iontorrent

Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems

USER GUIDE

for use with:
Ion ReproSeq™ PGS Kit with Ion 510™ Chips (16 samples/run)
Ion ReproSeq™ PGS Kit with Ion 520™ Chips (24 samples/run)
Ion ReproSeq™ PGS Kit with Ion 530™ Chips (96 samples/run)
Ion AmpliSeq™ Polyploidy Panel Kit with Ion 520™ Chips (80 Samples)
Ion AmpliSeq™ Polyploidy Panel Kit with Ion 530™ Chips (480 Samples)

Catalog Numbers A34899, A34900, A34901, GS45690, GS45691
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Revision H

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0016712 H (English)

Revision	Date	Description
H	28 May 2024	• Added instructions for changing the chip type in a run template. See "Create a new run template to change chip types for a planned run" on page 141. • Added instructions to create libraries using the Ion AmpliSeq™ Polyploidy Panel Kits. See Chapter 3, "Prepare Ion AmpliSeq™ Polyploidy Panel libraries". • Added instructions for combining Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel libraries. See Chapter 4, "Combine Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel Libraries". • Updated instructions for creating planned runs to use the Ion AmpliSeq™ Polyploidy Panel Kits. See "Create a Planned Run Template for use with Ion AmpliSeq™ Polyploidy Panel Kits" on page 61. • Added instructions for reanalysis to view Ion AmpliSeq™ Polyploidy Panel library results from a combined library sequencing run. See "Reanalyze an Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel combined library run to view the Ion AmpliSeq™ Polyploidy Panel results" on page 123. • Added instructions for manually running the ReproSeqSnpAnalysis plugin. See "ReproSeqSnpAnalysis plugin" on page 125. • Updated Ion S5™ ExT Kit components Ion S5™ ExT Chef PGS Reagents Part No. A34676.
G.0	28 September 2023	Fix error in the instruction to create a planned run template.
F.0	31 July 2023	• Updated Ion S5™ ExT Chef Supplies (Part No. A30673) kit to include the PCR Plate Frame. • Updated instructions to include loading the PCR Plate Frame. See "Load the tip racks and PCR Plate" on page 69. • Updated instructions to include removing the PCR Plate Frame. See "Remove and dispose of used consumables" on page 98. • Updated dilution of libraries added to the Library Sample Tube. • Updated Ion SingleSeq™ Kit configuration.

The information in this guide is subject to change without notice.

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Product information

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CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems provide reagents and materials for high-throughput whole-genome amplification and sequencing to detect chromosomal aneuploidies, chromosome arm events (>48 Mbp), and copy number variations starting from a single cell. The kits are used with the Ion Chef™ System, and the Ion S5™/Ion S5™ XL System or one of the Ion GeneStudio™ S5 Systems, which enable automated template preparation, chip loading, and sequencing to minimize hands-on time.

The Ion SingleSeq™ Kit, a component of each Ion ReproSeq™ PGS Kit, includes reagents to extract, amplify, and barcode genomic DNA for preparing libraries from up to 24 or 96 samples. The Ion ReproSeq™ PGS Kits also include reagents to prepare templates and load four chips on the Ion Chef™ Instrument, and sequence the chips on an Ion S5™/Ion S5™ XL Sequencer or one of the Ion GeneStudio™ S5 Series Sequencers. Depending on the kit that is used, up to 384 samples can be sequenced per kit.

The Ion AmpliSeq™ Polyploidy Panel Kits (available separately) include 74 microhaplotype amplicons for human identification and 368 single SNP amplicons representing the population at large. With a total of 590 SNP sites covered, the Ion AmpliSeq™ Polyploidy Panel Kits unveil the unique genomic variations present in each embryo sample, facilitating premium PGT-A analysis. By leveraging SNPs, PGT-A

analysis gains valuable insights into triploidy 69,XXX, maternal contamination, and embryo identity, facilitating the selection of embryos for in vitro fertilization research.

Ion ReproSeq™ PGS Kit	Samples per kit
Ion ReproSeq™ PGS Kit with Ion 510™ Chips (16 samples/run)	64
Ion ReproSeq™ PGS Kit with Ion 520™ Chips (24 samples/run)	96
Ion ReproSeq™ PGS Kit with Ion 530™ Chips (96 samples/run)	384

Instrument compatibility

The Ion ReproSeq™ PGS Kits described in this user guide are compatible with the Ion S5™/Ion S5™ XL Sequencers and Ion GeneStudio™ S5 Series Sequencers. We recommend using the Ion S5™ XL, Ion GeneStudio™ S5 Plus, or Ion GeneStudio™ S5 Prime Sequencer for rapid turnaround between runs. Use of the kits with the Ion S5™ or Ion GeneStudio™ S5 Sequencer requires more analysis time between runs.

Software compatibility

- The instrument software is updated to the latest available version.
- The Ion Torrent™ Server to that is connected to, or included with, the instrument is updated to the latest available version of Torrent Suite™ Software. For more information, see Appendix C, "Connections between Ion Torrent™ Server and the Ion Chef™ Instrument".
- If using the Ion AmpliSeq™ Polyploidy Panel, the following target regions and hotspots files should be installed in Torrent Suite™ Software.
  - Target regions file — `Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed`
  - Hotspots file — `Ion_AmpliSeq_Polyploidy_Hotspots_w1.0.0.bed`
- To view data and analysis results for the Ion AmpliSeq™ Polyploidy Panel, the ReproSeqSnpAnalysis plugin is installed in Torrent Suite™ Software. For more information, see "Install or upgrade plugins" on page 140.

  Note: You can configure Torrent Suite™ Software to run the ReproSeqSnpAnalysis plugin automatically when you create a Planned Run. However, you must run this plugin manually if you want to include sibling QC analysis results in the plugin results. For more information, see "Run the plugin manually from the sequencing run report for Sibling QC data" on page 126.

- To visualize aneuploidy results, Ion Reporter™ Software 5.10 or later is required. However, for the latest features and analysis workflows, we recommend that you use Ion Reporter™ Software 5.20.

Kit contents and storage

Kit configurations

Three configurations of the Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems are available. The kits vary in the number and size of Ion SingleSeq™ Kits provided, the maximum number of samples processed per kit, and the type of Ion S5™ sequencing chip provided.

Ion ReproSeq™ PGS Kit	Amount per kit	Samples per kit
Ion ReproSeq™ PGS Kit with Ion 510™ Chips (16 samples/run); Cat. No. A34899		
Ion SingleSeq™ Kit (24 reactions)	3 boxes	64[1]
Ion S5™ ExT Chef Supplies	4 boxes	
Ion S5™ Chef Solutions	4 cartridges	
Ion S5™ ExT Chef PGS Reagents	4 cartridges	
Ion S5™ ExT Sequencing Solutions	1 box	
Ion S5™ ExT Sequencing Reagents	4 cartridges	
Ion 510™ Chip Kit	4 chips	
Ion ReproSeq™ PGS Kit with Ion 520™ Chips (24 samples/run); Cat. No. A34900		
Ion SingleSeq™ 96 Kit (96 reactions)	1 x 2 boxes	96
Ion S5™ ExT Chef Supplies	4 boxes	
Ion S5™ Chef Solutions	4 cartridges	
Ion S5™ ExT Chef PGS Reagents	4 cartridges	
Ion S5™ ExT Sequencing Solutions	1 box	
Ion S5™ ExT Sequencing Reagents	4 cartridges	
Ion 520™ Chip Kit	4 chips	
Ion ReproSeq™ PGS Kit with Ion 530™ Chips (96 samples/run); Cat. No. A34901		
Ion SingleSeq™ 96 Kit (96 reactions)	4 x 2 boxes	384
Ion S5™ ExT Chef Supplies	4 boxes	
Ion S5™ Chef Solutions	4 cartridges	
Ion S5™ ExT Chef PGS Reagents	4 cartridges	
Ion S5™ ExT Sequencing Solutions	1 box	

(continued)

Ion ReproSeq™ PGS Kit	Amount per kit	Samples per kit
Ion S5™ ExT Sequencing Reagents	4 cartridges	384
Ion 530™ Chip Kit	4 chips	

[1] We recommend loading 16 sample libraries per Ion 510™ Chip. However, 3 boxes of the Ion SingleSeq™ Kit provide reagents and barcode adapters to prepare 72 libraries. The leftover reagents and barcode adapters can be used with the next purchased kit.

Ion SingleSeq™ Kit

Use the Ion SingleSeq™ Kit (Part No. A28955; 24 reactions/kit) to extract, amplify, and barcode genomic DNA. Immediately before use, thaw tubes on ice if needed.

Component	Amount	Storage
Cell Extraction Buffer (green cap)	120 µL	–30°C to –10°C
Extraction Enzyme Dilution Buffer (violet cap)	115.2 µL	
Cell Extraction Enzyme (yellow cap)	4.8 µL	
Pre-Amplification Buffer (red cap)	115.2 µL	
Pre-Amplification Enzyme (white cap)	4.8 µL	
Amplification Buffer (orange cap)	648 µL	
Amplification Enzyme (blue cap)	12 µL	
Nuclease-free Water (clear cap)	432 µL	
Barcode Plate (Ion SingleSeq™ Barcode Adapters loaded in wells A1–H3 or wells A1–H12)	1 plate; 5 µL/well (Barcodes 1–24)	

Use the Ion SingleSeq™ 96 Kit (Part No. A34763; 96 reactions/kit) to extract, amplify, and barcode genomic DNA. Immediately before use, thaw tubes on ice if needed.

Component	Amount	Storage
Ion SingleSeq™ Reagents (Part. No. A52555)		
Cell Extraction Buffer (green cap)	480 µL	–25°C to –15°C
Extraction Enzyme Dilution Buffer (violet cap)	460.8 µL	
Cell Extraction Enzyme (yellow cap)	19.2 µL	
Pre-Amplification Buffer (red cap)	460.8 µL	
Pre-Amplification Enzyme (white cap)	19.2 µL	
Amplification Buffer (orange cap)	3 × 864 µL	
Amplification Enzyme (blue cap)	48 µL	

(continued)

Component	Amount	Storage
Nuclease-free Water (clear cap)	432 µL	–25°C to –15°C
Ion SingleSeq™ Barcode Plate Set 1-96 (Part. No. A52556)		
Barcode Plate (Ion SingleSeq™ Barcode Adapters loaded in wells A1–H3 or wells A1–H12)	1 plate; 5 µL/well (Barcodes 1–96)	–25°C to –15°C

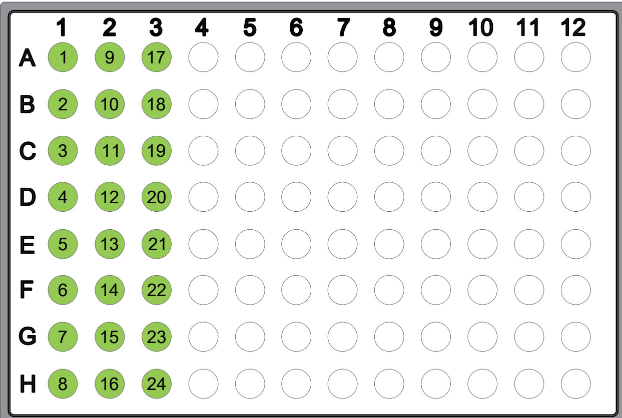
About the Ion SingleSeq™ Barcode Adapters

The Ion SingleSeq™ Kit is designed for high throughput applications and includes a 96-well Barcode Plate containing Barcode Adapters 1–24 or 1–96. Each well contains sufficient volume for a single use. The plate is sealed with foil that can be pierced with a pipet tip to collect the required amount of the Barcode Adapter for a reaction.

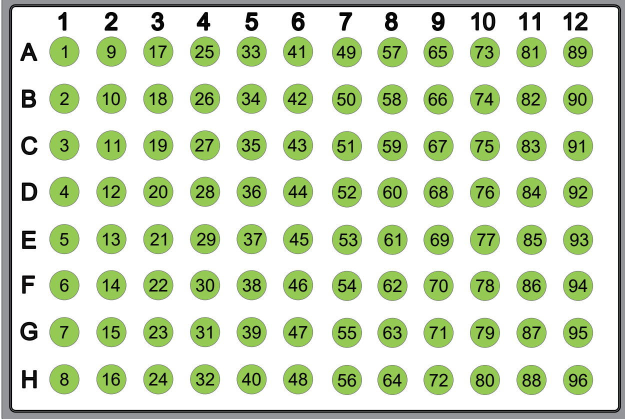
To minimize cross-contamination, either use all barcodes in a plate at once, or if you are using a subset, seal the pierced wells with laboratory tape.

IMPORTANT!

- Do not substitute barcode oligonucleotides from any other source for the Barcode Adapters contained in this kit.
- Avoid repeated freezing and thawing of the plate. Do not exceed 4 freeze/thaw cycles.



Barcode Plate containing Ion SingleSeq™ Barcodes 1–24. Twenty-four unique Barcode Adapters are loaded in wells A1 through H3.



Barcode Plate containing Ion SingleSeq™ Barcodes 1–96. Ninety-six unique Barcode Adapters are loaded in wells A1 through H12.

Note: Barcodes 1–24 in the Ion SingleSeq™ Barcodes 1–96 plate are identical to the Barcode Adapters in the Ion SingleSeq™ Barcodes 1–24 plate.

Ion S5™ ExT Kit components

IMPORTANT! Do not substitute components from any other Ion sequencing kits. This protocol was verified using these specific materials. Substitution can adversely affect system performance.

IMPORTANT! Store all consumables and cartridges under the recommended conditions and in an upright position. Do NOT store the Ion S5™ ExT Sequencing Reagents (Part No. A30522) on dry ice or in a closed environment where dry ice is present.

The Ion ReproSeq™ PGS 510, 520, and 530 Kits support 4 initializations, and contain all materials required for preparing and sequencing 4 Ion 510™, Ion 520™, or Ion 530™ Chips. On arrival, inspect all consumables and contact Technical Support if any of the products were damaged during shipping.

Contents	Amount/box	Storage
Ion S5™ ExT Chef Supplies (Part No. A30673)		
Chip Adapter	1	15°C to 30°C
Enrichment Cartridge v2	1	
Ion S5™ ExT Tip Cartridge	1	
PCR Plate	1	
PCR Plate Frame	1	
Frame Seal v2	1	
Recovery Station Disposable Lid v2	2	
Recovery Tube	12	
Ion S5™ Chef Solutions (Part No. A27754)		
Ion S5™ Chef Solutions	4 cartridges	15°C to 30°C
Ion S5™ ExT Chef PGS Reagents (Part No. A34676)		
Ion S5™ ExT Chef PGS Reagents	4 cartridges	–30°C to –10°C
Ion S5™ ExT Sequencing Solutions (Part No. A30521)		
Ion S5™ ExT Wash Solution	4 x 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	
Ion S5™ ExT Sequencing Reagents (Part No. A30522)		
Ion S5™ ExT Sequencing Reagents	4 cartridges	–30°C to –10°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Ion AmpliSeq™ Polyploidy Panel Kits	
Ion AmpliSeq™ Polyploidy Panel Kit with Ion 520™ Chips (80 Samples)	GS45691
Ion AmpliSeq™ Polyploidy Panel Kit with Ion 530™ Chips (480 Samples)	GS45690
Materials and equipment required for Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel library preparation	
ViiA™ 7 Real-Time PCR System with 96-Well Block (recommended), *or* Veriti™ 96-Well Thermal Cycler (0.2-mL block; for standard PCR amplification), *or* equivalent real-time or standard thermal cycler with heated lid	4453534 4452300
MicroAmp™ Optical 96-Well Reaction Plate MicroAmp™ Optical Adhesive Film (for real-time PCR) MicroAmp™ Clear Adhesive Film (for standard endpoint PCR)[1] *or equivalent*	N8010560 4360954 4306311
(Optional) MicroAmp™ Optical 8-Tube Strip, 0.2 mL[2] MicroAmp™ Optical 8-Cap Strip *or* MicroAmp™ Reaction Tube with Cap, 0.2 mL *or equivalent* 96-Well Tray/Retainer Set specific to your instrument	4316567 4323032 N8010540
Agencourt™ AMPure™ XP Reagent	Fisher Scientific NC9959336
DynaMag™-2 Magnet	12321D
1.5-mL Eppendorf™ DNA LoBind™ Microcentrifuge Tubes[3]	Fisher Scientific 13-698-791
Qubit™ 4 Fluorometer[4]	Q33238
Qubit™ dsDNA HS Assay Kit	Q32851 Q32854
Qubit™ Assay Tubes	Q32856
Ethanol	MLS
Low TE (10 mM Tris pH 8.0, 0.1 mM EDTA)	MLS
Phosphate-buffered saline (1X PBS, Ca^{2+}-free, Mg^{2+}-free, BSA-free)	MLS

(continued)

Item	Source
Nuclease-free Water	AM9932
SYBR™ Green I Nucleic Acid Gel Stain, 10,000X concentrate in DMSO[5]	S-7567
ROX™ Reference Dye[5]	12223-012
2, 10, 20, 200, 1000 µL pipettor set and filtered tips	MLS
Multichannel pipettors (2–20 µL and 20–200 µL; 8 channel)	MLS
(Optional) E-Gel™ Agarose Gels, 2%, and E-Gel™ iBase™ Power System, or E-Gel™ PowerBase™ System, *or equivalent*	Various
Additional materials required for templating and sequencing	
Ion Chef™ S5 Series Chip Balance	A29022
Non-interruptible Power Supply (UPS)[6]	MLS
Gloves, powder-free nitrile	MLS
Isopropanol, 70% solution	MLS
Wipes, disposable lint-free	MLS

[1] Recommended for all plate sealing other than real-time amplification reactions for easier application and removal.
[2] For standard end-point PCR amplification, standard tube strips and caps can be substituted.
[3] Can substitute 2.0-mL Eppendorf LoBind™ Tubes for 1.5-mL tubes.
[4] Qubit™ 2.0 Fluorometer and later are supported.
[5] Required if monitoring amplification by real-time PCR.
[6] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use a non-interruptible power supply that provides 2500 W output or higher.

Compatible Ion Chip kits

The following Ion Chip kits can be ordered separately for additional chips.

Description	Cat. No.	Quantity	Storage
Ion 510™ Chip Kit (4-pack)	A34292	4 chips	15°C to 30°C
Ion 520™ Chip Kit (4-pack)	A27761	4 chips	
Ion 530™ Chip Kit (4-pack)	A27763	4 chips	

Recommended materials for quantification and quality control

Item	Source
Qubit™ Flex Fluorometer or Qubit™ 4 Fluorometer[1] and the following kit. • Qubit™ dsDNA HS Assay Kit *(DNA samples)*	Q33327 Q33238 Q32851, Q32854
One of the following, or equivalent: • SimpliAmp™ Thermal Cycler • Veriti™ 96-Well Thermal Cycler[2] • VeritiPro™ Thermal Cycler, 96-well • ProFlex™ 96-well PCR System	Various
Ion Library TaqMan™ Quantitation Kit and real-time PCR instrument	4468802
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560, 4306737
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5-mL	13-698-791 (**fisherscientific.com**)
Agencourt™ AMPure™ XP Reagent	NC9933872
DynaMag™-2 Magnet	12321D
Nuclease-free Water	AM9932
Ethanol, Absolute, Molecular Biology Grade	050BP2818500 (**fisherscientific.com**)
Pipettors, 2–200 µL, and low-retention filtered pipette tips	(**fisherscientific.com**)
resDNASEQ™ Quantitative Human DNA Kit	A26366
For use with the Ion AmpliSeq™ Polyploidy Panel Kit with Ion 520™ Chips or Ion 530™ Chips[3]	
IonCode™ Barcode Adapters 1–384 Kit	A29751
DynaMag™–96 Side Magnet, or other plate magnet	12331D
Recommended sample control	
Control DNA (from CEPH Individual 1347-02)	403062

[1] Qubit™ 2.0 Fluorometer and later are supported.
[2] This product is supported but no longer available for purchase.
[3] For more information, see Chapter 3, "Prepare Ion AmpliSeq™ Polyploidy Panel libraries".

Recommended materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Recommended additional equipment	
One of the following Applied Biosystems™ real-time PCR instruments: • 7500 Real-Time PCR System • 7900HT Fast Real-Time PCR System[1] • StepOne™ Real-Time PCR System • StepOnePlus™ Real-Time PCR System • ViiA™ 7 Real-Time PCR System • QuantStudio™ 3 Real-Time PCR System • QuantStudio™ 5 Real-Time PCR System • QuantStudio™ 7 Flex Real-Time PCR System • QuantStudio™ 12K Flex Real–Time PCR System	Various
96-well plate centrifuge	**MLS**
Qubit™ 4 Fluorometer[2]	Q33238
Control DNA (CEPH 1347–02)	403062

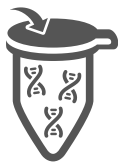
[1] Supported but no longer available for purchase.
[2] Qubit™ 2.0 Fluorometer or later are supported.

Workflow overview

Sample preparation workflow

Extract and prepare Ion SingleSeq™ library (page 22)

Extract gDNA from 1–10 cells using the extraction reagents provided with the kit, preamplify the gDNA, then amplify the gDNA with barcoded primers to generate libraries.

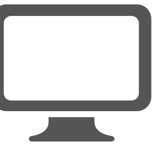


Pool, purify and quantify libraries (page 29)

Pool, purify and quantify libraries.

Create a planned run in Torrent Suite™ Software (page 48)

Planned Runs are digital instructions that are created in Torrent Suite™ Software for controlling the template preparation and sequencing instruments. Create a planned run to template and sequence the pooled libraries.



Template libraries (page 66)

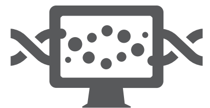
Template pooled libraries using the Ion Chef™ Instrument.

Sequence pooled libraries (page 92)

Sequence the pooled libraries using an Ion GeneStudio™ S5 Series Sequencer.

Analyze data (page 103)

Analyze data using Ion Reporter™ Software.



Prepare and pool Ion SingleSeq™ libraries

- Before you begin 21
- Extract and amplify the gDNA 22
- Pool, purify, and quantify the libraries 29

Before you begin

General procedural guidelines

- Use good laboratory practices to minimize cross-contamination of products. When designing the laboratory layout, consider the need for space separation of pre and post-amplification activities. Dedicate laboratory supplies and/or equipment to the appropriate space to reduce significantly the potential for contamination.
- MicroAmp™ Clear Adhesive Film is easier to apply and remove from amplification plates than MicroAmp™ Optical Adhesive Film, and is recommended for sealing plates in gDNA extraction, preamplification, and standard endpoint amplification reactions. Use MicroAmp™ Optical Adhesive Film to seal plates for real-time PCR amplification reactions.
- Pipet viscous solutions slowly and ensure complete mixing.
- Ensure that all reagents are completely thawed at room temperature, that is, no ice crystals are visible.
- Vortex all reagents, *except* for enzymes, for 5 seconds (mix enzymes by flicking the tube with your finger 4 times). Briefly centrifuge in a microcentrifuge for 3–5 seconds before use.

Guidelines for the number of libraries per run

The maximum number of Ion SingleSeq™ libraries you can pool and sequence on one chip depends on the type of chip you use. Follow these guidelines:

Ion Chip	Recommended maximum libraries per run
Ion 510™ Chip	16
Ion 520™ Chip	24
Ion 530™ Chip	96

Guidelines for sample handling

- Keep the cells on ice.
- For single cells or low-concentration control input gDNA, do not insert pipette tip into sample. The single cell could adhere to the tip and get removed from the sample tube.
- Add liquids above the top of liquid in a tube, do not submerge the tip.
- Do not vortex the cells.
- After adding extraction enzyme master mix to samples, do not mix. Vortexing or pipetting up and down can cause loss of cells on the wall of the well or pipette tip.
- Keep cell lysis and amplification reactions on ice or a cold block during reaction setup.
- Keep Ion SingleSeq™ libraries on ice during library pooling and quantification.

Extract and amplify the gDNA

Materials required

Provided in the Ion SingleSeq™ Kit (Part No. A28955 or A34763):

- Cell Extraction Buffer
- Extraction Enzyme Dilution Buffer
- Cell Extraction Enzyme
- Preamplification Buffer
- Preamplification Enzyme
- Amplification Buffer
- Amplification Enzyme
- Nuclease-free Water
- Barcode Plate containing Ion SingleSeq™ Barcodes 1–24 or Ion SingleSeq™ Barcodes 1–96

Other materials:

- *(If performing real-time PCR amplification)* MicroAmp™ 96-well Optical Reaction Plate and MicroAmp™ Optical Adhesive Film
- 0.2-mL MicroAmp™ 8-tube PCR tube strips, individual MicroAmp™ PCR tubes, caps, and MicroAmp™ 96-Well Tray/Retainer Set
- Pipettors, multichannel pipettors
- Pipette tips
- Real-time or standard PCR thermal cycler that is equipped with heated lid
- SYBR™ Green I and ROX dyes (Cat. Nos. S-7567 and 12223-012), if monitoring amplification by real-time PCR
- If performing troubleshooting, one of the following for diluting gDNA:
  - Low TE
  - PBS (Ca^{2+}-, Mg^{2+}-, BSA-free)
  - Nuclease-free Water

Extract genomic DNA

1. Prepare, by FACS or micro-manipulation, 1–10 cells per sample in up to 2.5 µL 1X PBS or Low TE, then pipet the cells into wells of a 96-well optical reaction plate.

   Note:
   - Instead of a 96-well reaction plate, a PCR tube strip, or individual PCR tubes can be used.
   - You can also sort cells by FACS directly into Cell Extraction Buffer at a density of 1–10 cells/5 µL.
   - If testing with the Ion AmpliSeq™ Polyploidy Panel, use 3–10 cells per sample.

   ---

2. *(Optional)* Set up one or more gDNA control reactions. Make serial dilutions of stock gDNA in low retention tubes immediately before use to avoid sample loss due to adsorption on to tube walls.

   Note: We recommend using Control DNA (from CEPH Individual 1347-02) to prepare gDNA control libraries. However, you can prepare control libraries from other accurately quantified, high quality, high molecular weight gDNA sources.

   ---

   a. Prepare a 1:100 dilution. Add 2 µL of stock solution (50 ng/µL) of Control DNA (from CEPH Individual 1347-02) to 198 µL of Low TE. Vortex to mix, then centrifuge briefly.

   b. Add 2 µL of 1:100 dilution to 131 µL of Low TE. Vortex to mix, then centrifuge briefly.
   The final concentration of Control DNA (from CEPH Individual 1347-02) is 7.5 pg/µL.

   c. Add 2 µL of diluted human gDNA (7.5 pg/µL) to an open plate well.

      Note: If testing with the Ion AmpliSeq™ Polyploidy Panel, use DNA at ≥37.5 pg/µL and confirm the concentration using the resDNASEQ™ Quantitative Human DNA Kit (Cat. No. A26366).

      ---

3. Add Cell Extraction Buffer (green cap) to each sample well to bring the total volume to 5 µL.

   Note:
   - If sorting cells by FACS directly into Cell Extraction Buffer (1–10 cells/5 µL), add 5 µL aliquots to each sample well.
   - If using gDNA for troubleshooting, add 3 µL of the Cell Extraction Buffer to the 2 µL of diluted gDNA added in step 2.

   ---

4. (Optional) Prepare a Non-Template Control (NTC) by adding 2.5 µL 1X PBS to 2.5 µL Cell Extraction Buffer.

5. Prepare an Extraction Enzyme master mix in a 1.5-mL tube on ice according to the following table. Scale the volume according to the number of samples (N) and NTCs you have. Vortex briefly, then centrifuge to collect liquid at the bottom of the tube.

   Note: Do not let the pipette tip touch the bottom of the well.

Component	Volume per reaction	Volume for N reactions[1]
Extraction Enzyme Dilution Buffer (violet cap)	4.8 µL	N × 4.8 µL × 1.1
Cell Extraction Enzyme (yellow cap)	0.2 µL	N × 0.2 µL × 1.1

   [1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

   Note: To dispense Extraction Enzyme master mix with an 8-channel multichannel pipettor, aliquot the master mix to 8 wells of a 96-well plate to serve as a reservoir. For example, aliquot 65 µL/well if you are processing 96 samples.

6. Add 5 µL Extraction Enzyme master mix to each cell or gDNA sample (10-µL final volume).

   IMPORTANT! Do not mix. Vortexing or pipetting up and down can cause loss of cells on the wall of the well or pipette tip.

7. Seal the plate with MicroAmp™ Clear Adhesive Film, then centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells.

8. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

Temperature	Time
75°C	10 minutes
95°C	4 minutes
22°C	Hold

9. Remove the plate, centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells, then place on ice or a cold block.

Pre-amplify the gDNA

1. Prepare a preamplification master mix in a 1.5-mL tube on ice according to the following table. Scale the volume according to the number of samples (N) and NTCs you have. Vortex briefly and centrifuge for 30 seconds to collect liquid at the bottom of tube.

Component	Volume per reaction	Volume for N reactions [1]
Preamplification Buffer (red cap)	4.8 µL	N × 4.8 µL × 1.1
Preamplification Enzyme (white cap)	0.2 µL	N × 0.2 µL × 1.1

   [1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

Note: To dispense the preamplification master mix with an 8-channel multichannel pipettor, aliquot the master mix to 8 wells of a 96-well plate to serve as a reservoir. For example, aliquot 65 µL/well if you are processing 96 samples.

2. Carefully remove the film seal from the plate, then add 5-µL preamplification master mix to each sample well (15-µL final volume).

IMPORTANT! Do not mix. Vortexing or pipetting up and down can cause loss of DNA on the wall of the tube or pipette tip.

3. Apply a new adhesive film, then centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells.

4. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program.

Stage	Step	Temperature	Time[1]
Hold	Activate the enzyme	95°C	2 minutes
Cycle (12 cycles)	Denature	95°C	15 seconds
	Anneal	15°C	50 seconds
		25°C	40 seconds
		35°C	30 seconds
	Extend	65°C	40 seconds
		75°C	40 seconds
Hold	—	4°C	Hold

[1] Cycling time is approximately 1 hour.

IMPORTANT! The thermal cycler must be equipped with a heated lid.

5. Remove the plate, centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells, then place on ice or a cold block.
   If you are not using the Ion AmpliSeq™ Polyploidy Panel, proceed to "Amplify the libraries" on page 26.

6. If you intend to use the Ion AmpliSeq™ Polyploidy Panel on preamplification material instead of final Ion SingleSeq™ libraries (recommended), transfer 6 µL of each sample reaction to a separate well in a new 96-well PCR plate.
   - To prepare Ion ReproSeq™ libraries, proceed to "Amplify the libraries" on page 26.
   - To prepare polyploidy libraries, proceed to Chapter 3, "Prepare Ion AmpliSeq™ Polyploidy Panel libraries".

Amplify the libraries

You can perform the amplification reaction on a real-time PCR instrument to monitor amplification during the run. Monitoring can give an early indication of amplification failure in a particular sample, or that a library is over- or under-represented before pooling.

1. If you are performing real-time PCR, prepare SYBR™ Green I/ROX dye mix. If you are performing standard endpoint PCR, proceed to step 2, then use Nuclease-free Water instead of the dye mix, as indicated in step 3.
   a. Dilute the stock SYBR™ Green I reagent 1:1000 with Low TE buffer to make a 10X working solution.
   b. Combine 10X SYBR™ Green I working solution and 25 µM ROX Reference Dye for the number of reactions (N) according to the following table:

Component	Volume per reaction	Volume for N reactions[1]
10X SYBR™ Green I working solution	0.5 µL	N × 0.5 µL × 1.1
25 µM ROX Reference Dye (or 2.5 µM, see below)	1.0 µL	N × 1.0 µL × 1.1
Nuclease-free Water (clear cap)	1.0 µL	N × 1.0 µL × 1.1

[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

IMPORTANT! Use ROX Reference Dye at 25 µM for the following real-time PCR instruments: 7300 Real-Time PCR System, 7900HT Real-Time PCR Instrument, StepOne™, StepOnePlus™, ABI PRISM™ 7000, and ABI PRISM™ 7700 instruments.

Dilute ROX Reference Dye to 2.5 µM with Nuclease-free Water and use 1.0 µL per reaction for the following instruments: 7500 Real-Time PCR Instrument and ViiA™ 7 instruments, and Agilent™ Mx3000P™, Mx3005P™, and Mx4000™ instruments.

2. Prepare the Ion SingleSeq™ Barcodes 1–24 plate:
   a. Thaw the plate for 10 minutes at room temperature.
   b. Centrifuge the plate briefly to collect contents at the bottom of the wells.
   c. Wipe the foil seal with 70% ethanol, then allow it to dry.

3. Prepare an amplification master mix in a 5-mL tube on ice according to the following table. Scale the volume according to the number (N) of samples and NTCs you have. Vortex briefly, then centrifuge to collect liquid at the bottom of tube.

Component	Volume per reaction		Volume for N reactions[1]
	Ion ReproSeq™ libraries only	Ion ReproSeq™ libraries and polyploidy libraries[2]	
Amplification Buffer (orange cap)	27 µL	27 µL	N × 27 µL × 1.1
Amplification Enzyme (blue cap)	0.5 µL	0.5 µL	N × 0.5 µL × 1.1
SYBR™ Green I/ROX dye mix[3]	2.5 µL	2.5 µL	N × 2.5 µL × 1.1
Nuclease-free water	0 µL	6 µL	N × 6 µL × 1.1
Total volume per reaction	**30 µL**	**36 µL**	

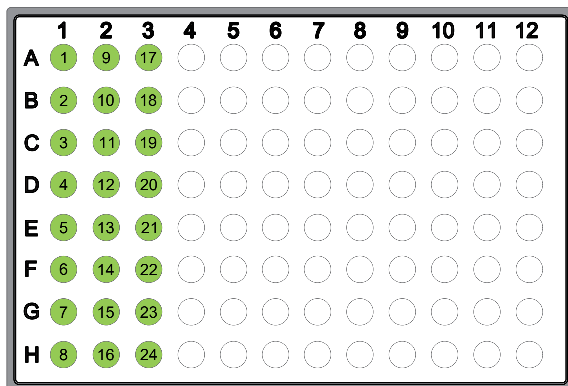
[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.
[2] An aliquot to prepare polyploidy libraries was removed after preamplification. (See step 6 of "Pre-amplify the gDNA" on page 24).
[3] Replace with Nuclease-free Water if performing endpoint PCR.

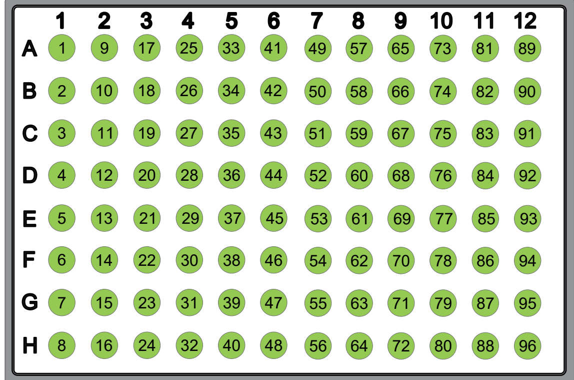
Note: To dispense the amplification master mix with an 8-channel multichannel pipettor, aliquot the master mix to eight wells of a 96-well plate to serve as a reservoir. For example, aliquot 396 µL/well if you are processing 96 samples.

4. Remove the film seal from the sample plate, then add amplification master mix to each well (45-µL final volume).
   - For samples being used to prepare Ion ReproSeq™ libraries only, add 30 µL of amplification master mix.
   - For samples being used to prepare both Ion ReproSeq™ libraries and polyploidy libraries, add 36 µL of amplification master mix.

5. Pierce the foil above the desired well of the Barcode Plate (see plate map) with a pipette tip. With a new tip, withdraw 5 µL of the Ion SingleSeq™ Barcode Adapter, then add to the appropriate sample (50-µL final volume). Repeat for each sample. Each Barcode Adapter is single-use only.

Note:
- To avoid sample misidentification later, be careful to track the correspondence of each sample and its Barcode Adapter.
- See "About the Ion SingleSeq™ Barcode Adapters" on page 14 for barcode plate handling guidelines.
- In the following plate map, Ion SingleSeq™ Barcode Plates containing Barcodes 1–24 and 1–96. Barcode Adapters are loaded in the indicated wells.





6. Adjust a pipettor to 30 µL, then mix the samples by pipetting up and down, using a new tip for each sample. Do not introduce bubbles into the samples.

   Note: If you used a non-optical reaction plate or PCR tube strip for cell lysis and preamplification, you can transfer samples to an optical plate at this point to perform real-time PCR amplification.

7. Apply a new film seal to the plate and briefly centrifuge to collect liquid at the bottom of the wells.

8. Place a MicroAmp™ Optical Film Compression Pad on the plate, load into the thermal cycler, then run the following program.

Step	Stage	Temperature	Time[1]
Hold	Activate the enzyme	95°C	3 minutes
Cycle (4 cycles)	Denature	95°C	20 seconds
	Anneal	50°C	25 seconds
	Extend	72°C	40 seconds
Cycle (12 cycles)[2]	Denature	95°C	20 seconds
	Anneal and Extend	72°C	55 seconds
Hold	—	4°C	Hold

[1] Cycling time is approximately 30 minutes.
[2] Acquire fluorescence data at this step if monitoring amplification in real-time.

9. Remove the plate, centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells, then place on ice or in a cold block.

10. *(Optional)* We recommend that new users run 10 µL of each amplified library on an E-Gel™ 2% Agarose Gel, or equivalent agarose gel, to check the quality of the amplified libraries.

    Note: Unpurified library fragments typically migrate nearer to 350 bp instead of the expected 250 bp.

Proceed to "Pool, purify, and quantify the libraries".

STOPPING POINT Sample libraries can be stored at –30°C to –10°C before proceeding to the next step.

Pool, purify, and quantify the libraries

Note: We recommend that you perform library pooling, purification, and quantification in one session. Do not store library pools before quantification.

