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Ion Torrent™ Technology

Run Validation and Introduction to the Torrent Browser

(Torrent Suite™ Software v4.0.2)

The world leader in serving science

Agenda

- Best places to find information
  - Ion Community
  - Torrent Browser
- Run report Analysis & validation
  - Live demo
  - Slides as reference

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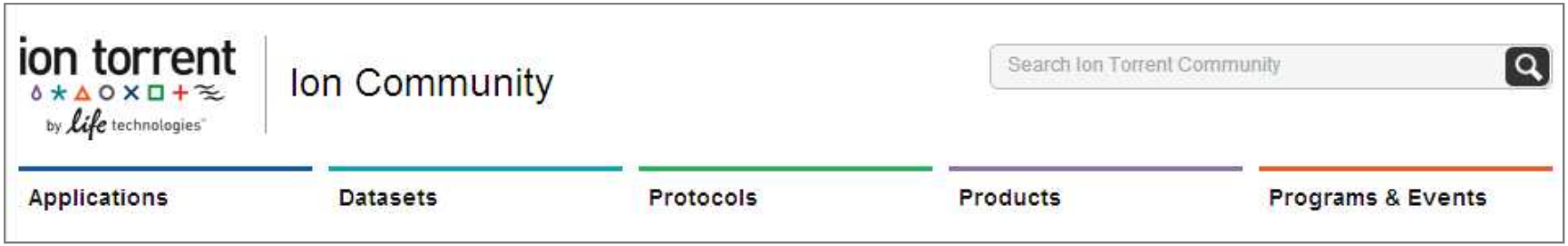
Ion Torrent™ Community



http://ioncommunity.lifetechnologies.com/welcome

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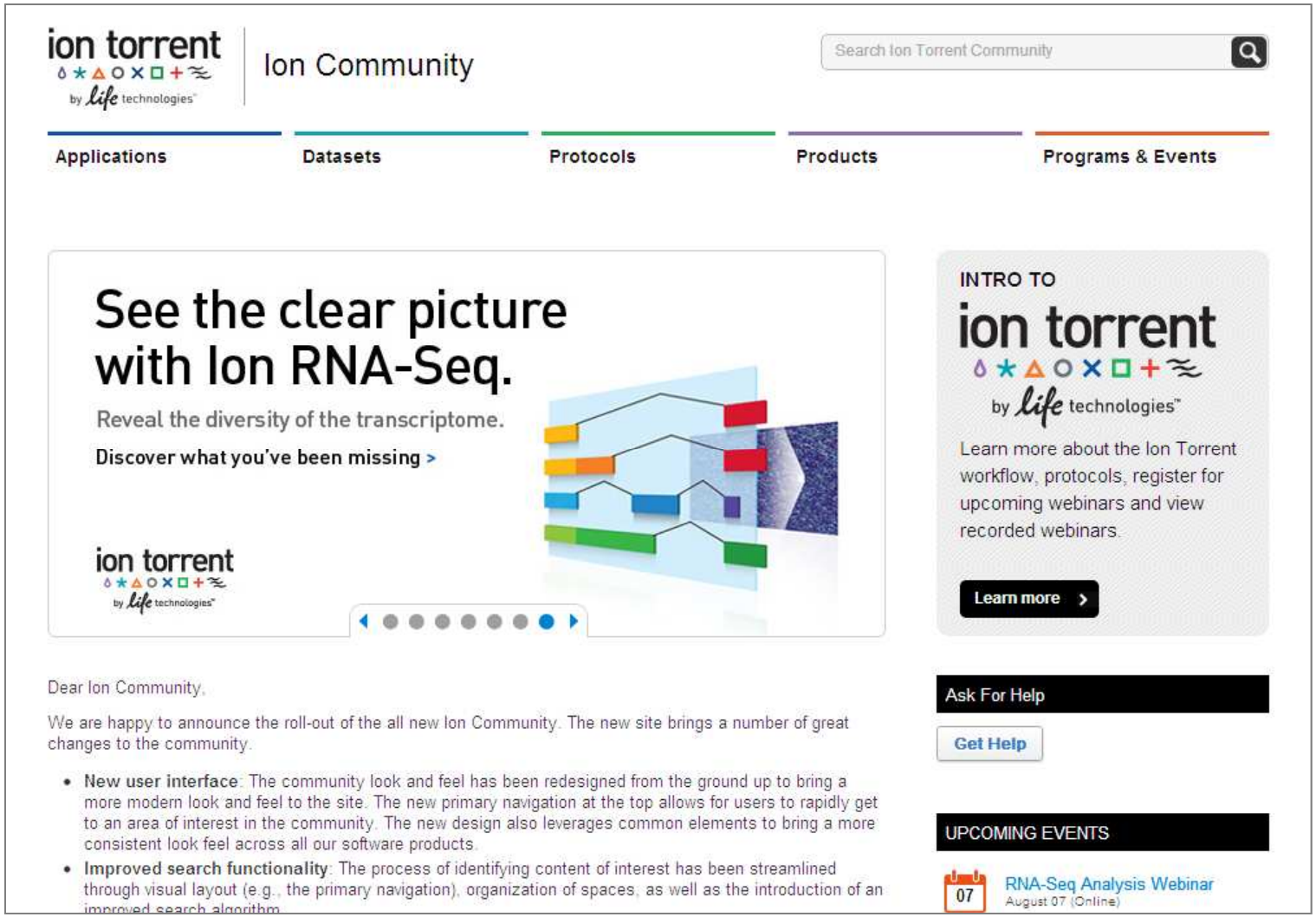
Ion Torrent™ Community



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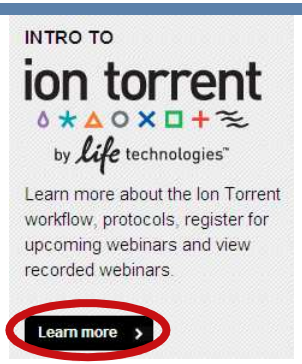
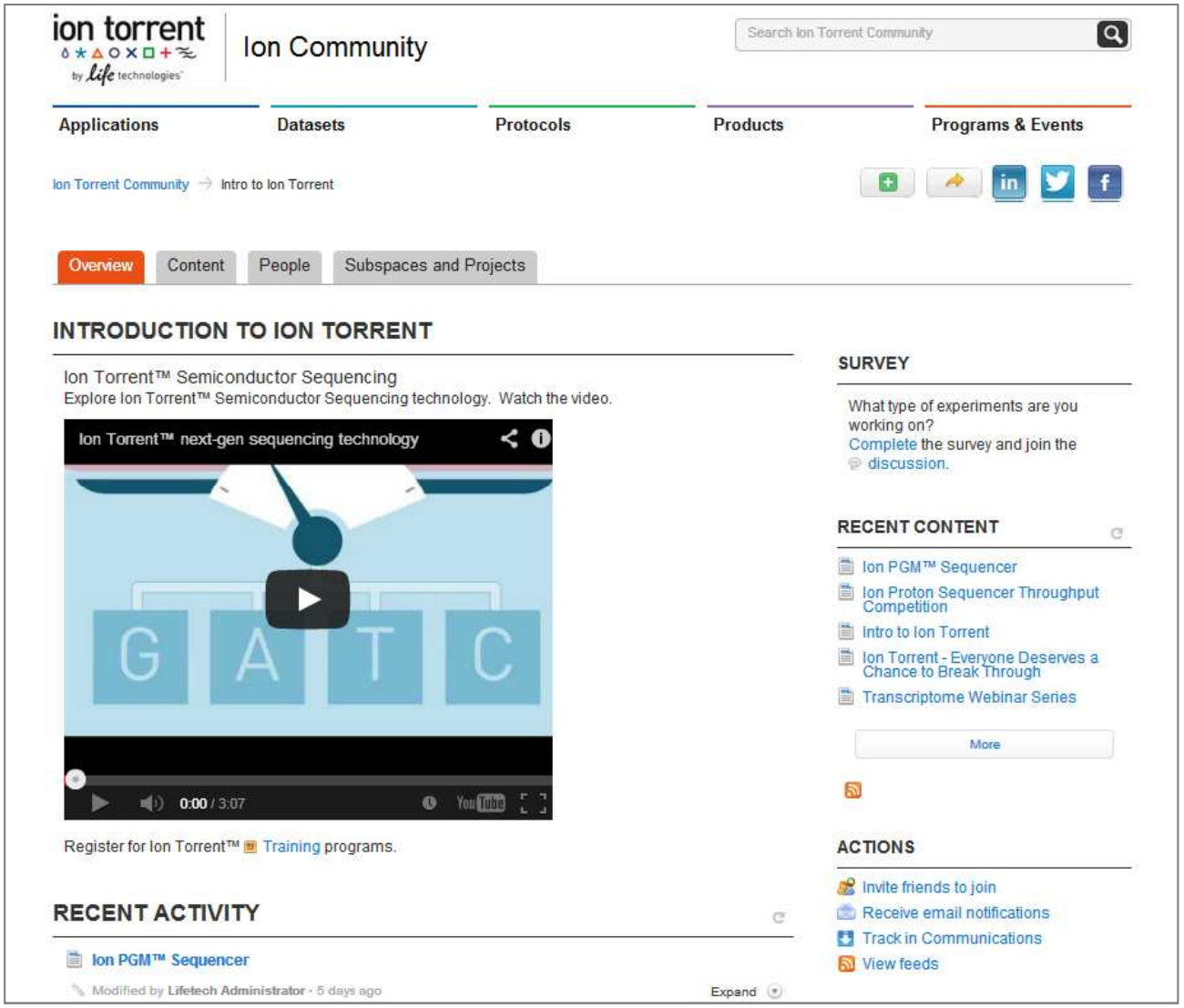
Ion Torrent™ Community





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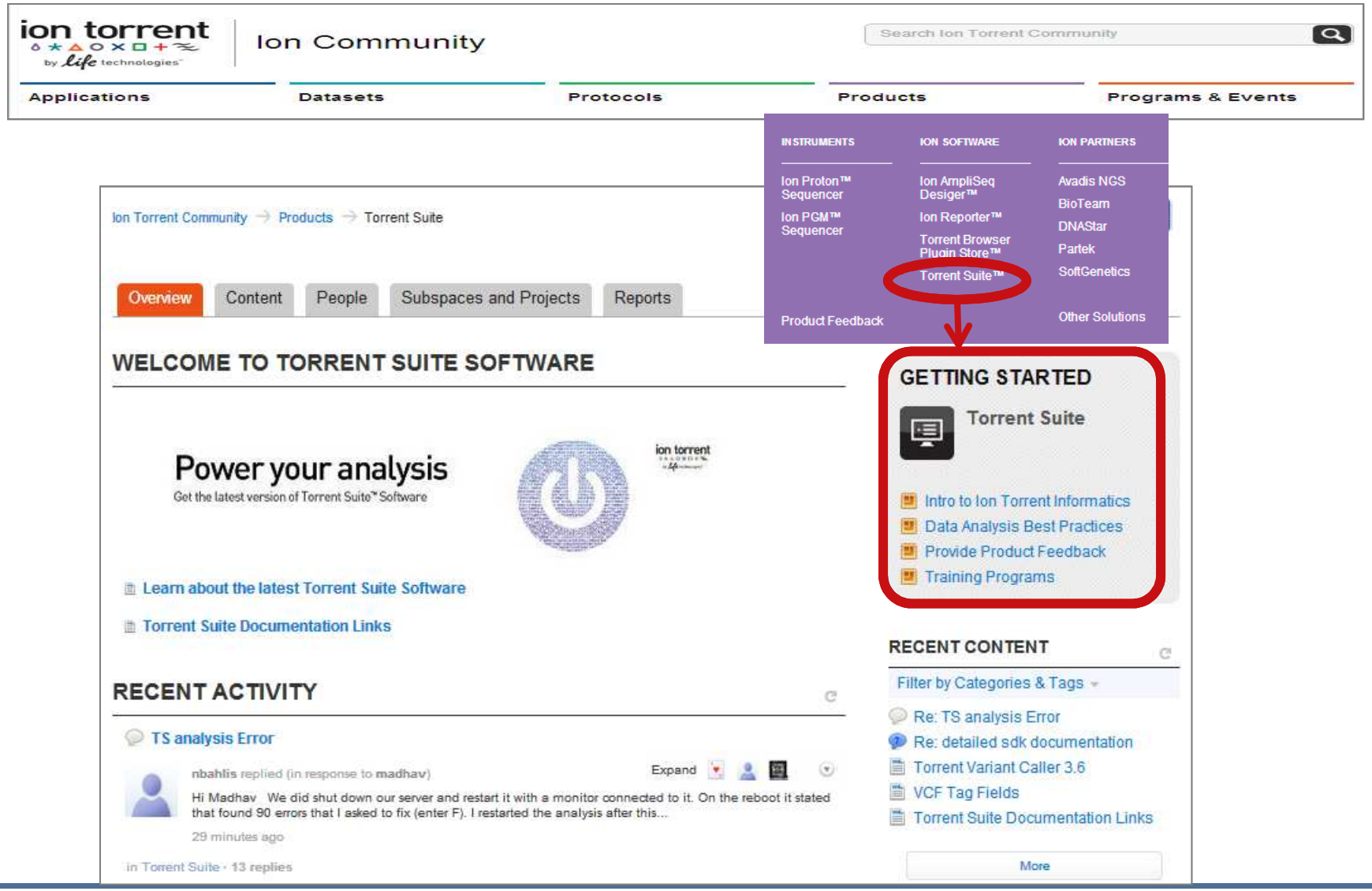
Ion Torrent™ Community



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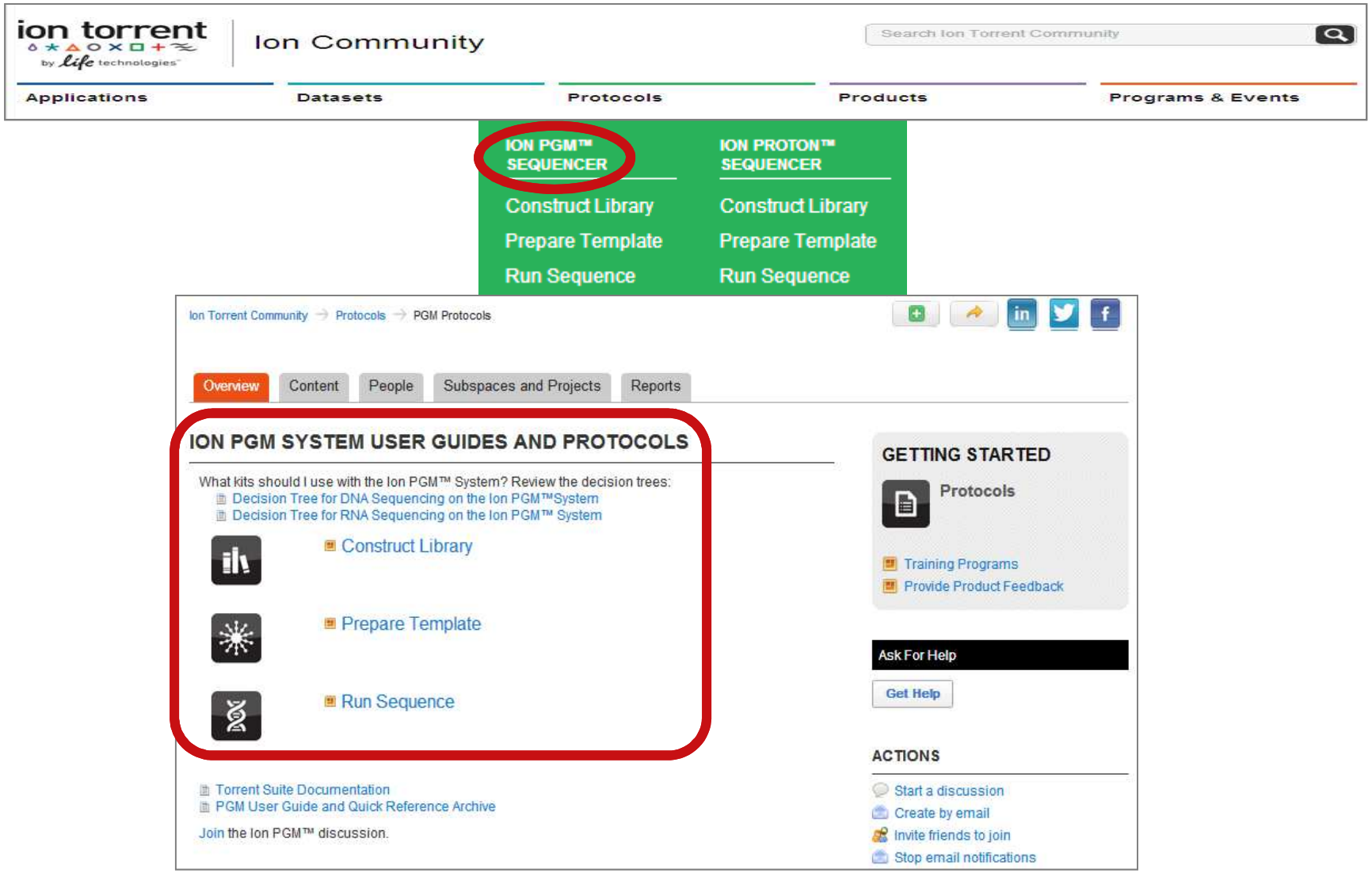
Ion Torrent™ Community



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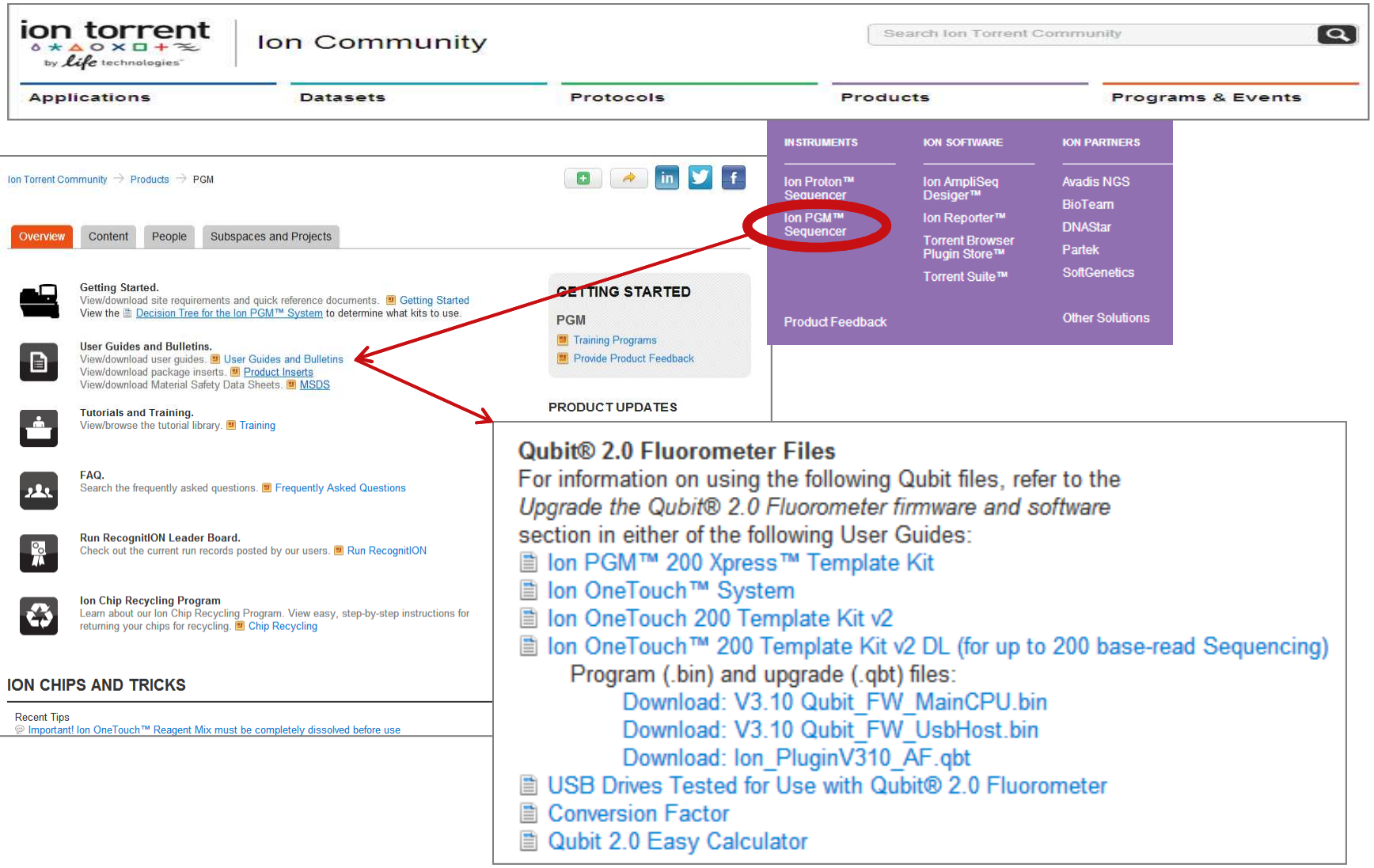
Ion Torrent™ Community



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“Best Practices”, Ion Community

Check for updates!

PRODUCT OVERVIEWS AND BEST PRACTICES (ION PGM SEQUENCER AND TORRENT SUITE)

Part I: Overview of Ion PGM™ System Protocols
Guidelines for library and template preparation, sequencing,

Part II: Overview of Torrent Sequencing and Alignment (v2
Sequence generation and alignment for Ion semiconductor d

Part III: Advanced Topics in Filtering of Ion Sequencing D
Options to reanalyze Ion sequencing data for improved data

Part IV: Overview of Variant Calling in Ion Sequencing Dat
Calling SNPs and indels in Ion semiconductor sequencing d

Part V: Advanced Topics in Variant Calling (v3.4.x)
Optimal sample-specific performance with the Torrent Varian

Part I: Overview of Ion PGM™ and Proton™ System Protocols

Version 24

created by sue on Aug 6, 2012 1:45 AM, last modified by Shauna Clark on Feb 15, 2013 2:18 PM

Guidelines for Ion library and template preparation, sequencing, and instrument handling

This document outlines the best practices for successful Ion library construction, template preparation, and sequencing for Ion validated kits and protocols. The recommendations in this guide are only a brief summary of important protocol points, and first-time users should familiarize themselves with the appropriate user guides prior to beginning a protocol. User guides and quick references may be found in the Protocols space on the Ion Community.

General guidance

We recommend using the latest library, template, and sequencing kits. For a list of the latest products and recommended kit compatibility, please see the Decision Tree for the Ion PGM™ System or Decision Tree for the Ion Proton™ System. Users should also register for the Ion Torrent Updates Group on the Ion Community to receive email notifications regarding all Ion Torrent upgrades. For questions, contact technical support in your region or your local FAS.

General laboratory guidance

- When designing the laboratory layout, consider the need for space separation of pre- and post-PCR activities. Separating the amplicon source, post-PCR activities from pre-PCR activities, and dedicating laboratory supplies and/or equipment to each space significantly reduces the potential for contamination

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

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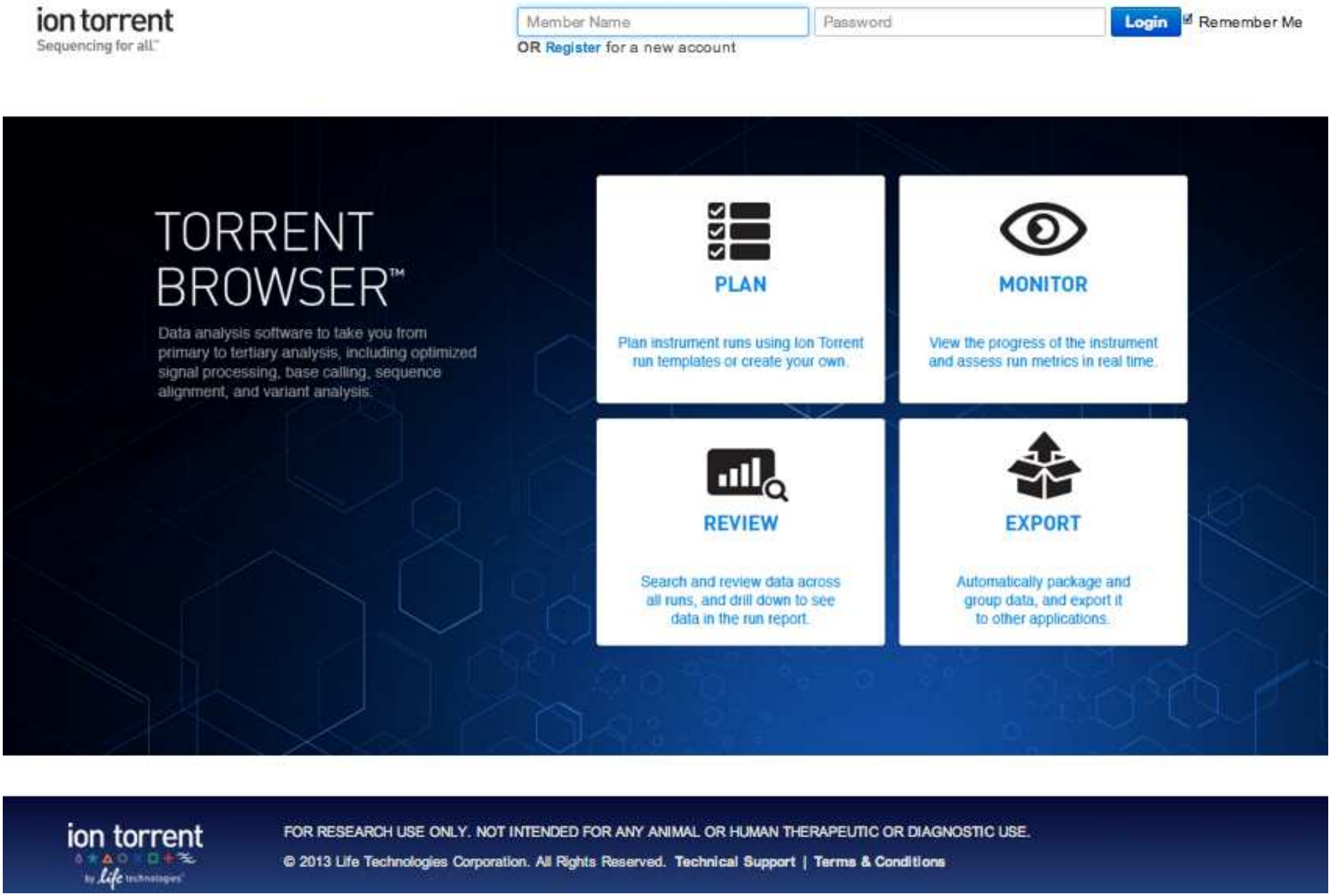

