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# Moving Implementation Mountains: Experiencing the Forensic Laboratory NGS and Bioinformatics Workflow Through Simulation

ISHI 2016 Workshop

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# An Introduction to the Ion S5™ and Ion Chef™ Technologies

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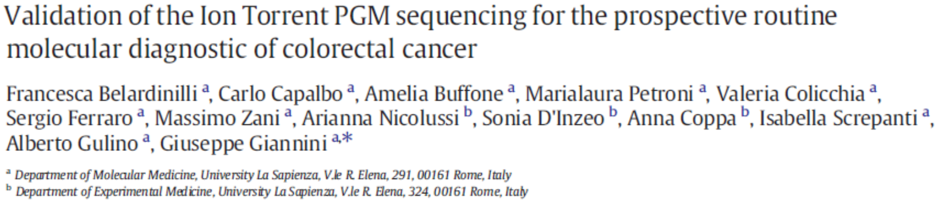
Department of Molecular and Medical Genetics
Institute of Applied Genetics
University of North Texas Health Science Center

# ADVANCEMENTS AND ADOPTION OF THE TECHNOLOGY

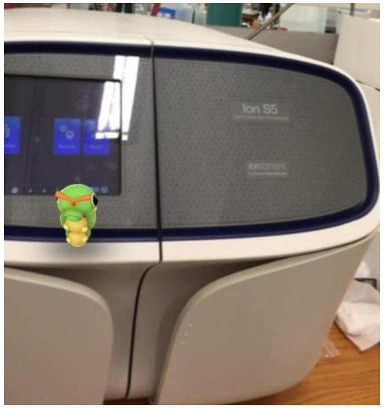
# Advancements and Adoption of the Technology



### Validation of the Ion Torrent PGM sequencing for the prospective routine molecular diagnostic of colorectal cancer

Francesca Belardinilli [a], Carlo Capalbo [a], Amelia Buffone [a], Marialaura Petroni [a], Valeria Colicchia [a], Sergio Ferraro [a], Massimo Zani [a], Arianna Nicolussi [b], Sonia D'Inzeo [b], Anna Coppa [b], Isabella Screpanti [a], Alberto Gulino [a], Giuseppe Giannini [a,*]

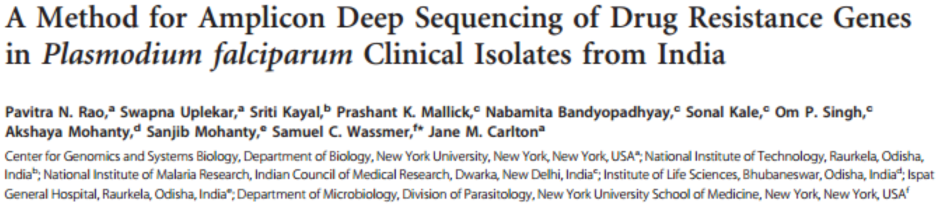
[a] *Department of Molecular Medicine, University La Sapienza, V.le R. Elena, 291, 00161 Rome, Italy*
[b] *Department of Experimental Medicine, University La Sapienza, V.le R. Elena, 324, 00161 Rome, Italy*



### A Next Generation Semiconductor Based Sequencing Approach for the Identification of Meat Species in DNA Mixtures

**Francesca Bertolini[1], Marco Ciro Ghionda[1,2], Enrico D'Alessandro[2], Claudia Geraci[1], Vincenzo Chiofalo[2,3], Luca Fontanesi[1]***

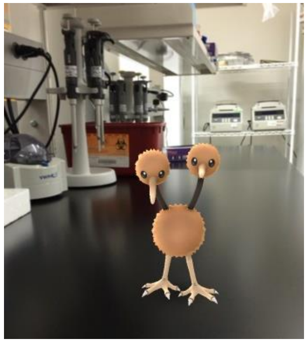
1 Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Viale Fanin 46, 40127, Bologna, Italy, 2 Department of Veterinary Sciences, Animal Production Unit, University of Messina, Polo Universitario dell'Annunziata, 98168, Messina, Italy, 3 Meat Research Consortium, Polo Universitario dell'Annunziata, 98168, Messina, Italy



### A Method for Amplicon Deep Sequencing of Drug Resistance Genes in *Plasmodium falciparum* Clinical Isolates from India

**Pavitra N. Rao,[a] Swapna Uplekar,[a] Sriti Kayal,[b] Prashant K. Mallick,[c] Nabamita Bandyopadhyay,[c] Sonal Kale,[c] Om P. Singh,[c] Akshaya Mohanty,[d] Sanjib Mohanty,[e] Samuel C. Wassmer,[f*] Jane M. Carlton[a]**

Center for Genomics and Systems Biology, Department of Biology, New York University, New York, New York, USA[a]; National Institute of Technology, Raurkela, Odisha, India[b]; National Institute of Malaria Research, Indian Council of Medical Research, Dwarka, New Delhi, India[c]; Institute of Life Sciences, Bhubaneswar, Odisha, India[d]; Ispat General Hospital, Raurkela, Odisha, India[e]; Department of Microbiology, Division of Parasitology, New York University School of Medicine, New York, New York, USA[f]



### The complete mitochondrial genome of the *Anabas testudineus* (Perciformes, Anabantidae) and its comparison with other related fish species

Bijay Kumar Behera ✉, Vishwamitra Singh Baisvar, Kavita Kumari, Ajaya Kumar Rout, Sudip Pakrashi, Prasenjet Paria, A. R. Rao & Anil Rai

# Advancements and Adoption of the Technology

Ion PGM™

Ion Proton™

Ion S5™

Ion OneTouch™ 2 and Ion OneTouch™ ES

Ion Chef™

# Advancements and Adoption of the Technology

- Available Panels:
  - Precision ID Ancestry Panel
  - Precision ID Identity Panel
  - Precision ID mtDNA Whole Genome Panel
  - Precision ID mtDNA Control Region Panel
  - Precision ID GlobalFiler NGS STR Panel

# Ion Torrent MPS Technologies

- Increasingly user-friendly
- Highly automated workflow
- Compatible with numerous genetic marker types
- Available data analysis options
- Accurate, reliable data

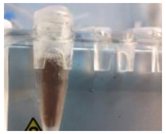
# ION TORRENT MPS WORKFLOW

# General MPS Workflow

**DNA** → **Library Preparation** → **Template Preparation** → **Sequencing** → **Analysis**

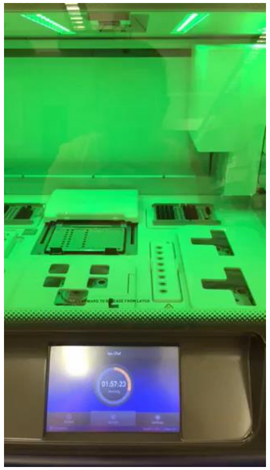
# Library Preparation on the Ion Chef™

**DNA** → **Library Preparation** → **Template Preparation** → **Sequencing** → **Analysis**



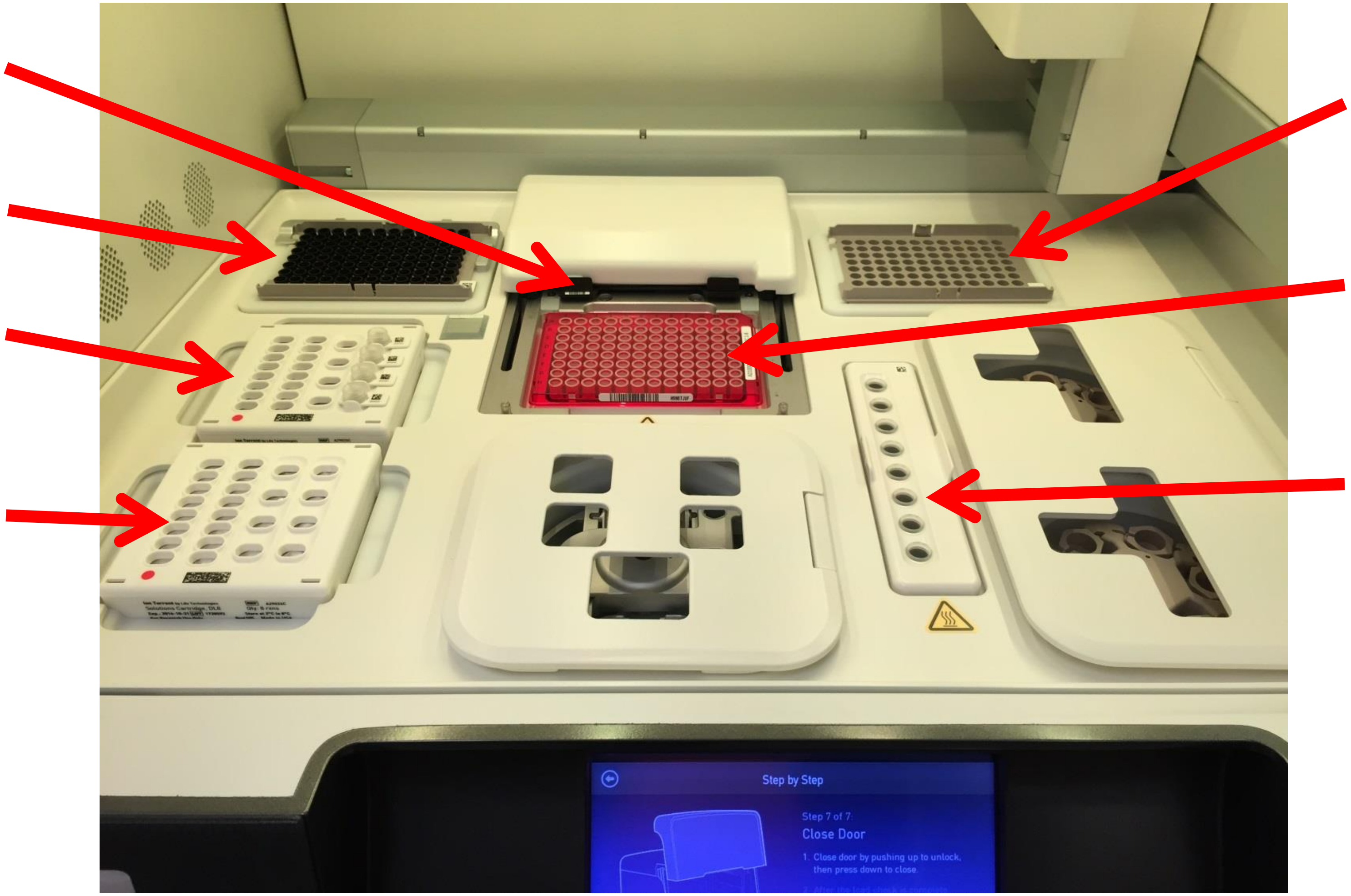
**Amplify Targets** → **Digest Primers** → **Ligate Adapters** → **Purify Libraries** → **Quantify Libraries**

# Library Preparation on the Ion Chef™



- Ion Chef™ automates the library preparation process
- Hours of hands-on steps ➡ Two pipetting steps
- Minimizes user-introduced variability
- Can work on other steps while Chef is running

