

# Precision ID mtDNA Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System

## APPLICATION GUIDE

for use with:

Precision ID mtDNA Whole Genome Panel

Precision ID mtDNA Control Region Panel

for use with:

Precision ID Library Kit

Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate

Precision ID DL8 Kit

Ion S5™ Precision ID Chef & Sequencing Kit

Ion 510™ Chip Kit

Ion 520™ Chip Kit

Ion 530™ Chip Kit

**Catalog Numbers** A30938, A31443

**Publication Number** MAN0017770

**Revision** D.0



Revision history: MAN0017770 D.0 (English)

Revision	Date	Description
D.0	5 October 2023	<ul style="list-style-type: none"><li>Updated instructions for creating a sample set. See “Create a sample set” on page 37.</li><li>Updated the library preparation instructions to include loading the PCR Plate Frame. See “Load the Ion Chef™ Instrument for library preparation” on page 41.</li><li>Updated the library preparation instructions to include removing the PCR Plate Frame. See “Unload the Ion Chef™ Instrument” on page 47.</li><li>Updated the template preparation instructions to include loading the PCR Plate Frame. See “Load the pipette tip racks and PCR Plate” on page 63.</li><li>Updated the template preparation instructions to include removing the PCR Plate Frame. See “Remove and dispose of used consumables” on page 78.</li><li>Updated the Torrent Suite™ Software version to v5.18.</li><li>Updated the Converge™ Software version to v.2.3.</li></ul>
C.0	18 August 2021	<ul style="list-style-type: none"><li>Update “Product description” on page 7.</li><li>Add procedural guidelines.</li><li>Include the VeritiPro™ Thermal Cycler for HID, 96-well in the list of HID-approved thermal cyclers.</li><li>Include the QuantStudio™ 5 Real-Time PCR System in the instrument list for library quantification.</li><li>For library preparation with the Ion Chef™ System, increase the amount of input gDNA to 0.3 ng and increase the sample concentration to 20 pg/μL. Add troubleshooting for low library concentration.</li><li>Update software version numbers.</li></ul>
B.0	16 January 2019	<ul style="list-style-type: none"><li>Correct the <i>Converge™ Software v2.1 Setup and Reference Guide</i> Pub. No.</li><li>Change the BED and JSON file versions to vX.X.</li><li>Updated the Torrent Suite™ Software version to v5.10.</li></ul>
A.0	9 October 2018	New Application Guide .

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

**DISCLAIMER:** TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

**Important Licensing Information:** These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

**Trademarks:** All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a trademark of Roche Molecular Systems, Inc., used under permission and license. Eppendorf, LoBind, and MixMate are trademarks of Eppendorf AG. Agencourt and AMPure are trademarks of Beckman Coulter, Inc.

©2018-2023 Thermo Fisher Scientific Inc. All rights reserved.

# Contents

■ CHAPTER 1	Product information	7
	Product description	7
	Software requirements	8
	Precision ID mtDNA panels	9
	Overview	9
	Developmental validation	10
	Product requirement	10
	Dual panel system and library amplification	11
	PCR methodologies for manual library preparation	12
	About the primers	13
	Degenerate primers	13
	Library preparation kits	14
	Precision ID Library Kit	14
	Precision ID DL8 Kit	15
	Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate	15
	Template preparation kits	16
	Required materials for manual library preparation, not supplied	16
	Required materials for library preparation on the Ion Chef™ System, not supplied	17
	DNA extraction and quantification kits	18
	Mitochondrial DNA extraction kits	18
	Mitochondrial DNA quantification kits	18
	Workflow	19
■ CHAPTER 2	Prepare libraries manually	20
	Workflow: prepare libraries manually	21
	Procedural guidelines	21
	Before each use of the kit	22
	Extract, then quantify input DNA	22
	Guidelines for mitochondrial DNA input per reaction (manual)	22
	Prepare the mtDNA target amplification reaction	22
	Amplify the targets	24
	Partially digest amplicons	25

Ligate adapters to the amplicons, then purify .....	26
Perform the ligation reaction .....	26
Purify the libraries .....	27
Elute the libraries .....	28
Quantify the libraries by qPCR .....	28
Dilute the libraries for quantification .....	28
Quantify the libraries .....	29
(Optional) Amplify and purify the libraries .....	31
Amplify the libraries .....	31
Purify the amplified libraries .....	31
Dilute, pool, and store the libraries .....	33
Dilute the libraries .....	33
(Optional) Pool the libraries .....	34
Store the libraries .....	34

■ **CHAPTER 3 Prepare libraries using the HID Ion Chef™ Instrument .....** 35

Software version requirements for library preparation .....	35
Workflow: Prepare libraries using the Ion Chef™ Instrument .....	36
Extract, then quantify input DNA .....	37
Guidelines for mitochondrial DNA input per reaction (Ion Chef™) .....	37
Dilute the gDNA samples .....	37
Thaw the reagents, then prepare the instrument .....	37
Create a sample set .....	37
Add Precision ID primer pools to Positions A and B of the Reagents cartridge .....	38
Add the DNA to the Precision ID DL8 IonCode™ Barcode Adapters .....	39
Load the Ion Chef™ Instrument for library preparation .....	41
Start the Ion Chef™ run .....	43
Unload the Ion Chef™ Instrument .....	47
Quantify the combined library by qPCR .....	48
Dilute the combined library for quantification .....	48
Quantify the combined library by qPCR .....	49
Dilute, pool, and store the combined libraries .....	50
Dilute the combined libraries .....	50
(Optional) Combine library pools .....	51
Store the libraries .....	51
Clean the Ion Chef™ Instrument .....	51
About the cleaning protocol .....	51
Clean the Ion Chef™ Instrument .....	52

■	<b>CHAPTER 4 Prepare the template on the HID Ion Chef™ Instrument .....</b>	<b>54</b>
	Software version requirements for template preparation .....	54
	Required materials .....	55
	Ion S5™ Precision ID Chef & Sequencing Kit components .....	55
	Compatible Ion Chip Kits .....	55
	Workflow: Template preparation on the Ion Chef™ Instrument .....	56
	Create an NGS mtDNA Planned Run .....	57
	Dilute the libraries for template preparation on the Ion Chef™ Instrument .....	60
	Prepare the libraries and consumables .....	60
	Load the Ion Chef™ System .....	61
	Load the pipette tip racks and PCR Plate .....	63
	Load the Reagents and Solutions cartridges .....	64
	Load the Recovery Tubes and Enrichment Cartridge v2 .....	66
	Load the Chip-loading centrifuge .....	68
	Confirm that consumables are correctly installed .....	70
	Single chip loading workflow .....	70
	Start the Ion Chef™ run .....	72
	Unload the chips for sequencing .....	76
	Clean the Ion Chef™ System .....	77
	About the cleaning protocol .....	77
	Materials required .....	77
	Clean the Ion Chef™ Instrument .....	77
■	<b>CHAPTER 5 Sequence on the HID Ion S5™/HID Ion GeneStudio™ S5 System .....</b>	<b>83</b>
	Software version requirements for sequencing .....	83
	Workflow: Sequencing on the HID Ion S5™/Ion GeneStudio™ S5 System .....	84
	HID Ion S5™/Ion GeneStudio™ S5 System component positions .....	84
	Before you begin .....	85
	Required sequencer cleanings .....	86
	Initialize the sequencer .....	86
	Start the sequencing run .....	88
■	<b>CHAPTER 6 Analyze the sequencing results .....</b>	<b>90</b>
	Review mtDNA run summary metrics .....	90
	Expected observations if recommended values are not met .....	97
	Confirm that data were exported to Converge™ Software .....	98
	<i>(If needed)</i> Manually export data to the Converge™ Software .....	99
	Related documentation for data analysis .....	99

■	<b>APPENDIX A</b>	<b>Troubleshooting</b>	<b>100</b>
		Manual library preparation	100
		Library preparation on the Ion Chef™ Instrument	103
		Ion Chef™ System: general operation	105
		Set up and operation of Ion S5™/Ion GeneStudio™ S5 Series System	108
		Instrument alarms and events	108
		Initialization—General errors	108
		Data transfer to Converge™ Software	109
■	<b>APPENDIX B</b>	<b>Supplemental procedures</b>	<b>110</b>
		Perform a manual cleaning of the sequencer	110
		Perform an instrument reset run with an initialized sequencer that is loaded with an unused Reagents cartridge	111
■	<b>APPENDIX C</b>	<b>Safety</b>	<b>112</b>
		Symbols on this instrument	112
		Conformity symbols	113
		Location of safety labels on this instrument	114
		Instrument safety	114
		General	114
		Physical injury	114
		Electrical safety	114
		Cleaning and decontamination	115
		Ultraviolet (UV) Safety	115
		Safety and electromagnetic compatibility (EMC) standards	115
		Safety compliance	115
		EMC	116
		Environmental design	116
		Instrument safety — HID Ion S5™/Ion GeneStudio™ S5 System	116
		Chemical safety	117
		Biological hazard safety	118
■	<b>APPENDIX D</b>	<b>Documentation and support</b>	<b>119</b>
		Related documentation	119
		Customer and technical support	120
		Limited product warranty	120
		<b>Glossary</b>	<b>121</b>



# Product information

- Product description ..... 7
- Software requirements ..... 8
- Precision ID mtDNA panels ..... 9
- Library preparation kits ..... 14
- Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate ..... 15
- Template preparation kits ..... 16
- Required materials for manual library preparation, not supplied ..... 16
- Required materials for library preparation on the Ion Chef™ System, not supplied ..... 17
- DNA extraction and quantification kits ..... 18
- Workflow ..... 19

## Product description

The Applied Biosystems™ Precision ID mtDNA Whole Genome Panel and Precision ID mtDNA Control Region Panel are next-generation sequencing (NGS) approaches to mitochondrial DNA (mtDNA) analysis for forensic applications. The Precision ID workflow for these panels enables automated library and template preparation using the Ion Chef™ System and sequencing and analysis with Ion GeneStudio S5 sequencers and Converge™ Software.

The Precision ID mtDNA Whole Genome Panel is a two-pool multiplex assay that targets the entire human mitochondrial genome (16,569 bp). Each pool contains 81 primer pairs, with minimal primer overlap between pools.

The Precision ID mtDNA Control Region Panel is a two-pool multiplex assay that targets the 1.2-kb control region of the human mitochondrial genome, which encompasses the hypervariable regions HV-I, II, and III.

This guide covers the following products:

Item	Cat. No.
<b>Panels</b>	
Precision ID mtDNA Whole Genome Panel	A30938
Precision ID mtDNA Control Region Panel	A31443

(continued)

Item	Cat. No.
<b>Library preparation kits</b>	
Precision ID Library Kit	A26435 (96 reactions) A30941 (384 reactions)
Precision ID DL8 Kit	A33212
<b>Barcode adapters</b>	
Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate	A33586
<b>Template preparation kits<sup>[1]</sup></b>	
Ion S5™ Precision ID Chef & Sequencing Kit (2 runs per initialization)	A33208
Ion S5™ Precision ID Chef & Sequencing Kit (1 run per initialization)	A35850
<b>Sequencing chips</b>	
Ion 510™ Chip Kit	A34292
Ion 520™ Chip Kit	A27762
Ion 530™ Chip Kit	A27764
<b>Library and template preparation system</b>	
HID Ion Chef™ System	A30070
<b>Sequencer System</b>	
HID Ion GeneStudio™ S5 System	A41431
HID Ion GeneStudio™ S5 Plus System	A41432
HID Ion GeneStudio™ S5 Prime System	A41433
<b>Sequencing kits <sup>[2]</sup></b>	
Ion S5™ Precision ID Chef & Sequencing Kit (2 runs per initialization)	A33208
Ion S5™ Precision ID Chef & Sequencing Kit (1 run per initialization)	A35850

<sup>[1]</sup> These kits also contain sequencing reagents for the HID Ion S5™ System and Ion GeneStudio™ S5 Systems.

<sup>[2]</sup> These kits also contain template preparation reagents.

## Software requirements

Analysis of Precision ID mtDNA panel sequencing results requires Converge™ Software v2.3 or later. See the *Converge™ Software Help* and the *Converge™ Software v2.3 Setup and Reference Guide* (Pub. No. 100039539) for further information. Also, ensure that you update Torrent Suite™ Software to version 5.18, or the latest HID-supported version.



# Precision ID mtDNA panels

## Overview

The Precision ID mtDNA Whole Genome Panel (Cat. No. A30938) and the Precision ID mtDNA Control Region Panel (Cat. No. A31443) contain pools of unlabeled Ion AmpliSeq™-designed PCR primers for preparing libraries from mitochondrial DNA (mtDNA). Both kits are dual-panel pool systems with small amplicon overlaps to cover the mtDNA genome.

Panel <sup>[1]</sup>	Cat. No.	Average amplicon size <sup>[2]</sup>	Amount	No. of primer pairs	Storage
Precision ID mtDNA Whole Genome Panel	A30938	163 bp	2 tubes <sup>[3]</sup>	81 <sup>[4]</sup>	-30°C to -10°C
Precision ID mtDNA Control Region Panel	A31443	153 bp	2 tubes <sup>[3]</sup>	7 <sup>[5]</sup>	

<sup>[1]</sup> For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures. For licensing and limited use restrictions visit [thermofisher.com/HIDlicensing](https://www.thermofisher.com/HIDlicensing).

<sup>[2]</sup> Libraries have an additional ~80 bp due to barcode adapters.

<sup>[3]</sup> Sufficient for 96 reactions (2-in-1 method) or 192 reactions (Conservative method) if preparing libraries manually, or 32 reactions if preparing libraries using the Ion Chef™ Instrument.

<sup>[4]</sup> There are 81 primer pairs per tube for a total of 162 primer pairs.

<sup>[5]</sup> There are 7 primer pairs per tube for a total of 14 primer pairs.

The Precision ID mtDNA Whole Genome Panel covers the entire mtDNA genome (16,569 bp; see Figure 1). Each pool in this panel contains 81 primer pairs to produce a total of 162 amplicons. This panel also contains degenerate primers to account for primer-binding single nucleotide polymorphisms (SNPs) that prevent amplicon dropouts.

The Precision ID mtDNA Control Region Panel only targets the Mitochondrial Control Region. This panel targets positions 15954–610 of the Control Region. The Control Region contains the non-coding Hypervariable (HV) Regions I, II, and III (see Figure 2). Each pool contains 7 primer pairs to produce a total of 14 amplicons. This panel also contains degenerate primers to account for primer-binding SNPs.

## Developmental validation

Developmental validation information for the Precision ID mtDNA Whole Genome Panel and the Precision ID mtDNA Control Region Panel can be found in the following documentation:

- Cihlar, J., Amory, C., Lagacé, R., Roth, C., Parson, W. and Budowle, B., 2020. Developmental Validation of a MPS Workflow with a PCR-Based Short Amplicon Whole Mitochondrial Genome Panel. *Genes*, 11(11), p.1345.
- Cihlar, J., Strobl, C., Lagacé, R., Muenzler, M., Parson, W. and Budowle, B., 2020. Distinguishing mitochondrial DNA and NUMT sequences amplified with the precision ID mtDNA whole genome panel. *Mitochondrion*, 55, pp.122–133.
- Strobl, C., Churchill Cihlar, J., Lagacé, R., Wootton, S., Roth, C., Huber, N., Schnaller, L., Zimmermann, B., Huber, G., Lay Hong, S., Moura-Neto, R., Silva, R., Alshamali, F., Souto, L., Anslinger, K., Egyed, B., Jankova-Ajanovska, R., Casas-Vargas, A., Usaquén, W., Silva, D., Barletta-Carrillo, C., Tineo, D., Vullo, C., Würzner, R., Xavier, C., Gusmão, L., Niederstätter, H., Bodner, M., Budowle, B. and Parson, W., 2019. Evaluation of mitogenome sequence concordance, heteroplasmy detection, and haplogrouping in a worldwide lineage study using the Precision ID mtDNA Whole Genome Panel. *Forensic Science International: Genetics*, 42, pp.244–251.

## Product requirement

These panels are designed for use with the Precision ID Library Kits (Cat. Nos. [A26435](#), [A30941](#)) and the Precision ID DL8 Kit (Cat. No. [A33212](#)).

## Dual panel system and library amplification

A dual panel system covers the mtDNA Genome and Control Region. The following figures are visual representations of the dual panels, Hypervariable Regions, and insert locations of the Precision ID mtDNA Control Region Panel.

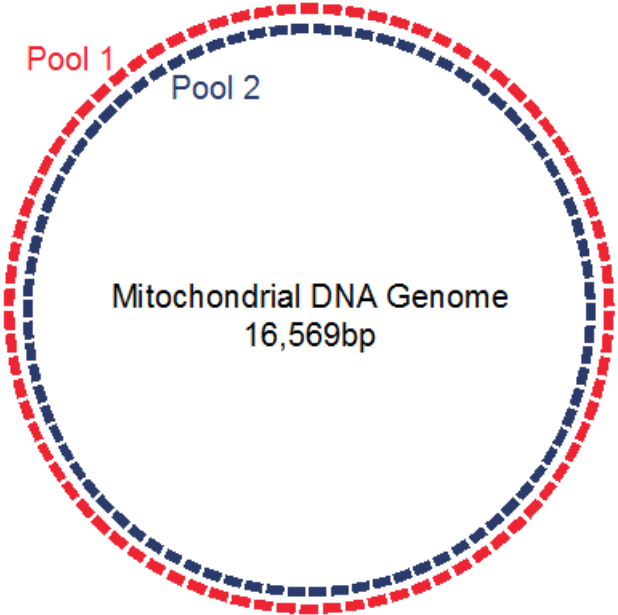


Figure 1 Visual representation of the dual panel system. It does not accurately display the actual number of amplicons.

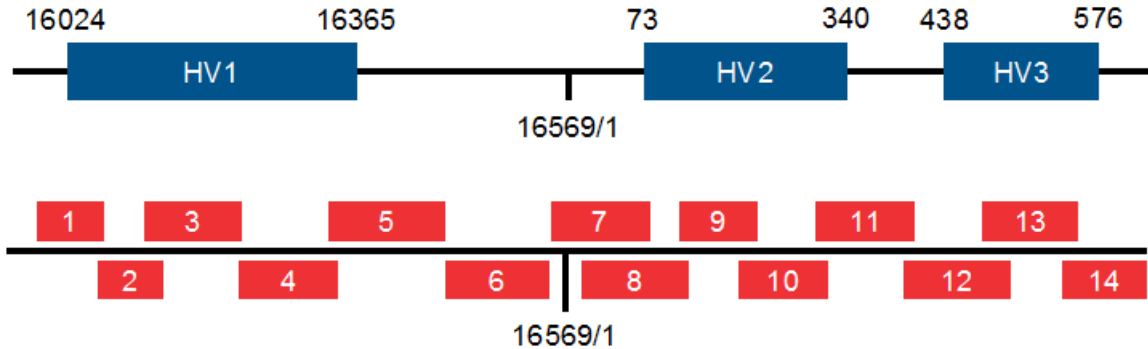


Figure 2 The Hypervariable Regions of the mtDNA Control Region and insert locations of the Precision ID mtDNA Control Region Panel.

## Insert locations of the Precision ID mtDNA Control Region Panel

Pool	Amplicon	Insert start	Insert end	Insert size	Insert overlap
1	1	15,954	16,069	116	14
2	2	16,056	16,131	76	22
1	3	16,110	16,225	116	4
2	4	16,222	16,341	120	3
1	5	16,339	16,458	120	11
2	6	16,448	16,552	105	11
1	7	16,542	80	108	65
2	8	16	119	104	1
1	9	119	248	130	1
2	10	248	329	82	31
1	11	299	411	113	27
2	12	385	480	96	21
1	13	460	543	84	25
2	14	519	610	92	N/A

## PCR methodologies for manual library preparation

Two PCR methodologies for manual library preparation for mtDNA are described in the following table. The methods are used to optimize reagent usage, and to optimize the integrity of input mtDNA, or resulting coverage. The procedure that uses these methods is found in “Prepare the mtDNA target amplification reaction” on page 22.

Method	Sample type	Reagent use	Amplification and ligation reactions
2-in-1	Low copy number samples	2X	Amplify each pool separately, then pool 10 $\mu$ L of each sample to create a new pool. Place 20 $\mu$ L of the new pool into a single well. Proceed as if processing one sample. In the barcode ligation reaction, use one barcode adapter.
Conservative	Non-degraded samples	1X	Amplify each pool in a 10- $\mu$ L half-reaction. Transfer one of the half-reactions into the other, then proceed as if processing one sample. In the barcode ligation reaction, use one barcode adapter.  <b>Note:</b> A new (clean) well is not required.

## About the primers

The dual-panel nature of the designs is necessary for whole genome sequencing.

- For the Precision ID mtDNA Whole Genome Panel, there is an average 11-bp amplicon overlap between the 2 pools. The average amplicon size is ~163 bp.
- For the Precision ID mtDNA Control Region Panel, there is an average 18-bp amplicon overlap between the 2 pools. The average amplicon size is ~153 bp.

## Degenerate primers

Degenerate primers account for the high frequency of variants of mtDNA. The number of degenerate primers for each pool is as follows:

Panel	Pool 1	Pool 2	Variant frequencies <sup>[1, 2]</sup>
Precision ID mtDNA Whole Genome Panel	81 primer pairs 119 degenerates	81 primer pairs 164 degenerates	1000 Genomes: >5% population frequency <a href="http://www.1000genomes.org">www.1000genomes.org</a>
Precision ID mtDNA Control Region Panel	7 primer pairs 45 degenerates	7 primer pairs 68 degenerates	MitoMap: >700 count <a href="http://www.mitomap.org">www.mitomap.org</a>

<sup>[1]</sup> Degenerates were designed to avoid dropouts caused by primer binding SNPs identified from these references.

<sup>[2]</sup> Additional degenerate primers were added after a round of global customer testing.

## Library preparation kits

### Precision ID Library Kit

The Precision ID Library Kit provides reagents for the rapid preparation of libraries from Precision ID panels. These library kits use a plate-based protocol for easy sample handling and tracking, and for compatibility with automation and high-throughput laboratories. When used with Precision ID mtDNA panels, this kit requires 0.1 ng of genomic DNA (gDNA) per target amplification reaction. DNA from various sources, including bodily fluid and bone samples, can be used as starting material.

The Precision ID Library Kit is tailored for human identification needs and provides reagents for 96 or 384 libraries.

Item	Amount		Storage
	Cat. No. <a href="#">A26435</a> (96 reactions) [1]	Cat. No. <a href="#">A30941</a> (384 reactions) <sup>[2]</sup>	
5X Ion AmpliSeq™ HiFi Mix (red cap)	384 µL	4 × 384 µL	–30°C to –10°C
FuPa Reagent (brown cap)	192 µL	4 × 192 µL	
Switch Solution (yellow cap)	384 µL	4 × 384 µL	
Platinum PCR SuperMix HiFi (black cap)	3 × 1.6 mL	12 × 1.6 mL	
Library Amplification Primer Mix (white cap)	192 µL	4 × 192 µL	
DNA Ligase (blue cap)	192 µL	4 × 192 µL	
Low TE (clear cap)	12 mL	4 × 12 mL	Room temperature

[1] Sufficient for 48 reactions (2-in-1 method) or 96 reactions (Conservative method) for each 96-reaction kit.

[2] Cat. No. [A30941](#) provides four 96-reaction kits.

## Precision ID DL8 Kit

The Precision ID DL8 Kit (Cat. No. [A33212](#)) contains materials sufficient for performing 4 Ion Chef™ runs, with up to 8 Precision ID libraries prepared per run. Upon arrival, inspect all consumables and contact Technical Support if any of the components have been damaged during shipping.