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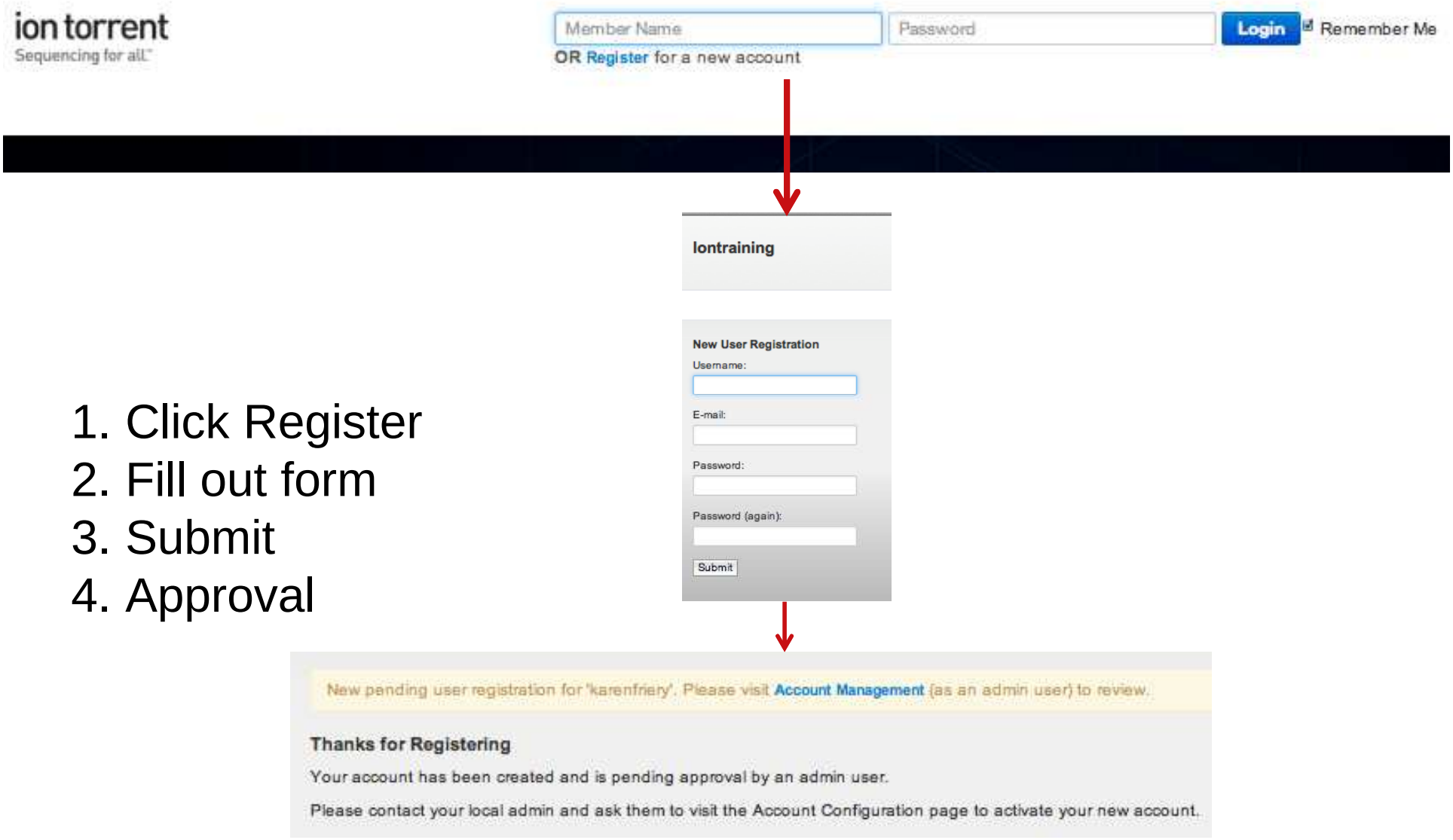
New Account Registration - Request



1. Click Register
2. Fill out form
3. Submit
4. Approval

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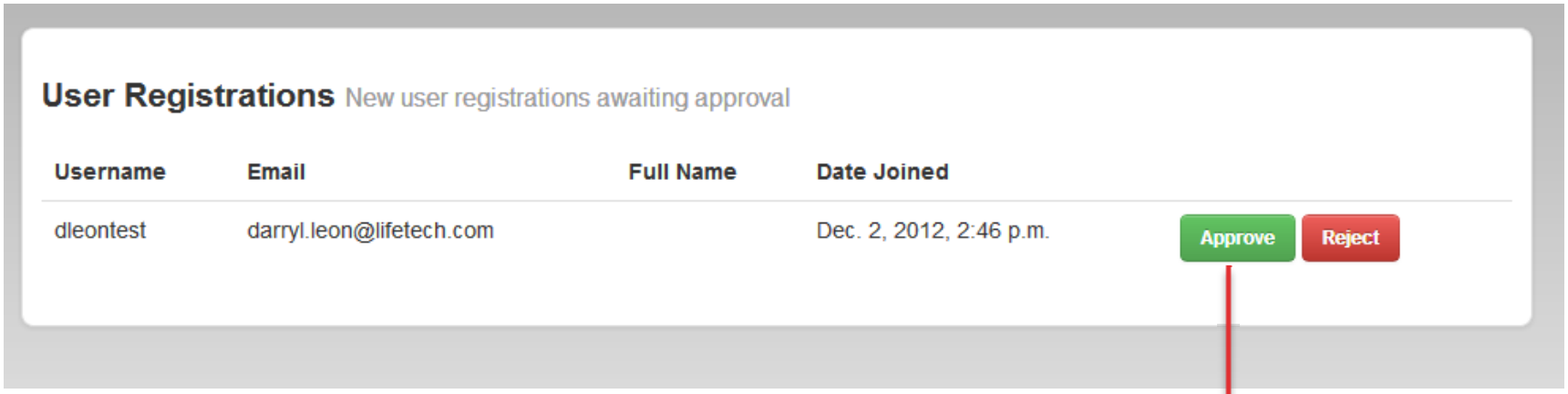
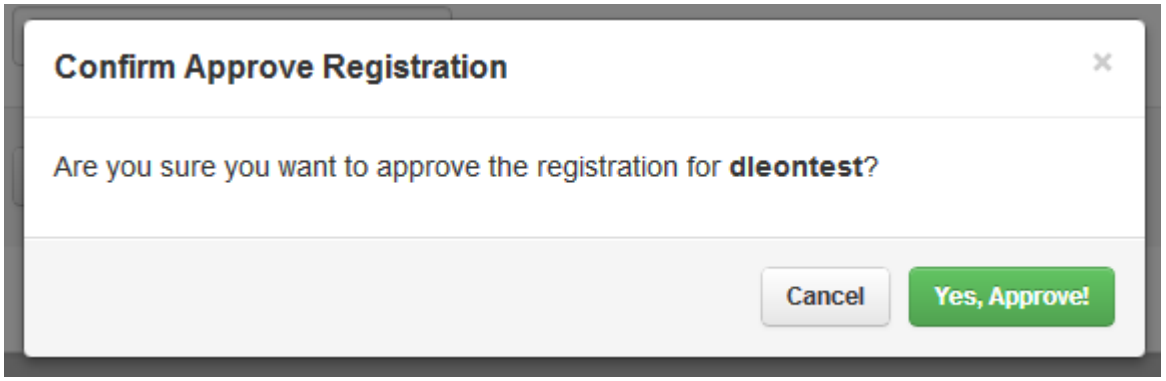
New Account Registration – Admin Approval



1. Login as Admin
2. Review request
3. Approve/Reject



*Note: Only administrators can reset user passwords.

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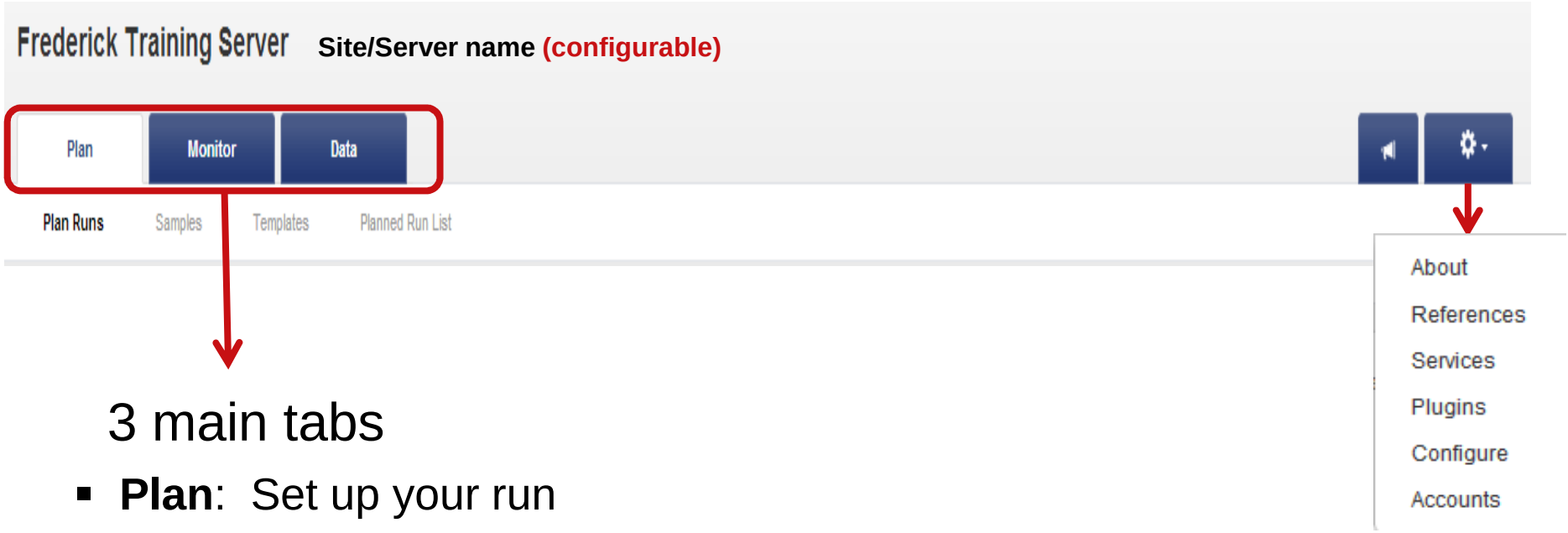
Log into Torrent browser



3 main tabs

- **Plan**: Set up your run
- **Monitor**: Check your run in real time
- **Data**: Analyze your run report(s)

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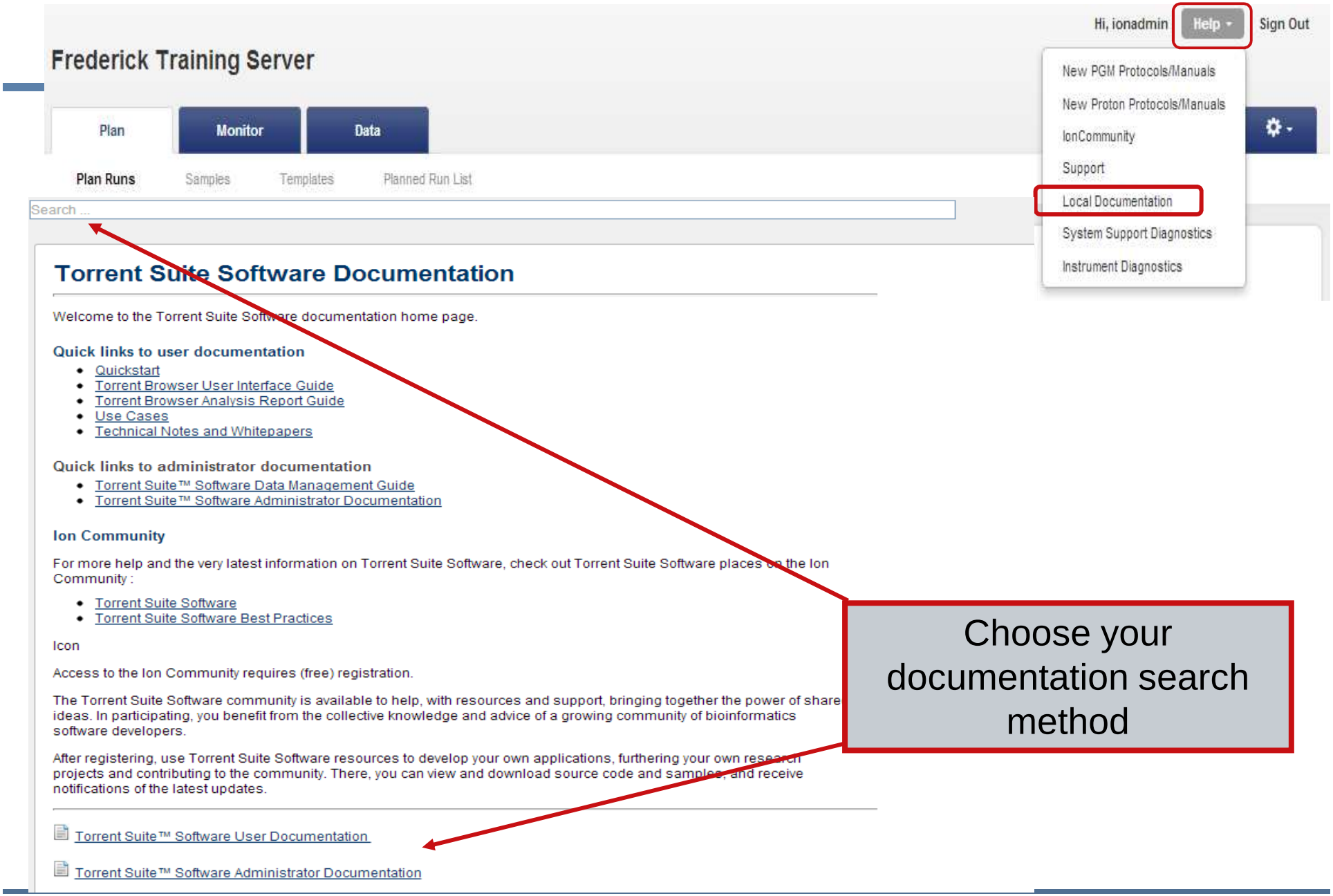

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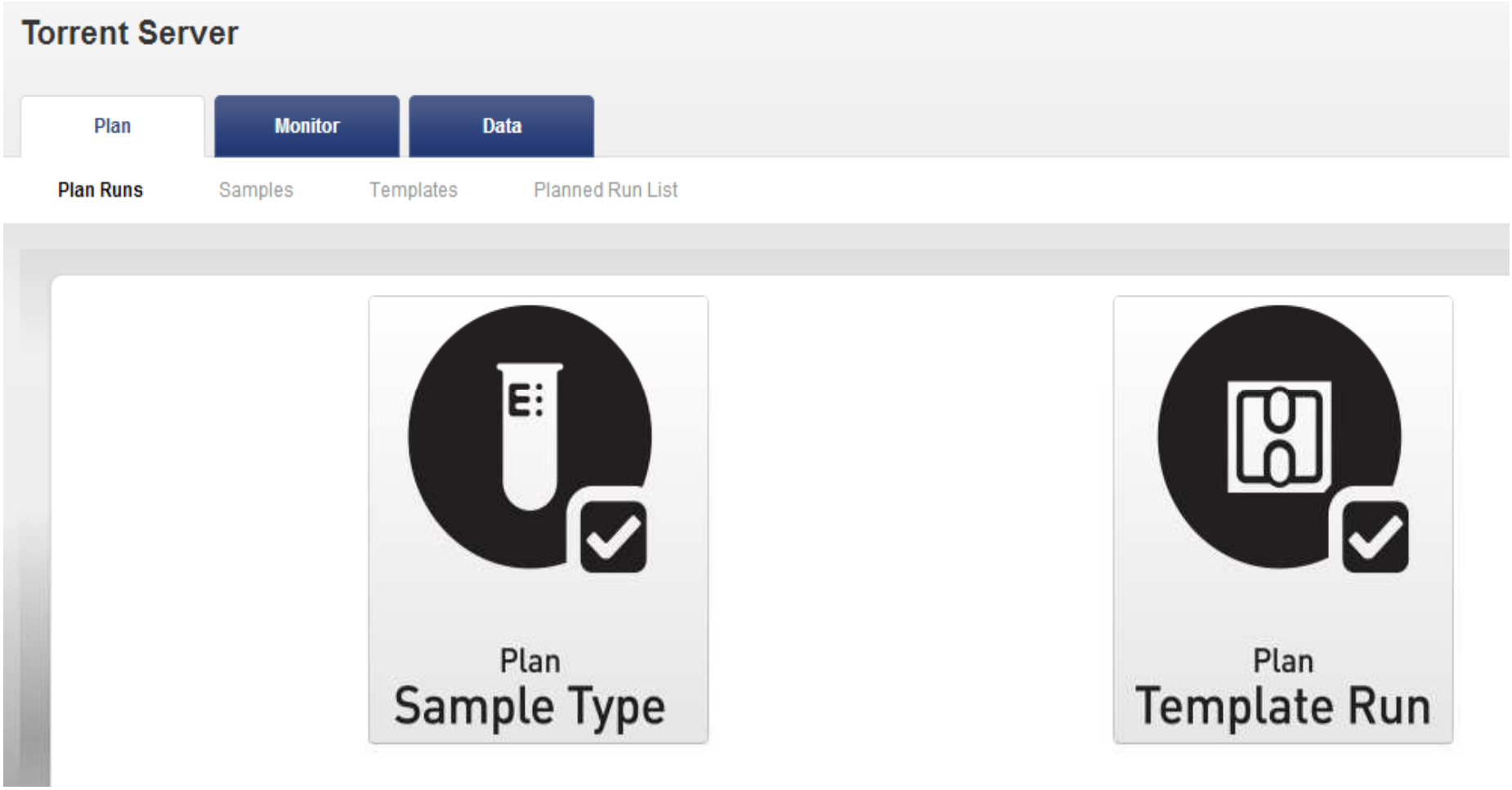
Planning a Run by Sample or Template



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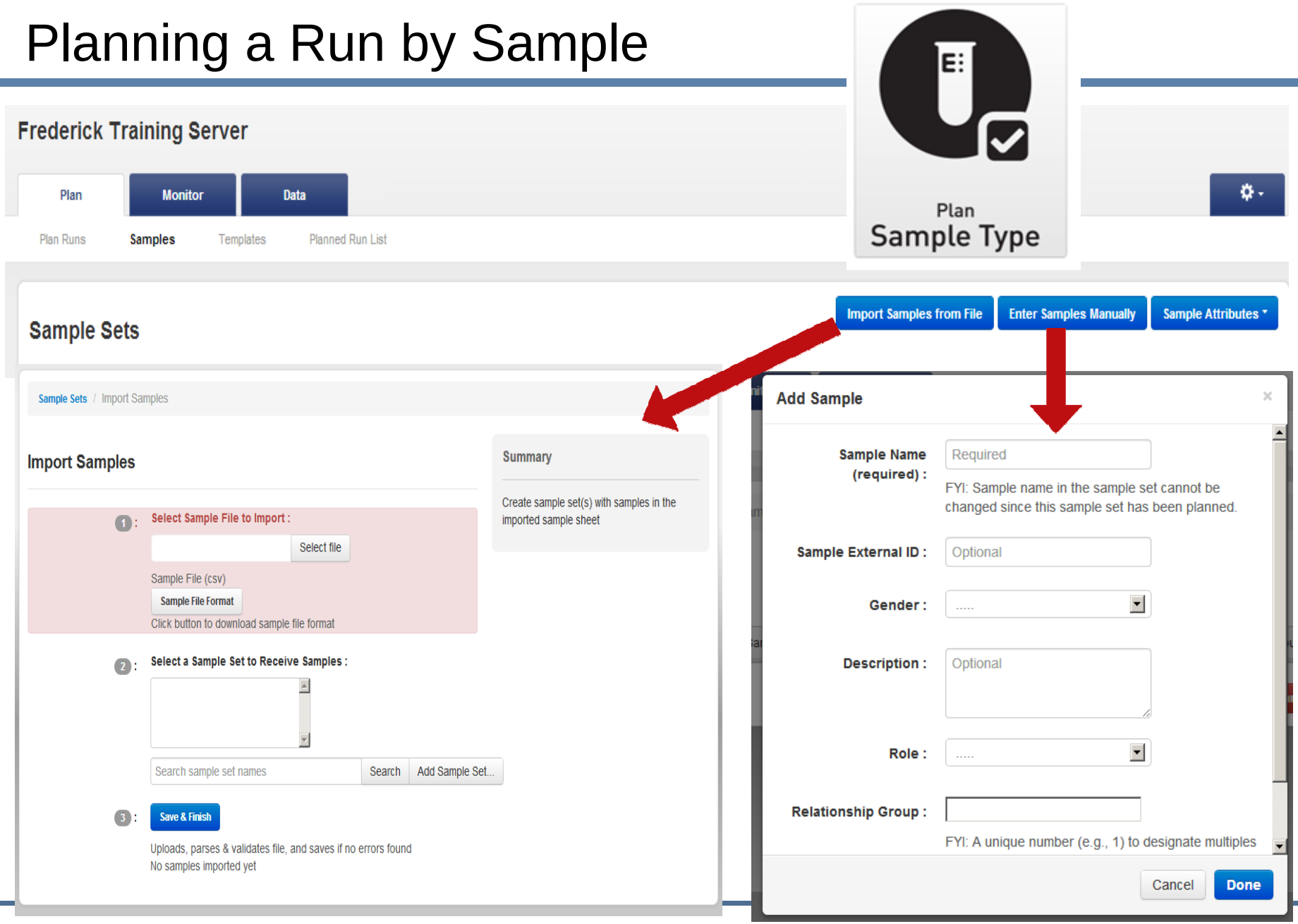
Planning a Run by Sample



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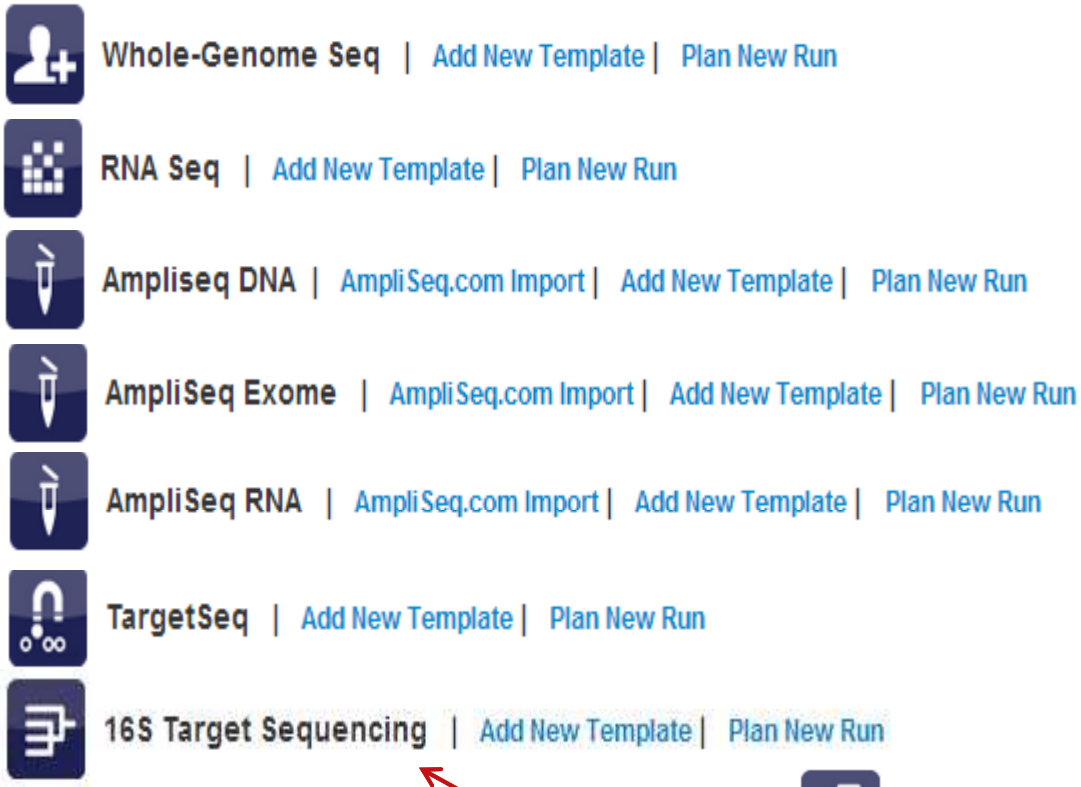
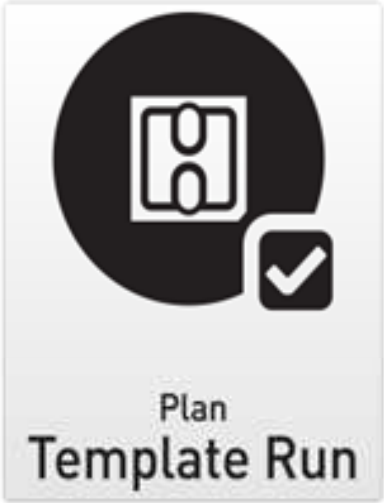
Planning a Template Run

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





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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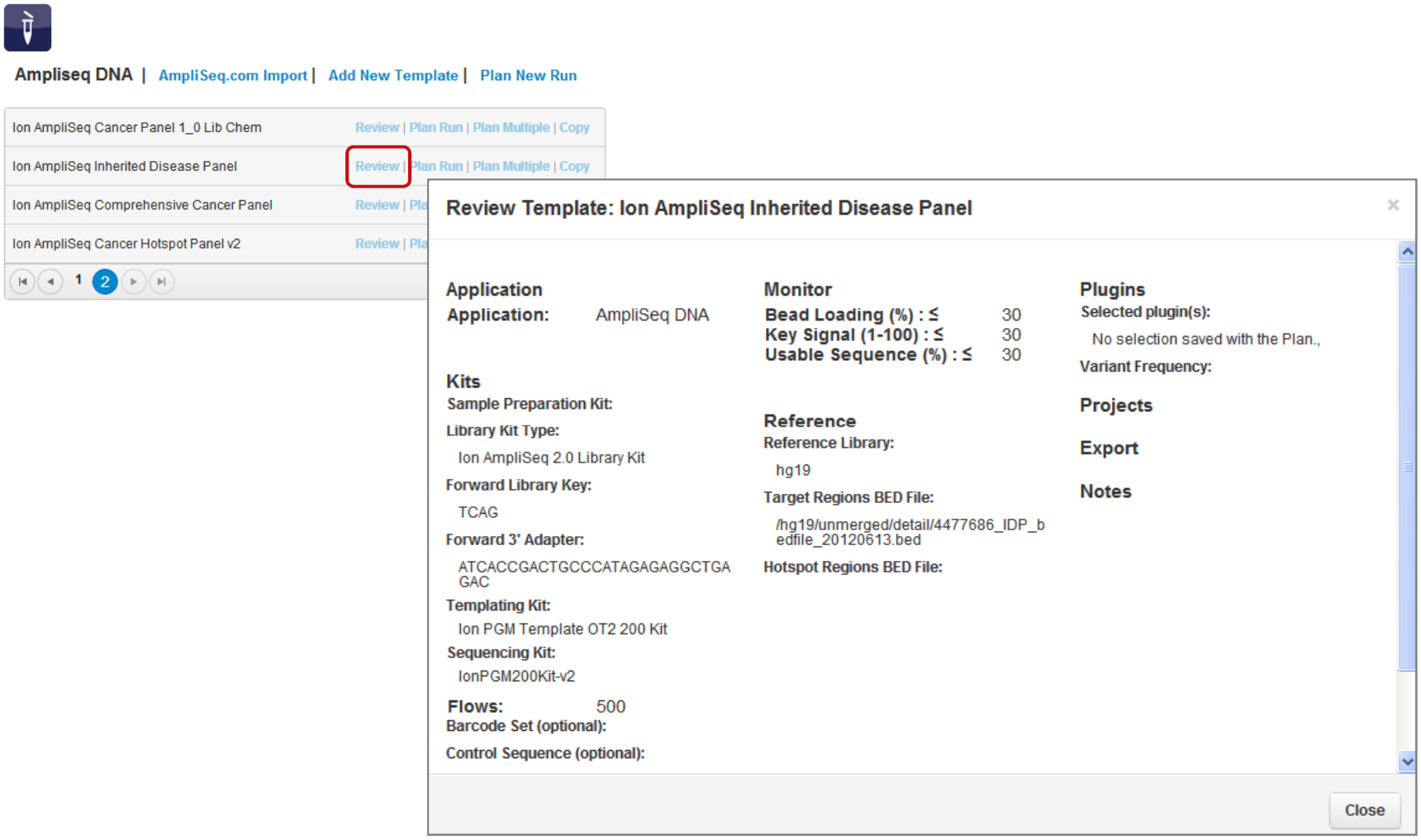
Review Settings of Pre-installed Template