Materials required

- 70% Ethanol - freshly prepared
- AMPure™ XP Reagent - warmed to room temperature
- DynaMag™-2 Magnet or DynaMag™–96 Side
- Low TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)
- Qubit™ 4 Fluorometer (Qubit™ 2.0 Fluorometer and later are supported)
- Qubit™ dsDNA HS (High Sensitivity) Assay Kit
- Qubit™ Assay Tubes

Warm AMPure™ XP Reagent

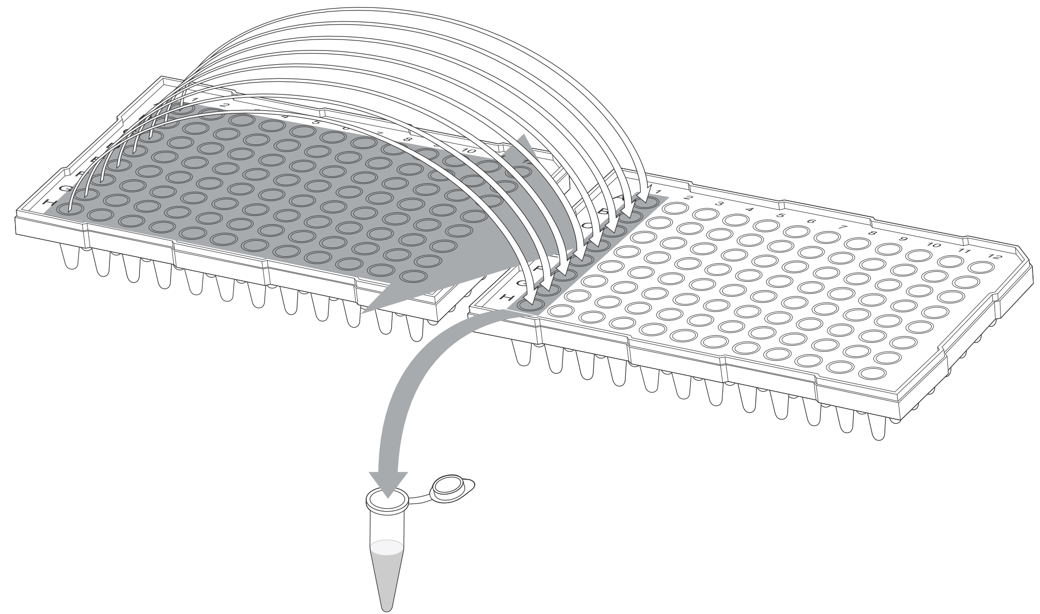
Warm AMPure™ XP Reagent at room temperature for 30 minutes.

Pool the libraries

1. Pool the libraries according to the PCR method you used to amplify the libraries. Before pooling, vortex the amplification reactions to mix, then centrifuge briefly to collect contents at the bottom of the wells or tubes.

If you used	Action
Standard endpoint PCR amplification	Add 5 µL of each library to a new 1.5-mL tube to create an equi-volume pool. Vortex the tube to mix and pulse-centrifuge to collect contents at the bottom of the tube.
Real-time PCR amplification	Calculate the median C_t value of the libraries you want to pool and adjust the volumes of libraries deviating from the median C_t following these guidelines: • Libraries that fall within 1 C_t of the median should be added at the normal volume (5 µL). • Libraries that fall >3 C_ts **later** than the median should not be included in the pool. It is likely that the input material was not present in the tube or well or was absorbed to the tube side. • Libraries that fall ≥1 but ≤3 C_ts **later** than the median should be added at 2X the normal volume (10 µL). • Do not adjust more than 25% of the libraries in a pool (excluding libraries > 3 C_ts later than median). If more than 25% of libraries fall 1 or more C_ts away from the median, adjust libraries with the most extreme C_t deviations until the 25% threshold is reached.

Note: You can use the following technique to rapidly pool 5-µL aliquots of a large number of libraries. First use an 8-channel pipettor to transfer aliquots from up to 96 libraries into 8 wells of a new plate, then use a standard pipettor to combine the contents of the 8 wells into a 1.5-mL Eppendorf™ LoBind™ tube. Use a new tip for every well when you transfer the 5-µL aliquots.



2. Transfer 40 µL of the library pool to a 0.2-µL tube for purification and quantification.

Note:
- When pooling fewer than 8 libraries, the pool volume is less than 40 µL. Add Nuclease-free Water to bring the final volume to 40 µL before library pool purification.
- When pooling more than 8 libraries, remove 40 µL from the pool for purification and quantification. If the remaining volume is ≥40 µL, store at –30°C to –10°C to use if a repeat purification is needed.

Purify the library pool

1. Transfer 40 µL of the library pool to a fresh PCR tube.
2. Heat the 40-µL aliquot of library pool in a thermal cycler using the following program.

Step	Temperature	Time	Number of cycles
1	70°C	2 minutes	1
2	22°C	Hold	1

3. Briefly pulse-centrifuge the tube to collect the contents, then transfer to a new 1.5-mL Eppendorf™ LoBind™ tube.
4. Add 40 µL (1X volume) of room temperature AMPure™ XP beads to the library pool.

5. Vortex briefly, pulse-centrifuge to collect contents, then incubate for 5 minutes at room temperature.

6. Place the tube in the DynaMag™-2 Magnet, then wait 5 minutes for beads to aggregate to the side of the tube or well of the plate.

7. Aspirate the supernatant carefully, then discard.

8. Wash beads with 250 µL of freshly prepared 70% ethanol while the tube or plate is still on the magnet.

9. Incubate for 30 seconds.

10. Aspirate the wash solution carefully, then discard.

11. Repeat step 8 through step 10, thoroughly removing all ethanol after the second wash.

12. Allow the beads to dry at room temperature for 3–4 minutes with the tube on the magnet.

13. Remove the tube from the magnet, add 40 µL of Low TE, then resuspend the beads by pipetting up and down.

14. Incubate the tube at room temperature for 1 minute.

15. Place the tube in the DynaMag™-2 Magnet, then wait 2–3 minutes for the beads to aggregate to the side of the tube.

16. Transfer 35 µL of the supernatant containing the purified library pool to a new 1.5-mL Eppendorf™ LoBind™ tube and place on ice. Avoid carryover of beads.

Quantify the library pool

Quantify the Ion SingleSeq™ library pool with the Qubit™ dsDNA HS (High Sensitivity) Assay Kit.

For details instructions, see the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326).

1. Prepare a Qubit™ working solution by diluting the Qubit™ dsDNA HS Reagent (Component A) 1:200 in Qubit™ dsDNA HS Buffer (Component B) in a plastic tube.

   Prepare sufficient working solution to quantify one or more purified pools plus two standards (volume required = (number of pools + 2) × 200 µL × 1.1).

   IMPORTANT! Do not prepare the working solution in a glass container.

2. Prepare the standards.
   a. Add 190 µL Qubit™ working solution to two labeled Qubit™ Assay Tubes used for standards.

   b. Add 10 µL of Qubit™ standard (Components C) to the one tube and 10 µL of Qubit™ standard (Components D) to the other tube.

   c. Mix by vortexing 2–3 seconds. Do not create bubbles.

3. Prepare the sample or library.
   a. Aliquot 198 µL Qubit™ working solution to labeled Qubit™ Assay Tubes used for samples.
   b. Add 2 µL of the library pool to the appropriate sample tube.
   c. Mix by vortexing 2–3 seconds. Do not create bubbles.
4. Incubate the tubes in the dark at room temperature for 2 minutes.
5. Measure standards first to generate a standard curve, then measure the library pool.
6. Convert ng/µL to nM by multiplying the ng/µL values obtained in step 5 by 6.06 nmol/mg.
   Example: Library pool concentration is 10 ng/µL
   10 ng/µL (or 10 mg/L) × 6.06 nmol/mg = 60.6 nmol/L = 60.6 nM

   Note: For DNA segments of 250-bp average length:
   MW = (250 bp × 660,000 mg/mol/bp) = 1.65×10^8 mg/mol
   Inverting 1.65×10^8 mg/mol and multiplying by 1×10^9 nmol/mol gives 6.06 nmol/mg.
7. Dilute the library pool to 1 nM.

   Note: In the preceding example, add 298 µL of Low TE to 5 µL of 60.6-nM pooled library stock. See "Library yield is low" on page 134 if library yield and concentration is lower than expected.

STOPPING POINT Diluted library pools and non-diluted library pool stock solutions can be stored for 1 week at 4°C. For longer term, store at -20°C.

Prepare Ion AmpliSeq™ Polyploidy Panel libraries

- Workflow . . . 34
- Materials required . . . 34
- Input samples . . . 34
- Prepare DNA target amplification reactions . . . 35
- Amplify the targets . . . 35
- Partially digest amplicons . . . 36
- Perform the ligation reaction . . . 36
- Purify the unamplified library . . . 37
- Quantify the unamplified library by qPCR . . . 38
- Quantify the amplified library using the Qubit™ Fluorometer . . . 41

Workflow

Polyploidy library procedure

Prepare and amplify targets (page 35**)**

Amplify target regions from DNA to generate libraries.

Partially digest amplicons (page 36**)**

Partially digest libraries to enable adapter ligation.

Ligate adapters and purify (page 36**)**

Ligate barcode adapters to libraries and purify.

Quantify the libraries (page 38 **or** page 41**)**

- Preamplification aliquot input—page 41
- Ion SingleSeq™ libraries—"Purify the unamplified library" on page 37 and "Quantify the unamplified library by qPCR" on page 38

Materials required

- Ion AmpliSeq™ Polyploidy Panel Kit with Ion 530™ Chips (480 Samples)
- IonCode™ Barcode Adapters 385-768 Kit
- Qubit™ Fluorometer and Qubit™ dsDNA HS Assay Kit or
  Ion Library TaqMan™ Quantitation Kit and real-time PCR instrument
- Other materials—See "Required materials not supplied" on page 16 and "Recommended materials" on page 19.

Input samples

The following input samples are compatible with Ion AmpliSeq™ Polyploidy Panel Kits.

- Aliquots removed after preamplification step ("Pre-amplify the gDNA" on page 24)—Recommended.
- Individual Ion SingleSeq™ libraries ("Pool the libraries" on page 29)

Prepare DNA target amplification reactions

IMPORTANT! Primer pools and 5X Ion AmpliSeq™ HiFi Mix are viscous. Pipet slowly and mix thoroughly. We recommend PCR setup on ice or a cold block.

1. For Ion SingleSeq™ library input samples, dilute each Ion SingleSeq™ aliquot 1:25 in water or Low TE.

   If you are using preamplification reaction input samples, proceed to step 2 without dilution.

2. Transfer 2 µL of each input sample to a 96-well plate.

3. Add the following components to each sample well. Prepare a master mix for multiple reactions.

Component	Volume per reaction	Volume for N reactions[1]
5X Ion AmpliSeq™ HiFi Mix(red cap)	4 µL	N × 4 µL × 1.1
5X Ion AmpliSeq™ Polyploidy Panel	4 µL	N × 4 µL × 1.1
Nuclease-free Water	10 µL	N × 10 µL × 1.1
Total	**18 µL**	

[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

4. Seal the plate with a MicroAmp™ Clear Adhesive Film.

   Note: To prevent evaporation during target amplification, use an applicator tool to press the film securely around each reaction well and around the perimeter of the plate.

5. Place a MicroAmp™ Optical Film Compression Pad on the plate, then load the plate into the thermal cycler.

Proceed to "Amplify the targets" on page 35.

Amplify the targets

To amplify target regions, run the following program.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
20 cycles	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold

Partially digest amplicons

IMPORTANT! FuPa Reagent is viscous. Pipet slowly and mix thoroughly. Perform this step on ice or a cold block, then quickly proceed to incubation.

1. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.
2. **Add 2 µL of FuPa Reagent** (brown cap) to each amplified sample. The total volume is ~22 µL.
3. Seal the plate with a clear adhesive film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
4. Place a compression pad on the plate, load in the thermal cycler, then run the following program:

Temperature	Time
50°C	10 minutes
55°C	10 minutes
60°C	20 minutes
10°C	Hold (for up to 1 hour)

5. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells.

STOPPING POINT Store plate at –20°C for longer periods.

Perform the ligation reaction

1. If there is visible precipitate in the Switch Solution or the tube cap after thawing, vortex or pipet up and down at room temperature to resuspend before pipetting.
2. Briefly centrifuge the plate to collect the contents.

3. Carefully remove the plate seal, then add the following components in the order that is listed to each well containing digested amplicons. If preparing multiple non-barcoded libraries, a master mix of Switch Solution and adapters can be combined before addition.

IMPORTANT!
- Add the DNA Ligase last. Do not combine DNA Ligase and adapters before adding to digested amplicons.
- You must use the IonCode™ Barcode Adapters. Do not use the Ion SingleSeq™ Barcode Plate Set GX.
- Use only one barcode (IonCode™ Barcode Adapter) for each sample.

Order of addition	Component	Volume
1	Switch Solution (yellow cap)	4 µL
2	IonCode™ Barcode Adapter	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume** (including ~22 µL of digested amplicon)	**~30 µL**

4. Seal the plate with a new MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

5. Place a MicroAmp™ Compression Pad on the plate, load in the thermal cycler, then run the following program:

Temperature	Time
22°C	30 minutes
68°C	5 minutes
72°C	5 minutes
10°C	Hold (for up to 24 hours)

STOPPING POINT Samples can be stored for up to 24 hours at 10°C on the thermal cycler. For longer periods, store at –20°C.

Purify the unamplified library

IMPORTANT! Bring Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.

Do NOT substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

1. Prepare 70% ethanol (350 µL × number of samples) fresh daily.

2. Briefly centrifuge the plate to collect the contents in the bottom of the wells.

3. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.

   Note: Visually inspect each well to ensure that the mixture is homogeneous.

4. Incubate the mixture for 5 minutes at room temperature.
5. Place the plate in a magnetic rack such as the DynaMag™-96 Side Magnet, then incubate for 2 minutes or until the solution clears. Carefully remove, then discard the supernatant without disturbing the pellet.
6. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet to wash the beads. Carefully remove, then discard the supernatant without disturbing the pellet.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down 5 times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.

7. Repeat step 6 for a second wash.
8. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes. Do not over dry.

   IMPORTANT! Residual ethanol drops inhibit library amplification. If needed, centrifuge the plate and remove remaining ethanol before air-drying the beads. Under conditions of low relative humidity, the beads air-dry rapidly. Do not over dry.

Proceed immediately to quantification.

- To quantify using qPCR, proceed to “Quantify the unamplified library by qPCR” on page 38.
- To quantify using the Qubit™ Fluorometer, proceed to “Quantify the amplified library using the Qubit™ Fluorometer” on page 41.

Quantify the unamplified library by qPCR

Elute the unamplified Ion AmpliSeq™ Polyploidy Panel library, then determine the concentration by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802). Unamplified libraries typically have yields of 250 pM–1 nM. After quantification, determine the dilution factor that results in a concentration of 250 pM.

Elute and dilute the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of Low TE to the pellet to disperse the beads.
2. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
3. Incubate at room temperature for at least 2 minutes.
4. Place the plate on the magnet for at least 2 minutes.

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C. We recommend transferring the supernatant to a new plate, then seal the plate with MicroAmp™ Clear Adhesive Film for long-term storage.

---

5. Prepare a 100-fold dilution for quantification. Remove 2 µL of supernatant, containing the library, then combine with 198 µL of Nuclease-free Water.

Proceed immediately to "Quantify library by qPCR and calculate the dilution factor" on page 39.

Quantify library by qPCR and calculate the dilution factor

Determine the concentration of each Ion AmpliSeq™ Polyploidy Panel library by qPCR with the Ion Library TaqMan™ Quantitation Kit using the following steps. Analyze each sample, standard, and negative control in duplicate 20-µL reactions.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Control Library (~68 pM; from the Ion Library TaqMan™ Quantitation Kit) at 6.8 pM, 0.68 pM, and 0.068 pM. Mark these tubes as standards, then use these concentrations in the qPCR instrument software.
2. Prepare reaction mixtures.
    - 96-well plate: For each sample, control, and standard, combine 20 µL of 2X Ion Library qPCR Master Mix and 2 µL of Ion Library TaqMan™ Quantitation Assay, 20X, then mix thoroughly. Dispense 11-µL aliquots into the wells of a 96-well PCR plate.
    - 384-well plate: For each sample, control, and standard, combine 10 µL of 2X Ion Library qPCR Master Mix and 1 µL of Ion Library TaqMan™ Quantitation Assay, 20X, then mix thoroughly. Dispense 5.5-µL aliquots into the wells of a 384-well PCR plate.
3. Add diluted library or control to each well.
    - 96-well plate: Add 9 µL of the diluted (1:100) Ion AmpliSeq™ Polyploidy Panel library or 9 µL of each control dilution to each well (two wells per sample as noted before), for a total reaction volume of 20 µL.
    - 384-well plate: Add 4.5 µL of the diluted (1:100) Ion AmpliSeq™ Polyploidy Panel library or 4.5 µL of each control dilution to each well (two wells per sample as noted before), for a total reaction volume of 10 µL.

4. Seal the plate with a MicroAmp™ Optical Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets.

5. Program your real-time instrument as follows:
    a. Enter the concentrations of the control library standards.
    b. Select ROX™ Reference Dye as the passive reference dye.
    c. Select a reaction volume of 20 µL.
    d. Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.
    e. The Ion Library qPCR Master Mix can be used on various instruments, as listed in the following table. The fast cycling program was developed using the StepOnePlus™ System in Fast mode.

IMPORTANT! When quantifying libraries made from panels with 275-bp or 375-bp designs, use standard qPCR cycling. Fast cycling can result in inaccurate quantification.

<table>
<tr><th>Real-time PCR System</th><th>Reaction plate</th><th>Run mode</th><th>Stage</th><th>Temperature</th><th>Time</th></tr>
<tr><td>7500 Fast</td><td>96-well Fast</td><td rowspan="5">Fast</td><td>Hold (UDG incubation)</td><td>50°C</td><td>2 minutes</td></tr>
<tr><td>7900 HT
7900 HT Fast</td><td rowspan="3">96-well Fast
384-well Standard</td><td>Hold (polymerase activation)</td><td>95°C</td><td>20 seconds</td></tr>
<tr><td>ViiA™ 7</td><td rowspan="3">Cycle (40 cycles)</td><td rowspan="2">95°C</td><td rowspan="2">1 second</td></tr>
<tr><td>QuantStudio™ 3, 5, or 7</td></tr>
<tr><td>StepOne™
StepOnePlus™</td><td>48-/96-well Fast</td><td>60°C</td><td>20 seconds</td></tr>
<tr><td>7300</td><td rowspan="5">96-well Standard</td><td rowspan="5">Standard</td><td>Hold (UDG incubation)</td><td>50°C</td><td>2 minutes</td></tr>
<tr><td>7500</td><td>Hold (polymerase activation)</td><td>95°C</td><td>2 minutes</td></tr>
<tr><td>7900 HT
7900 HT Fast</td><td rowspan="3">Cycle (40 cycles)</td><td>95°C</td><td>15 seconds</td></tr>
<tr><td>ViiA™ 7</td><td rowspan="2">60°C</td><td rowspan="2">1 minute</td></tr>
<tr><td>QuantStudio™ 3, 5, or 7</td></tr>
</table>

6. Following qPCR, calculate the average concentration of the undiluted Ion AmpliSeq™ Polyploidy Panel library by multiplying the concentration that is determined with qPCR by 100.

7. Based on the calculated library concentration, determine the dilution that results in a concentration of 150–250 pM.
For example:
    - The undiluted library concentration is 500 pM.
    - The dilution factor is 500 pM/250 pM = 2.
    - Therefore, 10 µL of library mixed with 10 µL of Low TE (1:2 dilution) yields 250 pM.

8. Dilute library to 250 pM, combine, then load onto the Ion S5™/Ion S5™ XL Sequencer or Ion GeneStudio™ S5 Series Sequencer.

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at -20°C.

If the libraries will be run with the Ion SingleSeq™ libraries, proceed to Chapter 4, "Combine Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel Libraries".

If running Ion AmpliSeq™ Polyploidy Panel libraries alone, proceed to Chapter 5, "Create a Planned Run".

Quantify the amplified library using the Qubit™ Fluorometer

Polyploidy libraries must be amplified before quantification with a Qubit™ Fluorometer to enrich amplifiable material and obtain sufficient material for accurate quantification. Amplify the library using Platinum™ PCR SuperMix High Fidelity, then purify. Quantify the library using a Qubit™ Fluorometer. After quantification, determine the dilution factor that results in a concentration of 150–250 pM.

Amplify the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of Platinum™ PCR SuperMix High Fidelity and 2 µL of Library Amplification Primer Mix to each bead pellet.
   - The Platinum™ PCR SuperMix High Fidelity is used to elute the libraries from the beads.
   - The Platinum™ PCR SuperMix High Fidelity and Library Amplification Primer Mix can be combined before addition.

2. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect the contents.

   Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

3. Place the plate back on the magnet for at least 2 minutes, then carefully transfer ~50 µL of supernatant from each well to a new well or a new plate without disturbing the pellet.

4. Seal the plate with MicroAmp™ Clear Adhesive Film, place a MicroAmp™ Optical Film Compression Pad on the plate, load in the thermal cycler, then run the following program.

Stage	Temperature	Time
Hold	98°C	2 minutes
5 cycles	98°C	15 seconds
	64°C	1 minute
Hold	10°C	Hold

STOPPING POINT Samples can be stored at –20°C.

Purify the amplified library

Perform a two-round purification process with the Agencourt™ AMPure™ XP Reagent.

- First round at 0.5X bead-to-sample-volume ratio—High molecular-weight DNA is bound to beads, while amplicons and primers remain in solution. Save the supernatant.
- Second round at 1.2X bead-to-original-sample-volume ratio—Amplicons are bound to beads, and primers remain in solution. Save the bead pellet and elute the amplicons from the beads.

IMPORTANT!

- Bring Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.
- Use freshly prepared 70% ethanol for the next steps. Combine 230 µL of ethanol with 100 µL of nuclease-free water per sample.
- Do NOT substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

First-round purification

1. Tap the plate gently on a hard flat surface, or briefly centrifuge to collect the contents, then remove the plate seal.

2. Add 25 µL (0.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each plate well containing ~50 µL of sample. Mix the bead suspension with the DNA thoroughly by pipetting up and down 5 times.

3. Incubate the mixture for 5 minutes at room temperature.

4. Place the plate in a magnet such as the DynaMag™–96 Side Magnet for at least 5 minutes, or until the solution is clear.

5. Carefully transfer the supernatant from each well to a new well of the 96-well PCR plate without disturbing the pellet.

   IMPORTANT! The supernatant contains the desired amplicons. Do not discard!

Second-round purification

1. To the supernatant (from “First-round purification” on page 42), add 60 µL (1.2X original sample volume) of Agencourt™ AMPure™ XP Reagent. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.

2. Incubate the mixture for 5 minutes at room temperature.

3. Place the plate in the magnet for 3 minutes or until the solution is clear. Carefully remove, then discard the supernatant without disturbing the pellet.

   IMPORTANT! The amplicons are bound to the beads. Save the bead pellet.

4. Add 150 µL of freshly prepared 70% ethanol to each well, then move the plate side to side in the magnet to wash the beads. Remove, then discard the supernatant without disturbing the pellet.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down five times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.

5. Repeat step 4 for a second wash.
6. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 2–5 minutes. Do not over dry.
7. Remove the plate from the magnet, then add 50 µL of Low TE to the pellet to disperse the beads.
8. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by setting a pipettor to 40 µL, then pipet the mixture up and down at least 5 times before sealing the plate.
9. Incubate at room temperature for at least 2 minutes.
10. Place the plate in the magnet for at least 2 minutes.

    IMPORTANT! The supernatant contains the desired amplicons. Do not discard!

Proceed immediately to "Elute and dilute the library" on page 43.

Elute and dilute the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of Low TE to the pellet to disperse the beads.
2. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
3. Incubate at room temperature for at least 2 minutes.
4. Place the plate on the magnet for at least 2 minutes.

   STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C. We recommend transferring the supernatant to a new plate, then seal the plate with MicroAmp™ Clear Adhesive Film for long-term storage.

5. Prepare a 100-fold dilution for quantification. Remove 2 µL of supernatant, containing the library, then combine with 198 µL of Nuclease-free Water.

Proceed immediately to "Qubit™ Fluorometer—Quantify the library and calculate the dilution factor" on page 44.

Qubit™ Fluorometer—Quantify the library and calculate the dilution factor

Analyze 10 µL of each amplified library using a Qubit™ Fluorometer and the Qubit™ dsDNA HS Assay Kit. Amplified libraries typically have concentrations of 300–1,500 ng/mL. Libraries below 300 ng/mL can still provide good quality sequences. We recommend the Qubit™ Flex Fluorometer when quantifying many samples. For more information, see the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326) or the *Qubit™ Flex Fluorometer User Guide* (Pub. No. MAN0009427).

1. Determine the concentration of each individual amplified library.
   a. Make a 1:200 working dilution of Qubit™ dsDNA HS reagent using the Qubit™ dsDNA HS Buffer.
   b. Combine 10 µL of the amplified Ion AmpliSeq™ library with 190 µL of dye reagent, mix well, then incubate for at least 2 minutes.
   c. Prepare each Qubit™ standard as directed in the user guide.
   d. Measure the concentration on the Qubit™ Fluorometer.
   e. *(Qubit™ 2.0 Fluorometer only)* Calculate the concentration of the undiluted library by multiplying by 20. Alternatively, use the "Calculate Stock Conc." feature on the instrument.
2. Based on the calculated library concentration (ng/mL), determine the dilution that results in a concentration of 250 pM.
   a. Convert the concentration in ng/mL to pM.

      $C_{pM} = ((C_{ng/mL} / D) / M) \times 10^6$

      Where:

      C_{pM} is the concentration in picomolar (pM)
      $C_{ng/mL}$ is the concentration in nanograms per milliliter (ng/mL)
      D is the length of the DNA sample in base pairs
      M is the molar mass of one base pair of DNA (660 grams/mol)

      Given an average amplicon size of 175 bp, then 1 ng/mL = 8.66 pM

      So, 250 pM/8.66 pM = 28.87 ng/mL
   b. Calculate the dilution factor (Y) for a given library concentration (X).

      X ng/mL / 28.87 ng/mL = Y

      For example:
      - The library concentration is 2,000 ng/mL.
      - The dilution factor is 2,000 ng/mL divided by 28.87 ng/mL = 69.3 (~70)
      - Therefore, adding 5 µL of library to 345 µL of Low TE (1:70 dilution) yields ~250 pM.
3. Dilute libraries to 250 pM, then combine.

STOPPING POINT Libraries can be stored at 4–8°C for up to 1 month. For longer term, store at –20°C.

Proceed to Chapter 4, “Combine Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel Libraries”.

Combine Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel Libraries

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- Barcode Compatibility 45
- Library Pooling 46
- Templating and sequencing of combined Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel libraries on Ion 530™ Chips 47

Overview of combining libraries

DNA libraries prepared with compatible Ion SingleSeq™ and IonCode™ Barcode Adapters are pooled at an appropriate ratio. The pooled libraries are templated and sequenced on an Ion 530™ Chip using the Ion Chef™ Instrument with the Ion S5™ ExT Chef PGS chemistry. Run plan parameters that are compatible with both library types are used. The run is automatically analyzed to produce PGS results from the Ion SingleSeq™ libraries. The run is then reanalyzed to produce results from the Ion AmpliSeq™ Polyploidy Panel libraries.

We recommend using only the Ion S5™ ExT Chef Reagents and Ion S5™ ExT Sequencing Reagents when combining Ion SingleSeq™and Ion AmpliSeq™ Polyploidy Panel libraries. Using other templating and sequencing chemistry can result in unstable ratios of read counts between the two library types. Only use the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef for templating and sequencing when running Ion AmpliSeq™ Polyploidy Panel libraries alone without combining with Ion SingleSeq™ libraries.

Barcode Compatibility

Ion SingleSeq™ Barcodes 1–96 share some sequences with IonCode™ Barcode Adapters 1–384 Kit (A29751). To avoid compatibility issues, the IonCode™ Barcode Adapters 385-768 Kit (A36546) or its component plates can be used.

Component plate	Part. No.
IonCode™ 0501–0596 in 96-well PCR Plate (red)	A36524
IonCode™ 0601–0696 in 96-well PCR Plate (yellow)	A36525
IonCode™ 0701–0796 in 96-well PCR Plate (green)	A36526
IonCode™ 0801–0896 in 96-well PCR Plate (blue)	A36527

Note: If using Ion SingleSeq™ Barcodes GX (101-196, 201-296, 301-396, or 401-496), IonCode™ Barcode Adapters 1–384 Kit (A29751) are compatible, while many barcodes from IonCode™ Barcode Adapters 385-768 Kit (A36546) are not compatible.

Library Pooling

For PGS results, it is desirable for each Ion SingleSeq™ PGS library to obtain >100,000 reads. For polyploidy results, it is desirable for each Ion AmpliSeq™ Polyploidy Panel library (IonCode™ Barcode Adapters) to obtain >200,000 reads. The total reads obtained from sequencing and the ratio of Ion AmpliSeq™ Polyploidy Panel libraries to Ion SingleSeq™ PGS libraries determine the allocation of reads in the run.

Ion SingleSeq™ libraries are equal *volume* pooled before purification and quantification using the Qubit™ Fluorometer. Ion AmpliSeq™ Polyploidy Panel libraries are quantitated either by qPCR (no library amplification) or Qubit™ Fluorometer and then combined in an equal *molar* pool. The instructions below describe how to combine these two pools into a single pool for templating and sequencing on the Ion Chef™ Instrument and Ion GeneStudio™ S5 Systems.

Combine Qubit™ Fluorometer quantitated Ion SingleSeq™ libraries with qPCR quantitated Ion AmpliSeq™ Polyploidy Panel libraries

1. Dilute the Ion SingleSeq™ library pool to 40–80 pM.
2. Dilute the Ion AmpliSeq™ Polyploidy Panel library pool so that its concentration is **2.5 times** the concentration of the Ion SingleSeq™ library pool.

   For example, if the Ion SingleSeq™ library pool is at 50 pM, dilute the Ion AmpliSeq™ Polyploidy Panel library pool to 125 pM.
3. Add 25 µL of diluted Ion SingleSeq™ library pool and 25 µL of diluted Ion AmpliSeq™ Polyploidy Panel library directly into the Library Sample Tube (50 µL total volume).
4. Vortex to mix, then centrifuge briefly.

Combine Qubit™ Fluorometer quantitated Ion SingleSeq™ libraries with Qubit™ Fluorometer quantitated Ion AmpliSeq™ Polyploidy Panel libraries

1. Dilute the Ion SingleSeq™ library pool to 40–80 pM.
2. Dilute the Ion AmpliSeq™ Polyploidy Panel library pool so that its concentration is **5 times** the concentration of the Ion SingleSeq™ library pool.

   For example, if the Ion SingleSeq™ library pool is at 50 pM, dilute the Ion AmpliSeq™ Polyploidy Panel library pool to 250 pM.
3. Add 25 µL of diluted Ion SingleSeq™ library pool and 25 µL of diluted Ion AmpliSeq™ Polyploidy Panel library directly into the Library Sample Tube (50 µL total volume).
4. Vortex to mix, then centrifuge briefly.

Templating and sequencing of combined Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel libraries on Ion 530™ Chips

The recommended maximum number of each library type in a combined library pool that can be sequenced per run on an Ion 530™ Chip is: 32 Ion SingleSeq™ libraries + 32 Ion AmpliSeq™ Polyploidy Panel libraries.

1. Prepare a run plan template including Ion SingleSeq™ Barcodes 1–96 and optional step 8 on page 51 increasing the number of **Flows** to 550. For more information, see "Create a Planned Run template for use with Ion ReproSeq™ PGS Kits" on page 49.
2. Use the run plan template to create a run plan that includes sample information. For more information, see "Create a Planned Run for use with Ion ReproSeq™ PGS Kits" on page 51.
3. Proceed to Chapter 6, "Run the Ion Chef™ System".

Create a Planned Run

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IMPORTANT! This sequencing kit is compatible with Torrent Suite™ Software 5.10 and later. Before proceeding, check for updates to Torrent Suite™ Software and the sequencing instrument software, and install the updates if available.

About Planned Runs

IMPORTANT! For more information about creating a Planned Run in Torrent Suite™ Software, including a complete description of each field in the **Create Plan** step, see the Torrent Suite™ Software help system or the Torrent Suite™ Software User Guide for the software version that you use.

Planned Runs are digital instructions that are created in Torrent Suite™ Software for controlling the template preparation and sequencing instruments. Planned Runs contain settings such as number of flows, kit types, barcodes, sample information, and reference files (if any). Planned Runs are also used to track samples, chips, and reagents throughout the workflow, from template preparation on the Ion Chef™ Instrument through sequencing on an Ion S5™/Ion S5™ XL Sequencer or Ion GeneStudio™ S5 Series Sequencer and subsequent data analysis.

Before you create a Planned Run template for use with Ion AmpliSeq™ Polyploidy Panel Kits, you must download the Ion AmpliSeq™ Polyploidy DNA Panel and the ReproSeqSnpAnalysis plugin plugin. The panel file includes the **Ion AmpliSeq Polyploidy DNA - Ion S5 System - 530** Planned Run template and analysis parameters that are used in this procedure. For more information, see “Upload panel files for Ion AmpliSeq™ Polyploidy Panel Kits” on page 56.

Create a Planned Run template for use with Ion ReproSeq™ PGS Kits

We recommend that you create a Planned Run template for reuse when the same conditions are used for multiple runs. Use this procedure to create a Planned Run template for use with Ion ReproSeq™ PGS Kits.

1. Sign in to Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, click **Reproductive** in the left navigation menu.
3. In the list of templates, find **Ion ReproSeq Aneuploidy – Ion S5 System**, then click ⚙ **(Actions)** ▸ **Copy**.
   The **Copy Template** screen opens to the **Save** step.
4. Enter or select the needed information.

Item	Action
Template Name	Enter a name for the Planned Run template.
(Optional) **Set as Favorite**	Select the **Set as Favorite** checkbox to add your custom template to the **Favorites** list.
Analysis Parameters	Ensure that **Default (Recommended)** is selected.
DNA Reference Library	Select **hg19 (Human (hg19))**.
Target Regions	Select **None**.
Hotspots Regions	Select **None**.
Monitoring Thresholds	Ensure that the values **Bead Loading**, **Key Signal**, **Usable Sequence** are each set to ***30.***

   As you make your selections, your settings are updated in the **Summary** pane.

5. *(Optional)* In the **Ion Reporter** step, complete the following fields if you plan to send data to Ion Reporter™ Software for further analysis after the sequencing run is complete.

Item	Selection
Ion Reporter Account	Select your Ion Reporter™ Software account.
Sample grouping	**Self** is preselected if only one Ion Reporter™ Software account or Ion Reporter™ Server is available. You can select another server, if appropriate.
Ion Reporter Upload Options	To transfer data automatically to Ion Reporter™ Software after the run is complete, select **Automatically upload to Ion Reporter after run completion.** To pause Torrent Suite™ Software before running Ion Reporter™ Software, select **Review results after run completion**, then upload data to Ion Reporter™ Software manually after the run.

Existing workflow	Select one of the following.
	• **ReproSeq PGS w1.1**
	• **ReproSeq No Gender PGS w1.1**
	• **ReproSeq Mosaic PGS w1.1**
	• **Note:**
	  . The ReproSeq Low-Coverage Whole-Genome Baseline is automatically used in the aneuploidy analysis workflows. As a result, there is no need to create a custom baseline for your Ion ReproSeq™ analyses.
	  . To customize one of the system-installed aneuploidy analysis workflows, see the software help system or the Ion Reporter™ Software User Guide for the software version that you use.
	  . The Low-pass whole-genome aneuploidy w1.0 workflow is used for non-Ion SingleSeq™ libraries only.

6. In the **Research Application** step, ensure that **DNA** is selected for the **Research Application** and **Whole Genome** is selected for **Target Technique**.

7. In the **Kits** step, ensure that **Ion Chef** is selected for the **Template Kit**, then complete the following fields.

Item	Selection
Instrument	**Ion GeneStudio™ S5 System**
Library Kit Type	**Ion SingleSeq Kit**
Template Kit	**Ion ReproSeq™ PGS Kits–Chef**
Sequencing Kit	**Ion S5 ExT Sequencing Kit**
Control sequence	Leave blank.
Chip Type	The chip type is set to the **Ion 530™ Chip** in the default run template and cannot be changed in the software. If you need to change the chip type to the Ion 510™ Chip or Ion 520™ Chip, you need to create a new template. See “Create a new run template to change chip types for a planned run” on page 141.

(continued)

Item	Selection
Barcode Set	Ion SingleSeq™ Barcodes 1–24 or Ion SingleSeq™ Barcodes 1–96.
Flows	250
Advanced Settings	**Use Recommended Defaults.**

8. *(Optional)* If sequencing combined Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel libraries. Set the number of **Flows** to 550.

9. Click **Next** to proceed to the **Plugins** step, then ensure that the **FilterDuplicates** plugin is selected. For more information about the **FilterDuplicates** plugin, see the Torrent Suite™ Software help system or the Torrent Suite™ Software User Guide for the software version that you use.

10. Click **Next** to proceed to the **Projects** step.

11. *(Optional)* In the **Projects** step, select the project appropriate to your run, then click **Next**.

12. In the **Save** step, click **Copy Template** to save the new Planned Run template.

The new template is now available in Torrent Suite™ Software, in the **Templates** screen under the **Reproductive** application.

Create a Planned Run for use with Ion ReproSeq™ PGS Kits

Ion ReproSeq™ PGS Kits require Ion Chef™ System be used for library preparation. Before you begin, your Ion Torrent™ Server must be connected to the Ion Chef™ Instrument and Ion S5™ System or Ion GeneStudio™ S5 System on which you will use the Planned Run. For more information, see "Prepare the Ion Chef™ System for use" on page 66.

1. Sign in to the Torrent Suite™ Software on a computer that is connected to your Ion Chef™ Instrument.

2. Under the **Plan** tab, in the **Templates** screen, click **Reproductive** in the research application list.

3. In the **Reproductive** list, click a customized Planned Run template name for use with Ion ReproSeq™ PGS Kits. Alternatively, click **(Actions)** ▸ **Plan Run** in the row that contains the Planned Run template.

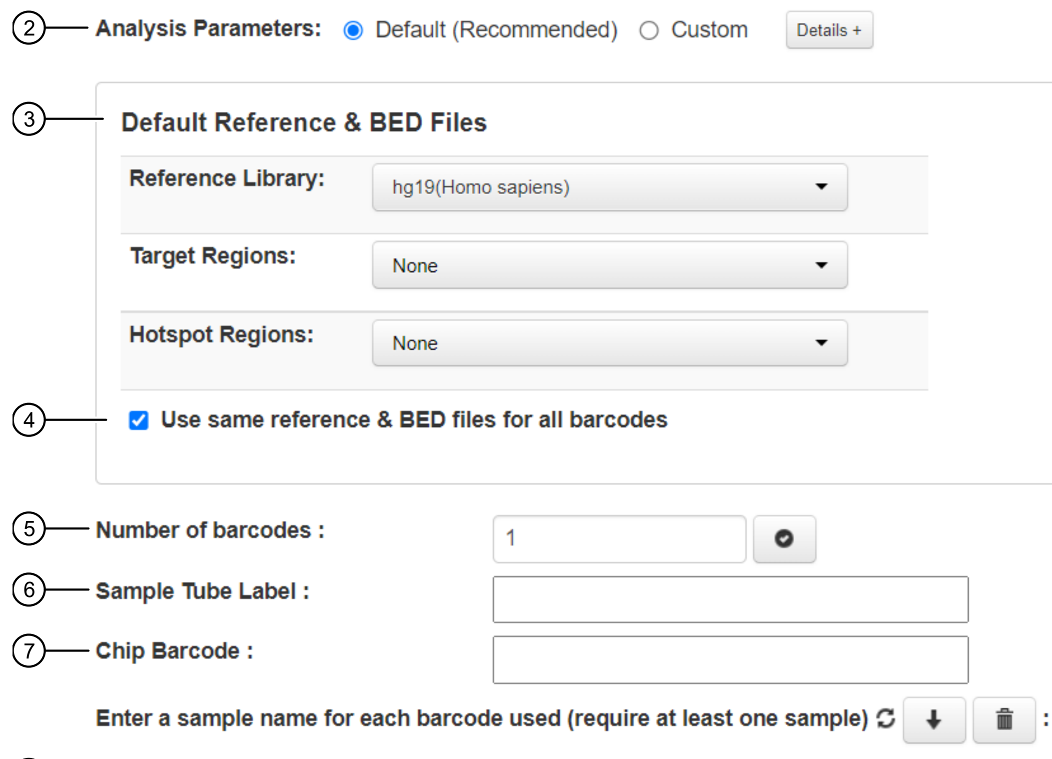
   The **Create Plan** screen opens to the **Plan** step.