# Library Preparation on the Ion Chef™



Foil seal

New tip rack

Reagent cartridge station

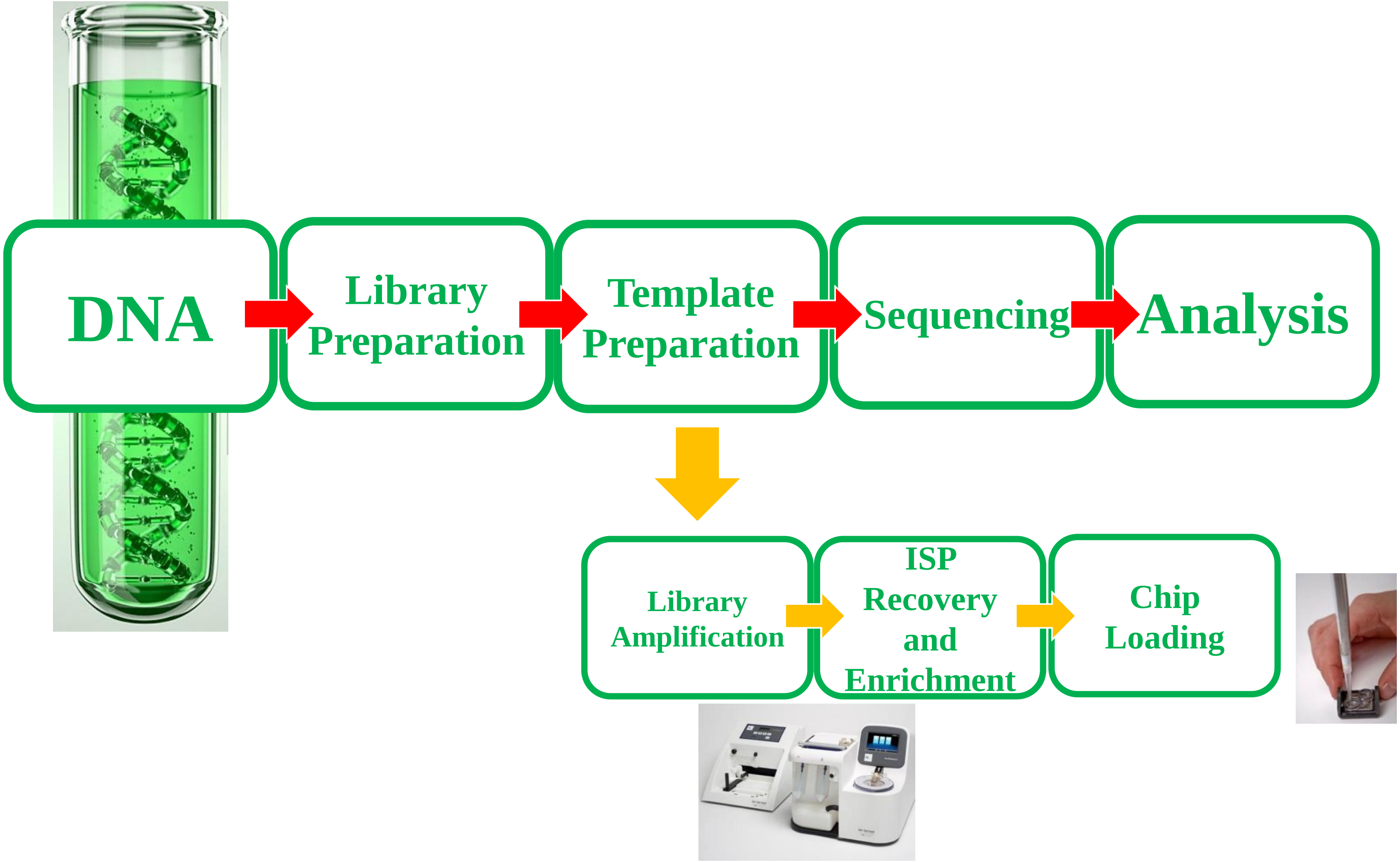
Solutions cartridge station

Used tip rack

96-well plate

Enrichment station

# Template Preparation on the Ion Chef™

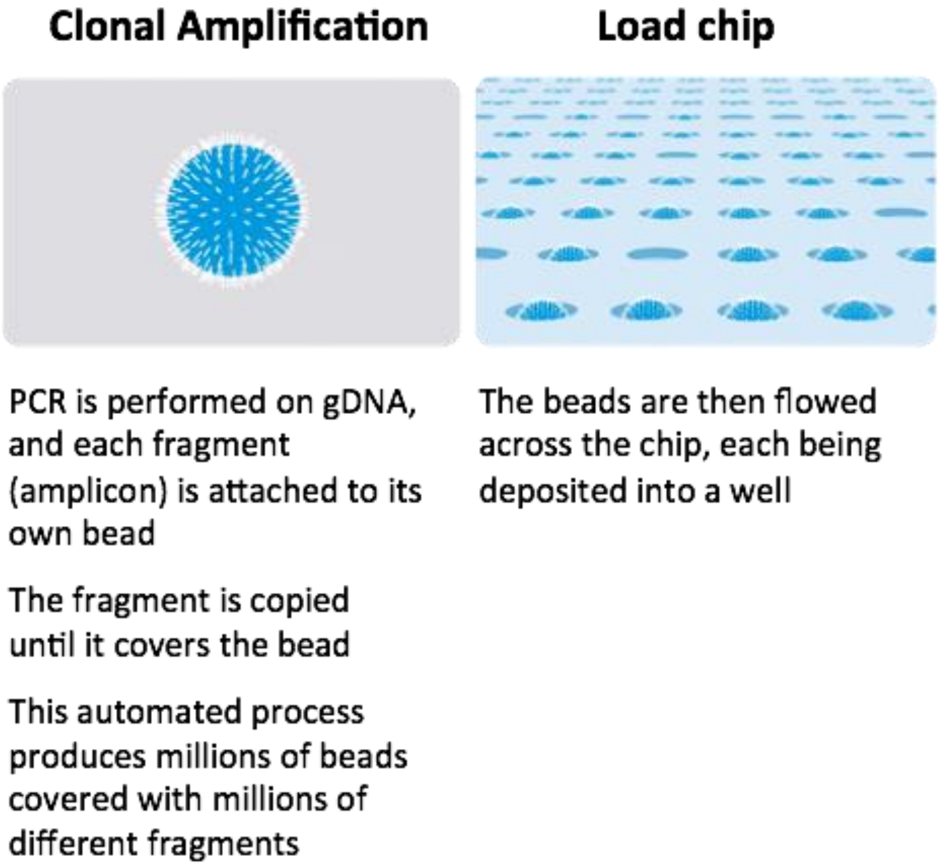


DNA → Library Preparation → Template Preparation → Sequencing → Analysis

Template Preparation ↓

Library Amplification → ISP Recovery and Enrichment → Chip Loading

# Ion Torrent Chemistry



**Clonal Amplification**

PCR is performed on gDNA, and each fragment (amplicon) is attached to its own bead

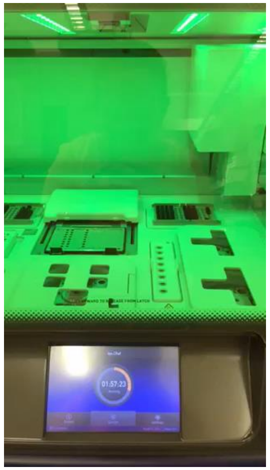
The fragment is copied until it covers the bead

This automated process produces millions of beads covered with millions of different fragments

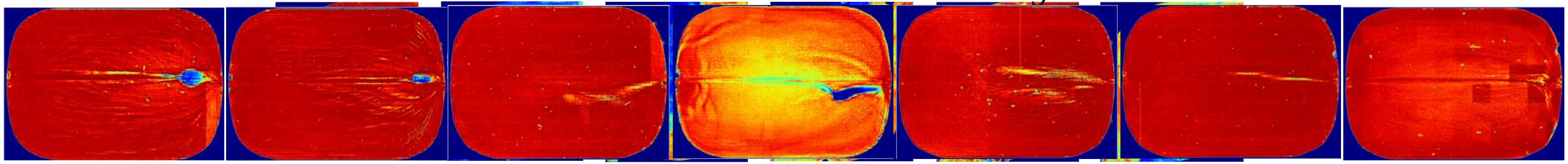
**Load chip**

The beads are then flowed across the chip, each being deposited into a well

# Template Preparation on the Ion Chef™

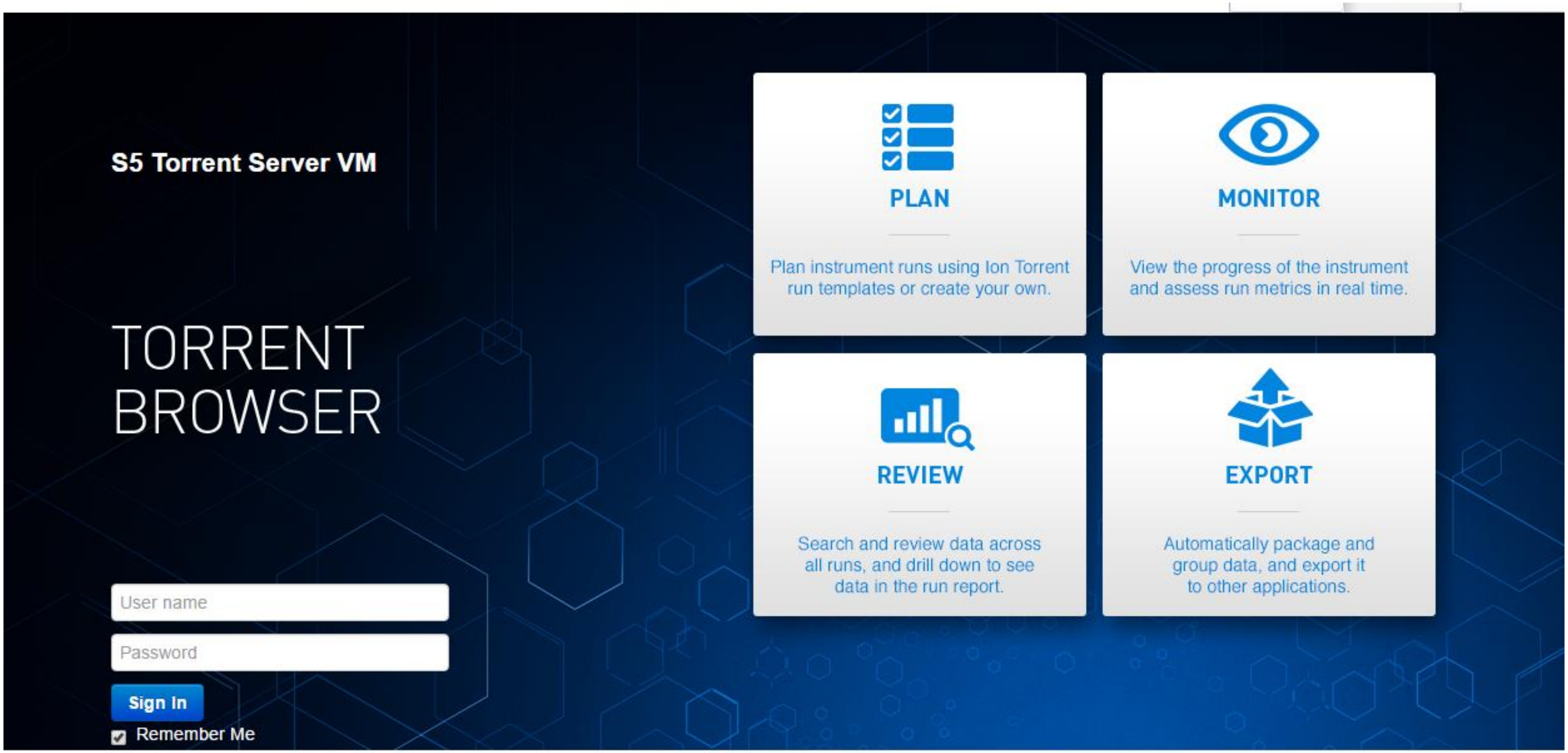


- Ion Chef™ automates the template preparation process
- Hours of hands-on steps → One pipetting step
- Multiple instruments → One instrument
- Minimizes user-introduced variability



- Can work on other steps while Chef is running

# Planning a Run



S5 Torrent Server VM

TORRENT BROWSER

**PLAN**

Plan instrument runs using Ion Torrent run templates or create your own.

**MONITOR**

View the progress of the instrument and assess run metrics in real time.

**REVIEW**

Search and review data across all runs, and drill down to see data in the run report.

**EXPORT**

Automatically package and group data, and export it to other applications.

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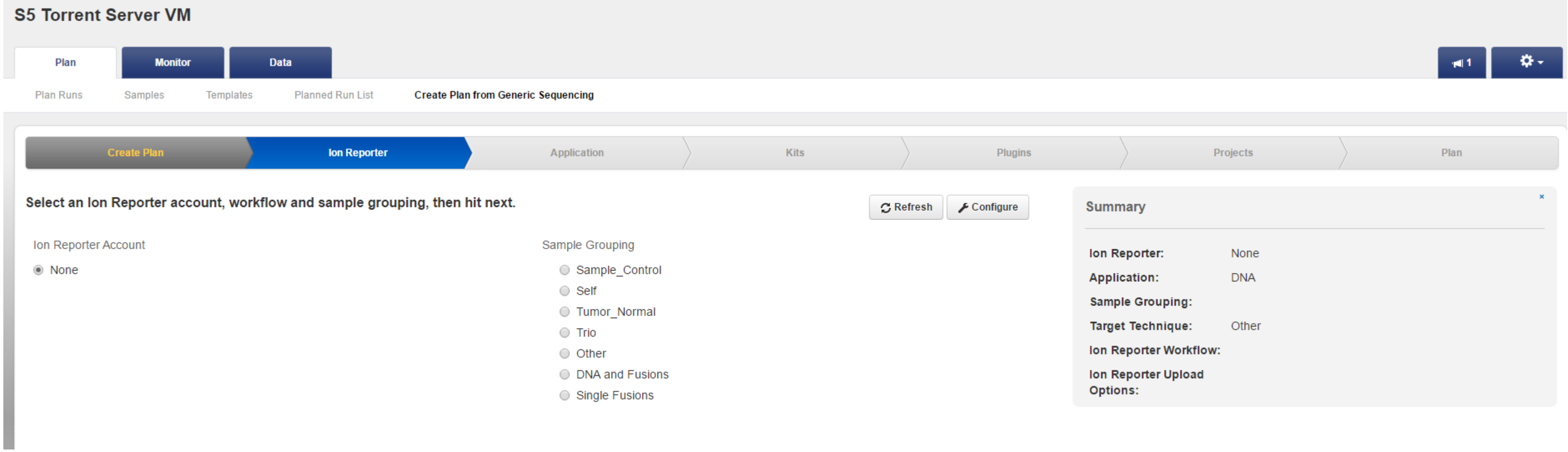
# Planning a Run



Select an Ion Reporter account, workflow and sample grouping, then hit next.

Ion Reporter Account

● None

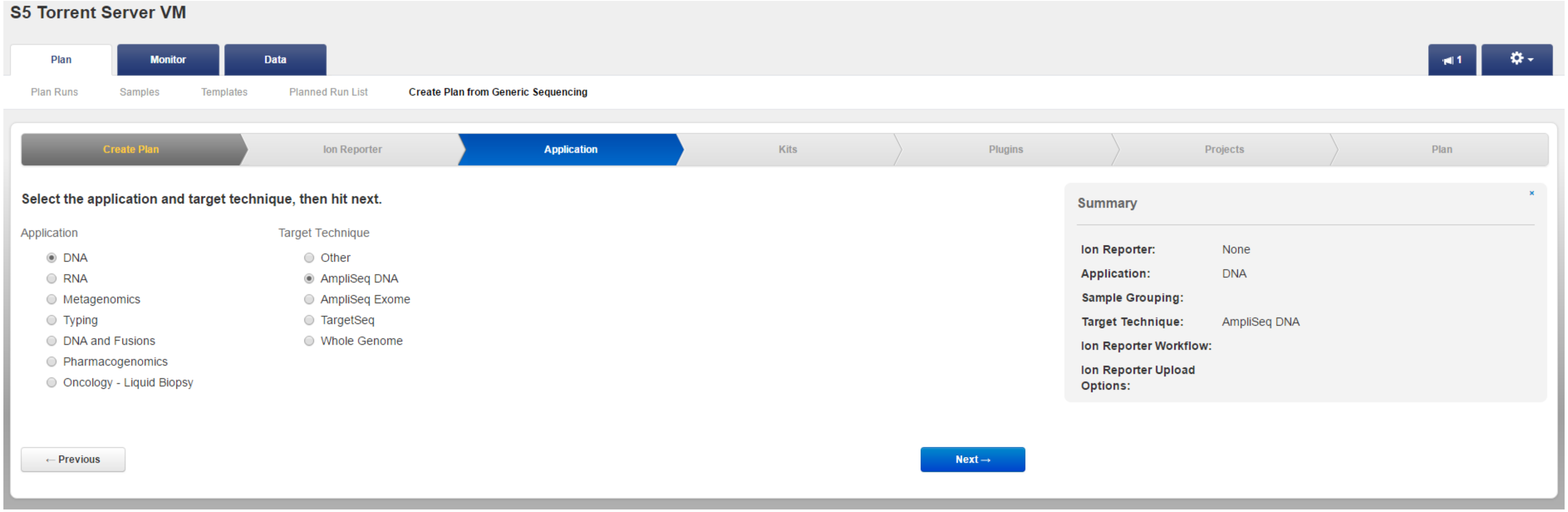
Sample Grouping

○ Sample_Control
○ Self
○ Tumor_Normal
○ Trio
○ Other
○ DNA and Fusions
○ Single Fusions

Summary

| | |
|---|---|
| **Ion Reporter:** | None |
| **Application:** | DNA |
| **Sample Grouping:** | |
| **Target Technique:** | Other |
| **Ion Reporter Workflow:** | |
| **Ion Reporter Upload Options:** | |