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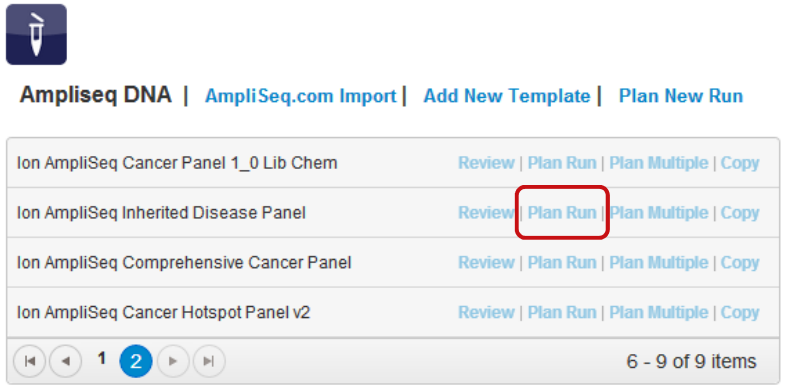
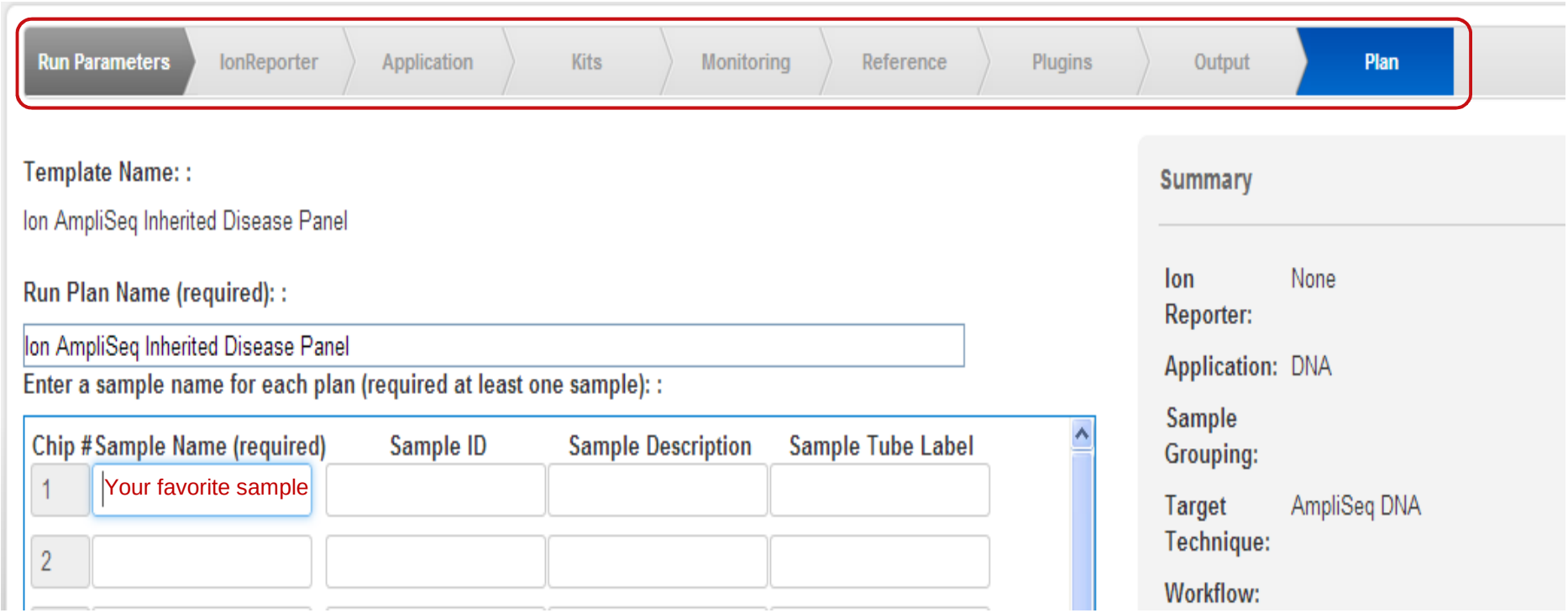
Plan Run from a Template





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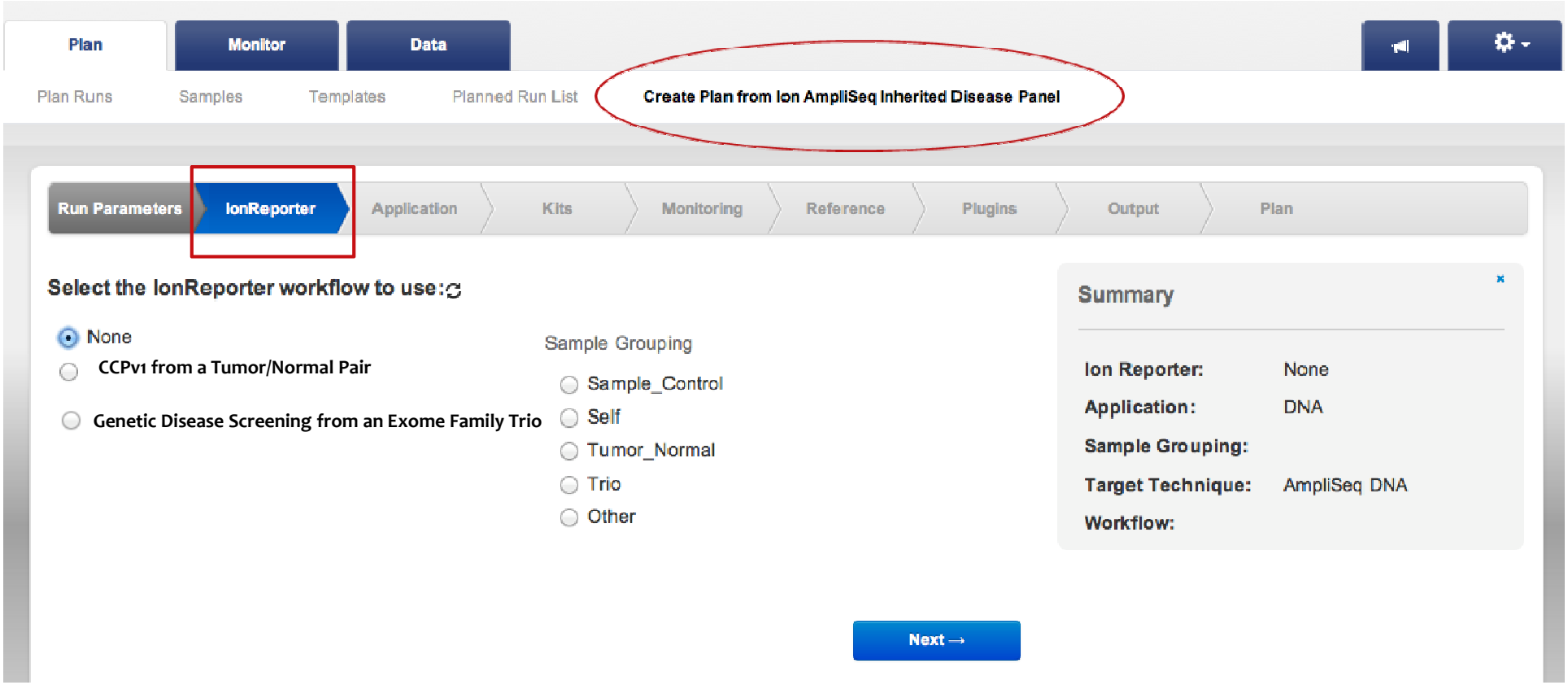
Ion Reporter Integration



Select the IR Workflow and Sample Grouping

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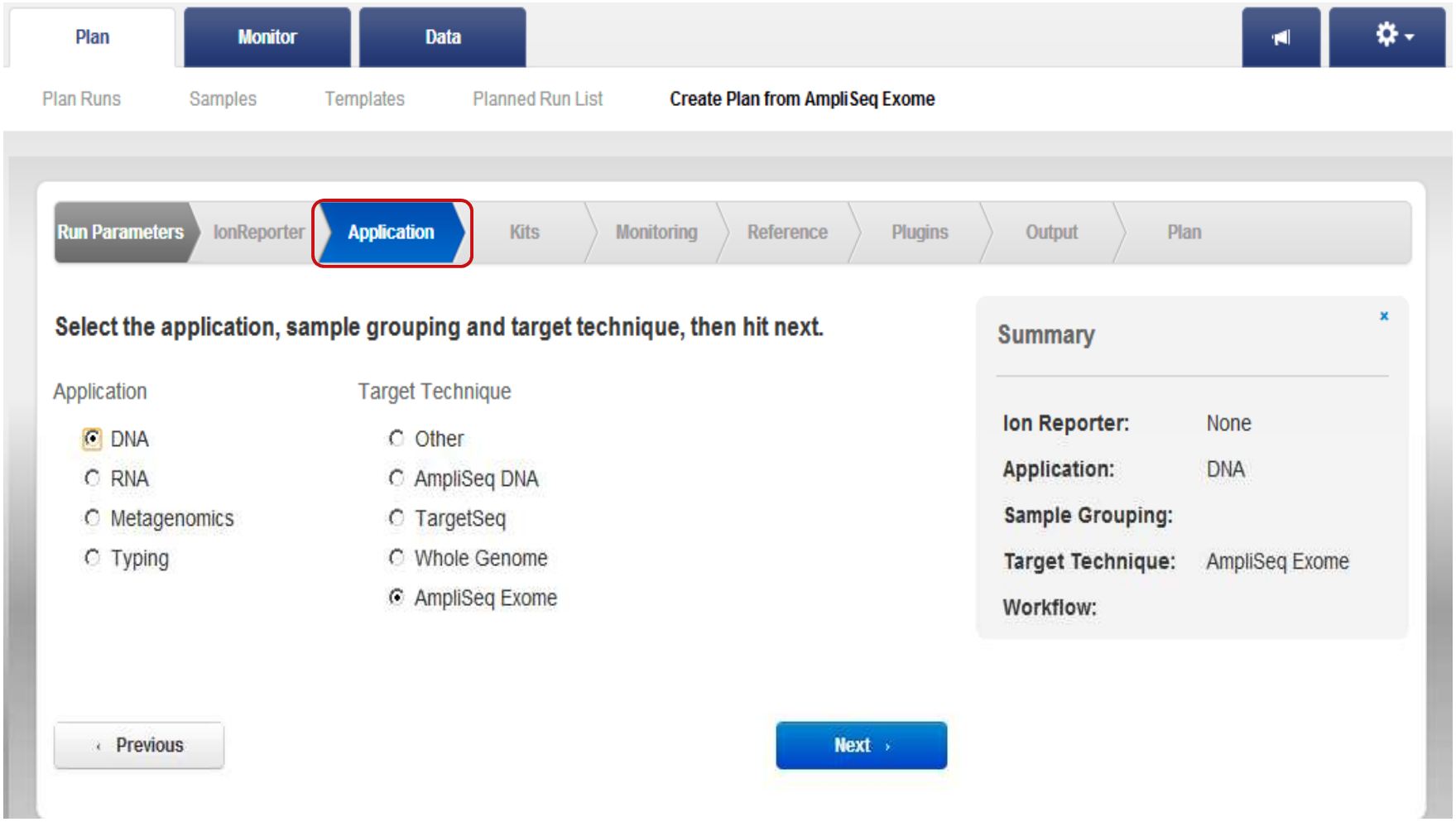
Template application



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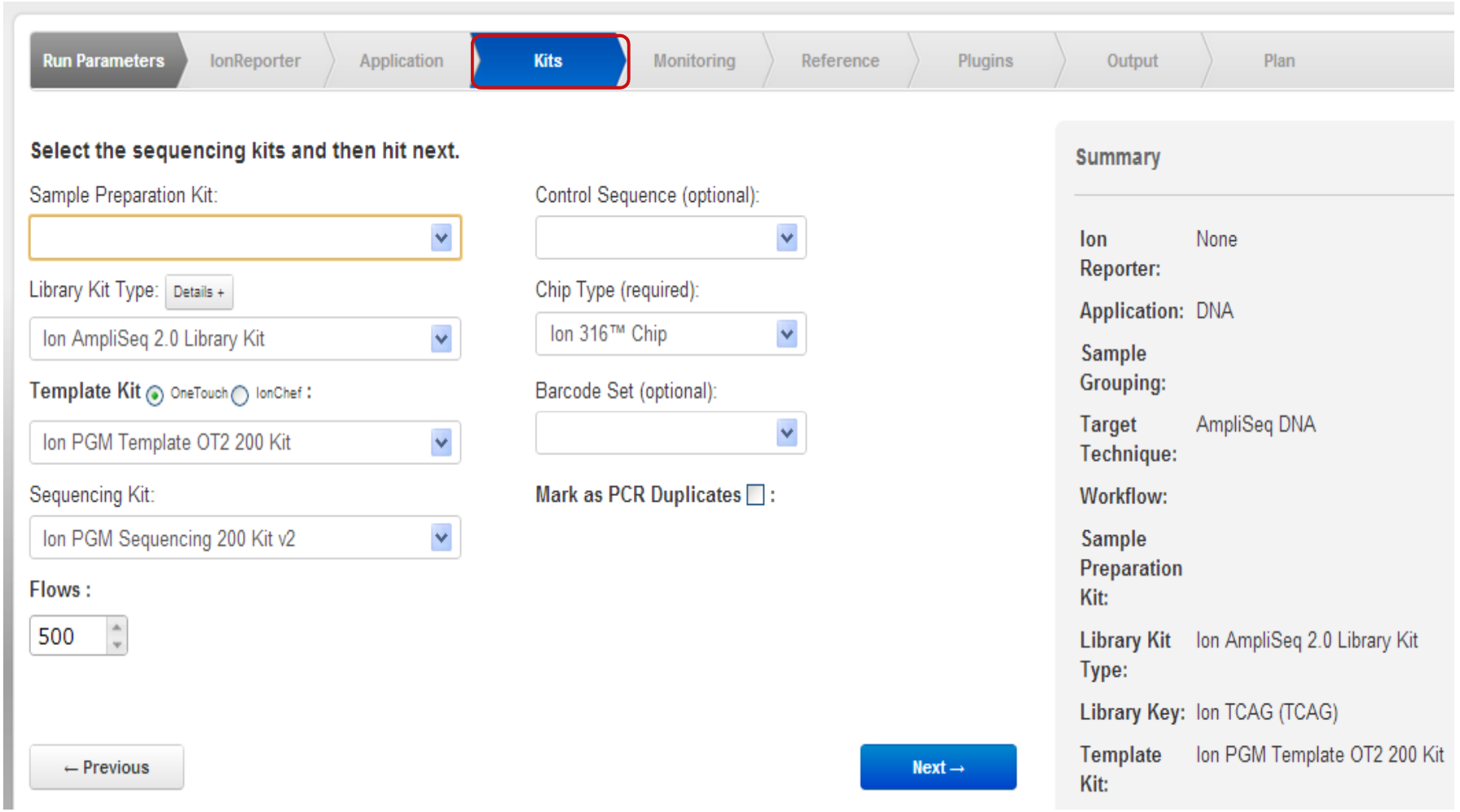
Plan Run from a Template - Kits



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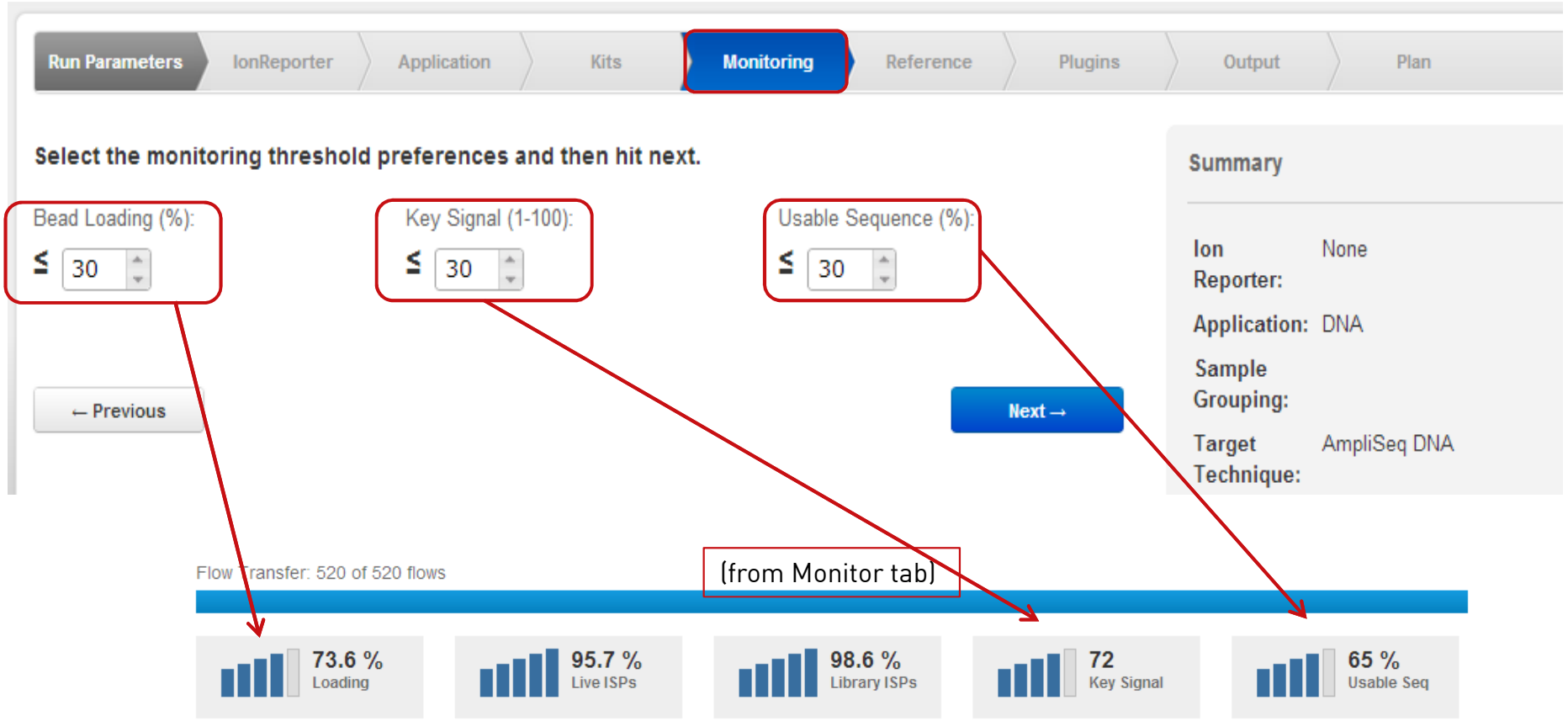
Plan Run from a Template - Monitor



Set thresholds for run, view in Monitor Tab:

- Blue bar = greater than threshold set
- Red bar = less than threshold set

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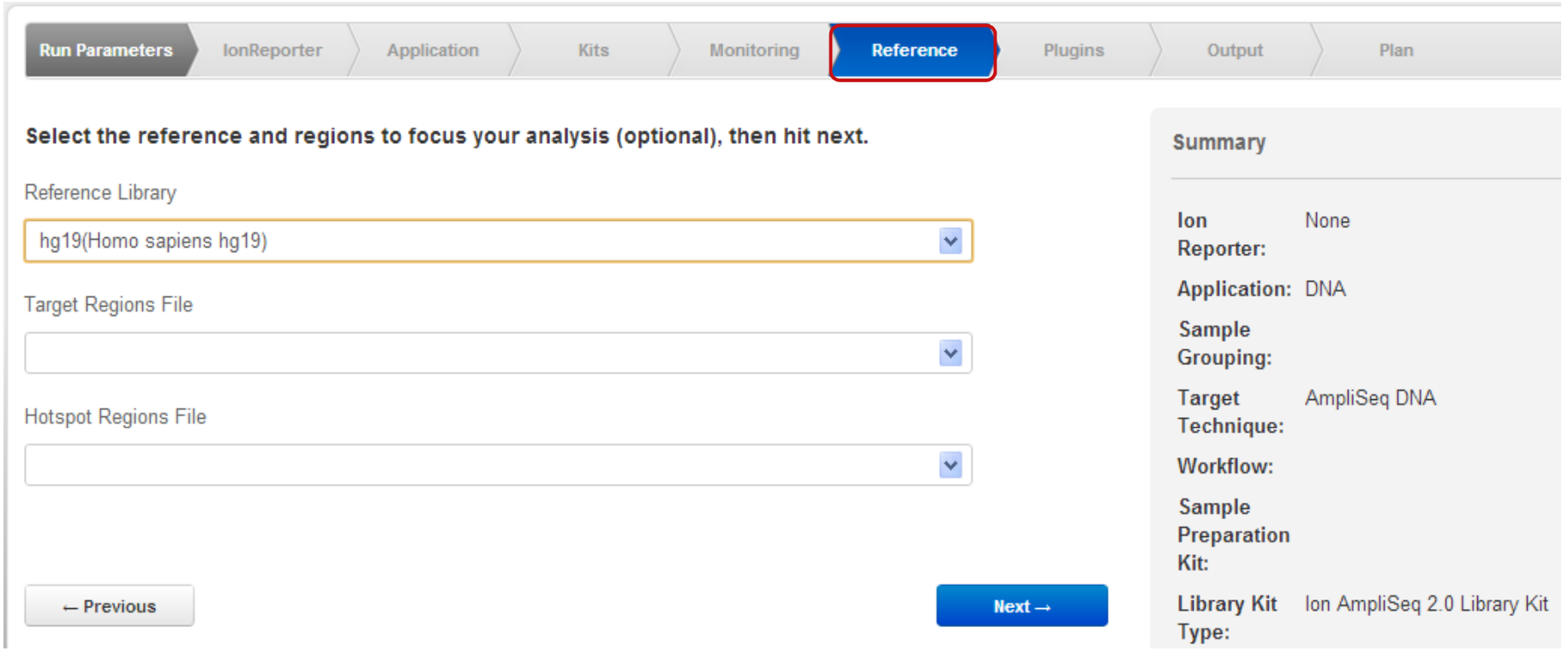
Plan a Run from a Template - Reference



References, sequences used in data analysis; Upload BED files

- Genome or other reference sequence
- Target Regions
- Hotspot Regions

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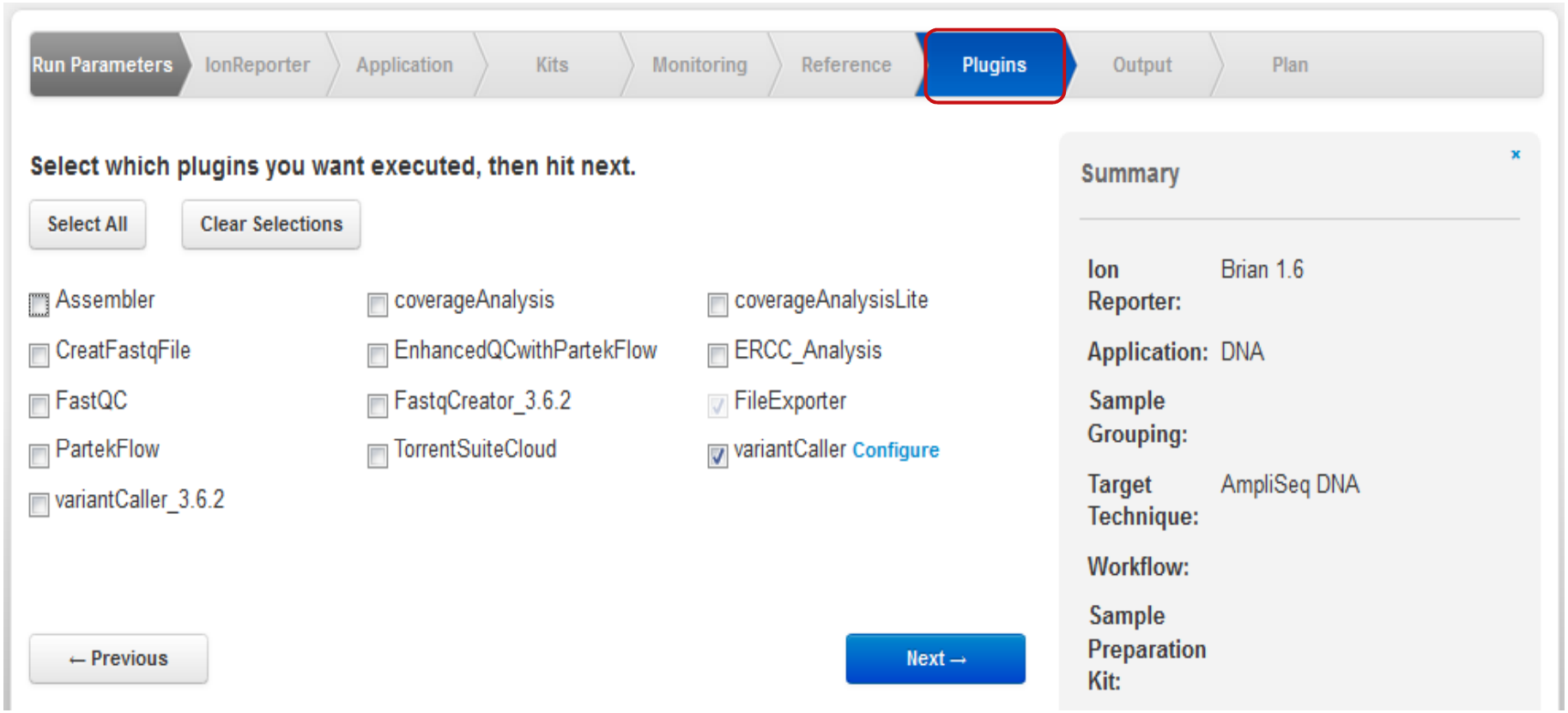
Plan a Run from a Template - Plugins



Plugins, specific analysis invoked for this run

- Commercial plugins
- Customer plugins
- Plugins with parameter settings (somatic/germline)