4. Enter, select, or verify the following information. Row numbers in the table correspond to the callouts in the following figure.

Callout	Item	Action
1	**Run Plan Name**	Enter a name for the Planned Run template.
2	**Analysis Parameters**	Ensure that **Default (Recommended)** is selected.

(continued)

Callout	Item	Action
3	**Default Reference & BED Files**	• Reference Library: **hg19 (hg19 from zip)** • Target Regions: **None** • Hotspot Regions: **None**
4	**Use same reference & BED files for all barcoces**	Ensure that the checkbox is selected.
5	**Number of barcodes**	Enter the number of barcodes to be used in this run, then click ✔.
6	**Sample Tube Label**	*(Optional)* Enter or scan the barcode of the Ion Chef™ sample tube to be used in the run.
7	**Chip Barcode**	*(Optional)* Enter or scan the chip barcode.
8	**Pre-Implantation Genetic Screening**	Select **Pre-Implantation Genetic Screening.**



5. *(Optional)* Enter notes for the sequencing run in **Add a note** and **Add LIMS Meta Data**.

6. Ensure that all **Monitoring Thresholds** are set to *30*.

7. *(Optional)* Click the **Ion Reporter** step, then complete the following fields if you plan to send data to Ion Reporter™ Software for further analysis after the sequencing run is complete.

Item	Selection
Ion Reporter Account	Select your Ion Reporter™ Software account.
Sample grouping	**Self** is preselected if only one Ion Reporter™ Software account or Ion Reporter™ Server is available. You can select another server, if appropriate.
Ion Reporter Upload Options	To transfer data automatically to Ion Reporter™ Software after the run is complete, select **Automatically upload to Ion Reporter after run completion.** To pause Torrent Suite™ Software before running Ion Reporter™ Software, select **Review results after run completion**, then upload data to Ion Reporter™ Software manually after the run.

Existing workflow	Select one of the following.
	• **ReproSeq PGS w1.1**
	• **ReproSeq No Gender PGS w1.1**
	• **ReproSeq Mosaic PGS w1.1**
	• **Note:**
	  ▪ The ReproSeq Low-Coverage Whole-Genome Baseline is automatically used in the aneuploidy analysis workflows. As a result, there is no need to create a custom baseline for your Ion ReproSeq™ analyses.
	  ▪ To customize one of the system-installed aneuploidy analysis workflows, see the software help system or the Ion Reporter™ Software User Guide for the software version that you use.
	  ▪ The Low-pass whole-genome aneuploidy w1.0 workflow is used for non-Ion SingleSeq™ libraries only.

8. In the **Research Application** step, ensure that **DNA** research application and **Whole Genome** target technique are selected.

9. In the **Kits** step, complete or verify the following fields.

Item	Selection
Instrument	**Ion GeneStudio™ S5 System.**
Chip Type	The chip type is set to the **Ion 530™ Chip** in the default run template and cannot be changed in the software. If you are using the default template and you need to change the chip type to the Ion 510™ Chip or Ion 520™ Chip, you need to create a new template. See "Create a new run template to change chip types for a planned run" on page 141. If you are using a new template, if needed, you can change the chip type in the software.

(continued)

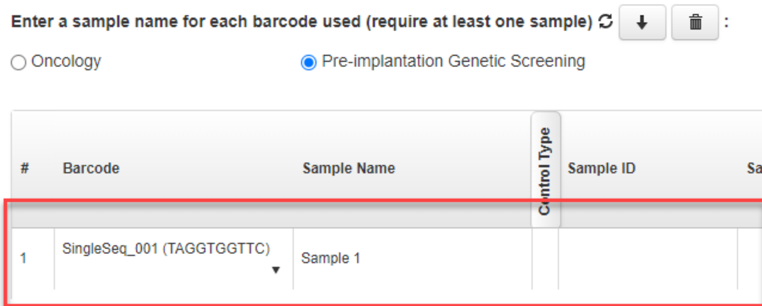
Item	Selection
(Optional) **Sample Preparation Kit**	Select the sample preparation kit used.
(Optional) **Control Sequence**	Leave deselected.
Library Kit Type	**Ion SingleSeq Kit**
Barcode Set	Select Ion SingleSeq™ Barcodes 1–24 or Ion SingleSeq™ Barcodes 1–96.
Template Kit	**IonChef**, and **Ion ReproSeq™ PGS Kits-Chef.**
Flows	250
Sequencing Kit	**Ion S5 ExT Sequencing Kit.**
Mark as Duplicate Reads	Leave deselected.
Enable Realignment	Leave deselected.
Advanced Settings	**Use Recommended Defaults.**

IMPORTANT! If sequencing combined Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel libraries, ensure the number of **Flows** is set to 550.

10. In the **Plugins** step, ensure that the **FilterDuplicates** plugin is selected. For more information about the **FilterDuplicates** plugin, see the software help system or the *Torrent Suite™ Software 5.18 User Guide* (Pub. No. MAN0026163).

11. *(Optional)* In the **Projects** step, select the project or projects that receive data from the runs that use this template, then click **Next**.

12. In the **Plan** step, In the **Plan** step for each sample enter sample information for each sample.



Item[1]	Action
Barcode	For each sample select the **Barcode** from the dropdown list.
Sample Name	Accept the auto-populated sample names or click in a field, then enter a unique sample name. We recommend sample names be unique even between runs.
Control Type	Click **Control Type** to view the **Control Type** column, then select **No Template Control** from the dropdown list to designate a sample as a no template control.
Sample ID	*(Optional)* Click in the field, then nter a sample ID. **Note:** Ensure that the sampleID is enabled in Torrent Suite™ Software. For more information, see the help system for your version of Torrent Suite™ Software.
Sample Description	*(Optional)* Click in the field, then enter a sample description.
Reference[2]	*(Optional)* Click **Reference** to view the **Reference** , **Target Regions**, and **Hotspot Regions** columns, then select the reference files for the barcode.
Annotations (expanded)	These columns appear after **Sample Receipt Date**. • *(Optional)* **Biopsy Days** • *(Optional)* **Cell Number** • *(Optional)* **Couple ID** • *(Optional)* **Embryo ID**
Sample Collection Date	*(Optional)* The date when the sample was collected.
Sample Receipt Date	*(Optional)* The date when the sample was received.
Ion Reporter Workflow	Select the workflow from the dropdown. Select **Show All Workflows** to display more workflows in the dropdown.
Relation	*(Optional)* Select the relation from the dropdown menu.
Gender	Select the gender from the dropdown menu.
Population	*(Optional)* Select the population from the dropdown menu.
Mouse Strains	Leave blank.
Witness	*(Optional)*
IR Set ID	Leave blank.

[1] Click vertical column headings (**Control Type**, **Reference**, **Annotations**) to reveal more columns.

[2] The Reference, Target Regions, and Hotspots Regions fields are displayed only if the checkbox for **Use same Reference & BED files for all barcodes** is selected (see step 4).

13. Click **Plan Run**.

The run is listed in the **Planned Run List** screen under the name that you specified and is automatically used by the Ion Chef™ Instrument when the associated Ion Chef™ Library Sample Tube is loaded on the instrument.

Note: If you have not entered the sample tube label (see step 4), you must select a run plan. You are prompted to do so when you set up the Ion Chef™ Instrument.

Upload panel files for Ion AmpliSeq™ Polyploidy Panel Kits

Before you create a Planned Run template or a Planned Run for use with Ion AmpliSeq™ Polyploidy Panel Kits, you must download the Ion AmpliSeq™ Polyploidy DNA Panel and the ReproSeqSnpAnalysis plugin from the Thermo Fisher™ Connect Platform at **Thermo Fisher Connect** (See "Install or upgrade plugins" on page 60). The panel file includes the **Ion AmpliSeq Polyploidy DNA - Ion S5 System - 530** Planned Run template and analysis parameters that are used in sequencing runs for the kits.

The following BED files must be uploaded.

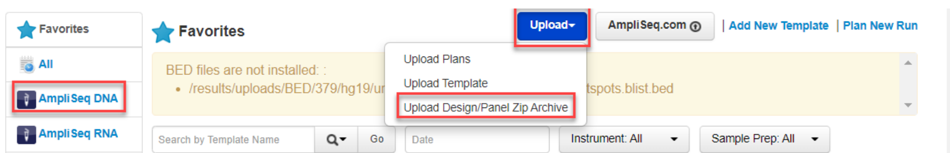
- Target regions file—**Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed**
- Hotspots file—**Ion_AmpliSeq_Polyploidy_Hotspots_w1.0.0.bed**

Import a Planned Run template

IMPORTANT! Do not restart the system during installation of planned run templates.

1. In the **Plan** tab, in the **Templates** screen, in the research application group pane on the left, select **AmpliSeq DNA**.

2. Click **Upload** ▸ **Upload Design/Panel ZIP Archive**.



3. In the **Import Panel from Zip Archive** dialog box, click **Select File**, select the following templates, then click **Upload Panel from Zip Archive**.

ZIP File	Description
Ion_AmpliSeq_Polyploidy_DNA_Panel.zip	Run Plan template for the Ion 530™ Chip.

The template is listed in the **AmpliSeq DNA** application group. Exported Planned Run templates have "exported" appended to the front of the original template name, unless the name has been edited in the CSV file.

Upload a target regions file

You can upload a target regions BED file from your computer to Torrent Suite™ Software to use with a reference sequence.

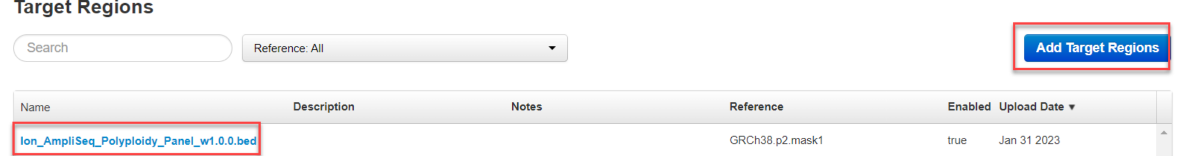
Target regions files are only BED files. Supported file extensions are .bed, .zip, and .bed.gz. During file upload, the software validates the BED file, then helps ensure that the coordinate regions of the BED file are valid for the selected reference genome. The new BED file is then available as an option when you create a Planned Run.

IMPORTANT! You must upload target regions files that match both the reference sequence and the reference sequence version. The uploader cannot always detect mismatch errors. It is your responsibility to avoid the following uploading errors:

- Uploading a BED file for a reference sequence of a different version (for example, an hg18 BED with an hg19 reference).
- Uploading a BED file for a different species.
- Uploading a hotspots BED file as a target regions BED file.

---

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Target Regions** in the left navigation menu.
2. In the **Target Regions** screen, click **Add Target Regions**.



3. In the **New Target Regions** screen, click **Select File**, then navigate to the file to be uploaded.
4. Select the reference sequence from the **Reference** list.

IMPORTANT!

- Be careful to select the correct reference sequence because the new target regions file can be used only with this reference.
- The reference sequence must be uploaded and available for selection before a target regions file can be uploaded.

---

5. *(Optional)* Add a description and notes.
6. Click **Upload Target Regions File**.

   Wait while the file is validated. The status updates to **Successfully Completed** after the upload finishes. Errors are reported in the **Processing Log** pane.

```
Pre Processing /results/uploads/BED/1/Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed
Dealing with the upload file
Compressed:     No
Updating Meta
Content:        Target regions file in BED format
()
Validating target file: Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed
()
Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed: 443 lines have non-unix line endings (showing first 10 warning).
Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed: line     1:           Warning  DOS line ending
```

For large files, validation can take several minutes. Refresh your browser to check that validation is complete.

The new file appears in the **Target Regions** list in the **Plan** step of the Planned Run workflow bar, and in the **Target Regions** table in the **References** tab.

Upload a hotspots file

You can upload a hotspots file from your computer to Torrent Suite™ Software to use with a reference sequence.

Hotspots files can be BED or VCF files. Supported file extensions are .bed, .vcf.gz, .zip, .bed.gz, and .vcf.gz. During file upload, the software validates the BED or VCF file, then helps ensure that the coordinate regions of the file are valid for the selected reference sequence. The new BED file is then available as an option when you create a Planned Run or configure the variantCaller plugin.

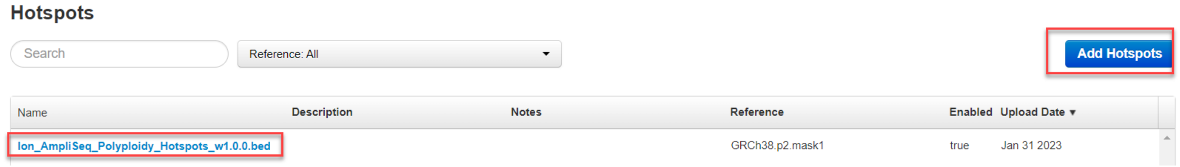
IMPORTANT! Upload BED or VCF files that match both the reference sequence and the reference sequence version. The uploader cannot always detect mismatch errors. It is your responsibility to avoid the following uploading errors:

- Uploading a BED or VCF file to a reference sequence of a different version (for example, an hg18 BED file with an hg19 reference).
- Uploading a BED or VCF file for a different species.
- Uploading a target regions BED file as a hotspots BED file.
- Uploading a hotspots file listing loci not included in a target regions file.

---

1. Click ⚙ **(Settings)** ▸ **Reference Sequences**, then click **Hotspots** in the left navigation menu.

2. In the **Hotspots** screen, click **Add Hotspots**.



3. In the **New Hotspots** screen, click **Select File**, then navigate to the file to be uploaded.

4. Select the reference sequence from the **Reference** list.

   IMPORTANT!
   - Ensure that you select the correct reference sequence because the new hotspots file can be used only with this reference.
   - The reference sequence must be uploaded and available for selection before a hotspots file can be uploaded.

5. *(Optional)* Add a description and notes.

6. Click **Upload Hotspots File**.

   The status updates to **Successfully Completed** after the upload finishes. Errors are reported in the **Processing Log** pane.

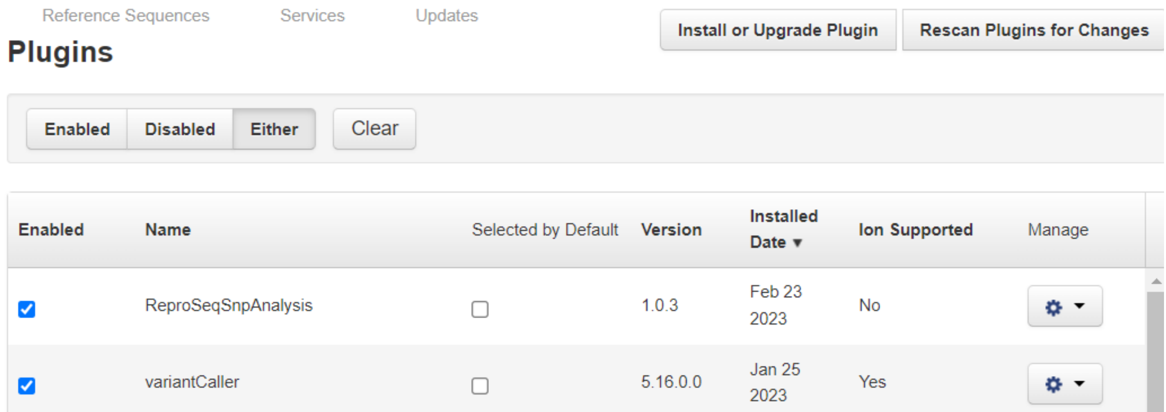
Note:
- For large files, validation can take a few minutes. Refresh your browser to check that validation is complete.
- If you selected a VCF file for upload, the software validates it and converts it to a BED file.

The new file appears in the **Hotspots** list in the **Plan** step of the Planned Run workflow bar, and in the **Hotspots** table in the **References** tab.

Enable an installed plugin

After installing a plugin, you must enable the plugin to make the plugin available in Torrent Suite™ Software.

1. Click ⚙ **(Settings)** ▸ **Plugins**.



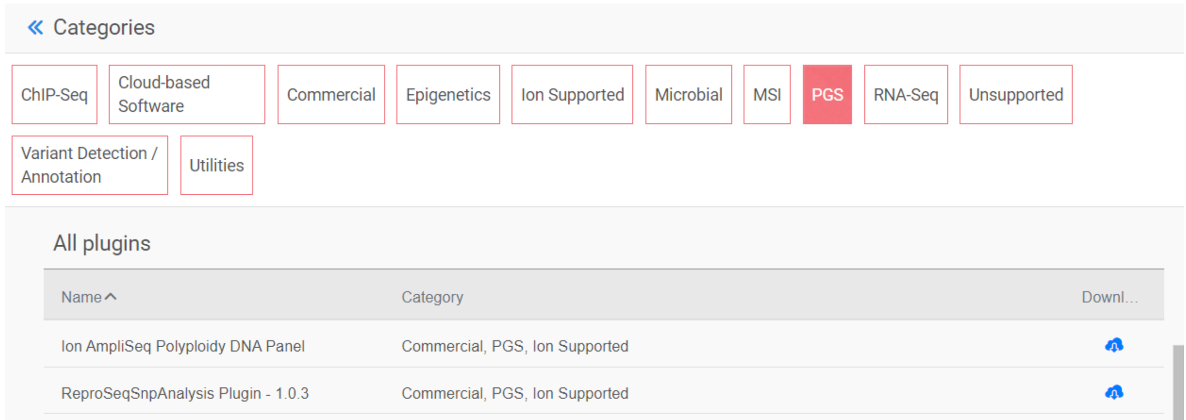
2. To make the plugin available, in the Plugins list, select the **Enabled** checkbox next to the ReproSeqSnpAnalysis.
   The plugin is immediately available.

Install or upgrade plugins

On Thermo Fisher™ Connect Platform, an administrator can install or upgrade the following:

1. Sign into **Thermo Fisher Connect**.
2. Click the **Apps** icon (▦).
3. In **AppConnect**, under **Resource Libraries**, click **Plugins**.
4. *(Optional)* Click **PGS** at the top of screen.
   The list of plugins is narrowed to only the plugins included that are in the selected category.

5. Download plugin and panel files.



   a. Click to download **Ion AmpliSeq Polyploidy DNA Panel**. Select the checkbox to indicate that you agree to the terms and conditions, then click **Download Plugin**.

   b. Click to download **ReproSeqSnpAnalysis Plugin - 1.0.3**. Select the checkbox to indicate that you agree to the terms and conditions, then click **Download Plugin**.

   Either a compressed directory or a debian file that contains the plugin is downloaded to your local machine.

6. In Torrent Suite™ Software, click **(Settings)** ▶ **Plugins** ▶ **Install or Upgrade Plugin**.

7. Click **Select File**, browse to the location where you downloaded the plugin file, select the file, then click **Open**.

8. In the **Install or Upgrade Plugin** dialog box, click **Upload and Install**.

The plugin is now visible in Torrent Suite™ Software.

Create a Planned Run Template for use with Ion AmpliSeq™ Polyploidy Panel Kits

We recommend setting up a customized Planned Run template for reuse when the same conditions are used for multiple runs. Use this procedure to create a Planned Run template for use with Ion AmpliSeq™ Polyploidy Panel Kits.

1. Sign in to Torrent Suite™ Software.

2. In the **Plan** tab, in the **Templates** screen, click **Polyploidy** in the left navigation menu.

3. In the **Reproductive** table, select **Ion AmpliSeq Polyploidy DNA - Ion S5 System - 530**, then click **Settings** ▶ **Copy** in the row of the template.
   The **Copy Template** screen opens to the **Save** step.

4. Enter or select the required information in each field in the **Save** step.

Field	Action
Template Name	Enter a name for your custom Planned Run template.
(Optional) **Set as Favorite**	Select the **Set as Favorite** checkbox to add your custom template to the **Favorites** list.
Analysis Parameters	Ensure that **Default (Recommended)** is selected.
DNA Reference Library	Ensure that **GRCh38.p2.mask1(GRCh28.p2.mask1)**is selected.
DNA Target Regions	Ensure that **Ion_AmpliSeq_Polyploidy_Panel_w1.0.0.bed** is selected.
DNA Hotspot Regions	Ensure that **Ion_AmpliSeq_Polyploidy_Hotspots_w1.0.0.bed** is selected.
Monitoring Thresholds	Verify that the values **Bead Loading**, **Key Signal**, **Usable Sequence** are each set to *30.*

As you make your selections, your settings are updated in the **Summary** pane.

5. Click the **Ion Reporter** step, then ensure that **None** is selected for **Ion Reporter Account**.

   Sequencing runs for use with Ion AmpliSeq™ Polyploidy Panel Kits can be analyzed only by the **ReproSeqSnpAnalysis** in Torrent Suite™ Software. Therefore, configuration for automatic transfer of data to Ion Reporter™ Software is unnecessary.

6. Click **Next**.

7. In the **Research Application** step, ensure that **DNA** is selected for the **Research Application** and **AmpliSeq DNA** is selected for **Target Technique** selections are correct, then click **Next**.

8. In the **Kits** step, select the required information in each field.

Option	Selection
Instrument	**Ion GeneStudio™ S5 Series**
Chip Type	**Ion 530™ Chip**
Sample Preparation Kit	*(Optional)* Select the sample preparation kit used to prepare samples.
Control sequence	Leave blank.
Library Kit Type	**Ion AmpliSeq 2.0 Library Kit**
Barcode Set	**IonCode**
Template Kit Ensure that **IonChef** is selected.	**Ion 520™ or Ion 530™ Chip**
Flows	550
Sequencing Kit	**Ion S5™ Sequencing Kit**
Mark as Duplicate Reads	Leave deselected.

(continued)

Option	Selection
Enable Realignment	Leave deselected.
Advanced Settings	Select **Customize**, then select **Chef Protocol – 400** for the **Templating Protocol**.

9. Click **Next**.
10. In the **Plugins** step, verify that the **coverageAnalysis**, **ReproSeqSnpAnalysis**, and **variantCaller** plugins are pre-selected.
11. Click **Next**.
12. *(Optional)* In the **Projects** step, select a project to receive data from Planned Runs that use this template, then click **Next**.
13. In the **Save** step, enter a template name in the **Template Name (required)** field, then click **Copy Template** to save your custom Planned Run template.

The new template is now available in Torrent Suite™ Software, in the **Templates** screen under the **Reproductive** application.

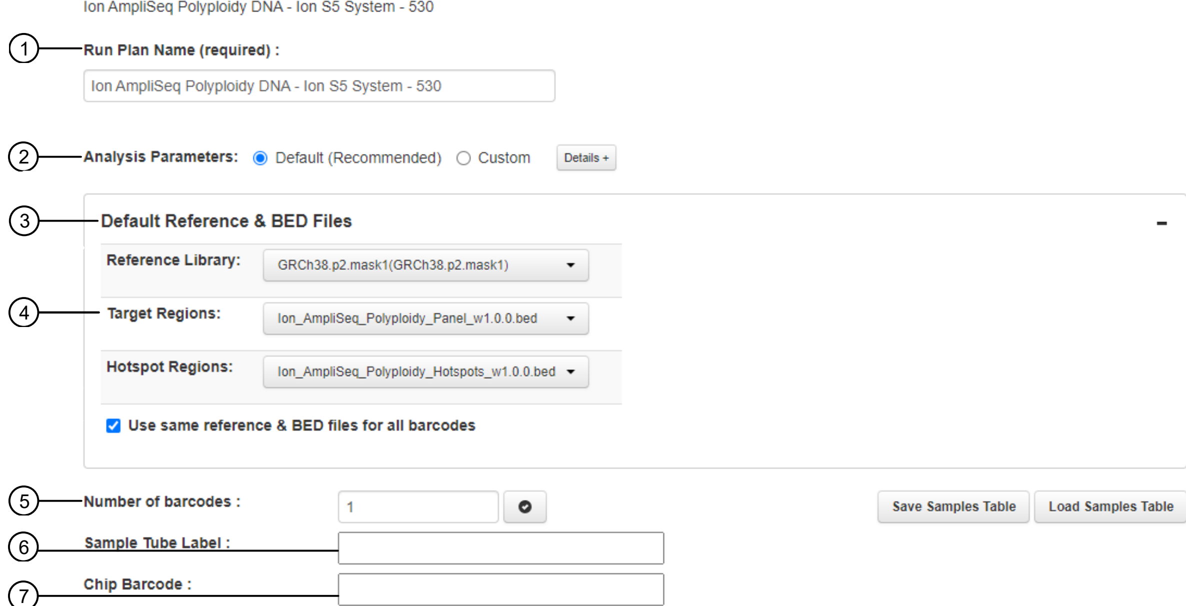
Create a Planned Run for use with Ion AmpliSeq™ Polyploidy Panel Kits

Ion ReproSeq™ PGS Kits require Ion Chef™ System be used for library preparation. Before you begin, your Ion Torrent™ Server must be connected to the Ion Chef™ Instrument and Ion S5™ System or Ion GeneStudio™ S5 System on which you will use the Planned Run. Fore more information, see "Prepare the Ion Chef™ System for use" on page 66.

1. Sign in to the Torrent Suite™ Software for the Ion Torrent™ Server that is connected to your Ion Chef™ Instrument.
2. In the **Plan** tab, in the **Templates** screen, click **Reproductive** in the left navigation menu.
3. In the **Template Name** column, click on your customized Planned Run template name.
   The **Create Plan** screen opens to the **Plan** step.

4. Enter or select the following information.

Callout	Field	Action
1	**Run Plan Name**	Enter a Planned Run name.
2	**Analysis Parameters**	Ensure that **Default (Recommended)** is selected.
3	**Default Reference & BED Files**	Select the default reference sequence file and target regions and hotspots BED files for your sequencing run.
4	**Use same reference & BED files for all barcodes**	Leave selected.
5	**Number of barcodes**	Enter the number of barcodes to be used in this run, then click ✔.
6	**Sample Tube Label**	Enter or scan the barcode of the Ion Chef™ sample tube to be used in the run.
7	**Chip Barcode**	*(Optional)* Enter or scan the chip barcode.



5. *(Optional)* Enter notes for the sequencing run in **Add a note** and **Add LIMS Meta Data**.

6. Ensure that all **Monitoring Thresholds** are set to *30*.

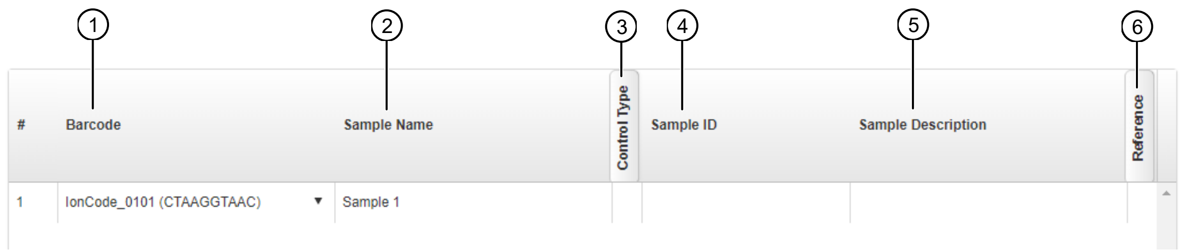
7. Click the **Ion Reporter** step, then ensure that **None** is selected for **Ion Reporter Account**.

   Sequencing runs for use with Ion AmpliSeq™ Polyploidy Panel Kits can be analyzed only by the **ReproSeqSnpAnalysis** in Torrent Suite™ Software. Therefore, configuration for automatic transfer of data to Ion Reporter™ Software is unnessary.

8. In the **Plan** step for each sample enter sample information for each sample.

Item[1]	Action
Barcode	For each sample select the **Barcode** from the dropdown list.
Sample Name	Accept the auto-populated sample names or click in a field, then enter a unique sample name. We recommend sample names be unique even between runs.
Control Type	Click **Control Type** to view the **Control Type** column, then select **No Template Control** from the dropdown list to designate a sample as a no template control.
Sample ID	*(Optional)* Click in the field, then enter a sample ID. **Note:** Ensure that the sampleID is enabled in Torrent Suite™ Software. For more information, see the help system for your version of Torrent Suite™ Software.
Sample Description	*(Optional)* Click in the field, then enter a sample description.
Reference[2]	*(Optional)* Click **Reference** to view the **Reference** , **Target Regions**, and **Hotspot Regions** columns, then select the reference files for the barcode.

[1] Click vertical column headings (**Control Type**, **Reference**, **Annotations**) to reveal more columns.
[2] The Reference, Target Regions, and Hotspots Regions fields are displayed only if the checkbox for **Use same Reference & BED files for all barcodes** is selected (see step 4).



9. Click **Plan Run**.

The run is listed in the **Planned Runs** screen under the name that you specified and is automatically used by the Ion Chef™ Instrument when the associated Ion Chef™ Library Sample Tube is loaded on the instrument.

Run the Ion Chef™ System

- Before you begin 66
- Materials required 67
- Prepare the consumables 67
- Add the library to the Library Sample Tube 68
- Load the Ion Chef™ System 68
- Start the Ion Chef™ run 80
- Unload the chip for sequencing 85

Before you begin

Library type

Depending on the library type you are preparing, chose from the following options:

- For Ion SingleSeq™ libraries only, proceed to "Prepare the Ion Chef™ System for use" on page 66.
- When combining Ion SingleSeq™ and Ion AmpliSeq™ Polyploidy Panel libraries, proceed to "Prepare the Ion Chef™ System for use" on page 66.
- For Ion AmpliSeq™ Polyploidy Panel libraries only, see the *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854).

Prepare the Ion Chef™ System for use

Before you use the Ion Chef™ Instrument:

- Ensure that the Ion Chef™ Instrument has been cleaned following the previous run. If not, clean the instrument *before* loading it with consumables.

  Note: For more information on the cleaning procedure, see Chapter 9, "Clean the Ion Chef™ System".

- Inspect the empty compartments of the Reagents and Solutions stations for condensation. Condensate can collect in these compartments, depending on temperature and humidity conditions. Before loading consumables into the instrument, wipe the compartments dry with a laboratory wipe or absorbent cloth, if needed.

- Ensure that the Ion Chef™ Instrument has a connection to your Ion Torrent™ Server. On the Ion Chef™ home touchscreen, touch **Settings**, then **Ion Torrent™ Server** to view the connection status of your instrument.

Note: If the instrument is not connected, see Appendix C, "Connections between Ion Torrent™ Server and the Ion Chef™ Instrument" for instructions on how to configure a direct or indirect network connection of the Ion Chef™ Instrument to an Ion Torrent™ Server.

Materials required

- Ion ReproSeq™ PGS Kit with
  Ion 510™, Ion 520™, or Ion 530™ Chip
- Ion Chef™ S5 Series Chip Balance
- Molecular-biology grade Nuclease-free Water
- P10 and P200 pipettors, and filtered tips
- Waste container

Prepare the consumables

1. Unbox the Ion S5™ ExT Chef Reagents cartridge 45 minutes before use, then allow it to warm to room temperature.

   IMPORTANT! The Reagents cartridge must sit at room temperature for 45 minutes before use.

2. Remove the other cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

   Prepare the following:
   - Chip Adapter and Chip Balance
   - Enrichment Cartridge v2
   - Ion S5™ ExT Tip Cartridge
   - PCR Plate, PCR Plate Frame, and Frame Seal v2
   - Recovery Station Disposable Lid v2 (2)
   - Recovery Tube (12)
   - Ion S5™ Chef Solutions cartridge
   - Ion S5™ ExT Chef Reagents cartridge (from step 1)

   IMPORTANT! Before use, gently tap the Reagents cartridge and Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.

   Note: When stored under normal conditions, a precipitate can form in some tubes of the Ion S5™ ExT Chef Reagents cartridge. If present, load the cartridge as directed. The precipitate dissolves when the reagents are mixed during instrument operation.

Add the library to the Library Sample Tube

1. Pipet 2–4 µL of the 1 nM pooled library into the Ion SingleSeq™ Library Sample Tube (barcoded tube) from Position A of the Reagents cartridge. Add Nuclease-free Water to 50 µL (final concentration of 40–80 pM). Pipet up and down 5 times to mix.

2. Cap the Library Sample Tube and store on ice until you are ready to load the tube into the Reagents cartridge and the Ion Chef™ Instrument.

Load the Ion Chef™ System

IMPORTANT!
- Rated centrifuge speeds are intended for operation only with the provided buckets and approved consumable chips, tubes, and sample preparation reagents.
- The Chip-loading centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chip, adapter, and chip balance. The centrifuge must be load-balanced. Proper care must be taken to load the bucket properly. If excessive vibrations arise, check to ensure that items are installed properly and rotors are load-balanced.
- Use only the materials supplied in the Ion ReproSeq™ PGS 510, 520, and 530 Kits to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets before each use to assure normal operation.
- Ensure that the instrument is powered on and has been cleaned following the last use.
- Ensure that all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure that the Reagents and Solutions station compartments are dry and free of condensate before loading components.

Follow the procedure described in the following sections to load the Ion Chef™ Instrument.

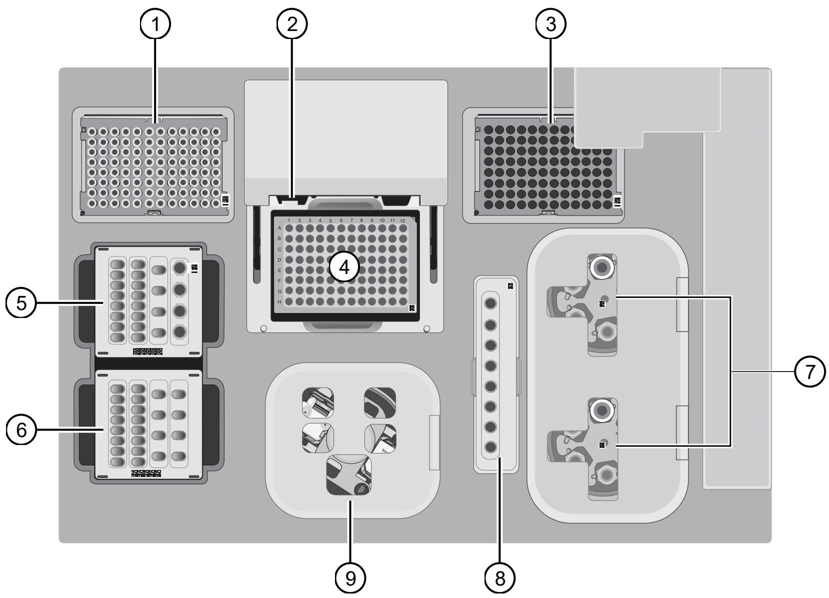


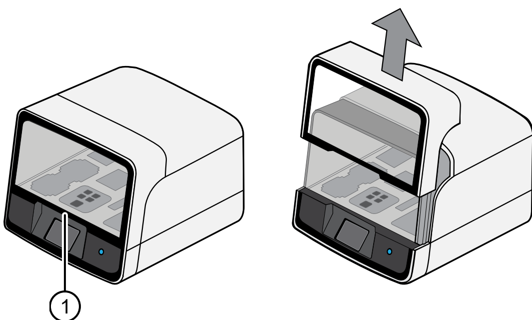
Figure 1 A schematic of a loaded Ion Chef™ Instrument.

① New Ion S5™ ExT Tip Cartridge
② Frame Seal v2 (inserted)
③ Empty tip rack (move from new Tip cartridge position)
④ PCR Plate and PCR Plate Frame
⑤ Ion S5™ ExT Chef Reagents cartridge
⑥ Ion S5™ Chef Solutions cartridge
⑦ Recovery centrifuges: Recovery Tubes and Recovery Station Disposable Lid v2
⑧ Enrichment Cartridge v2
⑨ Chip-loading centrifuge: Chip Adapter/Chip assembly and Chip Balance

Load the tip racks and PCR Plate

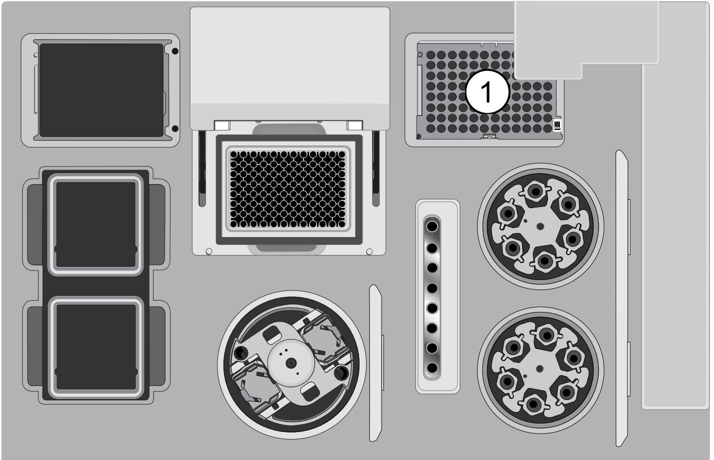
1. Tap ⏏ (Open Door) in the instrument touchscreen to open the instrument door, then wait for the latch to open.

2. Lift the instrument door to the top of the travel until the latch mechanism engages.



① Hold here, then lift

3. Load an empty pipette tip rack to the *Used* (Waste) Pipette Tip Position, then change gloves.



① Used Pipette Tip Position

IMPORTANT!

- Ensure that the pipette tip rack in the *Used* (Waste) Pipette Tip Position does not contain any tips. The instrument will abort the run if tips are present in the *Used* position.
- To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used* (Waste) Pipette Tip Position.

Note: A small amount of dried residue may be present in the tub of the empty pipette tip rack after a run. This will not affect the next run.

---

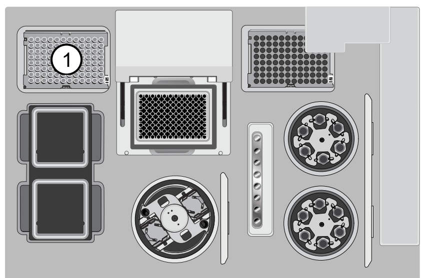
4. Unwrap a new Ion S5™ ExT Tip Cartridge and remove the cover to expose the pipette tips, then load it in the *New* Pipette Tip Position.