**IMPORTANT!** Store all consumables and cartridges under the recommended conditions and in an upright position. Precision ID DL8 Solutions cartridges are shipped at ambient temperature, but need to be stored at 2°C to 8°C upon arrival.

Component	Amount per kit	Storage
Precision ID DL8 Supplies (Part No. A30935) <ul style="list-style-type: none"> <li>• Ion AmpliSeq™ Tip Cartridge L8</li> <li>• PCR Plate Frame</li> <li>• Framed PCR Foil Seal</li> <li>• Enrichment Cartridge</li> </ul>	1 box with 4 inserts	15°C to 30°C
Precision ID DL8 Reagents (Part No. A32926)	4 cartridges	–30°C to –10°C
Precision ID DL8 Solutions (Part No. A30934)	4 cartridges	2°C to 8°C
Precision ID DL8 IonCode™ Barcode Adapters 1–32 for Chef DL8 in 96 Well PCR Plates (Part No. A33419) Set includes 4 PCR plates: <ul style="list-style-type: none"> <li>• Precision ID IonCode™ Barcode Adapters 1–8 for Chef DL8 in 96 Well PCR Plate (red)</li> <li>• Precision ID IonCode™ Barcode Adapters 9–16 for Chef DL8 in 96 Well PCR Plate (yellow)</li> <li>• Precision ID IonCode™ Barcode Adapters 17–24 for Chef DL8 in 96 Well PCR Plate (green)</li> <li>• Precision ID IonCode™ Barcode Adapters 25–32 for Chef DL8 in 96 Well PCR Plate (blue)</li> </ul>	1 set of 4 plates	15°C to 30°C

## Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate

The Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate (Cat. No. [A33586](#)) contains a set of 96 unique barcode adapters in a 96-well plate format for use in manual library preparation. When used in combination with the Precision ID Library Kit, this kit enables pooling of up to 96 libraries for multiplex sequence analysis.

Component	Quantity	No. of reactions	Storage
Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate	1 × 96-well plate (20 µL/well)	960 (10 reactions per barcode)	–30°C to –10°C

## Template preparation kits

For the template preparation kit for the Ion Chef™ System, see Chapter 4, “Prepare the template on the HID Ion Chef™ Instrument”, and see “Required materials” on page 55.

## Required materials for manual library preparation, not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

Item	Source
<b>Instruments and equipment</b>	
One of the following real-time PCR instruments: <ul style="list-style-type: none"> <li>7500 Real-Time PCR Instrument</li> <li>QuantStudio™ 5 Real-Time PCR System</li> </ul>	See web product pages
One of the following HID-approved thermal cyclers: <ul style="list-style-type: none"> <li>GeneAmp™ PCR System 9700 with silver or gold block.</li> <li>Veriti™ 96-Well Thermal Cycler</li> <li>VeritiPro™ Thermal Cycler for HID, 96-well<sup>[1]</sup></li> <li>ProFlex™ 96-well PCR System</li> </ul>	See web product pages
DynaMag™-96 Side Magnet, or other plate magnet	<a href="#">12331D</a>
Pipettes (2–200 µL)	MLS
<b>Plates, tubes, and other consumables</b>	
MicroAmp™ Optical 96-Well Reaction Plate	<a href="#">N8010560</a> <a href="#">4306737</a> (with barcode)
MicroAmp™ Clear Adhesive Film	<a href="#">4306311</a>
MicroAmp™ Optical Film Compression Pad	<a href="#">4312639</a>
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes (0.5 mL and 1.5 mL)	MLS
Low-retention, filtered pipette tips	MLS
<b>Accessories</b>	
(Optional) Eppendorf™ MixMate™ tool with 96 Tube holder (for library preparation and elution)	Eppendorf <a href="#">5353 000.014</a>



(continued)

Item	Source
<b>Reagents</b>	
Agencourt™ AMPure™ XP Reagent	Fisher Scientific <a href="#">NC9959336</a> or <a href="#">NC9933872</a> , or MLS
Ion Library TaqMan™ Quantitation Kit	<a href="#">4468802</a>
Nuclease-Free Water (not DEPC-Treated)	<a href="#">AM9932</a> , or equivalent
Absolute ethanol	MLS

<sup>[1]</sup> Internal validation data are available upon request.

## Required materials for library preparation on the Ion Chef™ System, 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
<b>Instruments and equipment</b>	
Uninterruptible power supply (UPS) <sup>[1]</sup>	MLS
Microcentrifuge <sup>[2]</sup>	MLS
Vortex mixer with a rubber platform	MLS
Pipettors (2–1000 µL)	MLS
<b>Tubes, plates, and other consumables</b>	
Microcentrifuge tubes (1.5 mL or 1.7 mL)	MLS
Filtered pipette tips	MLS
Wipes, disposable lint-free	MLS
Gloves, powder-free nitrile	MLS
<b>Reagents</b>	
Nuclease-Free Water (not DEPC-Treated)	<a href="#">AM9932</a> , or equivalent
Isopropyl alcohol, 70% solution	MLS

<sup>[1]</sup> For laboratories that experience frequent power outages or line voltage fluctuations, we recommend using an uninterruptible power supply that is compatible with 2500 W output or higher.

<sup>[2]</sup> Must fit standard 0.2- and 1.5 mL microcentrifuge tubes and generate 21,000 × g.

# DNA extraction and quantification kits

## Mitochondrial DNA extraction kits

We recommend the PrepFiler *Express* BTA™ Forensic DNA Extraction Kit (Cat. No. 4441351) for mtDNA extraction. If you are using this kit, follow the modification step listed in “Guidelines for mitochondrial DNA input per reaction (manual)” on page 22.

## Mitochondrial DNA quantification kits

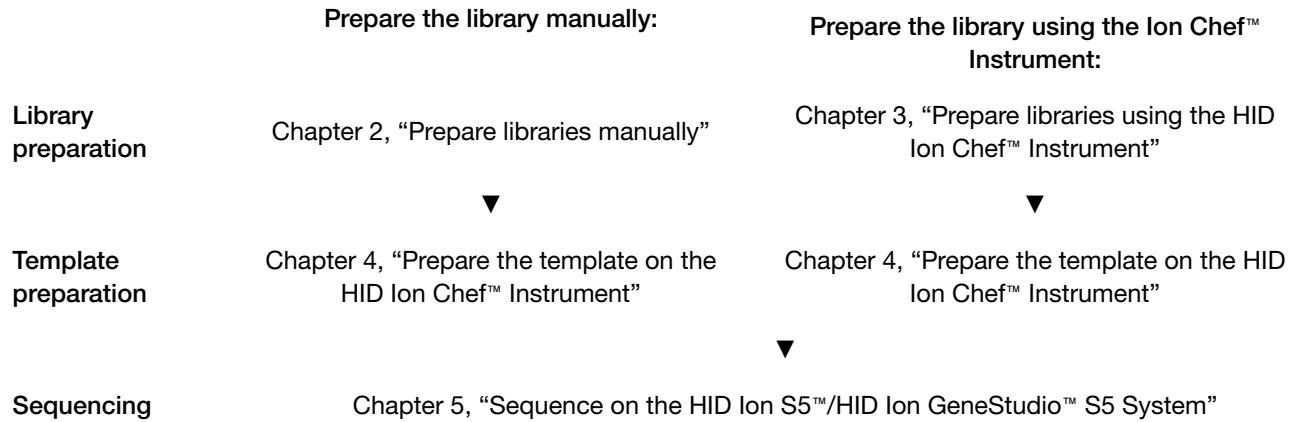
Use any in-house method to quantify mtDNA, or use the Quantifiler™ HP or Quantifiler™ Trio DNA Quantification Kit to approximate the amount of mtDNA in each sample.

- Quantifiler™ HP DNA Quantification Kit (Cat. No. [4482911](#))
- Quantifiler™ Trio DNA Quantification Kit (Cat. No. [4482910](#))

The Quantifiler™ Trio DNA Quantification Kit uses multiple-copy target loci for excellent detection sensitivity. The human-specific target loci (Small Autosomal, Large Autosomal, and Y-chromosome targets) each consist of multiple copies dispersed on various autosomal chromosomes (Small Autosomal and Large Autosomal), or multiple copies on the Y-chromosome. The primary quantification targets consist of relatively short amplicons (75 to 80 bases) to improve the detection of degraded DNA samples. In addition, this kit contains Large Autosomal targets with a longer amplicon (>200 bases) to help in determining if a DNA sample is degraded.

The Quantifiler™ HP DNA Quantification Kit is the same as the Quantifiler™ Trio DNA Quantification Kit, but without the Y-chromosome targets.

## Workflow



---

**Note:** Before starting, ensure that

- You have updated your Ion S5™, Ion S5™ XL, or Ion GeneStudio™ S5 Series System to Torrent Suite™ Software 5.18, or the latest HID-supported version.
  - You have installed appropriate BED and reference files into Torrent Suite™ Software.
-

# 2

## Prepare libraries manually

- Workflow: prepare libraries manually ..... 21
- Procedural guidelines ..... 21
- Before each use of the kit ..... 22
- Extract, then quantify input DNA ..... 22
- Prepare the mtDNA target amplification reaction ..... 22
- Amplify the targets ..... 24
- Partially digest amplicons ..... 25
- Ligate adapters to the amplicons, then purify ..... 26
- Quantify the libraries by qPCR ..... 28
- (Optional) Amplify and purify the libraries ..... 31
- Dilute, pool, and store the libraries ..... 33

To prepare libraries using the Ion Chef™ System, see Chapter 3, “Prepare libraries using the HID Ion Chef™ Instrument”.

## Workflow: prepare libraries manually

“Extract, then quantify input DNA” on page 22



“Prepare the mtDNA target amplification reaction” on page 22



“Amplify the targets” on page 24



“Partially digest amplicons” on page 25



“Ligate adapters to the amplicons, then purify” on page 26



“Quantify the libraries by qPCR” on page 28



“Dilute, pool, and store the libraries” on page 33

## Procedural guidelines

- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is free of amplicon contamination. Always change pipette tips between samples.
- Use a calibrated thermal cycler specified in “Required materials for manual library preparation, not supplied” on page 16.
- Pipet viscous solutions, such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, Switch Solution, DNA Ligase, and panels, slowly and ensure complete mixing by vortexing or pipetting up and down several times.

## Before each use of the kit

- Thaw components that contain enzymes—such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, DNA Ligase, and Platinum™ PCR SuperMix HiFi—on ice, and keep on ice during procedure. All other components, including primer pools, can be thawed at room temperature. Gently vortex and centrifuge before use.
- If there is visible precipitate in the Switch Solution after thawing, vortex or pipet up and down at room temperature to resuspend.
- Bring the Agencourt™ AMPure™ XP Reagent to room temperature.

---

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

---

## Extract, then quantify input DNA

### Guidelines for mitochondrial DNA input per reaction (manual)

- Use 0.1 ng of input gDNA per target amplification reaction for the mitochondrial panels.
- If you are using the Quantifiler™ HP or Quantifiler™ Trio DNA Quantification Kit, estimate the mtDNA input by using 10% of the gDNA Small Amplicon (SA) quantity. For example, for non-degraded samples use 0.1 ng of gDNA.
- See “Mitochondrial DNA extraction kits” on page 18 for a recommended mtDNA extraction kit.
- **IMPORTANT!** If you are using the PrepFiler Express BTA™ Forensic DNA Extraction Kit to extract mtDNA from non-BTA substrates such as blood or buccal, perform this modification: During the lysis, incubate the column/tube assembly at 56°C, then shake at 750 rpm for 40 minutes.

## Prepare the mtDNA target amplification reaction

---

**IMPORTANT!** 5X Ion AmpliSeq™ HiFi Mix is viscous. Pipet slowly, then mix thoroughly.

---

1. Use the following table to choose the amplification method that is based on your sample type.

Sample type	Method	Go to
Low copy number samples	2-in-1	step 2
Non-degraded samples	Conservative	step 3

---

**Note:** For descriptions of the methods, see “PCR methodologies for manual library preparation” on page 12.

---

2. For the 2-in-1 method, prepare two master mixes, one for each pool:
- Prepare the master mix for Precision ID mtDNA panel Pool 1, then add to each well of a 96-well plate:

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Precision ID mtDNA panel Pool 1	10 µL
gDNA, 0.1 ng <sup>[1]</sup>	X µL <sup>[2]</sup>
Nuclease-free water	6 – X µL
<b>Total</b>	<b>20 µL</b>

<sup>[1]</sup> 0.1 ng ≈ 2,900 mtDNA copies. gDNA quantifications were used to extrapolate the copy number of mtDNA. If more than 0.1 ng of gDNA is used, appropriately adjust the number of PCR cycles in “Amplify the targets” on page 24.

<sup>[2]</sup> ≤6 µL

- Prepare the master mix for Precision ID mtDNA panel Pool 2, then add to each well of a 96-well plate:

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Precision ID mtDNA panel Pool 2	10 µL
gDNA, 0.1 ng <sup>[1]</sup>	X µL <sup>[2]</sup>
Nuclease-free water	6 – X µL
<b>Total</b>	<b>20 µL</b>

<sup>[1]</sup> 0.1 ng ≈ 2,900 mtDNA copies. gDNA quantifications were used to extrapolate the copy number of mtDNA. If more than 0.1 ng of gDNA is used, appropriately adjust the number of PCR cycles in “Amplify the targets” on page 24.

<sup>[2]</sup> ≤6 µL

- Go to step 4.

3. For the Conservative method, prepare two master mixes, one for each pool:

- Prepare the master mix for Precision ID mtDNA panel Pool 1, then add to each well of a 96-well plate:

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	2 µL
Precision ID mtDNA panel Pool 1	5 µL
gDNA, 0.1 ng <sup>[1]</sup>	X µL <sup>[2]</sup>
Nuclease-free water	3– X µL
<b>Total</b>	<b>10 µL</b>

<sup>[1]</sup> 0.1 ng ≈ 2,900 mtDNA copies. gDNA quantifications were used to extrapolate the copy number of mtDNA. If more than 0.1 ng of gDNA is used, appropriately adjust the number of PCR cycles in “Amplify the targets” on page 24.

<sup>[2]</sup> ≤3 µL

- b. Prepare the master mix for Precision ID mtDNA panel Pool 2, then add to each well of a 96-well plate:

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	2 µL
Precision ID mtDNA panel Pool 2	5 µL
gDNA, 0.1 ng <sup>[1]</sup>	X µL <sup>[2]</sup>
Nuclease-free water	3 – X µL
<b>Total</b>	<b>10 µL</b>

<sup>[1]</sup> 0.1 ng ≈ 2,900 mtDNA copies. gDNA quantifications were used to extrapolate the copy number of mtDNA. If more than 0.1 ng of gDNA is used, appropriately adjust the number of PCR cycles in “Amplify the targets”.

<sup>[2]</sup> ≤3 µL

- c. Go to step 4.
- Seal the plate with a MicroAmp™ Clear Adhesive Film. To prevent evaporation, create a tight seal by applying pressure with an applicator.
  - Vortex the plate thoroughly, then centrifuge to collect droplets. Place a MicroAmp™ Compression Pad on the plate, then go to “Amplify the targets”.

## Amplify the targets

The cycle number for target amplification depends on the panel and the amount of input DNA. Cycle numbers can be increased if the quality or quantity of input DNA is uncertain.

**IMPORTANT!** When amplifying multiple samples in a single PCR plate, ensure that the input DNA across the samples is roughly equivalent, or the PCR cycle number is based on the sample with the *lowest* quantity. This ensures that the selected cycle number for target amplification is optimal for all the samples in the run.

Cycle numbers for each panel depending on input DNA

Panel	Amount of input gDNA	Number of cycles
Precision ID mtDNA Whole Genome Panel <i>or</i>	0.1 ng (~2,900 mtDNA copies) <sup>[1]</sup>	21 cycles
Precision ID mtDNA Control Region Panel	<0.1 ng	21 cycles + 1 to 5 cycles

<sup>[1]</sup> gDNA quantification was used to extrapolate the copy number of mtDNA. The actual number of mtDNA copies varies from sample source (for example bone, blood, saliva, hair, etc.).



- To amplify target regions, run the following program:

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycle number (see preceding table)	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold <sup>[1]</sup>

<sup>[1]</sup> Store reactions at 10°C overnight on the thermal cycler. For longer-term storage, store covered at -20°C for up to one month.

- Proceed using one of the following options:

Method	Action
2-in-1 method	Transfer 10 µL from each pool into a new well, for a total of 20 µL. Continue the library preparation as if you are processing one sample. Use one barcode adapter.
Conservative method	Transfer 10 µL from Pool 2 into the well containing Pool 1, for a total of 20 µL. Continue the library preparation as if you are processing one sample. Use one barcode adapter.

**STOPPING POINT** For either method, the target amplification reactions can be stored at 10°C overnight on the thermal cycler. For longer-term storage, store covered at -20°C for up to one month.

## 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.

- Remove the plate seal, then **add 2 µL of FuPa Reagent** (brown cap) to each amplified sample. The total volume is ~22 µL.
- Seal the plate with a clear adhesive film, vortex thoroughly, then spin down to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
- Load in the thermal cycler, then setup and run the following thermal cycling conditions:

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

**STOPPING POINT** Store the plate at -20°C.

## Ligate adapters to the amplicons, then purify

You *must* ligate a different barcode to each library when:

- sequencing multiple libraries on a single chip
- sequencing multiple replicates of DNA libraries from the same sample on a single chip

The barcode adapters included in the Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate are provided at the appropriate concentration, and include forward and reverse adapters in a single well. No further handling is necessary.

---

**IMPORTANT!** When handling barcode adapters, avoid cross-contamination. After use, reseal the barcode adapter plate with adhesive film and store at  $-30^{\circ}\text{C}$  to  $-5^{\circ}\text{C}$ .

---

### Perform the ligation reaction

---

**IMPORTANT!** If there is visible precipitate in the Switch Solution, vortex or pipet up and down at room temperature to resuspend.

---

1. Carefully remove the plate seal, then add the following components to each well containing digested amplicons in the order listed.

**IMPORTANT!** Add the DNA Ligase last. Do not combine DNA Ligase and adapters before adding to digested amplicons.

Order of addition	Component	Volume
1	Switch Solution (yellow cap)	4 $\mu\text{L}$
2	Precision ID IonCode™ Barcode Adapter	2 $\mu\text{L}$
3	DNA Ligase (blue cap)	2 $\mu\text{L}$
—	<b>Total volume</b> (including ~22 $\mu\text{L}$ of digested amplicon)	<b>~30 <math>\mu\text{L}</math></b>

**Note:**

- 2-in-1 method: use one barcode
  - Conservative method: use one barcode
-

- Seal the plate with a new MicroAmp™ 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.
- Load the plate in the thermal cycler, then perform the ligation reaction using the following temperature program:

Panel	Temperature	Time
Precision ID mtDNA Whole Genome Panel or Precision ID mtDNA Control Region Panel	22°C	30 minutes
	68°C	10 minutes
	10°C	Hold (for up to 1 hour)

**STOPPING POINT** Samples can be stored overnight at 10°C on the thermal cycler. For longer periods, store at –20°C.

## Purify the libraries

### IMPORTANT!

- Bring Agencourt™ AMPure™ XP Reagent to room temperature, then vortex thoroughly to disperse the beads before use. Pipet the solution slowly.
- Freshly prepare 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.

- 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, then incubate the mixture for 5 minutes at room temperature.  
Alternatively, use a plate mixer (such as the Eppendorf™ MixMate™ mixer with the 96 × 0.2-mL PCR tube holder) to mix the bead suspension. Seal the plate, mix for 5 minutes at 2,000 rpm at room temperature, then centrifuge the plate briefly to collect droplets.
- Place the plate in a magnetic rack (such as the DynaMag™–96 Side Magnet; Cat. No. [12331D](#)), then incubate for 2 minutes or until solution clears.
- Carefully remove, then discard the supernatant without disturbing the pellet.
- 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. 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). Return the plate to the magnet, then incubate for 2 minutes or until the solution clears.

- Repeat step 5 for a second wash.

7. Ensure that all ethanol droplets are removed from the wells. Keep the plate in the magnet, then air-dry the beads at room temperature for 5 minutes. Do not overdry.

---

**Note:** Residual ethanol inhibits PCR amplification. If needed, centrifuge the plate, then remove residual ethanol before air-drying the beads.

---

## Elute the libraries

1. Remove the plate containing the library from the magnet, then add 50  $\mu$ L of Low TE to the pellet to disperse the beads.
2. Seal the plate with a MicroAmp™ Clear Adhesive Film, then vortex thoroughly.
3. Incubate for 5 minutes at room temperature, then centrifuge to collect droplets.  
Alternatively, use a plate mixer (such as the Eppendorf™ MixMate™ mixer with the 96  $\times$  0.2-mL PCR tube holder) to mix the bead suspension. Seal the plate, mix for 5 minutes at 2,000 rpm at room temperature, then centrifuge to collect droplets.

---

**IMPORTANT!** For maximum recovery, ensure that the suspension incubates for at least 5 minutes at room temperature.

---

4. Place the plate on the magnet for at least 2 minutes.

---

**STOPPING POINT** Samples can be stored with beads at 4°C for up to one month. For long-term storage at –20°C, place the plate in the magnet, then transfer the sample supernatants to a new plate. Do not store libraries at –20°C in the presence of beads.

---

## Quantify the libraries by qPCR

After eluting each Precision ID library, determine concentration by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. [4468802](#)).

### Dilute the libraries for quantification

1. If samples have been stored at 4°C, vortex the plate, then centrifuge to collect droplets.
2. Place the plate in the magnetic rack for 2 minutes, or until the supernatant clears.
3. Prepare 1:100 dilutions by removing 2  $\mu$ L of supernatant, then combine with 198  $\mu$ L of Nuclease-free Water.
4. After removing the aliquots, store the plate at 4°C.

## Quantify the libraries

Use the Ion Library TaqMan™ Quantitation Kit to analyze each sample, standard, and negative control in duplicate 20- $\mu$ L reactions.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Control Library (~68 pM; provided in the kit) at the concentrations listed in the following table. Label them as standards, then use these concentrations in the qPCR experiment setup.

Standard	Control Library volume	Nuclease-free water volume	Concentration
1	5 $\mu$ L (undiluted)	45 $\mu$ L	6.8 pM
2	5 $\mu$ L Std 1	45 $\mu$ L	0.68 pM
3	5 $\mu$ L Std 2	45 $\mu$ L	0.068 pM

2. Prepare sufficient reaction mixture for replicate reactions for each sample, negative control, and control library dilution. Add an extra reaction to compensate for pipetting error. For each reaction, combine 10  $\mu$ L of Ion Library qPCR Master Mix and 1  $\mu$ L of Ion Library TaqMan™ Quantitation Assay, 20X in a tube, then mix thoroughly.

Component	Volume (1 reaction)
Ion Library TaqMan™ qPCR Mix	10 $\mu$ L
Ion Library TaqMan™ Quantitation Assay, 20X	1 $\mu$ L

3. Aliquot 11  $\mu$ L into each reaction well (two wells per reaction) of a PCR plate.
4. Add 9  $\mu$ L of the diluted (1:100) sample library, each control library dilution, or negative control to reaction wells, for a total reaction volume per well of 20  $\mu$ L.
5. Set up the real-time PCR instrument.
  - a. Enter the concentrations of the control library standards.
  - b. Select ROX™ Reference Dye as the passive reference dye.
  - c. Enter a reaction volume of 20  $\mu$ L.
  - d. Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.

e. Enter the following run parameters, depending on your system.