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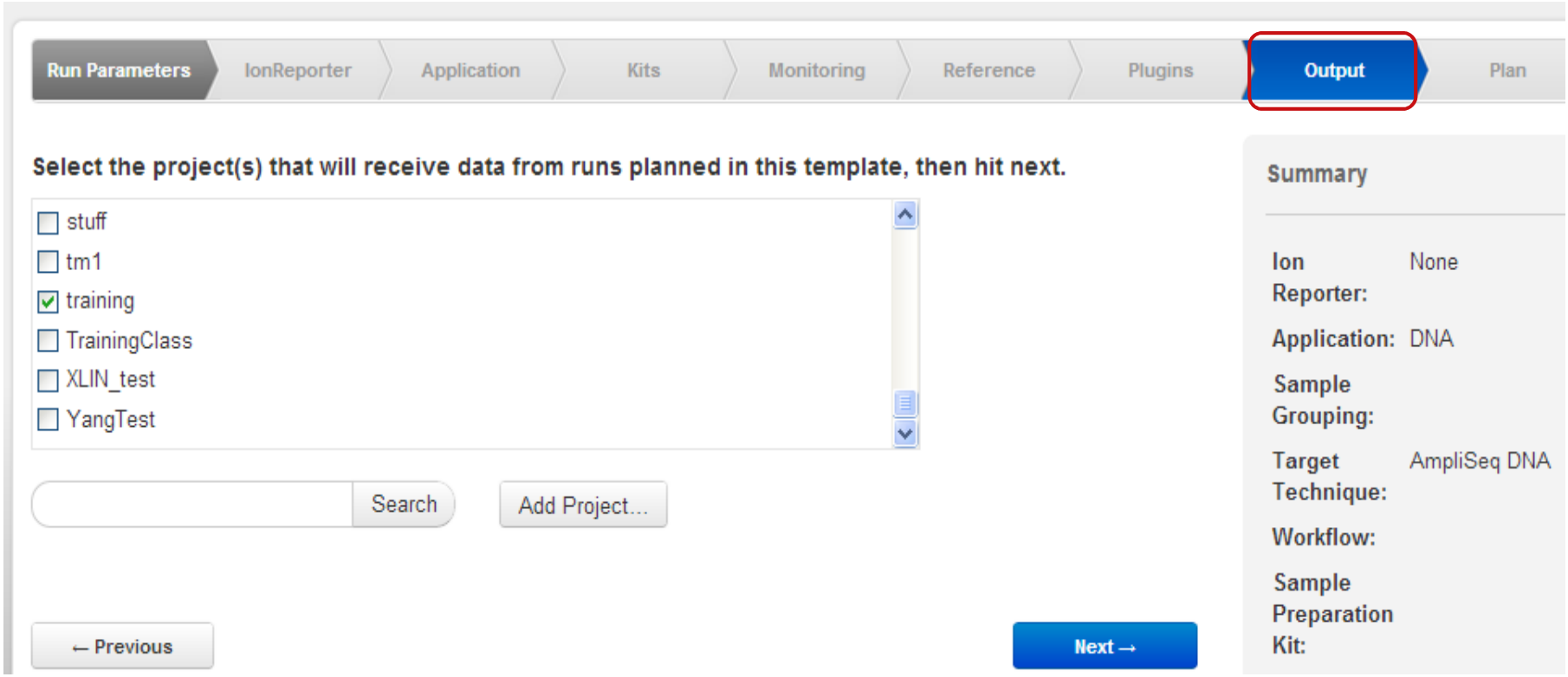
Plan Run from a Template - Output



Projects, ways to mark data into groups

- Data sets can be in many projects
- Can group data from a cohort together
- Data in projects can be combined

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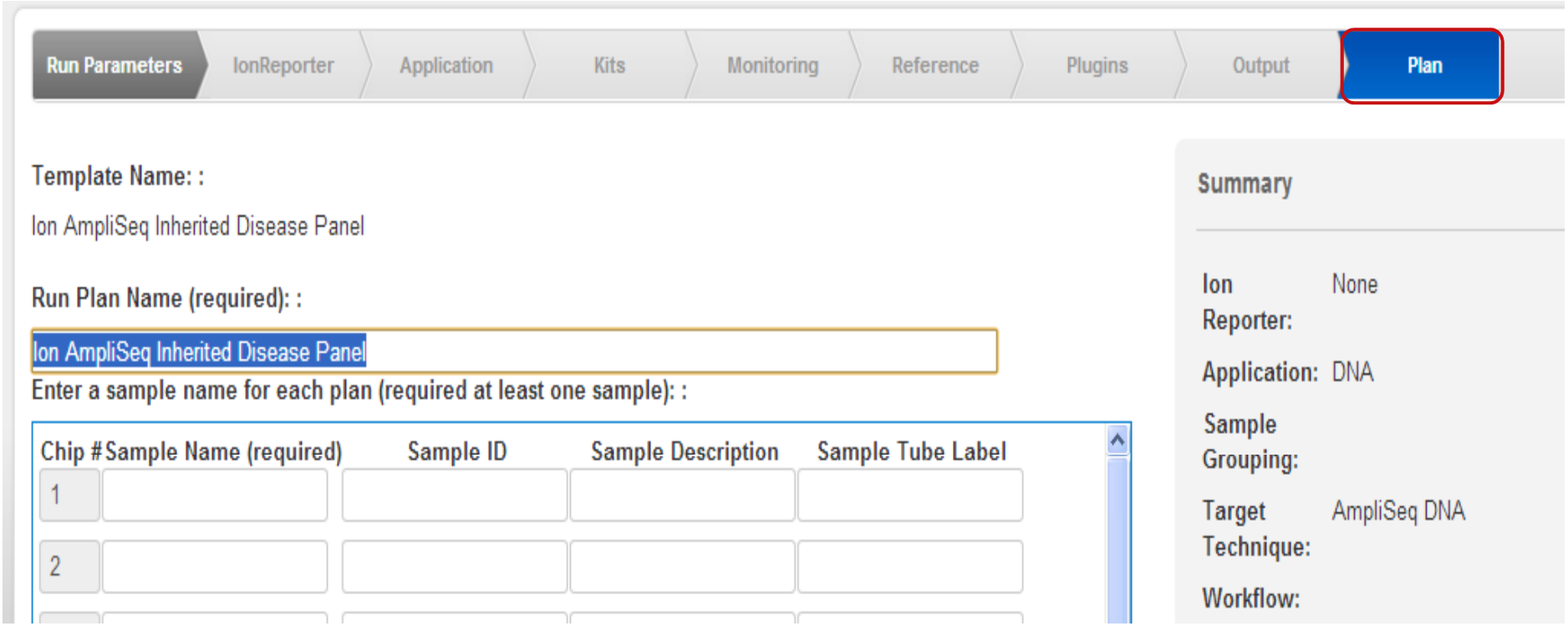
Plan Run from a Template



Plan Runs

- Accept template defaults and plan in seconds
- Add sample names to barcodes
- Plan for a group in one action

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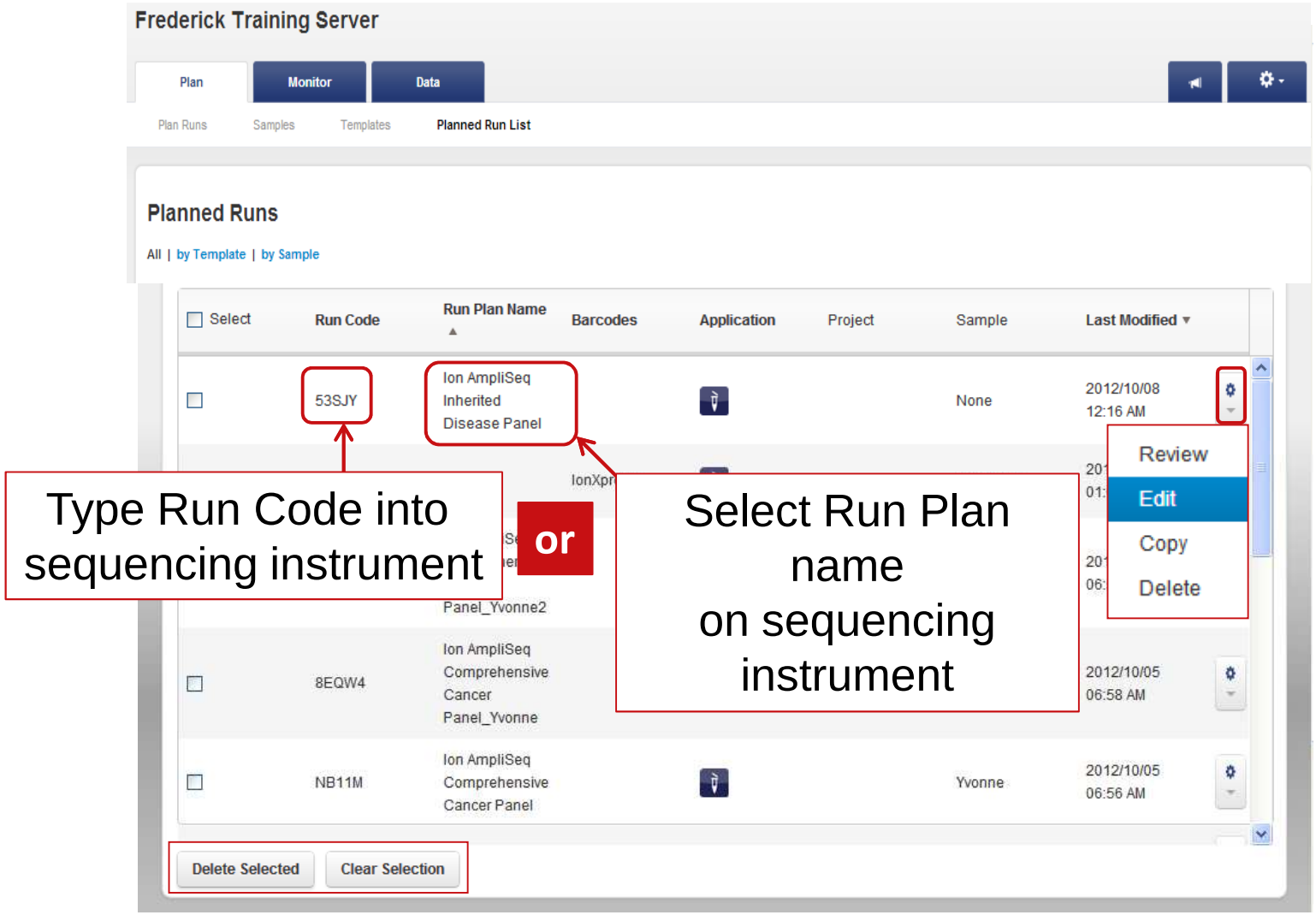
Planned Runs List



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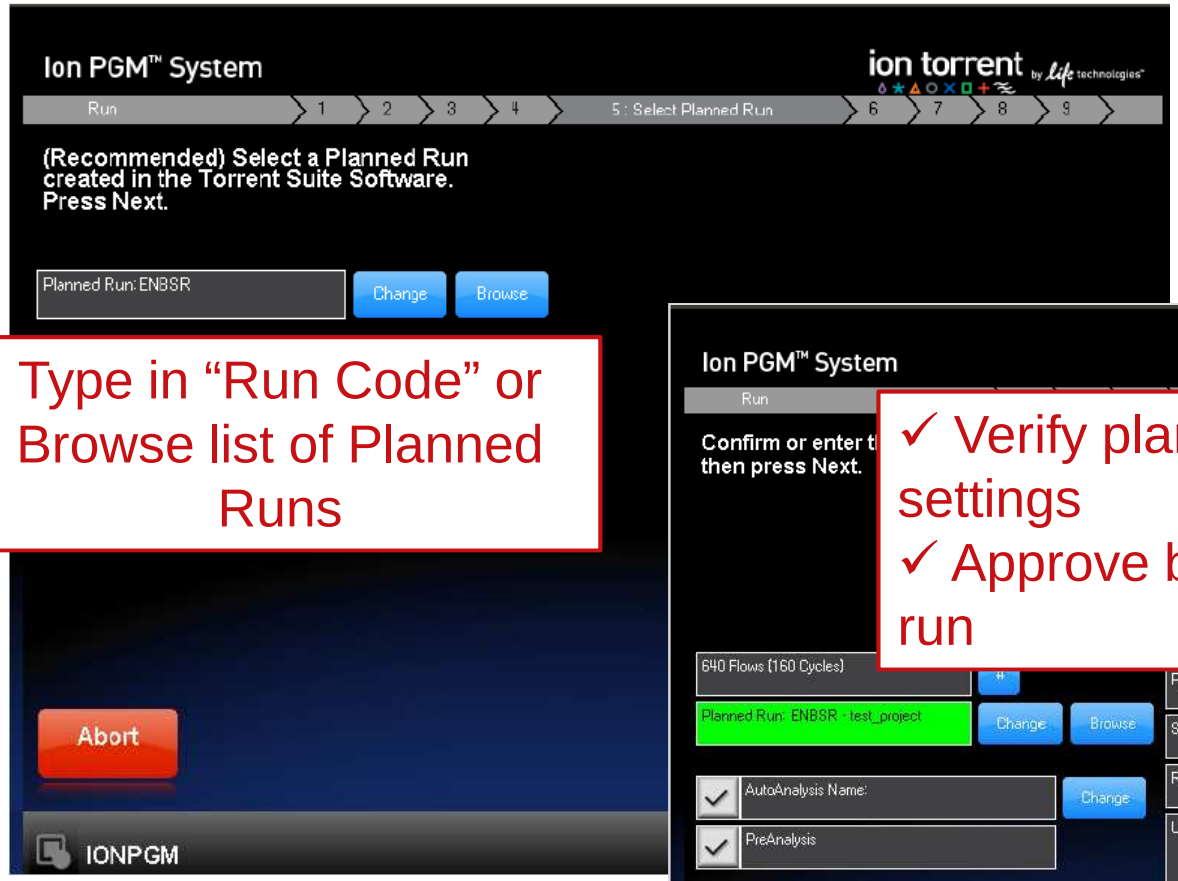
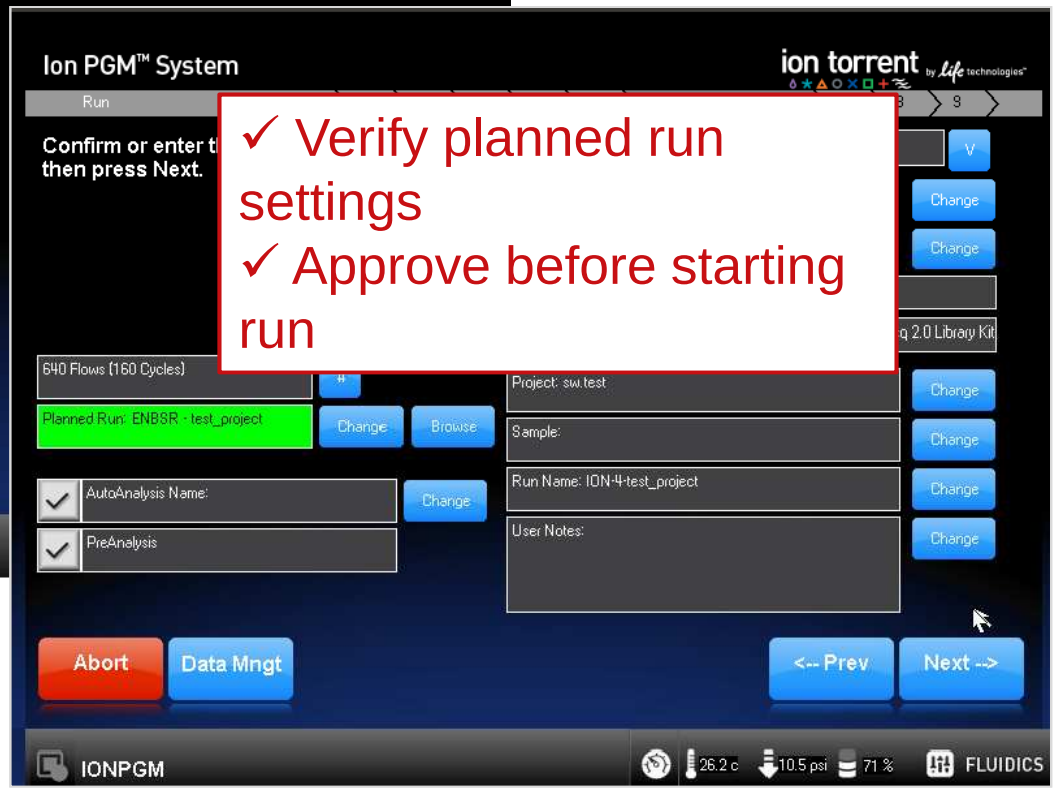
Pulling in a Planned Run to the PGM™ Sequencer



Type in “Run Code” or Browse list of Planned Runs



✓ Verify planned run settings
✓ Approve before starting run

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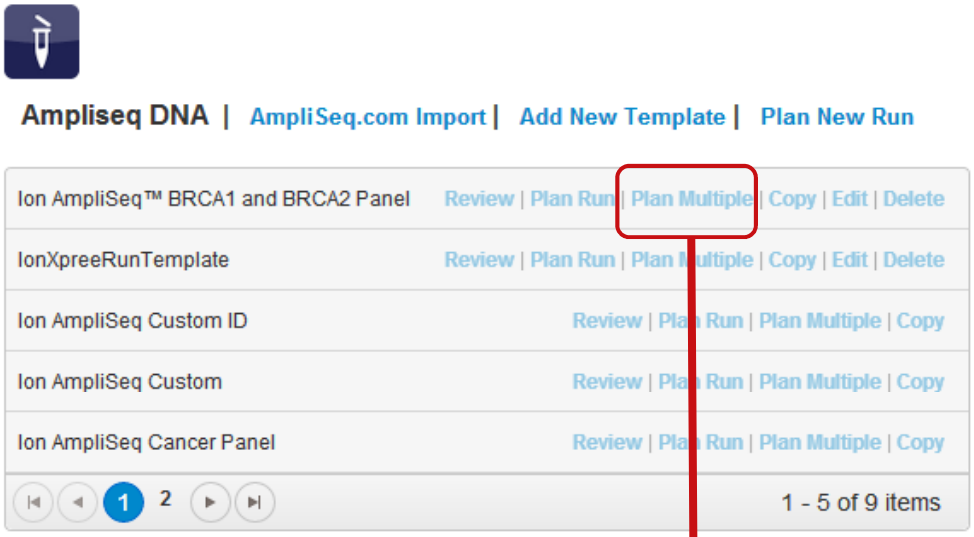
Easily Upload Multiple Plans - Overview

Download Plan Multiple Runs File



Review Uploaded Planned Runs

batchPlanning_2012_11_27_20_49_58.csv [Read-Only]

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Template	Plan name	Sample	Sample pr	Library kit	Templating	Control se	Sequence	Chip type	Flows	Bead loadi	Key signal	Usable se	Reference	Target regi	Hotspot re	Plugins
2	Ion AmpliSeq Fan_Nov	212012			Ion Xpress	Ion OneTouch 200 Te		(200bp) Ion PGM(tm)		500	30	30	30	hg19			
3	Ion AmpliSeq Fan_Nov	212012			Ion Xpress	Ion OneTouch 200 Te		(200bp) Ion PGM(tm)		500	30	30	30	hg19			
4	Ion AmpliSeq Fan_Nov	212012			Ion Xpress	Ion OneTouch 200 Te		(200bp) Ion PGM(tm)		500	30	30	30	hg19			
5																	

Modify File for Planning Multiple Runs

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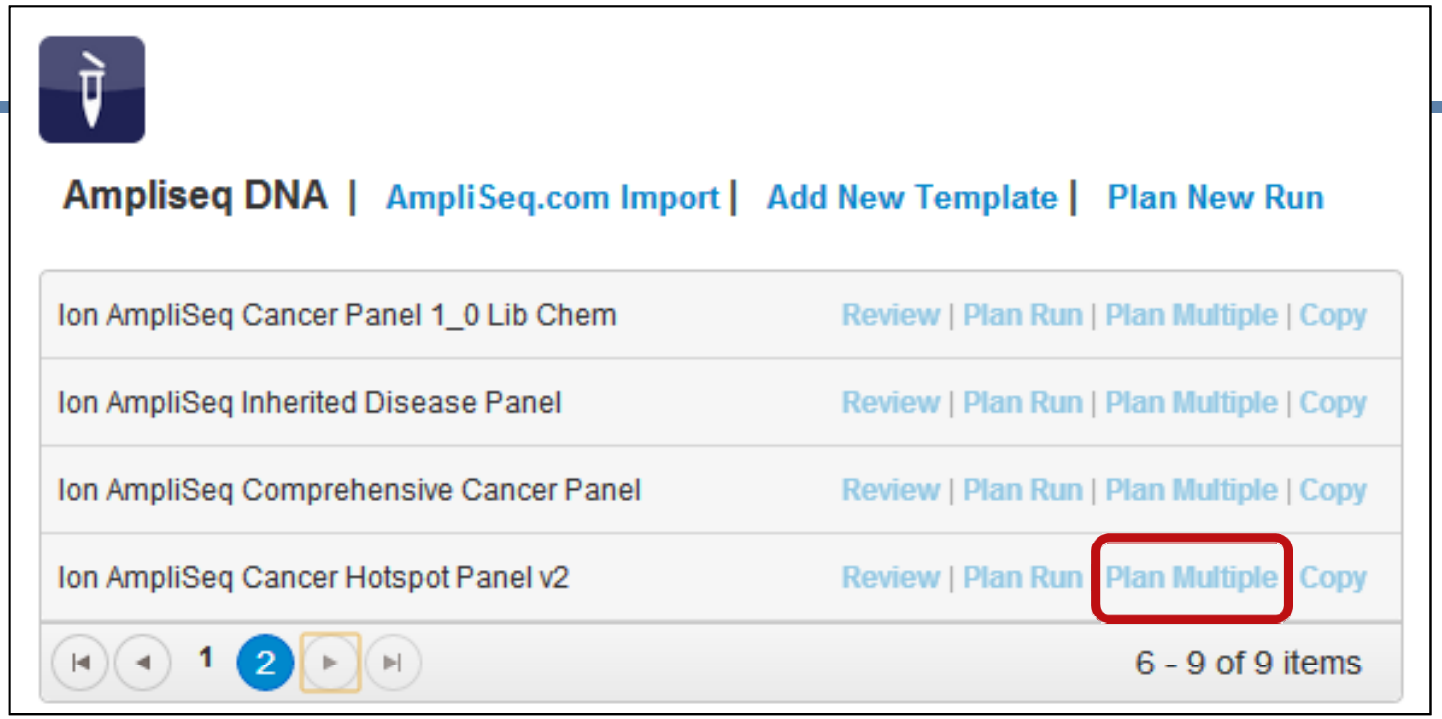
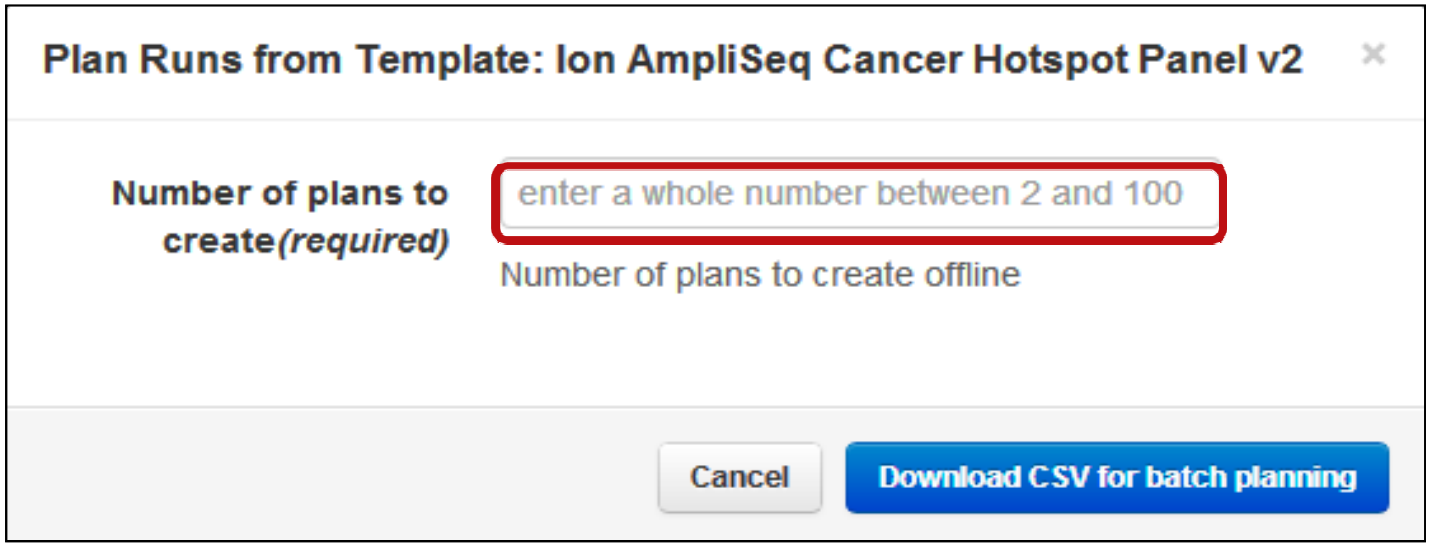
Select Plan Multiple



Enter Number of Plans to Create



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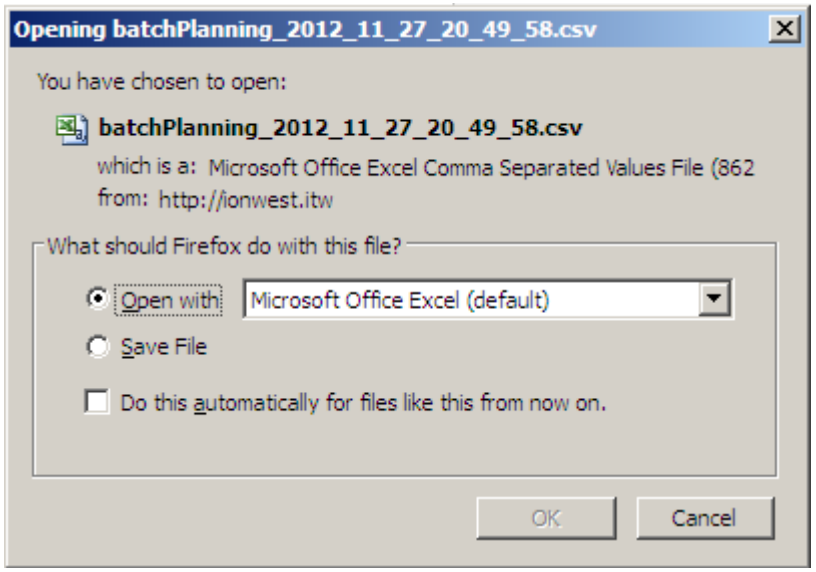
Save CSV File and Edit



batchPlanning_2012_11_27_20_49_58.csv [Read-Only]

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Template r	Plan name	Sample	Sample pr	Library kit	Templating	Control se	Sequence	Chip type	Flows	Bead loadi	Key signal	Usable se	Reference	Target regi	Hotspot re	Plugins
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3	Ion AmpliSeq Fan_Nov	212012			Ion Xpress	Ion OneTouch 200 Te		(200bp) Ion PGM(tm)		500	30	30	30	hg19			
4	Ion AmpliSeq Fan_Nov	212012			Ion Xpress	Ion OneTouch 200 Te		(200bp) Ion PGM(tm)		500	30	30	30	hg19			
5																	