# Planning a Run



**Select the application and target technique, then hit next.**

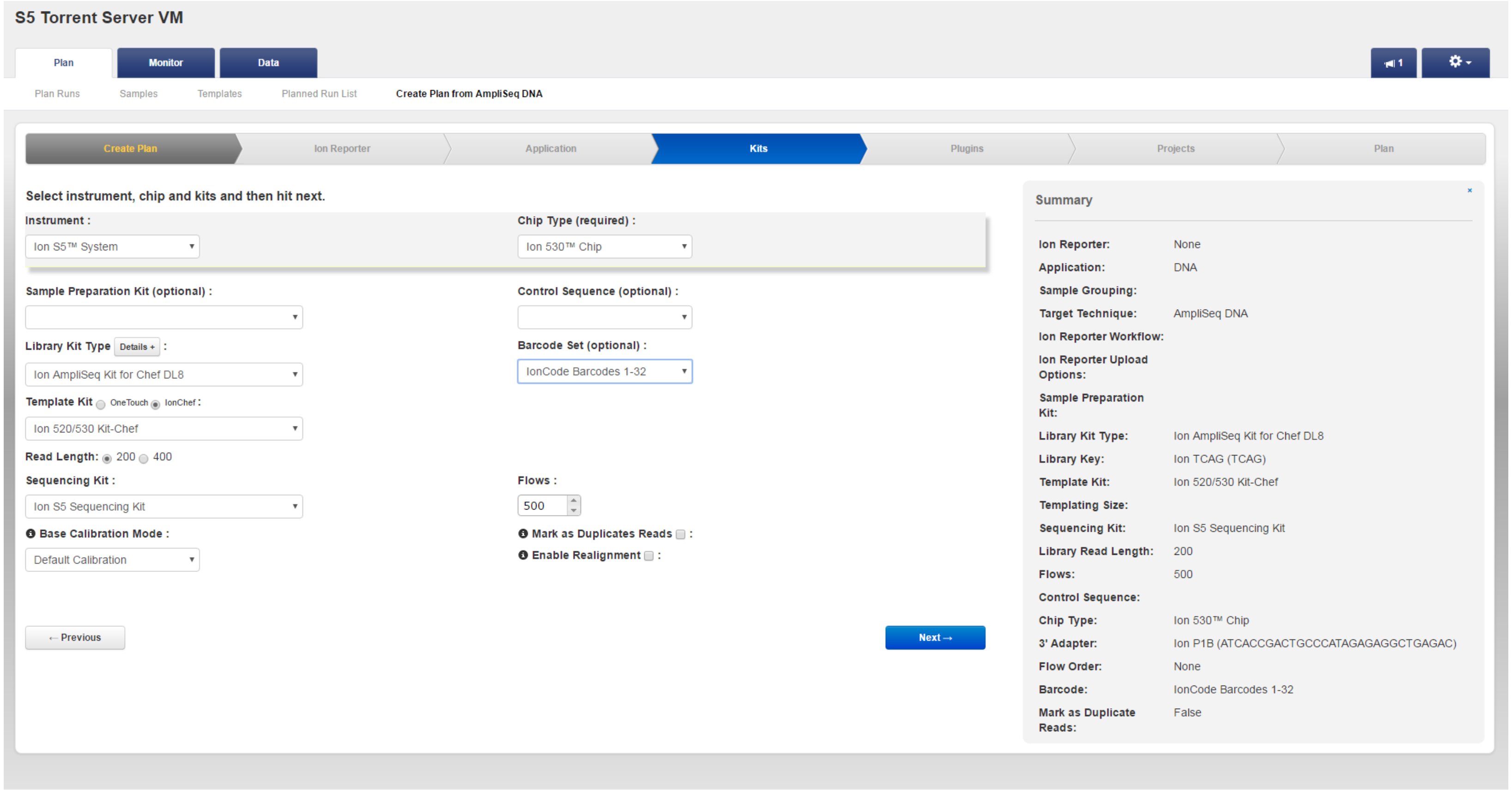
Application
- ● DNA
- ○ RNA
- ○ Metagenomics
- ○ Typing
- ○ DNA and Fusions
- ○ Pharmacogenomics
- ○ Oncology - Liquid Biopsy

Target Technique
- ○ Other
- ● AmpliSeq DNA
- ○ AmpliSeq Exome
- ○ TargetSeq
- ○ Whole Genome

Summary

**Ion Reporter:** None
**Application:** DNA
**Sample Grouping:**
**Target Technique:** AmpliSeq DNA
**Ion Reporter Workflow:**
**Ion Reporter Upload Options:**

# Planning a Run



Select instrument, chip and kits and then hit next.

**Instrument :** Ion S5™ System

**Chip Type (required) :** Ion 530™ Chip

**Sample Preparation Kit (optional) :**

**Control Sequence (optional) :**

**Library Kit Type :** Ion AmpliSeq Kit for Chef DL8

**Barcode Set (optional) :** IonCode Barcodes 1-32

**Template Kit** ○ OneTouch ● IonChef **:** Ion 520/530 Kit-Chef

**Read Length:** ● 200 ○ 400

**Sequencing Kit :** Ion S5 Sequencing Kit

**Flows :** 500

**Base Calibration Mode :** Default Calibration

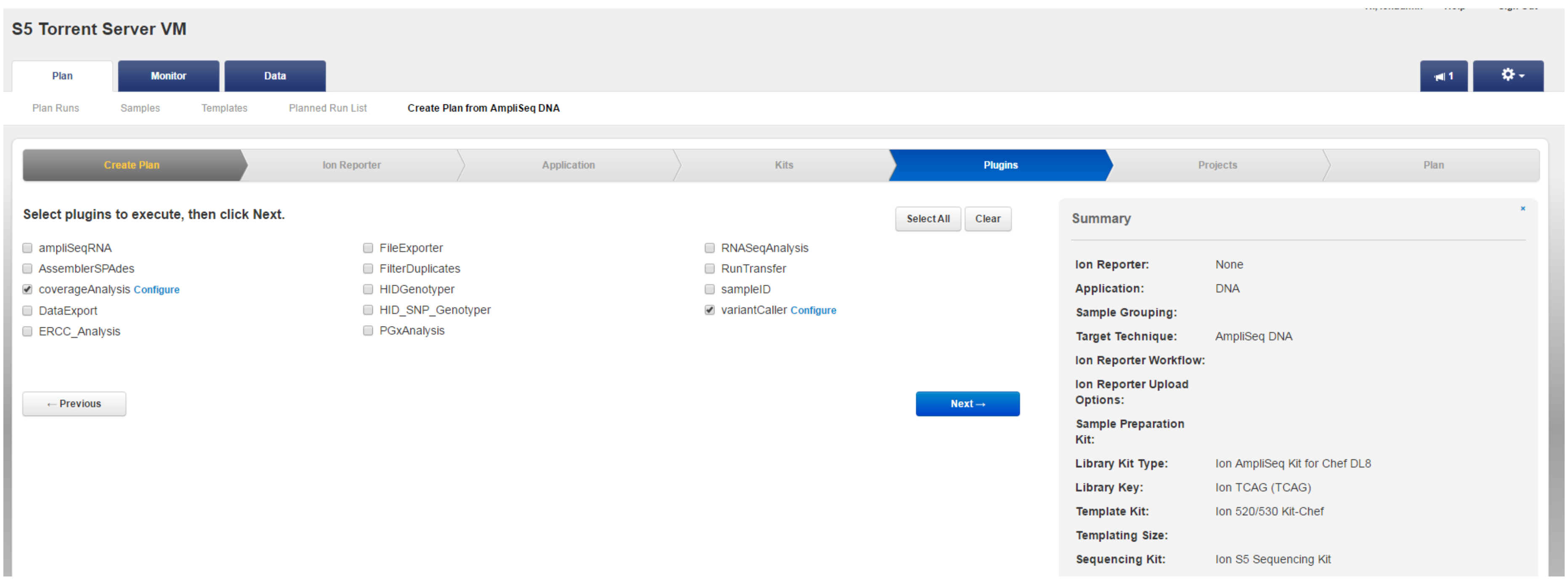
**Mark as Duplicates Reads** ☐ **:**

**Enable Realignment** ☐ **:**

**Summary**

| | |
|---|---|
| **Ion Reporter:** | None |
| **Application:** | DNA |
| **Sample Grouping:** | |
| **Target Technique:** | AmpliSeq DNA |
| **Ion Reporter Workflow:** | |
| **Ion Reporter Upload Options:** | |
| **Sample Preparation Kit:** | |
| **Library Kit Type:** | Ion AmpliSeq Kit for Chef DL8 |
| **Library Key:** | Ion TCAG (TCAG) |
| **Template Kit:** | Ion 520/530 Kit-Chef |
| **Templating Size:** | |
| **Sequencing Kit:** | Ion S5 Sequencing Kit |
| **Library Read Length:** | 200 |
| **Flows:** | 500 |
| **Control Sequence:** | |
| **Chip Type:** | Ion 530™ Chip |
| **3' Adapter:** | Ion P1B (ATCACCGACTGCCCATAGAGAGGCTGAGAC) |
| **Flow Order:** | None |
| **Barcode:** | IonCode Barcodes 1-32 |
| **Mark as Duplicate Reads:** | False |

# Planning a Run



Select plugins to execute, then click Next.

☐ ampliSeqRNA
☐ AssemblerSPAdes
☑ coverageAnalysis Configure
☐ DataExport
☐ ERCC_Analysis
☐ FileExporter
☐ FilterDuplicates
☐ HIDGenotyper
☐ HID_SNP_Genotyper
☐ PGxAnalysis
☐ RNASeqAnalysis
☐ RunTransfer
☐ sampleID
☑ variantCaller Configure

**Summary**

|  |  |
|---|---|
| **Ion Reporter:** | None |
| **Application:** | DNA |
| **Sample Grouping:** | |
| **Target Technique:** | AmpliSeq DNA |
| **Ion Reporter Workflow:** | |
| **Ion Reporter Upload Options:** | |
| **Sample Preparation Kit:** | |
| **Library Kit Type:** | Ion AmpliSeq Kit for Chef DL8 |
| **Library Key:** | Ion TCAG (TCAG) |
| **Template Kit:** | Ion 520/530 Kit-Chef |
| **Templating Size:** | |
| **Sequencing Kit:** | Ion S5 Sequencing Kit |

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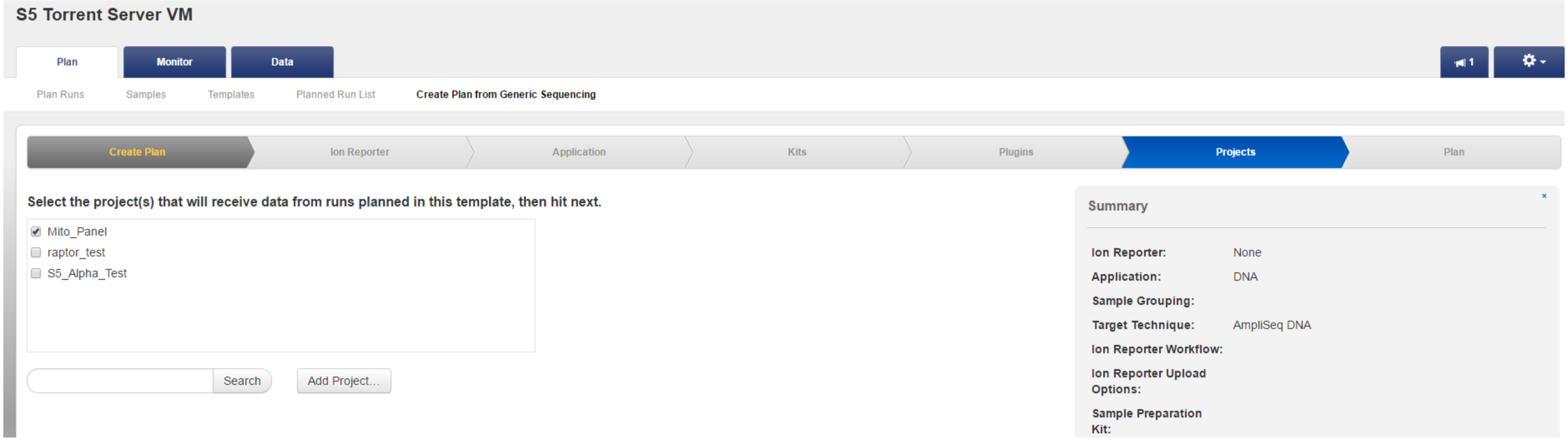
# Planning a Run



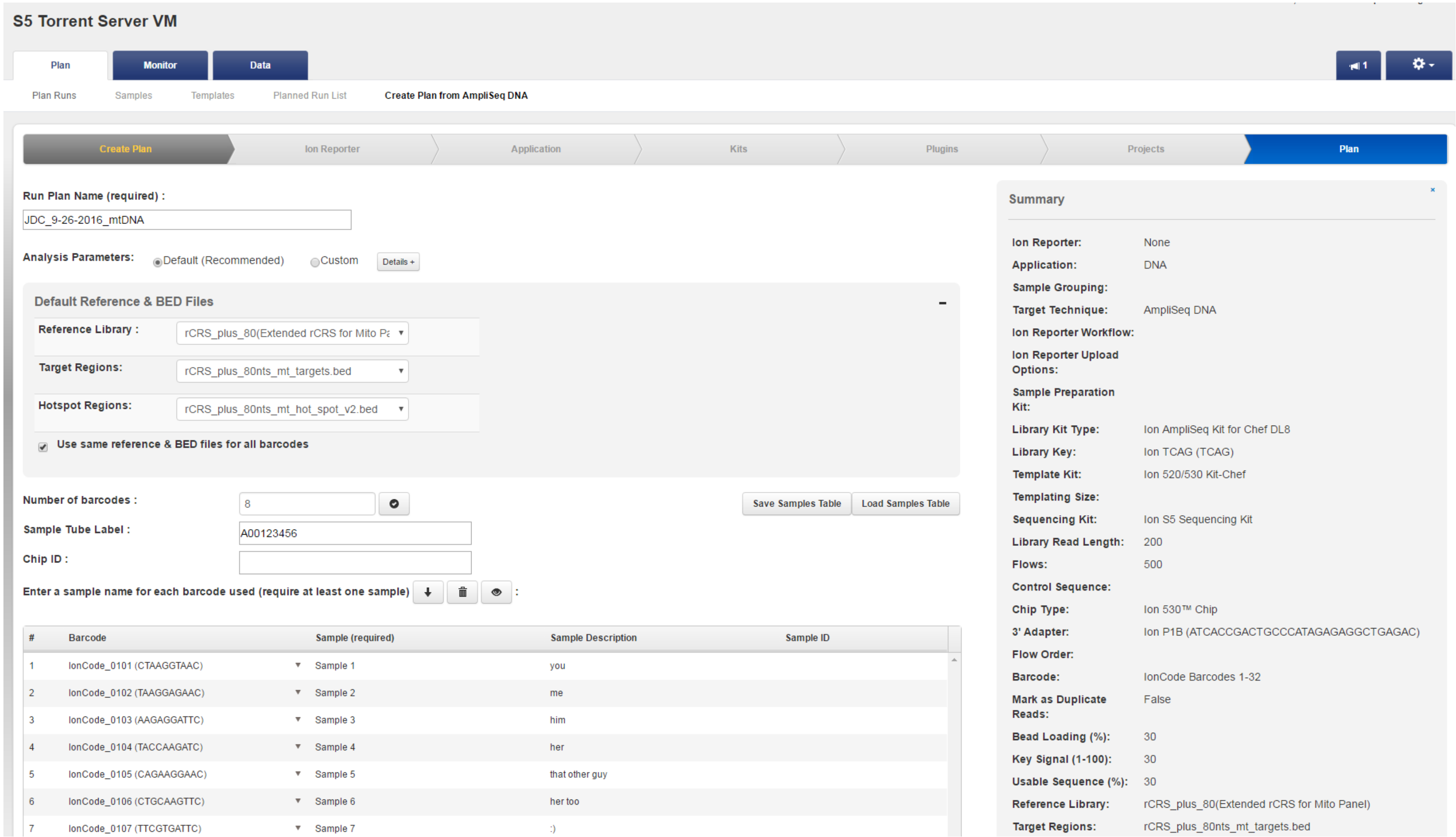
Select the project(s) that will receive data from runs planned in this template, then hit next.