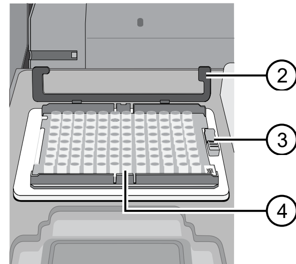
Note:

- Two Ion Chef™ Piercing Tips are pre-loaded into tip positions G7 and H7 on the Ion S5™ ExT Tip Cartridge.
- Keep the cover for later use.

---

5. Slide the catch forward to allow the locking bracket to pivot upward. Load the Ion S5™ ExT Tip Cartridge into the *New* Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the cartridge in place.



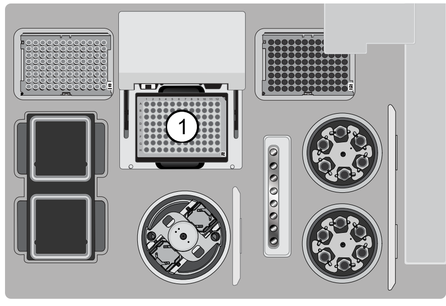


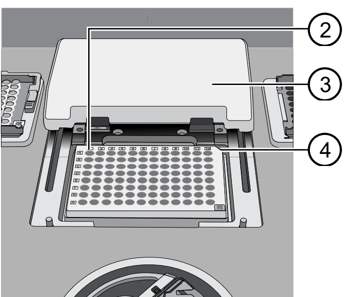
① New Pipette Tip Position
② Bracket
③ Catch
④ New Ion S5™ ExT Tip Cartridge

6. Load a new PCR plate into the thermal cycler sample block.

7. With the white dot on the PCR Plate Frame facing upward, load the PCR Plate Frame into the thermal cycler sample block pressing down firmly on each corner, then insert a new Frame Seal v2 underneath the automated heated cover. Ensure that the PCR Plate Frame is pressed completely down onto the thermal cycler block and that the PCR Plate Frame sits lower than the PCR Plate.

IMPORTANT! When the Frame Seal v2 is positioned correctly, its tabs project upward and contact the heated cover.





① Thermal cycler sample block
② Well A1
③ Cover
④ Keyed corner

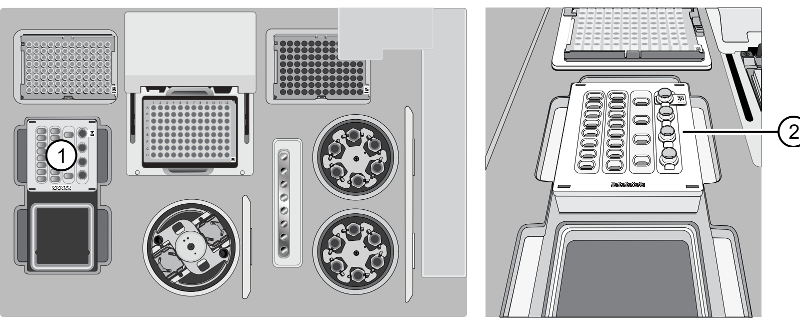
Load the Reagents and Solutions cartridges

IMPORTANT! Thaw the Reagents cartridge at room temperature for 45 minutes before use.

1. Gently tap the Ion S5™ ExT Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.

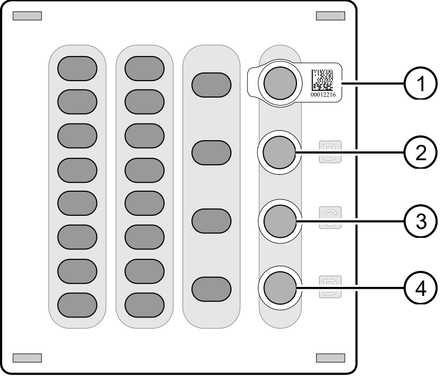
2. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

   IMPORTANT! Do not force the Ion Chef™ cartridges into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, confirm that you are loading the correct cartridge in the correct orientation.



① Reagents station (4°C)
② Ion S5™ ExT Chef Reagents cartridge

3. Uncap, then load the Library Sample Tube containing 50 µL of diluted library into Position A on the Reagents cartridge.



① Position A (Library Sample Tube)
② Position B (2M NaOH)
③ Position C (Ion S5™ ExT Pellet)
④ Position D (Empty tube)

IMPORTANT! Orient the Library Sample Tube so that the barcode is visible and oriented to the right.

4. Uncap the tube of 2M NaOH in Position B, the tube containing the Ion S5™ ExT Pellet in Position C, and the empty tube in Position D.

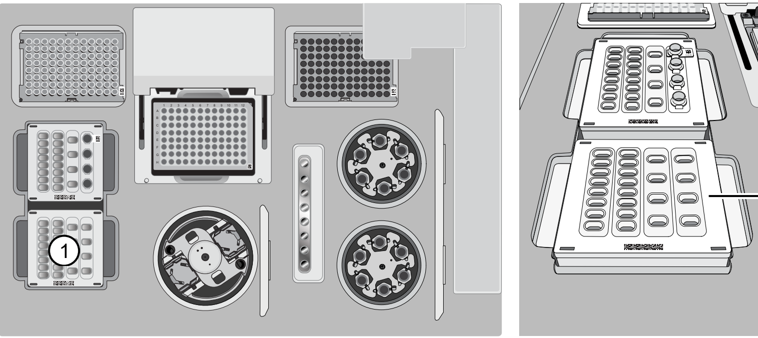
   IMPORTANT! When the Reagents cartridge is loaded:

   - Press down on the Library Sample Tube to ensure that it is firmly seated in the cartridge.
   - Ensure that *all* four tubes are uncapped.

   ---

5. Gently tap the Ion S5™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.

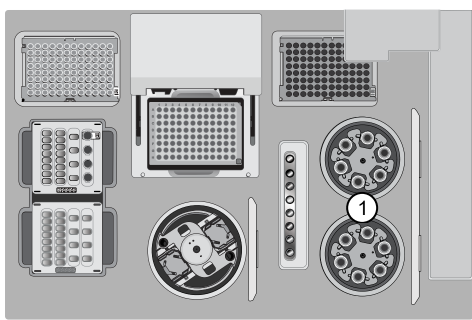
6. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.

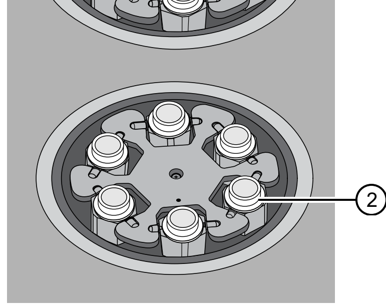


① Solutions station (room temperature)
② Ion S5™ Chef Solutions cartridge

Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes (v2) into each Recovery centrifuge.





① Recovery centrifuges
② Recovery Tube v2

Before sealing each centrifuge, confirm that:

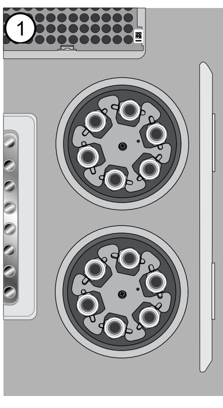
- The centrifuge is load-balanced with all required consumables.

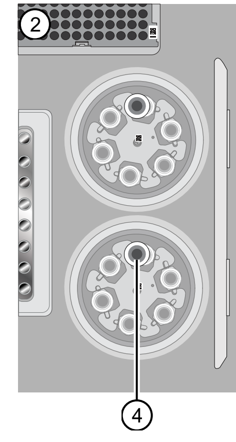
  IMPORTANT! The centrifuge must be load-balanced.

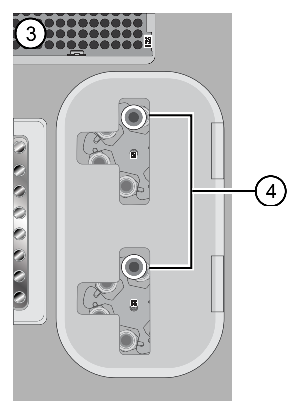
- The buckets are securely seated in the centrifuge rotors.
- The buckets are oriented correctly in the centrifuge so that they pivot outwards.

2. Place a Recovery Station Disposable Lid v2 over each centrifuge by lining up the tab with the depression on the deck, then snap into place. Ensure that the lids snap completely into place by applying firm downward pressure along the lid perimeter.

3. Close the hinged cover of the Recovery centrifuges. Confirm that the port of each disposable lid is positioned toward the rear of the instrument.





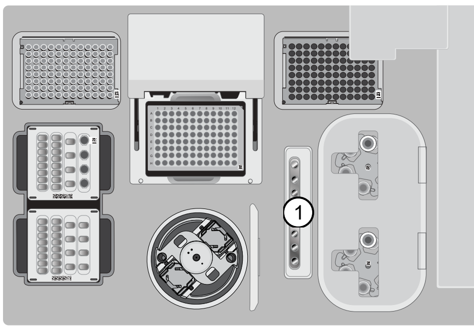


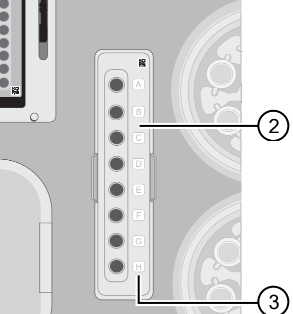
① Recovery Tubes installed
② Recovery Station Disposable Lids installed
③ Recovery centrifuge cover closed
④ Port

IMPORTANT!
- Do not obstruct or place any object on top of the Recovery centrifuge cover.
- Use only the supplied materials, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. To facilitate normal operation, inspect the buckets before each use.

4. Load the Enrichment Cartridge v2, then press down on the cartridge to ensure that it is level with the instrument deck.

IMPORTANT! Ensure that the Enrichment Cartridge v2 is loaded so that the lettering on the cartridge is right-side-up.



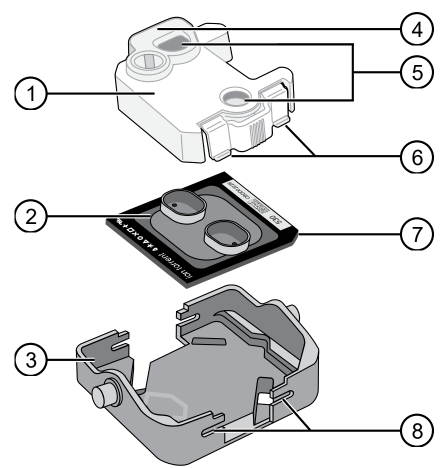


① Enrichment station
② Enrichment Cartridge v2
③ Lettering

Load the Chip-loading centrifuge

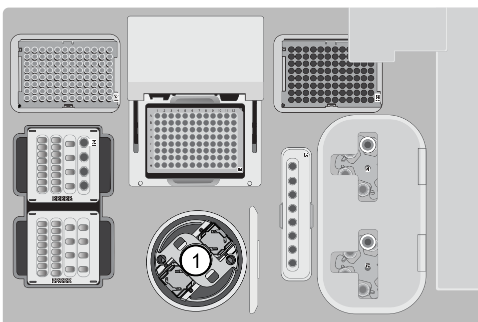
Note: If desired, you can label the tops of chips to distinguish them. Do not obstruct or overwrite the chip barcode with the label.

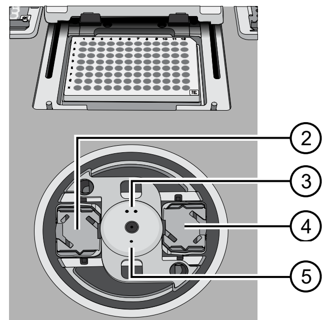
1. Load the chip that you will use for templating and sequencing into a centrifuge bucket, then attach a Chip Adapter to the assembly.
   a. Place the chip in the chip-loading bucket, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.
   b. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.
   c. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.
   d. Confirm that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket. Loading can fail if the adapter is not attached securely.



① Chip Adapter
② Ion Chip™
③ Bucket
④ Reservoir end of Chip Adapter
⑤ Ports (align with chip)
⑥ Tabs
⑦ Keyed corner (align with bucket)
⑧ Slots

2. Load the adapter/chip/bucket assembly into Position 1 of the Chip-loading centrifuge.

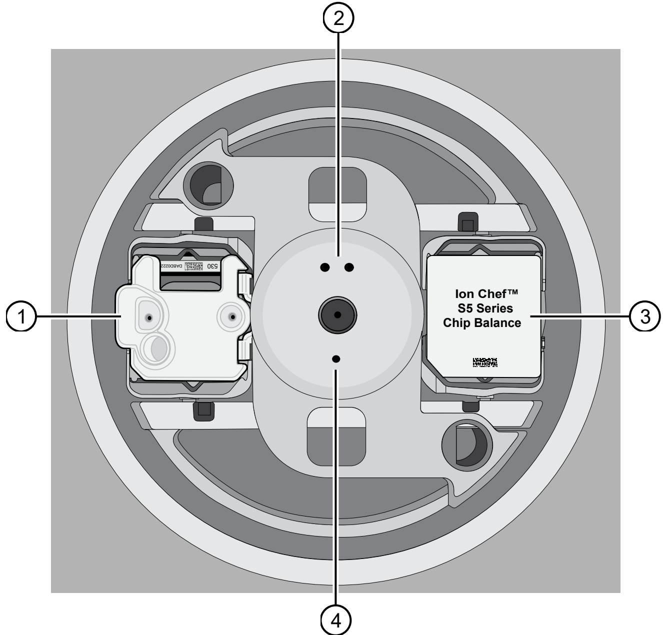


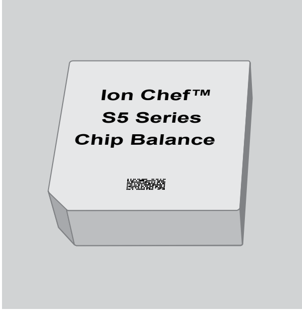


① Chip-loading centrifuge
② Position 1
③ Position 2 marker holes
④ Position 2
⑤ Position 1 marker hole

Note: Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest. The chip loaded in Position 1 is loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position A of the Reagents cartridge.

3. Load an Ion Chef™ S5 Series Chip Balance into Position 2 of the Chip-loading centrifuge.





① Position 1 (Chip)
② Position 2 marker holes
③ Position 2 (Chip Balance)
④ Position 1 marker hole

IMPORTANT! When the Chip-loading centrifuge is loaded, ensure that the Chip Adapter is firmly attached to its bucket, and that each bucket is securely seated in the centrifuge rotors.

---

4. Ensure the centrifuge is load-balanced, and the chip buckets are securely seated and oriented correctly in the centrifuge so that they pivot 90° outwards when touched. Then close the lid of the Chip-loading centrifuge.

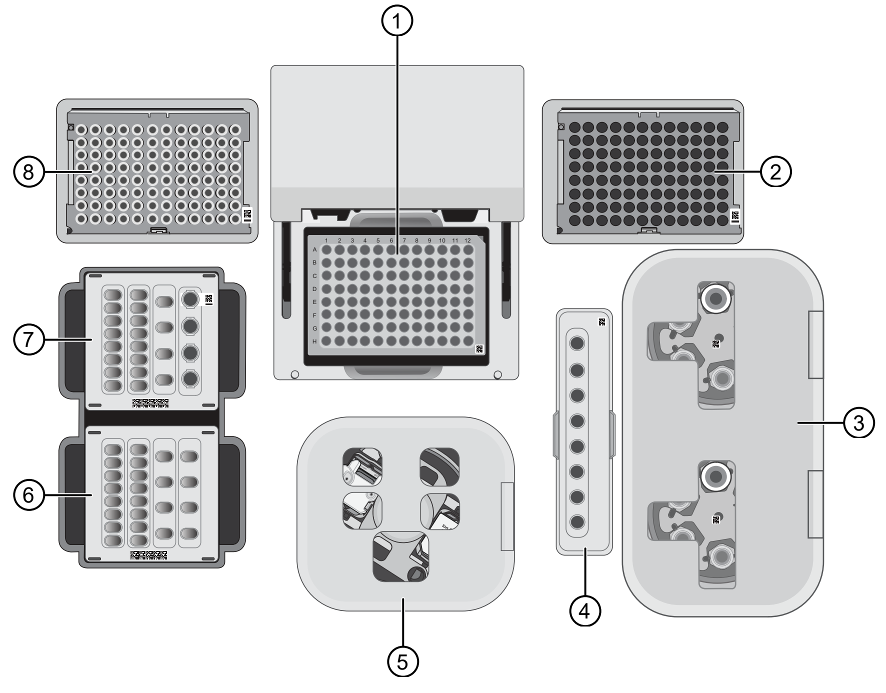
IMPORTANT! Do not obstruct or place any object on top of the lid.

Confirm that consumables are correctly installed

- Confirm that each cartridge is at the correct location and in the correct orientation.
- Press down on all cartridges to confirm that they are firmly pressed into place.
- Confirm that all tubes in the Ion S5™ ExT Chef Reagents cartridge, including the tube of NaOH in Position C, are uncapped and firmly pressed into place.
- Confirm that the centrifuge lids are installed correctly so that the port is oriented toward the rear of the instrument.
- Confirm that the tube and chip buckets are seated securely in the rotor arms of the Chip-loading and Recovery centrifuges, and that the consumables they contain are correctly installed.

CAUTION! To facilitate correct and safe instrument operation, you must confirm that all consumables are installed correctly to the deck before you start a run. The Ion Chef™ Instrument does not verify all aspects of the consumable setup before beginning each run.

Ion Chef™ pre-run checklist



✓	Checklist item
① Thermal cycler sample block	
☐	A new PCR Plate is loaded in the thermal cycler sample block.
☐	A PCR Plate Frame is loaded in the thermal cycler sample block.
☐	A Frame Seal v2 is in place and oriented under the heated cover.
② Used Pipette Tip position	
☐	The tip rack from previous run is transferred from the New Pipette Tip position to the Used Pipette Tip position.
③ Recovery centrifuge	
☐	Recovery Tubes (v2) are securely seated in the Recovery centrifuge buckets.
☐	Recovery centrifuges are load-balanced.
☐	Each Recovery Station Disposable Lid v2 is positioned so that the port is oriented toward the rear of the instrument.
④ Enrichment station	
☐	Enrichment Cartridge v2 is pressed into place at the Enrichment station so that the cartridge is **firmly seated** and **level** with the deck.
☐	Lettering on the cartridge is right-side up and positioned to the right of the enrichment tubes.

(continued)

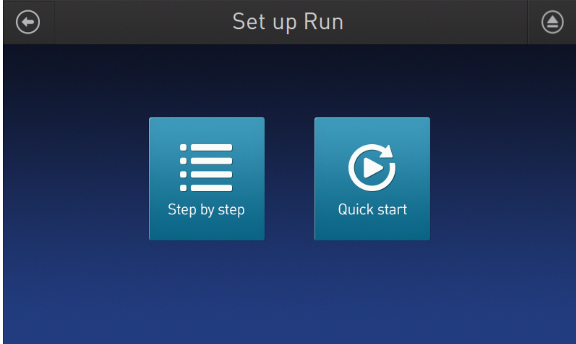
✓	Checklist item
⑤ Chip-loading centrifuge	
☐	Adapter/chip assembly is correctly seated in the Chip-loading centrifuge bucket loaded in Position 1, with the adaptor clips inserted into the bucket slots.
☐	Centrifuge buckets containing the adapter/chip assembly and Chip Balance, loaded in Position 2, are securely seated in the centrifuge rotor, and freely pivot 90° outwards.
⑥ Solutions station	
☐	Solutions cartridge is pressed into place at the Solutions station so that the cartridge is firmly seated and level with the deck.
⑦ Reagents station	
☐	Reagents cartridge contents have been thawed at room temperature for 45 minutes.
☐	Reagents cartridge tubes are uncapped and pressed into place at the Reagents station so that the tubes are **firmly seated** and **level** with the deck.
☐	Library Sample tube is **firmly seated** and in the correct orientation with barcode facing the PCR Plate and cap removed.
⑧ New Pipette Tip position	
☐	A new Ion S5™ ExT Tip Cartridge is loaded in the New Pipette Tip position.
☐	Bracket is pulled downward to lock the tip cartridge in place.

Start the Ion Chef™ run

1. Ensure that you have loaded the instrument with all kits and consumables.

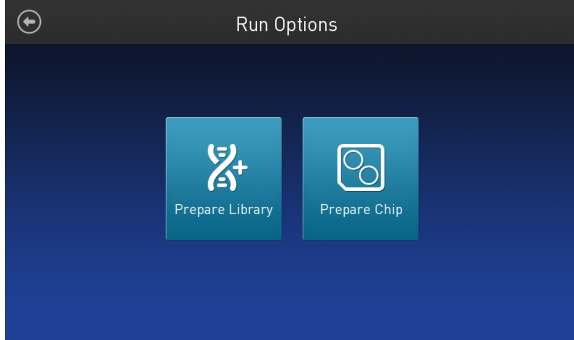
2. On the Ion Chef™ Instrument home touchscreen, tap **Set up run**.

3. Tap **Step by step** to have the instrument lead you through the instrument setup, or tap **Quick Start** to skip the instrument setup screens and proceed to Deck Scan.



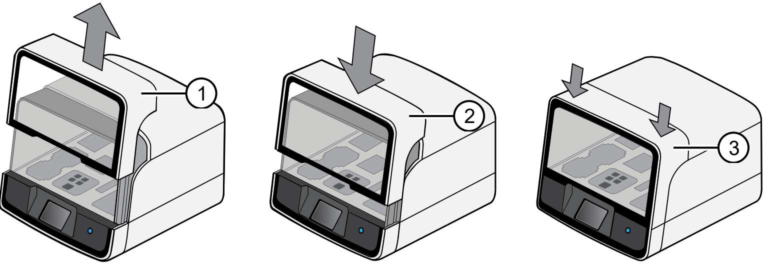
If you selected **Quick Start**, proceed to step 5, otherwise proceed to step 4.

4. *(**Step by step** setup only)* In the **Run Options** screen, tap **Prepare Chip** to select the templating run option.



5. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

   IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.

   ① Lift door first
   ② Lower
   ③ Press down to lock

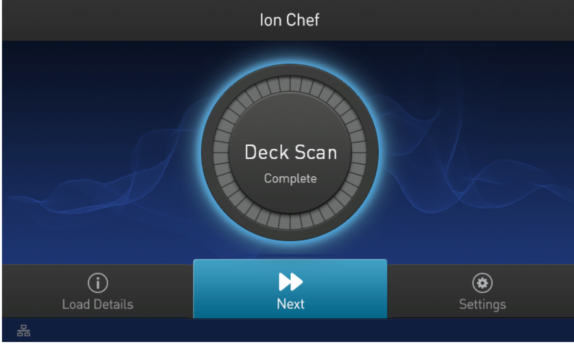
   After the door closes, the instrument vision system activates.

6. When prompted, tap **Start check** to begin Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility.

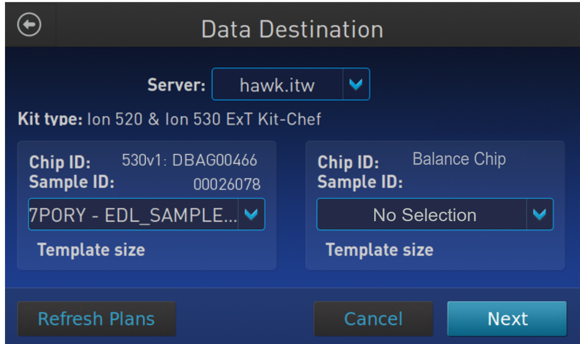
   During Deck Scan, the touchscreen displays warnings if the Ion Chef™ Instrument detects missing or incompatible consumables. You must address all warnings before the run can start. After you address each condition, tap **Yes** to continue.

   IMPORTANT! The Deck Scan function is not a substitute for manual inspection of the reagents and consumables on the Ion Chef™ Instrument before starting a run. To facilitate proper and safe instrument operation, ensure that all consumables are installed correctly before you continue.

7. When the Deck Scan is complete, tap **Next** to display the **Data Destination** screen.

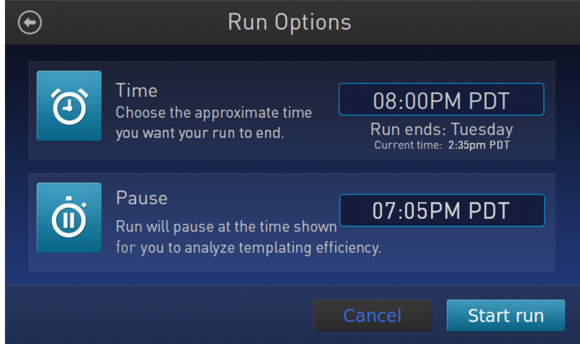
   

8. Ensure that the instrument displays the correct kit type, chip type, chip barcode, and Planned Run. If the correct Planned Run does not display, tap the dropdown list to select the appropriate Planned Run, then tap **Next.**



IMPORTANT! If the kit and chip type are not correct, confirm that you are using the correct kit and chip. If you are using the correct kit and chip, and an incorrect kit or chip type appears on the screen, contact Technical Support.

9. On the **Run Options** screen, tap the appropriate option to complete the run, then enter the desired time of run completion if needed.



The Ion Chef™ Instrument provides two options for obtaining a quality control (QC) sample that can be used to evaluate templating efficiency. Depending on your selection, the QC sample will be made available either during or after the run. In either case, you can obtain unenriched sample from the Library Sample Tube at Position A on the Reagents cartridge, or enriched sample from Position E on the Enrichment Cartridge v2.

By selecting	You can obtain the QC samples	Approximate time after run start
Time	immediately after the run ends, at the time you specify:	4 hours 50 minutes
Pause	when the instrument pauses operation before the chip loading step:	4 hours 15 minutes[1]

[1] An additional 1 hour is required for chip loading to complete the run. Total run time is longer because an extra centrifugation step is required with the Pause option.

Note: Select **Pause** if you are uncertain of library quality and want to evaluate templating efficiency before the chip is loaded. If you do not pause the run, you can collect QC samples after the run. Save the samples until sequence analysis is complete to have them available for troubleshooting.

10. On the **Run Options** screen, tap **Start run** to start the run.

Note: If you need to stop the run for any reason, tap **Cancel**, then tap **Yes** to confirm the cancellation.

If the Ion Chef™ Instrument encounters a problem during the run, it aborts the run and displays the error on the instrument touchscreen. If a run fails, perform the following tasks.

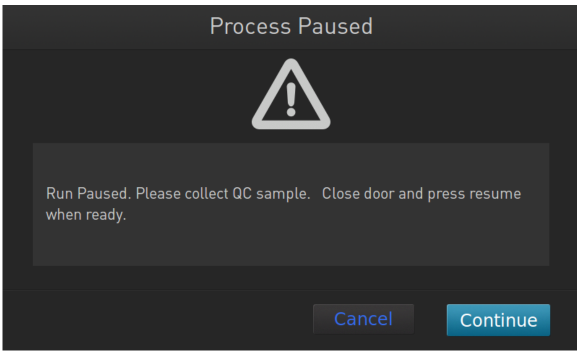
   a. Remove the consumables from the deck, then clean the instrument. If possible, retain the consumables for troubleshooting.

   b. Reset, then attempt to start the run again. If the run fails again, contact Technical Support to troubleshoot the problem. Record the error message for reference.

11. Initialize the sequencer at least 50 minutes before the Ion Chef™ System finishes chip loading. See Chapter 7, "Initialize the sequencer".

12. If you chose to pause the run to analyze the templating efficiency, remove the sample for testing when prompted to do so by the Ion Chef™ Instrument (approximately 4 hours 15 minutes into the run).

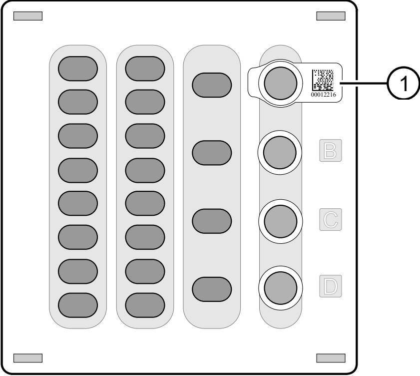
   a. When prompted to remove the QC sample, open the instrument door.



b. Transfer the entire volume of the QC sample from Position A of the Ion S5™ ExT Chef Reagents cartridge on the instrument deck to a new labeled microcentrifuge tube.

IMPORTANT!

- Do not remove the Library Sample Tubes from the Ion S5™ ExT Chef Reagents cartridge.
- If you unintentionally close the instrument door before you obtain the QC sample, you must wait until the end of the run before you can collect it. You cannot pause the run or open the door after it has been closed.



① Position A (QC sample)

c. If you are performing quality assessment of an enriched sample, transfer the QC sample from Position E of the Enrichment Cartridge v2 to a new labeled microcentrifuge tube.

d. Analyze the QC sample. For more information, see "Quality control of ISPs" on page 149.

e. Close the instrument door, then tap **Continue** to complete the run.

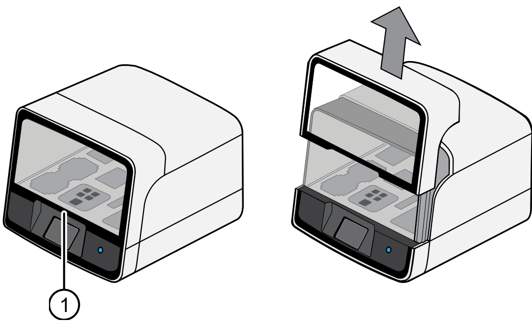
13. When the run is complete, unload the Ion Chef™ Instrument and sequence the chip immediately. You can collect QC samples from the Reagents and/or Enrichment cartridges if you have not done so already.

IMPORTANT! Liquid may be present in the chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.

Note: If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

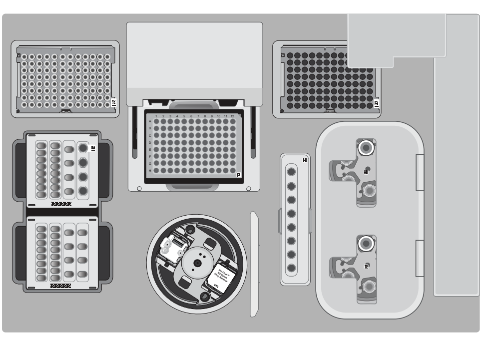
Unload the chip for sequencing

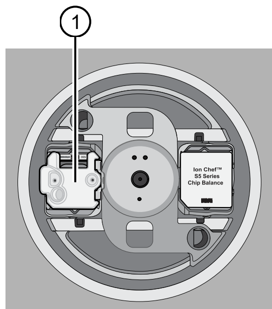
1. Open the instrument door.
   a. In the instrument touchscreen, tap ⏏ **(Open Door)**, then wait for the latch to open.
   b. Lift the instrument door to the top of the travel until the latch mechanism engages.



① Hold here, then lift

2. Open the lid of the Chip-loading centrifuge, then unload the adapter/chip/bucket assembly from the instrument.

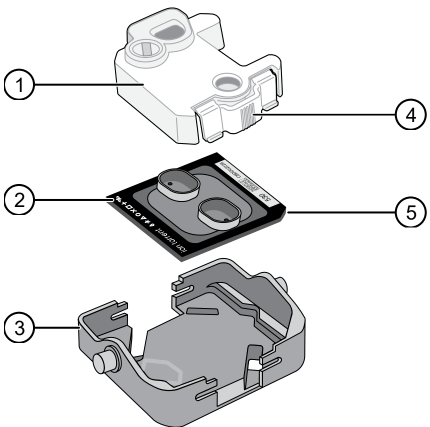




① Remove the adapter/chip/bucket assembly

3. Unload the chip from the adapter/chip/bucket assembly.
   a. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.

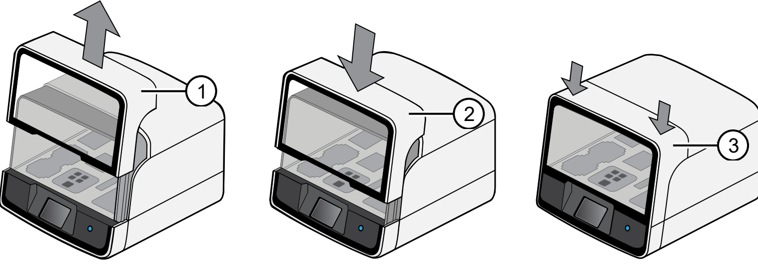
b. Grasp the chip by its edges, carefully lift the chip out of the bucket, then set it aside on a clean, static-free surface. Return the bucket to the Chip-loading centrifuge.



① Chip Adapter
② Ion Chip™
③ Bucket
④ Press, then remove adapter
⑤ Remove from bucket carefully

4. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.



① Lift door first
② Lower
③ Press down to lock

5. Load the chip into a sequencer, then promptly start the sequencing run.

   If you cannot sequence a loaded chip immediately or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

   IMPORTANT!

   - Liquid may be present in chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.
   - If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

Initialize the sequencer

- Ion S5™ and Ion GeneStudio™ S5 System component positions 88
- Before you begin 89
- When a manual cleaning of the sequencer is required 89
- Initialize the sequencer 90

Initialization takes ~50 minutes.

Note: The instructions in this chapter also apply to both Ion S5™ Systems and Ion S5™ XL Systems.

Ion S5™ and Ion GeneStudio™ S5 System component positions

Note: These positions also apply to Ion S5™ Systems and Ion S5™ XL Systems.

① Touchscreen
② Power button
③ Ion S5™ Sequencing Reagents cartridge
④ Chip clamp
⑤ Ion S5™ Wash Solution bottle. The waste reservoir is located behind the Ion S5™ Wash Solution bottle (shown on the right).
⑥ Ion S5™ Cleaning Solution bottle
⑦ Waste reservoir

Note:

- The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting you to replace the reagent before performing the run.
- RFID regulatory information is on the main screen under **Options ▸ Regulatory info**.

Before you begin

The Ion S5™/Ion S5™ XL and Ion GeneStudio™ S5 Series Sequencers are equipped to verify the compatibility of each chip and consumable that is loaded during initialization and sequencing, and that these components do not exceed their expiration date. To avoid exceptions during initialization, inspect this information for each consumable before installing onto the instrument.

- Unbox the Ion S5™ ExT Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.

  Do not remove the Ion S5™ ExT Sequencing Reagents cartridge from its packaging until immediately before loading, so that you can return the unused cartridge to storage if your sequencing run is delayed.

- Unbox the Ion S5™ ExT Wash Solution bottle. Invert the bottle 5 times within its vacuum-sealed bag, then swirl at an angle to mix thoroughly.

- Remove the Ion S5™ ExT Wash Solution bottle from its vacuum-sealed bag, then remove the red cap from the Ion S5™ ExT Wash Solution and Ion S5™ Cleaning Solution bottles immediately before installing on the instrument.

When a manual cleaning of the sequencer is required

The Ion S5™/Ion S5™ XL and Ion GeneStudio™ S5 Series Sequencers require that a cleaning be performed before initialization. This is normally performed automatically at the completion of the previous sequencing run. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run. However, if the "Enable post-run clean" checkbox is unchecked to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. See “Perform a manual cleaning of the sequencer” on page 150 for more information on how to perform a manual cleaning.

If a sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. See “Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge” on page 151 for more information.

Initialize the sequencer

1. In the instrument touchscreen main menu, tap **Initialize**.

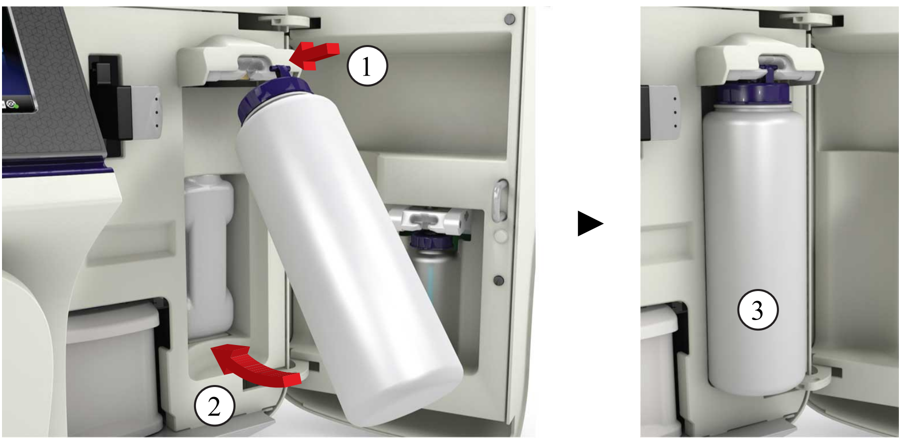
   The door, chip, and Reagent cartridge clamps unlock.

2. When prompted, remove the Ion S5™ ExT Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.

3. Reinstall the empty waste reservoir.

4. Replace the expended Ion S5™ ExT Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.

5. Ensure the new Ion S5™ ExT Wash Solution bottle is thoroughly mixed. Then remove the red cap and install.



6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.

7. If necessary, install a new Ion S5™ Cleaning Solution bottle.

   Note: The Ion S5™ Cleaning Solution bottle contains sufficient reagent to complete 4 cleanings.

8. Close the door, then tap **Next**.

   The instrument confirms that the consumables and chip are properly installed and that the Ion S5™ Cleaning Solution contains sufficient reagent to perform the post-run clean. Follow all on-screen recommendations to facilitate proper installation of required consumables.

   IMPORTANT! If the allowed number of post-run cleans has been met, the instrument prompts the user to replace the Ion S5™ Cleaning Solution bottle.

   Note: If a **Checking Reagent: Failed** warning appears, see “Bottle leak check fails” on page 138.

9. When initialization is complete (~50 minutes), tap **Home**.

The instrument is now ready for a sequencing run.

For information on recycling and disposal of used components, see “Reagent consumables disposal” on page 95.

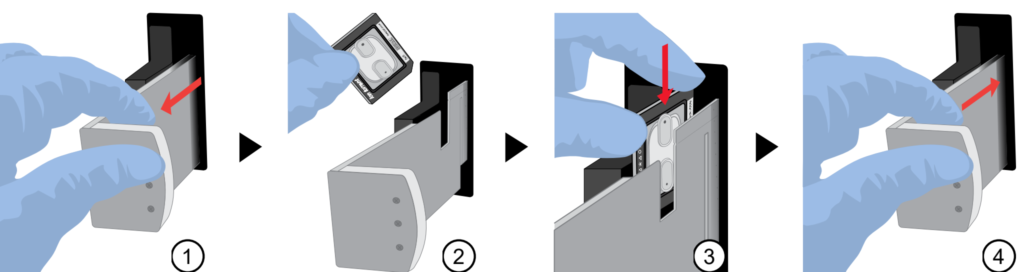
Start the sequencing run

- Chip handling guidelines 92
- Start the sequencing run 93
- Maintain the sequencer 95
- Reagent consumables disposal 95

Note: The instructions in this chapter also apply to both Ion S5™ Systems and Ion S5™ XL Systems.

Chip handling guidelines

To place a chip in the chip clamp, perform the steps that are described in the following figure:



① Slide the chip clamp out.

② Remove the chip currently in the clamp.

③ Place the appropriate loaded chip in the chip clamp with the chip notch in the bottom-front corner.

Note: Do not force the chip into the clamp. If the chip does not fit easily in the clamp, confirm that the notch is oriented as shown in the drawing.