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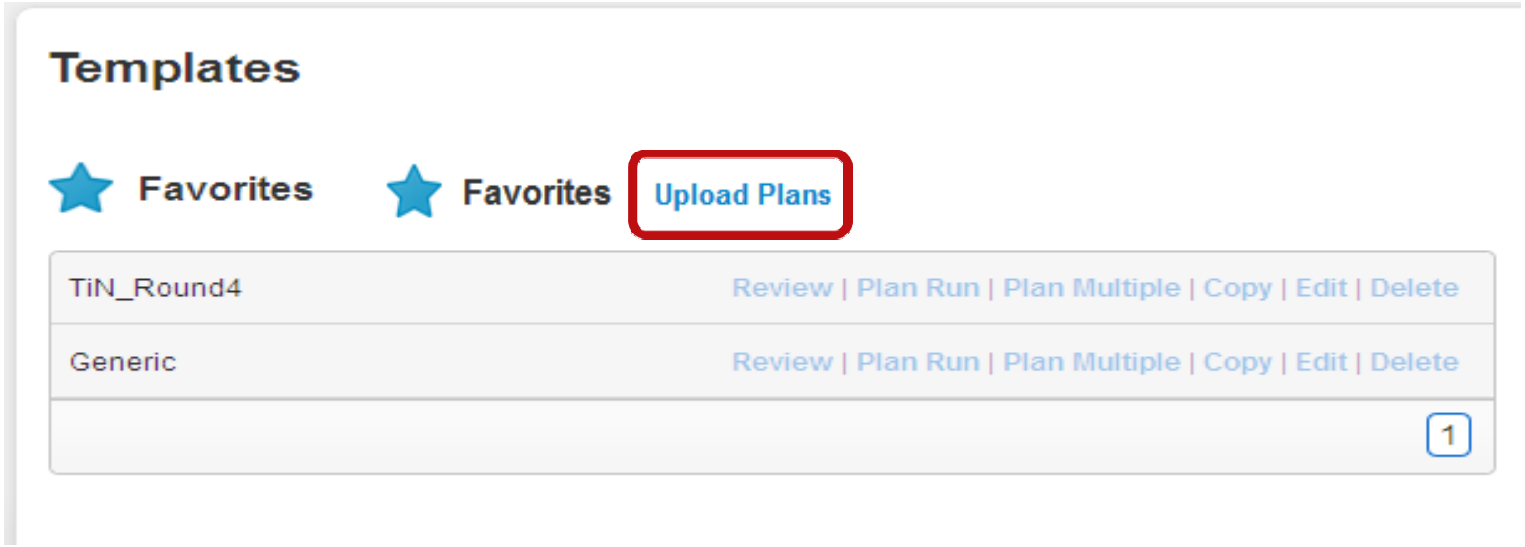
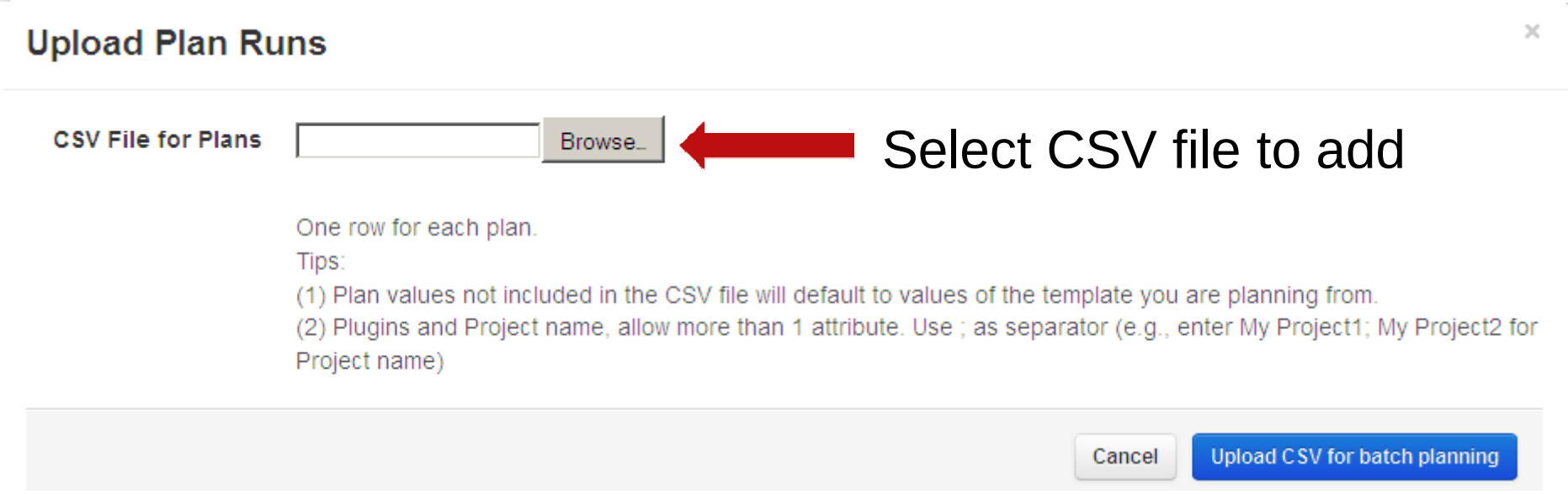
Upload Plans





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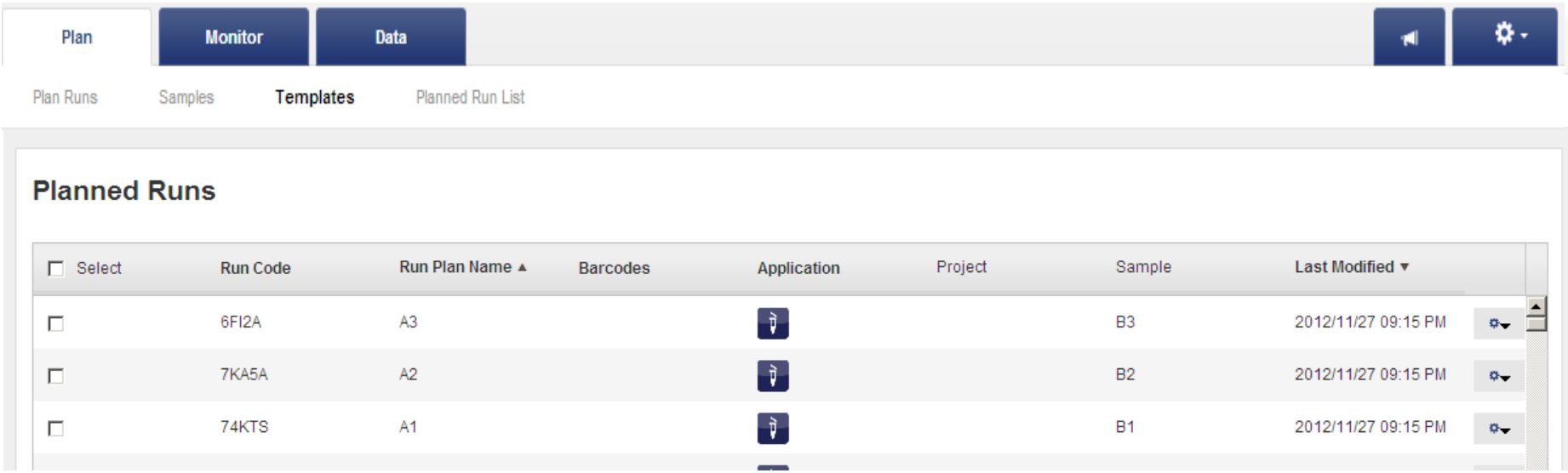
Review uploaded Planned Runs



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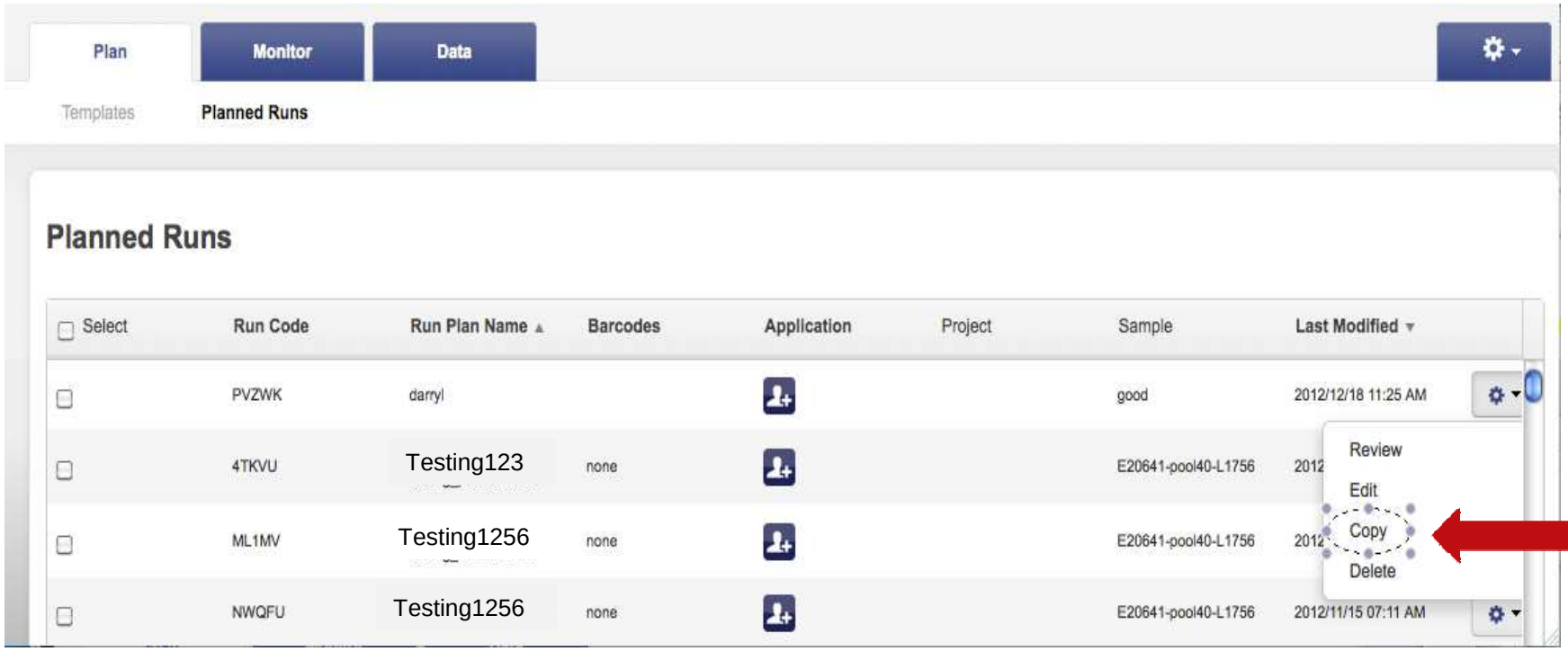
Plan cloning: Copy



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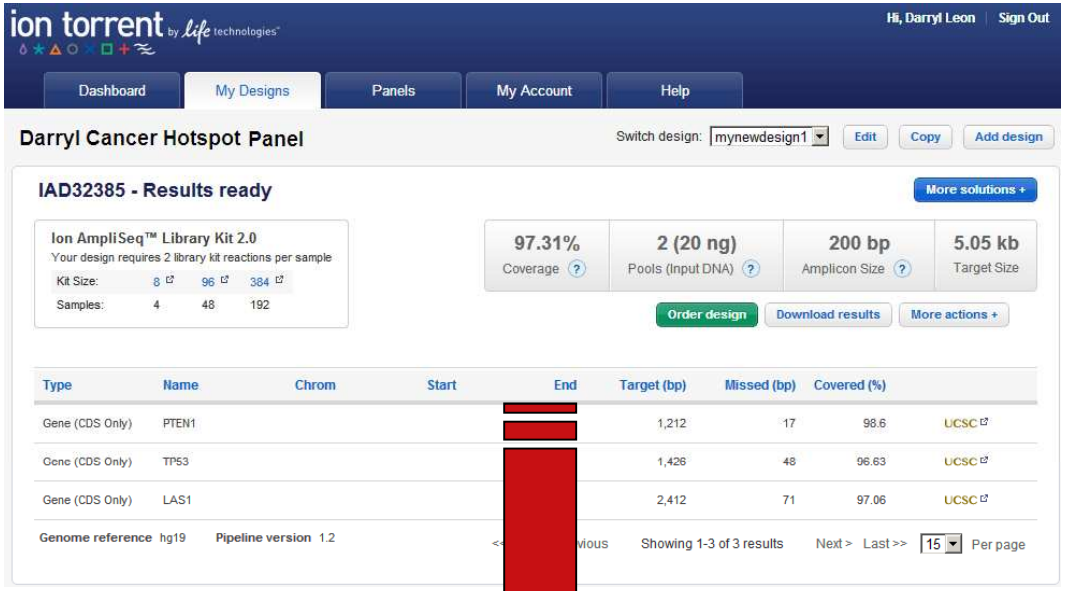
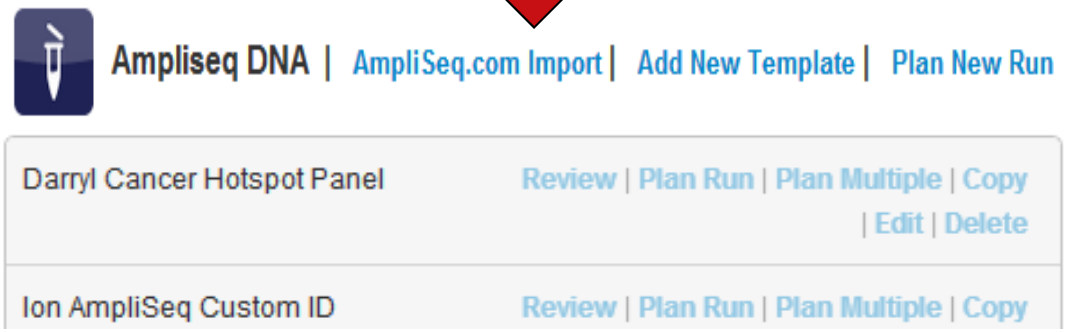
Easily Create Run Template from Ion AmpliSeq™ Designer downloaded files

Ion AmpliSeq™ Designer



Torrent Browser



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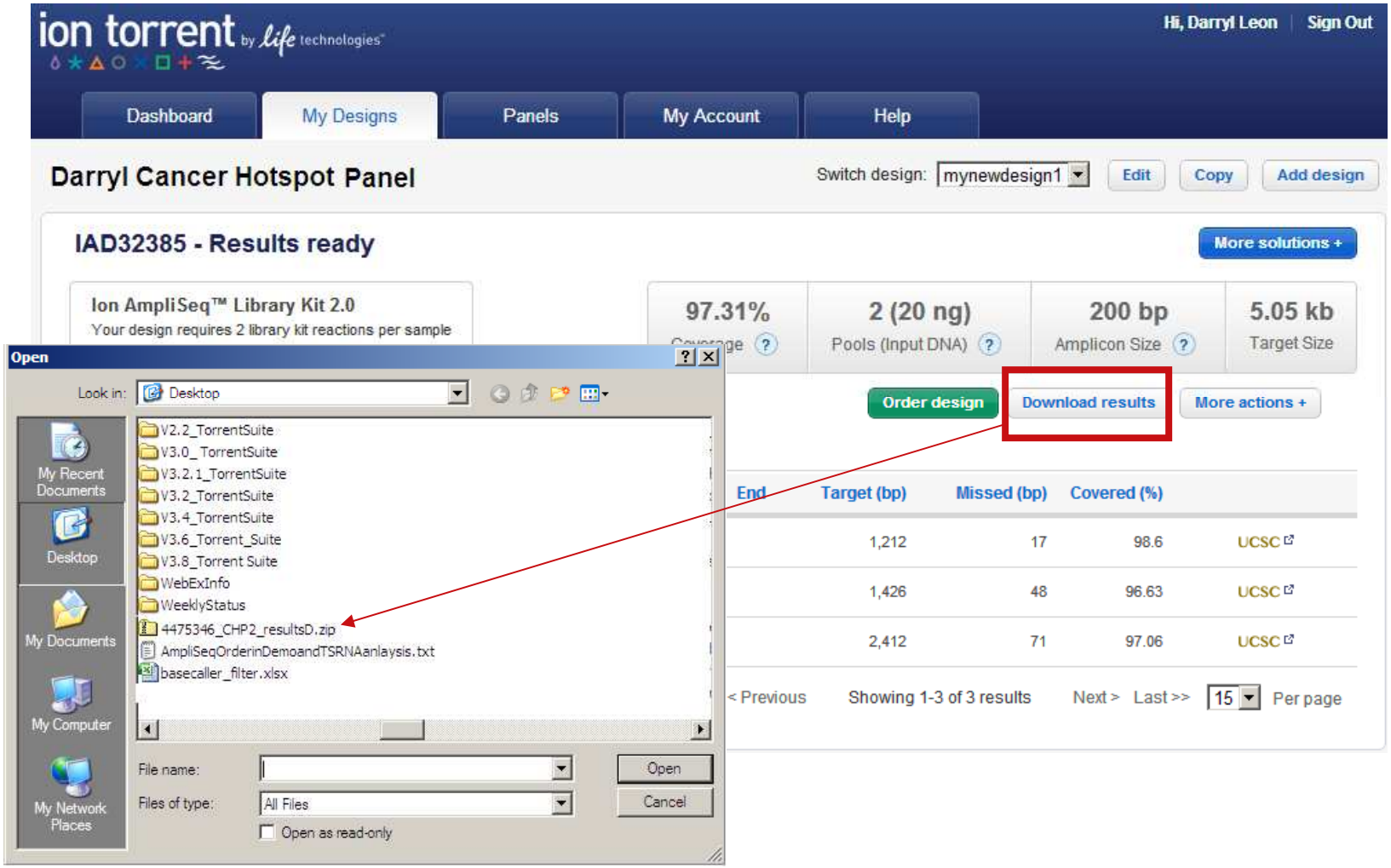
Download zip file from Ion AmpliSeq™ Designer to local computer



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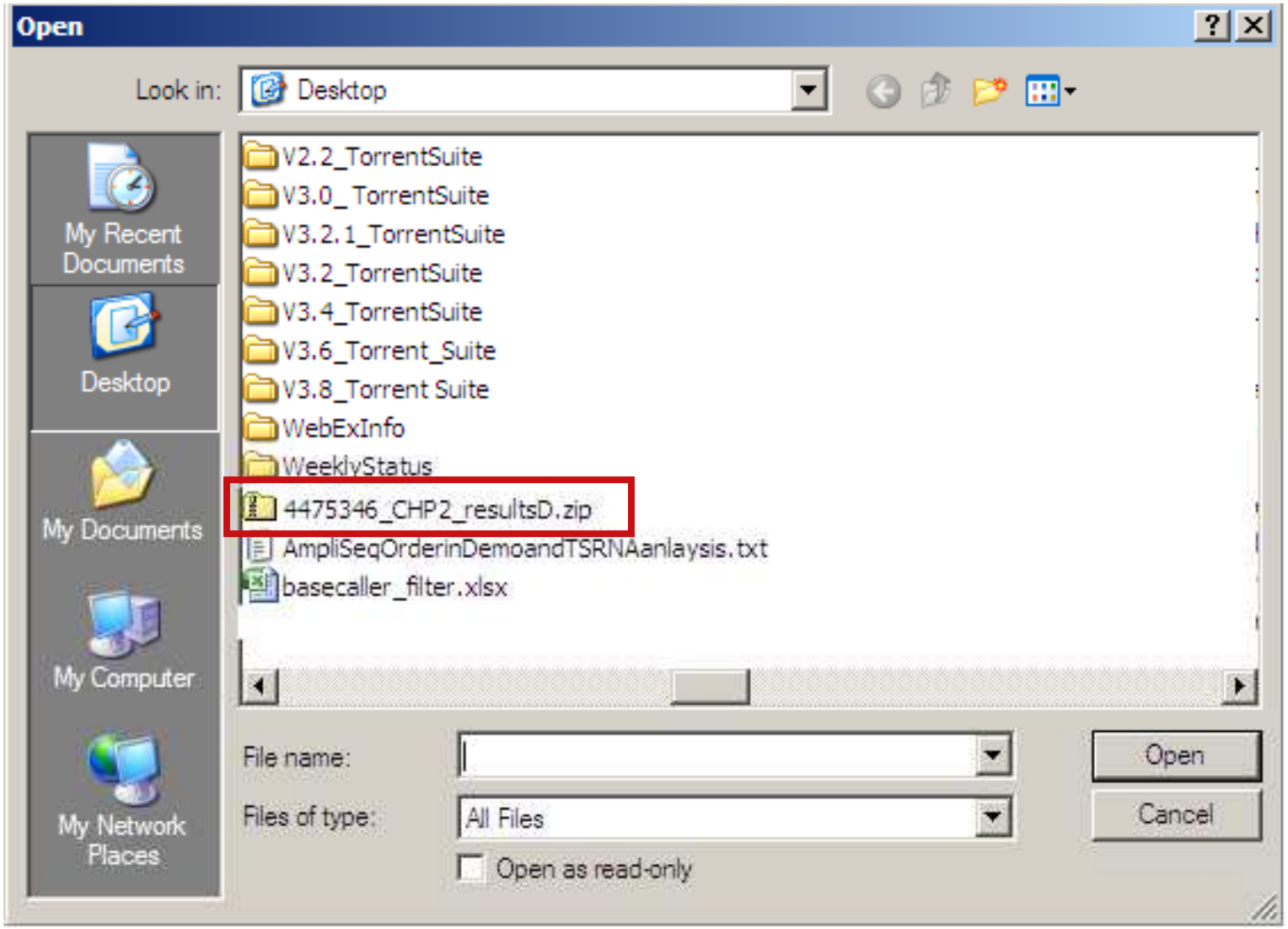
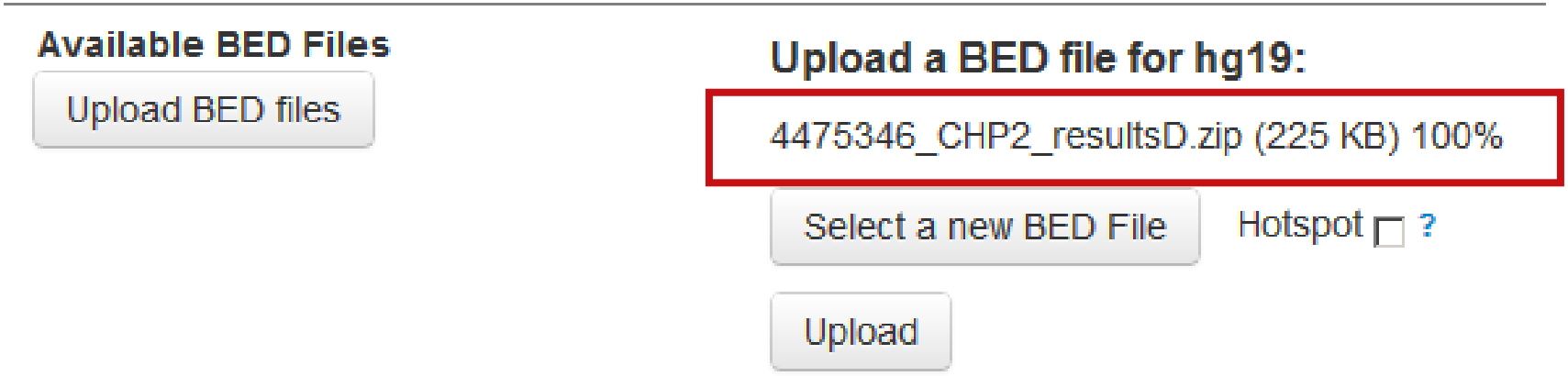
Navigate to Ion AmpliSeq™ Designer Zipped file and Upload



ThermoFisher SCIENTIFIC

Verify Zipped BED File is Added



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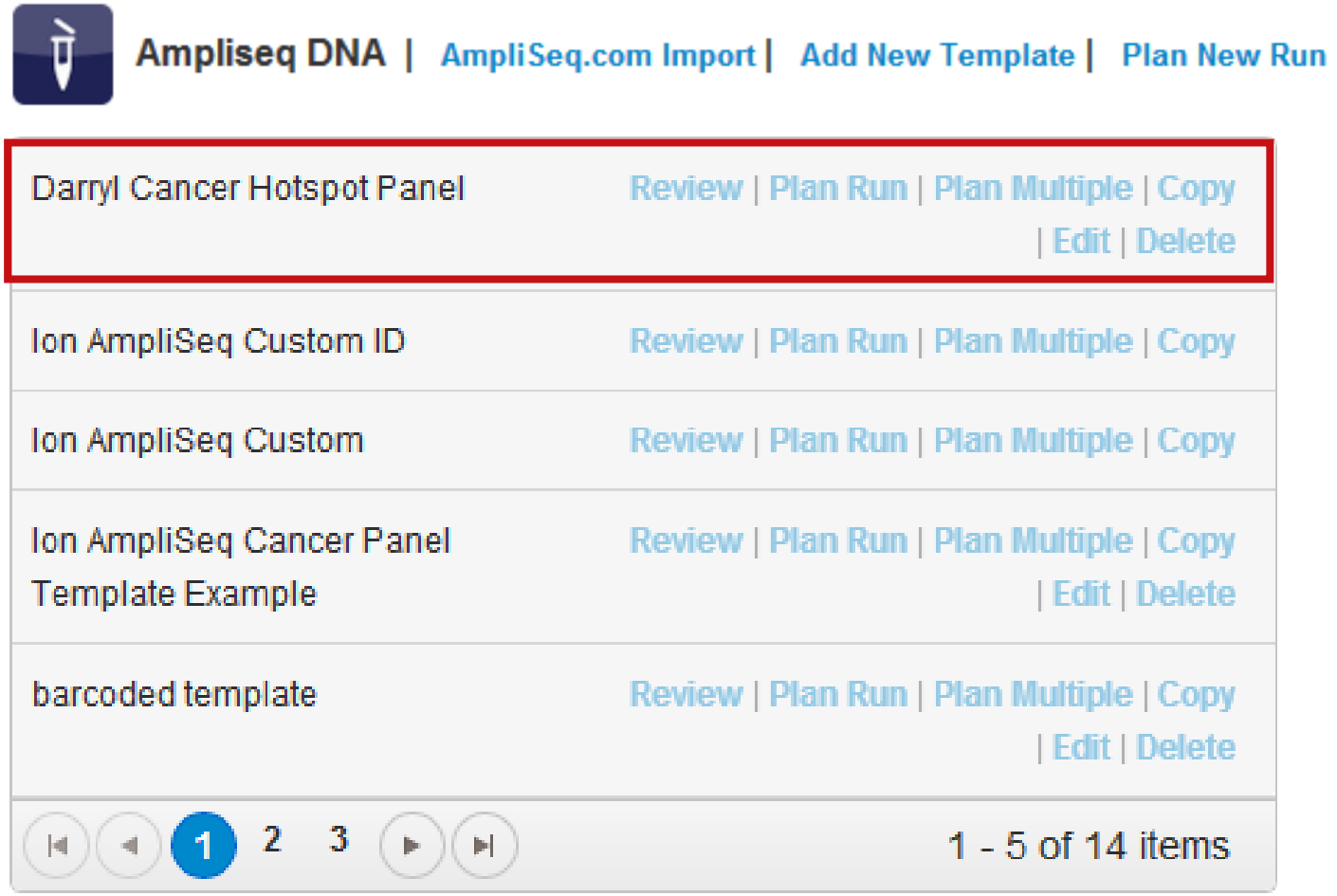
Verify Template is Automatically Generated



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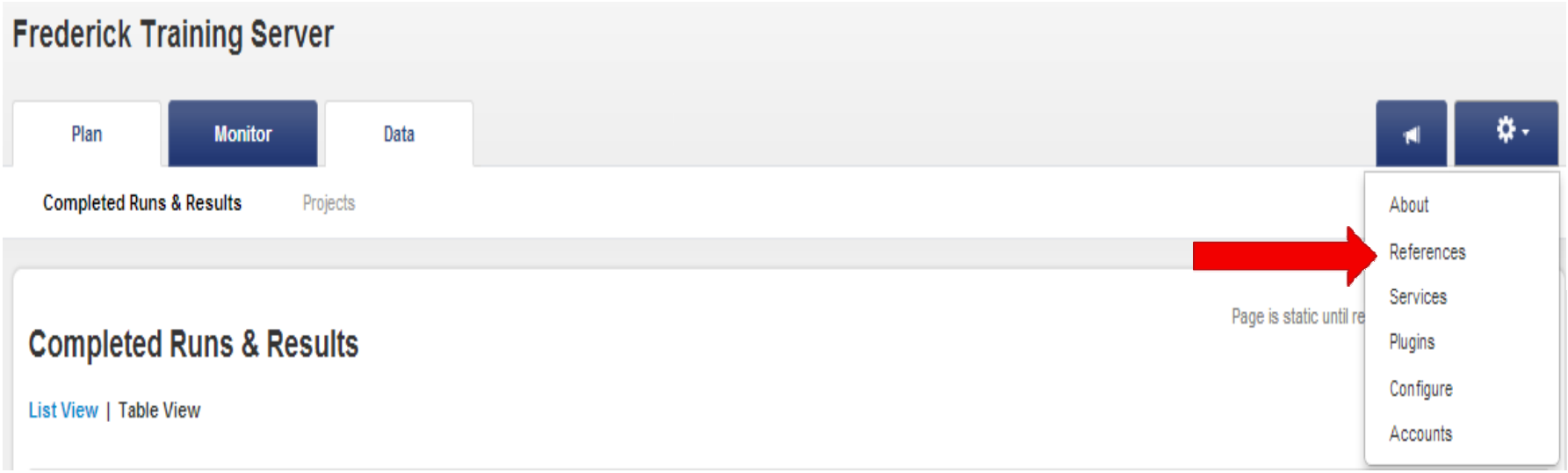
Select References in Torrent Browser