☑ Mito_Panel
☐ raptor_test
☐ S5_Alpha_Test

Summary

**Ion Reporter:** None
**Application:** DNA
**Sample Grouping:**
**Target Technique:** AmpliSeq DNA
**Ion Reporter Workflow:**
**Ion Reporter Upload Options:**
**Sample Preparation Kit:**

# Planning a Run



**Run Plan Name (required) :** JDC_9-26-2016_mtDNA

**Analysis Parameters:** ● Default (Recommended) ○ Custom

**Default Reference & BED Files**

**Reference Library :** rCRS_plus_80(Extended rCRS for Mito Pa

**Target Regions:** rCRS_plus_80nts_mt_targets.bed

**Hotspot Regions:** rCRS_plus_80nts_mt_hot_spot_v2.bed

☑ **Use same reference & BED files for all barcodes**

**Number of barcodes :** 8

**Sample Tube Label :** A00123456

**Chip ID :**

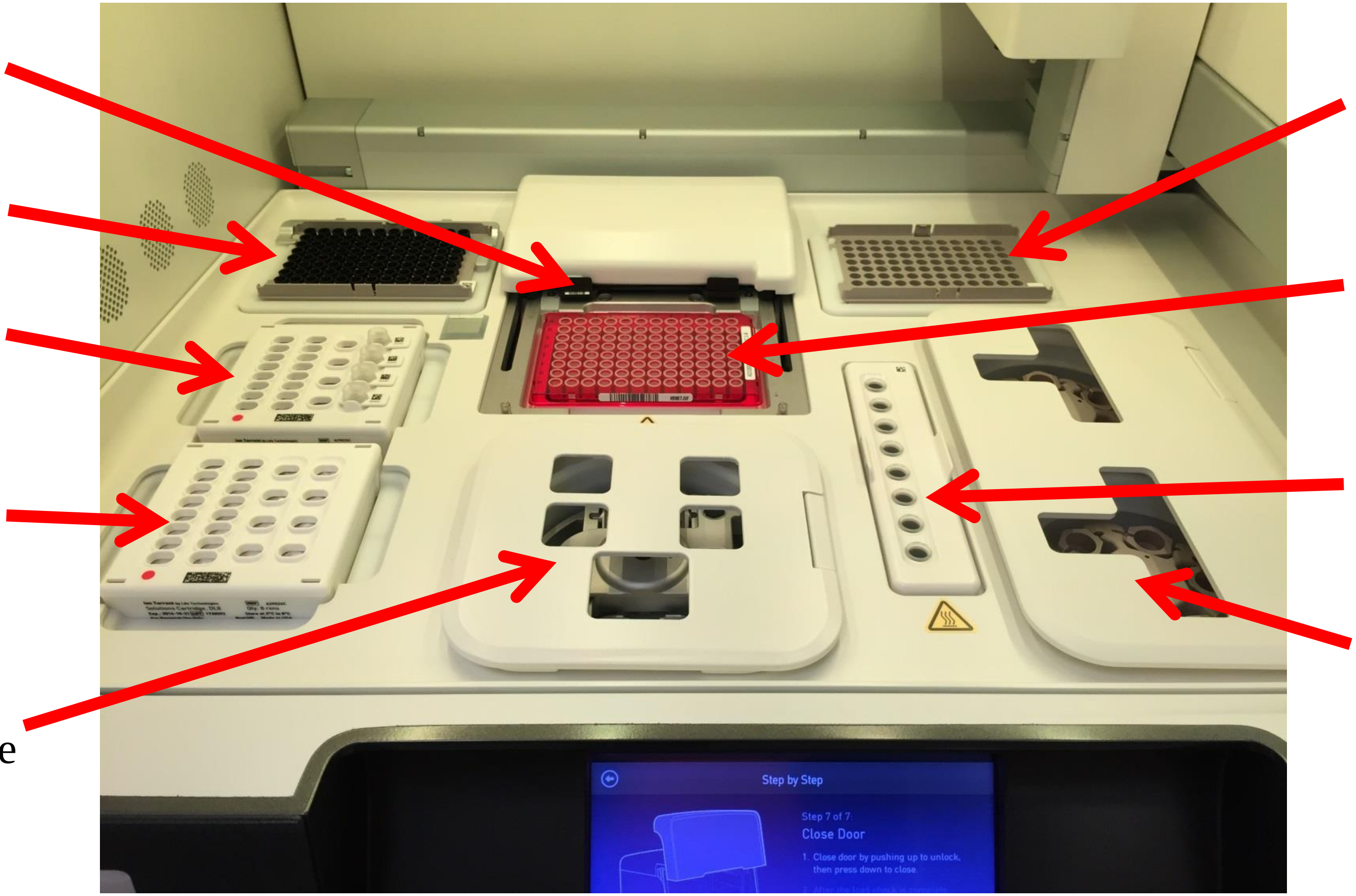
**Enter a sample name for each barcode used (require at least one sample)** :

| # | Barcode | Sample (required) | Sample Description | Sample ID |
|---|---|---|---|---|
| 1 | IonCode_0101 (CTAAGGTAAC) | Sample 1 | you | |
| 2 | IonCode_0102 (TAAGGAGAAC) | Sample 2 | me | |
| 3 | IonCode_0103 (AAGAGGATTC) | Sample 3 | him | |
| 4 | IonCode_0104 (TACCAAGATC) | Sample 4 | her | |
| 5 | IonCode_0105 (CAGAAGGAAC) | Sample 5 | that other guy | |
| 6 | IonCode_0106 (CTGCAAGTTC) | Sample 6 | her too | |
| 7 | IonCode_0107 (TTCGTGATTC) | Sample 7 | :) | |

**Summary**

| | |
|---|---|
| **Ion Reporter:** | None |
| **Application:** | DNA |
| **Sample Grouping:** | |
| **Target Technique:** | AmpliSeq DNA |
| **Ion Reporter Workflow:** | |
| **Ion Reporter Upload Options:** | |
| **Sample Preparation Kit:** | |
| **Library Kit Type:** | Ion AmpliSeq Kit for Chef DL8 |
| **Library Key:** | Ion TCAG (TCAG) |
| **Template Kit:** | Ion 520/530 Kit-Chef |
| **Templating Size:** | |
| **Sequencing Kit:** | Ion S5 Sequencing Kit |
| **Library Read Length:** | 200 |
| **Flows:** | 500 |
| **Control Sequence:** | |
| **Chip Type:** | Ion 530™ Chip |
| **3' Adapter:** | Ion P1B (ATCACCGACTGCCCATAGAGAGGCTGAGAC) |
| **Flow Order:** | |
| **Barcode:** | IonCode Barcodes 1-32 |
| **Mark as Duplicate Reads:** | False |
| **Bead Loading (%):** | 30 |
| **Key Signal (1-100):** | 30 |
| **Usable Sequence (%):** | 30 |
| **Reference Library:** | rCRS_plus_80(Extended rCRS for Mito Panel) |
| **Target Regions:** | rCRS_plus_80nts_mt_targets.bed |

# Template Preparation on the Ion Chef™



Foil seal

New tip rack

Reagent cartridge station

Solutions cartridge station

Chip Centrifuge

Used tip rack

96-well plate

Enrichment station

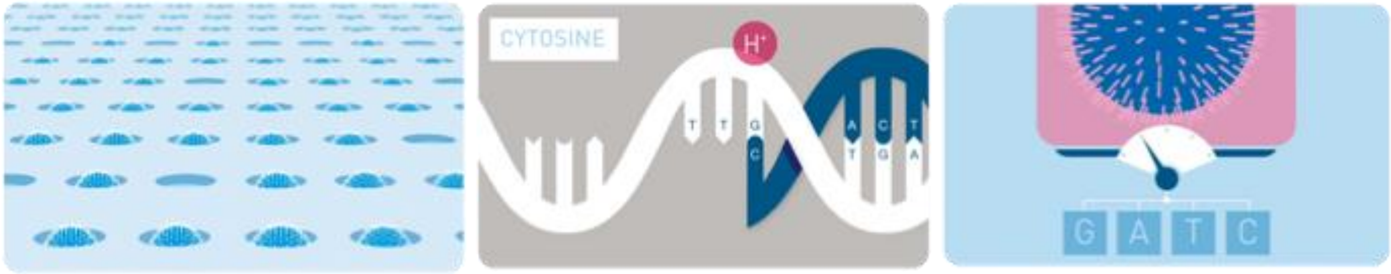
Tube Centrifuges

# Massively Parallel Sequencing on the Ion S5™

**DNA** → **Library Preparation** → **Template Preparation** → **Sequencing** → **Analysis**

**Sequencing** ↓ **Load Sequencer**

# Ion Torrent Chemistry



### Load chip

The beads are then flowed across the chip, each being deposited into a well

Then the chip is flooded with one of the four nucleotides

### Incorporate nucleotide

Whenever a nucleotide is incorporated into a single strand of DNA, a hydrogen ion is released changing the pH of the surrounding solution

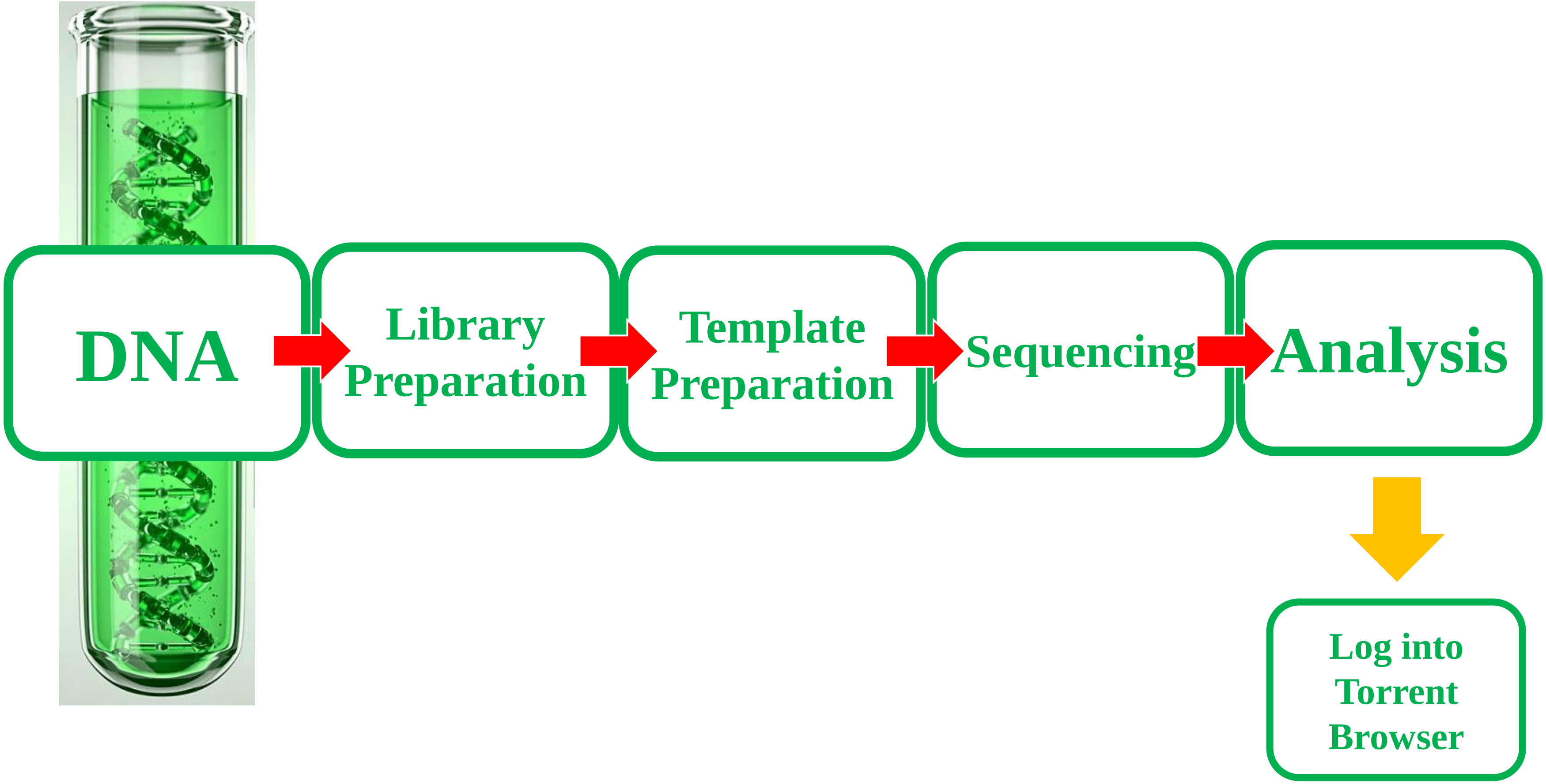
### Detect and call

Ion semiconductor sequencing chip is able to detect this change in pH and convert it directly to base calls

# Massively Parallel Sequencing on the Ion S5™

- Thaw reagents
- Empty waste container
- Load reagents
- Initialize instrument
- Load chip
- Start sequencing run

# Data Analysis

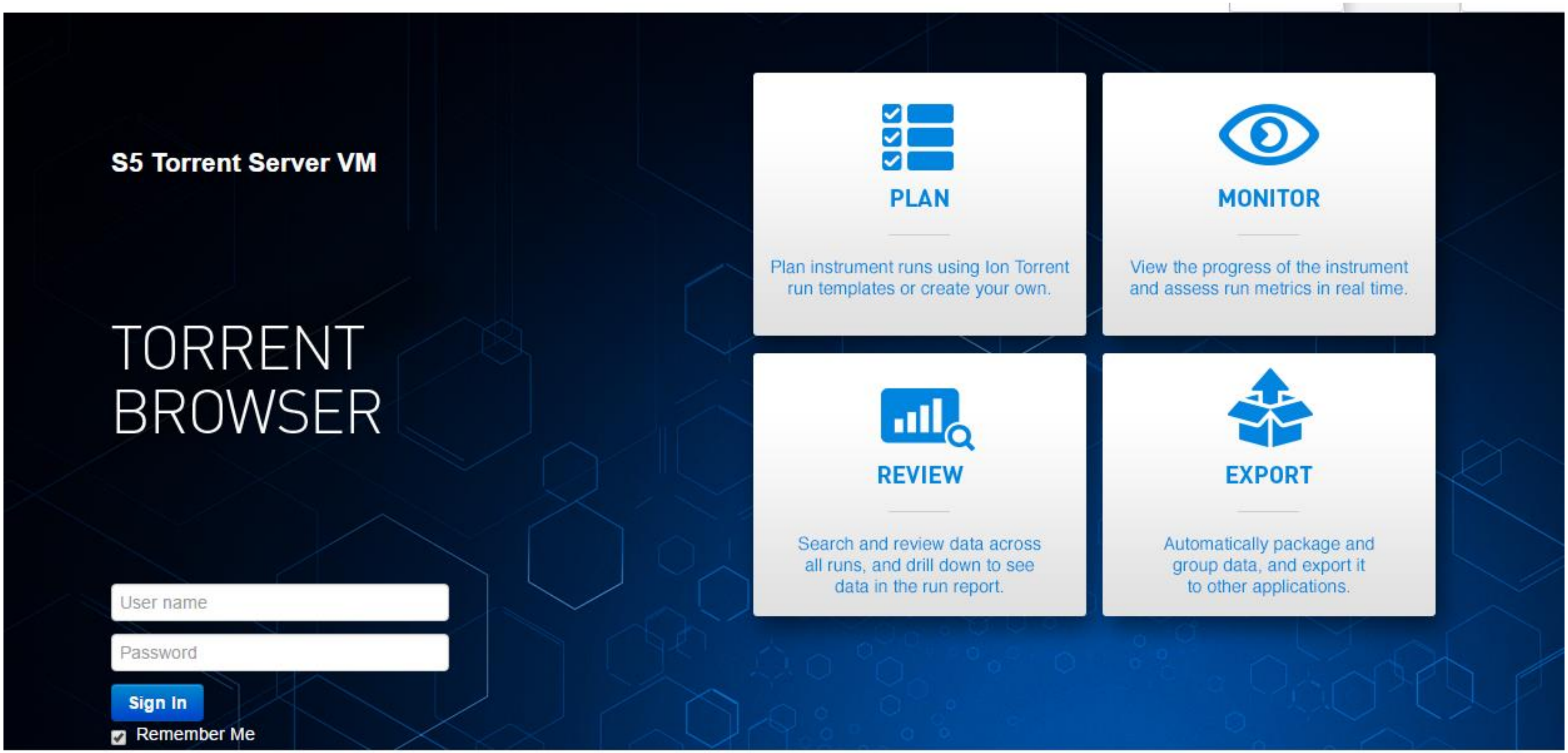


DNA → Library Preparation → Template Preparation → Sequencing → Analysis

↓

Log into Torrent Browser

# Data Analysis



S5 Torrent Server VM

TORRENT
BROWSER

**PLAN**

Plan instrument runs using Ion Torrent run templates or create your own.