④ Slide the metal tab in fully to engage the clamp, then close the instrument door.

Start the sequencing run

We recommend that you start a sequencing run as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

IMPORTANT!

- Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.
- We recommend that you verify the status of the Planned Run you will use as "Planned" before inserting the loaded chip. If your Planned Run does not advance to the Planned status in Torrent Suite™ Software after successful completion of an Ion Chef™ run, see “Planned Run status does not advance to "Planned"” on page 137.

---

1. After completion of initialization, tap **Run** in the instrument touchscreen. The door and chip clamp unlock.

2. Remove the used sequencing chip, then secure a chip loaded with template-positive Ion Sphere™ Particles in the chip clamp.

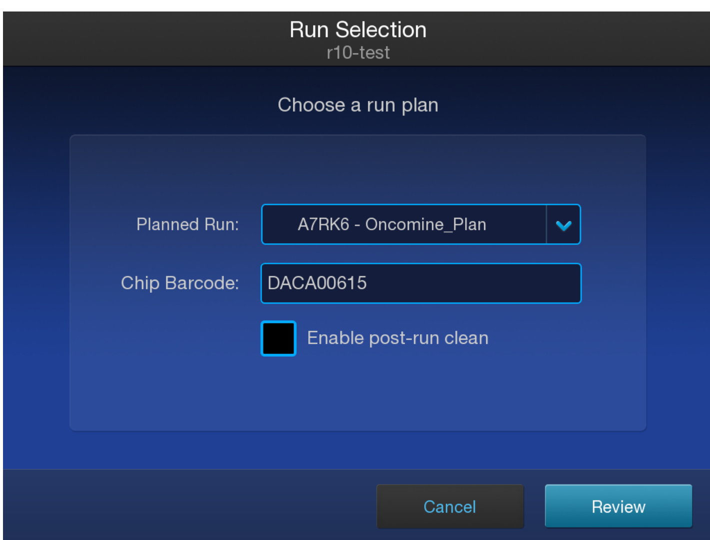
3. Push the chip clamp all the way in to engage, close the instrument door, then tap **Next**.

   Note: Do not remove the chip from the chip clamp until completion of the run. Removing and reinserting the chip risks introducing air bubbles in the chip.

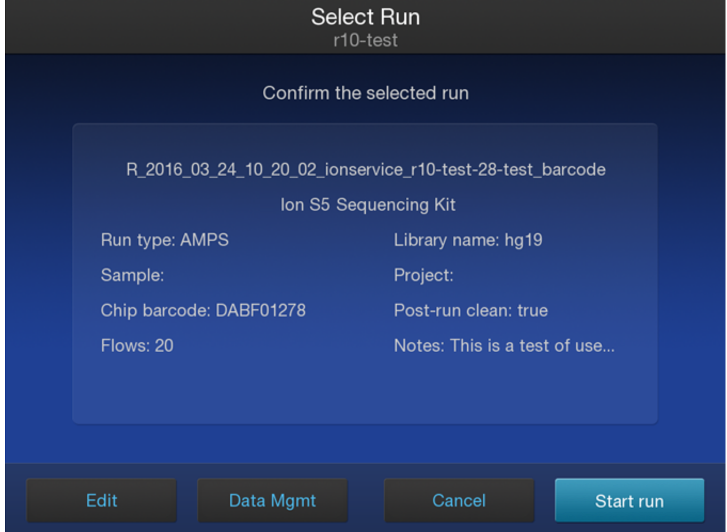
4. Confirm that the correct Planned Run has auto-populated. If this run is the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then tap **Review**.

   IMPORTANT!

   - Failure to deselect the checkbox results in a cleaning performed automatically after the first run. A second run is not available.
   - When starting the second sequencing run on a single initialization, ensure that the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.



5. Confirm that the remaining pre-populated settings are correct, or tap **Edit** to make changes if needed.



6. Confirm that the instrument door is closed, then tap **Start run** to begin the sequencing run.

IMPORTANT! During a run, do not open the instrument door, and avoid touching the instrument. Touching the instrument during the sequencing run can reduce the quality of the measurements.

When the sequencing run is complete, the instrument automatically performs the cleaning procedure unless the **Enable post-run clean** checkbox was deselected. After cleaning, the touchscreen returns to the main menu. Use Torrent Suite™ Software to review the results.

If you are sequencing a second chip on a single initialization, start the second run within 24 hours of start of initialization.

Maintain the sequencer

Materials required

- Lint-free wipes
- 70% isopropanol
- *(Optional)* 10% bleach solution

Clean or decontaminate the sequencer

In the event of a spill or leak on or inside the instrument, clean and decontaminate the sequencer.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Remove the Ion S5™ ExT Wash Solution bottle, then remove and empty the waste reservoir.
2. Remove the Ion S5™ ExT Sequencing Reagents cartridge.
3. Inspect the waste and nucleotide reagent bays for liquid.
4. Use absorbent paper to soak up as much liquid as possible, then wash the affected area with 10% bleach solution.
5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

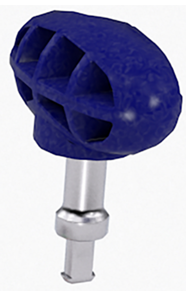
Reagent consumables disposal

IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Ion S5™ reagent consumables.

CO_2 scrubber removal and disposal

To properly discard the CO_2 scrubber, you must first remove it from the Ion S5™ Sequencing Reagents cartridge.

1. Invert the Ion S5™ Sequencing Reagents cartridge over an appropriate receptacle to drain all remaining liquid.
2. Wearing gloves, insert the Ion S5™ Cartridge Tool firmly into the CO_2 scrubber until the flange stops on the top of the cartridge.



Ion S5™ Cartridge Tool

3. Pull straight up on the tool while holding the nucleotide reagent cartridge down.

4. Remove the scrubber from the cartridge tool, then discard the scrubber according to applicable hazardous waste regulations.

The remaining nucleotide reagent cartridge should be disposed of appropriately.

Recycle Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles

The Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles are made of recyclable plastic.

1. Open the expended bottle by unscrewing the cap.
2. Remove the cap, sipper, and filter, then pour any residual liquid into an appropriate receptacle.
3. Rinse the empty bottle with water. Pour out the rinse water into the same liquid waste receptacle.
4. Recycle or dispose of the clean bottle, cap, sipper, and filter according to applicable regulations.

Clean the Ion Chef™ System

- About the cleaning protocol 97
- Materials required 97
- Clean the Ion Chef™ Instrument 97

About the cleaning protocol

The Ion Chef™ System includes an automated cleaning function that must be performed following every run. The cleaning routine is initiated from the Ion Chef™ Instrument touchscreen and is designed to minimize potential contamination. During the routine, the instrument irradiates the deck with ultraviolet light for 1 minute after all consumables have been removed from the instrument.

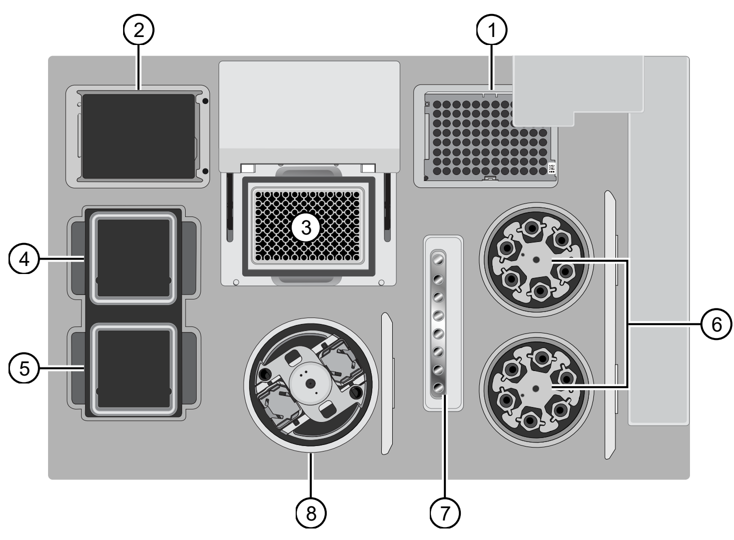
IMPORTANT! Although the Ion Chef™ Instrument cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries for use or when preparing the Ion Chef™ Instrument, make certain to observe sterile laboratory procedures at all times to help ensure minimal contamination.

Materials required

- Gloves, powder-free nitrile
- Isopropanol, 70% solution
- Wipes, lint-free

Clean the Ion Chef™ Instrument

IMPORTANT! Clean the Ion Chef™ Instrument as described in the following pages after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.



Ion Chef™ Instrument stations

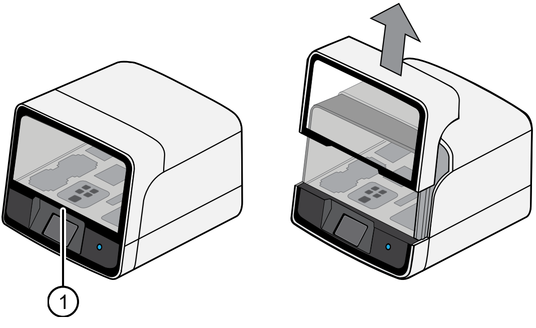
① Waste pipette tip position
② Empty Tip Cartridge v2: move to waste pipette tip station
③ Thermal cycler sample block
④ Reagents station
⑤ Solutions station
⑥ Recovery centrifuges
⑦ Enrichment station
⑧ Chip-loading centrifuge

Remove and dispose of used consumables

IMPORTANT!

- Do not discard the empty Ion S5™ ExT Tip Cartridge.
- Make sure to transfer the QC samples before you remove and discard the Reagents cartridge.

1. Tap ⏏ (Open Door) in the instrument touchscreen, then wait for the latch to open.
2. Lift the instrument door to the top of the travel until the latch mechanism engages.



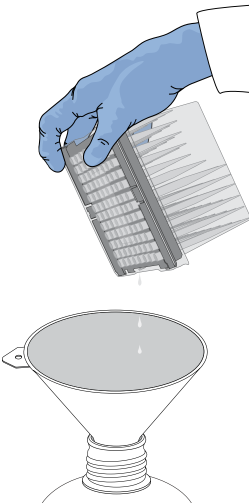
① Hold here, then lift

3. Remove, then discard the PCR Plate with the PCR Plate Frame and Frame Seal v2 from the thermal cycler sample block in unison.

   IMPORTANT! Do not attempt to separate the PCR Plate Frame from the PCR Plate and Frame Seal v2, as this may cause PCR product to splash and contaminate the instrument deck.

4. Remove, then discard the box of used pipette tips from the waste tip position.

   IMPORTANT! Handle the disposable reservoir in the waste tip position with care. During the run, liquid waste collects in the reservoir. Dispose of the liquid waste by tipping the reservoir on one corner and pouring the waste into an appropriate waste container:

   IMPORTANT! Do not reuse the waste pipette tip rack. Always move the empty Ion S5™ ExT Tip Cartridge from the new tip position to the waste tip position.

5. Move the empty Ion S5™ ExT Tip Cartridge to the waste tip position.

6. Remove, then discard the
   - Ion S5™ ExT Chef Reagents cartridge
   - Ion S5™ Chef Solutions cartridge
   - Enrichment Cartridge v2

7. Remove, then discard the consumables from the Recovery centrifuges, including the:
   - Recovery Station Disposable Lid v2
   - Recovery Tubes v2

8. Remove the Chip Balance from the Chip-loading centrifuge. Do not discard.

9. Close the Chip-loading centrifuge lid.

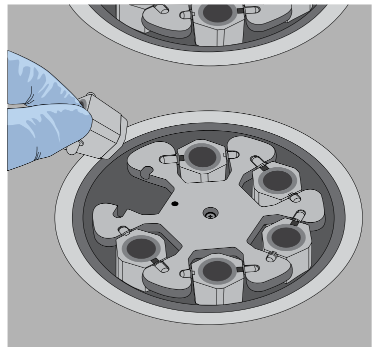
Inspect and clean the Recovery centrifuges and buckets

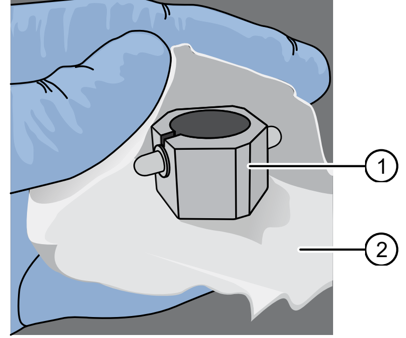
1. Inspect the Recovery centrifuges, then clean the components if excess liquid is present.

Is liquid present?	Action
No	Proceed to “Start the cleaning” on page 101.
Yes	Clean the centrifuge bowl and buckets as described below. **IMPORTANT!** Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do *not* need to clean the centrifuges after every run.

IMPORTANT! Wear powder-free, nitrile gloves when cleaning the Recovery centrifuge.

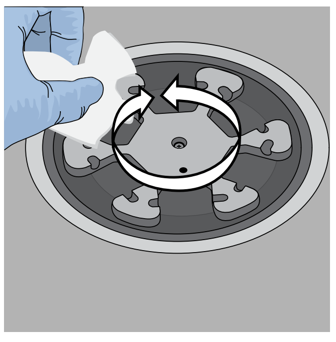
2. Remove the buckets from the Recovery centrifuges. Clean the inside and outside of each bucket using a lint-free wipe, then place the buckets on a clean, dry surface while you clean the centrifuge.

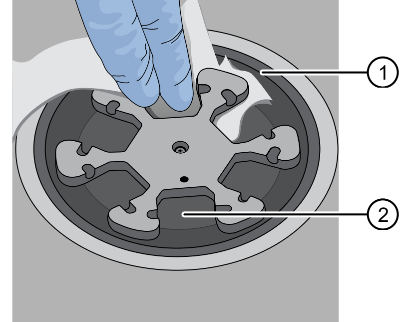




① Bucket ② Lint-free wipe

3. Use lint-free wipes to remove all fluid from inside the centrifuge bowl.



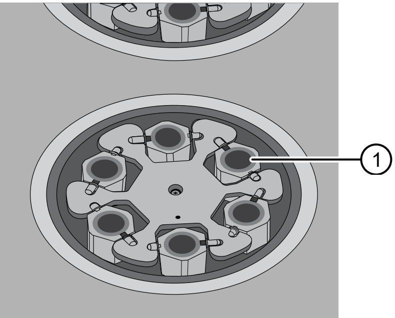


① Inside rim of the centrifuge ② Bottom of the centrifuge bowls

4. Use lint-free wipes treated with 70% isopropanol to clean the following parts.
   - Inside rim of the centrifuge.
   - Bottom of the centrifuge bowl.
   - Outside and inside of the centrifuge buckets.

5. Dry the centrifuge and buckets with lint-free wipes.

6. Install the centrifuge buckets, then close the Recovery centrifuge cover.



① Buckets (cleaned and installed)

Start the cleaning

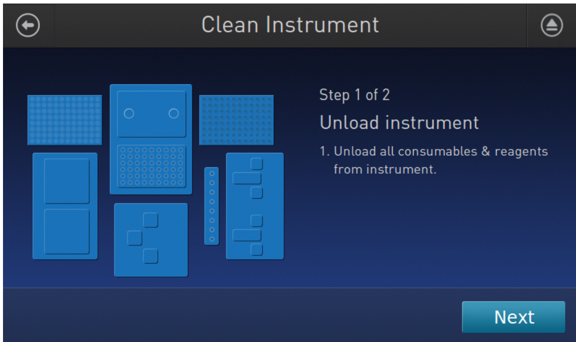
1. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.

   IMPORTANT! Before closing the door, ensure the covers of the Chip-loading and Recovery centrifuges are closed.

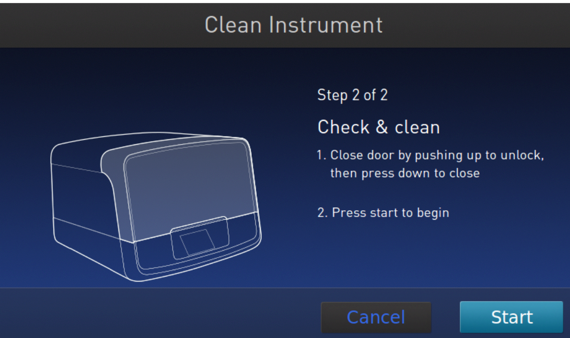
2. To start the cleaning, tap **Next** on the Ion Chef™ Instrument touchscreen that appears after run completion.

Note: You can also clean the instrument at any time starting from the home touchscreen. Tap **Settings ▸ Clean Ion Chef**.

3. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except the empty pipette tip rack in the waste tip position, then tap **Next**.



4. With the door closed, tap **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.



CAUTION! The Ion Chef™ Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.

Data analysis

- Manually launch an Ion Reporter™ Software analysis workflow 103
- Visualize aneuploidy analysis results 105
- Copy and edit an Ion Reporter™ Software analysis workflow 112
- Create and add a new filter chain 119
- Reanalyze an Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel combined library run to view the Ion AmpliSeq™ Polyploidy Panel results 123
- ReproSeqSnpAnalysis plugin 125

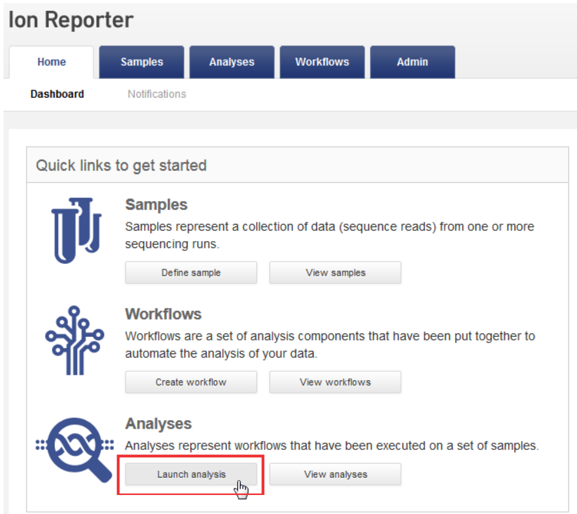
Manually launch an Ion Reporter™ Software analysis workflow

If you planned your run for automatic analysis with Ion Reporter™ Software, proceed to step 6.

1. Import your samples into Ion Reporter™ Software.

   See the Ion Reporter™ Software help system for detailed instructions for importing your samples and defining them in Ion Reporter™ Software.

2. In the Ion Reporter™ Software **Home** tab, click **Launch analysis** after the IonReporterUploader plugin run is complete.



3. In the **Workflow** step, select **Reproductive** from the **Research Category** menu to view the aneuploidy workflows. Select a ReproSeq w1.1 workflow from the list, then click **Next**.
   - ReproSeq PGS w1.1
   - ReproSeq No Gender PGS w1.1
   - ReproSeq Mosaic PGS w1.1

Note:
- The ReproSeq Low-Coverage Whole-Genome baseline is automatically loaded in the aneuploidy analysis workflows, making creation of a custom baseline for your Ion ReproSeq™ analyses unnecessary.
- To create a new aneuploidy analysis workflow, see "Copy and edit an Ion Reporter™ Software analysis workflow" on page 112.
- The Low-pass whole-genome aneuploidy w1.0 analysis workflow should be used for non-Ion SingleSeq™ libraries only.

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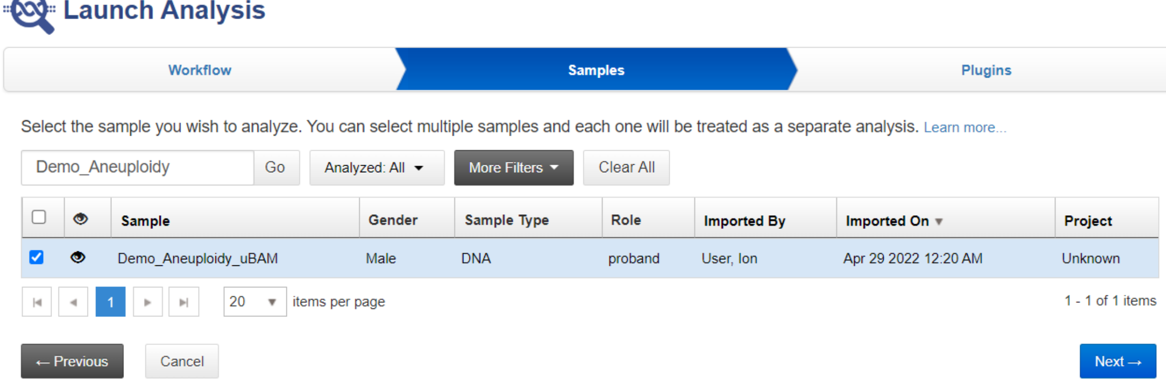
4. In the **Samples** step, select one or more samples.



5. Click **Next** twice to advance to the **Confirm & Launch** step.

6. In the **Confirm & Launch** step, you can enter a name for the analysis to change the default name, then click **Launch Analysis**.

7. Click **Analysis ▸ Overview** and select the analysis from the **Analyses** list to review results of the manually launched analysis. For more information, see the Ion Reporter™ Software help system.

Visualize aneuploidy analysis results

You can view interactive aneuploidy analysis results and customize visualizations in the Ion Reporter™ Genomic Viewer (IRGV). With this viewer, you can visualize multiple analyzes at once, zoom in on chromosomes of interest, and generate reports of the data that you view.

You must have IRGV set as the default viewer for this procedure. For more information, see "Set IRGV or IGV as the default viewer" on page 148.

Note: When you use aneuploidy analysis workflows, if there are not enough mapped reads to calculate **Called Gender**, the analysis will complete successfully and the sample coverage section in the `analysis.log` file contains information on why the gender calling failed.

1. Under the **Analyses** tab, click **Overview**.
2. Select the **Workflow** filter and enter ***ReproSeq*** to find analyses that used a **ReproSeq** analysis workflow, or select a specific analysis workflow filter, such as **ReproSeq No Gender PGS w.1.1**.
3. Select one or more analyses then click **Visualize**.
   The **Analysis Visualization** screen opens in the **IRGV & Generate Report** tab.

4. Use the interactive view to customize the visualization and generate reports, as needed.

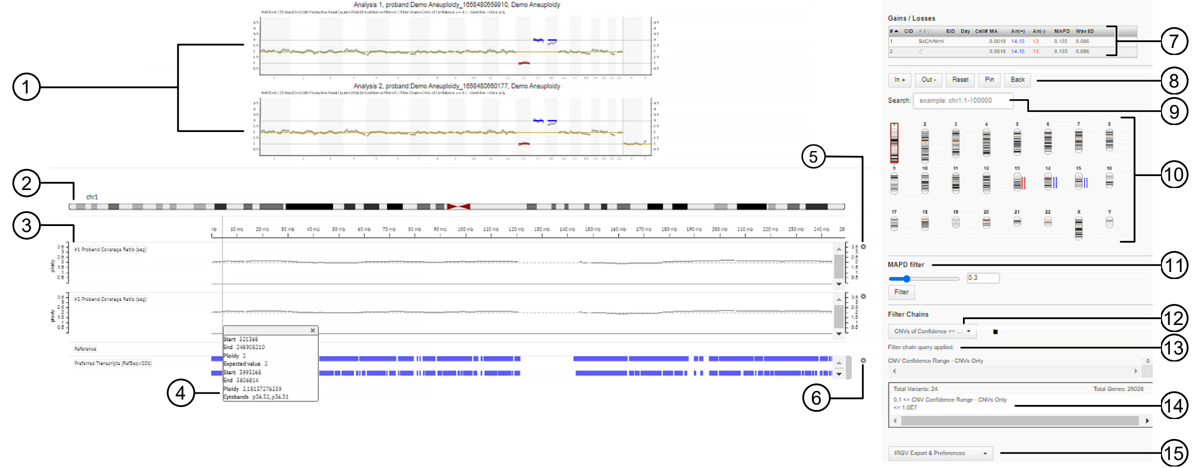


Figure 2 Aneuploidy analysis results

① Analysis whole genome view. Provides a genome-wide perspective of the sample data. A chart or graph is shown for each selected analysis. Click on the chart to zoom in on a region of the genomic data, or a chromosome, and view more information about your selection throughout the visualization.

② Click on this IRGV track to view details about the selected chromosome and view more information about the chromosome in the tracks below. Or, select data in the whole genome view or Karyogram to view data reflected in each track below.

③ Click **Shift** and drag across the x-axis of the ploidy scale to zoom in on a region of the scale and change the other data in the visualization to reflect the selection.

④ Hover over a tile in the visualization to get the following information.

- **Start:** Start coordinate for the copy number call region.
- **End:** End coordinate for the copy number call region.
- **Ploidy:** Called ploidy value. The highest allowable ploidy value calculated by the software is 10.
- **Expected value:** Expected ploidy value for this chromosome. The value is 2 for autosomes and chrX for females. It is 1 for chrX and chrY for males.
- **Start:** Start position of the selected tile.
- **End:** End position of selected tile.
- **Ploidy:** The calculated ploidy value of the tile. The highest value that will be shown by the software is 11.
- **Cytobands:** The cytoband range of the tile.

⑤ Adjustable ploidy scale for selected chromosomes or chromosome regions. Change the track name or track height for the histogram, or hide the track. See "Adjust IRGV BAM tracks" on page 148 for more information.

⑥ Adjustable **Reference** and **Preferred Transcript** tracks. Change the BAM track name, track height, and track color, or collapse, squish or expand the track. See "Adjust IRGV BAM tracks" on page 148 for more information.

⑦ Click in a row of the **Gains/Losses** table to open a detailed view of a single sample visualization. See "View single sample aneuploidy details" on page 107 for more information.

The **Gains/Losses** table shows the following information.

CID (Couple ID): An identifier for the parents of an embryo.

EID (Embryo ID): An identifier for the embryo biopsied and run through the ReproSeq workflow)

Cell# (Cell Num): The number of cells in the sample, if known.

MA: A calculated result the ratio of Mitochondrial DNA and Autosomal DNA.

An: Aneuploidy, shown as An(+) for a copy number gain, and An(-) for a copy number loss. The affected chromosomes are shown in a comma-separated list.

MAPD (Median Absolute Pairwise Difference): An estimate of coverage variability between adjacent amplicons.

WaveSD (Waviness Standard Deviation): Standard deviation of all log2 read count ratios after subtraction of the contribution of pairwise differences.

⑧ Zoom in and out of the chromosome Karyogram, and click **Reset** to return to the default chromosome view.

⑨ Enter a set of chromosomal coordinates in the **Search** field to zoom into chromosome data, or click on a chromosome in the Karyogram to view its chromosomal coordinates. Or, view the chromosomal coordinates when you change the selections in the whole genome chart, IRGV, or Karyogram.

⑩ Chromosome Karyogram.

⑪ Adjust the MAPD filter.

⑫ Select a filter chain to apply filters to the data. If you select multiple analyses generated from analysis workflows with different filter chains that use different confidence settings, the software will apply the filter chain with the lowest confidence setting to all selected analyses.

⑬ View the filter change, or filter chain query that is applied to the visualization.

⑭ View details about the filter chain, or filter chain query, the total number of genes, and the variants that were filtered in.

⑮ Export the visualization, or change the IRGV preferences. See "Set IRGV preferences" on page 146 for more information.

View single sample aneuploidy details

You can view an interactive view of details from a single sample aneuploidy analysis in the Ion Reporter™ Genomic Viewer (IRGV). This visualization provides a view of the genomic data that you can zoom in on to view individual chromosomal data, and a table of events that were called. You can adjust the list of events to show only events that were filtered in for the analysis by the applied filter chain.

You must have IRGV set as the default viewer for this procedure. For more information, see "Set IRGV or IGV as the default viewer" on page 148.

1. In the **Analyses** tab, click **Overview**.
2. Select the **Workflow** filter and enter ***ReproSeq*** to find analyses that used a **ReproSeq** analysis workflow, or select a specific analysis workflow filter, such as **ReproSeq No Gender PGS w.1.1**.
3. Select one or more analyses then click **Visualize**.
   The **Analysis Visualization** screen opens in the **IRGV & Generate Report** tab.
4. Click in a row of the **Gains/Losses** table.
   The **IRGV Details** screen opens in a new browser tab.

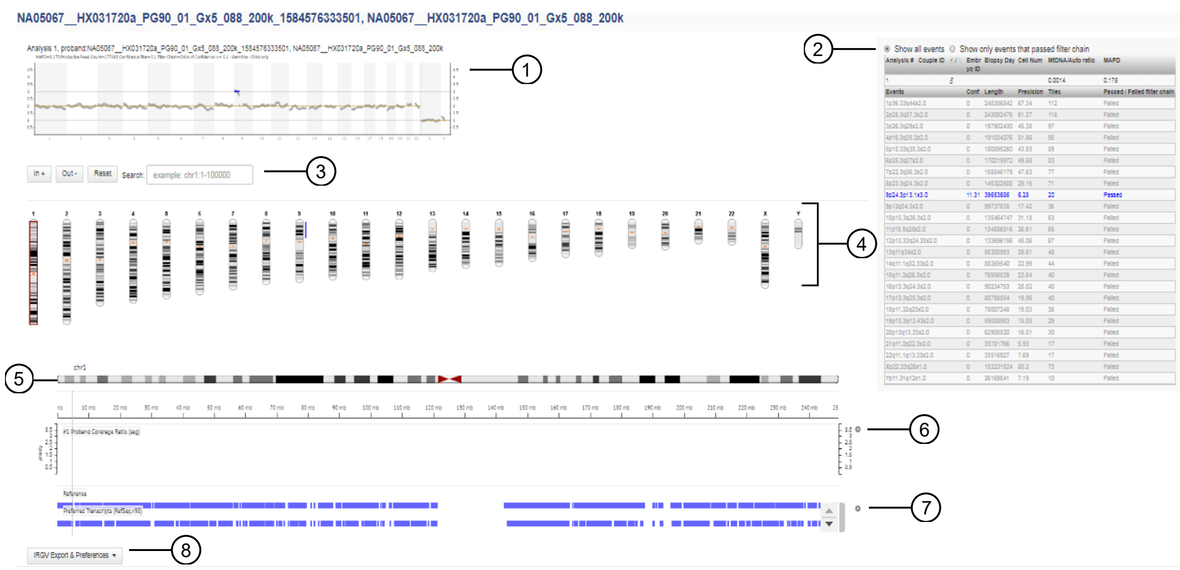


Figure 3 IRGV Details screen

① Analysis Whole Genome View. Provides a genome-wide perspective of the data. Metrics above the view are:

ⓐ Productive Read Count The sum of mapped reads from a sample that align to the autosomes and chrX in females or to the autosomes and chrX + chrY in males. If the CNV finding parameter ***Remove Duplicates*** is set to ***True***, then the **Productive Read Count** metric excludes duplicates. Productive reads are the specific reads that are used to establish the relative coverage of tiles across the sample.

ⓑ Confidence

ⓒ Filter Chain The filter chain that was applied to the analysis.

② Click in a row of the **Gains/Losses** table to open a visualization with the data charts and a table of the events in a separate tab. Select an option in the table to see all events, or only events that passed the applied filter chain.

③ Enter a chromosome coordinate in the **Search** field to zoom into chromosome data, or click on a chromosome in the Karyogram to view its chromosome coordinate. Zoom in and out of the Karyogram, and click **Reset** to return to the default chromosome view.

④ Select a chromosome on the Karyogram to the chromosome coordinate, narrow the focus of the whole genome view, and update the IRGV tracks to reflect the selection.

⑤ IRGV tracks. Each track updates dynamically as you select data in the whole genome view or the Karyogram view.

⑥ Adjustable ploidy scale for selected chromosomes or chromosome regions. Change the track name or track height for the histogram, or hide the track. See "Adjust IRGV BAM tracks" on page 148 for more information.

⑦ Adjustable reference and preferred transcript tracks. Change the BAM track name, track height, and track color, or collapse, squish or expand the track. See "Adjust IRGV BAM tracks" on page 148 for more information.

⑧ Export the visualization, or change the IRGV preferences. See "Set IRGV preferences" on page 146 for more information.

Change Y-axis height in Whole Genome aneuploidy visualization

You can expand the height of the Y-axis when you view whole genome aneuploidy analysis results in Ion Reporter™ Genomic Viewer (IRGV). By default the image of the data is 300 pixels high. IRGV includes the option to double this height to 600 pixels. The expanded view is useful when you review mosaic data, because more data is shown on a single plot and more space is available between data points, making the mosaic data more pronounced.

1. Under the **Analyses** tab, click ***Overview***.
2. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.
3. In the **IRGV & Generate Report** tab, scroll to the bottom of the visualization, then click **IRGV/Export & Preferences ▸ Show IRGV preferences** to expand the **Preferences** section.

Option	Description
Expanded	Select this option to increase the height of the Whole Genome view to 600 pixels.
Compressed	This is the default setting. Select this option to set the height of the Whole Genome view to 300 pixels.

Smoothing, no gender, and mosaicism results in IRGV

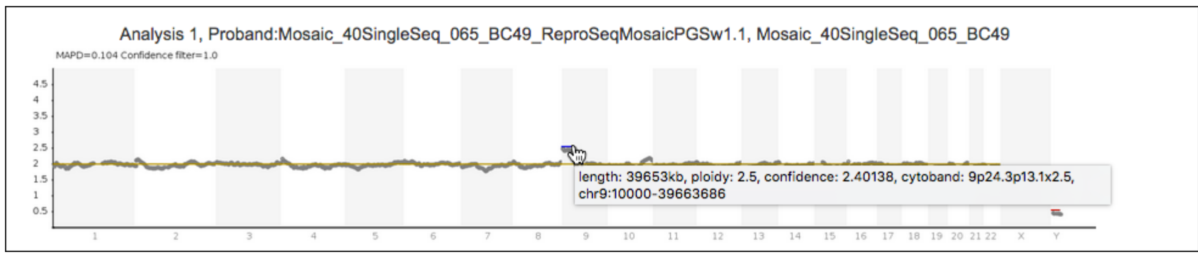


Figure 4 **Example of a mosaic sample**

The following Ion Reporter™ Genomic Viewer (IRGV) plot shows non-integer ploidy for the short arm of chromosome 9, indicating that the sample is mosaic.

This image shows five analyses run with various Ion Reporter™ Software aneuploidy analysis workflows that show how smoothing, no gender, and mosaicism appear in IRGV visualizations. Visualization of aneuploidy detection is enhanced by tile-by-tile data points that appear as easier-to-see circles in the whole-genome views. All results from IRGV aneuploidy analysis workflows use this enhanced view.

Note: Analyses 2 through 5 have smoothing of the discrete tile data turned on by default. Smoothing of the discrete data have no effect on the calls that are made by the aneuploidy pipeline in the software. Smoothing can be turned on or off in any analysis workflow.

Figure 5 Examples of smoothing, No Gender and mosaicism

① An analysis launched with the Low Pass Whole-Genome Aneuploidy analysis workflow and the Demo Aneuploidy sample that is available in the software, with a loss of one copy of chr13 and single copy gains of chrs 14 and 15. This analysis was run with the earlier version of an Ion Reporter™ Software analysis workflow version that does not include smoothing of the discrete tile data.

② An analysis launched with the default ReproSeq analysis workflow and the Demo Aneuploidy sample in Ion Reporter™ Software.

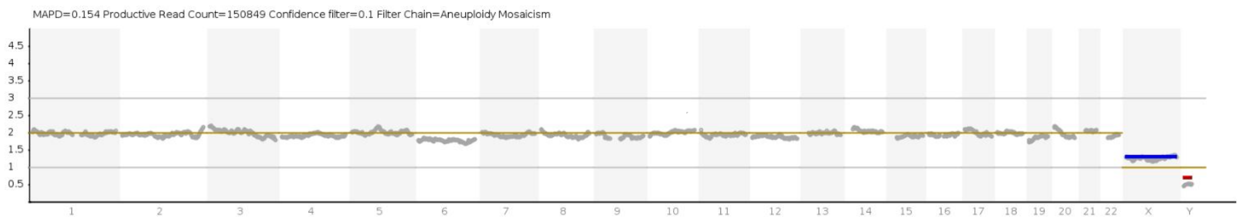
③ An analysis launched with the ReproSeq No Gender analysis workflow and the Demo Aneuploidy sample. Use of the no gender analysis workflow generates analysis results that do not record or show the called gender of the sample in the software or in data files. Instead, a called gender value of SxChrNrml is given when the sample is either a normal XY male or a normal XX female with no sex chromosome aberrations. A gender call is SxChrAbnrml when the sex chromosomes deviate from normal male or normal female by either whole-chromosome or subchromosomal aneuploid events. Aneuploidies on autosomes do not affect the called gender value, and can be present in samples with normal sex chromosomes.

④ An analysis launched with the ReproSeq Mosaic analysis workflow and the Demo Aneuploidy sample. Some chromosomes can look like they were called aneuploid if the copy number is called as CN loss = ploidy 1.95, or CN Gain = ploidy 2.05 (as shown in this example on chr1), and on the Y chromosome as a slight ploidy gain. Gain or Loss events with ploidy very near expected normal ploidy can be a result of slight differences in normalization instead of true biological ploidy changes.

⑤ An analysis of a spiked-in sample that was mixed to have a chr9p event of relative copy number ploidy of 2.5 and analyzed through the ReproSeq Mosaic analysis workflow. The two mixed samples consisted of one male and one female, one of which had an integer copy number gain of ploidy=3 for chr 9, although the other sample was normal ploidy=2 for chr 9. The mixing of two samples of different genders can be seen in the observed ploidies of the X and Y chromosomes.

Visualization of triploid samples

Mosaic workflows call triploid male biopsies (69, XXY). Mosaic events on chrX and chrY are called in 69, XXY samples since three copies of autosomes will be normalized to 2N at a ratio of 1.5 (3/2 = 1.5), thus 2N chrX should be called 1.33 (2/1.5 = 1.33) and 1N chrY should be called 0.66 (1/1.5 = 0.66).



Example genome view plot and table from a 69, XXY sample with chrX called ploidy 1.3 and chrY called ploidy 0.7

View X and Y chromosome data in low-read samples

Aneuploidy samples with low total read counts can yield analysis results where X and Y chromosome ploidies are not displayed and gender is not called. There are two options you can try to visualize X and Y chromosome data in this situation, although we recommend that you interpret with caution any analysis results showing high MAPD values.

- A low-read sample that results in no display of X and Y chromosome data can be analyzed in a custom aneuploidy workflow with a lower value entered for the parameter "CNV Gender Min Autosomes Count" (default = 25000). Copy your existing ReproSeq PGS workflow, then edit this parameter by navigating to **Parameters ▸ Cnv Finding ▸ Advanced**.

Gender calling

CNV Gender Caller Enable Flag. (Do not enable for non-Aneuploidy workflows. For other workflows, called gender results may be inaccurate)

Flag to indicate whether Gender caller should be invoked.