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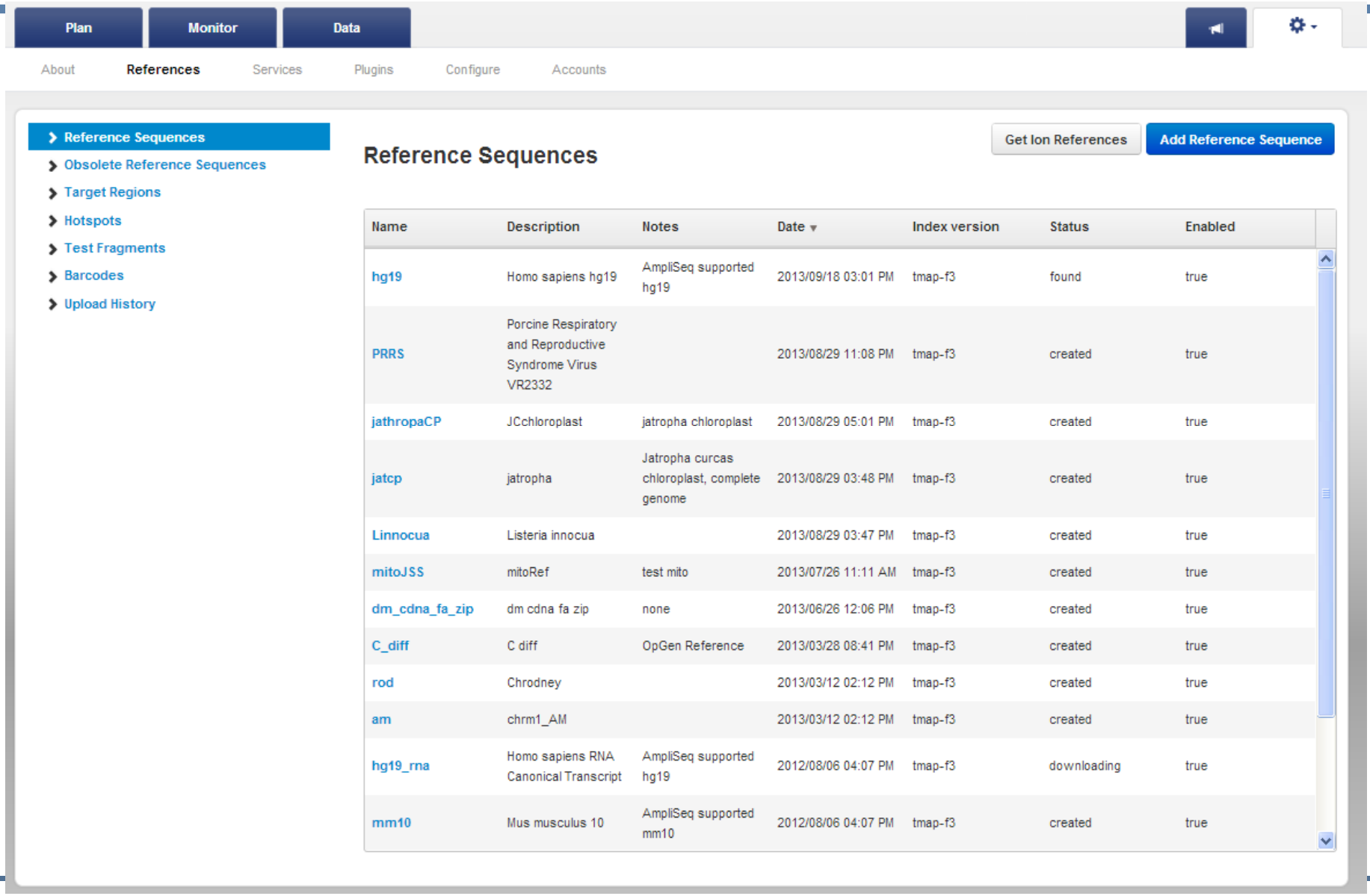
Select Reference Genome



Name	Description	Notes	Date	Index version	Status	Enabled
hg19	Homo sapiens hg19	AmpliSeq supported hg19	2013/09/18 03:01 PM	tmap-f3	found	true
PRRS	Porcine Respiratory and Reproductive Syndrome Virus VR2332		2013/08/29 11:08 PM	tmap-f3	created	true
jathropaCP	JCchloroplast	jatropha chloroplast	2013/08/29 05:01 PM	tmap-f3	created	true
jatcp	jatropha	Jatropha curcas chloroplast, complete genome	2013/08/29 03:48 PM	tmap-f3	created	true
Linnocua	Listeria innocua		2013/08/29 03:47 PM	tmap-f3	created	true
mitoJSS	mitoRef	test mito	2013/07/26 11:11 AM	tmap-f3	created	true
dm_cdna_fa_zip	dm cdna fa zip	none	2013/06/26 12:06 PM	tmap-f3	created	true
C_diff	C diff	OpGen Reference	2013/03/28 08:41 PM	tmap-f3	created	true
rod	Chrodney		2013/03/12 02:12 PM	tmap-f3	created	true
am	chrm1_AM		2013/03/12 02:12 PM	tmap-f3	created	true
hg19_rna	Homo sapiens RNA Canonical Transcript	AmpliSeq supported hg19	2012/08/06 04:07 PM	tmap-f3	downloading	true
mm10	Mus musculus 10	AmpliSeq supported mm10	2012/08/06 04:07 PM	tmap-f3	created	true

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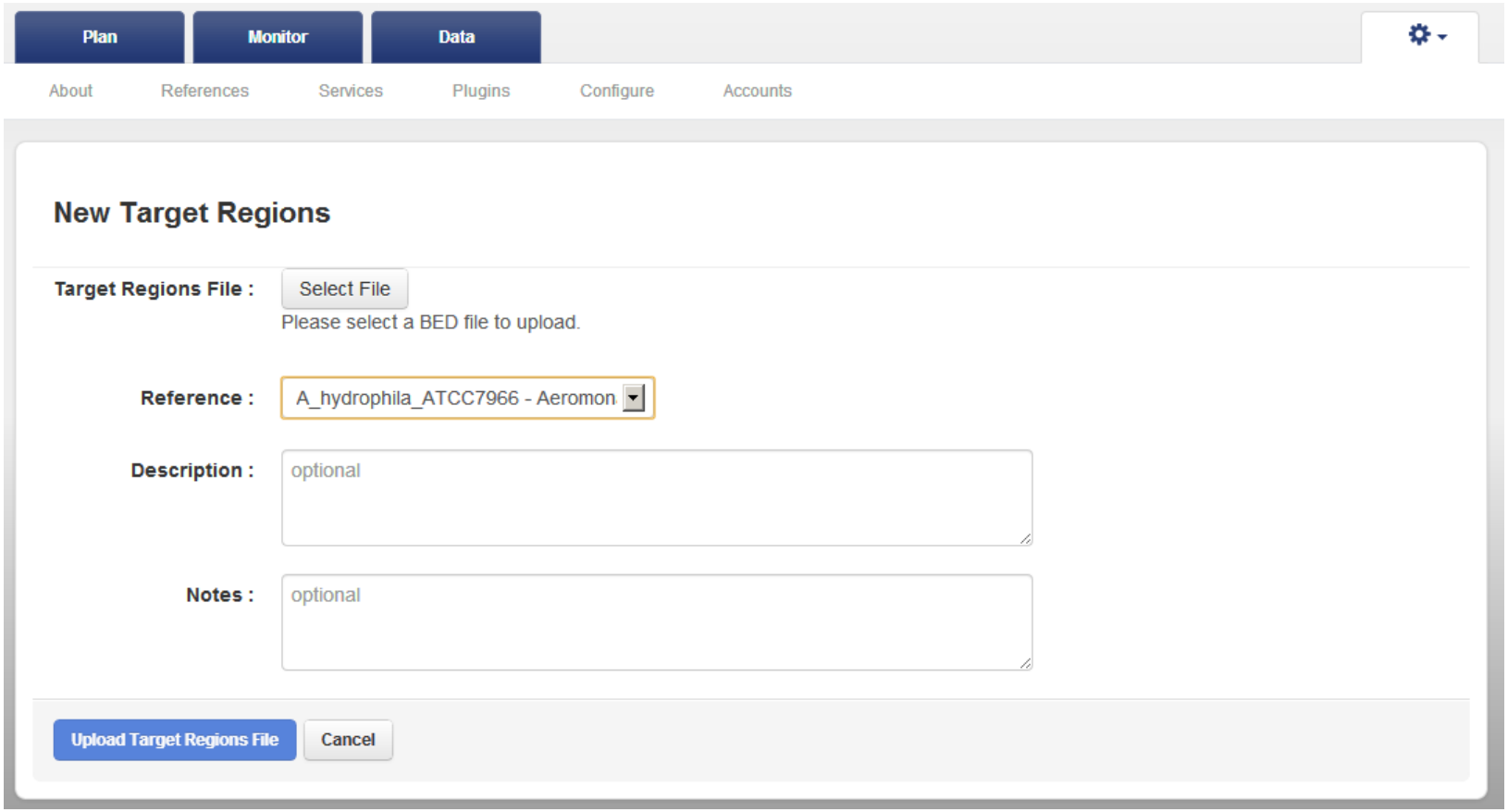
Target File Selection



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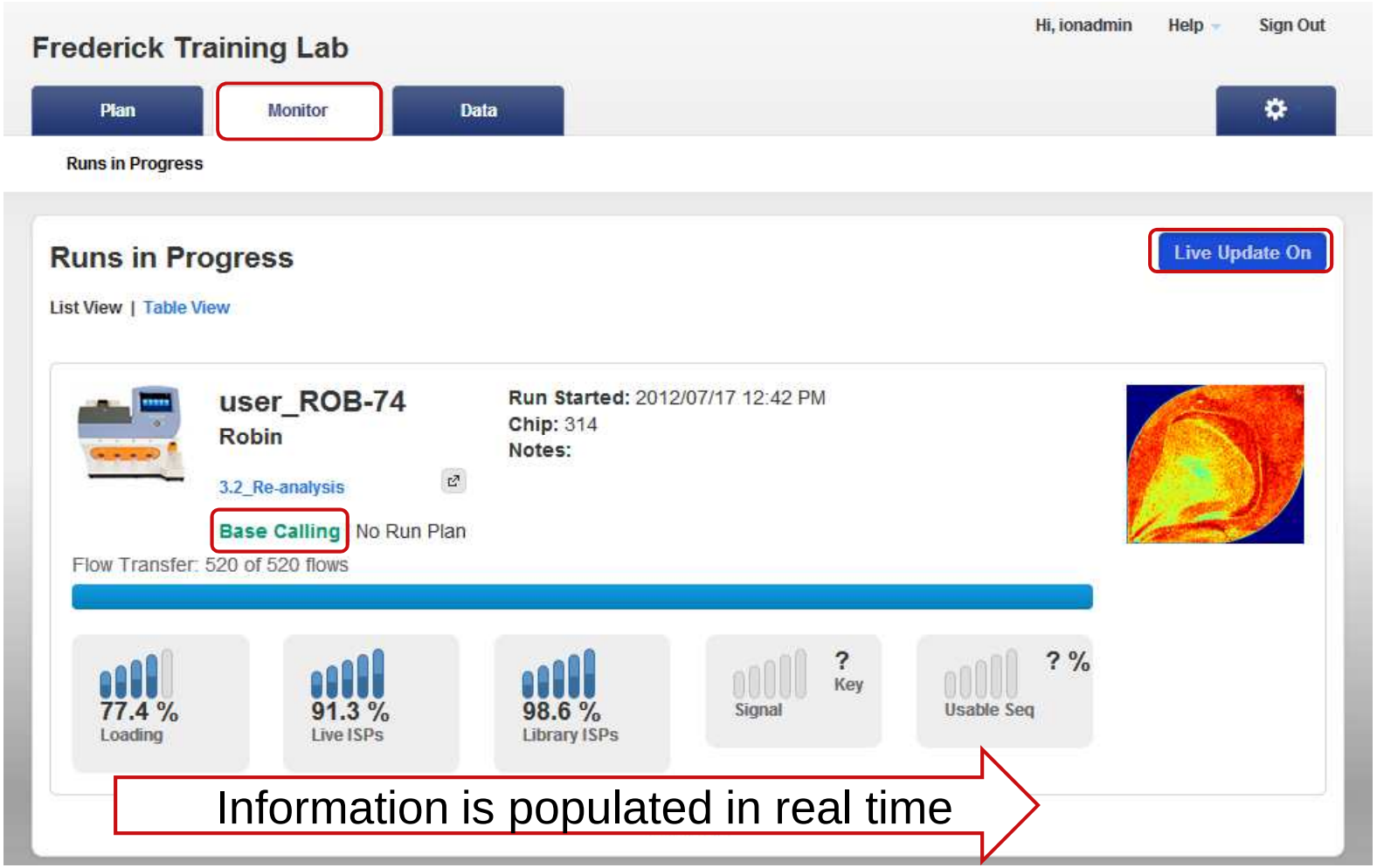
Monitor Tab



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Data Tab – Table View & List View

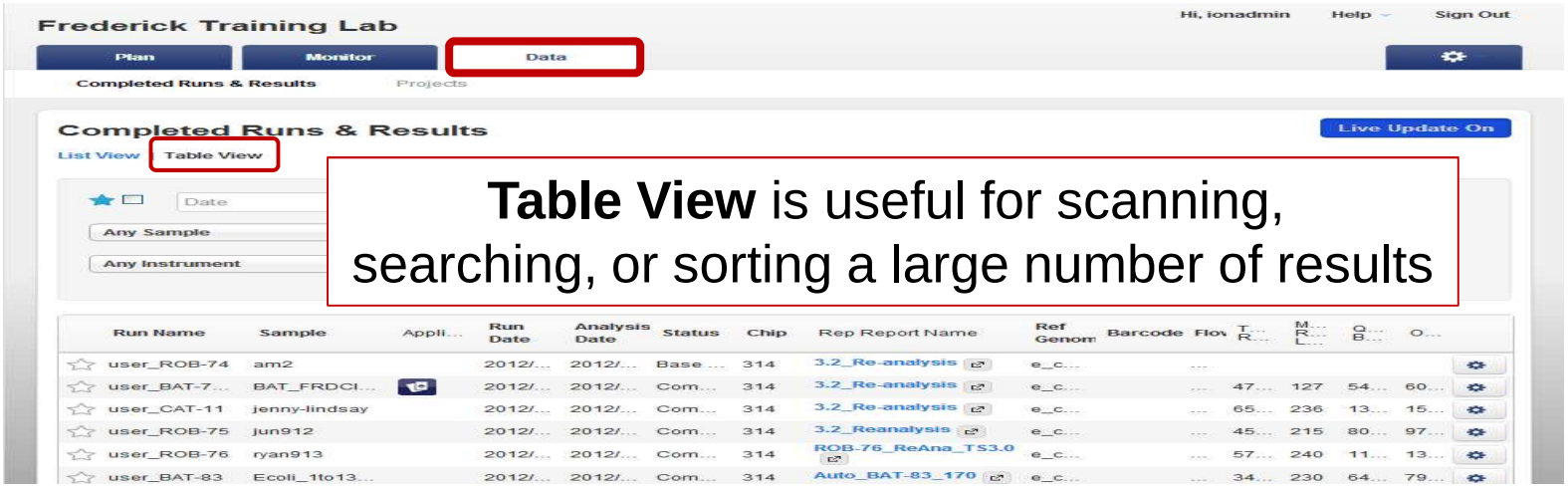
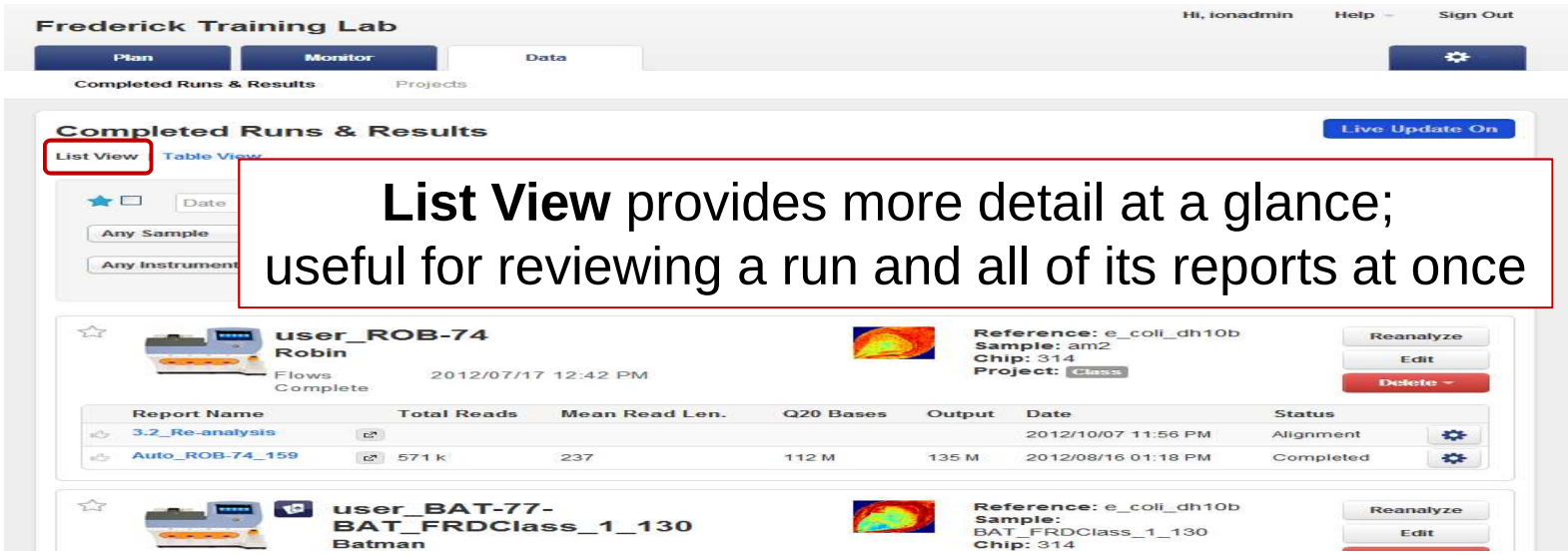


Table View is useful for scanning, searching, or sorting a large number of results



List View provides more detail at a glance; useful for reviewing a run and all of its reports at once

Screen shot is from TS 3.2; other versions may vary.

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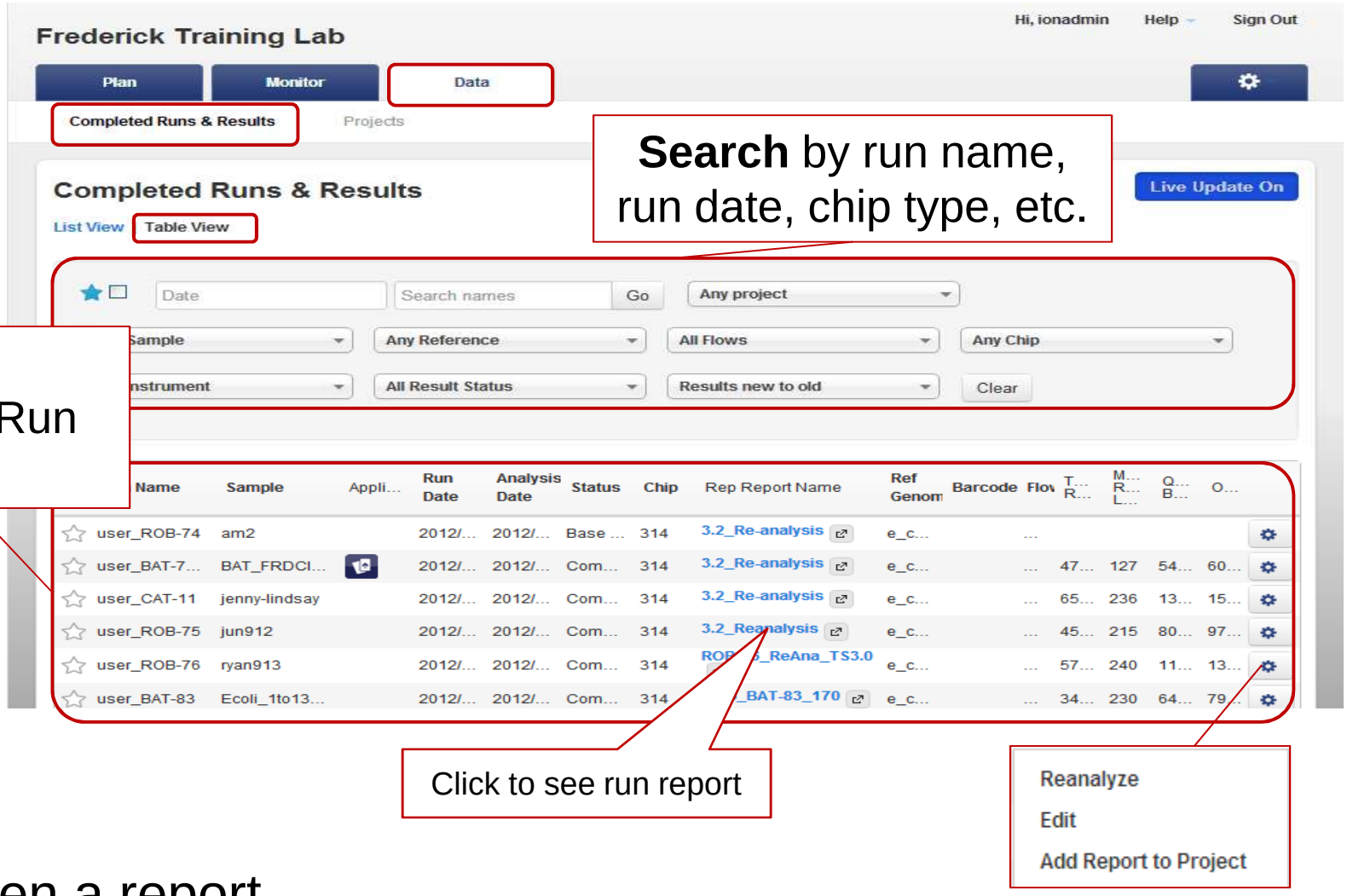
Data Tab



Open a report

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Run Report - Overview

Navigation bar

Barcoding table (if applicable)

Unaligned/ Aligned metrics

Plugin summaries

Output files

Screen shot is from TS 3.2; other versions may vary.

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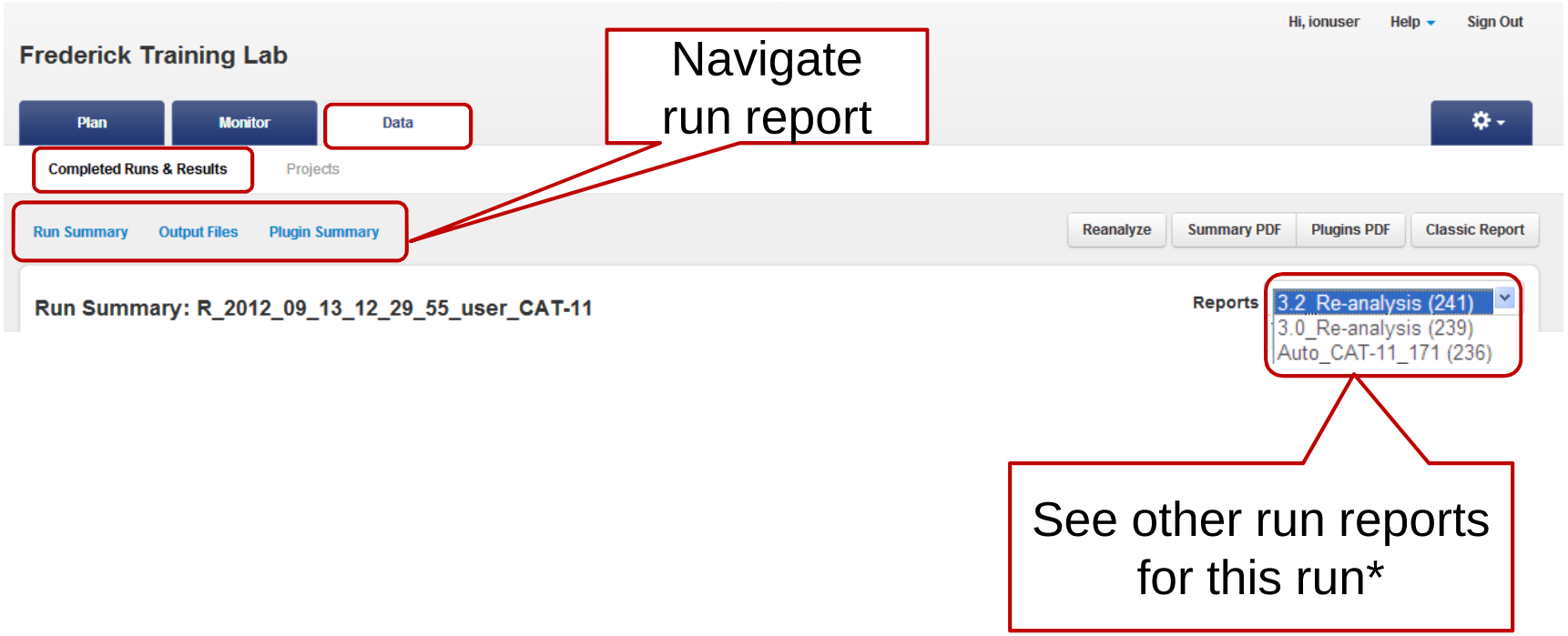
Run Report – Top



* Most recent analysis defaults as representative run report. User can change representative run report.