**MONITOR**

View the progress of the instrument and assess run metrics in real time.

**REVIEW**

Search and review data across all runs, and drill down to see data in the run report.

**EXPORT**

Automatically package and group data, and export it to other applications.

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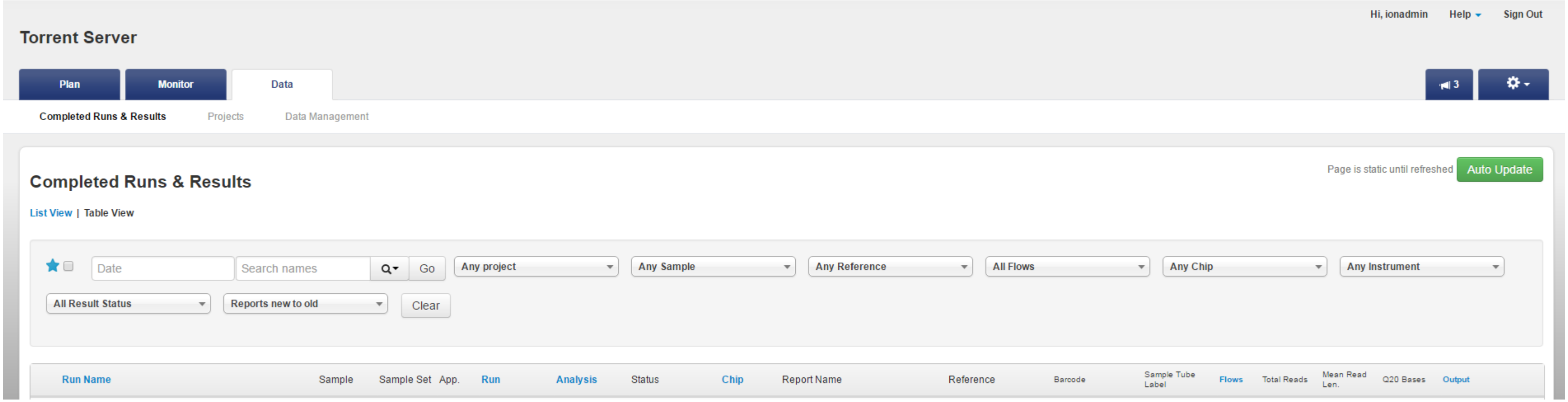
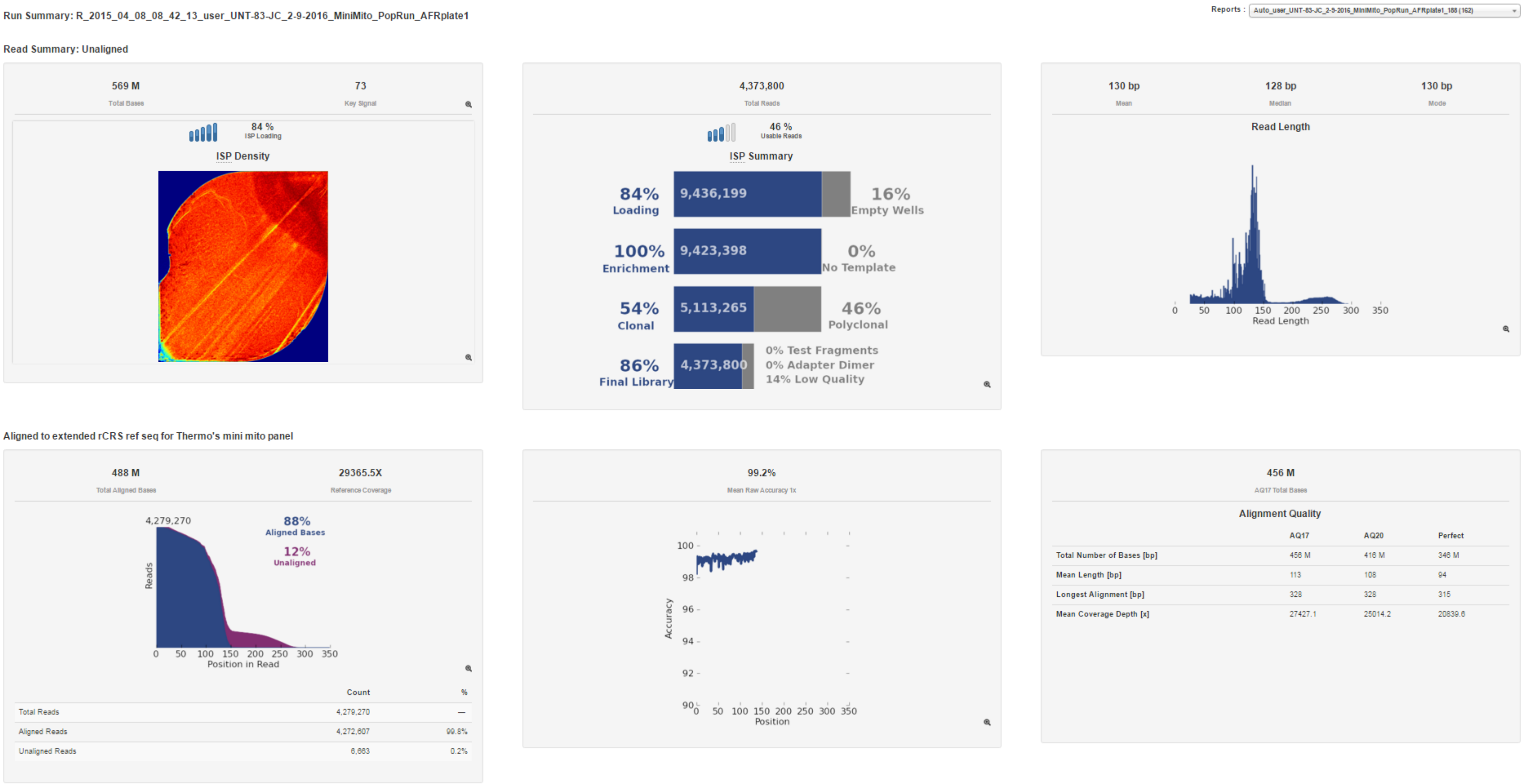
# Data Analysis



## Completed Runs & Results

| Run Name | Sample | Sample Set | App. | Run | Analysis | Status | Chip | Report Name | Reference | Barcode | Sample Tube Label | Flows | Total Reads | Mean Read Len. | Q20 Bases | Output |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

# Data Analysis



Run Summary: R_2015_04_08_08_42_13_user_UNT-83-JC_2-9-2016_MiniMito_PopRun_AFRplate1

Reports : Auto_user_UNT-83-JC_2-9-2016_MiniMito_PopRun_AFRplate1_188 (162)

Read Summary: Unaligned

569 M Total Bases

73 Key Signal

84 % ISP Loading

4,373,800 Total Reads

46 % Usable Reads

130 bp Mean

128 bp Median

130 bp Mode

Aligned to extended rCRS ref seq for Thermo's mini mito panel

488 M Total Aligned Bases

29365.5X Reference Coverage

99.2% Mean Raw Accuracy 1x

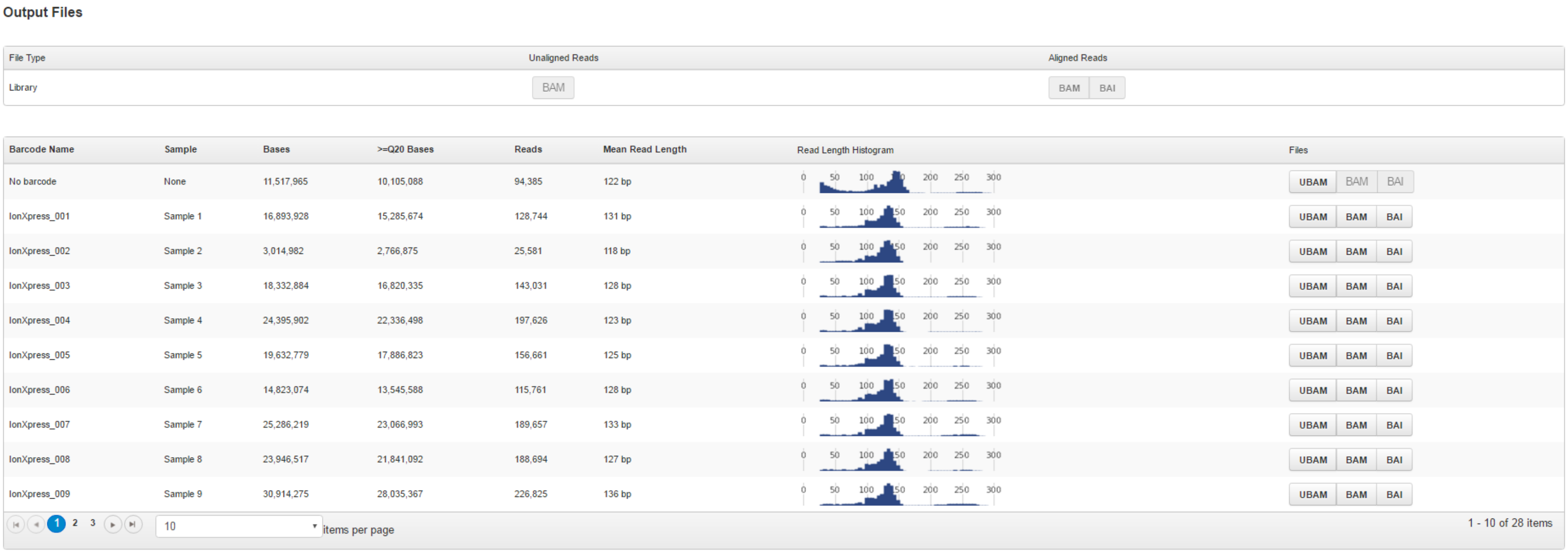
456 M AQ17 Total Bases

| | Count | % |
|---|---|---|
| Total Reads | 4,279,270 | — |
| Aligned Reads | 4,272,607 | 99.8% |
| Unaligned Reads | 6,663 | 0.2% |

Alignment Quality

| | AQ17 | AQ20 | Perfect |
|---|---|---|---|
| Total Number of Bases [bp] | 456 M | 416 M | 346 M |
| Mean Length [bp] | 113 | 108 | 94 |
| Longest Alignment [bp] | 328 | 328 | 315 |
| Mean Coverage Depth [x] | 27427.1 | 25014.2 | 20839.6 |

# Data Analysis

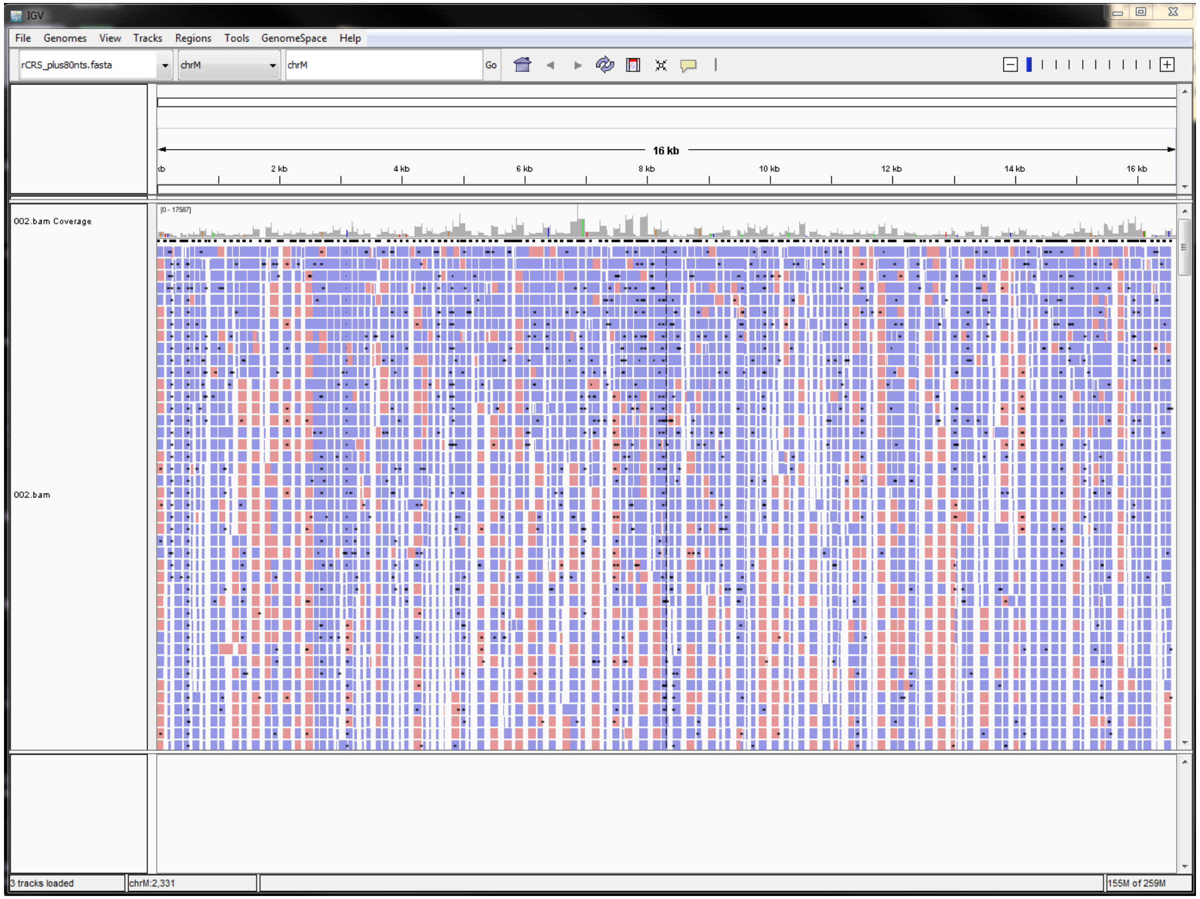


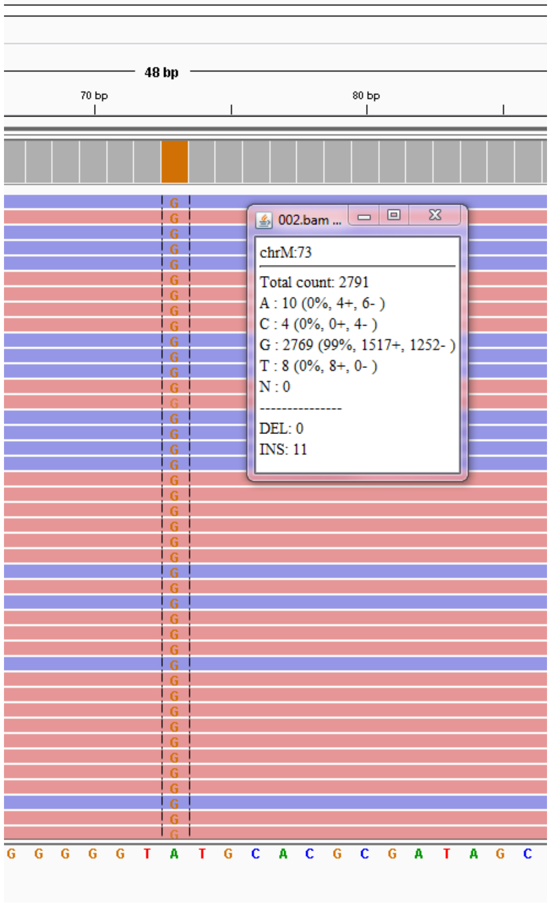
**Output Files**

| File Type | Unaligned Reads | Aligned Reads |
|---|---|---|
| Library | BAM | BAM BAI |