Real-time PCR System	Stage	Temperature	Time
7500 Real-Time PCR Instrument with SDS Software v1.2.3	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	32 seconds
7500 Real-Time PCR Instrument with HID Real-Time PCR Analysis Software v1.1 or later	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	30 seconds
QuantStudio™ 5 Real-Time PCR System	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	30 seconds

6. Run the reactions, then collect the real-time data.

See “Dilute the libraries” on page 33 for library concentrations required for template preparation. Depending on your quantification results, proceed with one of the following options:

- If sufficient library was prepared, continue to “Dilute the libraries” on page 33.
- If insufficient library was prepared, continue to “(Optional) Amplify and purify the libraries” on page 31.
- Continue with less than optimal library concentration and proceed to “Dilute the libraries” on page 33. See Appendix A, “Troubleshooting” for effects of using low library concentration.

## (Optional) Amplify and purify the libraries

A library that yields less than the recommended concentration can be rescued by library amplification. Amplified libraries need to be purified before quantification and use.

### Amplify the libraries

1. Combine 25  $\mu\text{L}$  of each unamplified library (total undiluted library is  $\sim 50 \mu\text{L}$ , from “Elute the libraries” on page 28) with 72  $\mu\text{L}$  of Platinum™ PCR SuperMix HiFi and 3  $\mu\text{L}$  of Library Amplification Primer Mix from the Precision ID Library Kit in one well of a 96-well PCR plate.

---

**Note:** The Platinum™ PCR SuperMix HiFi and Library Amplification Primer Mix can be combined before addition.

---

2. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge briefly 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. Load the plate in a thermal cycler, then run the following program:

Stage	Temperature	Time
Hold	98°C	2 minutes
5–10 cycles	98°C	15 seconds
	64°C	1 minute
Hold	10°C	Hold (for up to 24 hours)

---

**STOPPING POINT** Samples can be held overnight or up to 24 hours at 10°C on the thermal cycler. For longer periods, store at  $-20^{\circ}\text{C}$ .

---

### Purify the amplified libraries

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  $\mu\text{L}$  of ethanol with 100  $\mu\text{L}$  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 centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.
2. **Add 50  $\mu\text{L}$  (0.5X sample volume) of Agencourt™ AMPure™ XP Reagent** to each plate well containing  $\sim 100 \mu\text{L}$  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 32 above, **add 120  $\mu\text{L}$  (1.2X original sample volume) of Agencourt™ AMPure™ XP Reagent**. Mix the bead suspension with the DNA thoroughly by pipetting up and down 5 times.
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  $\mu\text{L}$  of freshly prepared 70% ethanol** to each well, then move the plate side to side in the magnet to wash the beads. Remove and 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 mix by pipetting up and down 5 times (with the pipettor set at  $100 \mu\text{L}$ ), 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 overdry.**
7. Remove the plate from the magnet, then **add 50  $\mu\text{L}$  of Low TE** to the pellet to disperse the beads.



8. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge to collect droplets.  
Alternatively, use a plate mixer (such as the Eppendorf™ MixMate™ mixer with the 96 × 0.2-mL PCR tube holder) to mix the bead suspension. Seal the plate, mix for 5 minutes at 2,000 rpm at room temperature, then centrifuge to collect droplets.
9. Incubate at room temperature for at least 2 minutes.
10. Place the plate in the magnet for at least 2 minutes, then analyze an aliquot of the supernatant as described in “Quantify the libraries by qPCR” on page 28.

---

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

---

## Dilute, pool, and store the libraries

### Dilute the libraries

1. After the run is complete, calculate the average concentration of each undiluted library using the following equation:

Avg concentration of undiluted library = (qPCR mean quantity) × (library dilution)

For example:

- qPCR mean quantity: 3 pM
- Sample library dilution: 100

The average concentration of the undiluted library: (3 pM) × (100) = 300 pM

2. Dilute libraries as described in the following table.

Recommended library dilutions for the Ion Chef™ System

Panel	Dilute to	Minimum volume	Templating size in Planned Run setup
Precision ID mtDNA Whole Genome Panel <i>or</i> Precision ID mtDNA Control Region Panel	30 pM	25 µL	200 bp

---

**Note:** To ensure accurate dilution of sample library, avoid pipetting volumes of 1 µL or less. For the example of a 1:15 dilution, dilute 2 µL of sample library in 28 µL of Low TE or nuclease-free water.

---

## (Optional) Pool the libraries

---

**IMPORTANT!** The quality of sequencing data relies on achieving the correct concentration of starting library.

---

After diluting the sample library to its target concentration (pM), pool equal volumes of multiple diluted libraries. Use the pooled libraries in template preparation reactions on the Ion Chef™ Instrument.

Use the following recommendations for the number of manually-prepared sample libraries loaded per chip. The recommendations are based on full mitochondrial genome coverage for whole genome, and with at least 100X coverage. You may need to adjust the number of samples per chip based on your individual coverage requirements, sample quality, and throughput.

---

**Note:** Ensure that the volume of the pooled library, or single library, is  $\geq 25$   $\mu\text{L}$ .

---

Panel	Samples per Ion S5™ Chip		
	Ion 510™ Chip <sup>[1]</sup>	Ion 520™ Chip	Ion 530™ Chip <sup>[2]</sup>
Precision ID mtDNA Control Region Panel	37	56	—
Precision ID mtDNA Whole Genome Panel	—	25	32

<sup>[1]</sup> Ion 510™ Chip not supported with Precision ID mtDNA Whole Genome Panel.

<sup>[2]</sup> Ion 530™ Chip not supported with Precision ID mtDNA Control Region Panel.

## Store the libraries

Store both diluted and undiluted libraries at 2°C to 8°C for up to 1 month. For long-term storage, store libraries at -30°C to -10°C.

---

**Note:** Ensure that Agencourt™ AMPure™ XP beads are removed before storing libraries at -30°C to -10°C.

---

# 3

## Prepare libraries using the HID Ion Chef™ Instrument

- Software version requirements for library preparation ..... 35
- Workflow: Prepare libraries using the Ion Chef™ Instrument ..... 36
- Extract, then quantify input DNA ..... 37
- Dilute the gDNA samples ..... 37
- Thaw the reagents, then prepare the instrument ..... 37
- Create a sample set ..... 37
- Add Precision ID primer pools to Positions A and B of the Reagents cartridge ..... 38
- Add the DNA to the Precision ID DL8 IonCode™ Barcode Adapters ..... 39
- Load the Ion Chef™ Instrument for library preparation ..... 41
- Start the Ion Chef™ run ..... 43
- Unload the Ion Chef™ Instrument ..... 47
- Quantify the combined library by qPCR ..... 48
- Dilute, pool, and store the combined libraries ..... 50
- Clean the Ion Chef™ Instrument ..... 51

This chapter contains brief procedures for automated HID library preparation on the Ion Chef™ Instrument. For complete instrument procedures, troubleshooting and maintenance information, see *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) and *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854).

If you are preparing libraries manually, see Chapter 2, “Prepare libraries manually”.

### Software version requirements for library preparation

Panel	Software version required
Precision ID mtDNA Control Region Panel	Torrent Suite™ Software 5.18, or the latest HID-supported version
Precision ID mtDNA Whole Genome Panel	

## Workflow: Prepare libraries using the Ion Chef™ Instrument

“Extract, then quantify input DNA” on page 22



“Dilute the gDNA samples” on page 37



“Thaw the reagents, then prepare the instrument” on page 37



“Create a sample set” on page 37



“Add Precision ID primer pools to Positions A and B of the Reagents cartridge” on page 38



“Add the DNA to the Precision ID DL8 IonCode™ Barcode Adapters” on page 39



“Load the Ion Chef™ Instrument for library preparation” on page 41



“Start the Ion Chef™ run” on page 43



“Unload the Ion Chef™ Instrument” on page 47



“Dilute the combined libraries” on page 50

## Extract, then quantify input DNA

### Guidelines for mitochondrial DNA input per reaction (Ion Chef™)

- Use 0.3 ng of input gDNA per sample well for the mitochondrial panels.
- If you are using the Quantifiler™ HP or Quantifiler™ Trio DNA Quantification Kit, estimate the mtDNA input by using 10% of the gDNA Small Amplicon (SA) quantity. For example, for non-degraded samples use 0.3 ng of gDNA with the Ion Chef™.
- See “Mitochondrial DNA extraction kits” on page 18 for a recommended mtDNA extraction kit.
- **IMPORTANT!** If you are using the PrepFiler Express BTA™ Forensic DNA Extraction Kit to extract mtDNA from non-BTA substrates such as blood or buccal, perform this modification: During the lysis, incubate the column/tube assembly at 56°C, then shake at 750 rpm for 40 minutes.

### Dilute the gDNA samples

Dilute samples to 20 pg/μL with nuclease-free water. Prepare 15 μL of each diluted sample (0.3 ng total) to prepare up to 8 libraries per Ion Chef™ run.

### Thaw the reagents, then prepare the instrument

1. Before the run, thaw one Precision ID DL8 Reagents cartridge at room temperature for 20 minutes.
2. If needed, thaw Precision ID primer pools.
3. If not performed after a previous run, unload, then clean the Ion Chef™ Instrument (see “Clean the Ion Chef™ Instrument” on page 51).
4. Verify that the Ion Chef™ Instrument has a connection to the Torrent Server. On the Ion Chef™ home touchscreen, tap **Settings**, then **Torrent Server** to view the connection status of your instrument.

**Note:** If the instrument is not connected, see the *Ion Chef™ and Torrent Server Network Setup User Guide* (Pub. No. MAN0013444) for instructions on how to configure a direct or indirect network connection of the Ion Chef™ Instrument to a Torrent Server.

### Create a sample set

1. Sign into the Torrent Suite™ Software.
2. In the **Plan** tab, click **Samples**, then click **Add or Update Sample Set/Samples**.

3. Click **Enter New Sample**.

a. In the **Add Sample** dialog box, enter the following information for each sample in the library preparation.

- **Sample Name**—enter a sample name.
- **PCR Plate Position**—select a plate position of A to H.
- **Barcode Kit**—enter *IonCode Barcodes 1–32*.
- **Barcode**—enter the barcode for the appropriate IonCode Plate and plate position. For example, *IonCode\_0101 for Red plate, well A*.

b. Click **Done**.

Your new samples and sample attributes appear in the **Enter Samples** list.

c. Repeat for each sample in the library preparation.

4. Click **Create Sample Set** to add the samples to a sample set.

a. Enter the following information for the Sample Set.

- **Sample Set Name**—enter a name for the sample set.
- **Group Type**—select **DNA and Fusions**.
- **Library Prep Type**—enter **AmpliSeq on Chef**.
- **Library Prep Kit**—**Precision ID Chef DLB**.

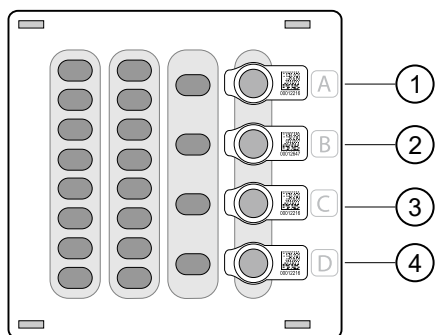
b. Click **Save Sample Set**.

## Add Precision ID primer pools to Positions A and B of the Reagents cartridge

1. Uncap all 4 tubes in positions A, B, C, and D in the Precision ID DL8 Reagents cartridge. Save the caps.
2. Add primer pools to the Precision ID DL8 Reagents cartridge using the following guidelines (even if processing fewer than 8 samples):

If you are using the	Action
Precision ID mtDNA Whole Genome Panel or Precision ID mtDNA Control Region Panel	Pipet 150 µL of Pool 1 into the Position A tube, and 150 µL of Pool 2 into the Position B tube. <sup>[1]</sup>

<sup>[1]</sup> The Ion Chef™ Instrument performs similarly to the 2-in-1 method, as described on page “PCR methodologies for manual library preparation” on page 12 .



- ① Position A (150  $\mu$ L Pool 1)
- ② Position B (150  $\mu$ L Pool 2)
- ③ Position C (Empty tube)
- ④ Position D (Output tube)

---

**Note:**

- When the run is complete, the tube in Position D contains 700  $\mu$ L of combined barcoded libraries at a concentration of approximately 100 pM with 0.3 ng of input DNA.
  - If input DNA is <0.3 ng, then library concentration can be <100 pM, and library quantification with qPCR is required.
- 

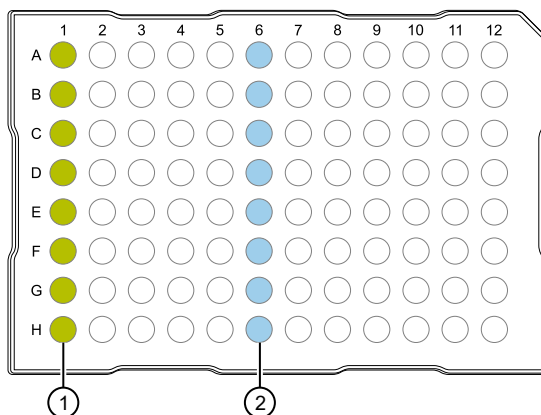
## Add the DNA to the Precision ID DL8 IonCode™ Barcode Adapters

1. Remove the plate seal from a Precision ID DL8 IonCode™ Barcode Adapters Plate (provided), then discard.
2. Pipet 15  $\mu$ L of each DNA sample (20 pg/ $\mu$ L, 0.3 ng total) into wells A1 to H1 of the plate as shown in the following figure.

---

**IMPORTANT!** Carefully inspect each well for air bubbles. Remove any air bubbles by gentle pipette mixing. Alternatively, centrifuge the plate briefly in a plate centrifuge.

---



- ① Column 1 wells containing 15  $\mu$ L of each diluted DNA sample (20  $\mu$ g/ $\mu$ L).
- ② Column 6 wells containing 8 dried-down IonCode™ barcodes. Lowest number is in A6 and highest is in H6. All appear light blue in the actual plates.

---

**Note:** If processing fewer than 8 samples, it is preferable to add replicates or positive control samples to the run. Otherwise, pipet 15  $\mu$ L of nuclease-free water into column 1 wells that do not contain a DNA sample.

---

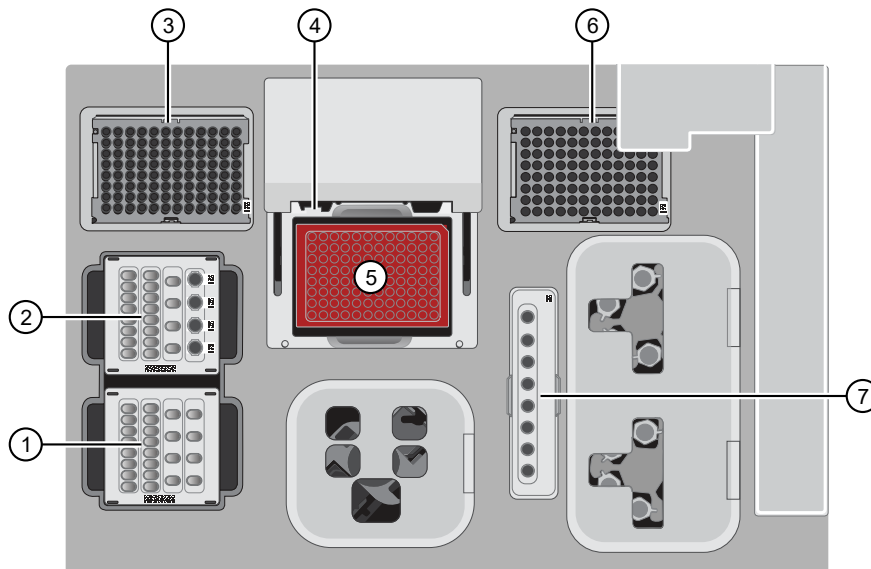
**Note:** If processing 5 or fewer samples, quantify your output combined library by qPCR to ensure that an optimal concentration is used in templating reactions.

---




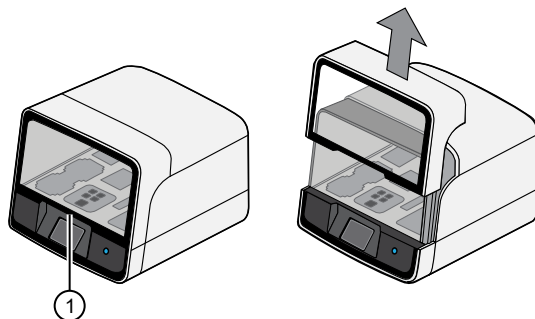
## Load the Ion Chef™ Instrument for library preparation

Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure.



- ① Precision ID DL8 Solutions cartridge
- ② Precision ID DL8 Reagents cartridge
- ③ Ion AmpliSeq™ Tip Cartridge L8
- ④ Framed PCR Foil Seal
- ⑤ Precision ID DL8 IonCode™ Barcode Adapters 96 Well Plate and PCR Plate Frame
- ⑥ Empty Tip Cartridge L8
- ⑦ Enrichment Cartridge

1. Open the instrument door:
  - a. On 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 and lift

2. Load the Precision ID DL8 Solutions cartridge into the Solutions station.
  - a. Gently tap the cartridge on the bench to force the reagents to the bottoms of the tubes.

- b. Load the cartridge into the Solutions station so that it snaps into place, and is level on the deck.
3. Load the Precision ID DL8 Reagents cartridge into the Reagents station.
  - a. To force the reagents to the bottoms of the tubes, gently tap the cartridge on the bench and verify that all the liquid is at the bottom, and not splashed on the side of the tubes.
  - b. Load the cartridge into the Reagents station so that it snaps into place, and is level on the deck.

---

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

---

---

**IMPORTANT!** Ensure that 4 flagged tubes are uncapped, then loaded in Positions A–D of the Reagents cartridge, and Primer Pools are loaded in Positions A and B.

---

4. Load a new Ion AmpliSeq™ Tip Cartridge L8 into the New Pipette Tip station (left side of deck).
  - a. Unwrap the Ion AmpliSeq™ Tip Cartridge L8, then remove the cover to expose the pipette tips.
  - b. Pull the tip station catch backwards to open the locking bracket. Load the Ion AmpliSeq™ Tip Cartridge L8, then push the locking bracket closed.

---

**IMPORTANT!** If you do not close the locking bracket, the run will fail.

---

5. Load an empty tip cartridge from a previous run into the Used Tip station.
6. Load the Precision ID DL8 IonCode™ Barcode Adapters 96 Well Plate containing gDNA onto the thermal cycler block and press down to seat it.
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.

---

8. Load the Enrichment Cartridge into the Enrichment station, then press down on the cartridge to ensure that it is level with the instrument deck.
9. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door so that the lower locks engage.

---

**IMPORTANT!** After closing the door, confirm that both sides of the door are locked down.

---

## Start the Ion Chef™ run

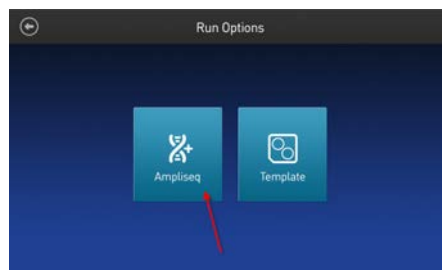
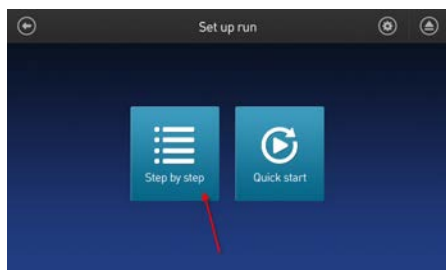
Perform the following steps to start an Ion AmpliSeq™ run on the Ion Chef™ Instrument.

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



2. Tap **Step by step**, then tap **AmpliSeq** on the **Run Options** screen.

**Note:** To bypass the step by step deck loading guide, tap **Quick start**.



3. Ensure that you have loaded the Ion Chef™ deck with Precision ID DL8 Kit consumables by advancing through the step by step deck loading steps on the instrument touchscreen.
4. Tap **Start check** on the **Close Door** screen. The Ion Chef™ Instrument performs a Deck Scan.

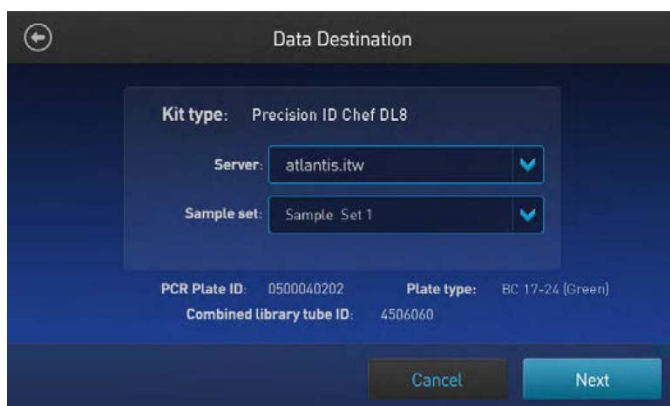


---

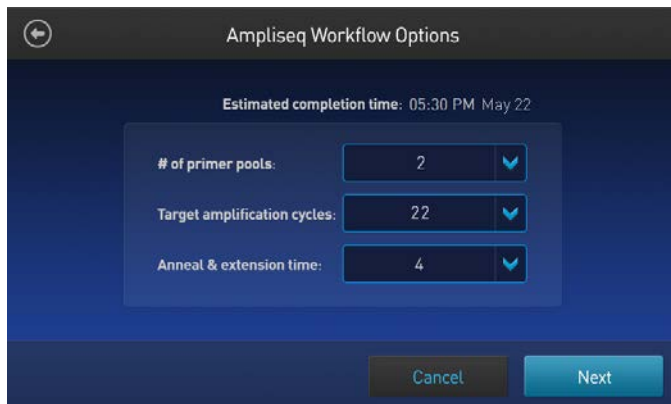
**Note:** If the PCR plate is not recognized, select the appropriate plate when prompted. Because no sample set was selected or planned in the Torrent Server, the following warning appears: "No sample Set detected. Do you want to continue?" Tap **OK**.

---

5. After Deck Scan completes (~3 minutes), tap **Next**.
6. In the **Data Destination** screen perform the following actions, then tap **Next**.
  - Verify the server information.
  - Choose the sample set created on page 37.



- Enter the appropriate number of primer pools, target amplification cycles, and an anneal/extension time for your run, then tap **Next**.



Panel recommendations for Ion AmpliSeq™ Workflow Options shown above.

Panel	Amount of input gDNA added	# of primer pools	Cycle number	Anneal & extension time
Precision ID mtDNA Whole Genome Panel or Precision ID mtDNA Control Region Panel	0.3 ng (~8,700 copies) <sup>[1]</sup>	2	22 cycles	4
	<0.3 ng	2	22 cycles + 1 to 5 cycles	4

<sup>[1]</sup> gDNA quantifications were used to extrapolate the copy number of mtDNA. The actual number of mtDNA copies varies with sample source (for example; bone, blood, saliva, hair).

- Review panel and amplification entries for the run in the **AmpliSeq Confirmation Window**, then tap **Start Run**.
- After approximately 7 hours, return to the Ion Chef™ Instrument. On the **Run Complete** screen, tap **Next** to go to the unloading and cleaning steps.



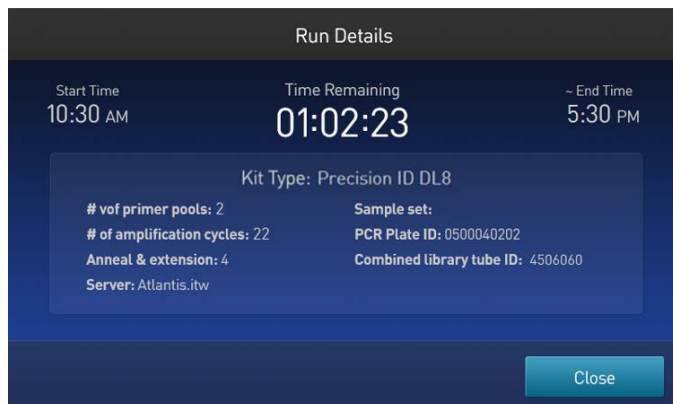
---

**IMPORTANT!** After a run, the Ion Chef™ Instrument holds the barcoded libraries in the tube that is loaded in Position D of the Reagents cartridge. To avoid fluid loss due to evaporation, remove, then cap the tube of combined barcoded libraries as soon as possible after run completion. Do not leave the tube in the instrument longer than 24 hours after the start of the run. After 24 hours from the start of the run, the instrument chiller will stop actively cooling, and the sample will be held at 27°C.