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Run Report – Barcoding information (if used)

Barcode Name	Sample	Bases	>=Q20 Bases	Reads	Mean Read Length	Read Length Histogram	Files
No barcode	None	1,855,688	1,516,254	25,940	71 bp		BAM BAI
IonXpress_001	None	29,580	24,580	268	110 bp		BAM BAI
IonXpress_002	None	24,070	20,293	219	109 bp		BAM BAI
IonXpress_003	None	54,162	44,683	485	111 bp		BAM BAI
IonXpress_004	None	1,927,550	1,580,007	14,833	129 bp		BAM BAI

For each Barcode, we show…

- Total output (bases)
- AQ20 Bases
- AQ20 reads
- Mean AQ20 read length

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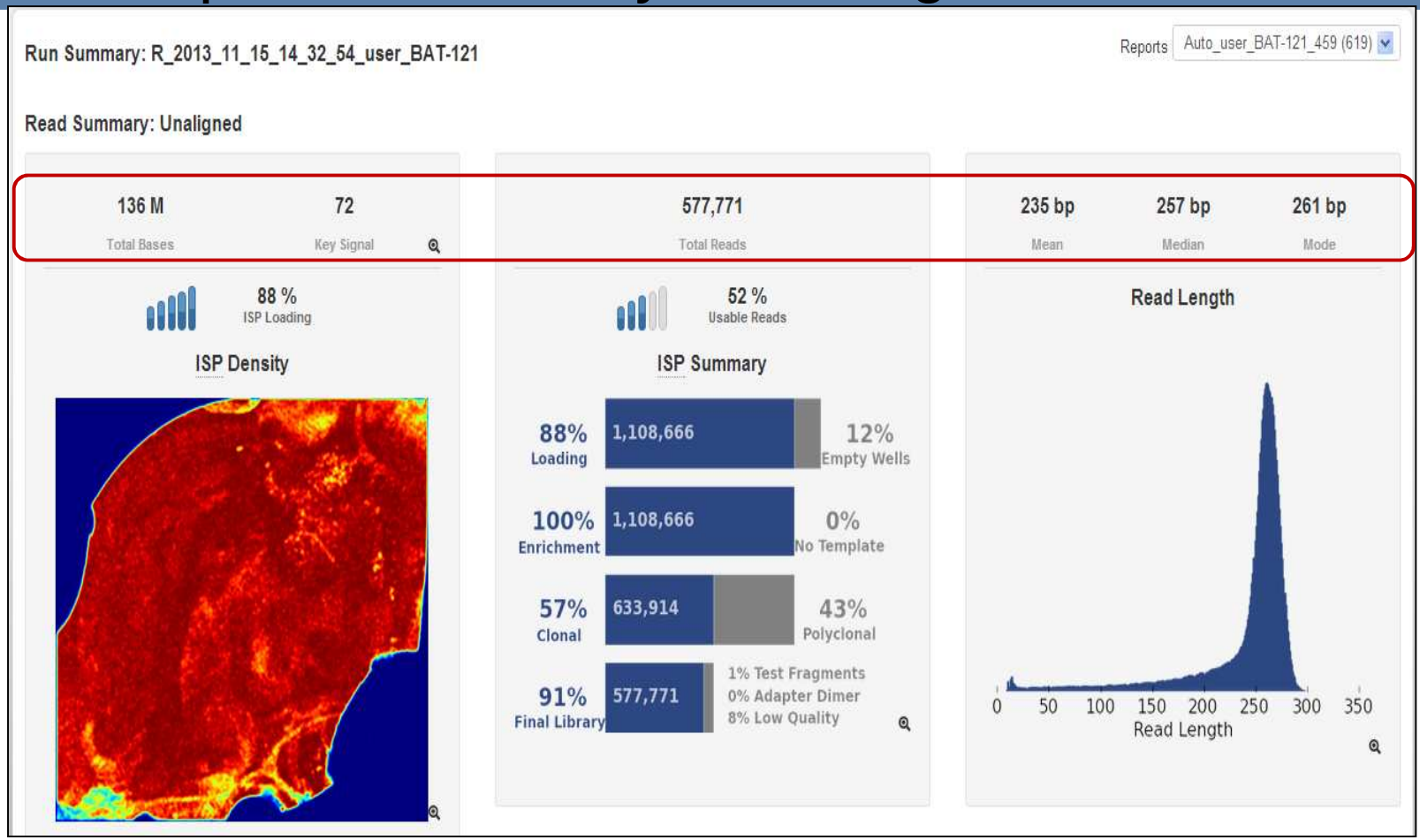
Run Report – Summary of Unaligned reads



Efficiency of chip loading

Percentage of wells that generated reads that passed filtering

Average read lengths

Information about bases and reads *before* mapping

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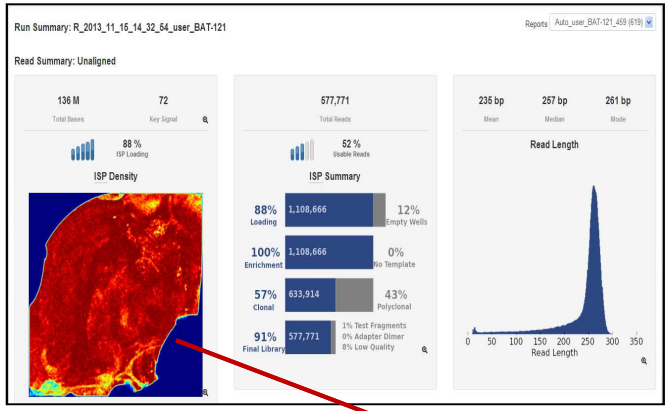
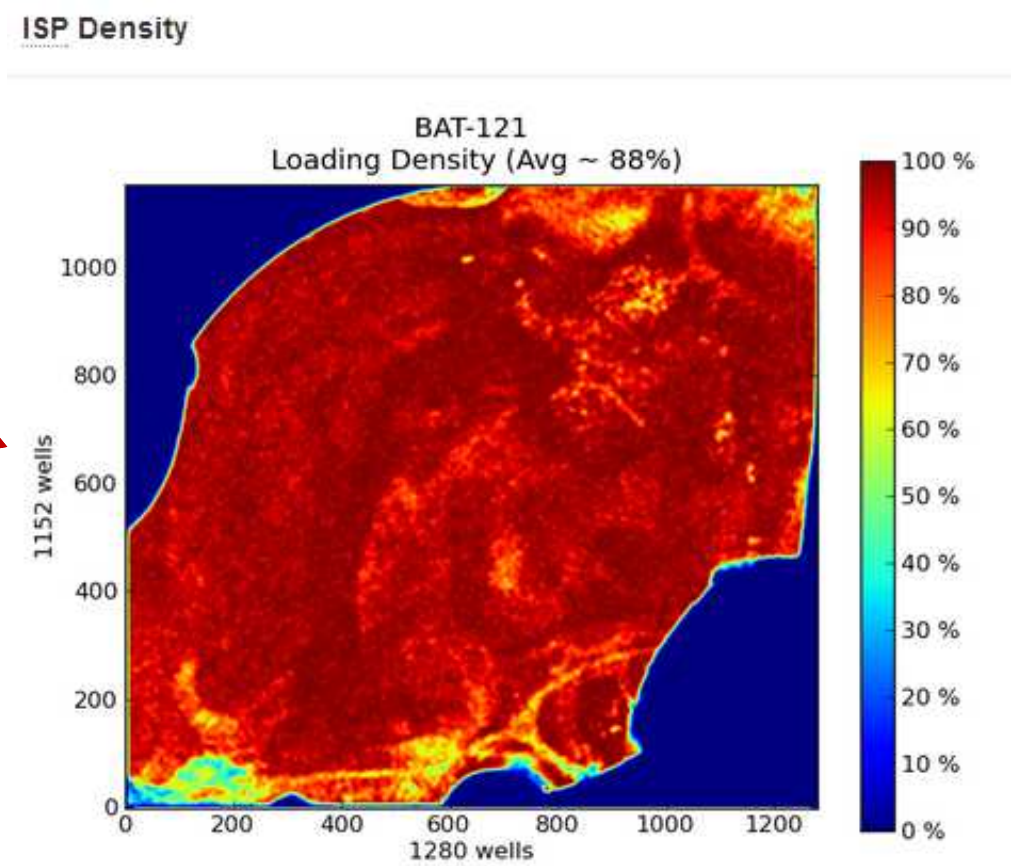
Chip Loading Image: Very Good Loading Example





Ion 314™v2 chip heat map, indicating percent loading across the physical surface

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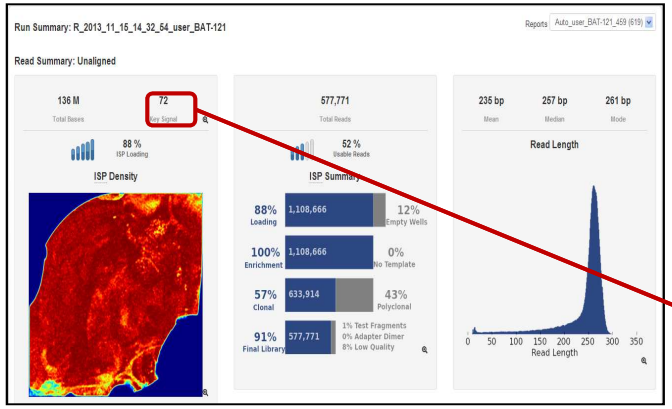
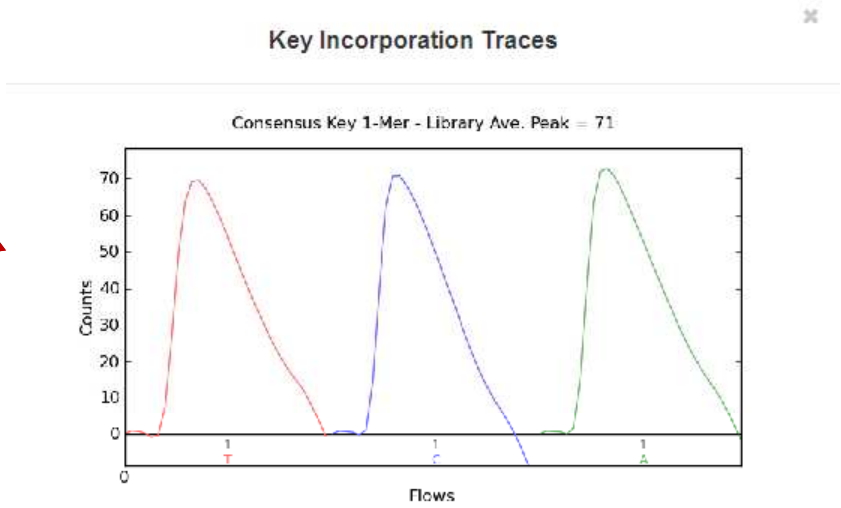
Run Report validation – Key Signal





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+ 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.

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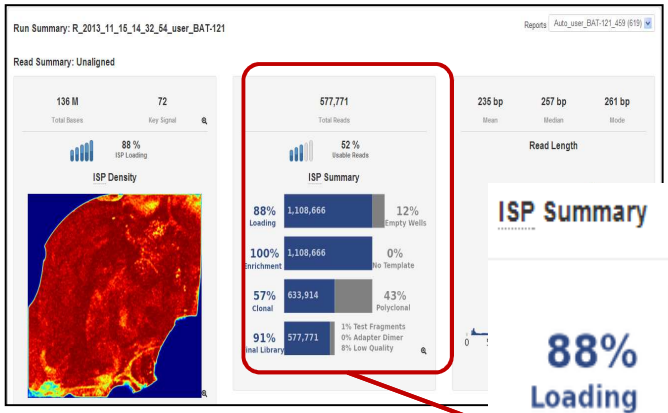
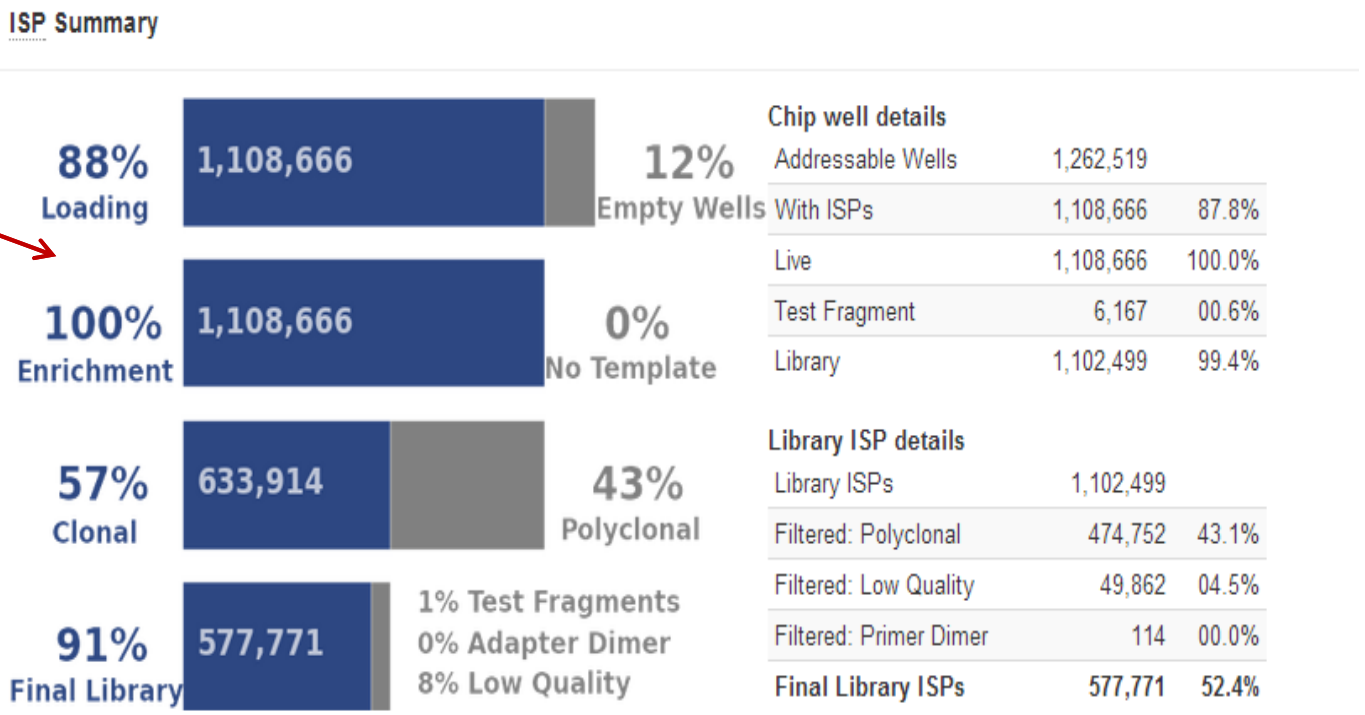
Run Report – Chip Well Details





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

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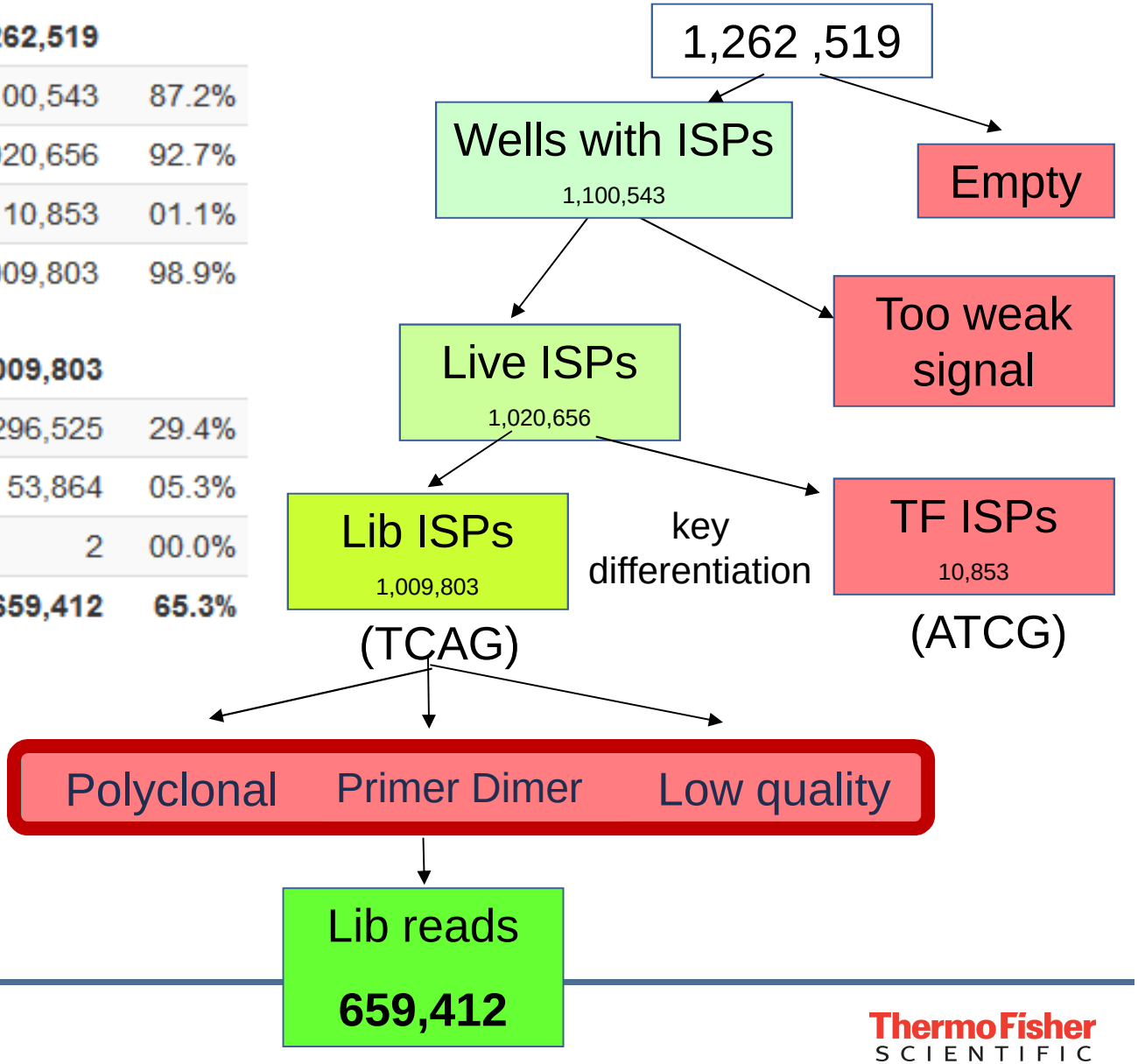
Ion Sphere™ Particle (ISP) Identification Summary

Addressable Wells	**1,262,519**	
With ISPs	1,100,543	87.2%
Live	1,020,656	92.7%
Test Fragment	10,853	01.1%
Library	1,009,803	98.9%
Library ISPs	**1,009,803**	
Filtered: Polyclonal	296,525	29.4%
Filtered: Low Quality	53,864	05.3%
Filtered: Primer Dimer	2	00.0%
Final Library ISPs	**659,412**	**65.3%**

Percentage of sequence available for analysis after filtering

Mouse over for help



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Ion Sphere™ Particle (ISP) Identification Enrichment Calculation

Addressable Wells	**1,262,519**	
With ISPs	1,100,543	87.2%
Live	1,020,656	92.7%
Test Fragment	10,853	01.1%
Library	1,009,803	98.9%
Library ISPs	**1,009,803**	
Filtered: Polyclonal	296,525	29.4%
Filtered: Low Quality	53,864	05.3%
Filtered: Primer Dimer	2	00.0%
Final Library ISPs	**659,412**	**65.3%**

$$\%\ \text{enrich} = \frac{\text{Lib ISPs}}{(\text{Wells with ISPs} - \text{TF ISPs})}$$

Lib ISPs: 1,009,803

Wells with ISPs: 1,100,543

TF ISPs: 10,853

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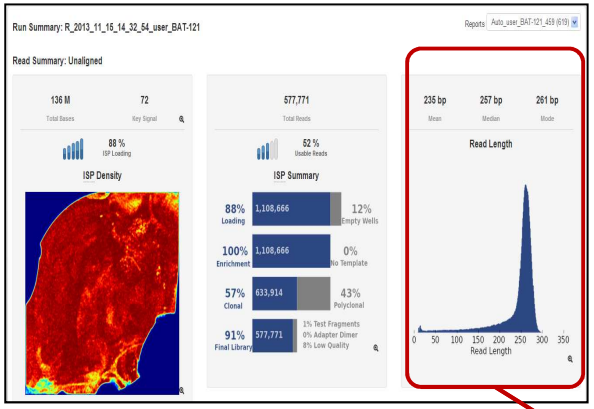
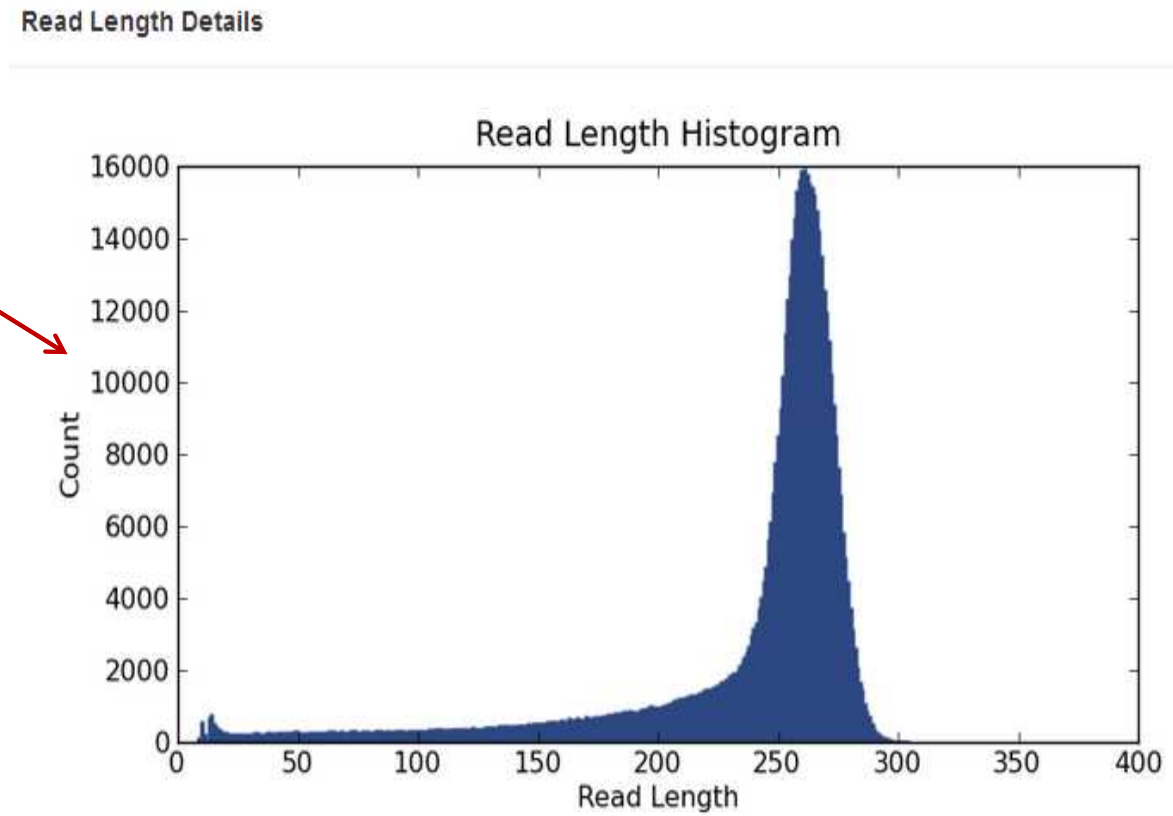
Run Report – Read Length Details



Histogram of read length for all reads in output files



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What does Q20 mean?

Predicted Quality

Q: What does Q17/Q20 mean?

A: The **Q20 length** value attributed to a read is an estimate of the read length at which the predicted total error rate in the read will correspond to a Phred-scale quality score of 20. The Phred scale is defined as ***-10×log10(error probability)***, so Q20 corresponds to an error rate of 1% and Q17 corresponds to an error rate of 2%. Please, consult the following link for a more complete description of Phred values: http://en.wikipedia.org/wiki/Phred_quality_score.

The **Q20 length** is determined by looking at the per-base quality scores to estimate the total read error rate at every position in the read. For example, if the first base had a predicted quality score of 20 and the second base had a predicted quality score of 17, then the estimated total read error for the first two bases would be ***0.5×1% + 0.5×2% = 1.5%***. This total read error rate is evaluated at every position in the read, after which the maximal position at which the total read error rate is 1% or less is identified. This position defines the Q20 length of the read.

The Q20 length is derived entirely from the predicted per-base quality scores. The predicted quality scores are somewhat conservative and in cases where alignment to a reference sequence is also possible users will generally find that the predicted Q17 lengths tend on average to be shorter than the corresponding AQ17 lengths which are based on the actual as opposed to predicted errors (see the following section on AQ20/AQ17)

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What does AQ20 mean?

- Alignment of reads is a process to map the read to a reference
- Any discrepancy in alignment to a reference is listed as a mismatch, and described by a mapping quality score
- In Torrent Browser, throughput reads are >= AQ20 reads
- AQ20 length of a read is the longest length at which the aligned read has a mismatch rate of 1% or less
- AQ20 content is the sum of all the AQ20 lengths

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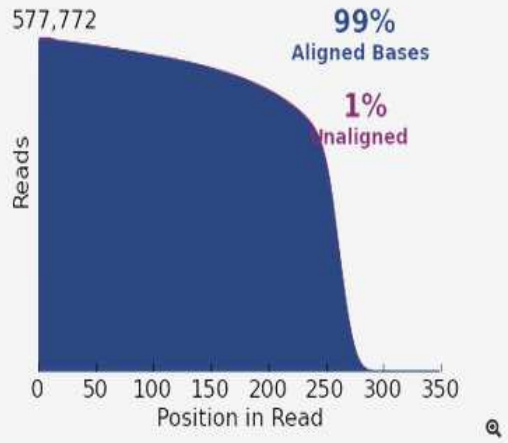
Run Report – Reads Aligned to Reference

Aligned to E. coli DH10B

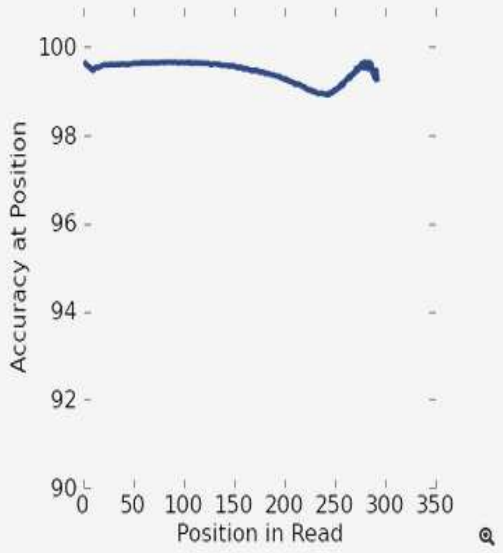
135 M
Total Aligned Bases

28.8X
Reference Coverage



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

99.5%
Mean Raw Accuracy 1x



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

Shows metrics gathered *after* mapping to reference.

