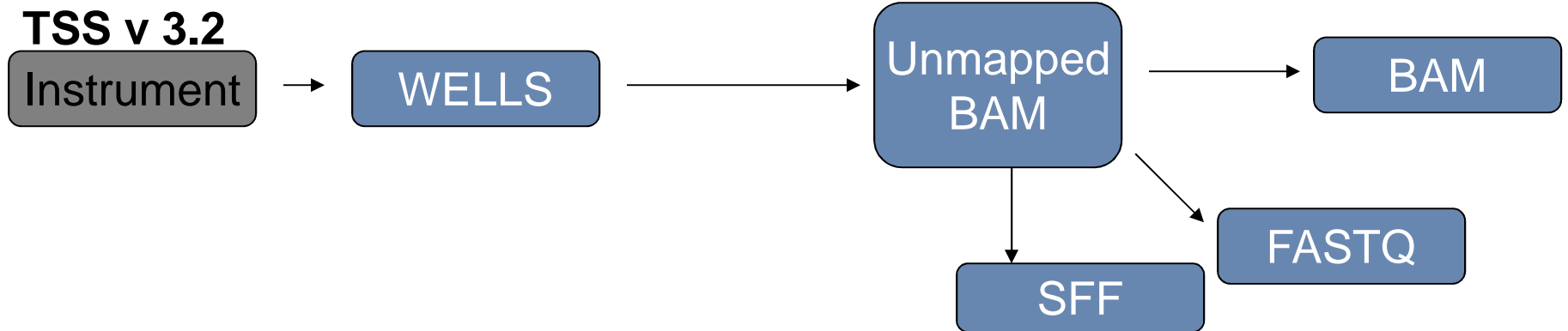
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Read Trimming

Each reported read is subject to removing lower quality bases according to the following rules:
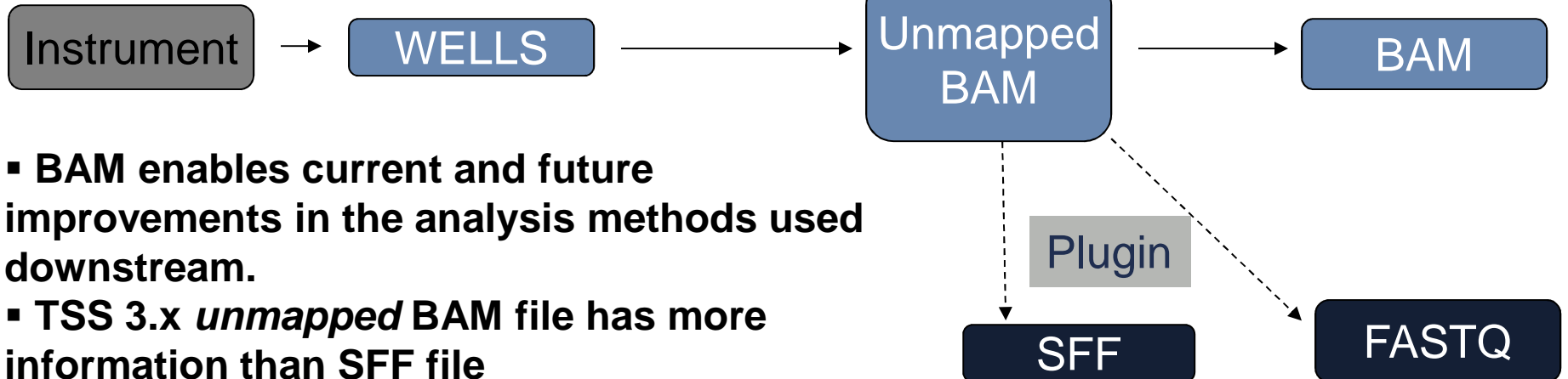