---

**Note:** View the run information in the **Run Details** screen.

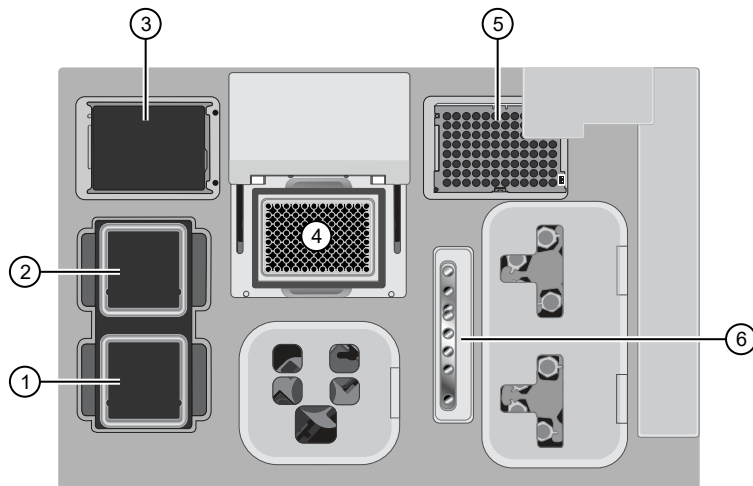
---



You can also monitor your run in Torrent Suite™ Software by navigating to **Monitor ▶ Ion Chef** and viewing the **Library Prep Progress** bar and **Library Prep Status** associated with your sample.


## Unload the Ion Chef™ Instrument

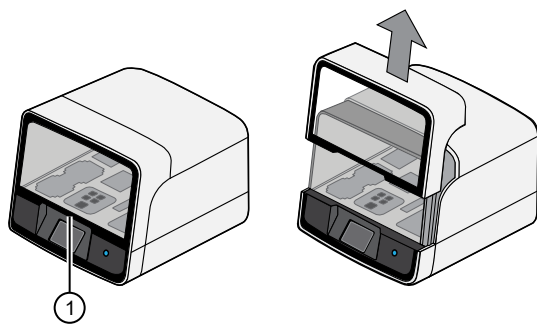
Remove used consumables from the instrument from the indicated stations, and remove the tube containing the combined library from the Reagents cartridge.



- ① Solutions station
- ② Reagents station
- ③ New Pipette Tip station: move the empty Tip Cartridge to the Used Pipette Tip station
- ④ Thermal cycler sample block
- ⑤ Used Pipette Tip station
- ⑥ Enrichment station

**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 and lift

- 2. Remove the Precision ID DL8 Reagents cartridge. Remove and cap the combined library tube from Position D, then discard the cartridge.
- 3. Remove, then discard the Precision ID DL8 Solutions cartridge.

4. Remove, then discard the Precision ID DL8 IonCode™ Barcode Adapters 96 Well Plate with the PCR Plate Frame and Frame Seal v2 from the PCR sample block in unison.

---

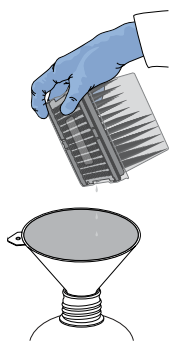
**IMPORTANT!** Do not attempt to separate the PCR Plate Frame from the PCR Plate and Frame Seal v2, as this may cause PCR product to splash and contaminate the instrument deck.

---

5. 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. Appropriately discard the liquid waste by tipping the reservoir on one corner, then pouring the waste into a waste container:



---

**IMPORTANT!** Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge L8 from the new tip position to the waste tip position.

---

6. Move the empty Tip Cartridge L8 from the New Pipette Tip station to the Used Pipette Tip station.

---

**IMPORTANT!** Do not discard the empty Tip Cartridge L8.

---

7. Remove, then discard the Enrichment Cartridge.

## Quantify the combined library by qPCR

After unloading the Ion Chef™ Instrument, determine the concentration of the combined library pools by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. [4468802](#)).

### Dilute the combined library for quantification

1. If the combined library tube has been stored at 4°C, vortex the tube, then centrifuge briefly to collect droplets.
2. Prepare a 1:100 dilution by combining 2 µL with 198 µL of nuclease-free water.
3. After removing the aliquot, store the tube at 4°C.



## Quantify the combined library by qPCR

Use the Ion Library TaqMan™ Quantitation Kit to analyze the combined sample library, 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; provided in the kit) at the concentrations listed in the following table. Label them as standards, then use these concentrations in the qPCR experiment setup.

Standard	Control Library volume	Nuclease-free water volume	Concentration
1	5 µL (undiluted)	45 µL	6.8 pM
2	5 µL Std 1	45 µL	0.68 pM
3	5 µL Std 2	45 µL	0.068 pM

2. Prepare sufficient reaction mixture for replicate reactions for each sample, negative control, and control library dilution. Add an extra reaction to compensate for pipetting error. For each reaction, combine 10 µL of Ion Library qPCR Master Mix and 1 µL of Ion Library TaqMan™ Quantitation Assay, 20X in a tube, then mix thoroughly.

Component	Volume (1 reaction)
Ion Library TaqMan™ qPCR Mix	10 µL
Ion Library TaqMan™ Quantitation Assay, 20X	1 µL

3. Aliquot 11 µL into each reaction well (two wells per reaction) of a PCR plate.
4. Add 9 µL of the diluted (1:100) sample library, control library dilution, or negative control to reaction wells, for a total reaction volume per well of 20 µL.
5. Set up the real-time PCR instrument.
  - a. Enter the concentrations of the control library standards.
  - b. Select ROX™ Reference Dye as the passive reference dye.
  - c. Enter a reaction volume of 20 µL.
  - d. Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.

- e. Enter the following run parameters, depending on your system.

Real-time PCR System	Stage	Temperature	Time
7500 Real-Time PCR Instrument with SDS Software v1.2.3	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	32 seconds
7500 Real-Time PCR Instrument with HID Real-Time PCR Analysis Software v1.1 or later	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	30 seconds
QuantStudio™ 5 Real-Time PCR System	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	30 seconds

6. Run the reactions, then collect the real-time data.

Proceed to “Dilute, pool, and store the combined libraries”.

## Dilute, pool, and store the combined libraries

### Dilute the combined libraries

**IMPORTANT!** Dilute the combined libraries to the optimal input concentration before proceeding to template preparation. The quality of sequencing data relies on achieving the correct concentration of starting library.

Dilute libraries as described in the following table. Then use polyclonality and low-quality filter results from a sequencing run performed with ISPs templated at the starting concentration to titrate up or down to achieve optimal concentrations, if needed.

Panel	Dilute to	Minimum volume	Templating size in Planned Run setup
Precision ID mtDNA Whole Genome Panel <i>or</i> Precision ID mtDNA Control Region Panel	30 pM	25 µL	200 bp

## (Optional) Combine library pools

After diluting library pools prepared on the Ion Chef™ Instrument to the target concentration (pM), you can "super-pool" libraries by combining equal volumes of the library pools, provided the pools were prepared with different Precision ID DL8 IonCode™ Barcode Adapters. Use the combined library pools in template preparation reactions on the Ion Chef™ Instrument.

Use the following recommendations for the number of Ion Chef™-prepared sample libraries loaded per chip. The recommendations are based on full mitochondrial genome coverage for whole genome, and at least 100X coverage. You may need to adjust the number of samples per chip based on your individual coverage requirements, sample quality, and throughput.

Panel	Samples per Ion S5™ Chip		
	Ion 510™ Chip <sup>[1]</sup>	Ion 520™ Chip	Ion 530™ Chip <sup>[2]</sup>
Precision ID mtDNA Control Region Panel	32 <sup>[3]</sup>	32 <sup>[3]</sup>	—
Precision ID mtDNA Whole Genome Panel	—	25	32 <sup>[3]</sup>

<sup>[1]</sup> Ion 510™ Chip not supported with Precision ID mtDNA Whole Genome Panel.

<sup>[2]</sup> Ion 530™ Chip not supported with Precision ID mtDNA Control Region Panel.

<sup>[3]</sup> The number of Precision ID DL8 IonCode™ Barcode Adapters is currently limited to 32.

## Store the libraries

Store both diluted and undiluted libraries at 2°C to 8°C for up to 1 month. For long-term storage, store libraries at –30°C to –10°C.

## Clean the Ion Chef™ Instrument

### 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 ensure minimal contamination.

---

## Clean the Ion Chef™ Instrument

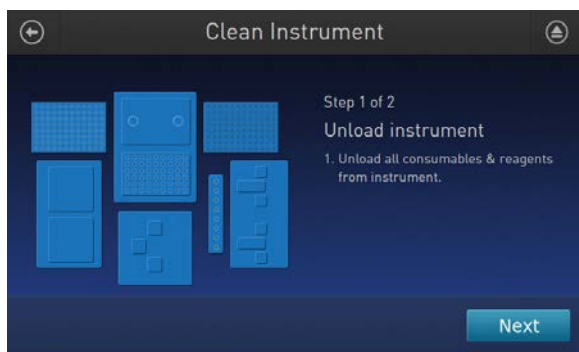
**IMPORTANT!** Clean the Ion Chef™ Instrument after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.

1. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.
2. On the Ion Chef™ Instrument touchscreen that appears after run completion, tap **Next**.



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

3. Ensure that you have removed all consumables from the Ion Chef™ Instrument, 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 and illuminates the ultraviolet (UV) light in the instrument.



**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.

# 4

## Prepare the template on the HID Ion Chef™ Instrument

- Software version requirements for template preparation ..... 54
- Required materials ..... 55
- Workflow: Template preparation on the Ion Chef™ Instrument ..... 56
- Create an NGS mtDNA Planned Run ..... 57
- Dilute the libraries for template preparation on the Ion Chef™ Instrument ..... 60
- Prepare the libraries and consumables ..... 60
- Load the Ion Chef™ System ..... 61
- Start the Ion Chef™ run ..... 72
- Unload the chips for sequencing ..... 76
- Clean the Ion Chef™ System ..... 77

This chapter contains brief procedures for automated HID template preparation. During template preparation on the Ion Chef™ Instrument, target sequences in a sample library are clonally amplified on Ion Sphere™ Particles (ISPs), which are then loaded by the instrument onto a sequencing chip. For complete instrument procedures, troubleshooting and maintenance information, see the *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854).

### Software version requirements for template preparation

Panel	Software version required
Precision ID mtDNA Control Region Panel	Torrent Suite™ Software 5.18, or the latest HID-supported version
Precision ID mtDNA Whole Genome Panel	

## Required materials

### Ion S5™ Precision ID Chef & Sequencing Kit components

Component	Part No.	Quantity per kit	
		A33208	A35850
Ion S5™ Precision ID Chef Supplies	A33209	4 boxes	4 boxes
Ion S5™ Precision ID Chef Reagents	A32882	1 box	1 box
Ion S5™ Precision ID Chef Solutions	A33210	1 box	1 box
Ion S5™ Precision ID Sequencing Reagents	A32883	1 box	2 boxes
Ion S5™ Precision ID Sequencing Solutions	A33211	1 box	2 boxes

### Compatible Ion Chip Kits

Chip	Cat. No.	Quantity	Storage
Ion 510™ Chip Kit (2 × 4-pack)	A34292	8 chips	15°C to 30°C
Ion 520™ Chip Kit (2 × 4-pack)	A27762	8 chips	
Ion 530™ Chip Kit (2 × 4-pack)	A27764	8 chips	

## Workflow: Template preparation on the Ion Chef™ Instrument

“Create an NGS mtDNA Planned Run” on page 57



“Dilute the libraries for template preparation on the Ion Chef™ Instrument” on page 60



“Prepare the libraries and consumables” on page 60



“Load the Ion Chef™ System” on page 61



“Start the Ion Chef™ run” on page 72



“Unload the chips for sequencing” on page 76



“Clean the Ion Chef™ System” on page 77



## Create an NGS mtDNA Planned Run

**Note:** A Planned Run must be created for each chip.

1. Sign in to Torrent Suite™ Software.
2. Select **Plan ▶ Templates**.
3. In the navigation pane, click **Human Identification**, then click the appropriate panel template.
  - **Precision ID mtDNA Control Region Panel - S5**
  - **Precision ID mtDNA Whole Genome Panel - S5**

The Planned Run template opens to the **Plan** screen.

4. In the **Plan** screen, complete each option according to the following table.

**Note:** Sample name information is transferred to Converge™ Software with the results.

Option	Action	
	Precision ID mtDNA Control Region Panel	Precision ID mtDNA Whole Genome Panel
Run Plan Name (required)	Enter a name for the run plan.	
Analysis Parameters	Select <b>Default (Recommended)</b> .	
Reference Library	Select PrecisionID_mtDNA_rCRS(Mito)	
Target Regions	Select Precision_ID_mtDNA_Control_Region_Panel_Targets_vX.X.bed.	Select Precision_ID_mtDNA_Whole_Genome_Panel_Targets_vX.X.bed.
Hotspot Regions	Select <b>None</b> .	
Use same reference & BED files for all chips	Leave the option selected.	
Number of chips	Enter the appropriate number of chips.	
Sample (required)	Enter a name for each sample.	
Sample ID	If not including a control, <b>Sample ID</b> is optional. Controls are not supported for Precision ID mtDNA panels in the current release of Converge™ Software.	
Sample Description	<i>(Optional)</i> If you have already created a case for this sample in Converge™ Software, enter the Case ID.	

(continued)

Option	Action	
	Precision ID mtDNA Control Region Panel	Precision ID mtDNA Whole Genome Panel
Reference	Leave blank.	
Sample Tube Label	Leave blank (recommended); the information is auto-populated during the Ion Chef™ template preparation run.	
Chip Barcode	Leave blank; the information is auto-populated after the Ion Chef™ template preparation run.	
Bead Loading (%)	Use default (30)	
Key Signal (1-100)	Use default (30)	
Usable Sequence (%)	Use default (30)	

- Click the **Ion Reporter** step, confirm the following options, then click **Next**.
  - Ion Reporter Account**—None (default)
  - Sample Grouping**—Self (default)
- In the **Research Application** screen, confirm the following options, then click **Next**.
  - Research Application**—Human Identification (default)
  - Target Technique**—AmpliSeq DNA (default)
- In the **Kits** screen, confirm or enter information for each option according to the following table, then click **Next**.

---

**Note:** Many fields are auto-populated after selection of the panel Planned Run template.

---

Option	Action
Instrument	Select the appropriate instrument: Torrent Suite™ Software 5.18, or the latest HID-supported version—Ion GeneStudio™ S5 System
Chip Type	Select the appropriate chip: Ion 510™ Chip, Ion 520™ Chip, or Ion 530™ Chip.
Sample Preparation Kit	Leave blank.
Control Sequence	Leave blank.
Library Kit Type	Select the appropriate library kit: <ul style="list-style-type: none"> <li>For manual library preparation—Select Precision ID Library Kit.</li> <li>For Ion Chef™ library preparation—Select Precision ID Chef DL8.</li> </ul>
Barcode Set	Confirm or select IonCode.

(continued)


Option	Action
Template Kit	Confirm or select <b>IonChef</b> , then select <b>Precision ID Chef Reagents</b> from the dropdown list.
Sequencing Kit	Confirm or select <b>Precision ID S5 Sequencing Kit</b> .
Flows	Auto-populated.
Advanced Settings	Use the recommended defaults.

8. In the **Plugins** screen, click **Clear**, select **HIDGenotyper-x**, then click the blue **Configure** link.

9. In the **Genotyper** tab of the **Configure Plugin** dialog, select the following options.

Option	Action	
	Precision ID mtDNA Control Region Panel	Precision ID mtDNA Whole Genome Panel
References	Select <b>PrecisionID_mtDNA_rCRS(Mito)</b> .	
Regions to analyze	Select one of the following: <ul style="list-style-type: none"> <li>• <b>HV1 &amp; HV2 control regions</b>— regions 16024–16365 and 73–340</li> <li>• <b>HV1, HV2 &amp; HV3 control regions</b>—regions 16024–16365, 73–340, and 438–576</li> <li>• <b>Control region (16024–576)</b></li> </ul>	Select <b>whole genome</b> .

10. (Optional) Upload the default/recommended analysis settings.


- a. Click  **Load parameters from file** in the top-right corner of the **Configure Plugin** dialog.
- b. Click **Choose File**, then follow the instructions on the screen to upload the **Precision\_ID\_mtDNA\_Panel\_AnalysisParams\_vX.X.json** file.

11. In the **Mito** tab of the **Configure Plugin** dialog, review the default plugin analysis settings. (If needed) Edit the **Coverage**, **General**, and/or **Thresholds** settings.

---

**Note:** For detailed information on the plugin analysis settings, see the *Converge™ Software Help*.

---

12. (Optional) If you edited any settings, click  **Download parameters to file** in the top-right corner of the **Configure Plugin** dialog to save the changes to an **analysisParams.json** file for future import.

13. Click **Save Changes** to close the **Configure Plugin** dialog, then click **Next**.

14. (Optional) In the **Projects** screen, assign a project, then click **Next**.

- Select an existing project from the list, or
- Click **Add Project**, then enter one or more project names

15. In the **Plan** screen, confirm the information.
16. Click **Plan Run**, then continue with template preparation and sequencing.

## Dilute the libraries for template preparation on the Ion Chef™ Instrument

Ensure that each sample library (or pooled sample libraries) has been previously diluted. If you prepared the libraries manually, see “Dilute the libraries” on page 33. If you prepared the libraries using the Ion Chef™ Instrument, see page 50.

### Prepare the libraries and consumables

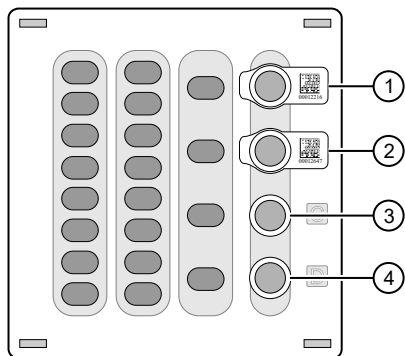
1. Unpack the Ion S5™ Precision ID Chef Reagents cartridge 45 minutes before use, then allow it to warm to room temperature.

---

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

---

2. Pipet 25 µL of diluted library pool (see “Dilute the libraries” on page 33 or “Dilute the combined libraries” on page 50) to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes in the following figure).



**Figure 3** Ion S5™ Precision ID Chef Reagents cartridge

- ① Position A (DNA library pool)
- ② Position B (DNA library pool)
- ③ Position C (NaOH)
- ④ Position D (Empty tube)

---

**Note:** If running the Human CEPH Control 200 Library from the Ion S5™ Controls Kit (Cat. No. A27760), prepare the control library for use by diluting 1 µL of stock library into 52-µL nuclease-free water.

---

3. Cap, then store the two diluted DNA libraries on ice until you are ready to load them onto the Ion Chef™ Instrument.

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

Prepare the following:

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

---

**IMPORTANT!** Before use, gently tap the Solutions and Reagents cartridges 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™ Precision ID Chef Reagents cartridge. If present, load the cartridge as directed – the precipitate dissolves when the reagents are mixed during instrument operation.

---

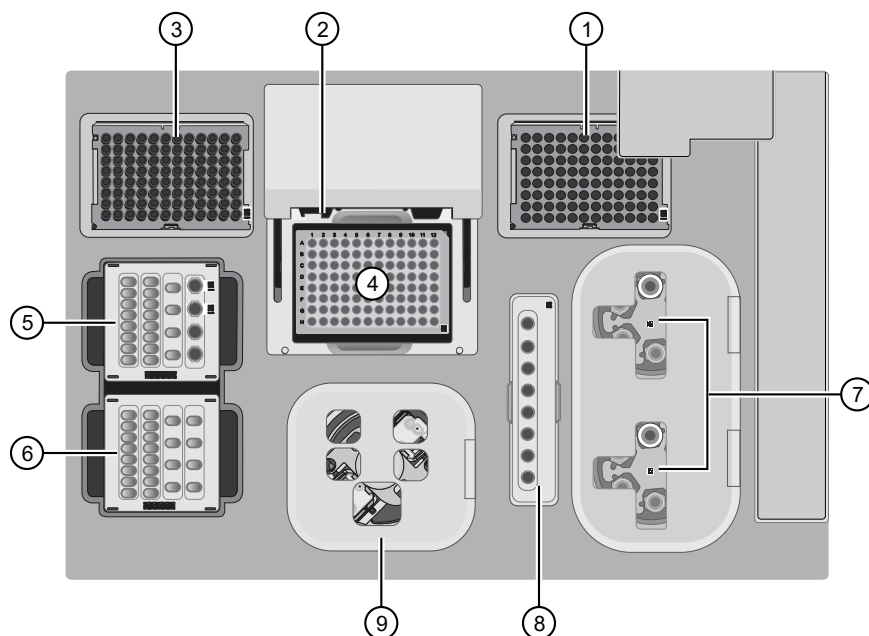
## Load the Ion Chef™ System

---

### IMPORTANT!

- Rated centrifuge speeds are intended only for operation 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, chips, and adapters. The centrifuge must be load-balanced. Proper care must be taken to load the buckets properly. If excessive vibrations arise, check that items are installed properly and rotors are load-balanced.
  - Use only the materials supplied in the Ion S5™ Precision ID Chef & Sequencing Kit 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.
  - Confirm that the instrument is powered on and was 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 free of condensate before loading components.
- 


Follow the procedures in this section to load the Ion Chef™ Instrument.



**Figure 4 A schematic of a loaded Ion Chef™ Instrument**

- |   |   |
|---|---|
| ① Empty tip rack (move from new Tip Cartridge position) | ⑥ Ion S5™ Precision ID Chef Solutions cartridge         |
| ② Frame Seal v2   | ⑦ Recovery Tubes and Recovery Station Disposable Lid v2 |
| ③ New Tip Cartridge v2                                  | ⑧ Enrichment Cartridge v2                               |
| ④ PCR Plate and PCR Plate Frame                         | ⑨ Chip Adapter/Chip assemblies                          |
| ⑤ Ion S5™ Precision ID Chef Reagents cartridge          |   |

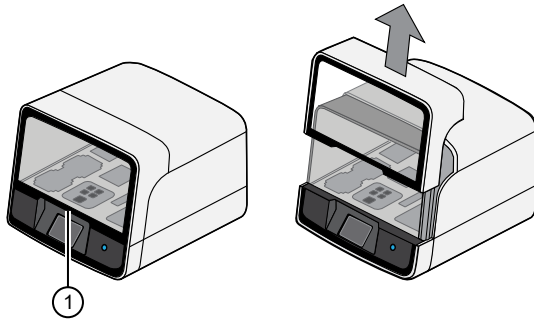
## Load the pipette tip racks and PCR Plate

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.

---

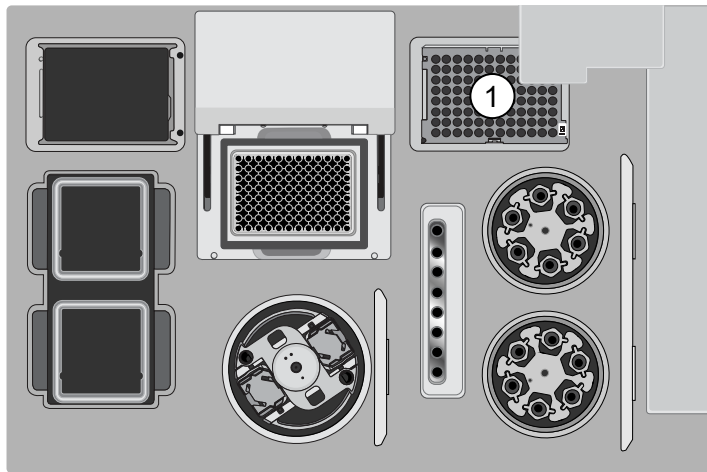
**IMPORTANT!** Lift the door from the center.