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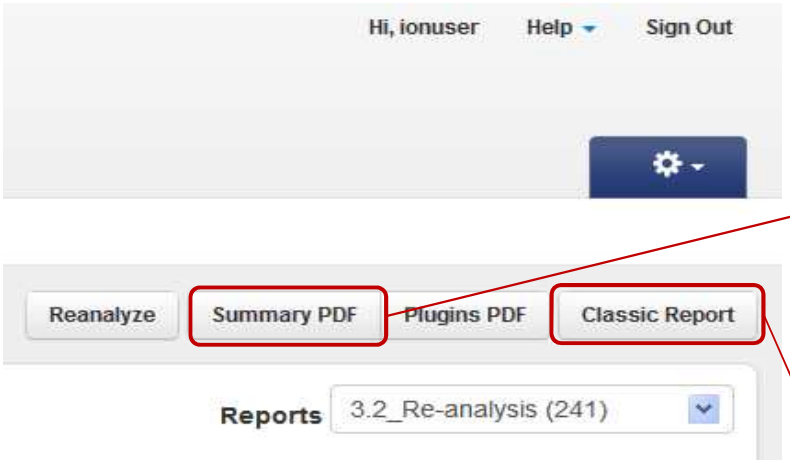
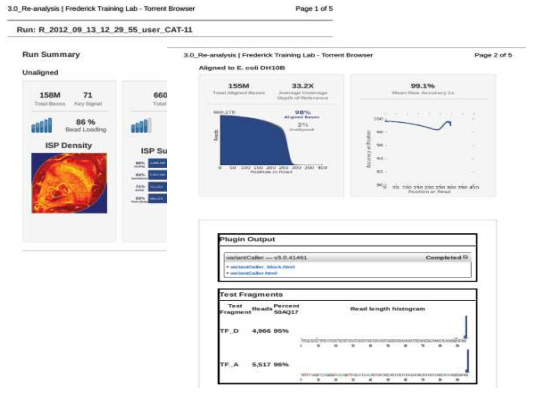
Run Report – Output Files





File format of older version

Output Files

File Type	Unaligned Reads			Aligned Reads	
Library	BAM	SFF	FASTQ	BAM	BAI
Barcodes	BAM	SFF	FASTQ	BAM	BAI

Barcode Name	Sample	Bases	>=Q20 Bases	Reads	Mean Read Length	Read Length Histogram	Files
No barcode	None	3,919,092	3,098,417	42,495	92 bp		BAM BAI
IonXpress_007	None	809,623,089	671,266,731	4,673,571	173 bp		BAM BAI

1 | 10 items per page | 1 - 2 of 2 items

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

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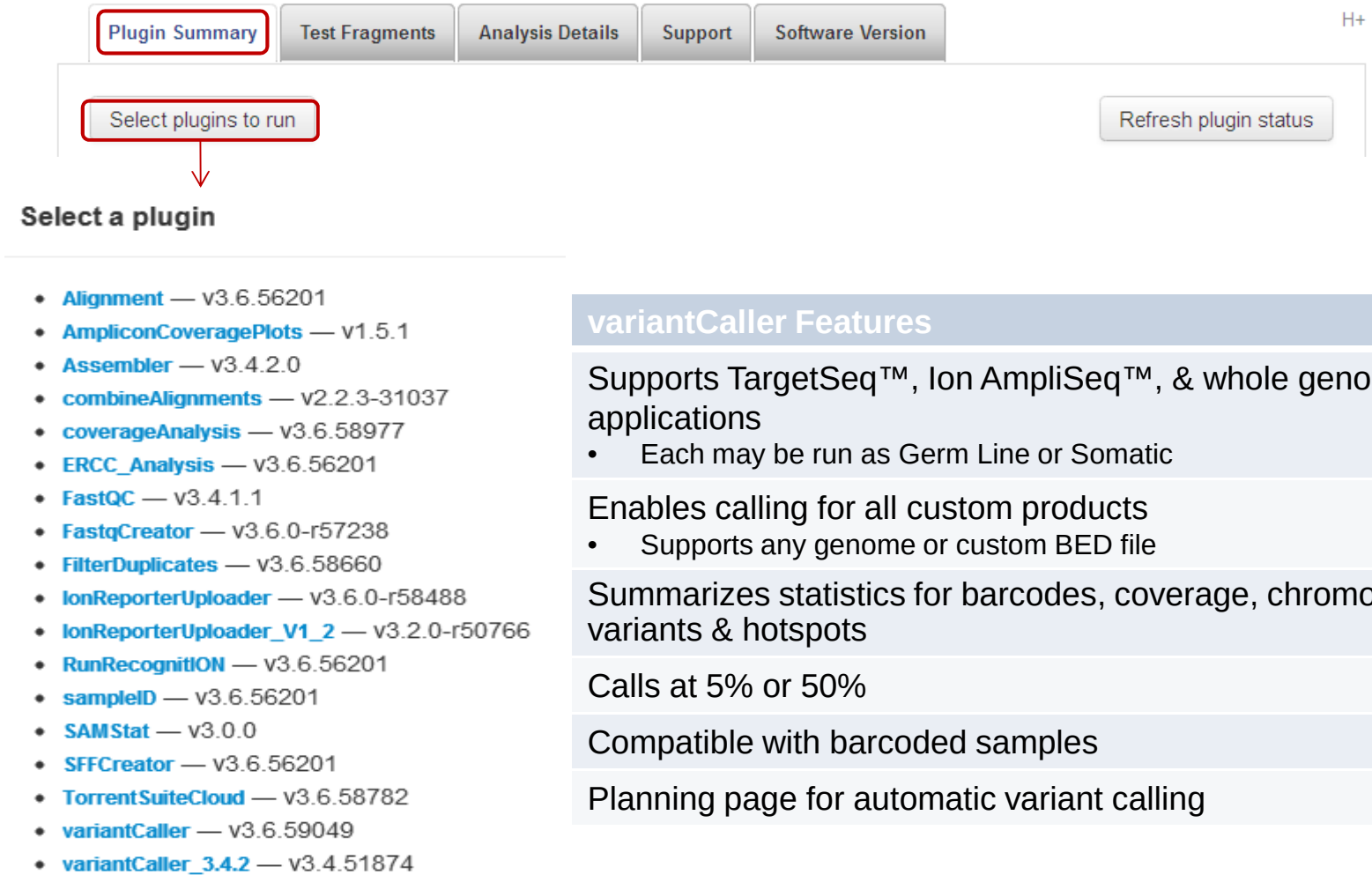
Plugin Summary



variantCaller Features
Supports TargetSeq™, Ion AmpliSeq™, & whole genome applications • Each may be run as Germ Line or Somatic
Enables calling for all custom products • Supports any genome or custom BED file
Summarizes statistics for barcodes, coverage, chromosomes, variants & hotspots
Calls at 5% or 50%
Compatible with barcoded samples
Planning page for automatic variant calling

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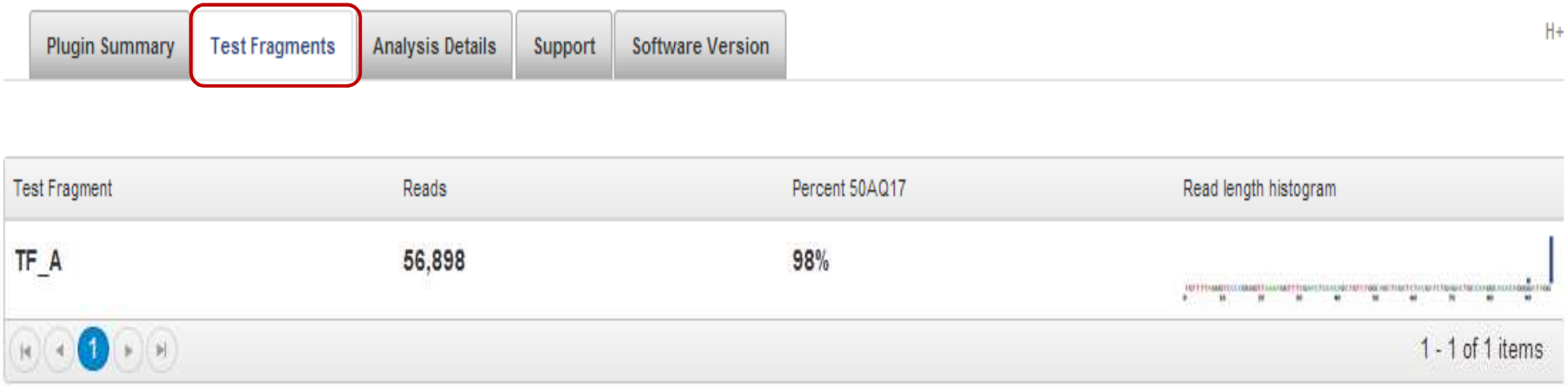
Test Fragment Summary



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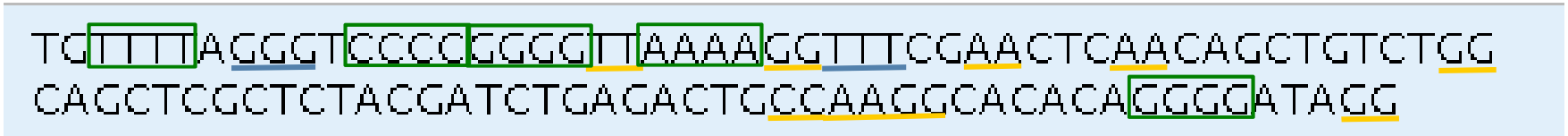
Run Report Validation: Test fragment Summary

TF_A



- Test Fragment designed with variable homopolymeric challenges (2mer, 3mer and 4mer)
- TFs are used for install validation
- TFs are spiked into sample for run validation
- No specification on test fragment sequencing performance
- Specific kit type may have different TFs

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Report Information

Plugin Summary | Test Fragments | **Analysis Details** | Support | Software Version

Run Name	R_2013_11_15_09_36_24_user_BAT-120
Run Date	Nov. 15, 2013, 9:36 a.m.
Run Flows	500
Projects	training
Sample	e.coli
Sample Tube Label	None
Reference	e_coli_dh10b
Instrument	Batman
Flow Order	TACGTACGTCTGAGCATCGATCG
Library Key	TCAG
TF Key	ATCG
Chip Check	Passed
Chip Type	314R
Chip Data	single
Barcode Set	
Analysis Name	Auto_user_BAT-120_456
Analysis Date	Nov. 15, 2013, 2:38 p.m.
Analysis Flows	500
runID	LXTUK

Plugin Summary | Test Fragments | Analysis Details | Support | **Software Version**

Torrent_Suite	4.0.1
host	68JKKQ1
ion-analysis	4.0.5-1
ion-dbreports	4.0.21-1
ion-gpu	4.0.0-1
ion-pipeline	4.0.6-1
ion-plugins	4.0.21-1
ion-torrentr	4.0.4-1
Script	21.5.5
LiveView	545
DataCollect	462
OS	20
Graphics	35

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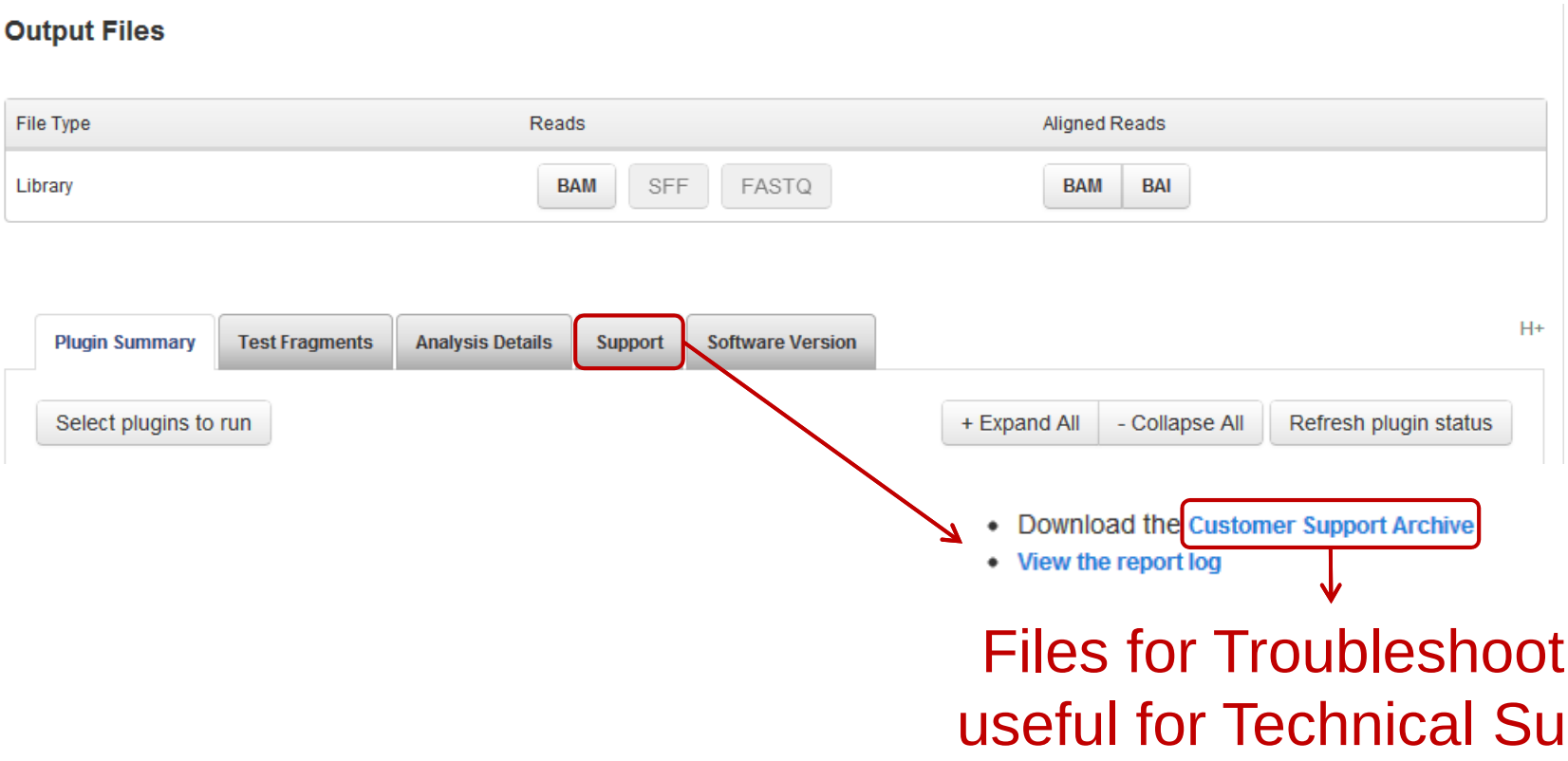
Customer Support Archive (CSA)



If you need troubleshooting /support, send in your CSA file (have it ready!)

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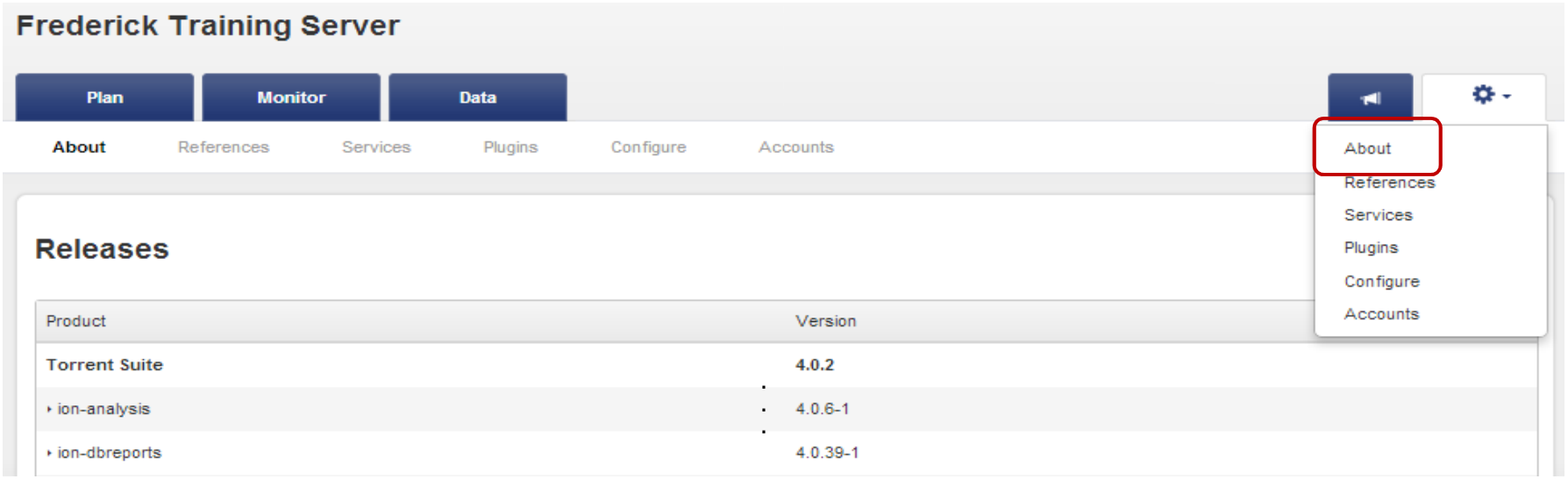
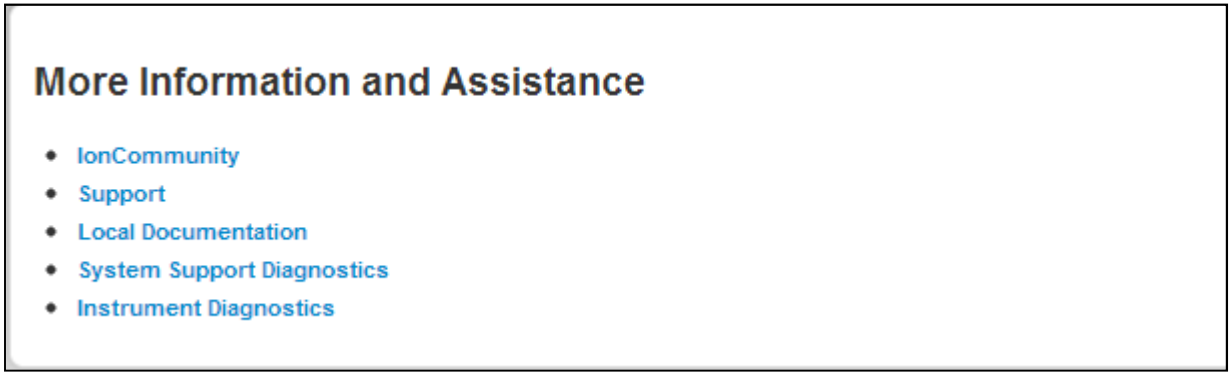
About Link





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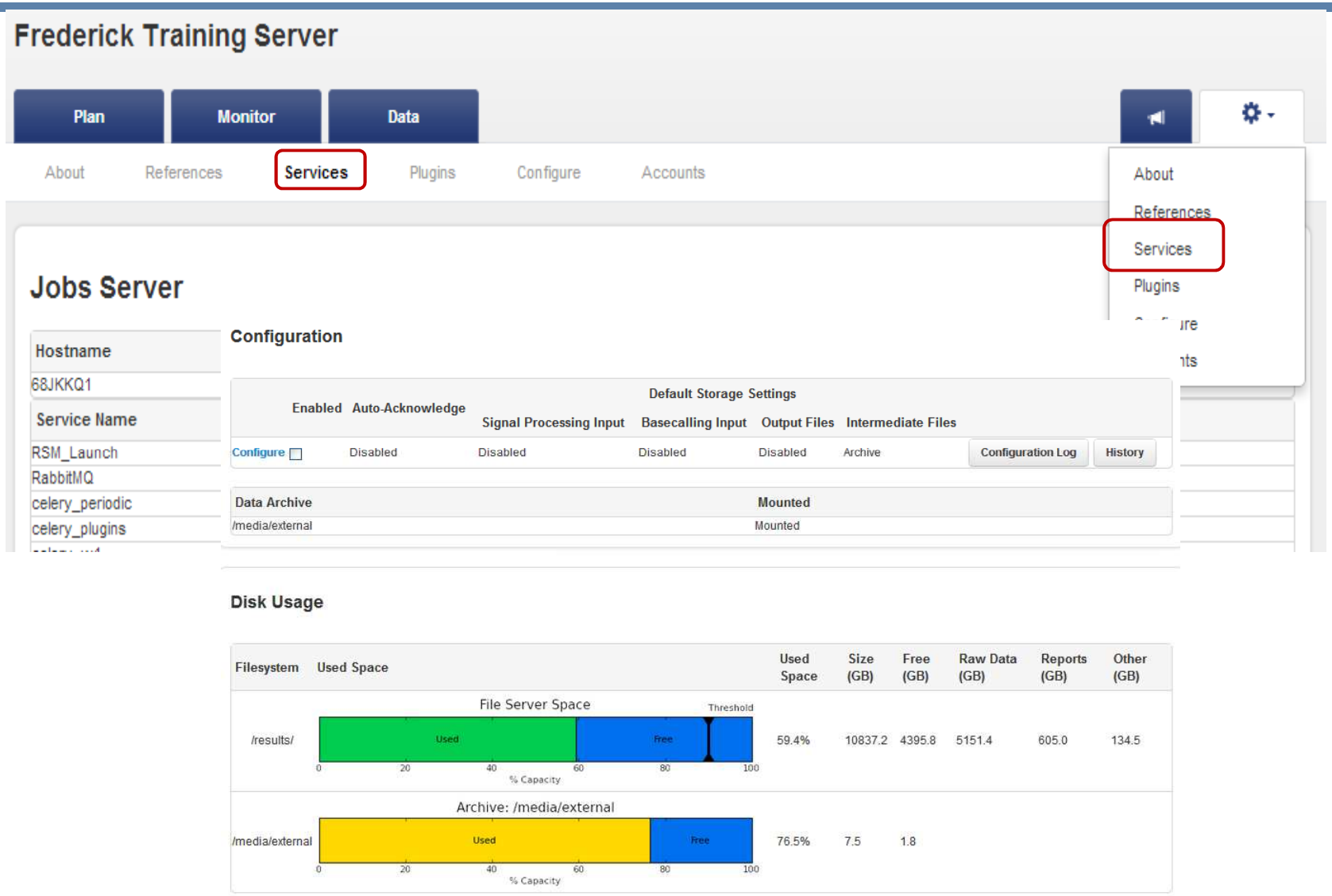
Services Link



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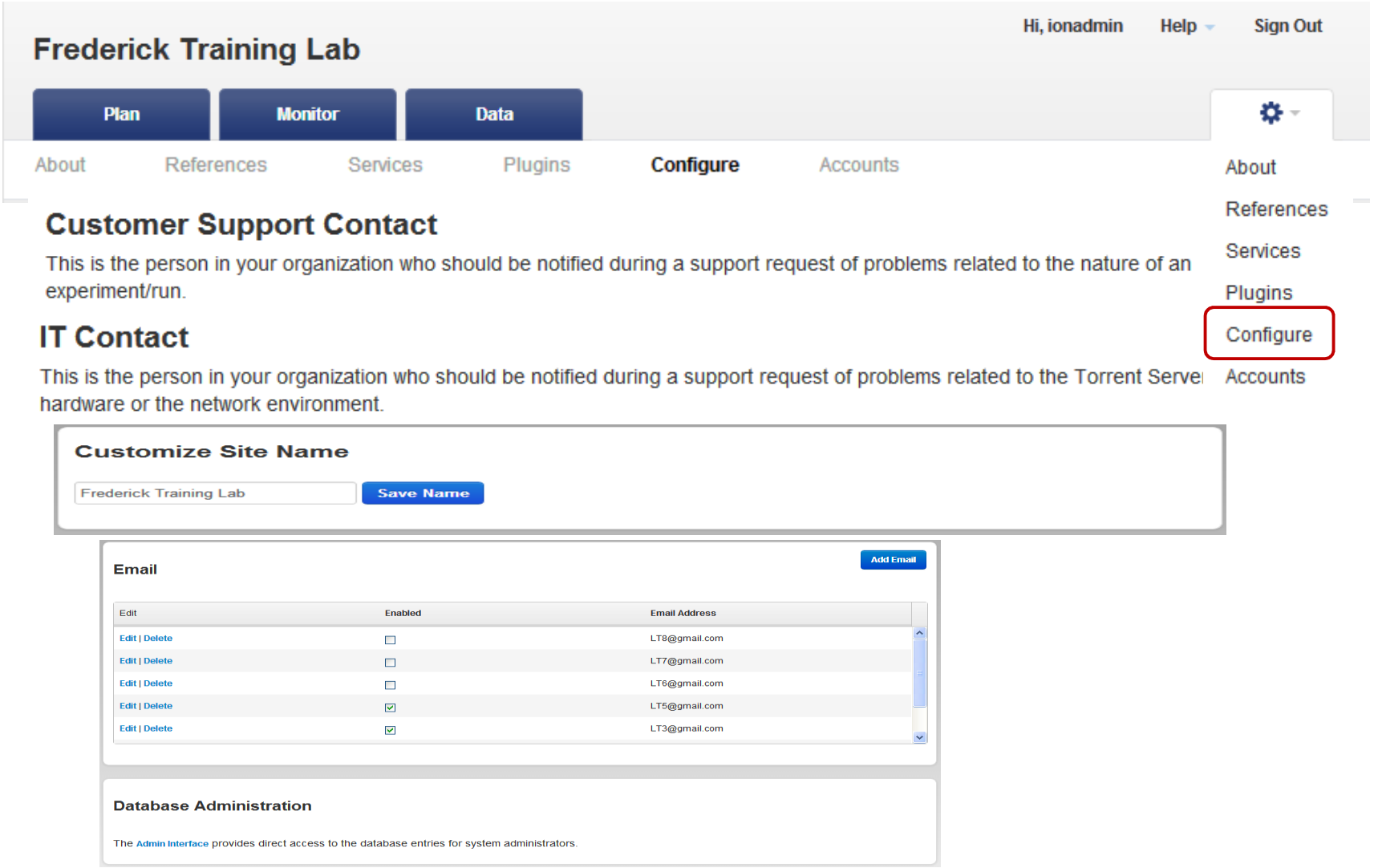
Configure Link



Screen shot is from TS 3.6; other versions may vary.

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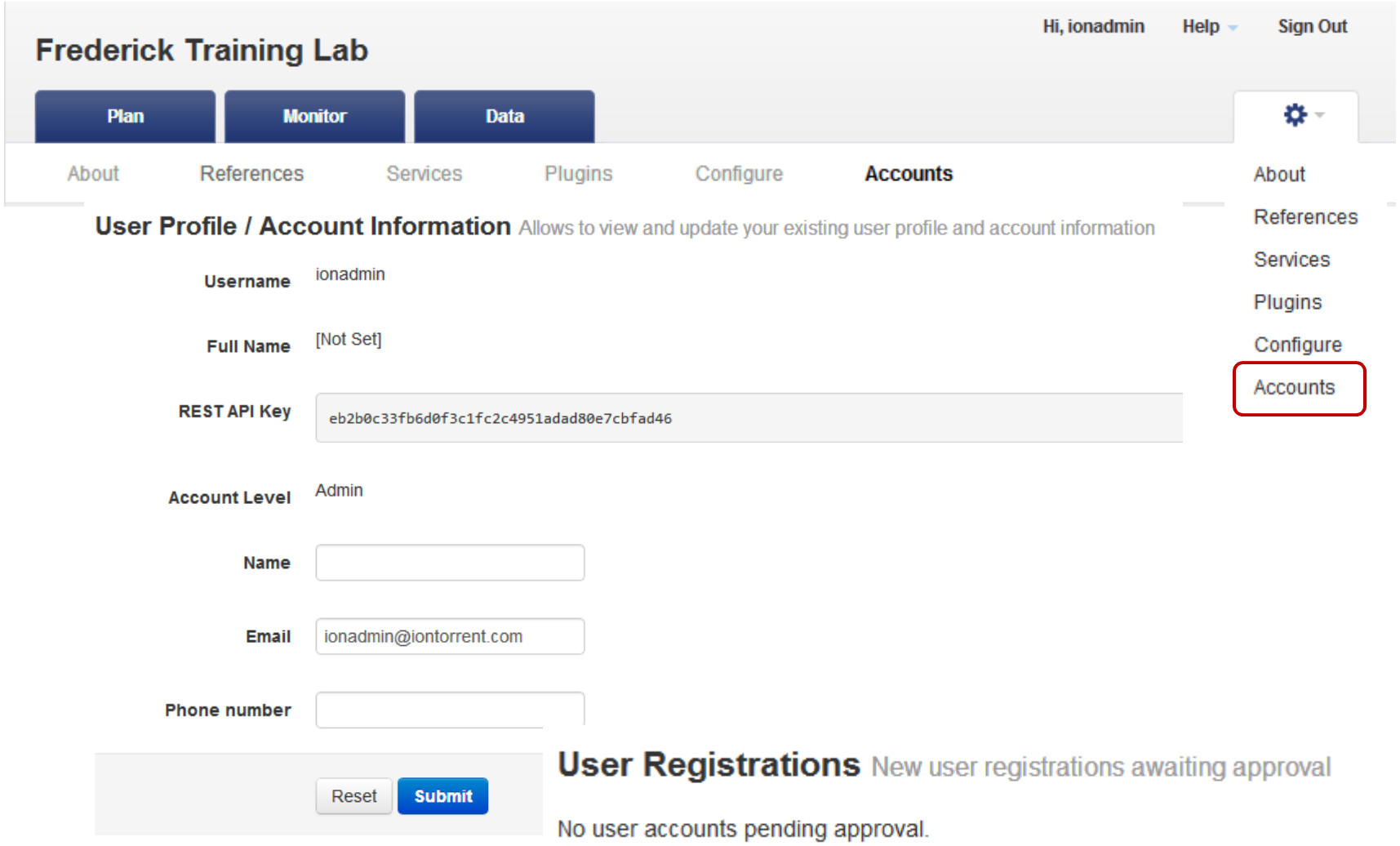
Accounts Link



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