| Barcode Name | Sample | Bases | >=Q20 Bases | Reads | Mean Read Length | Read Length Histogram | Files |
|---|---|---|---|---|---|---|---|
| No barcode | None | 11,517,965 | 10,105,088 | 94,385 | 122 bp | | UBAM BAM BAI |
| IonXpress_001 | Sample 1 | 16,893,928 | 15,285,674 | 128,744 | 131 bp | | UBAM BAM BAI |
| IonXpress_002 | Sample 2 | 3,014,982 | 2,766,875 | 25,581 | 118 bp | | UBAM BAM BAI |
| IonXpress_003 | Sample 3 | 18,332,884 | 16,820,335 | 143,031 | 128 bp | | UBAM BAM BAI |
| IonXpress_004 | Sample 4 | 24,395,902 | 22,336,498 | 197,626 | 123 bp | | UBAM BAM BAI |
| IonXpress_005 | Sample 5 | 19,632,779 | 17,886,823 | 156,661 | 125 bp | | UBAM BAM BAI |
| IonXpress_006 | Sample 6 | 14,823,074 | 13,545,588 | 115,761 | 128 bp | | UBAM BAM BAI |
| IonXpress_007 | Sample 7 | 25,286,219 | 23,066,993 | 189,657 | 133 bp | | UBAM BAM BAI |
| IonXpress_008 | Sample 8 | 23,946,517 | 21,841,092 | 188,694 | 127 bp | | UBAM BAM BAI |
| IonXpress_009 | Sample 9 | 30,914,275 | 28,035,367 | 226,825 | 136 bp | | UBAM BAM BAI |

# Data Analysis





chrM:73

Total count: 2791
A : 10 (0%, 4+, 6- )
C : 4 (0%, 0+, 4- )
G : 2769 (99%, 1517+, 1252- )
T : 8 (0%, 8+, 0- )
N : 0
---------------
DEL: 0
INS: 11

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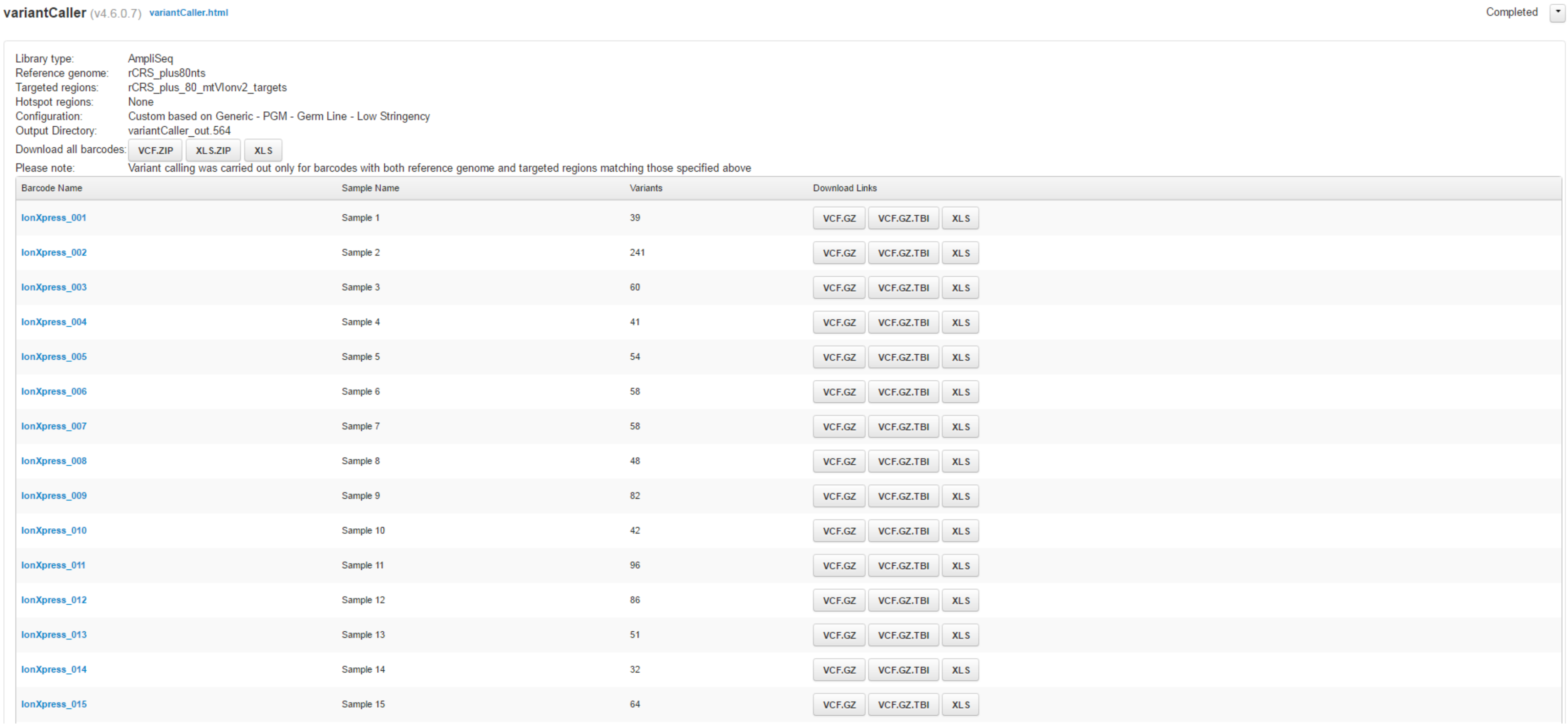
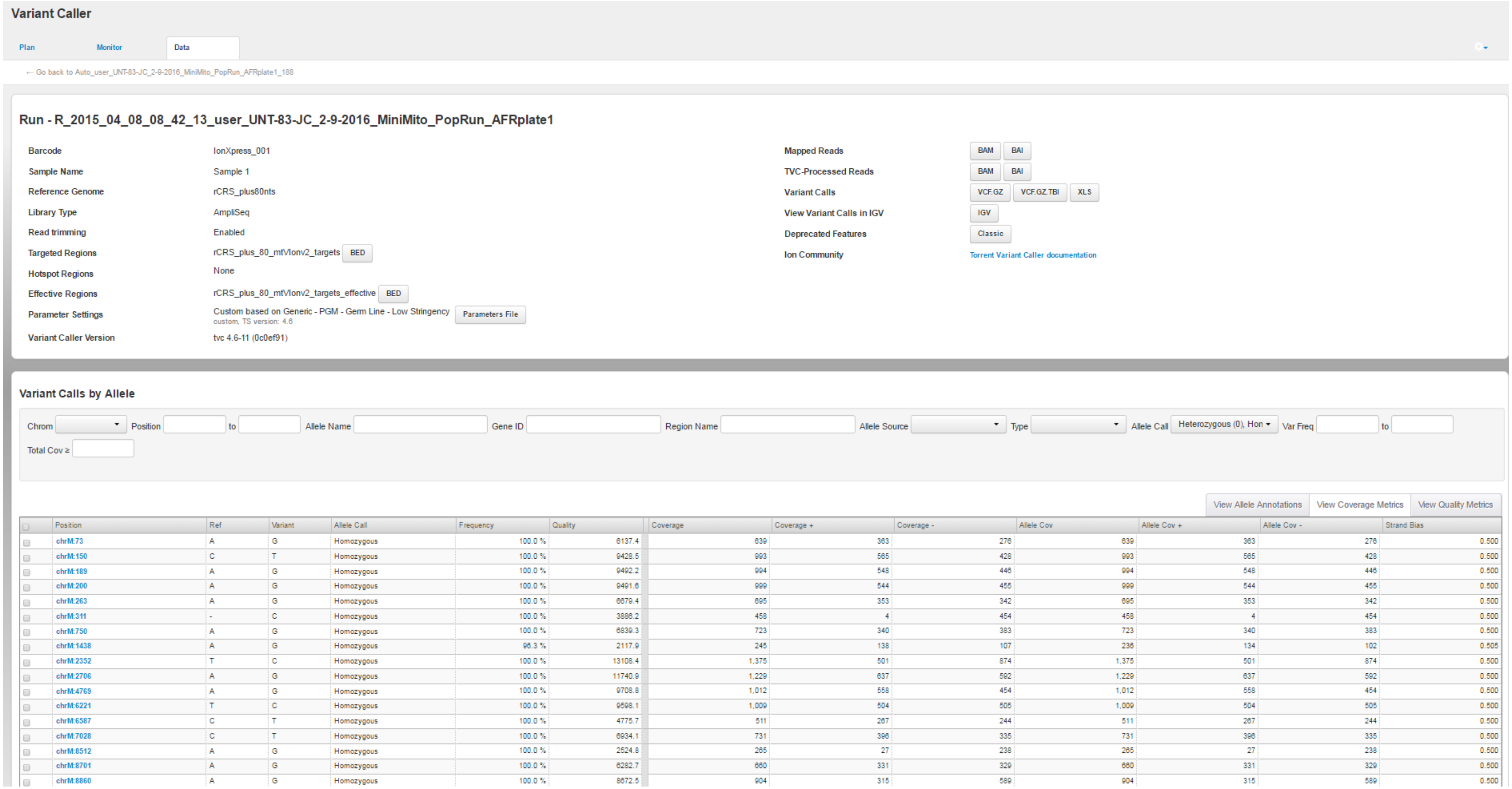
# Data Analysis



Library type: AmpliSeq
Reference genome: rCRS_plus80nts
Targeted regions: rCRS_plus_80_mtVIonv2_targets
Hotspot regions: None
Configuration: Custom based on Generic - PGM - Germ Line - Low Stringency
Output Directory: variantCaller_out.564
Download all barcodes: VCF.ZIP XLS.ZIP XLS
Please note: Variant calling was carried out only for barcodes with both reference genome and targeted regions matching those specified above

| Barcode Name | Sample Name | Variants | Download Links |
|---|---|---|---|
| IonXpress_001 | Sample 1 | 39 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_002 | Sample 2 | 241 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_003 | Sample 3 | 60 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_004 | Sample 4 | 41 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_005 | Sample 5 | 54 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_006 | Sample 6 | 58 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_007 | Sample 7 | 58 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_008 | Sample 8 | 48 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_009 | Sample 9 | 82 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_010 | Sample 10 | 42 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_011 | Sample 11 | 96 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_012 | Sample 12 | 86 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_013 | Sample 13 | 51 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_014 | Sample 14 | 32 | VCF.GZ VCF.GZ.TBI XLS |
| IonXpress_015 | Sample 15 | 64 | VCF.GZ VCF.GZ.TBI XLS |

# Data Analysis



## Variant Caller

← Go back to Auto_user_UNT-83-JC_2-9-2016_MiniMito_PopRun_AFRplate1_188

### Run - R_2015_04_08_08_42_13_user_UNT-83-JC_2-9-2016_MiniMito_PopRun_AFRplate1

| | |
|---|---|
| **Barcode** | IonXpress_001 |
| **Sample Name** | Sample 1 |
| **Reference Genome** | rCRS_plus80nts |
| **Library Type** | AmpliSeq |
| **Read trimming** | Enabled |
| **Targeted Regions** | rCRS_plus_80_mtVIonv2_targets |
| **Hotspot Regions** | None |
| **Effective Regions** | rCRS_plus_80_mtVIonv2_targets_effective |
| **Parameter Settings** | Custom based on Generic - PGM - Germ Line - Low Stringency<br>custom, TS version: 4.6 |
| **Variant Caller Version** | tvc 4.6-11 (0c0ef91) |

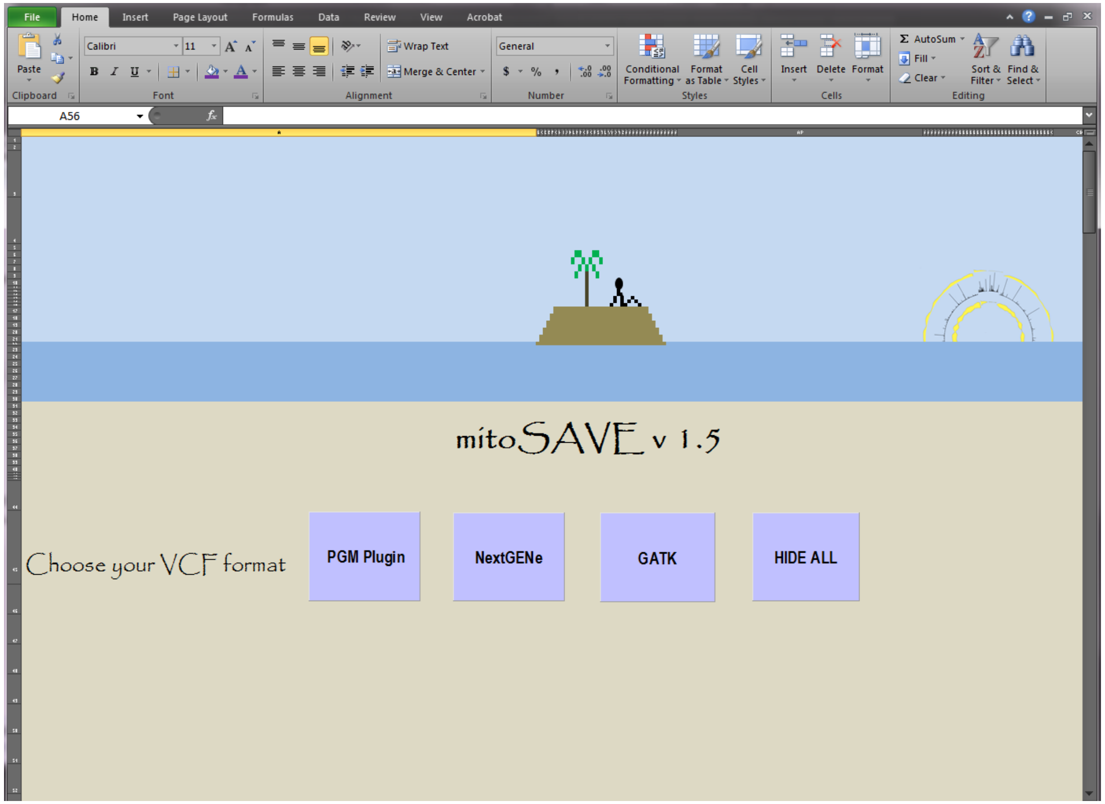
| | |
|---|---|
| **Mapped Reads** | BAM BAI |
| **TVC-Processed Reads** | BAM BAI |
| **Variant Calls** | VCF.GZ VCF.GZ.TBI XLS |
| **View Variant Calls in IGV** | IGV |
| **Deprecated Features** | Classic |
| **Ion Community** | Torrent Variant Caller documentation |