---



① Center of door

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



① Used Pipette Tip Position

---

**IMPORTANT!**

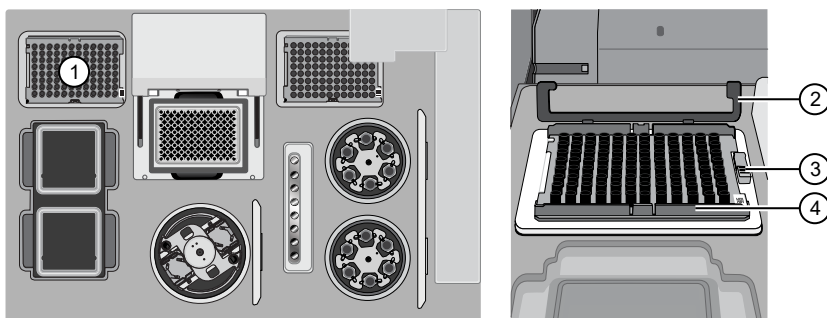
- Ensure that the pipette tip rack in the *Used (Waste)* Pipette Tip Position does not contain any tips. The instrument aborts the run if tips are present in the *used* position.
  - To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used (Waste)* Pipette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

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

---

4. Unwrap a new Tip Cartridge v2 and remove the cover to expose the pipette tips, then load it in the *New Pipette Tip Position*. See the figure in step 5.

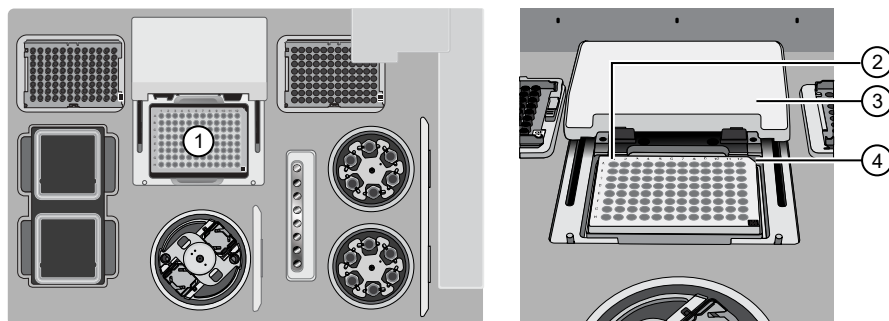
- Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge v2 into the New Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the bracket and cartridge in place.



- ① New Pipette Tip Position  
② Bracket  
③ Catch  
④ New Tip Cartridge v2

- Load a new PCR Plate into the thermal cycler sample block.
- 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

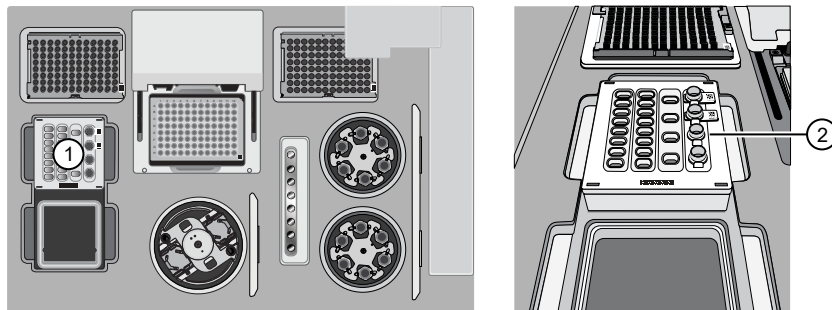
**IMPORTANT!** Thaw the Reagents cartridge at room temperature for 45 minutes before use.

- Gently tap the Ion S5™ Precision ID Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
- If bubbles are present below the surface of the liquid, repeat step 1.



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

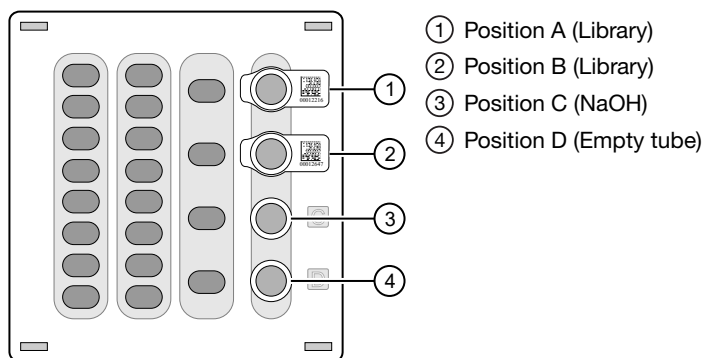
**IMPORTANT!** Do not force the Ion Chef™ cartridges into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, confirm that you are loading the correct cartridge in the correct orientation.



① Reagents station (4°C)

② Ion S5™ Precision ID Chef Reagents cartridge

- Uncap, then load the two Library Sample Tubes, each containing 25 µL of diluted library or combined library pool, into Positions A and B on the Reagents cartridge.



**IMPORTANT!**

- Orient the sample tubes so that the barcodes are visible and oriented to the right.
- Remove the caps from each Library Sample Tube before proceeding.
- Because 200- and 400-base-read libraries require different run parameters, do not load a 200-base-read library and 400-base-read library in a single Ion Chef™ run. Both libraries that are loaded in a run must have a similar read length.

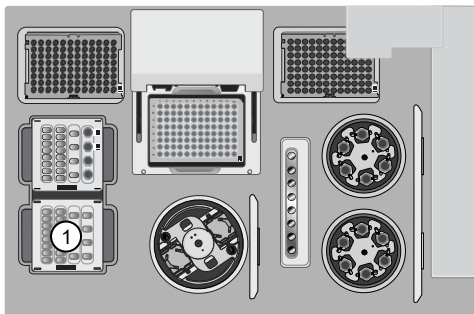
- Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.

**IMPORTANT!** When the Reagents cartridge is loaded:

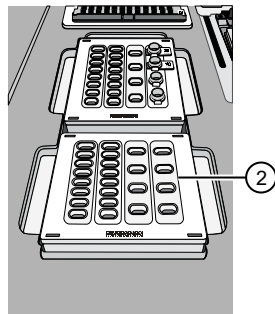
- Press down on each Library Sample Tube to ensure that they are firmly seated in the cartridge.
- **Ensure that *all* tubes are uncapped, including the tube at Position D.**

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

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



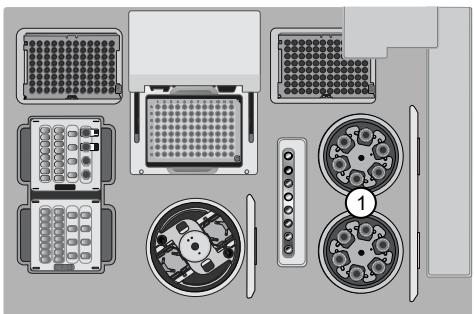
① Solutions station (room temperature)



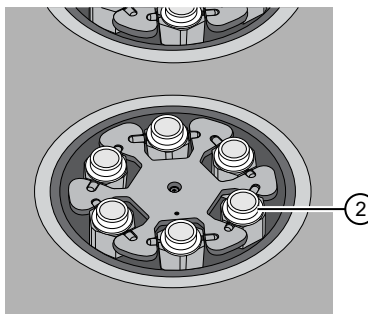
② Ion S5™ Precision ID Chef Solutions cartridge

## Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes 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.

---

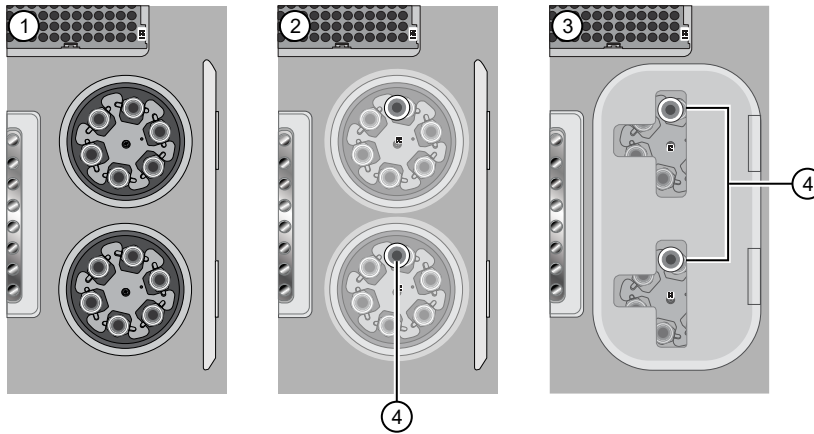
**IMPORTANT!** The centrifuge must be load-balanced.

---

- 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.



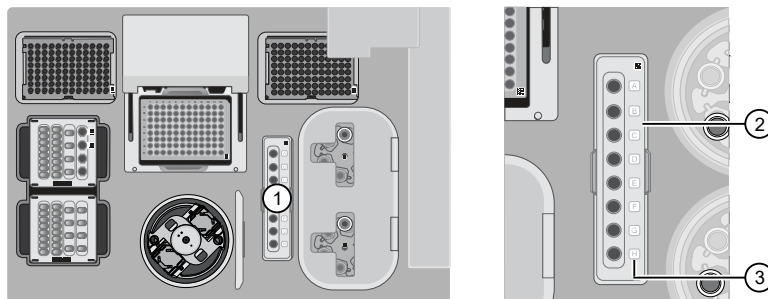
- ① Recovery Tubes installed
- ② Recovery Station Disposable Lid v2 installed
- ③ Recovery centrifuge cover closed
- ④ Port

**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 ensure 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.

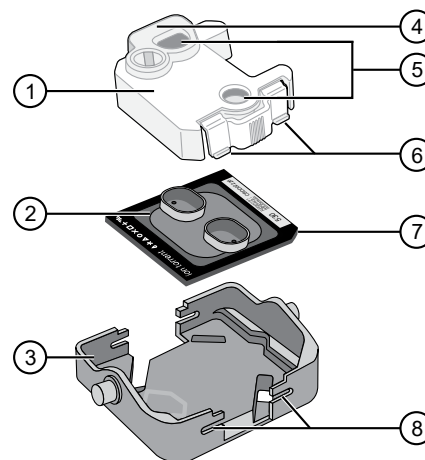
**IMPORTANT!** Confirm that the Enrichment Cartridge v2 is loaded so that the lettering on the cartridge is right-side-up.



- ① Enrichment station
- ② Enrichment Cartridge v2
- ③ Lettering

## Load the Chip-loading centrifuge

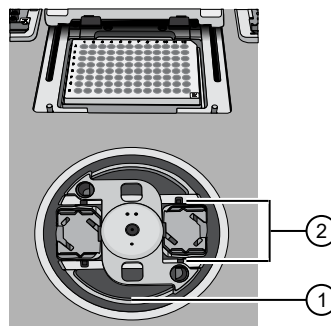
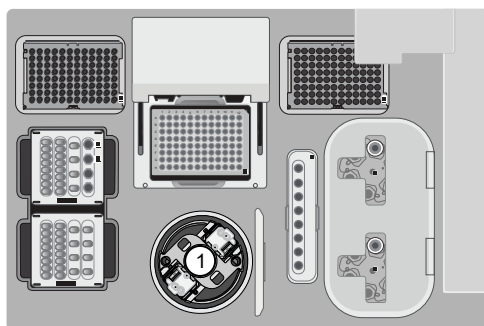
1. Load each chip that you will use for templating and sequencing into a centrifuge bucket, then attach a Chip Adapter to the assembly.
  - a. Place the chip in the chip-loading bucket with the keyed corners of the chip and bucket aligned, then align the wells of the Chip Adapter to the wells of the 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. Ensure 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 Flexible tabs
- ⑦ Keyed corner (align with bucket)
- ⑧ Slots

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

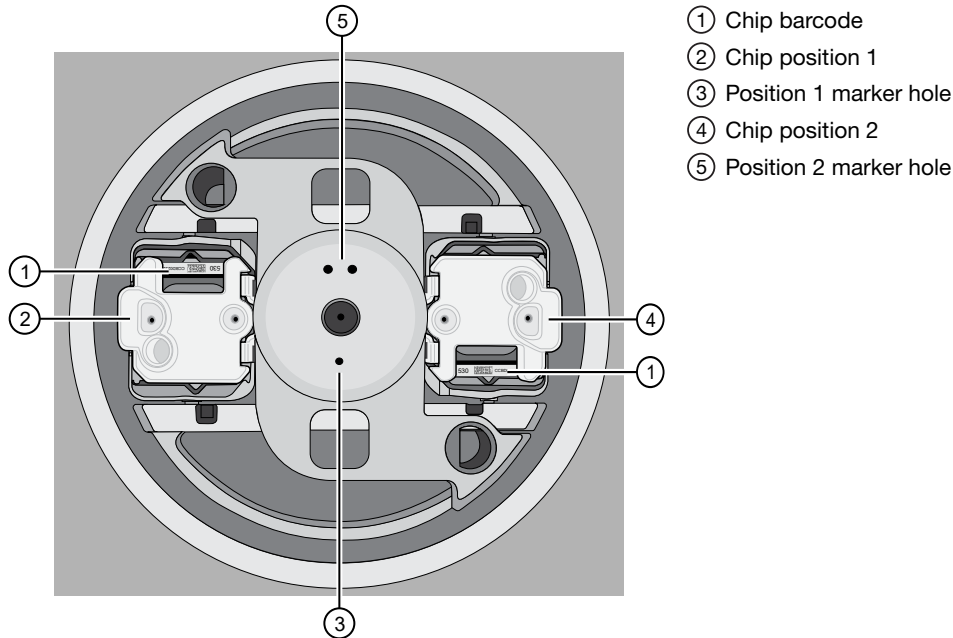
2. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.



① Chip-loading centrifuge

② Mounting grooves

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



---

**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 that is loaded in Position 1 is loaded with ISPs prepared from the DNA library in the Ion Chef™ Library Sample Tube that is loaded in Position A of the Reagents cartridge.
- The chip that is loaded in Position 2 of the centrifuge is loaded with ISPs prepared from the DNA library in the Ion Chef™ Library Sample Tube that is loaded in Position B of the Reagents cartridge.
- If you are performing a mixed chip run, ensure that the chips are loaded in the appropriate positions of the centrifuge.

- 
3. Ensure that 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™ Precision ID 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 ensure 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.

## Single chip loading workflow

You can set up an Ion Chef™ run to load a single chip instead of two, using the Ion Chef™ S5 Series Chip Balance loaded opposite to the sequencing chip in the Chip-loading centrifuge. The Ion Chef™ S5 Series Chip Balance is provided in the Ion S5™ Installation Kit.

Load the Ion Chef™ Instrument as you would normally load the system. For single chip loading, perform the following steps:

1. Add the single diluted library to an Ion Chef™ Library Sample Tube, then load the tube into Position A of the Reagents cartridge.
2. Load an empty Ion Chef™ Library Sample Tube into Position B of the Reagents cartridge. Uncap both tubes.
3. Load the chip in Position 1 and the Ion Chef™ S5 Series Chip Balance in Position 2 of the Chip-loading centrifuge.



Ion Chef™ S5 Series Chip Balance

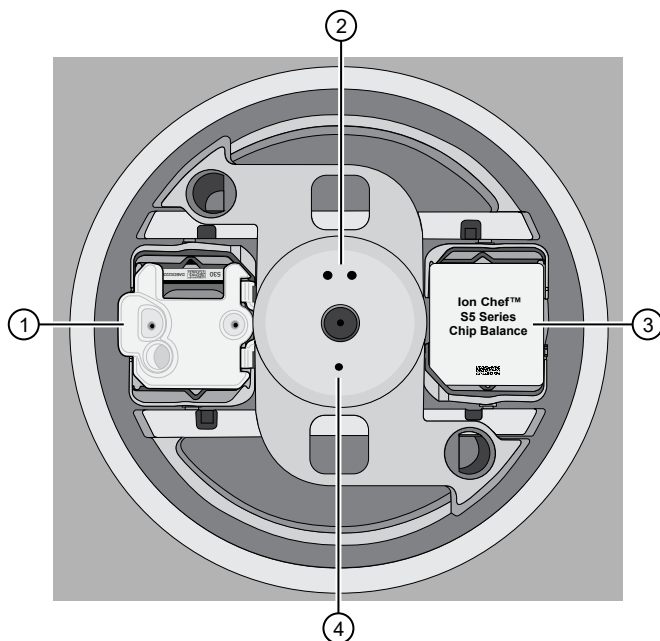
---

**IMPORTANT!** Do not use Ion Chef™ 314, 316/318, or P-Series versions of the chip balance with Ion S5™ Series chips. Each chip balance is weight-matched to the chip (and corresponding chip adapter) specified on the chip balance label.

---

**Note:** Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest.

---



- ① Position 1 (chip)
- ② Position 2 marker holes
- ③ Position 2 (Chip Balance)
- ④ Position 1 marker holes

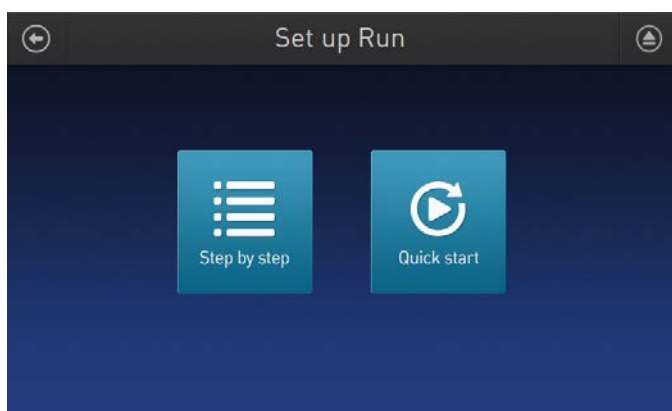
4. Resume the normal workflow in “Load the Chip-loading centrifuge” at step 3. The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.

## Start the Ion Chef™ run

1. Verify 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.



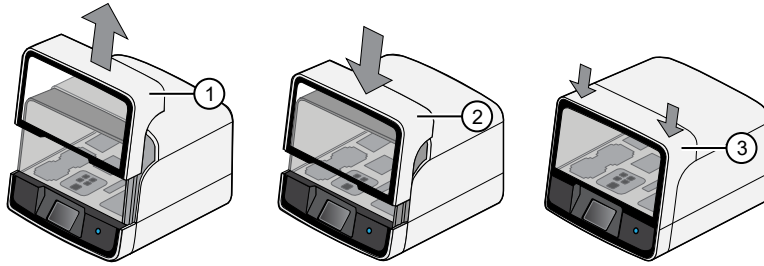
4. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage. After the door closes, the instrument vision system activates.

---

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

---





- ① Lift door first
- ② Lower
- ③ Press down to lock

5. When prompted, tap **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to verify their presence and compatibility. During Deck Scan, the touchscreen can show warnings if the Ion Chef™ Instrument detects missing or incompatible consumables. Address all warnings before the run can start. After you address each condition, tap **Yes** to continue.


---

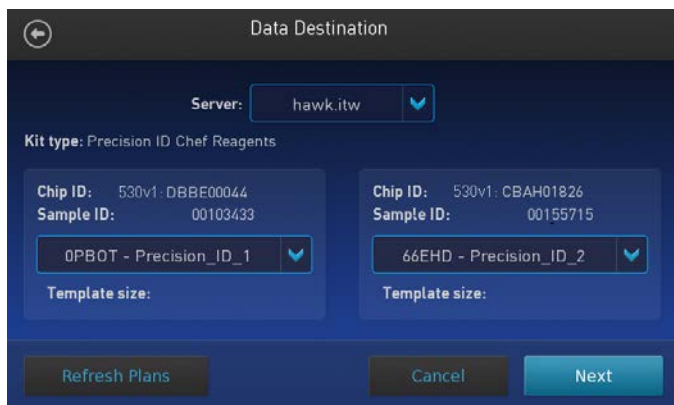
**IMPORTANT!** The Deck Scan function is not a substitute for manual inspection of the reagents and consumables on the Ion Chef™ Instrument before starting a run. To ensure proper and safe instrument operation, verify that all consumables are installed correctly before you continue.

---

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



- Verify that the instrument displays the correct kit type, chip type, chip barcodes, and Planned Run. If the correct Planned Runs do not display, tap the dropdown list  to select the Planned Run for each chip, then tap **Next**.

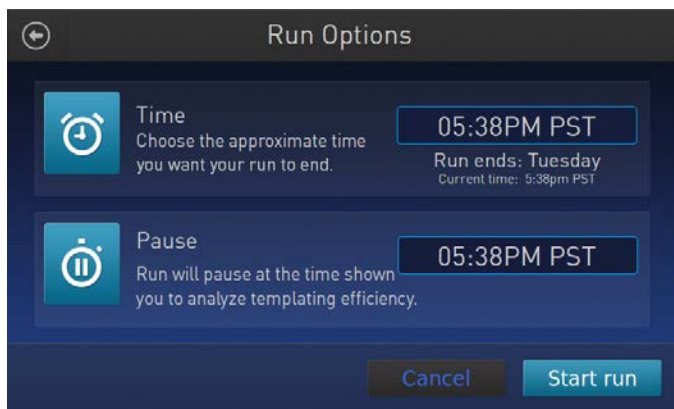



---

**IMPORTANT!** If the kit and chip type are not correct, verify 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.

---

- 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 quality control (QC) samples to evaluate templating efficiency.

By selecting	You can obtain QC samples
Time	immediately after the run ends, at the time you specify (14 hours 38 minutes after run start).
Pause	when the instrument pauses operation before the chip loading step (approximately 13 hours into the run).

---

**Note:** If you are unsure when you will sequence the loaded chips, select the Pause option.

---

You can obtain unenriched samples from the corresponding Library Sample Tubes at Positions A and B on the Reagents cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge v2.

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

---

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

If the Ion Chef™ Instrument encounters a problem during the run, it aborts the run, then displays the error on the instrument touchscreen. If a run fails:

1. Remove the consumables from the deck, then clean the instrument. If possible, retain the consumables for troubleshooting.
2. Reset, then reattempt the run. If the run fails again, contact Technical Support to troubleshoot the problem.

- 
10. Initialize the Ion S5™, Ion S5™ XL, or Ion GeneStudio™ S5 Series Sequencer or at least 40 minutes before the Ion Chef™ System finishes chip loading.

Initialization of the instrument can be performed up to 24 hours before starting a sequencing run. If the intent is to perform two sequencing runs per initialization, the first run must be completed and the second run must be started within the 24-hour period. By initializing the sequencer before completion of chip loading, you ensure that the chips can be sequenced as soon as possible after loading is complete.