- First four bases of the 5' end that contain the key are removed.
- No quality trimming occurs at the 5' end.
- At the 3' end, if sequencing occurs through the insert to the P1 adapter, then the P1 adapter is removed.
- Remove lower quality base calls based on signal intensities
- The 3' end of the read is trimmed to the point where the average quality score is at least Q15 across a moving 30 bp window

http://ioncommunity.lifetechnologies.com/docs/DOC-7011 (p. 326)

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Transition from SFF to BAM
for Torrent Suite™ Software 3.4 and later

TSS v 3.2

Instrument → WELLS → Unmapped BAM → BAM

Unmapped BAM → SFF

Unmapped BAM → FASTQ

TSS v 3.4 and later

Instrument → WELLS → Unmapped BAM → BAM

- **BAM enables current and future improvements in the analysis methods used downstream.**
- **TSS 3.x *unmapped* BAM file has more information than SFF file**
- **TSS 3.x *unmapped* BAM file typically less than half the size of the corresponding SFF file.**

Plugin

Unmapped BAM → SFF

Unmapped BAM → FASTQ

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SFFCreator and FASTQCreator Plugins

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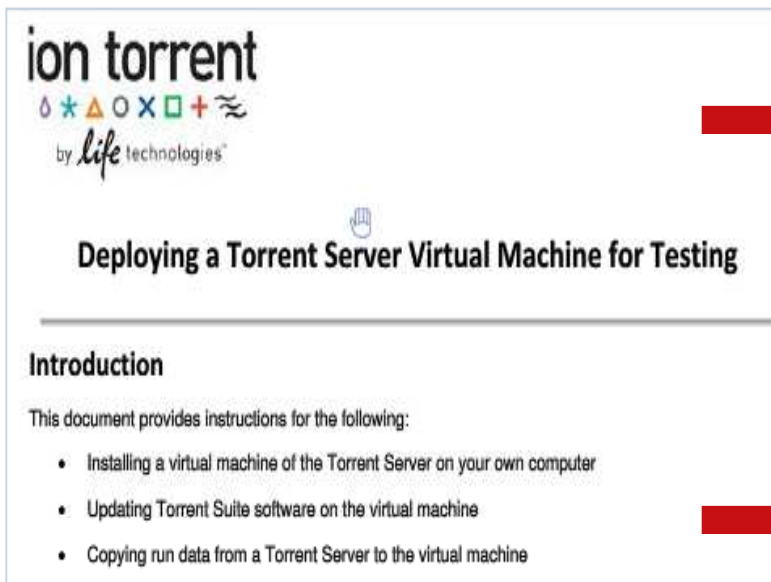
Testing Software Updates

ion torrent
by *life* technologies

Deploying a Torrent Server Virtual Machine for Testing

Introduction

This document provides instructions for the following:

- Installing a virtual machine of the Torrent Server on your own computer
- Updating Torrent Suite software on the virtual machine
- Copying run data from a Torrent Server to the virtual machine

Try Torrent Browser on the cloud

We recommend reviewing this new software on the cloud prior to download

1. Please watch this video, which shows how to use the cloud instance, **first**.
   The link is here How To Set Up A Torrent Suite Cloud Account
2. If you are running version TB 2.2, or earlier, on your local server please also watch this video showing how to modify your server to run the cloud plugin
   Setting Up Your Torrent Server for the Torrent Suite Cloud Plugin
3. Link for cloud registration is here https://torrentsuite.lifetechnologies.com/
4. Link for Plugin Store is here Torrent Browser Plugin Store
5. For any technical issues use this e-mail address pod@penguincomputing.com

http://ioncommunity.lifetechnologies.com/docs/DOC-6825

Experience the new version using a virtual machine

Download the VM for 3.0 HERE
The Instructions are here Torrent Server Virtual Machine
Watch this Video showing how to set up a VM for complete instructions around this process

Watch the Torrent Browser 3.0 Videos (also applicable to Torrent Browser 3.2)

We have prepared several videos that review how the new Torrent Browser Software user interface works and we also have provided an HTML model which allows you to review the design of the new software; please review and let us know what you think:

Below are a set of videos to introduce you to the streamlined user interface of Torrent Browser 3.0
Planning a Run in Torrent Browser 3.0
Monitoring in Torrent Browser 3.0
Data in Torrent Browser 3.0

You can review the upcoming version of Torrent Browser Software that was shown in the videos above using the clickable HTML model (recommended browsers Firefox or Chrome).
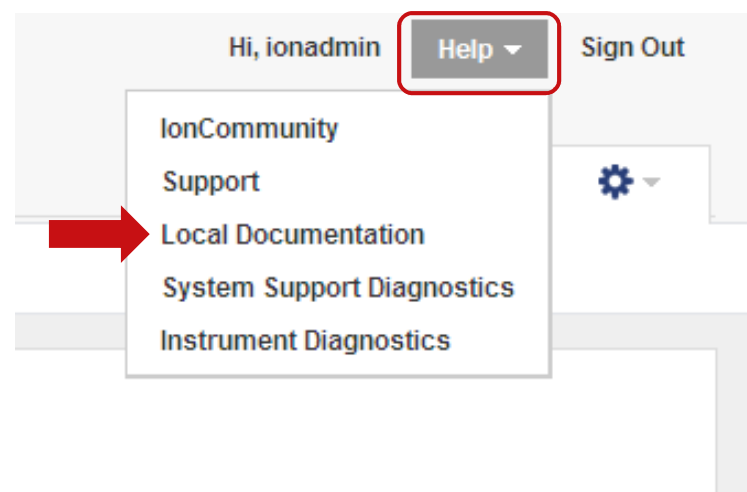
Torrent Server Virtual Machine

Safe environment for testing, i.e., plugins, software updates

http://ioncommunity.lifetechnologies.com/docs/DOC-1921

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Ion Torrent™ Software Support Policies

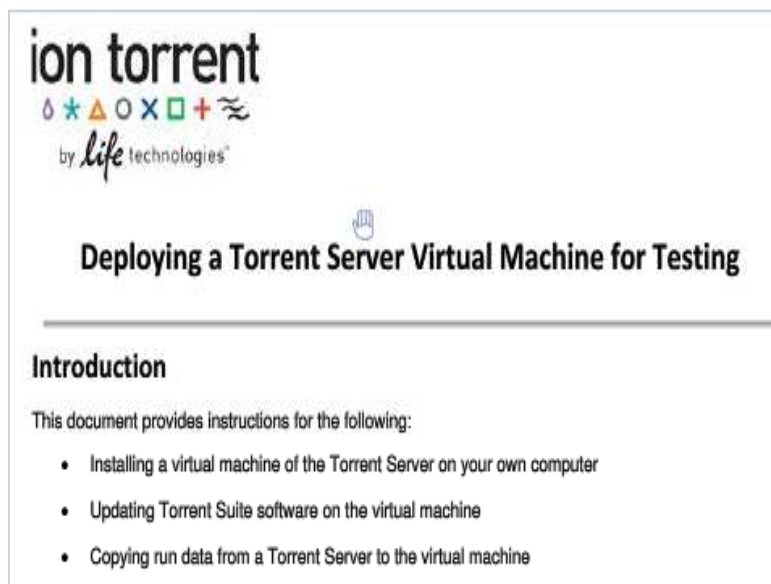**ThermoFisher**
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Available Help Documents

- **Torrent Suite™ Software User Documentation**
  - Torrent Browser User Interface Guide (aka tab guide)
  - Torrent Browser Analysis Report Guide
- **Torrent Suite ™ Software Administrator Documentation**
  - Developing Your System
  - Maintaining Your System
  - Administration
  - Troubleshooting
- **Release Notes**

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Available Resources

Torrent Server Virtual Machine

Safe environment for testing,

i.e., plugins, software updates

http://ioncommunity.lifetechnologies.com/docs/DOC-1921

Example data sets

Ion Community > Datasets >

Datasets by Chip

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Included Torrent Server / Torrent Suite™ Software Support