### Variant Calls by Allele

Chrom: (blank)
Position: (blank) to (blank)
Allele Name: (blank)
Gene ID: (blank)
Region Name: (blank)
Allele Source: (blank)
Type: (blank)
Allele Call: Heterozygous (0), Hon
Var Freq: (blank) to (blank)
Total Cov ≥ (blank)

| ☐ | Position | Ref | Variant | Allele Call | Frequency | Quality | Coverage | Coverage + | Coverage - | Allele Cov | Allele Cov + | Allele Cov - | Strand Bias |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ☐ | chrM:73 | A | G | Homozygous | 100.0 % | 6137.4 | 639 | 363 | 276 | 639 | 363 | 276 | 0.500 |
| ☐ | chrM:150 | C | T | Homozygous | 100.0 % | 9428.5 | 993 | 565 | 428 | 993 | 565 | 428 | 0.500 |
| ☐ | chrM:189 | A | G | Homozygous | 100.0 % | 9492.2 | 994 | 548 | 446 | 994 | 548 | 446 | 0.500 |
| ☐ | chrM:200 | A | G | Homozygous | 100.0 % | 9491.6 | 999 | 544 | 455 | 999 | 544 | 455 | 0.500 |
| ☐ | chrM:263 | A | G | Homozygous | 100.0 % | 6679.4 | 695 | 353 | 342 | 695 | 353 | 342 | 0.500 |
| ☐ | chrM:311 | - | C | Homozygous | 100.0 % | 3886.2 | 458 | 4 | 454 | 458 | 4 | 454 | 0.500 |
| ☐ | chrM:750 | A | G | Homozygous | 100.0 % | 6839.3 | 723 | 340 | 383 | 723 | 340 | 383 | 0.500 |
| ☐ | chrM:1438 | A | G | Homozygous | 96.3 % | 2117.9 | 245 | 138 | 107 | 236 | 134 | 102 | 0.505 |
| ☐ | chrM:2352 | T | C | Homozygous | 100.0 % | 13108.4 | 1,375 | 501 | 874 | 1,375 | 501 | 874 | 0.500 |
| ☐ | chrM:2706 | A | G | Homozygous | 100.0 % | 11740.9 | 1,229 | 637 | 592 | 1,229 | 637 | 592 | 0.500 |
| ☐ | chrM:4769 | A | G | Homozygous | 100.0 % | 9708.8 | 1,012 | 558 | 454 | 1,012 | 558 | 454 | 0.500 |
| ☐ | chrM:6221 | T | C | Homozygous | 100.0 % | 9598.1 | 1,009 | 504 | 505 | 1,009 | 504 | 505 | 0.500 |
| ☐ | chrM:6587 | C | T | Homozygous | 100.0 % | 4775.7 | 511 | 267 | 244 | 511 | 267 | 244 | 0.500 |
| ☐ | chrM:7028 | C | T | Homozygous | 100.0 % | 6934.1 | 731 | 396 | 335 | 731 | 396 | 335 | 0.500 |
| ☐ | chrM:8512 | A | G | Homozygous | 100.0 % | 2524.8 | 265 | 27 | 238 | 265 | 27 | 238 | 0.500 |
| ☐ | chrM:8701 | A | G | Homozygous | 100.0 % | 6282.7 | 660 | 331 | 329 | 660 | 331 | 329 | 0.500 |
| ☐ | chrM:8860 | A | G | Homozygous | 100.0 % | 8672.5 | 904 | 315 | 589 | 904 | 315 | 589 | 0.500 |

# Data Analysis



mitoSAVE v 1.5

Choose your VCF format

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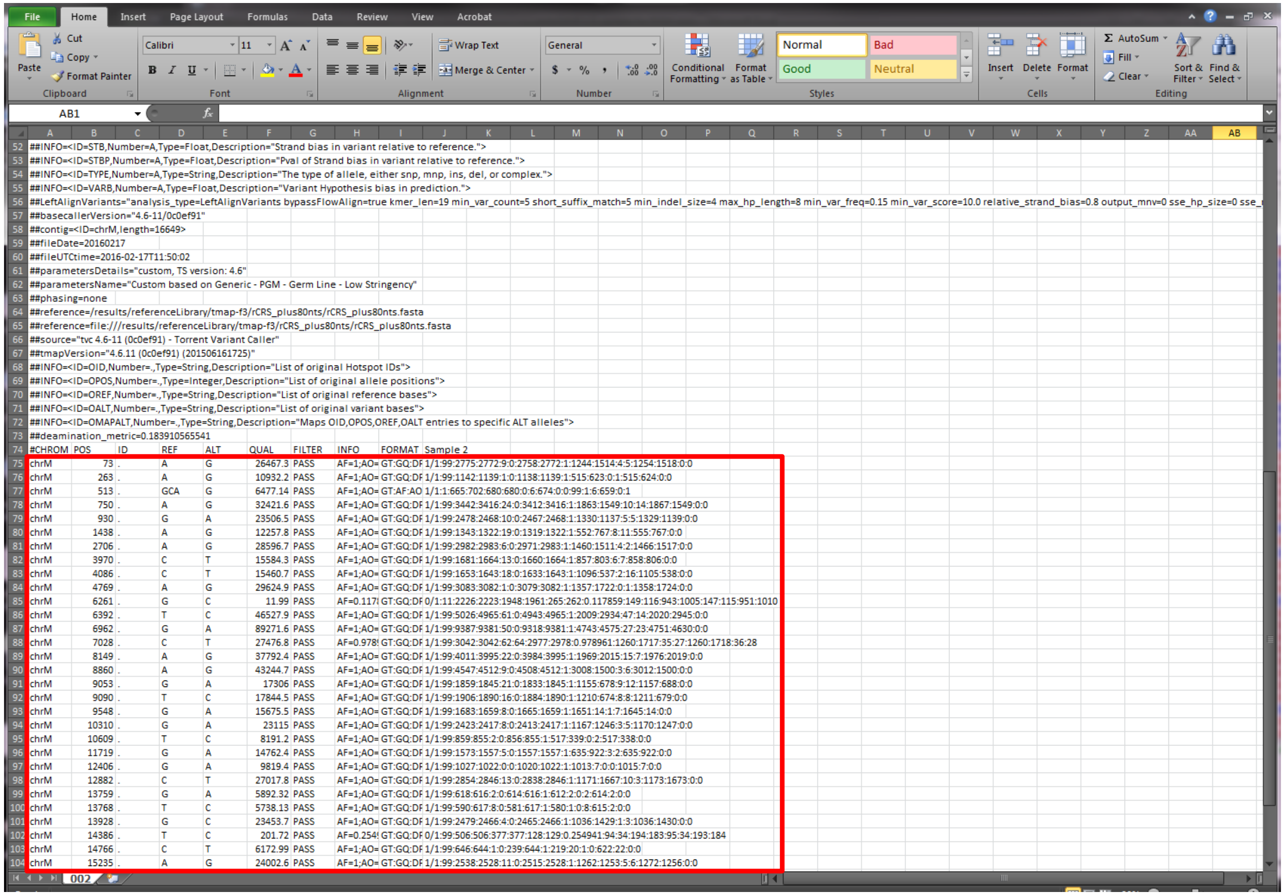
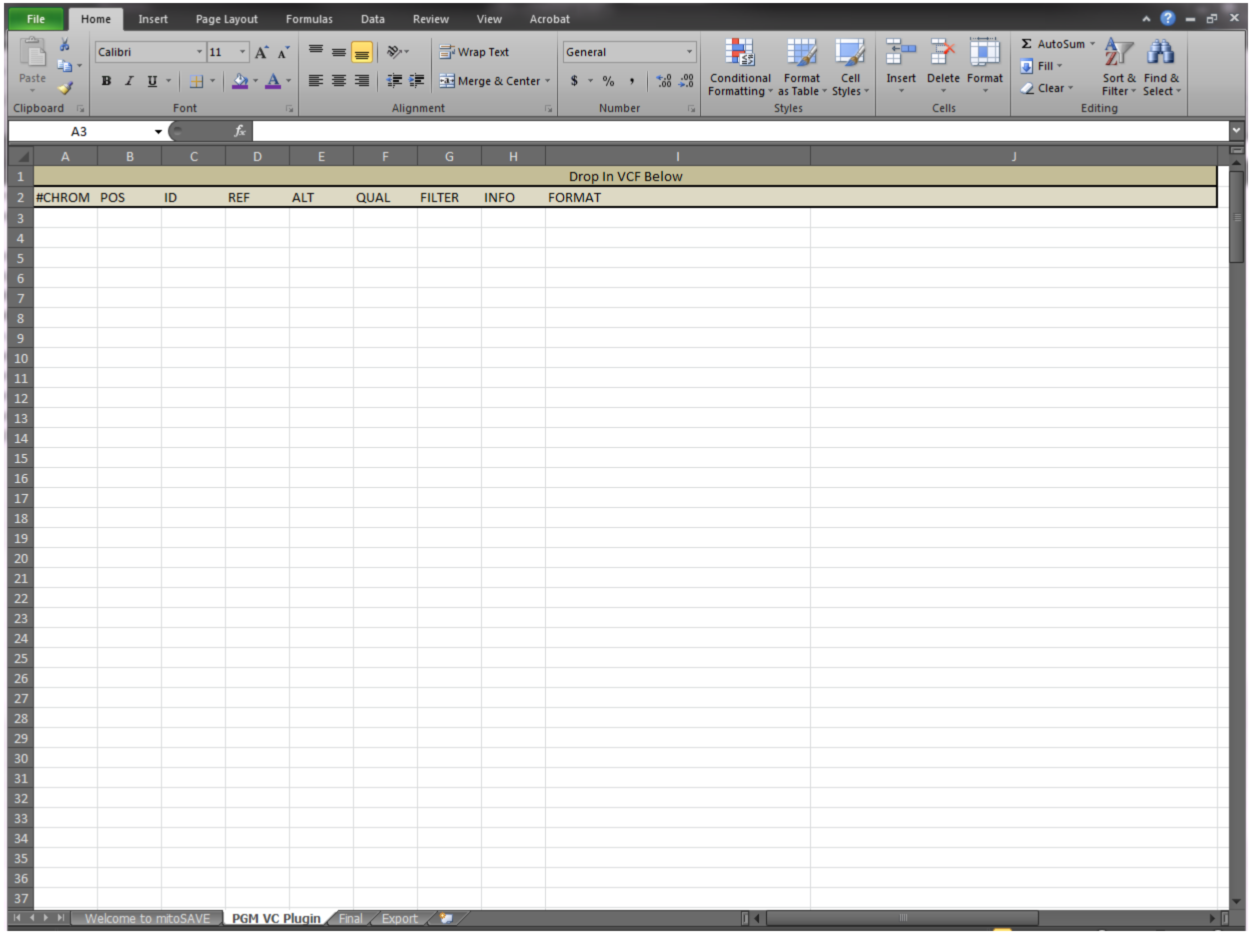
# Data Analysis



```
##INFO=<ID=STB,Number=A,Type=Float,Description="Strand bias in variant relative to reference.">
##INFO=<ID=STBP,Number=A,Type=Float,Description="Pval of Strand bias in variant relative to reference.">
##INFO=<ID=TYPE,Number=A,Type=String,Description="The type of allele, either snp, mnp, ins, del, or complex.">
##INFO=<ID=VARB,Number=A,Type=Float,Description="Variant Hypothesis bias in prediction.">
##LeftAlignVariants="analysis_type=LeftAlignVariants bypassFlowAlign=true kmer_len=19 min_var_count=5 short_suffix_match=5 min_indel_size=4 max_hp_length=8 min_var_freq=0.15 min_var_score=10.0 relative_strand_bias=0.8 output_mnv=0 sse_hp_size=0 sse_
##basecallerVersion="4.6-11/0c0ef91"
##contig=<ID=chrM,length=16649>
##fileDate=20160217
##fileUTCtime=2016-02-17T11:50:02
##parametersDetails="custom, TS version: 4.6"
##parametersName="Custom based on Generic - PGM - Germ Line - Low Stringency"
##phasing=none
##reference=/results/referenceLibrary/tmap-f3/rCRS_plus80nts/rCRS_plus80nts.fasta
##reference=file:///results/referenceLibrary/tmap-f3/rCRS_plus80nts/rCRS_plus80nts.fasta
##source="tvc 4.6-11 (0c0ef91) - Torrent Variant Caller"
##tmapVersion="4.6.11 (0c0ef91) (201506161725)"
##INFO=<ID=OID,Number=.,Type=String,Description="List of original Hotspot IDs">
##INFO=<ID=OPOS,Number=.,Type=Integer,Description="List of original allele positions">
##INFO=<ID=OREF,Number=.,Type=String,Description="List of original reference bases">
##INFO=<ID=OALT,Number=.,Type=String,Description="List of original variant bases">
##INFO=<ID=OMAPALT,Number=.,Type=String,Description="Maps OID,OPOS,OREF,OALT entries to specific ALT alleles">
##deamination_metric=0.183910565541
```