11. When the run is complete, unload the Ion Chef™ Instrument and sequence the chips immediately.

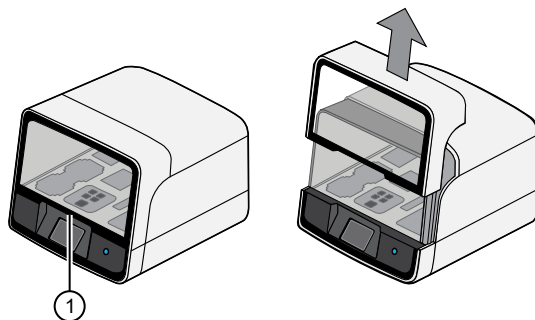
---

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

---

## Unload the chips 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.



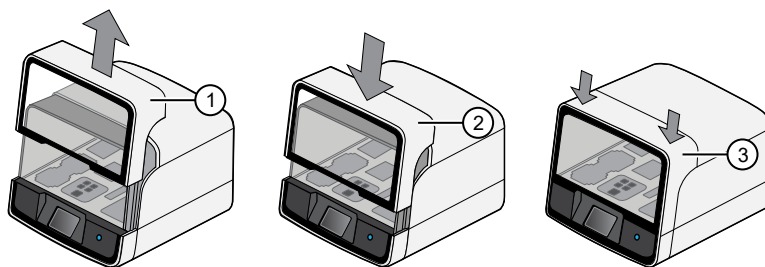
① Hold here and lift

2. Remove the chip/bucket assemblies from the Chip-loading centrifuge. Remove the Chip Adapter from the chip, then discard the adapter. Carefully remove the chip from each bucket, then set the chips aside on a clean, static-free surface. Return the buckets to the Chip-loading centrifuge.
3. 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. 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

4. Load one or both chips into sequencers and promptly start the sequencing runs.

---

**Note:** Start sequencer initialization ahead of time so that the sequencer is ready to load when the Ion Chef™ Instrument run completes.

---

## Clean the Ion Chef™ System

### 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 ensure minimal contamination.

---

### Materials required

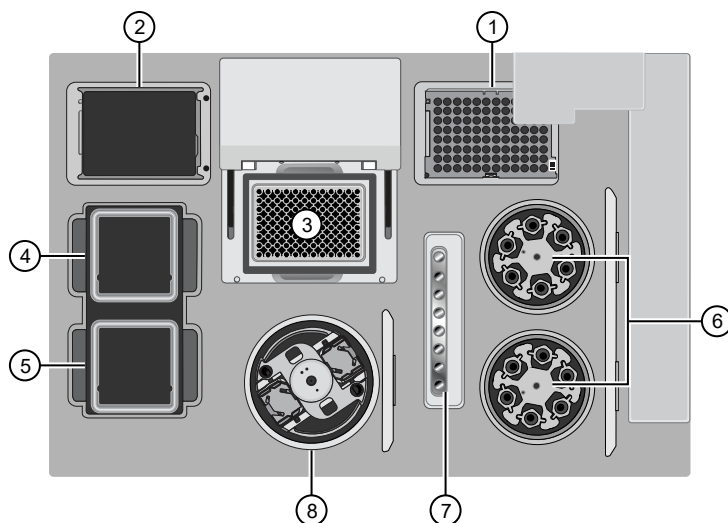
- Gloves, powder-free nitrile
- Isopropanol, 70% solution
- Wipes, lint-free

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

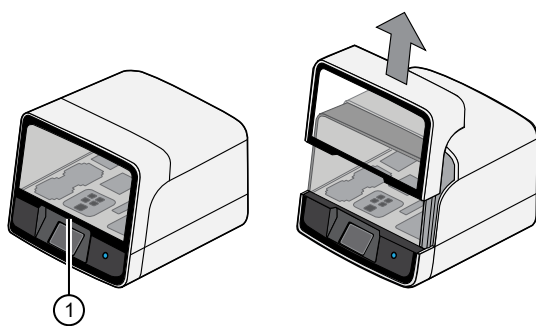
## Remove and dispose of used consumables

### IMPORTANT!

- Do not discard the empty Tip Cartridge v2.
- 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 by the door handle to the top of the travel until the latch mechanism engages.

**IMPORTANT!** Lift the door from the center.



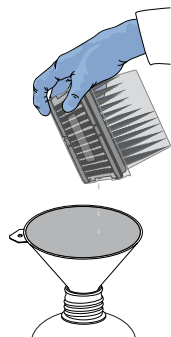
① Center of door

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 Tip Cartridge v2 from the new tip position to the waste tip position.

5. Move the empty Tip Cartridge v2 to the waste tip position.
6. Remove, then discard the
  - Ion S5™ Precision ID Chef Reagents cartridge
  - Ion S5™ Precision ID 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. Close the Chip-loading centrifuge cover.

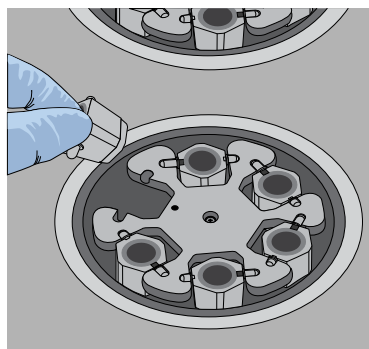
### Inspect and clean the Recovery centrifuges and buckets

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

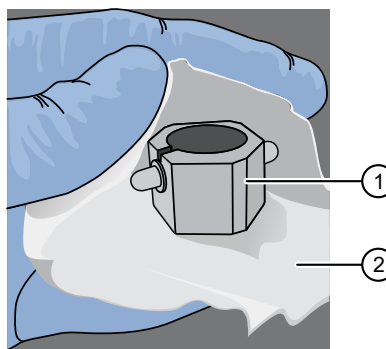
Is liquid present?	Action
No	Proceed to “Start the cleaning” on page 80.
Yes	Clean the centrifuge bowl and buckets as described below. <b>IMPORTANT!</b> Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do <i>not</i> need to clean the centrifuges 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.

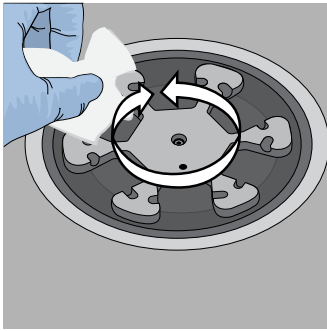


① Bucket

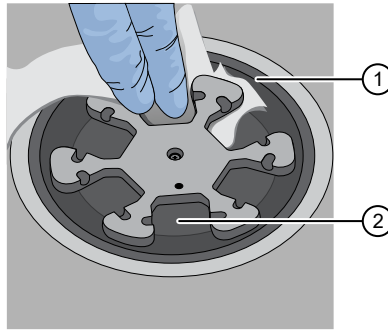


② Lint-free wipe

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

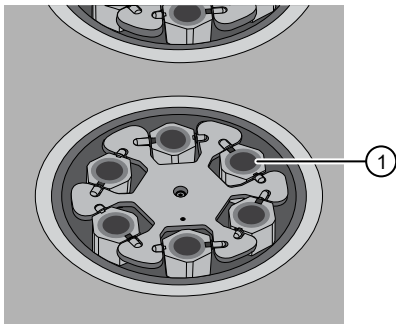


① Inside rim of the centrifuge



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.



# Sequence on the HID Ion S5™/HID Ion GeneStudio™ S5 System

- Software version requirements for sequencing ..... 83
- Workflow: Sequencing on the HID Ion S5™/Ion GeneStudio™ S5 System ..... 84
- HID Ion S5™/Ion GeneStudio™ S5 System component positions ..... 84
- Before you begin ..... 85
- Required sequencer cleanings ..... 86
- Initialize the sequencer ..... 86
- Start the sequencing run ..... 88

This chapter contains brief procedures for sequencing Precision ID libraries on an HID Ion S5™/Ion S5™ XL System, or an HID Ion GeneStudio™ S5 Series System. For complete instrument procedures, troubleshooting and maintenance information, see the *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854), the *Ion S5™ and Ion S5™ XL Instrument User Guide* (Pub. No. MAN0010811), or the *Ion GeneStudio™ S5 Instrument User Guide* (Pub. No. MAN0017528).

## Software version requirements for sequencing

Panel	Software version required
Precision ID mtDNA Control Region Panel	Torrent Suite™ Software 5.18, or the latest HID-supported version
Precision ID mtDNA Whole Genome Panel	

## Workflow: Sequencing on the HID Ion S5™/Ion GeneStudio™ S5 System

“Initialize the sequencer” on page 86



“Start the sequencing run” on page 88

### HID Ion S5™/Ion GeneStudio™ S5 System component positions



- ① Touchscreen
- ② Power button
- ③ Ion S5™ Precision ID Sequencing Reagents cartridge
- ④ Chip clamp
- ⑤ Precision ID Wash Solution bottle. Waste reservoir located behind the Precision ID Wash Solution bottle (shown on the right).
- ⑥ Precision ID 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 the user to replace the reagent before performing the run.

**Note:** RFID regulatory information can be found on the main screen under **Options ▶ Regulatory info.**

## Before you begin

The HID Ion S5™/Ion S5™ XL Sequencers and HID 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™ Precision ID Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.  
Do not remove the Ion S5™ Precision ID 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 Precision ID Wash Solution bottle. Invert the bottle 5 times within its vacuum-sealed bag, then swirl at an angle to mix thoroughly.
- Remove the Precision ID Wash Solution bottle from its vacuum-sealed bag, then remove the red cap from the Precision ID Wash Solution and Precision ID Cleaning Solution bottles immediately before installing on the instrument.

## Required sequencer cleanings

HID Ion S5™/Ion S5™ XL Sequencers and HID Ion GeneStudio™ S5 Series Sequencers require cleaning before initialization. Cleaning 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 deselected 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 110 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 111 for more information.

## Initialize the sequencer

1. In the instrument touchscreen main menu, tap **Initialize**. The door, chip, and Reagent cartridge clamps unlock.



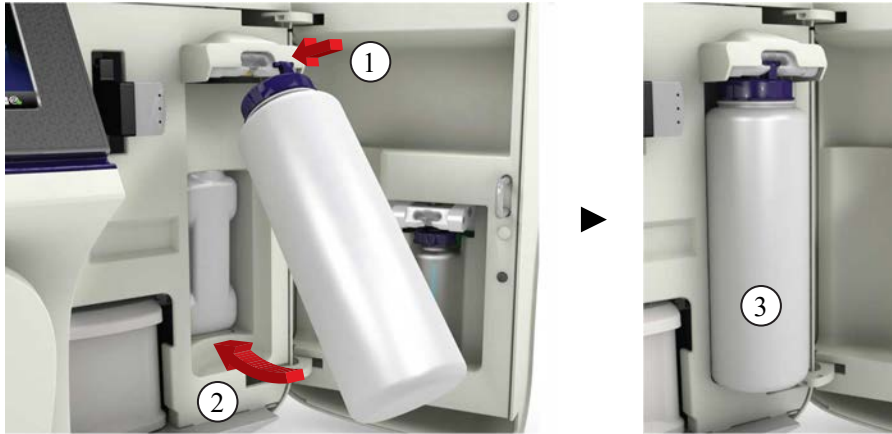
2. When prompted, remove the Precision ID Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.
3. Reinstall the empty waste reservoir.
4. Replace the expended Ion S5™ Precision ID Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.

---

**Note:** Dispose of used reagents appropriately.

---

5. Ensure that the new Precision ID Wash Solution bottle has been thoroughly mixed. If not, invert the bottle 5 times, then swirl the bottle at an angle to mix thoroughly. 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 Precision ID Cleaning Solution bottle.

---

**Note:** The Precision ID 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 Precision ID Cleaning Solution bottle contains enough reagent to perform the post-run clean. Follow all on-screen recommendations to ensure proper installation of required consumables.

---

**IMPORTANT!** If the allowed number of post-run cleaning procedures has been met, the instrument will prompt the user to replace the Precision ID Cleaning Solution bottle.

---

9. When initialization is complete (~30–40 minutes), tap **Home**.

The instrument is now ready for a sequencing run.

## Start the sequencing run

We recommend that you sequence loaded chips on a sequencer 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.

---

**Note:** Do *not* press the power button during a run. Interrupting power to the instrument during a run may result in sequencing run failure and loss of sample.

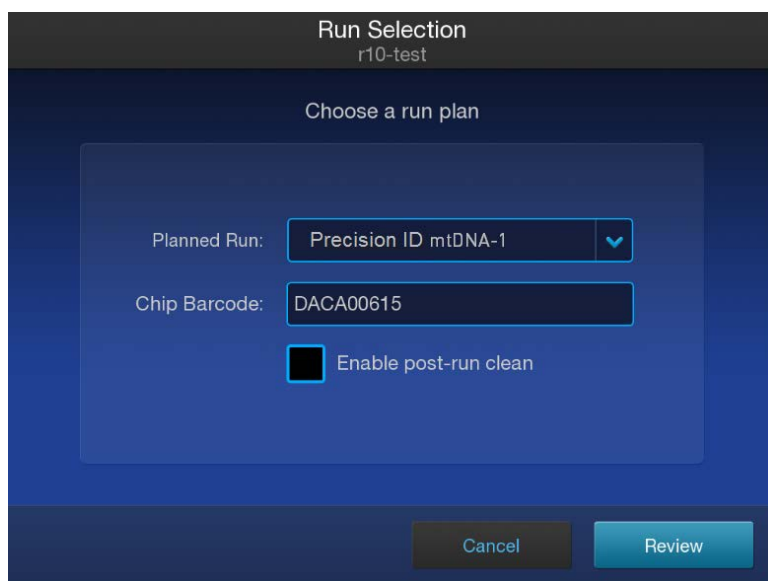
---

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

---

**Note:** When starting the second sequencing run on a single initialization, ensure the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.

---



6. Confirm the pre-populated settings are correct, or make changes using the buttons and dropdown lists if necessary.
7. 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 may 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.



# Analyze the sequencing results

## Review mtDNA run summary metrics

Review the run summary metrics to confirm data quality before proceeding to data analysis and review in Converge™ Software v2.1 or later.

1. Sign in to the Torrent Server via Torrent Suite™ Software.
2. Select **Data ▶ Completed Runs & Results**, then click a **Report Name** to view the run summary.
3. Compare the results to the following recommended values. See “Expected observations if recommended values are not met” on page 97 for possible impact to the Converge™ Software data analysis results.


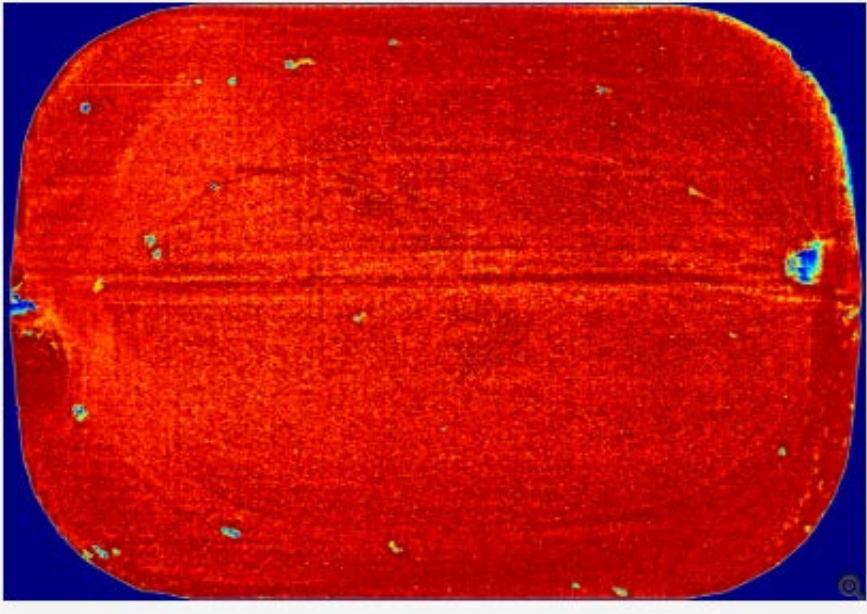
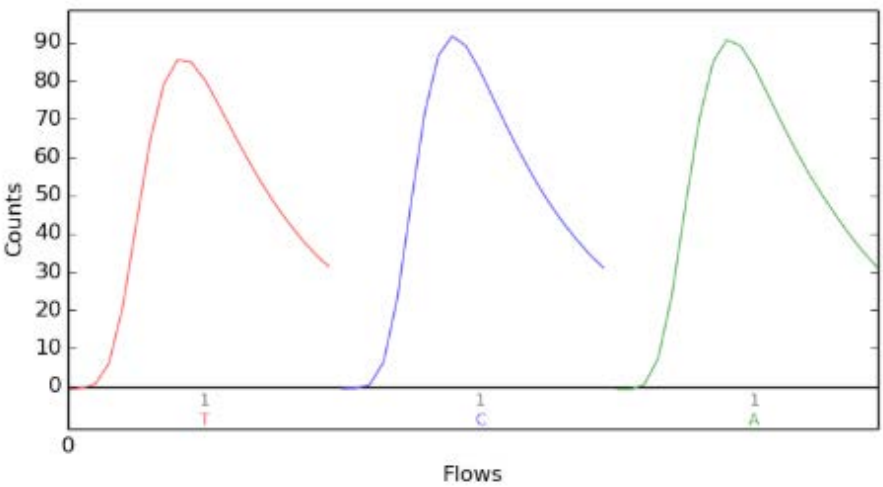
---

**Note:** For more information about the run report metrics, see *Torrent Suite™ Software Help*. For additional troubleshooting information, see Appendix A, “Troubleshooting”.

---

### Recommended values for sequencing run report metrics

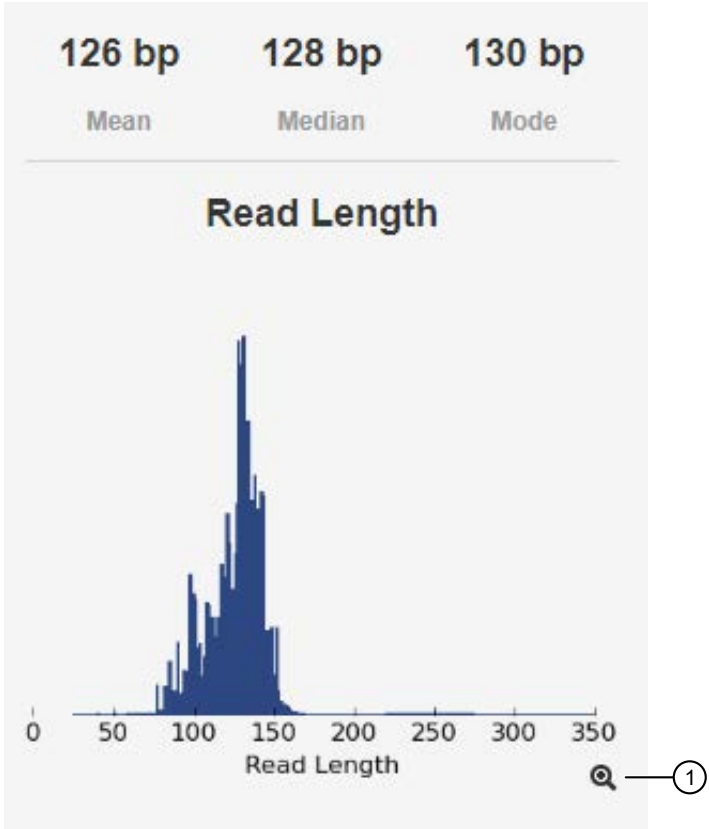
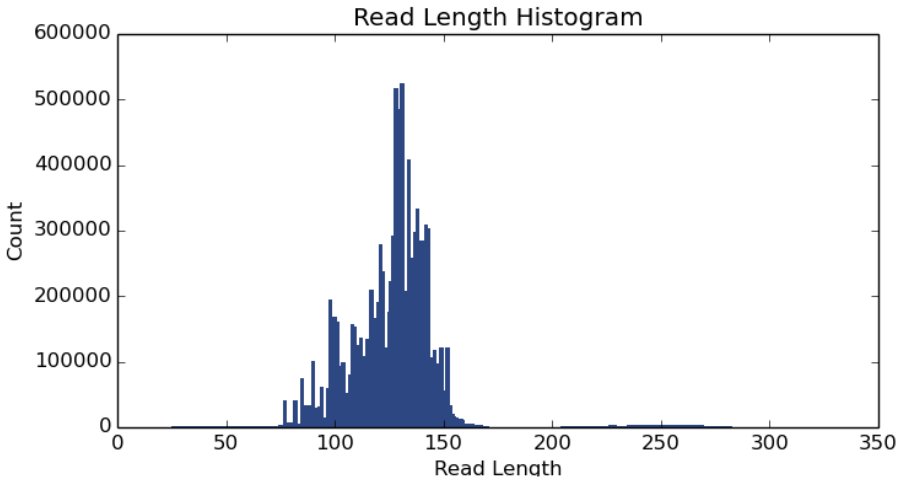
Recommended values for sequencing run report metrics for the Precision ID mtDNA Whole Genome Panel and the Precision ID mtDNA Control Region Panel.