◉ True ○ False

CNV Gender Threshold

Specifies threshold ratio of chrY to Autosomes for taking male/female call

0 <= 7

CNV Gender Min Mapping QV

Specifies min mapping qv of reads to consider in gender calling

0 <= 30 <= **255**

CNV Gender Min Autosomes Count

Specifies min number of required filtered reads in autosomes

0 <= 25000

CNV CHRM To Autosomes Ratio Min Mapping QV

Specifies min mapping qv of reads to consider in calculating chrM A Ratio

0 <= 30 <= **255**

- Create a custom aneuploidy workflow with the CNV parameter "Plot Y chromosome for Female or Unknown Gender" enabled. Copy your existing ReproSeq PGS workflow, then enable this parameter by navigating to **Parameters ▸ Cnv Finding ▸ Advanced** and selecting **True** for this parameter.

  Analysis

  Plot Y chromosome for Female or Unknown Gender?

  Plot Y chromosome for Female or Unknown Gender.

  Note: With this parameter enabled, the analysis displays X and Y chromosome data, but does not attempt to call the gender. Also, the software calls X and Y chromosome reads as CNV gains by default, regardless of read count.

See “Copy and edit an Ion Reporter™ Software analysis workflow” for further information on how to copy and edit an Ion Reporter™ aneuploidy workflow.

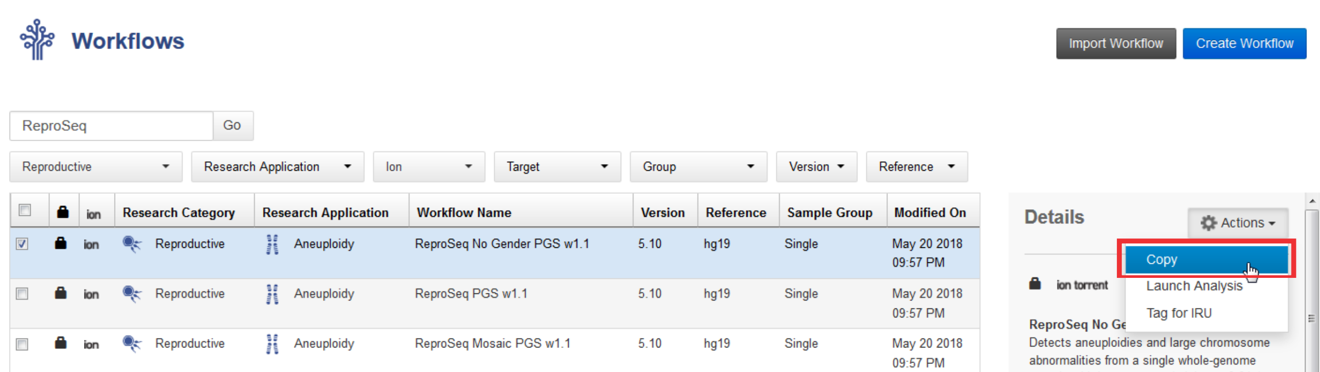
Download additional examples of Ion ReproSeq™ PGS data

Additional examples of Ion ReproSeq™ PGS datasets and analyses can be downloaded at **https://apps.thermofisher.com/apps/publiclib/#/datasets**. Sign in is required.

Copy and edit an Ion Reporter™ Software analysis workflow

You can copy and edit an existing Ion Reporter™ Software aneuploidy analysis workflow to create a new analysis workflow. For example, if you copy the ReproSeq No Gender PGS w1.1 analysis workflow, you can modify the settings to enable mosaicism detection, change the CNV sensitivity, or disable smoothing.

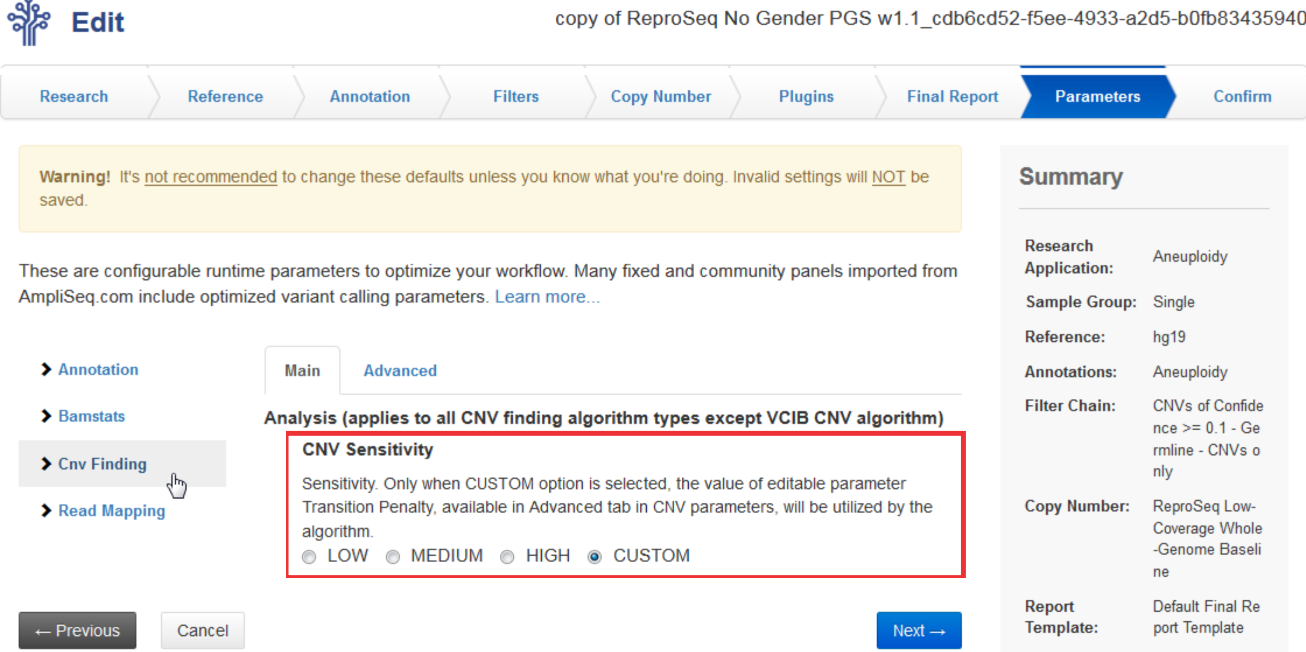
1. On the Ion Reporter™ Software **Home** tab, click **View workflows**, and select the **Reproductive** research category, then select the **Aneuploidy** research application. Select the analysis workflow of interest, then in the **Details** section, click **Actions ▸ Copy**.



2. In the **Edit** screen, click **Next**, and make any changes that are needed in each step.

3. In the **Parameters** step, click **Cnv Finding**, then change the **CNV Sensitivity** setting.

   Note: Low sensitivity results in fewer false positives, but more false negatives. High sensitivity results in fewer false negatives, but more false positives. High sensitivity is required to make segmental aneuploidy calls of ~20 Mbp. Custom sensitivity allows you to change the CNV Transition Penalty parameter to detect even smaller segmental CNV events more sensitively. See "Improve detection of small segmental CNV events" on page 115 for further information.



4. Click the **Advanced** tab to access more analysis parameter settings.

5. Scroll to the **Analysis (applies only to Aneuploidy workflows)** section, then make desired changes.
   For example, you can select **True** under **Enable Mosacism Detection** to enable mosaicism detection. When mosaicism is enabled, the software reports a CNV event as a decimal ploidy value instead of an integer value.

Analysis (applies only to Aneuploidy workflows)

Remove Duplicates

Removes duplicate reads

(•) True ( ) False

Enable Mosaicism Detection

Enable Mosaicism Detection

(•) True ( ) False

Enable Smoothing

Enable Smoothing

(•) True ( ) False

Hide called gender

Hide gender called by CNV gender calling

(•) True ( ) False

To enable mosaicism detection and/or smoothing in a No Gender analysis workflow, copy and edit the ReproSeq No Gender w1.1 analysis workflow as described. You cannot enable gender hiding in the other ReproSeq analysis workflows because the **Hide called gender** setting is locked as **False** (gender is called), and cannot be set to **True**.

Analysis (applies only to Aneuploidy workflows)

Remove Duplicates

Removes duplicate reads

(•) True ( ) False

Enable Mosaicism Detection

Enable Mosaicism Detection

(•) True ( ) False

Enable Smoothing

Enable Smoothing

(•) True ( ) False

Hide called gender

Hide gender called by CNV gender calling

( ) True (•) False

To show Y chromosome data for every sample, whether it is female, male, or unknown, set **Plot Y chromosome for Female or Unknown Gender** to **True**.

The default value for this setting is **False**, which shows Y chromosome data only if the sample is male.

Analysis

Plot Y chromosome for Female or Unknown Gender?

Plot Y chromosome for Female or Unknown Gender.

6. Click the **Annotation**, **Bamstats**, and **Read Mapping** links to access additional analysis workflow parameters. When you have completed your edits, click **Next** to proceed to the **Confirm** step.

   Note: Do not change parameters from the default settings unless you understand how the change can affect your analysis.

   ---

7. Rename the analysis workflow, then click **Save Workflow**.
   The analysis workflow appears in the list of available Ion Reporter™ Software analysis workflows.

Improve detection of small segmental CNV events

You can improve the detection of small segmental CNV events if you adjust the `CNV Transition Penalty` parameter in Ion ReproSeq™ analysis workflows for use with aneuploidy research. The CNV Transition Penalty parameter establishes the trade-off between false-positive and false-negative rates. The transition penalty is a probability that the copy-number state changes for any given random tile. A smaller probability results in calling of only larger CNV segment sizes, or calling of only segments that include greater support for the changed state, that is, a greater difference in copy number value from the current ploidy state.

Low sensitivity results in fewer false positives, but more false negatives. High sensitivity results in fewer false negatives, but more false positives. High sensitivity is required to make segmental aneuploidy calls of ~20 Mbp. **Custom CNV Sensitivity** allows you to change the CNV Transition Penalty parameter to detect even smaller segmental CNV events more sensitively.

IMPORTANT! Do not change parameters from the default settings unless you understand how the change can affect your analysis.

You can change the CNV Transition Penalty parameter to any value that does not exceed the highest allowable for this parameter:

- –1.05 for nonmosaic analysis workflows
- –2.31 for mosaic analysis workflows

1. In the **Workflows** tab, click **Overview**.
2. In the list of analysis workflows, select **Aneuploidy** from the **Research Application** list, select the software version that you use, then sort the list by predefined analysis workflows.
3. Select the analysis workflow of interest, such as the Ion ReproSeq™ PGS analysis workflow, then in the **Workflows** tab, click **Overview**, then in the **Workflows** table, click the row for an analysis workflow that you want to copy, then click **(Actions) ▸ Copy**.
4. In the **Create Workflow** screen, advance to the **Parameters** step, then click the **Cnv Finding** link. In the **Analysis** section **Main** tab, change the **CNV Sensitivity** setting to **Custom**, then enter new values for the parameter.
5. Click **Advanced**, scroll to the **CNV Transition Penalty** parameter, then enter the desired value.

   The approximate recommended values are:

   - -2.33 for mosaic ploidy calling
   - -15 for low sensitivity
   - -5 for medium sensitivity
   - -2.0 for nonmosaic ploidy calling
   - -3.0 for high sensitivity in mosaic and nonmosaic analysis workflows
6. In the **Confirm** step, click **Save Workflow**.

   The following results show a sample analyzed twice with two customized workflows—one with the CNV Transition Penalty parameter set to -3, and a second with the parameter set to -2.

   In this visualization, a 7.9-Mb deletion that is called on chromosome 6 is visible with the slightly higher transition penalty.

Adjust the tile size

Further improvement in sensitivity to detect small segmental CNV events can be obtained by decreasing the tile size. This adjustment must be accompanied by selection or creation of a CNV Baseline with a corresponding smaller tile size. Pre-built CNV baselines corresponding to smaller tile sizes are included in Ion Reporter™ Software and are ready to use in customized aneuploidy analysis workflows. Follow these steps to adjust the tile size parameter in an aneuploidy workflow.

1. In the **Create Workflow** or **Edit** workflow screen, navigate to **Parameters ▸ CNV Finding**. Under the **Advanced** tab, scroll to the **Analysis (applies only to Aneuploidy workflows)** section.

    Analysis (applies only to Aneuploidy workflows)

    Remove Duplicates

    Removes duplicate reads

    ◉ True ○ False

    Enable Mosaicism Detection

    Enable Mosaicism Detection

    ○ True ◉ False

    Enable Smoothing

    Enable Smoothing

    ◉ True ○ False

    Set Tile Size for Aneuploidy Workflow

    Set Tile Size for Aneuploidy Workflow. The tileSize used for creating the Aneuploidy Baseline must match the tileSize selected here.

    1 <= [ ] <= **100000000**

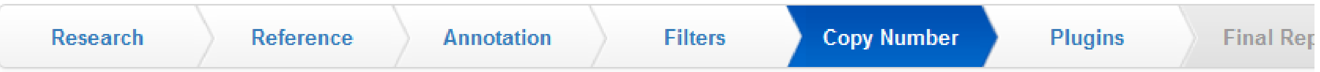
    Hide called gender

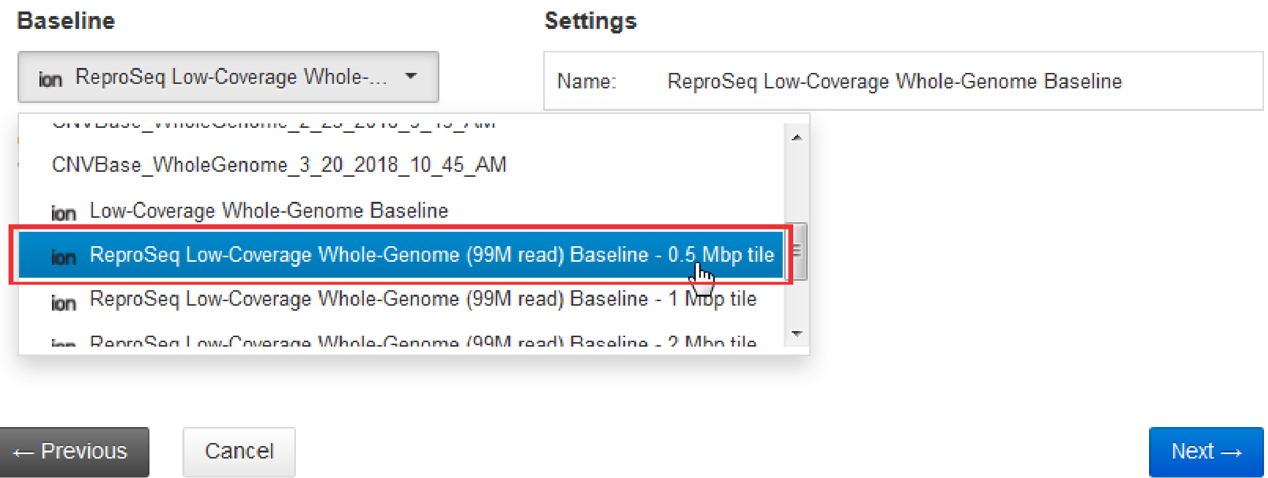
    Hide gender called by CNV gender calling

    ○ True ◉ False

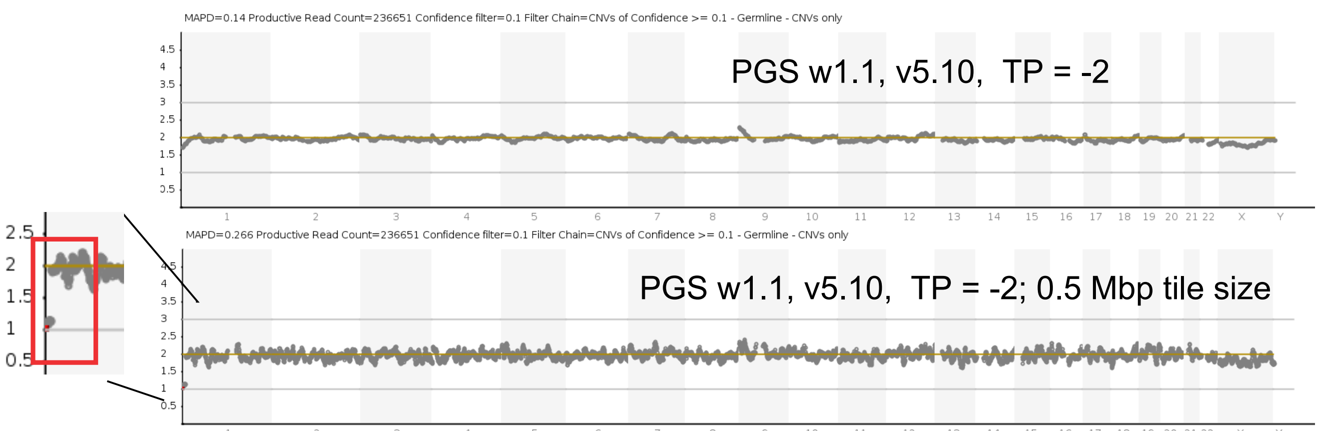
2. For a tile size of 0.5 Mbp, enter 500000 in the **Set Tile Size for Aneuploidy Workflow** field.

3. Navigate back to the **Copy Number** step of the workflow screen, then select **ReproSeq Low-Coverage Whole-Genome (99M read) Baseline - 0.5 Mbp tile** from the **Baseline** dropdown list.





4. In the **Confirm** step, click **Save Workflow**.
   Example of increased sensitivity: a 4.5-Mb deletion is called on chromosome 1 with tile size set to 0.5 Mbp.

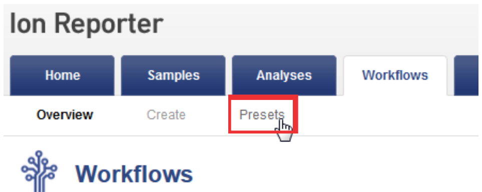


Create and add a new filter chain

Modify the CNV Confidence Range

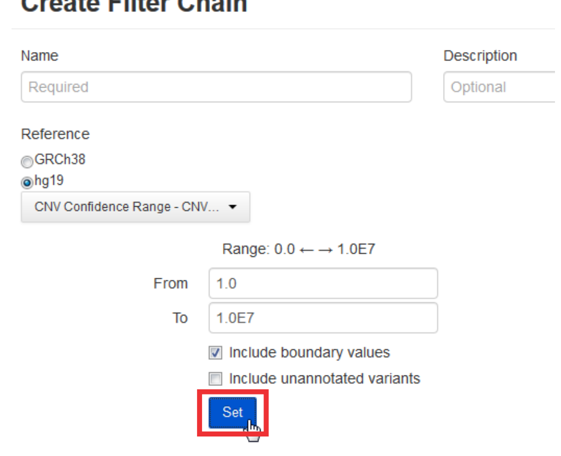
ReproSeq aneuploidy analysis workflows have the CNV Confidence Range set at 0.1 to 1.0E7 as the default setting. To specify another range for more stringent (1.0 to 1.0E7) or less stringent (0.01 to 1.0E7) CNV filtering, you must first create a new filter chain, then edit an analysis workflow to include the filter chain.

1. In Ion Reporter™ Software, in the **Workflows** tab, click **Presets**.

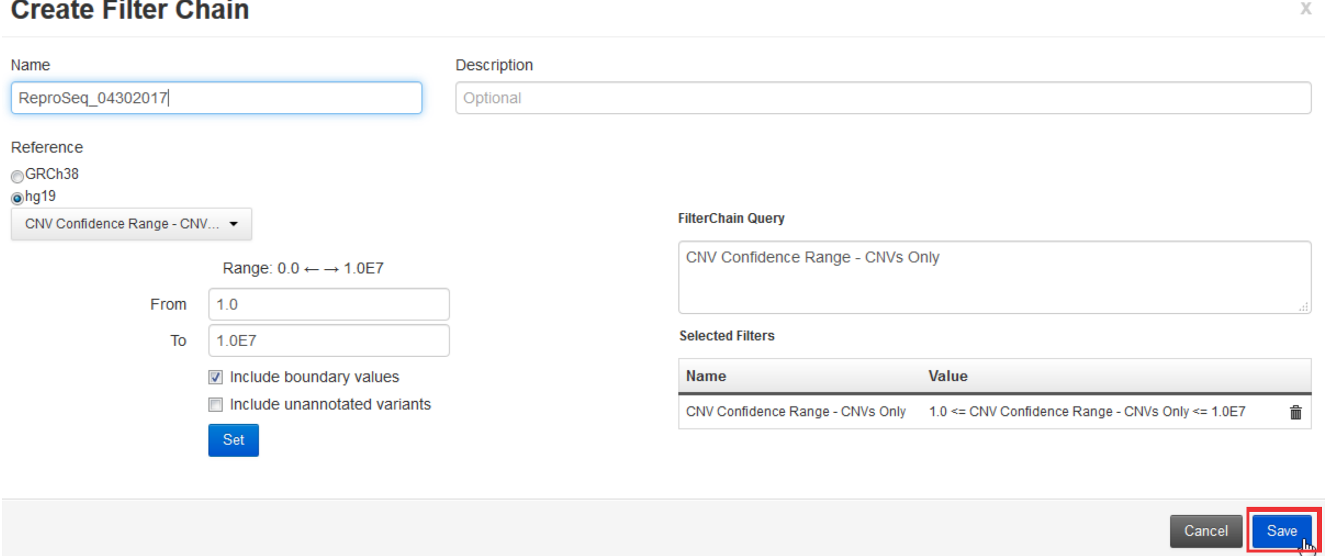


2. Click **Create Preset ▸ Filter Chain**.

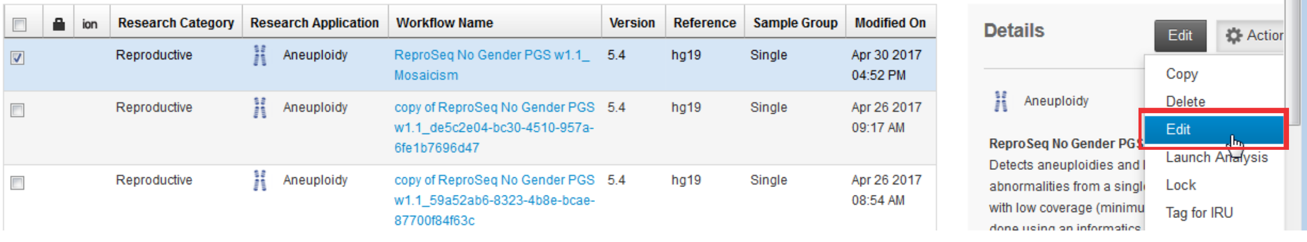
3. In the **Create Filter Chain** dialog box, select **CNV Confidence Range - CNVs Only** from the dropdown list, and enter the confidence range that the filter chain will use, then click **Set**.



4. Enter a name for the filter chain, then click **Save**.

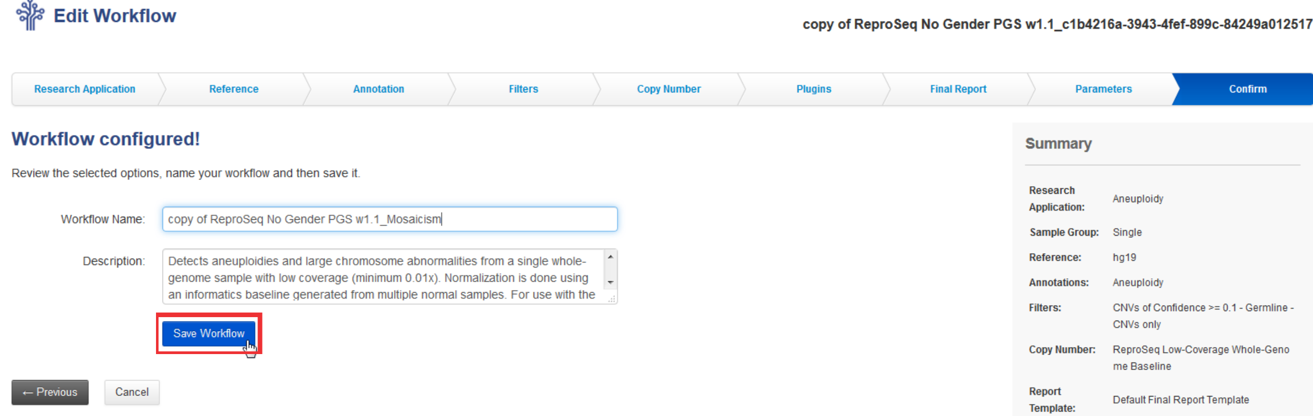


5. Select the analysis workflow that you want to edit to add the new filter chain from the list in the Ion Reporter™ Software **Workflows** tab, then click **Edit ▸ Actions** in the **Details** section.



6. In the **Filters** step of the **Edit Workflow** screen, select the filter chain that you want to add to the analysis workflow, then click **Next**.

7. Click through the remaining steps to make any additional edits, then name the edited analysis workflow in the **Confirm** step, and click **Save Workflow**.



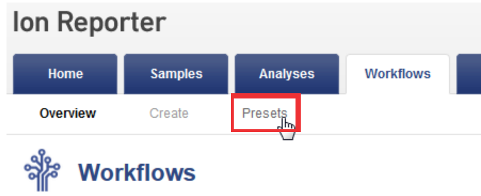
For more information, see Ion Reporter™ Software help system.

Create an Expected Normal Ploidy Buffer filter chain

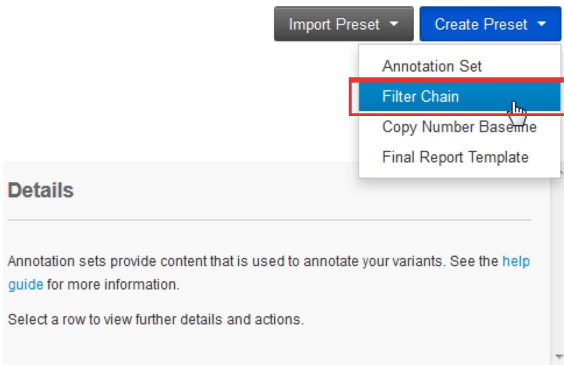
Changing the transition penalty parameter can increase sensitivity of the analysis to detect segmental mosaic CNV events. However, a side effect of this enhanced sensitivity is increased frequency of false positives. To filter false positives, you can create an Expected Normal Ploidy Buffer (ENPB) filter chain and add it to your Ion Reporter™ workflow.

By default, the ENPB filter is set to filter out all copy number variant segments of gain or loss within 0.2 ploidy value of expected normal. With this setting, ploidy changes between 2.2 and 1.8 on autosomes and female X chromosomes are filtered, as are changes between 1.2 and 0.8 on male X and Y chromosomes. You can customize the amount of buffer by adjusting the range values when you configure the filter chain. The confidence parameter in the ENPB filter overrides any other confidence filtering in effect within the buffer zone. ENPB filter chains can be selected in the **Filter Chain** dropdown list in the IRGV visualization tab.

1. In the Ion Reporter™ **Workflows** tab, click **Presets**.

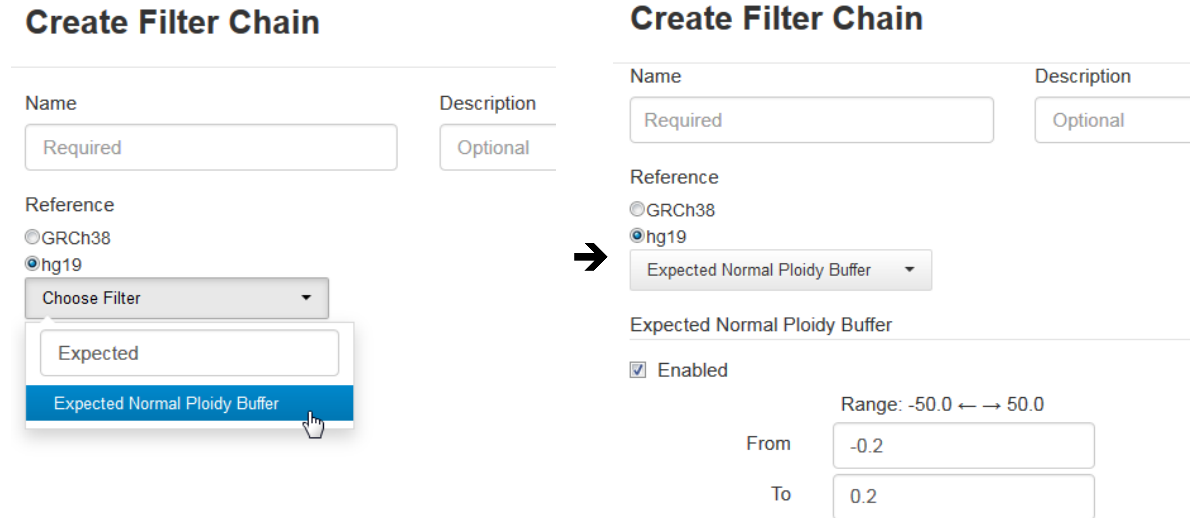


2. In the **Create Preset** dropdown list, select **Filter Chain**.

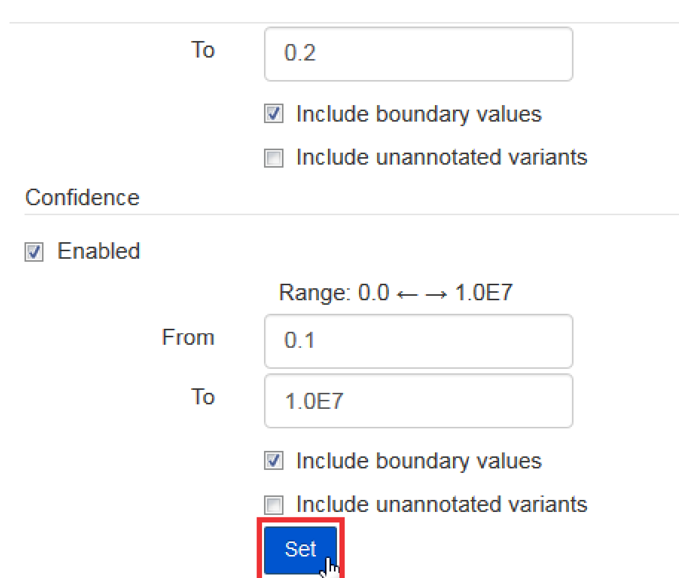


3. In the dialog, select **Expected Normal Ploidy Buffer** from the dropdown list on the left, then adjust the values in the **From** and **To** fields, if needed.

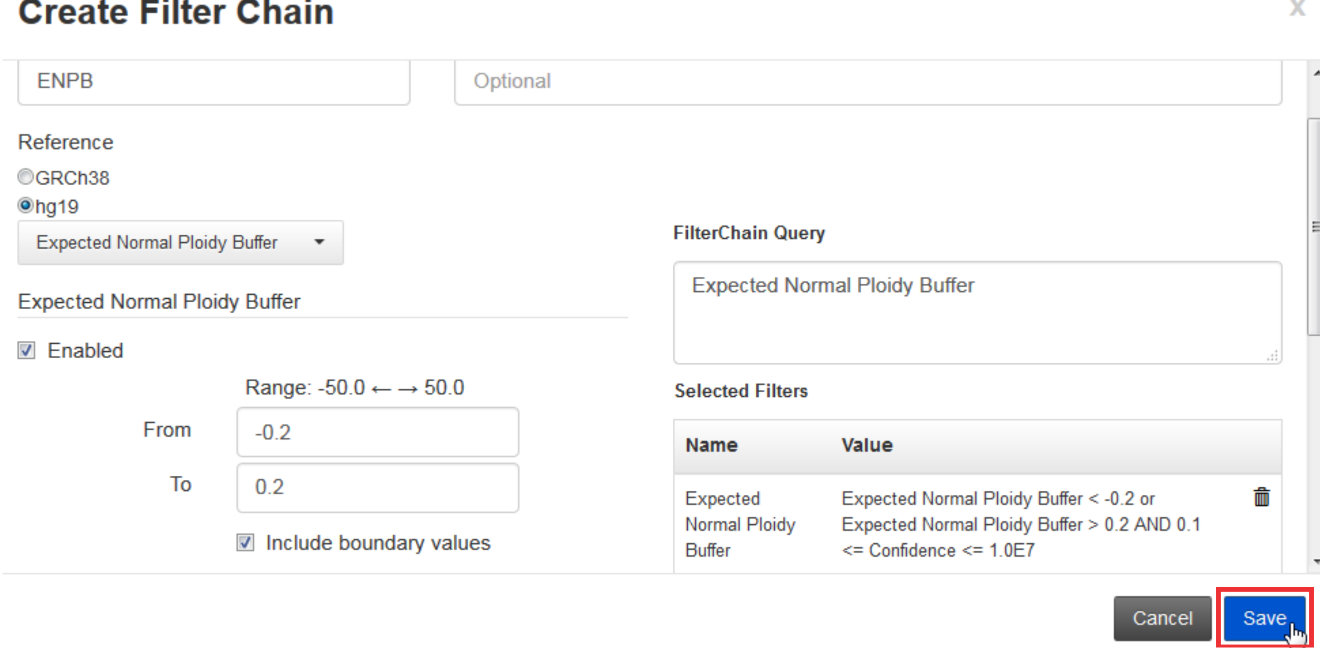
Note: You can type "Expected" in the **Choose Filter** field to do a quick search of the dropdown list.



4. Scroll to the **Confidence** section of the dialog, adjust the confidence range parameters, if needed, then click **Set**.



5. Enter a name for the filter chain, click **Save**.



6. Go to step 5 on page 120 in "Modify the CNV Confidence Range" to add the filter chain to your workflow.

Reanalyze an Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel combined library run to view the Ion AmpliSeq™ Polyploidy Panel results

After completion of a combined Ion SingleSeq™ & Ion AmpliSeq™ Polyploidy Panel library sequencing run, you need to reanalyze the results in Torrent Suite™ Software to view the Ion AmpliSeq™ Polyploidy Panel library results. To reanalyze the sequencing results you need to edit the Run Plan to include Ion AmpliSeq™ Polyploidy Panel library information, then start a reanalysis.

1. Sign in to Torrent Suite™ Software.
2. In the **Data** tab, click **Completed Runs & Reports**.
3. Search, filter, or sort the list to find the run report of interest.
4. Click the report name link for your completed sequencing run to open the **Run Report**.

5. Click **Report Actions ▸ Edit Run Plan**, then edit the run plan.
   a. For each run planning step complete or verify the following fields.

Item	Action
Research Application step	Set **Target Technique** to **AmpliSeq DNA**.
Kits step	1. Set **Library Kit Type** to **Ion AmpliSeq Library Kit Plus**. 2. Set the **Barcode Set** to **IonCode**.
Plugins step	1. Ensure the following plugins are selected. • coverageAnalysis • variantCaller • ReproSeqSnpAnalysis 2. Ensure the **FilterDuplicates** plugin is deselected.

   b. Configure the variantCaller plugin. Click **Configure** next to the plugin name, set the following values, then click **Save Plugin Settings**.
      - Chip Type – 530
      - Library Type – AmpliSeq
      - Variant Frequency – Germ Line
      - Parameter Settings – Generic - S5/S5XL (510/520/530) - Germ Line - Low Stringency

6. In the **Save** step, make the following choices.

Item	Selection
Analysis Parameters:	Custom
Reference Library:	**GRCh38.p2.mask1**
Target Regions:	**Ion_AmpliSeq_Polyploidy_Panel_w1.0.0**
Hotspot Regions:	**Ion_AmpliSeq_Polyploidy_Hotspots_w1.0.0**
Use same reference & BED files for all barcodes	Ensure this is selected.

7. Click **Custom** to view the detailed custom analysis parameters. Then edit the **Pre-BaseCaller for calibration** and **BaseCaller** parameters to remove: `--extra-trim-left 30`

8. Update each sample library with an IonCode barcode assignment.
   If you have many samples, you can update the existing sample set by importing the sample information using a CSV file. For more information, see “Create a Sample Set by importing samples from a CSV file” on page 142.

9. Click **Update Run & Reanalyze**.

ReproSeqSnpAnalysis plugin

After a sequencing run is complete, you can manually run the ReproSeqSnpAnalysis plugin in Torrent Suite™ Software to perform an analysis, regardless of whether the plugin was run automatically after a run. You may need do this to run an updated version of the plugin or to configure the plugin for use with Sibling QC.

For general information about manually running the ReproSeqSnpAnalysis plugin, see "Run the plugin manually from the sequencing run report" on page 125.

For detailed instructions about running the ReproSeqSnpAnalysis plugin for Sibling QC, see "Run the plugin manually from the sequencing run report for Sibling QC data" on page 126.

Run the plugin manually from the sequencing run report

This procedure provides general instructions for manually running the ReproSeqSnpAnalysis plugin.

For detailed instructions about running the ReproSeqSnpAnalysis plugin for Sibling QC, see "Run the plugin manually from the sequencing run report for Sibling QC data" on page 126.

1. Sign in to Torrent Suite™ Software.
2. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for the completed sequencing run.
3. Click **Plugins ▸ Select Plugins to Run**, then click ReproSeqSnpAnalysis plugin.
4. In the **Configure Plugin** screen, the following plugin options are displayed. If needed, change the options, then click **Submit** to start the analysis.
   - **Reference Genome**: GRCh38.p2.mask1. (Prepopulated from the run and cannot be changed.)
   - **variantCaller run**: Prepopulated with the last run of the variantCaller plugin. *(Optional)* If the prepopulated run is not the correct run, select a different run from the dropdown list.
   - **Target Regions**: Ion_AmpliSeq_Polyploidy_Panel_w1.0.0 (Prepopulated from Run Plan.)
   - **Target Hotspots**: Ion_AmpliSeq_Polyploidy_Hotspots_w1.0.0 (Prepopulated from Run Plan.)
   - **Barcode to Couple ID**: Used when Sibling QC is required from a run. See "Run the plugin manually from the sequencing run report for Sibling QC data" on page 126.

   If the plugin does not require configuration, analysis starts immediately without a confirmation screen.

Run the plugin manually from the sequencing run report for Sibling QC data

When Sibling QC information is needed from a run, a Couple ID file must be uploaded for the sample. This can only be done manually when the run is complete.

The Couple ID CSV file is a comma-separated table that specifies the barcodes to use with the corresponding family name (Couple ID) for the sample.

Before manually running the ReproSeqSnpAnalysis plugin, ensure that a Couple ID file for the run with the following information has been created. Use a text editor to create the CSV file that contains a header with "***Barcode,Couple ID***", followed by rows that include barcodes and the couple ID with which they are associated as shown in the following example.

	A	B
1	Barcode	Couple ID
2	IonCode_0201	V104
3	IonCode_0202	V104
4	IonCode_0208	U1463
5	IonCode_0216	U1463
6	IonCode_0217	U13291
7	IonCode_0218	U13291
8	IonCode_0219	U13291

Note: If viewed using a spreadsheet program, the comma-separated fields of the file are displayed in separate cells as shown in the preceding figure.