| #CHROM | POS | ID | REF | ALT | QUAL | FILTER | INFO | FORMAT | Sample 2 |
|---|---|---|---|---|---|---|---|---|---|
| chrM | 73 | . | A | G | 26467.3 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2775:2772:9:0:2758:2772:1:1244:1514:4:5:1254:1518:0:0 |
| chrM | 263 | . | A | G | 10932.2 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1142:1139:1:0:1138:1139:1:515:623:0:1:515:624:0:0 |
| chrM | 513 | . | GCA | G | 6477.14 | PASS | AF=1;AO= | GT:AF:AO | 1/1:1:665:702:680:680:0:6:674:0:0:99:1:6:659:0:1 |
| chrM | 750 | . | A | G | 32421.6 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:3442:3416:24:0:3412:3416:1:1863:1549:10:14:1867:1549:0:0 |
| chrM | 930 | . | G | A | 23506.5 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2478:2468:10:0:2467:2468:1:1330:1137:5:5:1329:1139:0:0 |
| chrM | 1438 | . | A | G | 12257.8 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1343:1322:19:0:1319:1322:1:552:767:8:11:555:767:0:0 |
| chrM | 2706 | . | A | G | 28596.7 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2982:2983:6:0:2971:2983:1:1460:1511:4:2:1466:1517:0:0 |
| chrM | 3970 | . | C | T | 15584.3 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1681:1664:13:0:1660:1664:1:857:803:6:7:858:806:0:0 |
| chrM | 4086 | . | C | T | 15460.7 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1653:1643:18:0:1633:1643:1:1096:537:2:16:1105:538:0:0 |
| chrM | 4769 | . | A | G | 29624.9 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:3083:3082:1:0:3079:3082:1:1357:1722:0:1:1358:1724:0:0 |
| chrM | 6261 | . | G | C | 11.99 | PASS | AF=0.117 | GT:GQ:DF | 0/1:11:2226:2223:1948:1961:265:262:0.117859:149:116:943:1005:147:115:951:1010 |
| chrM | 6392 | . | T | C | 46527.9 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:5026:4965:61:0:4943:4965:1:2009:2934:47:14:2020:2945:0:0 |
| chrM | 6962 | . | G | A | 89271.6 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:9387:9381:50:0:9318:9381:1:4743:4575:27:23:4751:4630:0:0 |
| chrM | 7028 | . | C | T | 27476.8 | PASS | AF=0.978 | GT:GQ:DF | 1/1:99:3042:3042:62:64:2977:2978:0.978961:1260:1717:35:27:1260:1718:36:28 |
| chrM | 8149 | . | A | G | 37792.4 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:4011:3995:22:0:3984:3995:1:1969:2015:15:7:1976:2019:0:0 |
| chrM | 8860 | . | A | G | 43244.7 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:4547:4512:9:0:4508:4512:1:3008:1500:3:6:3012:1500:0:0 |
| chrM | 9053 | . | G | A | 17306 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1859:1845:21:0:1833:1845:1:1155:678:9:12:1157:688:0:0 |
| chrM | 9090 | . | T | C | 17844.5 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1906:1890:16:0:1884:1890:1:1210:674:8:8:1211:679:0:0 |
| chrM | 9548 | . | G | A | 15675.5 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1683:1659:8:0:1665:1659:1:1651:14:1:7:1645:14:0:0 |
| chrM | 10310 | . | G | A | 23115 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2423:2417:8:0:2413:2417:1:1167:1246:3:5:1170:1247:0:0 |
| chrM | 10609 | . | T | C | 8191.2 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:859:855:2:0:856:855:1:517:339:0:2:517:338:0:0 |
| chrM | 11719 | . | G | A | 14762.4 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1573:1557:5:0:1557:1557:1:635:922:3:2:635:922:0:0 |
| chrM | 12406 | . | G | A | 9819.4 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:1027:1022:0:0:1020:1022:1:1013:7:0:0:1015:7:0:0 |
| chrM | 12882 | . | C | T | 27017.8 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2854:2846:13:0:2838:2846:1:1171:1667:10:3:1173:1673:0:0 |
| chrM | 13759 | . | G | A | 5892.32 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:618:616:2:0:614:616:1:612:2:0:2:614:2:0:0 |
| chrM | 13768 | . | T | C | 5738.13 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:590:617:8:0:581:617:1:580:1:0:8:615:2:0:0 |
| chrM | 13928 | . | G | C | 23453.7 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2479:2466:4:0:2465:2466:1:1036:1429:1:3:1036:1430:0:0 |
| chrM | 14386 | . | T | C | 201.72 | PASS | AF=0.254 | GT:GQ:DF | 0/1:99:506:506:377:377:128:129:0.254941:94:34:194:183:95:34:193:184 |
| chrM | 14766 | . | C | T | 6172.99 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:646:644:1:0:239:644:1:219:20:1:0:622:22:0:0 |
| chrM | 15235 | . | A | G | 24002.6 | PASS | AF=1;AO= | GT:GQ:DF | 1/1:99:2538:2528:11:0:2515:2528:1:1262:1253:5:6:1272:1256:0:0 |

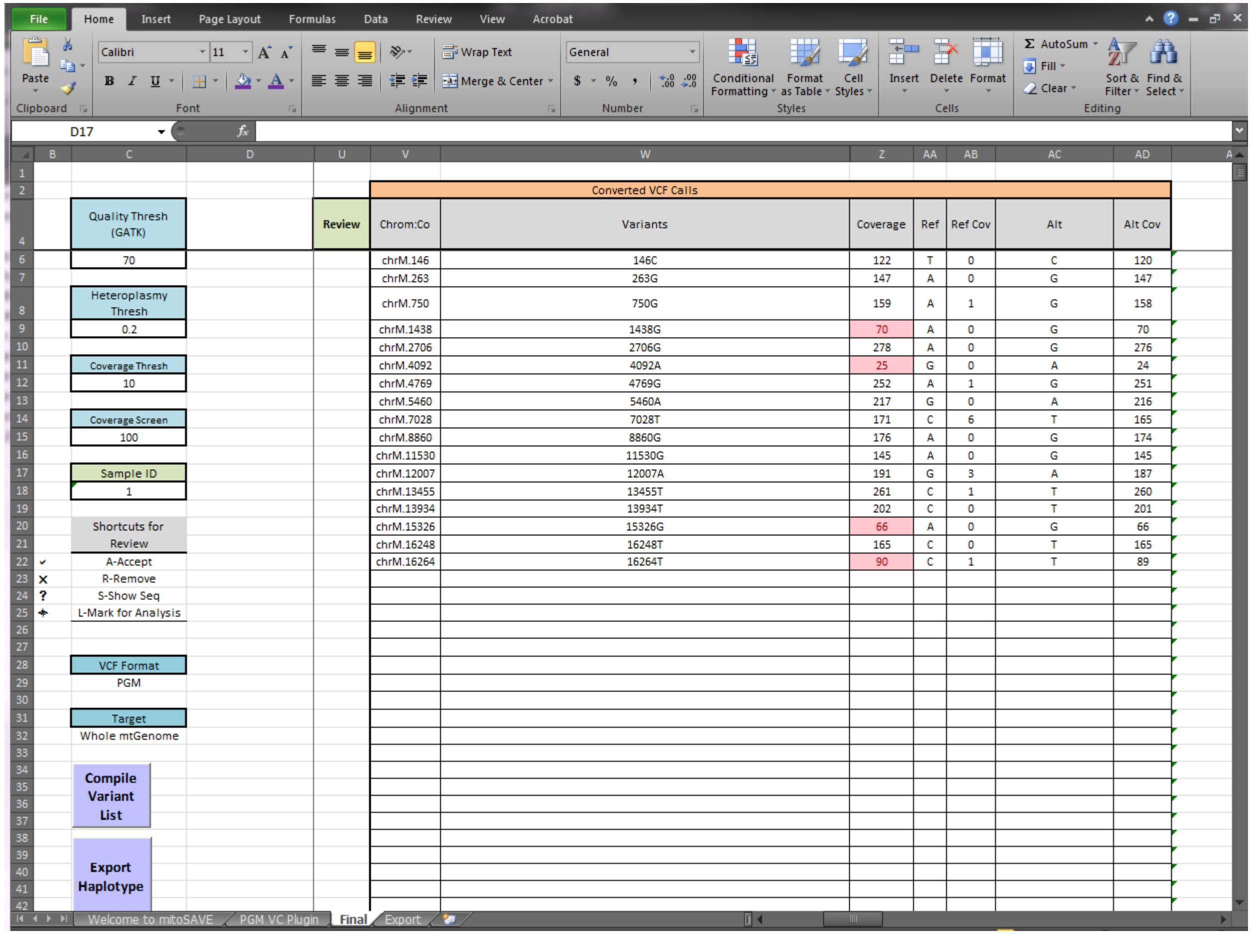
# Data Analysis



Drop In VCF Below

| #CHROM | POS | ID | REF | ALT | QUAL | FILTER | INFO | FORMAT |
|---|---|---|---|---|---|---|---|---|

# Data Analysis



Quality Thresh (GATK)
70

Heteroplasmy Thresh
0.2

Coverage Thresh
10

Coverage Screen
100

Sample ID
1

Shortcuts for Review
✓ A-Accept
✗ R-Remove
? S-Show Seq
✣ L-Mark for Analysis

VCF Format
PGM

Target
Whole mtGenome

Converted VCF Calls

| Review | Chrom:Co | Variants | Coverage | Ref | Ref Cov | Alt | Alt Cov |
|---|---|---|---|---|---|---|---|
| | chrM.146 | 146C | 122 | T | 0 | C | 120 |
| | chrM.263 | 263G | 147 | A | 0 | G | 147 |
| | chrM.750 | 750G | 159 | A | 1 | G | 158 |
| | chrM.1438 | 1438G | 70 | A | 0 | G | 70 |
| | chrM.2706 | 2706G | 278 | A | 0 | G | 276 |
| | chrM.4092 | 4092A | 25 | G | 0 | A | 24 |
| | chrM.4769 | 4769G | 252 | A | 1 | G | 251 |
| | chrM.5460 | 5460A | 217 | G | 0 | A | 216 |
| | chrM.7028 | 7028T | 171 | C | 6 | T | 165 |
| | chrM.8860 | 8860G | 176 | A | 0 | G | 174 |
| | chrM.11530 | 11530G | 145 | A | 0 | G | 145 |
| | chrM.12007 | 12007A | 191 | G | 3 | A | 187 |
| | chrM.13455 | 13455T | 261 | C | 1 | T | 260 |
| | chrM.13934 | 13934T | 202 | C | 0 | T | 201 |
| | chrM.15326 | 15326G | 66 | A | 0 | G | 66 |
| | chrM.16248 | 16248T | 165 | C | 0 | T | 165 |
| | chrM.16264 | 16264T | 90 | C | 1 | T | 89 |

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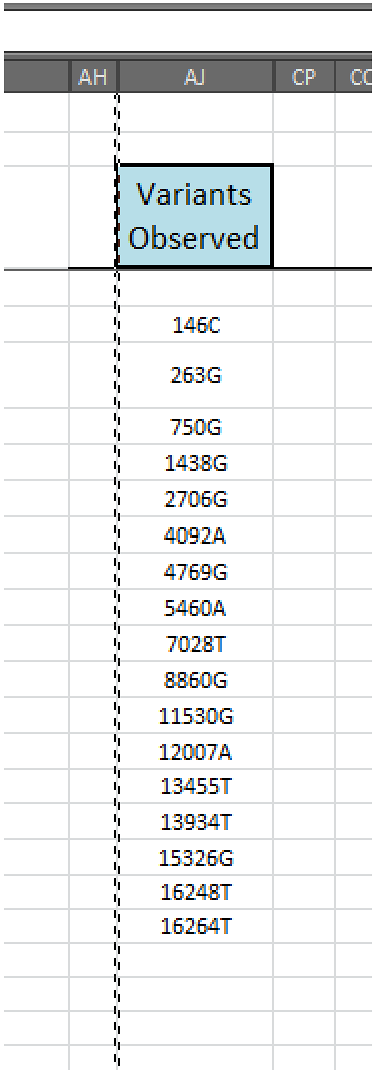
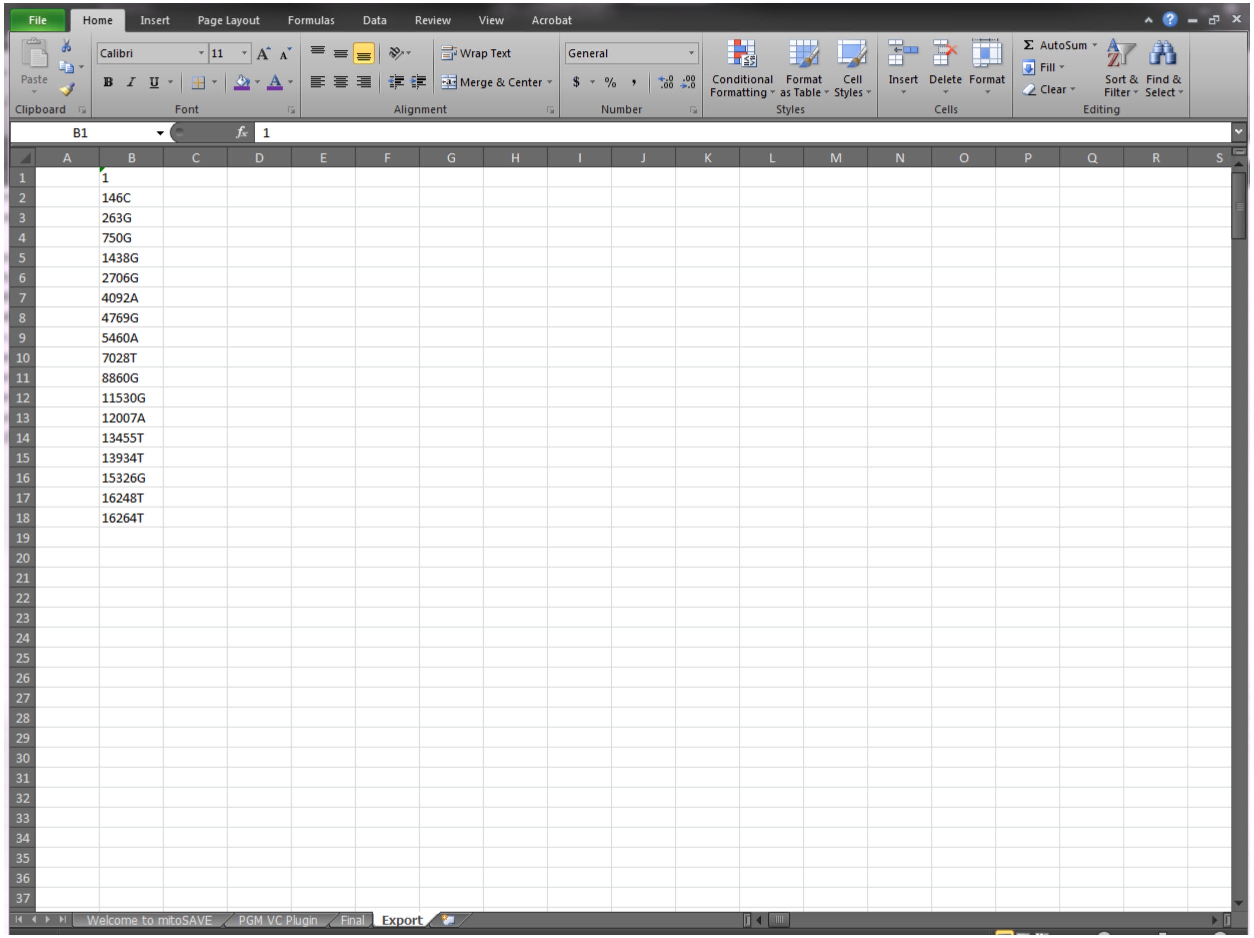
# Data Analysis



| Variants Observed |
|---|
| 146C |
| 263G |
| 750G |
| 1438G |
| 2706G |
| 4092A |
| 4769G |
| 5460A |
| 7028T |
| 8860G |
| 11530G |
| 12007A |
| 13455T |
| 13934T |
| 15326G |
| 16248T |
| 16264T |

# Data Analysis



B1: 1

| | B |
|---|---|
| 1 | 1 |
| 2 | 146C |
| 3 | 263G |
| 4 | 750G |
| 5 | 1438G |
| 6 | 2706G |
| 7 | 4092A |
| 8 | 4769G |
| 9 | 5460A |
| 10 | 7028T |
| 11 | 8860G |
| 12 | 11530G |
| 13 | 12007A |
| 14 | 13455T |
| 15 | 13934T |
| 16 | 15326G |
| 17 | 16248T |
| 18 | 16264T |

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# QUESTIONS?

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# Acknowledgements

- The Budowle Lab
- Thermo Fisher HID R&D Team

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