Torrent Suite™ Software is open source software

- Ion Torrent Field Bioinformatics Specialists will support Torrent Suite™ software, but *not* source code
- Source code is available on Ion Community, Torrent Dev section
- If working on source code, must install on platform other than Torrent Server

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Ion Trainings

http://ioncommunity.lifetechnologies.com/community/programs_and_events/training

Bioinformatics Training and Services

lifetechnologies.com/biofxservices

Item	Bioinformatics data analysis	Time (hrs)
1	Targeted Resequencing	3
2	Amplicon Sequencing	3
3	Exome Sequencing	3
4	Whole Genome*	3
5	AmpliSeq	3

Item	IT professional services	Time (hrs)
18	IT Consulting Services	1
19	Remote Software Updates for Torrent Server	1

20 hours to use—however you choose

Ordering information
SKU: ZGPCSCIONBFX

Item	Bioinformatics training	Time (hrs)
6	Ion Data Analysis Essentials (Life Technologies site)	14
7	Intro to Torrent Suite™ Software	3
8	Ion Variant Calling Data Analysis***	3
9	Ion AmpliSeq™ DNA Data Analysis	3
10	Ion AmpliSeq™ RNA Data Analysis	3
11	Ion Reporter™ Software Workflow	3
12	Variant Calling Analysis & Interpretation	2
13	RNA-Seq Data Analysis**	2
14	smRNA Data Analysis**	1
15	De novo Assembly*	1
16	Targeted Sequencing Introduction / Overview**	1
17	Linux Basics	3

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Introduction to Torrent Suite™ Software:
An Interactive Web Course

- 3 hour interactive, online session
- Instructor led
- Practice with data sets

Who Should Attend?

- Ion PGM™ or Proton™ Users with no previous experience with Torrent Suite™ Software and are interested in a data and software-focused class with hands-on experience with Torrent Suite™ Software

- New Ion Users who have taken the Ion PGM™ or Proton™ Systems Course and would like a refresher and the opportunity to for additional hands-on experience with the Torrent Suite Software

Prerequisites:

Ownership of a Ion PGM™ or Proton™ System or affiliation with a laboratory that has purchased a system. Browser Requirements for interactivity: Firefox or Google Chrome

Course Description:

Jump-start your Ion Torrent data analysis with this interactive web course which will introduce and provide hands-on training for the Torrent Suite™ Software. Each attendee will be given access to the Torrent Suite™ Software and data sets with exercises to guide them through hands-on scenarios.

Time Zone Conversion: Not located in the US/Pacific time zone? See what time it is in your local time zone.

Click here for current training promotions>>

Tell a colleague about this course. Copy the following URL:
https://learn.lifetechnologies.com/courses/view/id/303

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Torrent Server IT Service Contract

- Using Torrent Suite Software is as simple as using any web interface
- Keeping it updated and managing the data requires some Linux® software experience.
- If you don't have it, the BioTeam can help.
- Order it from the Web Store.

Product	Part #
3 month support contract	4465993
12 month support contract	4465994

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Bioinformatics Services

Service Product	Description	Part Number
Variant Caller Annotation	Data analysis for enhanced variant annotation	4473258
IT cluster install (new)	High Perf Cluster Install Svc	SV000212
Bioinformatics Consulting (new)	Remote or on-site service for data analysis and troubleshooting	SV000211
IT professional services	Consulting for software installation	SV000206
On-site software upgrades for Torrent Server	Service for Torrent Server software upgrades	SV000010

- Provided by Field Bioinformatics Specialists or Field Service Engineers
- Contact your sales representative

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Ion Community - Torrent Suite™ Software

http://ioncommunity.lifetechnologies.com/community/products/torrent_suite

- Sign up to receive automatic update notifications

- Monitor discussion threads

- Monitor new content

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

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