Parameter	Recommended values
ISP Loading and Key Signal	<div style="text-align: center;"> <p><b>1.45 G</b> <span style="margin-left: 200px;"><b>90</b></span></p> <p>Total Bases <span style="margin-left: 200px;">Key Signal</span></p> <hr/> <p> <b>90 %</b> <span style="margin-left: 20px;">ISP Loading</span></p> <p><b>ISP Density</b></p>  <p><b>Consensus Key 1-Mer - Library Ave. Peak = 90</b></p>  </div> <p>① Click to view the <b>Key Signal</b> <span style="margin-left: 200px;">③ Key Signal: &gt;60</span></p> <p>② ISP Loading: &gt;50%</p>

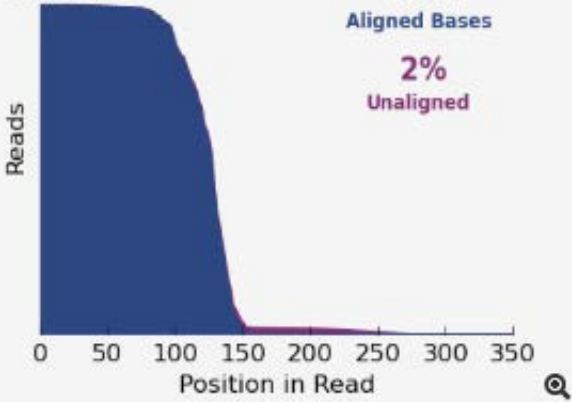
Recommended values for sequencing run report metrics (continued)

Parameter	Recommended values
ISP Summary	<p>With an ISP Loading &gt;50%:</p> <p><b>11,498,449</b> Total Reads</p> <p><b>34 %</b> Usable Reads</p> <p><b>ISP Summary</b></p> <p><b>90%</b> Loading</p> <p><b>33,896,396</b></p> <p><b>10%</b> Empty Wells</p> <p><b>100%</b> Enrichment</p> <p><b>33,890,152</b></p> <p><b>0%</b> No Template</p> <p><b>58%</b> Clonal</p> <p><b>19,537,306</b></p> <p><b>42%</b> Polyclonal</p> <p><b>59%</b> Final Library</p> <p><b>11,498,449</b></p> <p>1% Test Fragments 0% Adapter Dimer 40% Low Quality</p> <p>① Enrichment: ≥98% ② Total Usable Reads: ~30–40% ③ Polyclonal: 25–50% ④ Test Fragment: 1% Adapter Dimer: ≤1% Low Quality: ≤50%</p>

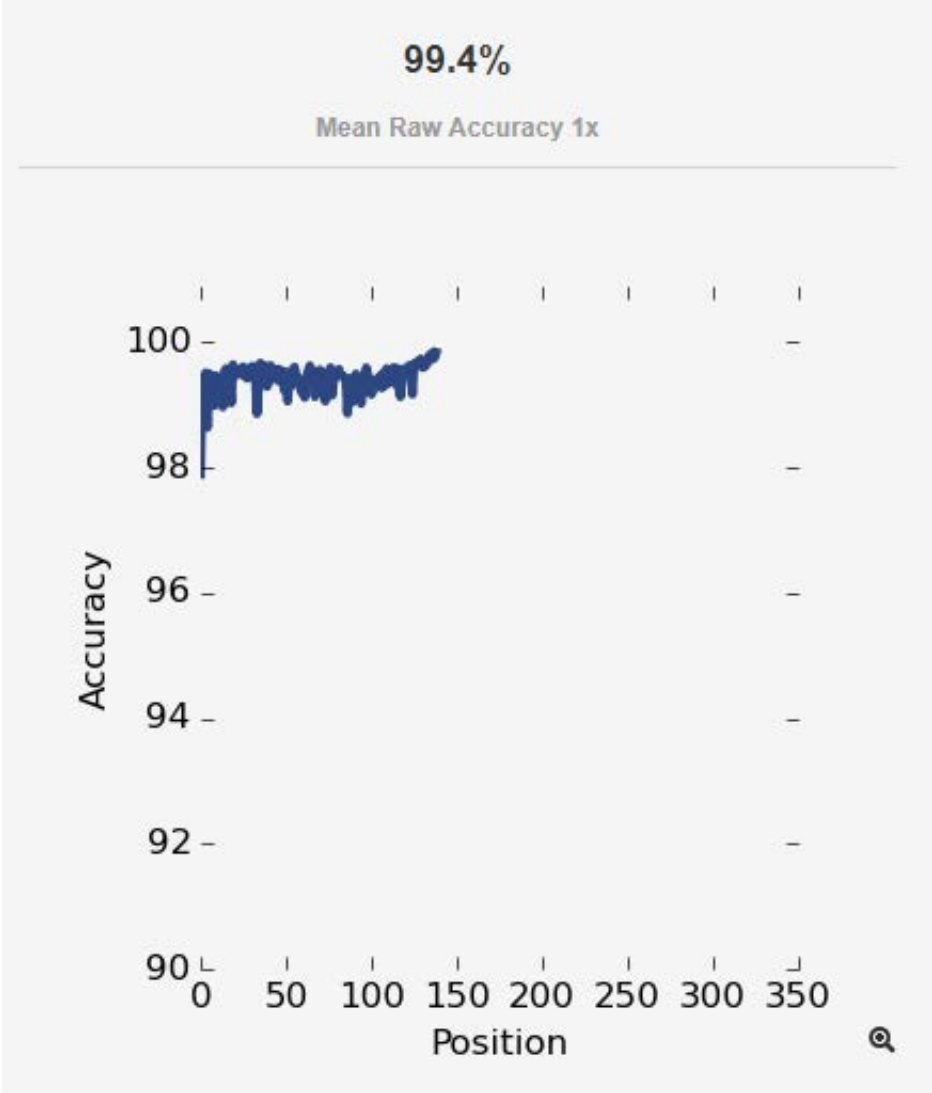
Recommended values for sequencing run report metrics (continued)

Parameter	Recommended values
Read Histogram	<p data-bbox="456 306 1451 401">A typical read histogram and counts for each fragment for 32 samples prepared with the Precision ID mtDNA Whole Genome Panel using the Ion Chef™ Instrument for library and template preparation, and the Ion S5™ Instrument for sequencing.</p> <div data-bbox="634 411 1338 1234">  <p data-bbox="690 443 1214 548">126 bp      128 bp      130 bp Mean      Median      Mode</p> <p data-bbox="834 600 1068 642">Read Length</p> <p data-bbox="656 1121 1247 1184">0    50    100    150    200    250    300    350 Read Length</p> <p data-bbox="1219 1167 1338 1199">🔍 ①</p> </div> <div data-bbox="472 1247 1365 1724">  <p data-bbox="821 1251 1110 1276">Read Length Histogram</p> <p data-bbox="472 1268 581 1507">600000 500000 400000 300000 200000 100000 0</p> <p data-bbox="574 1667 1360 1724">0    50    100    150    200    250    300    350 Read Length</p> </div> <p data-bbox="518 1755 862 1787">① Click to see histogram details.</p>

## Recommended values for sequencing run report metrics (continued)

Parameter	Recommended values												
Alignment	<div data-bbox="462 310 1107 1333"> <p><b>1.36 G</b>      <b>81541.9X</b></p> <p>Total Aligned Bases      Reference Coverage</p> <hr/> <p>11,037,435      <b>98%</b> <span style="border: 1px solid black; border-radius: 50%; padding: 2px;">1</span></p> <p>                                 Aligned Bases</p> <p>                                 <b>2%</b></p> <p>                                 Unaligned</p>  <p>Reads</p> <p>0 50 100 150 200 250 300 350</p> <p>Position in Read</p> <table border="1" data-bbox="487 1008 1071 1302"> <thead> <tr> <th></th> <th>Count</th> <th>%</th> </tr> </thead> <tbody> <tr> <td>Total Reads</td> <td>11,037,435</td> <td>—</td> </tr> <tr> <td>Aligned Reads</td> <td>11,028,621</td> <td>99.9%</td> </tr> <tr> <td>Unaligned Reads</td> <td>8,814</td> <td>0.1%</td> </tr> </tbody> </table> </div> <p><span style="border: 1px solid black; border-radius: 50%; padding: 2px;">1</span> Total Aligned Bases: <math>\geq 95\%</math></p>		Count	%	Total Reads	11,037,435	—	Aligned Reads	11,028,621	99.9%	Unaligned Reads	8,814	0.1%
	Count	%											
Total Reads	11,037,435	—											
Aligned Reads	11,028,621	99.9%											
Unaligned Reads	8,814	0.1%											

Recommended values for sequencing run report metrics (continued)

Parameter	Recommended values
Mean Raw Accuracy	<p data-bbox="456 306 943 338">Example of normal trend for Raw Accuracy.</p> <div data-bbox="456 348 1382 1434">  <p data-bbox="854 390 964 426"><b>99.4%</b></p> <p data-bbox="768 464 1052 491">Mean Raw Accuracy 1x</p> <p data-bbox="529 894 565 1056">Accuracy</p> <p data-bbox="881 1367 1019 1402">Position</p> </div>

Recommended values for sequencing run report metrics (continued)

Parameter	Recommended values																																																																																
Alignment Quality	<p>Example of typical output for Alignment Quality in the primary analysis.</p> <div style="border: 1px solid #ccc; padding: 10px; background-color: #f9f9f9;"> <p style="text-align: center;"><b>1.3 G</b></p> <p style="text-align: center;">AQ17 Total Bases</p> <hr/> <p style="text-align: center;"><b>Alignment Quality</b></p> <table style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th></th> <th style="text-align: center;">AQ17</th> <th style="text-align: center;">AQ20</th> <th style="text-align: center;">Perfect</th> </tr> </thead> <tbody> <tr> <td style="border-top: 1px solid #ccc;"><b>Total Number of Bases [bp]</b></td> <td style="border-top: 1px solid #ccc; text-align: center;">1.3 G</td> <td style="border-top: 1px solid #ccc; text-align: center;">1.21 G</td> <td style="border-top: 1px solid #ccc; text-align: center;">1 G</td> </tr> <tr> <td><b>Mean Length [bp]</b></td> <td style="text-align: center;">121</td> <td style="text-align: center;">116</td> <td style="text-align: center;">103</td> </tr> <tr> <td><b>Longest Alignment [bp]</b></td> <td style="text-align: center;">321</td> <td style="text-align: center;">321</td> <td style="text-align: center;">319</td> </tr> <tr> <td><b>Mean Coverage Depth [x]</b></td> <td style="text-align: center;">78164.5</td> <td style="text-align: center;">72395.0</td> <td style="text-align: center;">60120.9</td> </tr> </tbody> </table> </div>		AQ17	AQ20	Perfect	<b>Total Number of Bases [bp]</b>	1.3 G	1.21 G	1 G	<b>Mean Length [bp]</b>	121	116	103	<b>Longest Alignment [bp]</b>	321	321	319	<b>Mean Coverage Depth [x]</b>	78164.5	72395.0	60120.9																																																												
	AQ17	AQ20	Perfect																																																																														
<b>Total Number of Bases [bp]</b>	1.3 G	1.21 G	1 G																																																																														
<b>Mean Length [bp]</b>	121	116	103																																																																														
<b>Longest Alignment [bp]</b>	321	321	319																																																																														
<b>Mean Coverage Depth [x]</b>	78164.5	72395.0	60120.9																																																																														
Individual sample results	<p>Example of results for 8 Whole Genome mtDNA libraries run on one Ion 530™ Chip.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Barcode Name</th> <th>Sample</th> <th>Bases</th> <th>&gt;=Q20 Bases</th> <th>Reads</th> <th>Mean Read Length</th> <th>Read Length Histogram</th> <th>Files</th> </tr> </thead> <tbody> <tr> <td>No barcode</td> <td>None</td> <td>64,159,070</td> <td>57,960,342</td> <td>460,493</td> <td>139 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0109</td> <td>DL8_Mt-WG_CHR_Rep1</td> <td>153,183,534</td> <td>143,061,858</td> <td>1,216,134</td> <td>125 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0110</td> <td>DL8_Mt-WG_CHR_Rep2</td> <td>164,587,063</td> <td>153,609,654</td> <td>1,310,292</td> <td>125 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0111</td> <td>DL8_Mt-WG_GM09947A_Rep1</td> <td>188,553,387</td> <td>176,178,381</td> <td>1,514,486</td> <td>124 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0112</td> <td>DL8_Mt-WG_GM09947A_Rep2</td> <td>169,270,940</td> <td>158,067,887</td> <td>1,361,686</td> <td>124 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0113</td> <td>DL8_Mt-WG_HL60_Rep1</td> <td>192,366,788</td> <td>179,826,572</td> <td>1,553,722</td> <td>123 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0114</td> <td>DL8_Mt-WG_HL60_Rep2</td> <td>195,096,470</td> <td>182,843,551</td> <td>1,576,525</td> <td>123 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0115</td> <td>DL8_Mt-WG_NA10742A_Rep1</td> <td>170,262,505</td> <td>158,119,496</td> <td>1,309,518</td> <td>130 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> <tr> <td>IonCode_0116</td> <td>DL8_Mt-WG_NA10742A_Rep2</td> <td>154,006,475</td> <td>143,290,210</td> <td>1,195,072</td> <td>128 bp</td> <td></td> <td>UBAM BAM BAI</td> </tr> </tbody> </table> <p style="text-align: center;">10 Items per page <span style="float: right;">1 - 9 of 9 items</span></p>	Barcode Name	Sample	Bases	>=Q20 Bases	Reads	Mean Read Length	Read Length Histogram	Files	No barcode	None	64,159,070	57,960,342	460,493	139 bp		UBAM BAM BAI	IonCode_0109	DL8_Mt-WG_CHR_Rep1	153,183,534	143,061,858	1,216,134	125 bp		UBAM BAM BAI	IonCode_0110	DL8_Mt-WG_CHR_Rep2	164,587,063	153,609,654	1,310,292	125 bp		UBAM BAM BAI	IonCode_0111	DL8_Mt-WG_GM09947A_Rep1	188,553,387	176,178,381	1,514,486	124 bp		UBAM BAM BAI	IonCode_0112	DL8_Mt-WG_GM09947A_Rep2	169,270,940	158,067,887	1,361,686	124 bp		UBAM BAM BAI	IonCode_0113	DL8_Mt-WG_HL60_Rep1	192,366,788	179,826,572	1,553,722	123 bp		UBAM BAM BAI	IonCode_0114	DL8_Mt-WG_HL60_Rep2	195,096,470	182,843,551	1,576,525	123 bp		UBAM BAM BAI	IonCode_0115	DL8_Mt-WG_NA10742A_Rep1	170,262,505	158,119,496	1,309,518	130 bp		UBAM BAM BAI	IonCode_0116	DL8_Mt-WG_NA10742A_Rep2	154,006,475	143,290,210	1,195,072	128 bp		UBAM BAM BAI
Barcode Name	Sample	Bases	>=Q20 Bases	Reads	Mean Read Length	Read Length Histogram	Files																																																																										
No barcode	None	64,159,070	57,960,342	460,493	139 bp		UBAM BAM BAI																																																																										
IonCode_0109	DL8_Mt-WG_CHR_Rep1	153,183,534	143,061,858	1,216,134	125 bp		UBAM BAM BAI																																																																										
IonCode_0110	DL8_Mt-WG_CHR_Rep2	164,587,063	153,609,654	1,310,292	125 bp		UBAM BAM BAI																																																																										
IonCode_0111	DL8_Mt-WG_GM09947A_Rep1	188,553,387	176,178,381	1,514,486	124 bp		UBAM BAM BAI																																																																										
IonCode_0112	DL8_Mt-WG_GM09947A_Rep2	169,270,940	158,067,887	1,361,686	124 bp		UBAM BAM BAI																																																																										
IonCode_0113	DL8_Mt-WG_HL60_Rep1	192,366,788	179,826,572	1,553,722	123 bp		UBAM BAM BAI																																																																										
IonCode_0114	DL8_Mt-WG_HL60_Rep2	195,096,470	182,843,551	1,576,525	123 bp		UBAM BAM BAI																																																																										
IonCode_0115	DL8_Mt-WG_NA10742A_Rep1	170,262,505	158,119,496	1,309,518	130 bp		UBAM BAM BAI																																																																										
IonCode_0116	DL8_Mt-WG_NA10742A_Rep2	154,006,475	143,290,210	1,195,072	128 bp		UBAM BAM BAI																																																																										



## Expected observations if recommended values are not met

Primary analysis results observation	Indicates	Expected observations in Converge™ Software data analysis <sup>[1]</sup>
<p>One or more of following:</p> <ul style="list-style-type: none"> <li>• Low ISP loading (<math>\leq 50\%</math>)</li> <li>• Low total usable reads (<math>\leq 30\%</math>)</li> <li>• Low polyclonal ISPs (<math>&lt; 25\%</math>)</li> <li>• High polyclonal ISPs (<math>&gt; 50\%</math>)</li> <li>• High adapter dimer ISPs (<math>&gt; 1\%</math>)</li> <li>• High percentage of low quality ISPs (<math>&gt; 50\%</math>)</li> <li>• Total aligned bases <math>&lt; 95\%</math></li> </ul>	<p>Less than optimal sample coverage on the chip.</p> <p><b>Note:</b> High polyclonal, adapter dimer, and/or low-quality ISPs may result in higher total ISP loading, but these reads are filtered from the results, resulting in a lower number of usable reads.</p>	<p>Lower coverage (reads per allele).</p> <p>If using relative thresholds for mtDNA analysis, lower coverage can affect the proper calling of stutters, artefacts, and noise.</p>
<p>One or both of following:</p> <ul style="list-style-type: none"> <li>• Low key signal (<math>\leq 60\%</math>)</li> <li>• Low enrichment (<math>&lt; 98\%</math>)</li> </ul>	<p>Less than optimal number of sample fragments were sequenced.</p> <p>The key signal is the percentage of live ISPs with a key sequence that is identical to the library key sequence. The key sequence is the first set of bases that are sequenced and is used to identify the library fragments. If the key is not sequenced, then the barcode and insert may not be properly sequenced.</p>	<p>More noise in results.</p>
<p>One or both of following:</p> <ul style="list-style-type: none"> <li>• Low test fragment (positive sequencing control) ISPs (significantly less than 1%)</li> <li>• Atypical mean raw accuracy/ Low raw accuracy</li> </ul>	<p>Issues during library, template, or sequencing preparation.</p>	<p>We do not recommend that you continue to Converge™ Software data analysis.</p>
<p>One or both of following:</p> <ul style="list-style-type: none"> <li>• Atypical histogram</li> <li>• Atypical sample results</li> </ul>	<p>Low DNA integrity (for example, sample degradation or presence of inhibitor) and/or low sample input.</p>	<p>Lower coverage, imbalanced profiles, and/or allelic dropout.</p>

<sup>[1]</sup> See Appendix A, “Troubleshooting” for recommended actions for addressing these issues in future runs.

## Confirm that data were exported to Converge™ Software

**Note:** If you have not configured the HID Genotyper Plugin to export results automatically to the Converge™ Software, see “(If needed) Manually export data to the Converge™ Software” on page 99.

1. Sign in to Torrent Suite™ Software.
2. Select **Data ▶ Completed Runs & Reports**, then click a **Report Name** to view the run summary.
3. Click **Plugins**, click **HIDGenotyper-X.X**, select the appropriate plugin run from the dropdown list (if more than one), confirm that the status is **Completed**, then click **results.html**.



4. In the **HID Genotyper Result** window, confirm that the **Upload Status** column displays "Completed" and the **Description** displays "Successful" in all rows.

**Note:** If these two conditions are not met, see “Export from Torrent Server to Converge™ Software Server failed” on page 109.

5. Click the **Batch ID** link to view the results in the Converge™ Software.

### HID Genotyper Result

Run ID: JCU1X Analysis Settings: [↓](#)

Run Name: R\_2018\_07\_27\_08\_56\_37\_user\_S5XL-00298-318-Cera280\_Mendel\_Mt-WG\_Test\_1

Analysis Name: Auto\_user\_S5XL-00298-318-Cera280\_Mendel\_Mt-WG\_Test\_1\_641

Notes: TSS v5.10.0 / HIDGenotyper (2.1-6f66507)

Batch ID: [Batch-1032](#)

**Sample Data** [↑](#) [↓](#)

Page 1 of 2 10

Sample Name	Barcode ID	Upload Status	Description	Error
ID8	IonCode_0102	Completed	Successful	

- ① View results in the Converge™ Software
- ② Download individual files
- ③ Manually export sequencing results to the Converge™ Software
- ④ Status of data upload to Converge™ Software
- ⑤ Status of HID Genotyper Plugin vX.X analysis

## (If needed) Manually export data to the Converge™ Software

Manually export the sequencing results:




- If an error occurs during automatic export (see also “Export from Torrent Server to Converge™ Software Server failed” on page 109).
- If you have not configured the HID Genotyper Plugin to export results automatically to the Converge™ Software. See the Converge™ Software v2.3 (Pub. No. 100039539).

1. Sign in to the Torrent Server via the Torrent Suite™ Software.
2. Select **Data ▶ Completed Runs & Reports**, then click a **Report Name** to view the run report.
3. Click **Plugins**, click **HIDGenotyper-X.X**, then click **results.html**.



**Note:** If there are multiple plugin runs on the run report, select the appropriate run from the dropdown list, then click **results.html**. The **Batch External ID** from the Converge™ Software is appended to the run name.

4. In the **Sample Data** pane of the **HID Genotyper Result** window, perform the appropriate action:

Option	Action
If an error occurred during automatic export	Click  to try to export again. If results are still not exported to Converge™ Software, follow the instructions for manual export in the following row.
If you are not configured for automatic export	<ol style="list-style-type: none"> <li>a. Click  to download the results (&lt;Experiment Name&gt;.bef file).</li> <li>b. In the Converge™ Software <b>Batch Dashboard</b>, select  ▶ <b>Import Batch</b>, select the BEF file to import, then click <b>Upload</b>.</li> </ol>

## Related documentation for data analysis

See the following documents to analyze sequencing data from the Precision ID mtDNA panels and Custom Ion AmpliSeq™ SNP Panels:

- Converge™ Software v2.3 (Pub. No. 100039539)
- *Converge™ Software Help*



# Troubleshooting

This appendix contains brief information for troubleshooting manual library preparation, library and template preparation on Ion Chef™ Instrument, and sequencing on the Ion S5™/Ion S5™ XL Sequencer, and Ion GeneStudio™ S5 Series Sequencers.

For complete troubleshooting information about	See
Library preparation on the Ion Chef™ Instrument	<i>Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide</i> (Pub. No. MAN0013432)
Template preparation on the Ion Chef™ Instrument	<i>Ion 510™ &amp; Ion 520™ &amp; Ion 530™ Kit – Chef User Guide</i> (Pub. No. MAN0016854)
Sequencing on the Ion S5™ or Ion S5™ XL Instrument	<i>Ion S5™ and Ion S5™ XL Instrument User Guide</i> (Pub. No. MAN0010811)
Sequencing on an Ion GeneStudio™ S5 Series Instrument	<i>Ion GeneStudio™ S5 Instrument User Guide</i> (Pub. No. MAN0017528)

## Manual library preparation

Observation	Possible cause	Recommended action
The library concentration is less than recommended	Input DNA was inaccurately quantified.	Re-quantify input DNA using one of the Quantifiler™ kits. See “Mitochondrial DNA quantification kits” on page 18.
	Residual ethanol in sample DNA inhibited target amplification.	Carefully remove all drops, using an additional centrifugation and removal step, if necessary.
	Less than 0.1 ng of input DNA was used.	Add more DNA, or add up to 4 target amplification cycles. Alternatively, amplify the library as described in “Amplify the libraries” on page 31.
	PCR, digestion, or ligation was inefficient.	Ensure proper dispensing and mixing of viscous components at each step.
	AMPure™ XP Beads were over-dried.	Do not dry the AMPure™ XP Beads for more than 10 minutes. If the bead surface appears cracked, then either continue with the protocol, and expect a higher percentage of low quality DNA, or repeat the library preparation.

Observation	Possible cause	Recommended action
Amplicons are lost	The library was poorly purified.	Vortex the AMPure™ XP Reagent thoroughly before use, and dispense the full volume.  Increase the AMPure™ XP Reagent volume from 1.5X to 1.7X. See “Purify the libraries” on page 27.
	Denaturation of the digested amplicon occurred.	Verify the use of the 60°C/20-minute temperature incubation during the primer digestion step. See “Partially digest amplicons” on page 25.
Barcoded library representation is uneven	Library was inaccurately quantified.	Ensure that the sample library concentrations are within the control library concentration range (within the standard curve) as measured by qPCR.
	Libraries were inaccurately combined.	Dilute the libraries to the target concentration, then combine equal volumes.
Uniformity is poor, AT-rich amplicons are under-represented	PCR was inefficient.	Double the anneal and extend time.
Uniformity is low	Amplicons were under-represented.	Follow “(Optional) Amplify and purify the libraries” on page 31 to reamplify the libraries.
Low key signal (<60%) The key signal is the percentage of live ISPs with a key signal that is identical to the library key signal. Low key signal indicates incorrect signal processing.	<ul style="list-style-type: none"> <li>An error occurred during library preparation and/or sequencing.</li> <li>Barcode adapters did not ligate properly during library preparation, so no key is available to sequence.</li> <li>Sequencing flow and raw signal acquisition errors occurred.</li> </ul>	Re-start procedure from library preparation.
Percentage of test fragment ISPs is low (significantly <<1%)	Test fragment was not added, or another error occurred during Ion Chef™ template preparation.	Repeat Ion Chef™ template preparation.
Percentage of adapter dimer ISPs is high (>1%)  <b>Details:</b> Increased chip loading observed, but subsequently lower total reads because the adapter dimers will be filtered out.	The unamplified library was inefficiently purified.	Decrease the AMPure™ XP Reagent volume from 1.5X to 1.0X. See “Purify the libraries” on page 27.
	Barcode adapter dimer formation occurred.	Do not combine Switch Solution, diluted barcode adapter mix, and DNA ligase before adding to the ligation reaction.
	The barcode adapter concentration was too high.	Ensure that barcode adapters are diluted properly.
High polyclonal ISPs (>50%)	The library input for template preparation was too high.	Decrease the amount of sample library for templating preparation by 50%.