1. Sign in to Torrent Suite™ Software.
2. In the **Data** tab, click **Completed Runs & Reports**, then click the report name link for the completed sequencing run.
3. Click **Plugins ▸ Select Plugins to Run**, then click ReproSeqSnpAnalysis plugin.
4. In the **Configure Plugin** screen, the following plugin options are displayed. Change the options if needed, then click **Submit** to start the analysis.
   - **Reference Genome**: GRCh38.p2.mask1 (Prepopulated from the run and cannot be changed.)
   - **variantCaller run**: Prepopulated with the last run of the variantCaller plugin. *(Optional)* If the prepopulated run is not the correct run, select a different run from the dropdown list.
   - **Target Regions**: Ion_AmpliSeq_Polyploidy_Panel_w1.0.0 (Prepopulated from Run Plan.)
   - **Target Hotspots**: Ion_AmpliSeq_Polyploidy_Hotspots_w1.0.0 (Prepopulated from Run Plan.)
   - **Barcode to Couple ID**: To upload the Couple ID file, click **Choose File**, navigate to the location of the Couple ID file, then click **Open**.

Review ReproSeqSnpAnalysis plugin results

Results from the ReproSeqSnpAnalysis plugin are included in the sequencing run report for the Ion AmpliSeq™ Polyploidy Panel in Torrent Suite™ Software, if the plugin was configured to run automatically in the Planned Run.

On the run report screen, in the **ReproSeqSnpAnalysis** section you can:

- Review the summary of the **ReproSeqSnpAnalysis** plugin run for each barcode used.
- Review the **Sibling QC**, **Status**, **Polyploidy**, **Contamination** (maternal contamination), **Ploidy Confidence**, **Contamination Level** (maternal contamination), and **MHAP 3rd Alleles**.
- Download data files for all barcodes and each individual barcode or sample.

You can also access the detailed **ReproSeqSnpAnalysis** plugin summary report for each barcode or sample from the **ReproSeqSnpAnalysis** section on the run report screen. In the detailed **ReproSeqSnpAnalysis** plugin summary report screen, you can:

- View Polyploidy analysis for each barcode.
- Review **Sibling Similarity Dendrogram** and **Sibling Similarity Heatmap**.
- Download **Barcode Summary Report**, **Sibling QC Cluster Summary**, **Sibling QC Pairwise Similarity Matrix**, and **Sibling QC k-Cluster Analysis Matrix**.

This procedure applies only to sequencing runs that use Ion AmpliSeq™ Polyploidy Panel Kits.

1. Sign into Torrent Suite™ Software.
2. In the **Data** tab, click **Completed Runs & Reports**.
3. In the list of runs, locate the run of interest, then click the link in the **Report Name** column.
4. In the left navigation menu, click ReproSeqSnpAnalysis plugin to view the plugin results.
   - Review the following data for each sample in the run.

Information for barcodes in ReproSeqSnpAnalysis plugin results

Column	Description
Barcode name	The barcode used for the sample.
Sample	A unique identifier for a specific sample.
Couple ID	An optional attribute to identify a group of biologically related samples. Sibling QC results depend upon on identification of the sibling groups through the Couple ID attribute. For example, this attribute can indicate a group of sibling samples or a repeat sample from the same source. If the Couple ID attribute is not set, the sample name is used as the default value. Any unrelated samples used as controls should include a distinct value, or no value, for Couple ID. The value **Excluded** can be assigned to indicate that this sample should be ignored for Sample QC analysis. In this case the Sample QC result will also show **Excluded**. **Note:** Couple IDs are added when the run is planned.

Information for barcodes in ReproSeqSnpAnalysis plugin results *(continued)*

Column	Description
SiblingQC	Conclusion about the sibling QC based on alleotypical clustering that is compared to the expected family grouping that is based on the Couple ID attribute. This result is not available until all samples have been individually analyzed. A single sample that is incorrectly annotated for Couple ID, or is otherwise not the expected sample, such as specifying a Couple ID on an unrelated or distant family member sample, might have a large distribution on the Sibling QC analysis. Values are **Pass**, **Merge**, **Split**, and **Fail**. Samples with a value of **Pass** are those samles that are included in a single similarity cluster with all other expected family members. Samples with a value of **Merge** are also are included in a single similarity cluster with all other expected family members, but also contain samples from other families. Samples with a value of **Split** are members of the majority family within their cluster but one or more expected family members are within other clusters. Samples with a value of **Fail** are not in the majority family of those in which the sample is clustered.
Status	Sample normality status conclusion. Values are **Normal Abnormal**, and **NOCALL**.
Polyploidy	A polyploidy call based on consolidated analysis methods. Values can be: **Diploid**, **Triploid**, **Haploid**, or **NOCALL**.
Contamination	Contamination level summary call. Values are **High**, **Low**, **None,** and **NOCALL**.
Ploidy Call Confidence	Qualative ploidy call confidence based on the difference in **Assignment probability** scores, agreement with curve fitting and normaility assessment, mean allele reads, and the number of heterozygous SNP alleles frequencies that are called. Values are **High**, **Low** and **Very Low**.
Contamination Level	Predicted contamination based on 2x median MHAP third allele frequencies.
MHAP 3rd Alelles	Microhapolotype targets with a third allele detected, after coverage and frequency thresholds are applied.

- Click a barcode to review a **Single Sample Report**

Metrics of sample run quality in ReproSeqSnpAnalysis plugin results

Column	Description
Sample Run QC Metrics	
Sample Status	Overall call status for the sample that is based on run quality metrics, anueploidy and/or contamination analysis. The software uses a default value of 100 minimum number of reads. A low value, for example, below 50%, is a general indicator of poor run quality and might reflect the final contamination call. Values are **Normal**, **Abnormal**, **Contaminated** and **NOCALL**. A **NOCALL** means there is insufficient data to make a confident call, which indicates that the sample might need to be retested.

Metrics of sample run quality in ReproSeqSnpAnalysis plugin results *(continued)*

Column	Description
Amplicons with sufficient reads	Percentage of all panel amplicons that had at least the minimum number reads. The software uses a default value of 100 minimum number of reads. A low value, for example, below 50%, is a general indicator of poor run quality and might reflect the final cohtamination call.
Alleles mean read depth	Mean read depth over all targeted SNP and Microhaplotype alleles. A low value indicates a low quality run.
Maternal Contamination Report	
Contamination status	Overall contamination assessment that is based on detection of additional alleles. Values can be **High**, **Low**, **None** or **NOCALL**. A HIgh call is based on having both a **Predicted contamination** of at least 30% with at least 3 **Third alleles detected.** A **None** call means there is no significant evidence for contamination, whereas a **Low** call means some contamination is detected but not enough to meet both thresholds for a **High** call. A **NOCALL** means there was insufficient microhaplotype target coverage for a confident call.
Predicted contamination	**Predicted contamination** that is based on 2x median allele frequency. Analysis assumes that the mother is the most likely source for DNA contamination. Other sources are likely to produce more alternative alleles and a higher contamination prediction.
Third alleles detected	Number of **Third alleles detected**, after read and frequency filters are applied. When default settings are used, a third allele is detected if at least 5% of the reads are for the alternative allele.
Amplicons with sufficient reads	Percentage of all microhaplotype (MHAP) amplicons with at least 50 reads. Only amplicons that meet the threshold value of a minimum number of 50 reads are used for contamination prediction. If fewer than 50% of the panel amplicons meet this threshold, the result will be a **NOCALL**.
Polyploidy Report	
Polyploidy Status	Summary polyploidy call. Values are **Diploid** (normal), **Triploid** or **Haploid**. Higher levels of polyploidy, for example tetraploidy, are not specifically assessed but are likley to result in **Triploid** calls. The call is primarily based on the **Assignment probability** call but might be a **NOCALL** if the **Call confidence** is very low, based on margins, reads and consensus in complementary analysis. Triploid samples are very likely to also be assessed as maternally contaminated.
Call confidence	Qualative ploidy call confidence based on the difference in **Assignment probability** scores, agreement with curve fitting and normaility assessment, mean allele reads, and the number of heterozygous SNP alleles frequencies that are called. Values are **High**, **Low** and **Very Low**.
Hom allele calls	Total number homozygous SNP hotspots called by the variantCaller software module and haplotype calls from contamination analysis, filtered by AF range and quality.

Metrics of sample run quality in ReproSeqSnpAnalysis plugin results *(continued)*

Column	Description
Het allele calls used	Total number heterozygous SNP hotspots called by the variantCaller software module, filtered by AF range and quality.
Hets AF mean	The mean average of the variant heterozygous SNP allele frequencies. This value is expected to be close to 50% unless there are very few reads or the sample is **Haploid**.
Sharpiro test	Call based on the Shapiro-Wilk Test p.value. The null hypothesis is that the data is distributed normally so a value of 0.05 or greater is used to indicate the distribution is probably normal.
Hets AF StdDev	The standard deviation in heterozygous SNP allele frequencies. Indicator of both quality of the run and/or normality of the distribution.
Normal vs. GMM	Call made based on fitting the MAF frequency histogram to a normal curve or 2 gaussian curves to the data at expected 1/3 and 2/3 mean variant allele frequencies.
Assignment probability	Triploidy call based on the mean probablisic assignment of individual allele frequencies to diploid or triploid samples.
Heterozygous Allele Frequency Distribution and Normality Assessment Metrics	
IonCode_0101.NormalDist Fit p-value: 0.000000 Fit LogLik: -1361.2 AsgnProb: 1.1991	Allele frequency histogram for filtered heterozygous calls with a normal fit curve that is superimposed.
IonCode_0101.GMM Delta LogLik: 19.4 Fit LogLik: -1341.8 AsgnProb: 1.2464	Allele frequency histogram for filtered heterozygous calls with a double-gaussian fit curve that is superimposed. The software uses expected variant frequencies at 33.3% and 66.7% to generate this graphic.
Download RepreSeq Analysis Report summary statistics	Click to download a text summary of the combined **Alleleic Imbalance Report** statistics presented above.

Metrics of sample run quality in ReproSeqSnpAnalysis plugin results *(continued)*

Column	Description
Download a summary table of all allele calls by chromosome	Click to download a tab-sepatated text table of all variants call summary for by chromosome, which includes the additonal Microhyplotype allele calls. The UPD90 is a call for evidence of UPD per chromosome and is "Yes" if there are at least 90% homozygous allele calls (50% is expectation) and at least 10 allele calls. **Note:** Calls for UPD are not supported by software.
Download a table of detetected alleles from contamination analysis	Click to download a tab-sepatated text table of alleles and their counts for each amplicon.

- Download and review the following reports, graphs and matrixes.

Item	Description
Barcode Summary Report	A table of the barcode summary that is shown in the ReproSeqSnpAnalysis plugin results.
Sibling Similarity Dendrogram	Graphic that shows alleleotypic similarity of hierarchial clustering for sibling QC results.
Sibling Similarity Heatmap	Heatmap that shows alleleotypic similarity clustering.
SiblingQC Cluster Summary	Table that compares alleleotypic similarity analysis to expected family groupings.
SiblingQC Pairwise Similarity Matrix	Table of alleleotypic similarity values between each pair of samples.
SiblingQC k-Cluster Analysis Matrix	Table that shows sibling alleltype cluster analysis at all target numbers of families. The expected number is that defined by the number of individual sample Couple IDs that are assigned. This output shows samples that are grouped into subgroups by similarity, as shown by the dendrogram heirarchy. It is intended for use with troubleshooting results in which Sibling QC indicates that a sample does not have a **Pass** status, or for samples in which expected family relationships are uncertain. A divergence of family groupings between samples at lower cluster (k) sizes will often highlight samples that are inconsistent.

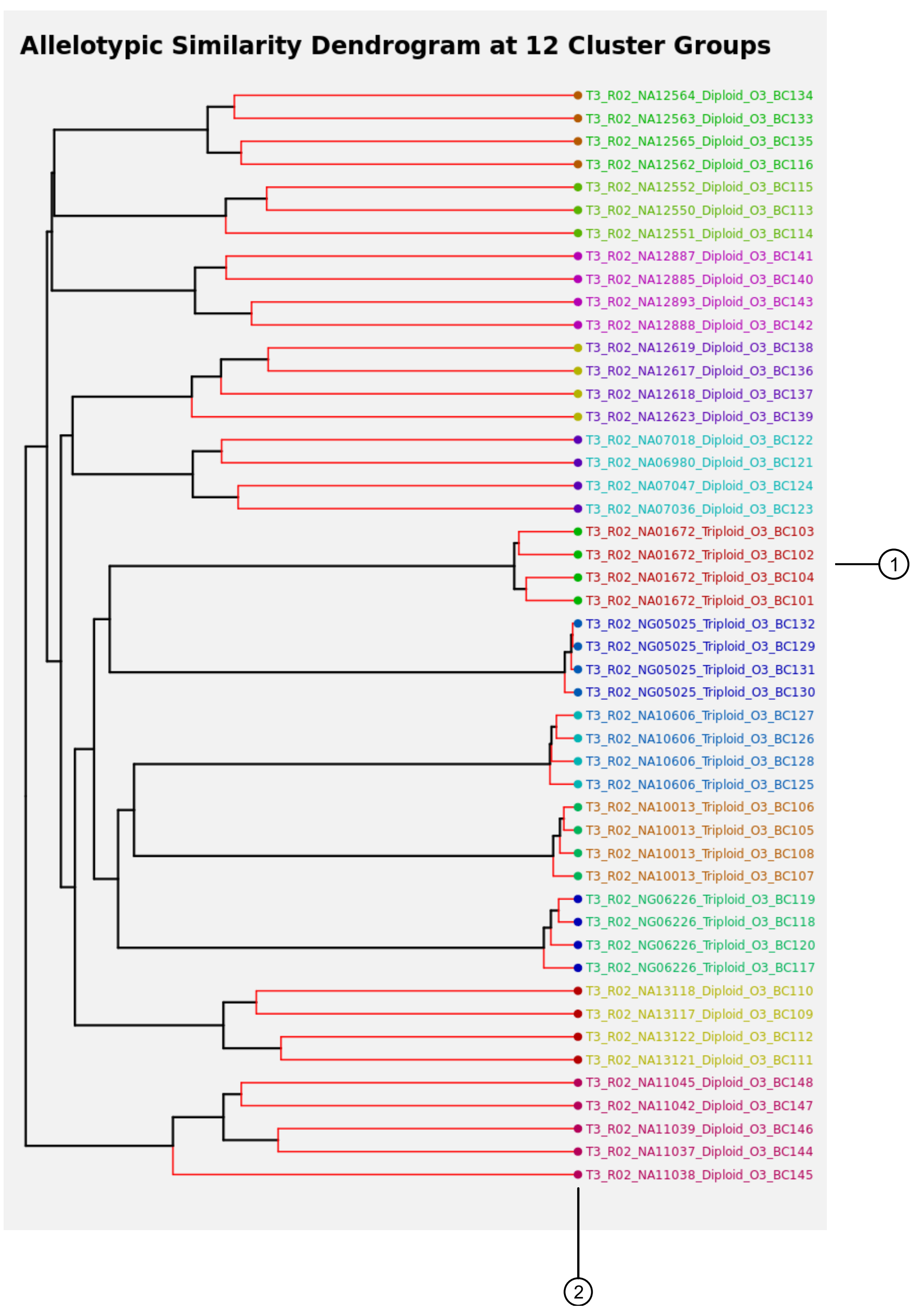
Dendogram view

You can download a dendogram diagram that shows the alleotypic similarity of hierarchial clustering for sibling QC results.

In the run plan for this examle, 12 Couple ID values were assigned as sample attributes. The Couple IDs define either sibling sets or technical replicates.

The relatively short length of the lines for a clustered set of samples demonstrates the high similarity of sample replicates.

Example dendogram



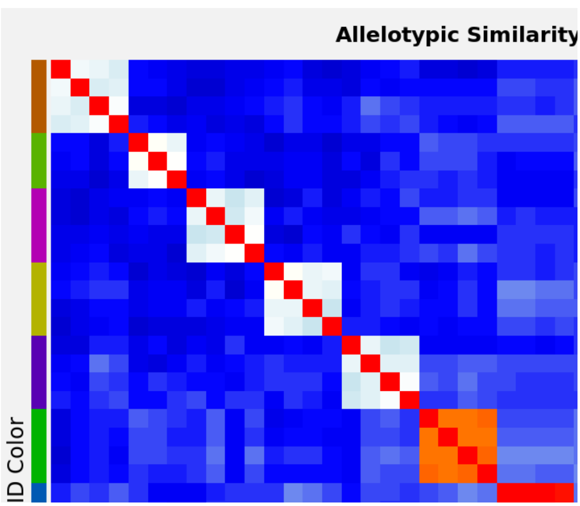
① In this example, the dendogram diagram shows 12 clusters, and each cluster is identified by a unique font color.

② The colored dots at the start of the sample name is associated with the expected family groupings that are based on the Couple ID.

Heatmap example

Degrees of similarity between all possible sample pairs are shown by a heatmap.

Example heatmap



The colored bar on the left side of the heatmap signifies the Couple ID group for the sample that is listed on the right side. Similarities are represented by the row of colors that correspond to each other sample, and each is identified by the index of the corresponding sample that is listed on the right side.

Troubleshooting

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- Set up and operation of Ion S5™/Ion GeneStudio™ S5 Series System 138
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Ion SingleSeq™ library preparation

Observation	Possible cause	Recommended action
Library yield is low	Genomic DNA was amplified inefficiently.	Use the Human CEPH Genomic DNA Control at 15–60 pg input as a positive control in pre-amplification and amplification reactions to determine whether DNA is amplifiable in your system.
		Use real-time PCR to monitor amplification.
		Check the quality and quantity of positive control and sample libraries after amplification by running 10 µL aliquots on 2% agarose gels.
	Amount of starting material was insufficient due to loss or mishandling of cells.	Increase the number of cells in your sample.
		Keep the cells on ice.
		Do not insert pipette tip into sample – cell(s) may adhere to the tip after addition of reagent.
	Agencourt™ AMPure™ XP Reagent was over-dried during cleanup.	Do not dry the Agencourt™ AMPure™ XP Reagent more than 4 minutes.
	Percentage of ethanol in wash of Agencourt™ AMPure™ XP Reagent was less than 70%.	Prepare 70% ethanol from newly-opened ethanol reagent bottle.
		If preparing from an ethanol reagent bottle that has been frequently used and has possibly absorbed water from air, increase the percentage of ethanol in the wash from 70% to 75%.
Barcode balance in a pool is poor.	Variation in gDNA input in a pool of samples was too high.	Avoid pooling single cell samples with multiple cell samples; that is, pool single-cell samples together and pool multiple-cell samples in a second pool if possible.

Setup and operation of the Ion Chef™ System

Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.
The instrument door cannot be opened	An obstruction was present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error occurred.	Contact Technical Support to report the problem and for further assistance.
The instrument stops during a run	The instrument encountered an internal error.	1. Record the error displayed on the instrument display, then tap **OK**. 2. Contact Technical Support to report the problem and for further assistance.
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.
Liquid residue is present in the Recovery centrifuge following a run	During normal instrument operation, a noticeable coating of liquid can collect on the bowl and buckets of the Recovery centrifuge following repeated runs.	Remove the residue as instructed in “Clean the Ion Chef™ Instrument” on page 97.

<table>
<tr><th>Observation</th><th>Possible cause</th><th>Recommended action</th></tr>
<tr><td rowspan="2">Instrument will not begin a run</td><td>The instrument has encountered a Deck Scan error (one or more consumables are absent or loaded improperly).</td><td>1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the error(s) displayed, replace the missing consumable as directed, tap No when prompted then tap Next to cancel the run. After returning to the home screen, restart the run.
2. If the error persists, confirm that:
• All buckets are seated correctly in the rotors of the Recovery and Chip-Loading Centrifuges.
• All cartridges are loaded correctly and are level on the instrument deck.
• The barcodes of the Ion Chef™ Library Sample Tubes are visible and positioned correctly.
• All tubes are both present and uncapped on the Ion S5™ ExT Chef PGS Reagents (sample tubes, NaOH tube, and the empty tube).
3. If the error persists after you check the consumables on the instrument deck, do one of the following:
• If you are confident that the Ion Chef™ Instrument is set up correctly and you are comfortable disregarding the warnings, tap YES following Deck Scan to proceed with the run.
• If the instrument cannot begin the run, contact Technical Support for further assistance.</td></tr>
<tr><td>The instrument has encountered an internal error.</td><td>1. Record the error displayed on the instrument display, then tap OK.
2. Contact Technical Support to report the problem and for further assistance.</td></tr>
<tr><td>Instrument displays one or more alerts during a run</td><td>The instrument detected one or more problems during the run.</td><td>After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot.

IMPORTANT! The detected problem might impact the performance of the sequencing run.</td></tr>
</table>

Observation	Possible cause	Recommended action
Instrument displays one or more alerts during a run *(continued)*	• Network connection to the server was interrupted. • User name or password was incorrect.	1. Tap the Instrument status button to view the alert(s). 2. In the Instrument status screen, confirm that the name of the Torrent Server connection is red. 3. Contact your network administrator to confirm that: • The Torrent Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection. • The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials. 4. If the alert persists, capture an image of the alert or error message, if possible, to help troubleshoot, then contact Technical Support for further assistance.
Planned Run status does not advance to "Planned" **Details:** The status of a successfully completed run remains listed as "Reserved" in Torrent Suite™ Software.	Connectivity was temporarily lost or interrupted between the Ion Chef™ Instrument and Torrent Suite™ Software.	Manually change the status of the run to "Planned". 1. Sign in to the Torrent Suite™ Software. 2. In the **Plan** tab, click **Planned Runs**. 3. For the Planned Run of interest, click ⚙ ▸ **Completed on Chef**. The status changes to "Planned".

Set up and operation of Ion S5™/Ion GeneStudio™ S5 Series System

Instrument alarms and events

Observation	Possible cause	Recommended action
Red "Alarms" and/or "Events" message in Main Menu	• Available software updates were detected. • Connectivity issues were detected. • Instrument did not detect required files or hardware.	Tap the red **Alarms** icon to see detailed messages. • If a message states "Newer Software Available": **IMPORTANT!** After updates are installed, the instrument must be restarted. a. In the main menu, tap **Settings ▸ Check for Updates**. b. Select the **Released Updates** checkbox, then tap **Update**. c. When installation is complete, follow the onscreen prompts to restart the instrument. **Note:** In some cases, the instrument restarts automatically after software installation. • If a message states "No Connectivity to Torrent Server", "No Connectivity to ftp server", or "Network Manager not connected", disconnect and re-connect the ethernet cable, confirm that the router is operational, and verify that the network is up and running. • For any other messages: a. Power off the instrument: On the home screen, tap **Settings ▸ System Tools ▸ Shut Down ▸ Shut Down**. b. Wait 30 seconds, then press the button on the side of the instrument to power on the instrument. • If the red "Alarms" and/or "Events" message still appears in the main menu, contact Technical Support.

Initialization—General errors

Observation	Possible cause	Recommended action
Bottle leak check fails	• Bottle seal was not tight. • Bottle was damaged or defective.	1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.

Ion Reporter™ Software results

Observation	Possible cause	Recommended action
High MAPD value (>0.3) is observed	Low library read representation (<50,000 reads per sample) occurred.	Sequence poorly represented samples to a higher depth by increasing their proportional concentration in the sample library pool.
		Reduce the number of samples run per chip.
	Library read representation was normal (>100,000 reads per sample), but library quality was poor.	Repeat preparation of sample libraries.
Chromosome X and Y data are missing in IRGV plots, preventing gender call These samples also tend to show high MAPD values. See "View X and Y chromosome data in low-read samples" on page 111 for further information.	The sample exhibited very low total read counts, resulting in no display of X and Y chromosome data.	Sequence poorly represented samples to a higher depth.
		Reduce the number of samples run per chip.
		Navigate to **Parameters** ▸ **CNV Finding** ▸ **Advanced** ▸ **Gender section** and lower the value of the "CNV Gender Min Autosomes Count" parameter.
		Navigate to **Parameters** ▸ **CNV Finding** ▸ **Advanced** ▸ **Analysis** and set the "Plot Y chromosome for Female or Unknown Gender" parameter to **True**.

Supplemental procedures

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Install or upgrade plugins

On Connect, an administrator can install or upgrade the following.

- The ReproSeqSnpAnalysis plugin plugin for use with the Ion AmpliSeq™ Polyploidy Panel. This in Torrent Suite™ Software plugin is supported by Thermo Fisher Scientific but is not preinstalled in the Torrent Suite™ Software.

  You can download the Ion-supported ReproSeqSnpAnalysis plugin in Torrent Suite™ Software if you sign into an account on Connect, and click **View all apps**, then click the **Plugins** resource library.

1. Sign in to the **Thermo Fisher Connect**.
2. Click the **Apps** icon ( ).
3. In **AppConnect**, under **Resource Libraries**, click **Plugins**.
4. *(Optional)* Click a category at the top of screen.
   The list of plugins is narrowed to only the plugins included that are in the selected category.
5. Click to download the ReproSeqSnpAnalysis plugin plugin. Select the checkbox to indicate that you agree to the terms and conditions, then click **Download Plugin**.
   Either a compressed directory or a debian file that contains the plugin is downloaded to your local machine.
6. In Torrent Suite™ Software, click **(Settings)** ▸ **Plugins** ▸ **Install or Upgrade Plugin**.

7. Click **Select File**, browse to the location where you downloaded the plugin file, select the file, then click **Open**.

8. In the **Install or Upgrade Plugin** dialog box, click **Upload and Install**.

The plugin is now visible in Torrent Suite™ Software.

Create a new run template to change chip types for a planned run

The default run template provided with the system is locked and changes to the chip type cannot be made in the software.

Use this procedure to create a new template and change the chip type to be used in a Planned Run to a Ion 510™ Chip or Ion 520™ Chip for use with Ion ReproSeq™ PGS Kits.

Note: The new template is unlocked and future changes to the chip type can be made in the software.

---

1. Sign in to Torrent Suite™ Software.

2. In the **Plan** tab, in the **Templates** screen, click **Reproductive** in the left navigation menu.

3. In the list of templates, find **Ion ReproSeq Aneuploidy – Ion S5 System**, then click ⚙ **(Actions)** ▸ **Export**.

4. Save the CSV file to your computer.

5. Using a text editor, open the CSV file and make the following changes, then save the updated file.
   - On line 2, change the name of the template by editing the text that follows “Template name (required),exported Ion ReproSeq Aneuploidy - Ion S5 System”.
   - On line 8, edit the text “Chip type (required),530”, to change the chip number to 510, or 520.

6. From the Torrent Suite™ Software, **Plan Templates** page, click **Upload**, then click **Upload Templates**.

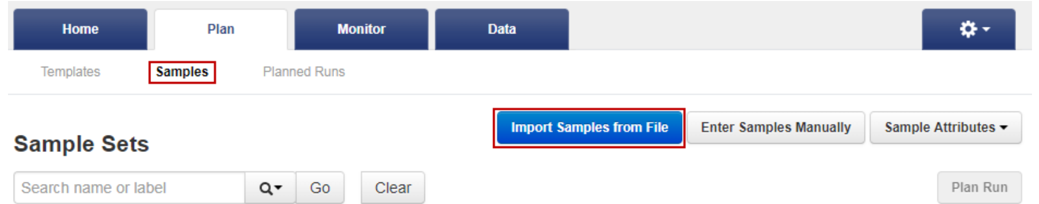
7. In the **Upload Templates** dialog, click **Select file**, navigate to select the updated template CSV file, then click **Load**.

The new template is now available in Torrent Suite™ Software, in the **Templates** screen under the **Reproductive** application.

Create a Sample Set by importing samples from a CSV file

If you have many samples, you can import new samples into Torrent Suite™ Software, or update existing samples, using a CSV file that contains sample information. If you do not yet have a samples file, you can create one from an available CSV template that is available in Torrent Suite™ Software. During this process, you can also create a new Sample Set for the new samples.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.



If you do not yet have a samples file, create a new samples file from an available samples CSV file. For more information, see "Create a samples file from a template" on page 142.

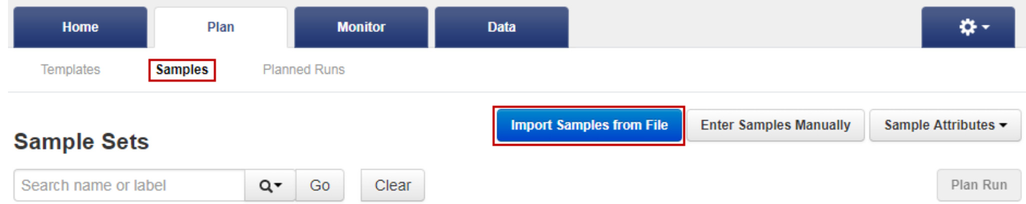
2. Upload the samples file, and optionally add a new Sample Set to receive the samples.
   a. In the **Import Samples** section, click **Select file**, then navigate to sample import file, then upload the sample import file.
   b. Select a Sample Set CSV file, then click **Open**.
   c. Select a Sample Set to receive the samples.
   (Optional) To add a new Sample Set to receive the samples, click **Add Sample Set**, then complete the Sample Set information.
   For information about how to define a Sample Set, see "Sample attributes" on page 143.

3. Click **Save & Finish**.
   The system loads, parses, and validates the file, then, if no errors are found, saves the samples and Sample Sets.

Create a samples file from a template

If you do not already have a samples file on your computer to use to import samples from a CSV file, then you can download a samples template and use it to create a samples file.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.



2. In step 1 of the **Import Samples** section, click **Sample File Format** to download a sample CSV template.

   The sample file format CSV contains the version of the CSV file in the top row, and sample attributes in separate columns.

3. To create a new CSV file, copy and paste the contents of your existing sample CSV file into the new file format.

4. Open the CSV template and enter sample information into the cells.

   For information on how to define the samples, see "Sample attributes" on page 143.

5. Save the file to your computer so that is available for use to create a Sample Set.

Sample attributes

When you create a Sample Set, you can enter sample attributes into Torrent Suite™ Software manually or import samples with a CSV file. Most of the sample information is optional except for **Sample Name**. However, some information is required if you transfer data to Ion Reporter™ Software, and those attributes required by Ion Reporter™ Software are indicated in the following table.

Attribute	Description
Sample Name (Required)	The unique name of the sample. Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_). This attribute is used by Ion Reporter™ Software. A 255-character limit must be followed for Ion Reporter™ Software name validation. If you are using Ion Reporter™ Software, and the actual sample name already exists in that software, a string such as _v1 or _v2, and so on, is added to the sample name.
Sample External ID	*(Optional)* If you manage samples in an external system, for example, a Laboratory Information Management System (LIMS), enter the identifier from that system.
PCR Plate Position	A unique identifier for the 96-well plate used for library preparation and templating. • Select a plate position of **A** to **H**.
Barcode Kit	The name of the barcode kit used to make a library from the sample. The same barcode kit must be used for all samples in a Sample Set.
Barcode	The name of the specific barcode in the selected barcode kit. Assign a unique barcode to each sample in a Sample Set.

(continued)

Attribute	Description
Sample ID	A unique identification code (SampleID) for each barcode in a sample. This helps to track samples or possibly identify misassignment between samples and barcodes in a sequencing run. The SampleID is passed to Ion Reporter™ Software. If you manage samples in an external system (for example, a LIMS), you can use the identifier from that system. This attribute cannot be changed. **Note:** This attribute is optional in Torrent Suite™ Software. However, this value can be required for planned runs that include automatic upload of data to Ion Reporter™ Software.
Control Type	The control type used when preparing the sample. If a value is selected, the sample is identified as a control sample. • **MSI On-Chip Control** • **No Template Control**
Basic Annotations	
Description	Typically, one or two sentences that describe the sample.
Nucleotide Type	The nucleic acid type or RNA variant type that is associated with the sample: • **Unspecified** • **DNA** • **RNA** • **TNA** • **Fusions**
Sample Source	The source from which the sample is extracted. • **Blood** • **FFPE** • **Other**
Panel Pool Type	The type of pool that is used by the assay. • **Dual Pool** • **Single Pool**

(continued)

Attribute	Description
Gender	The biological gender of the sample. This attribute is used by Ion Reporter™ Software. • **Female** • **Male** • **Unknown** **IMPORTANT!** If you are using Ion Reporter™ Software, do not leave this blank. Select **Unknown** if the gender is not known. Several analysis workflows in Ion Reporter™ Software, for example, copy number variation detection and genetic disease research, are limited when the gender is not known. The analysis workflows can return unexpected results when the gender is incorrectly specified for a sample.
Type	The relationship type for this sample. Type is used in conjunction with **Relationship Group**, described below. For example, a relationship group can contain two samples, one with a type **Tumor** and another with a type **Normal**. The following sample relationships are supported by Ion Reporter™ Software: • **Control** • **Father** • **Mother** • **Normal** • **Sample** • **Self**—Use **Self** for both a single sample and for the proband sample in a trio. A single sample is not related to other samples and is analyzed by itself. • **Tumor**
Relationship Group	Use **Relationship Group** to designate a group of multiple related samples within the same Sample Set. For example, DNA and RNA samples from the same sample would have the same **Relationship Group** number. Use a whole number to define a sample as part of a relationship group. It is used in conjunction with **Type**. For example, a Sample Set can contain 6 samples, consisting of 3 groups of 2 related samples each (of types **Tumor** and **Normal**). In this case, you would designate the two samples in each group as part of group 1, 2, or 3. This is identical to the Set ID in the IonReporterUploader plugin.
Extra Annotations (used for specialized applications, such as preimplantation genetic screening (PGS) research or oncology research)	
Sample Collection Date	The date that the blood sample was drawn.
Sample Receipt Date	The date that the laboratory received the blood sample.
Cancer Type	The type of cancer that is present in the sample.

(continued)

Attribute	Description
Population	The super population code assignment for a sample, as defined by the human 1000 genomes project (**www.internationalgenome.org/faq/which-populations-are-part-your-study/**). The population is relevant to the analysis of samples by the TCRB-LR assay workflow in Ion Reporter™ Software. The TCRB-LR analysis workflow produces a haplotype group assignment for samples having a population attribute of "European".
Mouse Strains	The name of a mouse strain. Choose from a select number of the most common strains. In Ion Reporter™ Software 5.12, the selected mouse strain will not affect Ion Reporter™ Software analysis workflows.
Cellularity %	The percentage of tumor cells in the sample. This is a whole number between 1 and 100.
Biopsy Days	The post-fertilization time at which the biopsy was taken from an embryo. This is a whole number.
Cell Number	The cell count of the biopsied material.
Couple ID	An identifier for the couple. For use with the Reproductive research application.
Embryo ID	An identifier for the embryo. For use with the Reproductive research application.
User-defined Attributes	
<user defined>	If you create additional sample attributes, each attribute is listed here and in the CSV file. Attributes that are marked as mandatory must be entered for each sample. If you create an attribute of type integer, only numeric characters (whole numbers) can be entered for that attribute.

Set IRGV preferences

You can modify the elements that are included in Ion Reporter™ Genomic Viewer (IRGV) analysis visualizations with preferences. To set the preferences, you must first visualize an analysis in the viewer.

1. Under the **Analyses** tab, click ***Overview***.
2. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.

3. In the **IRGV & Generate Report** tab, scroll to the bottom of the visualization, then click **IRGV/Export & Preferences ▸ Show IRGV preferences** to expand the **Preferences** section.

   You can set the following IRGV preferences.

Option	Description
Default mapd value	This value will be used as default value in the mapd filter.
Default sort order [1]	The preferred sort order for Aneuploidy analyses (NR, CID, EID or BIOPSY) for the summary table and tracks. NR sorts by the analysis nr, CID sorts by couple ID, EID sorts by embryo ID and BIOPSY sorts by biopsy days.
Analysis limit for BAM tracks	Maximum number of analysis that will include BAM tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Analysis limit for BED tracks	Maximum number of analysis that will include BED tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Limit for coverage data	By default, the maximum limit of 25000 is used. Reduce this number if you do not want to see the coverage data, or you have problems with the browser, such as freezing.
Max number tracks in karyo	By default, 5 tracks are shown. You can set a value of 1 to 10. When you increase the number of tracks, more space is used in the Karyo view.
Max Ploidy in Whole Genome View	Maximum value of the Y-axis (ploidy) in the Whole Genome View graphs.
Display Fixed Ploidy Lines	You can choose to display fixed ploidy lines at copy number=1 and copy number=3 across the Whole Genome View. • **true**—Select this default setting to display fixed ploidy lines at CN=1 and CN=3 across the Whole Genome View. • **false**—Select this setting to display ploidy lines that are not fixed.
Whole Genome View Height	You can double the height of the Y-axis when you view whole genome data in IRGV. The **Enlarged** view is useful to review mosaic data, because more data is shown on a single plot and more space is available between data points, so that the mosaic data more pronounced. • **Compressed**—Select this default setting for IRGV images that are 300 pixels high. • **Expanded**—Select this setting for IRGV images that are 600 pixels high.

[1] This preference can be set only when multiple analyses are selected.

4. For each value that you want to change in the **Preferences** section, click **Edit** next to the value that you want to change, then enter a new value into the **Edit Preferences** dialog box, then click **OK**.

5. When your edits are complete, click **Save Preferences**.

   The preferences are reset to the default settings if you click **Reset Preferences**.

The selected preferences are applied to all analyses that you visualize immediately after you save your changes.

Set IRGV or IGV as the default viewer

You can set a preference for your account that determines whether Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV) opens by default when you visualize analyses.

IGV is the genomic viewer that is made by the Broad Institute. IRGV is the faster-loading genomics viewer that is built into Ion Reporter™ Software.

1. Click ⚙ **(Settings)** ▸ **Account Preferences**.
2. In the **Account Preferences** dialog box, in the **IGV launch preference** row, select **IGV** or **IRGV**, then click **Save**.
   A visualization opens when you perform the following actions.

To view . . .	Do this . . .
A visualization of one or more analyses in IRGV.	1. In the **Analyses** tab, in the **Overview** screen, enable the checkbox in for the analysis you want to visualize, then click **Visualize** in the upper right corner of the screen. The visualization opens in the **Analysis Visualization** screen in **IRGV**.
A visualization of a gene of interest in a separate browser tab.	1. In the **Analysis Visualization** screen, click **Variants Table**, then click on the locus of interest in the **Locus** column. • If the **Account Preference** is set to IRGV, IRGV is launched in a separate browser window. • If the **Account Preference** is set to IGV, a JNLP file is downloaded when you click the locus in the **Variants Table**. Double-click the file to launch IGV as a standalone application on your computer.

Adjust IRGV BAM tracks

You can adjust the BAM read coverage track in an Ion Reporter™ Genomic Viewer (IRGV) visualization to make it easier to view the data. When you adjust the BAM read coverage track, it changes the amount of data that is shown in the plot. For example, you can see more BAM tracks when you use the **Squish** setting.

For best results, use the **Expanded** option to click and view hover help with details about variants and base calls that are visualized in each read coverage track.

Note: A maximum of 500 aligned reads are shown in each BAM track in Ion Reporter™ Genomic Viewer (IRGV). To see more than 500 aligned reads, use Integrative Genomics Viewer (IGV).

In an open **Analysis Visualization**, in the **IRGV & Generate Report** tab, click **Actions** next to the read coverage track, then select an option to adjust the view of the track.

Option	Description
Expanded	Select this option to view the read coverage track with the maximum visible height for each BAM track.
Squish	Select this option to view reads in the coverage track in a single line that has minimal height for each BAM track.
Collapse	Select this option to view no BAM tracks and view only the coverage density at the top of the BAM track.

Quality control of ISPs

Quality control using the Guava™ easyCyte 5 Benchtop Flow Cytometer

The Guava™ easyCyte 5 Benchtop Flow Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 400 to 600 base-read sequencing on an Ion S5™/Ion S5™ XL Sequencer or Ion GeneStudio™ S5 Series Sequencer.

- **Unenriched sample** – Obtain the QC sample from the Library Sample Tube on the Ion S5™ ExT Chef Reagents cartridge at position A.
- **Enriched sample** – Obtain enriched sample from the tube on the Enrichment Cartridge v2 at position E.

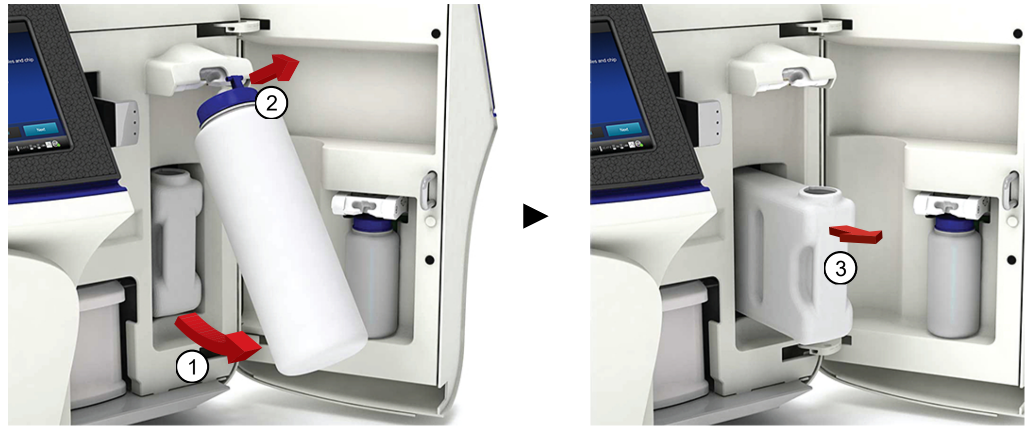
For details, see the *Ion Sphere™ Particles Quality Assessment for the Ion Proton™ and Ion S5™ Systems Using the Guava™ easyCyte 5 Benchtop Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at **thermofisher.com**.

Perform a manual cleaning of the sequencer

A cleaning protocol is normally performed automatically at the completion of each sequencing run. If a cleaning is necessary, perform the following procedure.

1. On the home screen, select **Settings ▸ Clean Instrument**.
   The instrument door unlocks allowing access to the consumables.

2. Remove the Ion S5™ ExT Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.



3. Reinstall the empty waste reservoir and a *used* Ion S5™ ExT Wash Solution bottle.

4. Ensure that the Ion S5™ ExT Sequencing Reagents cartridge and Ion S5™ ExT Wash Solution bottle are properly installed.

   IMPORTANT! Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

5. Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.

6. Close the instrument door, then tap **Next**.
   Cleaning takes ~35 minutes to complete. On completion the instrument door automatically unlocks and the chip and cartridge clamps disengage.

7. Proceed to Chapter 7, "Initialize the sequencer".

Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge

Cleaning is normally automatically performed at completion of a sequencing run. If an Ion S5™/Ion S5™ XL Sequencer or Ion GeneStudio™ S5 Series Sequencer is initialized, an instrument reset run is required to ensure proper cleaning before reinitialization in one of the following situations.

- A sequencing run is not started within 24 hours after initialization.
- A sequencing run is not completed due to a power failure or an abort, and <200 flows occurred before the stoppage.

Do NOT perform a manual cleaning with an unused, initialized Ion S5™ ExT Sequencing Reagents cartridge.

Note:
- If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning is sufficient (see "Perform a manual cleaning of the sequencer" on page 150).
- If the number of flows that occurred before a power failure or abort is unknown, perform an instrument reset run.

To perform an instrument reset run, use the following procedure before reinitialization.

1. In the instrument touchscreen main menu, tap **Run**.
   The instrument door unlocks and the chip clamp disengages.
2. Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.
3. Close the instrument door, then tap **Next**.
4. When prompted, select **Planned Run (none)**. Ensure that the **Enable post-run clean** checkbox is selected, then tap **Review**.
5. In the **Select Run** screen, tap **Edit**, then in the **Detail** screen set the number of flows to **200**. Ensure that the **Post-Run/Clean** checkbox is selected, then tap **Close**.
6. Tap **Start run**, then tap **Accept** to confirm that Post-Run Clean is enabled, and to start the run.

When the instrument reset run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.

Maintain the Ion Chef™ System

For further information on the following Ion Chef™ System maintenance procedures, see the *Ion Chef™ Instrument User Guide* (Pub. No. MAN0018668), available at **thermofisher.com**.

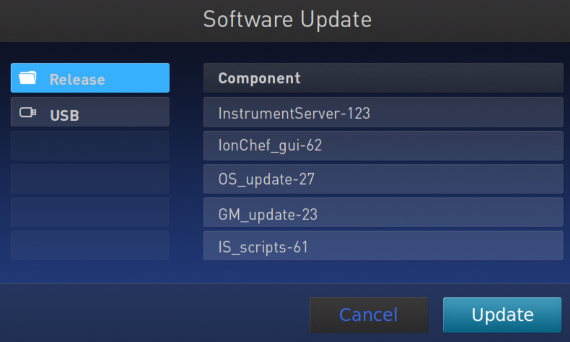
- Install a firmware update
- Change the instrument name
- Replace the ultraviolet lamp
- Perform an XYΘ calibration
- Perform a Z calibration

Note: You must install the Ion Chef™ Instrument firmware update in Torrent Suite™ Software 5.12 or later to access the XYΘ calibration and Z calibration routines.

Install a firmware update

To optimize proper operation of the Ion Chef™ Instrument, we recommend periodically confirming that your instrument is running the most current firmware. We occasionally release updates to the instrument firmware, which can include important changes to the Ion Chef™ System operation. To ensure that your instrument is running the most current firmware, check the firmware version, then update if needed.

1. On the Ion Chef™ Instrument touchscreen, tap **Settings**.
2. On the **Settings** screen, tap **Check for updates**.
3. On the **Software Update** screen, tap **Release** to search the server for updates to the Ion Chef™ Instrument firmware.



Note: Users are also notified of the availability of firmware updates on the Notifications screen.

4. Select the available component update, tap **Update**, then wait for the instrument to complete the update.

Note: If the Ion Chef™ Instrument is operating on an isolated network and cannot connect to our website, you must transfer the firmware update manually. To perform a manual update, transfer the firmware files to a USB drive and insert the drive into the USB port at the rear of the instrument.

Tap **USB** on the Software Update screen, select from the list of available software components, then tap **Update**.

When finished, the Ion Chef™ Instrument displays the update status.

5. Power off and on the Ion Chef™ Instrument to complete the update.

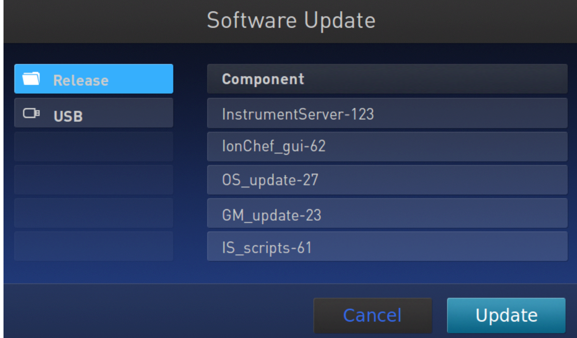
IMPORTANT! If you are updating the firmware manually, remove the USB drive before powering on the Ion Chef™ Instrument.

Check the Ion Chef™ Instrument firmware

1. On the Ion Chef™ Instrument touchscreen, press **Settings**.

2. On the **Settings** screen, press **Check for updates**.

3. On the **Software Update** screen, press **Release** to search the server for updates to the Ion Chef™ Instrument firmware.



Note: Users are also notified of the availability of firmware updates on the Notifications screen.

4. Select the available component update, press **Update**, then wait for the instrument to complete the update.

Note: If the Ion Chef™ Instrument is operating on an isolated network and cannot connect to our website, you must transfer the firmware update manually. To perform a manual update, transfer the firmware files to a USB drive and insert the drive into the USB port at the rear of the instrument. Press **USB** on the Software Update screen, select from the list of available software components, then press **Update**.

When finished, the Ion Chef™ Instrument displays the update status.

5. Power off and on the Ion Chef™ Instrument to complete the update.

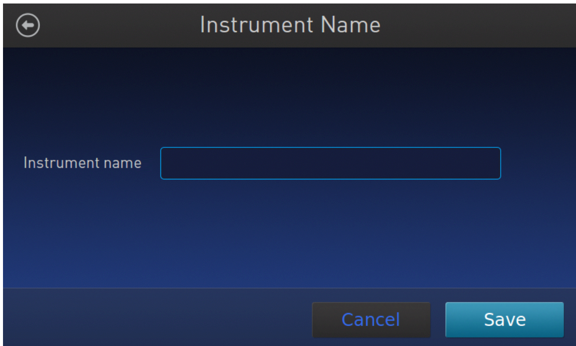
IMPORTANT! If you are updating the firmware manually, remove the USB drive before powering on the Ion Chef™ Instrument.

Change the instrument name

The following procedure describes how to change the name that is used to identify the Ion Chef™ Instrument both on the network and in the data that it generates.

1. In the Ion Chef™ Instrument home screen, tap **Settings**.
2. In the **Settings** screen, tap **Instrument settings**.
3. In the **Instrument Settings** screen, tap **Set Instrument Name**. Tap in the **Instrument name** field, enter a new instrument name using the keypad that appears, then tap **Save**.

   Note: Use only alphanumeric characters. Do not use special characters or spaces in the name.

4. In the next screen, tap **Save** again. Power off and on the Ion Chef™ Instrument to effect the name change.

Replace the ultraviolet bulb

Replace the ultraviolet (UV) bulb after about 500 hours of use. Contact your local field service engineer to schedule a bulb replacement.

Connections between Ion Torrent™ Server and the Ion Chef™ Instrument

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■ Ion Chef™ Instrument connection options 160
■ Edit the Ion Torrent™ Server firewall tables to allow the LAN port IP 164
■ Transfer a Planned Run 166
■ Undo a Planned Run transfer (administrator) 168
■ Undo a Planned Run transfer (user) 169

This chapter describes how to connect the standalone Ion Torrent™ Server component of the PGM™, Ion Proton™, Ion S5™ XL, or Ion GeneStudio™ S5 Prime System to an Ion Chef™ Instrument.

Overview

This document describes how to configure the Ion Chef™ Instrument to connect with a Ion Torrent™ Server. The guide also explains how to connect an Ion Chef™ Instrument to the internal Ion Torrent™ Server in Ion S5™, Ion GeneStudio™ S5, and Ion GeneStudio™ S5 Plus sequencers. When you connect to a Ion Torrent™ Server, you can use Torrent Suite™ Software to plan and monitor instrument runs, review run reports, and download run results from the connected instruments.

You can use the following types of network connections to connect a Ion Chef™ Instrument to an Ion Torrent™ Server or a Ion Torrent™ Server that is embedded in an Ion GeneStudio™ S5 Sequencer:

- A local area network (LAN) by DHCP IP connection
- A direct connection
- A local area network (LAN) by Static IP connection

If you add multiple Ion Torrent™ Servers to a network, you can configure connections between servers, if you use Torrent Suite™ Software 5.6 and later. Connections that use an Ion Mesh configuration, enable you to do the following:

- Share Planned Runs with Ion Torrent™ Servers that are included in the Ion Mesh configuration.
- Track reagent and cartridge usage across Ion Chef™ Instrument flexible workflows from different Ion Chef™ Instruments connected to different Ion Torrent™ Servers.
- View and monitor all runs of interest across multiple Ion Torrent™ Servers on the screen in Torrent Suite™ Software.

About the Ion Chef™ Instrument

The Ion Chef™ Instrument is factory-configured for IPv4 TCP/IP communication and includes a fast Ethernet adapter (100 Mbps) with a RJ45-type connector for integrating the device into a LAN. If the instrument is connected to a LAN, an active network port must be available/assigned in place before the scheduled installation date.

If you are activating a newly installed LAN port for your Ion Chef™ Instrument, ensure that the LAN has been configured to allow HTTP-443, SSH-22, and FTP-8021 traffic. For additional information, see the "Networking Requirements" section of the *Ion Chef™ Site Preparation Guide* (Pub. No. MAN0007956).

About the Ion Torrent™ Server

The Ion Torrent™ Server is an integral part of the system and includes a quad-port gigabit NIC for direct communication with the network or instruments such as Ion Chef™, Ion PGM™, Ion Proton™, or Ion S5™ XL/Ion GeneStudio™ S5 instruments. When the Ion Torrent™ Server is connected to the network, Ion Torrent™ Servers that are on the same software versions and subnet can transfer Planned Runs from one Ion Torrent™ Server to another.

Networking guidelines and best practices

We recommend that you consult a network administrator before connecting the Ion Chef™ Instrument to your laboratory network. See the *Ion Chef™ System Site Preparation Guide* (Pub. No. MAN0007956) for more information about the Ion Chef™ Instrument networking requirements.

Network setup workflow

Collect the required network information.

Connect the Ion Chef™ Instrument to the configured LAN port.

Configure the Ion Chef™ Instrument with the required information.

Monitor the Ion Chef™ Instrument to test the network connection.

Share Planned Runs and monitor consumables with Ion Mesh.

Network connection examples

This guide provides a basic description of how to integrate the Ion Torrent™ Server and the Ion Chef™ Instrument into several possible network architectures. Because your network can contain advanced features, such as a firewall or network domains, we recommend that you consult a network administrator before connecting the Ion Chef™ Instrument to your laboratory network. See the *Ion Chef™ System Site Preparation Guide* (Pub. No. MAN0007956) for more information.

This guide explains the following types of connections:

- DHCP IP (indirect connection)
- Direct connection
- Static IP (indirect connection)

Collect the required network information

Before you begin the procedures in this guide, obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP IP or static IP).
- If the network requires static IP addresses, obtain the static IP address, subnet mask, gateway address, and DNS server name for the LAN port.

Note: For the Planned Run sharing and file transfer protocol (FTP) functionality to be successful, the subnet must be the same on all Ion Torrent™ Servers and Ion Chef™ Instruments on the LAN.

Materials required

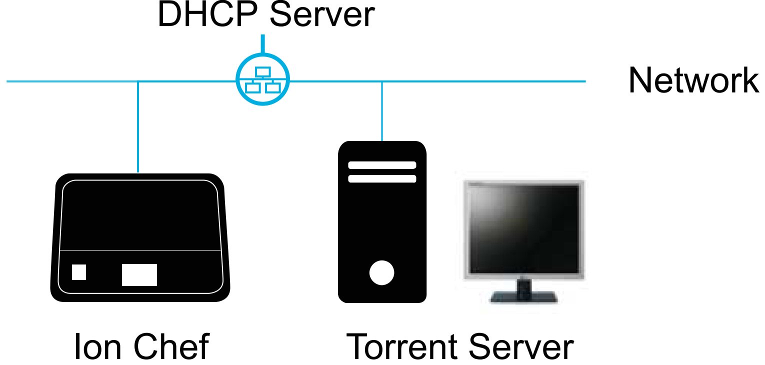
An ethernet cable with RJ45 connectors (CAT6 Ethernet cable for a 100Mbit/s network connection or a CAT5 for 100Mbit/s connection).

See *Ion Chef™ System Site Preparation Guide* (Pub. no. MAN0007956) for additional information.

General requirements

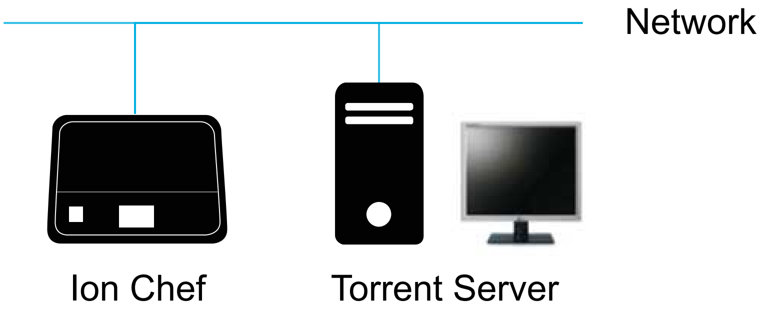
The following diagrams illustrate common network configurations to which an Ion Chef™ Instrument and Ion Torrent™ Server might be added. See the *Ion Chef™ System Site Preparation Guide* (Pub. No. MAN0007956) for more information.

DHCP IP



If using DHCP for the LAN port, make sure that the port is set to auto-populate when the Ion Chef™ instrument is connected and ensure that all the below requirements are met.

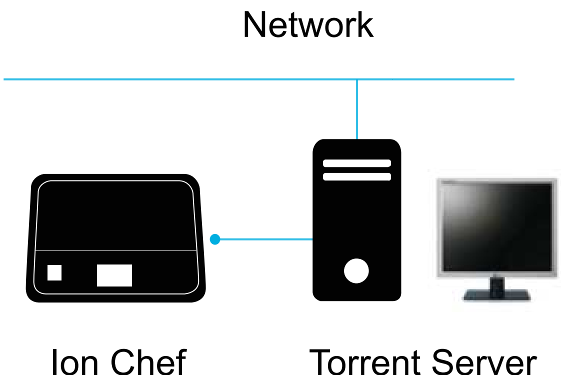
Static IP



If the network requires static IP addresses, obtain the static IP address, subnet mask, gateway address, and DNS server name for the LAN port.

For planned run sharing and FTP functionality to be successful, the subnet must be the same on all Ion Torrent™ Servers and Ion Chef™ instruments on the LAN.

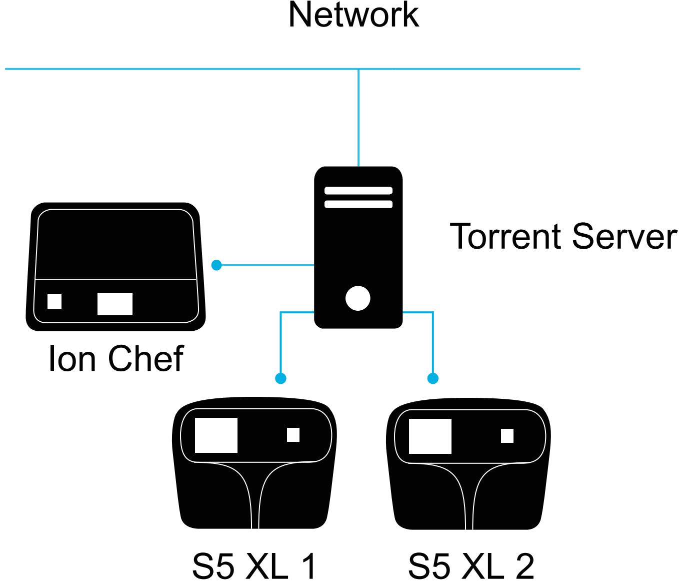
Direct connection



To configure the direct connection between an Ion Chef™ instrument and a Ion Torrent™ Server, you will need a Category 6 cable of sufficient length.

Another direct connection configuration option consists of one Ion Chef™ instrument, one Ion Torrent™ Server and two Ion S5™ XL Sequencers. However, this configuration is not recommended due to increased analysis times.

WARNING! The direct configuration shown below is known to result in lengthy analysis times.



Ion Chef™ Instrument connection options

When the Ion Chef™ Instrument is connected to a server, an icon (🖴) on the lower left side of the home screen appears.

Connect the Ion Chef™ Instrument to the server in one of three ways: DHCP IP (non-direct), Direct, or Static IP (non-direct).

Connect the instrument directly to the server

You can connect the Ion Chef™ Instrument directly the Ion Torrent™ Server.

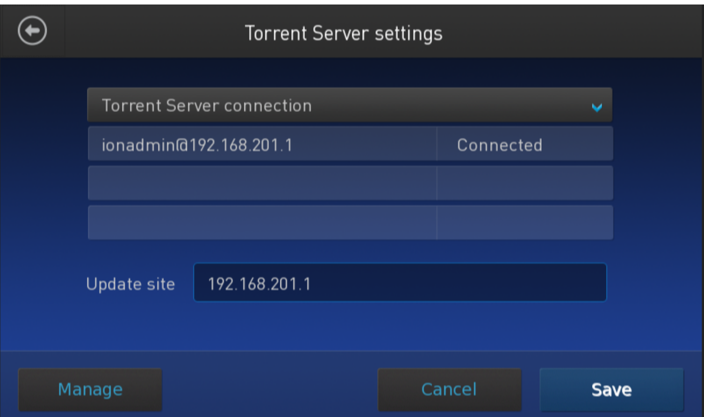
1. Verify the connection of the Ion Chef™ Instrument to the Ion Torrent™ Server.
   a. Plug the connector from the instrument directly into one of the available ports on the back of the server.
   The instrument is assigned a default IP address, for example 192.168.201.1, based on the server port that you connect to.

   b. On the Home screen, tap **Settings ▸ Instrument Settings ▸ About ▸ About Instrument**, then verify the IP address for the server connection on the instrument.

2. Connect the instrument.
   a. On the Home screen, tap **Settings ▸ Torrent Server**.

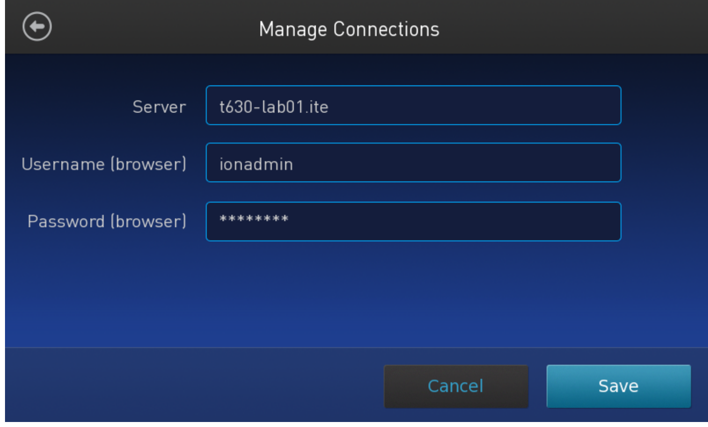
b. Verify that the server settings are correct. Ensure that the Torrent Suite™ Software password is correct.

The Torrent Suite™ Software URL is the IP address of the port you connected to on the back of the server (192.168.20x.1).



The server and instrument are now connected.

3. To set the password, tap **Manage**, then enter the password to the ionadmin account password on the selected Ion Torrent™ Server.



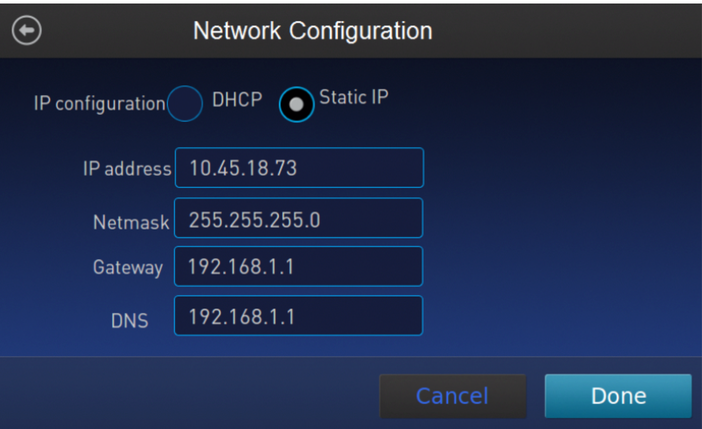
4. Tap **Save**.

The Ion Torrent™ Server is connected directly to the Ion Chef™ Instrument. An icon (▤) appears on the Home screen on the lower left side. You can now use Ion Mesh to share Planned Runs and monitor consumables.

Connect the instrument by static IP

If you connect to the Ion Chef™ Instrument to the Ion Torrent™ Server through the company LAN that is configured as a Static IP, you need to configure the Static IP, Gateway, Netmask, and DNS Server Names. (Only one DNS server is required for the instrument.) You also need to modify the server firewall settings. See "Edit the Ion Torrent™ Server firewall tables to allow the LAN port IP" on page 164 for more information.

1. Go to **Settings ▸ Instrument Settings ▸ Network ▸ Configuration**.
2. Select **Static IP**.
3. Enter the addresses and tap **Done**.



The Ion Chef™ Instrument is connected to the Ion Torrent™ Server.

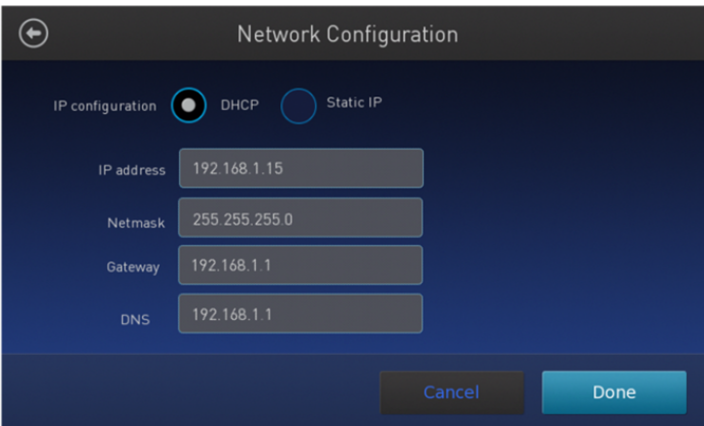
Connect by network to the Ion Torrent™ Server

To connect to the Ion Torrent™ Server through the company LAN, you need to target ServerPortIP (Eth1), see "Add the Ion Torrent™ Server IP address" on page 163 for more information. When the LAN is set to DHCP, the IP, Gateway, Netmask, and DNS Name Servers populate automatically. You will also need to modify the Ion Torrent™ Server firewall settings. See "Edit the Ion Torrent™ Server firewall tables to allow the LAN port IP" on page 164 for more information.

1. On the Home screen, tap **Settings ▸ Instrument Settings ▸ Network Configuration**.
2. Select the appropriate IP configuration:

Option	Description
DHCP	Dynamic Host Configuration Protocol is a client/server protocol that automatically provides an Internet Protocol (IP) host with its IP address and other related configuration information such as the subnet mask and the gateway.
Static IP	Static Internet Protocol (IP) address is a permanent number assigned to a computer by an Internet Service Provider (ISP).

3. Ensure that the information is correct, then tap **Done**.



The Ion Torrent™ Server is connected to the company LAN, an icon (icon) on the lower left side of the home screen appears. You can now use Ion Mesh to share Planned Runs and monitor consumables.

Add the Ion Torrent™ Server IP address

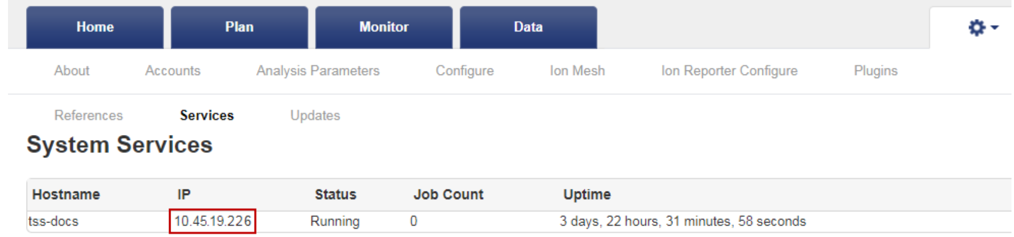
The first time that an instrument is connected to a Ion Torrent™ Server, or any time the connection configuration is changed, for example from a direct connection to a connection over a network, you must add the IP address of the server to the instrument. To add the Ion Torrent™ Server IP address from the Ion Chef™ Instrument. After you complete this procedure the IP address is available to select on the Ion Chef™ Instrument.

1. Sign in to Torrent Suite™ Software.

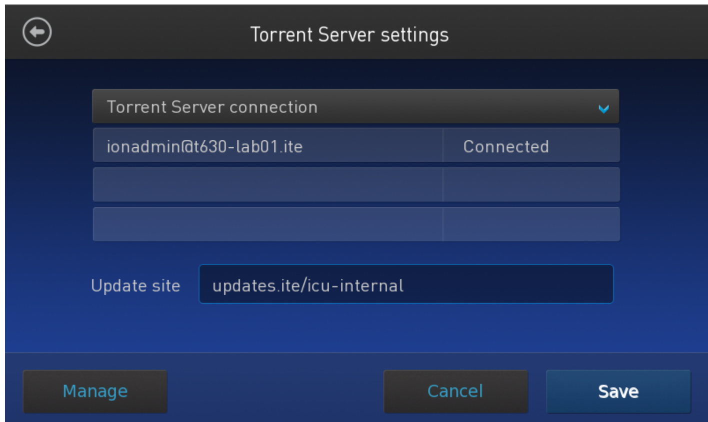
2. Click **(Settings)** ▸ **Services**.

3. Record the server IP address that is displayed on the **System Services** screen.

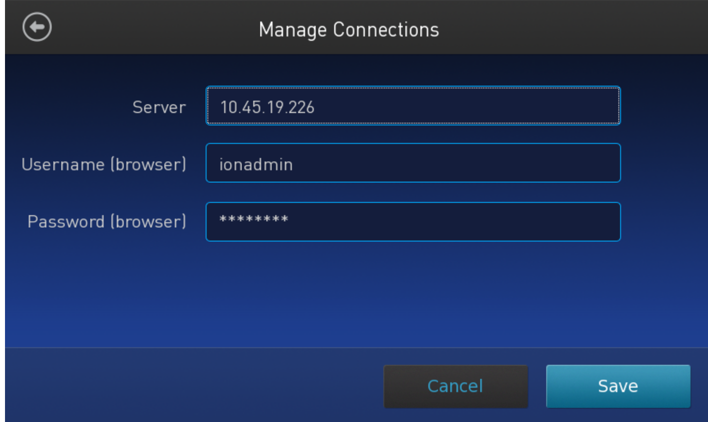
   Later you will enter the IP address on the instrument screen.



4. Return to the Ion Chef™ Instrument user interface, then tap **Manage**. The **Manage Connections** dialog opens.



5. Enter the server IP address or Ion Torrent™ Server host name in the **Server** field and repeat for **Update site**. Click **Save**.



The Ion Torrent™ Server host name or IP address is linked to the Ion Chef™ Instrument.

Edit the Ion Torrent™ Server firewall tables to allow the LAN port IP

When connecting through a LAN, the Ion Torrent™ Server firewall settings need to be modified for both DHCP and Static LAN connections.

Note: When directly connected, instruments are on the 192.168.20x.x subnet from which all traffic is enabled, so modification of firewall settings is not required.

Any exception would mean that editing the local firewall tables to include the LAN IP is required. Also, if the Ion Chef™ Instrument is getting a DHCP address from your network, you need to create an exception for the entire DHCP subnet.

Create a firewall exception for an Ion Torrent™ Server

Consult your system administrator or Thermo Fisher Field Service Engineer (FSE) or Field Application Specialist (FAS) for this procedure. On the Ion Torrent™ Server, edit the /etc/iptables.custom file and add a firewall rule to allow all traffic from the IP address of the Ion Chef™ Instrument. (This is a blank file initially and is intended for custom firewall configuration.)

IMPORTANT! Only edit the /etc/iptables.custom file and not /etc/iptables.rules, because /etc/iptables.rules is overwritten when software is updated.

1. Enter `sudo^nano^/etc/iptables.custom`, then add the three lines below:

```
*filter
-A INPUT -s xxx.xxx.xxx.xxx -j ACCEPT
COMMIT
```

Note: `-A INPUT -s xxx.xxx.xxx.xxx -j ACCEPT` adds the rule to the format. Enter the IP address of the Ion Chef™ instrument in place of "`xxx.xxx.xxx.xxx`". When finished editing, save changes by pressing **CTRL + O** and **CTRL + X**.

2. After this command, manually apply the change:

```
sudo^iptables-restore^--noflush^<^/etc/iptables.custom
```

Note: You can also list the firewall rules with: `sudo^iptables-L`.

If this command returns with no output, it means it succeeded.

3. If the Ion Chef™ Instrument is getting a DHCP address from your network, create the exception for the entire DHCP subnet.

The example below assumes that the DHCP address for the Ion Chef™ Instrument is 172.16.48.0 and the DHCP subnet range is 172.16.48.1 to 172.16.48.254. Add these three lines:

```
*filter
-A INPUT -s 172.16.48.0/24 -j ACCEPT
COMMIT
```

4. Restart the server.

5. *(Optional)* If you need to make corrections, repeat this process and completely drop all rules and start fresh. Enter the following:

```
sudo^iptables-F
sudo^iptables-restore^<^/etc/iptables.rules
sudo^iptables-restore--noflush^</^etc/iptables.custom
```

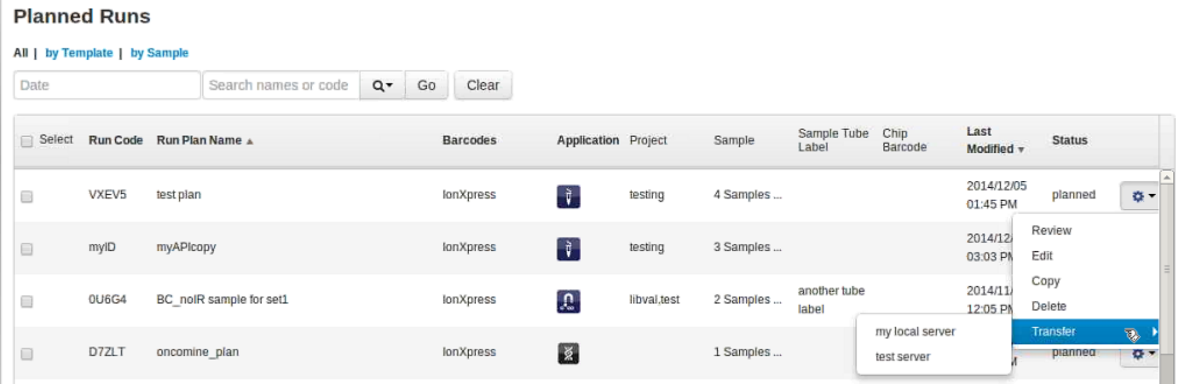
Removing a rule from either of these files would remove it from the active firewall rules, because the `iptables-restore` command does not overwrite rules, it adds them to all active rules.

Transfer a Planned Run

You can execute a Planned Run on the Ion Chef™ Instrument connected to the origin server, and then, when the run is complete, transfer the Planned Run to a destination server for sequencing.

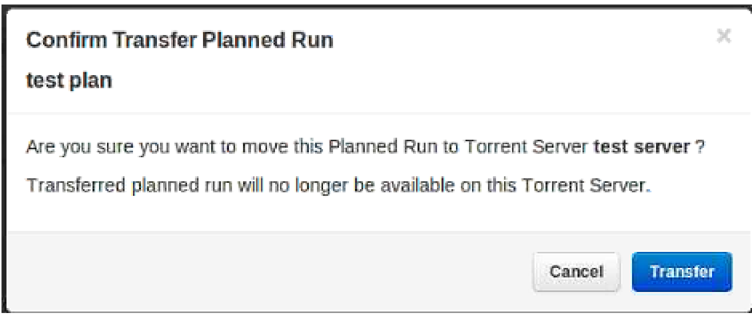
Alternatively, you can create the Planned Run on an origin server and then transfer it to a destination server for both the Ion Chef™ run and the sequencing run.

1. Sign into Torrent Suite™ Software on the *origin* Ion Torrent™ Server, then go to **Plan ▸ Planned Run List**.
2. Click **(Actions)** for the Planned Run you want to transfer, select **Transfer**, then select the *destination* Ion Torrent™ Server.



3. A confirmation dialog appears. Check the information, then click **Transfer**.

Note: You can no longer access this Planned Run on the origin server after it has transferred.



4. A status window displays the results of the transfer:
   - The green box lists the samples that are successfully processed, and the required target BED files that are on the destination server.
   - The red box lists any required BED files or plugins that are not present on the destination server. To perform the run successfully, you must edit the transferred Planned Run on the destination server and manually add the missing BED files or plugins.

test plan

Successfully created test plan on Torrent Server test server

....processed Samples: Sample 2, Sample 3, Sample 1

....found BED files: target.bed

....found IR account IonEast IR (Version: 4.2 | User: Ion User | Org: IR Org)

Planned run data is incomplete, please Edit test plan to fix the following errors

Unable to find bedfile: HSMv12.1_hotspots.bed for reference: hg19

Unable to find bedfile: atarget.bed for reference: hg19

Close

5. To edit the transferred Planned Run and add missing files:
   a. Download required files from the destination server.
   b. In the **References** tab, click **(Actions)** ▸ **Edit** from the dropdown list for the Planned Run.
   c. In the **Edit Plan** screen, select the files or plugins if needed, then click **Update Plan**.

Note: You can also navigate to the **Edit Plan** screen by clicking the **Edit test plan** link in the status page above.

Undo a Planned Run transfer (administrator)

1. On the *destination* Ion Torrent™ Server, delete the transferred Planned Run from either the **Planned Run** screen or the **admin** screen.

2. On the *origin* server, locate the plan on the , uncheck **PlanExecuted** and change **PlanStatus** to **Planned**.

Change planned experiment

PlanName: test_plan
PlanGUID: 97ef4510-b7c0-4b09-8a91-6fc8008d9aae
PlanShortID: VXEV5
PlanExecuted
PlanStatus: Planned
Username: ionadmin
PlanPGM:
Date: Date: 2014-12-05 Today | Time: 14:07:07 Now |
PlanExecutedDate: Date: Today | Time: Now |
MetaData: {"destination":"test server","location":"http://6wvvpq1.ite/rundb/api/v1/plannedexperiment/92/"}
ChipBarcode:
SeqKitBarcode:
ExpName:
UsePreBeadfind
UsePostBeadfind
Cycles:
AutoName:

Undo a Planned Run transfer (user)

If you transferred a Planned Run in error, you can transfer it back to the origin server or to another server.

1. On the destination Torrent Server, navigate to **Plan** ▸ **Planned Run List** and locate the transferred Planned Run.

2. In the row that contains the Planned Run for which you want to undo the transfer, click **(Settings)** ⚙ ▸ **Transfer**, then select the Ion Torrent™ Server to which you will transfer the run.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Instrument safety

General

CAUTION! **Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! **Moving Parts.** Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! **Ensure appropriate electrical supply.** For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! **Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

WARNING! **Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! **Cleaning and Decontamination.** Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Ultraviolet (UV) Safety

The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to help ensure that the UV lamp is not powered when the door is open.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety — Ion S5™ and Ion GeneStudio™ S5 System

For detailed information on Ion S5™ and Ion GeneStudio™ S5 System instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendix of the *Ion S5™ and Ion S5™ XL Sequencer User Guide* (Pub. No. MAN0010811), or the *Ion S5™ Sequencer User Guide* (Pub. No. MAN0017528), available at **thermofisher.com**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.