Observation	Possible cause	Recommended action
High polyclonal ISPs (>50%) (continued)	The library was inaccurately quantified.	Re-quantify library to ensure accurate quantification.
	The TDF was incorrectly calculated.	Ensure that the TDF is calculated correctly.
Low ISP loading (<50%) and high enrichment (>90%)	The liquid was not removed after the chip check.	Use a pipette to remove as much liquid as possible. Place the chip upside down in a centrifuge bucket, then perform a 5-second centrifugation with the chip tab pointing inward.
	Too much liquid is remaining in the chip after loading.	Perform a 5-second centrifugation with the chip tab pointing outward and remove any liquid. If some liquid remains in the chip after the centrifugation, lightly and rapidly tap the point of the chip tab against the benchtop a few times, then remove any liquid. Do NOT centrifuge the chip upside-down.
Percentage of low quality ISPs is high (>40%) Increased chip loading observed, but low quality ISPs are filtered, resulting in lower total reads and lower coverage.	Library input was low.	Double the volume of library used in template preparation.
	Library quality was low.	Use a fresh dilution of library prepared in a low-bind tube. Re-prepare library starting from re-quantified DNA.
ISP loading is low (<40%), and percentage of low quality ISPs is high (>60%)	Library input was low.	Increase the amount of sample library in template preparation.
	The library was inaccurately quantified.	Ensure that qPCR quantification is performed correctly.
	The TDF was incorrectly calculated.	Ensure that the TDF is calculated correctly.
	Library quality was low.	Re-prepare library starting from re-quantified DNA.
Extremely low ISP loading (<30%), then sequencing failure	The sequencing primer step was omitted.	If no template-positive, enriched ISPs were saved, start over at template preparation.
	The sequencing polymerase step was omitted.	If no template-positive, enriched ISPs were saved, start over at template preparation.
	There was no recovery of ISPs after enrichment.	Confirm OT2 and ES operated correctly. Verify the quantity and quality of the library that went into the OT2 amplification solution. Ensure that ~200 µL was present in the 0.2-mL PCR collection tube after the ES run completed.
Read histogram is atypical Sample results are atypical.	Quality of DNA used in library preparation was poor.	Re-isolate genomic DNA.
		Proceed with analysis, but expect dropouts and/or lower total coverage and imbalanced profile if sample is known to be degraded or to contain inhibitors.

Observation	Possible cause	Recommended action
Read histogram is atypical Sample results are atypical. <i>(continued)</i>	Amount of library used in template preparation was low.	Double the library input used in template preparation.
Percentage of total aligned bases is low (<95%) Expect lower total reads and coverage	Sample was contaminated with non-human DNA or inhibitors.	Re-extract DNA to remove inhibitors. Remove source of contaminating DNA.
	Non-specific amplification occurred caused by excessive number of PCR cycles.	Reduce the number of PCR amplification cycles used in library preparation.
	Incorrect reference library was used.	Use the correct reference library (hg19).
Mean raw accuracy is low (<95%) Extremely low accuracy indicates a sequencing issue.	Incorrect kits were used in template preparation, or sequencing reaction.	Use the correct kits, starting with template preparation, ensuring that correct Precision ID kits are used.
	pH of buffers (determined during sequencer initialization) was incorrect.	Use the correct sequencing kit. Do not store kit components near dry ice.
	Incorrect reference library was used.	Use the correct reference library (hg19).

## Library preparation on the Ion Chef™ Instrument

Observation	Possible cause	Recommended action
ISP loading is low (<40%) <b>Details:</b> Low total usable reads (<30%) Low polyclonal ISPs (<10%)	Too little library was added for Ion Chef™ template preparation.	Quantify library concentration by qPCR and repeat the Ion Chef™ templating run.
		Double the library input and repeat the Ion Chef™ templating run, or increase the number of amplification cycles.
		Continue to Converge™ analysis; expect lower overall coverage.
	DNA input integrity/library preparation was poor.	Re-isolate genomic DNA and repeat library preparation.
	Chip loading by the Ion Chef™ instrument was not optimal.	Repeat the Ion Chef™ templating run.

Observation	Possible cause	Recommended action
Key signal is low (<50%) The key signal is the percentage of live ISPs with a key signal that is identical to the library key signal. Low key signal indicates incorrect signal processing.	<ul style="list-style-type: none"> <li>An error occurred during library preparation and/or sequencing.</li> <li>Barcode adapters did not ligate properly during library preparation, so no key is available to sequence.</li> <li>Sequencing flow and raw signal acquisition errors occurred.</li> </ul>	Re-start procedure from library preparation.
		Continue with Converge™ analysis, but expect noisier results.
Uniformity is poor, AT-rich amplicons are under-represented	PCR was inefficient.	Double the anneal and extend time.
Percentage of polyclonal ISPs is high (>50%)	Library input was too high in template preparation.	Reduce library input and repeat template preparation.
	Library was mis-quantified.	Re-quantify library to ensure accurate quantification.
Percentage of test fragment ISPs is low (significantly <<1%)	Test fragment was not added, or another error occurred during Ion Chef™ template preparation.	Repeat Ion Chef™ template preparation.
ISP loading is low (<50%), and percentage of low quality ISPs is high (>50%)	Library input was low.	Increase the volume of library used in template preparation.
		Use a fresh dilution of library prepared in a low-bind tube.
	Library quality was low.	Re-prepare library starting from re-quantified DNA.
Read histogram is atypical Sample results are atypical.	Quality of DNA used in library preparation was poor.	Re-isolate genomic DNA.
		Proceed with analysis, but expect dropouts and/or lower total coverage and imbalanced profile if sample is known to be degraded or to contain inhibitors.
	Amount of library used in template preparation was low.	Double the library input used in template preparation.
Percentage of total aligned bases is low (<95%) Expect lower total reads and coverage	Sample was contaminated with non-human DNA or inhibitors.	Re-extract DNA to remove inhibitors.
	Non-specific amplification occurred caused by excessive number of PCR cycles.	Remove source of contaminating DNA. Reduce the number of PCR amplification cycles used in library preparation.



Observation	Possible cause	Recommended action
Percentage of total aligned bases is low (<95%) Expect lower total reads and coverage <i>(continued)</i>	Incorrect reference library was used.	Use the correct reference library (hg19).
Mean raw accuracy is low (<95%) Extremely low accuracy indicates a sequencing issue.	Incorrect kits were used in template preparation, or sequencing reaction.	Use the correct kits, starting with template preparation, ensuring that correct Precision ID kits are used.
	pH of buffers (determined during sequencer initialization) was incorrect.	Use the correct sequencing kit. Do not store kit components near dry ice.
	Incorrect reference library was used.	Use the correct reference library (hg19).

## Ion Chef™ System: general operation


Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	<ol style="list-style-type: none"> <li>1. Power off the instrument, wait 30 seconds, then power on the instrument.</li> <li>2. If the instrument fails again, contact Technical Support.</li> </ol>
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.

Observation	Possible cause	Recommended action
The instrument does not start a run	The instrument encountered a Deck Scan error (one or more consumables are absent or loaded improperly).	<ol style="list-style-type: none"> <li>1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the one or more errors displayed, replace the missing consumable as directed, tap <b>No</b> when prompted, then tap <b>Next</b> to cancel the run. After returning to the home screen, restart the run.</li> <li>2. If the error persists, ensure that: <ul style="list-style-type: none"> <li>• All cartridges and PCR plate are loaded correctly and are level on the instrument deck.</li> <li>• The barcodes of the 4 flagged tubes in Positions A–D of the Reagents cartridge are visible and positioned correctly.</li> <li>• All tubes are both present <i>and</i> uncapped in the Reagents cartridge.</li> </ul> </li> <li>3. If the error persists after you check the consumables on the instrument deck, do one of the following: <ul style="list-style-type: none"> <li>• If you are confident that the Ion Chef™ Instrument is set up correctly, and you are comfortable disregarding the warnings, tap <b>YES</b> following Deck Scan to proceed with the run.</li> <li>• If the instrument cannot start the run, contact Technical Support for further help.</li> </ul> </li> </ol>
	The instrument encountered an internal error.	<ol style="list-style-type: none"> <li>1. Record the error that is displayed on the instrument display, then tap <b>OK</b>.</li> <li>2. Contact Technical Support to report the problem and for further help.</li> </ol>
The instrument stops during a run	The instrument encountered an internal error.	<ol style="list-style-type: none"> <li>1. Record the error displayed on the instrument display, then tap <b>OK</b>.</li> <li>2. Contact Technical Support to report the problem and for further assistance.</li> </ol>
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.

Observation	Possible cause	Recommended action
Instrument displays one or more alerts during a run	The instrument detected one or more problems during the run.	After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot.  <b>IMPORTANT!</b> The detected problem might impact the performance of the sequencing run.
	<ul style="list-style-type: none"> <li>• Network connection to the server was interrupted.</li> <li>• User name or password was incorrect.</li> </ul>	<ol style="list-style-type: none"> <li>1. Tap the Instrument status button to view the alert(s).</li> <li>2. In the Instrument status screen, confirm that the name of the Torrent Server connection is red.</li> <li>3. Contact your network administrator to confirm that:                             <ul style="list-style-type: none"> <li>• The Torrent Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection.</li> <li>• The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials.</li> </ul> </li> <li>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.</li> </ol>
Liquid residue is present in the Recovery centrifuges following a run	During normal instrument operation, a noticeable coating of liquid collected on the bowl and buckets of the Recovery centrifuges after repeated runs.	Remove the residue as instructed in “Inspect and clean the Recovery centrifuges and buckets” on page 79.

# 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  	<ul style="list-style-type: none"> <li>Available software updates were detected.</li> <li>Connectivity issues were detected.</li> <li>Instrument did not detect required files or hardware.</li> </ul>	Tap the red <b>Alarms</b> icon to see detailed messages. <ul style="list-style-type: none"> <li>If a message states "Newer Software Available":               <p><b>IMPORTANT!</b> After updates are installed, the instrument must be restarted.</p> <ol style="list-style-type: none"> <li>In the main menu, tap <b>Settings</b> ▶ <b>Check for Updates</b>.</li> <li>Select the <b>Released Updates</b> checkbox, then tap <b>Update</b>.</li> <li>When installation is complete, follow the onscreen prompts to restart the instrument.</li> </ol> <p><b>Note:</b> In some cases, the instrument restarts automatically after software installation.</p> </li> <li>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.</li> <li>For any other messages:               <ol style="list-style-type: none"> <li>Power off the instrument: On the home screen, tap <b>Settings</b> ▶ <b>System Tools</b> ▶ <b>Shut Down</b> ▶ <b>Shut Down</b>.</li> <li>Wait 30 seconds, then press the button on the side of the instrument to power on the instrument.</li> </ol> </li> <li>If the red "Alarms" and/or "Events" message still appears in the main menu, contact Technical Support.</li> </ul>

## Initialization—General errors

Observation	Possible cause	Recommended action
Bottle leak check fails	<ul style="list-style-type: none"> <li>Bottle seal was not tight.</li> <li>Bottle was damaged or defective.</li> </ul>	<ol style="list-style-type: none"> <li>Finger-tighten the bottles.</li> <li>If the bottle continues to leak, replace the bottle.</li> <li>If leak check continues to fail, contact Technical Support.</li> </ol>

## Data transfer to Converge™ Software

Observation	Possible cause	Recommended action
Export from Torrent Server to Converge™ Software Server failed	Converge™ Software Server was not running at time of NGS run.	Confirm that Converge™ Software Server is running, then export results as described in “(If needed) Manually export data to the Converge™ Software” on page 99
	Appropriate Converge™ Software license is not installed (Error message CVG-04004: "The application module ngs x . x cannot be used. A valid license must be installed.")	Confirm that the necessary software license(s) are installed, then export results as described in “(If needed) Manually export data to the Converge™ Software” on page 99. For installation, renewal, and other license-related instructions, see the Converge™ Software v2.3 (Pub. No. 100039539).
	Temporary communication issues between servers.	Export results as described in “(If needed) Manually export data to the Converge™ Software” on page 99.



# Supplemental procedures

## 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 Precision ID Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.



3. Reinstall the empty waste reservoir and a *used* Precision ID Wash Solution bottle.
4. Ensure that the Ion S5™ Precision ID Sequencing Reagents cartridge and Precision ID 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 “Initialize the sequencer” on page 86.

## 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 HID Ion S5™, Ion S5™ XL, or HID 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™ Precision ID 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 [page 110](#)).
- 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.



**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](https://www.thermofisher.com/support).

## Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:




- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)








(continued)

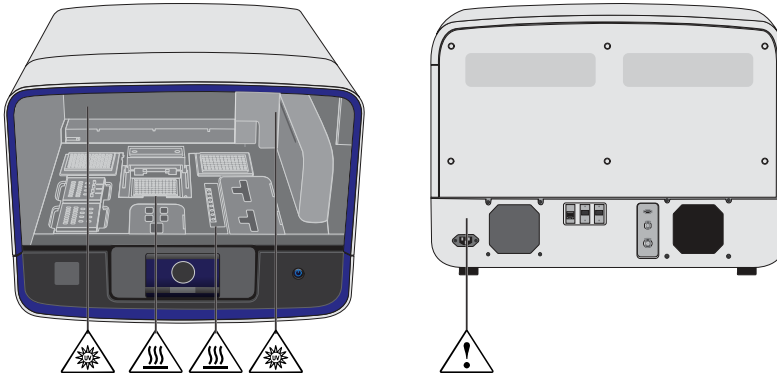
Symbol	English	Français
	<p>Do not dispose of this product in unsorted municipal waste</p> <p> <b>CAUTION!</b> To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.</p>	<p>Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.</p> <p> <b>MISE EN GARDE !</b> Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.</p>

## Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union Low Voltage Directive 2006/95/EC, EMC Directive 2014/30/EU, and R&TTE Directive 1999/5/EC.
	Indicates conformity with Australian standards for electromagnetic compatibility.



## Location of safety labels on this instrument



## 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 ensure that the UV lamp is not powered when the door is open.

## Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

### Safety compliance

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
IEC 61010-2-010 EN 61010-2-010	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</i>
IEC/EN 61010-2-020	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges</i>
IEC 61010-2-081 EN 61010-2-081	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes</i>



## EMC

Reference	Description
Directive 2014/30/EU	European Union “EMC Directive”
FCC Part 15	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS 2064	Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment
ICES-001, Issue 3	Industrial, Scientific and Medical (ISM) Radio Frequency Generators
US FCC CFR Title 47 Part 15.225, Subpart C	Operation within the band 13.110–14.010 MHz.
Industry Canada RSS 210, Issue 8, Annex 2 EN 302 291-1/2 V1.1.1	Licence-Exempt Radio Apparatus: Category I Equipment

## Environmental design

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive” – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment

## Instrument safety – HID Ion S5™/Ion GeneStudio™ S5 System

For detailed information on HID Ion S5™/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 Instrument User Guide* (Pub. No. MAN0010811), or the *Ion GeneStudio™ S5 Instrument User Guide* (Pub. No. MAN0017528), available at [thermofisher.com](http://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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf](http://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf)
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/publications/i/item/9789240011311](http://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Related documentation

Document	Publication number
<b>Planned Run setup and data analysis</b>	
<i>Torrent Suite™ Software Help</i>	MAN0017597
<i>Converge™ Software v2.3 Setup and Reference Guide</i>	100039539
<i>Converge™ Software Help</i>	Online only
<b>HID library preparation</b>	
<i>Precision ID mtDNA Panels: Manual Library Preparation Quick Reference</i>	MAN0017771
<i>Precision ID SNP Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide</i>	MAN0017767
<i>Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide</i>	MAN0016129
<i>Ion Library TaqMan™ Quantitation Kit User Guide</i>	MAN0015802
<b>Automated library preparation on the Ion Chef™ System</b>	
<i>Precision ID mtDNA Panels: Library Preparation on the HID Ion Chef™ System Quick Reference</i>	MAN0017772
<i>Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide</i>	MAN0013432
<i>Ion AmpliSeq™ Library Preparation on the Ion Chef™ System Quick Reference</i>	MAN0013433
<i>Ion Library TaqMan™ Quantitation Kit User Guide</i>	MAN0015802
<b>Template preparation on the Ion Chef™ System for S5</b>	
<i>Precision ID mtDNA Panels: Template Preparation and Sequencing Quick Reference</i>	MAN0017934
<i>Ion 510™ &amp; Ion 520™ &amp; Ion 530™ Kit – Chef User Guide</i>	MAN0016854
<i>Ion 510™ &amp; Ion 520™ &amp; Ion 530™ Kit – Chef Quick Reference</i>	MAN0016855
<b>Sequencer reference and maintenance</b>	
<i>Ion S5™ and Ion S5™ XL Instrument User Guide</i>	MAN0010811
<i>Ion GeneStudio™ S5 Instrument User Guide</i>	MAN0017528



## Customer and technical support

For support:

- **In North America**—Send an email to [HIDTechSupport@thermofisher.com](mailto:HIDTechSupport@thermofisher.com), or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to [thermofisher.com/support](http://thermofisher.com/support) to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) 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](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).



# Glossary

## aligned read length

The aligned length of a read at a given accuracy threshold is defined as the greatest position in the read at which the accuracy in the bases up to and including the position meets the accuracy threshold. For example, the AQ17 length of a read is the greatest length at which the read error rate is 2% or less. The "perfect" length is the longest perfectly aligned segment. For all of these calculations, the alignment is constrained to start from position 1 in the read—that is, no 5' clipping is allowed.

## aligned reads

The number of bases covered by reads aligned to the reference sequence.

## AQ score

An alignment quality (AQ) score defines read accuracy when compared to the reference sequence. The discrepancy can be biological (real variant) or technical (sequencing error). For example, an AQ score of 17 represents 2% aligned read error rate, while an AQ score of 20 represents 1% aligned read error rate when compared to the reference sequence.

## average base coverage depth

The average number of reads of all targeted reference bases.

## average base read depth

The average number of reads of all targeted reference bases that were read at least once.

## BAM file

A BAM (binary alignment map) file (.bam) is the binary version of a SAM (sequence alignment map) file (.sam). A SAM file is a tab-delimited text file that contains sequence alignment data. A BAM file contains aligned reads sorted by reference location.

## barcode

A barcode is a machine-readable code in the form of numbers and a pattern of parallel lines of varying widths, printed on and identifying a product.

There are several applications for barcodes. Libraries can be molecularly barcoded with unique nucleic acid sequence identifiers. Library barcodes are used during data analysis to sort the sequencing results from sequencing reactions that contain combined libraries. Chips and sample tubes also contain unique numeric barcodes that aid in the setup of the experimental analysis workflow.

## bead loading

The percentage of chip wells that contain live ISPs. The percentage value considers only potentially addressable wells. Bead loading is calculated as number of loaded ISPs divided by the number of potentially addressable wells.

## BED file

Browser Extensible Data file—BED file—defines chromosome positions or regions.

## clonal ISP

An ISP is clonal if all of its DNA fragments are cloned from a single original template. All fragments on such a bead are identical and respond in unison during a nucleotide flow across the chip.

## empty wells

Wells on a chip that do not contain an ISP.

## final library reads

Number (and percentage) of reads, passing all filters that are recorded in the unmapped BAM file. This value may be different from the total number of reads located in the Library Summary Section due to specifications associated with read trimming beyond a minimal requirement resulting in total number of reads being slightly less than Final Library Reads.

## flow

A flow is the event of exposing a chip to one particular dNTP ( deoxyribonucleotide triphosphate), followed by a washing step.

## flow order

The order in which a chip is exposed to each particular dNTP. The default Samba flow order consists of a 32-base sequence, repeated. This flow order resists phase errors by providing opportunities for out-of-phase molecules to catch up and is designed to sample all dimer (nucleotide pair) sequences efficiently. Samba is the default flow order because it improves sequencing accuracy for longer reads by resisting phase errors.

## hotspots file

A BED or a VCF file that defines regions in the gene that typically contain variants. Specifying a hotspots file to use in a run enables the Torrent Variant Caller (TVC) module to identify if a specific variant is present or absent. A hotspots file instructs the Torrent Variant Caller (TVC) module to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspots file does not affect other parts of the analysis pipeline.

## ISPs

Ion Sphere™ Particles (ISPs) are particles that contain bound copies of a single (ideally) DNA fragment amplified during template preparation.

**key signal**

Average 1-mer signal in the library key.

**library ISPs**

Live ISPs that have a key signal identical to the library key signal.

**library key**

A short known sequence of bases used to distinguish a library fragment from a test fragment (for example, "TCAG").

**live ISP**

An ISP with a signal of sufficient strength and composition to be associated with the library or test fragment key.

**low quality ISP**

An ISP with a low or unrecognizable signal.

**mapped reads**

Sequencing reads that have been mapped to the reference sequence.

**mean raw accuracy**

Average raw accuracy of 1-mers at a specific position in the read.

**MNP**

Multiple nucleotide polymorphism (MNP) is a genetic mutation in an allele that differs from the reference allele of the same length by >1 nucleotide.

**on target reads**

Sequencing reads mapped to any target region of the reference. A read is considered to be on target if at least one aligned base overlaps a target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5' bases of the read, is not counted.

**output files**

Output files consist of all BAM files, run reports, and plugin results.

**Planned Run**

A Planned Run is a file that contains executable instructions for individual runs. The file contains all the specifications, settings, and parameters for template preparation and chip loading on the Ion Chef™ Instrument, and sequencing on an Ion S5™/Ion S5™ XL or Ion GeneStudio™ S5 Series system.

**Planned Run template**

A reusable experimental design (digital protocol) for the sequencing instrument that holds specifications for sample preparation, sequencing, data export, and post-sequencing data analysis.

### **polyclonal ISP**

An ISP that carries clones from two or more library sequences.

### **preinstalled Planned Run template**

A Planned Run template that comes preinstalled with Torrent Suite™ Software on the Torrent Server. Preinstalled Planned Run templates are designed to help users create a Planned Run or a customized Planned Run template for a specific research application. Preinstalled Planned Run templates cannot be deleted from the server.

### **primer dimer ISP**

An ISP that carries an insert length of less than 8 base pairs.

### **Q score**

Phred quality score (Q score) is used to measure the accuracy of the nucleotide sequence generated by the sequencing instrument. The Q score represents the probability that a given base is called incorrectly by the sequencer.

### **Q17**

Predicted error rate of 2%.

### **Q17 bases**

The number of bases that have a Q Score of  $\geq 17$  in a given sequencing output.

### **Q20**

Predicted error rate of 1%.

### **Q20 bases**

The number of bases that have a Q Score of  $\geq 20$  in a given sequencing output.

### **read**

The sequence of a section of a unique fragment obtained after the end of the sequencing process.

### **read length**

The length of called reads measured in base pairs.

### **reference library**

A consensus nucleotide sequence that represents the genome of a particular species. The results from a sequencing run are compared to the reference library to identify sequence variants.

### **relationship group**

Defines related samples within a Sample Set. Related samples are designated by the same relationship group number.

## **SAM file**

A SAM (Sequence Alignment Map) file is a tab-delimited text file that contains sequence alignment data. It stores biological sequences aligned to a reference sequence.

## **sample**

Genetic material from one source (for example, DNA from one individual).

## **signal processing input files**

Signal processing input files consist of the raw voltage measurement data collected during the sequencing run. These files are required to reanalyze the run from signal processing.

## **SNP**

Single nucleotide polymorphism (SNP) is a genetic mutation in an allele that differs from the reference allele of the same length by one nucleotide.

## **STR**

Short Tandem Repeat (STR) is a repeated genomic sequence, variations in which are useful in human identification.

## **system SNR**

System signal-to-noise ratio.

## **target base coverage**

Summary statistics for targeted base reads of the reference. A base covered by multiple target regions is counted only once per sequencing read.

## **target regions file**

## **test fragment**

A test fragment (TF) is a known nucleotide sequence that is used to monitor system characteristics.

## **test fragment ISPs**

Live ISPs with a key signal that is identical to the test fragment key signal.

## **test fragment key**

A test fragment key (TF key) is the nucleotide sequence that is used to identify test fragment reads.

## **TF key peak counts**

Signal strength of the first three bases of the TF key.

## **total reads**

Total number of filtered and trimmed reads independent of length reported in the output BAM file.

### **uBAM file**

An unmapped BAM (uBAM) file is a variant form of the BAM file format in which the read data does not contain mapping information. This is basically an "off-label" use of the BAM format (which was specifically designed to contain mapping information) that is used for data management reasons. It allows you to attach metadata to the reads from as early on in the analysis process as possible.

### **unaligned reads**

Nucleotide bases covered by reads that are not aligned to the reference.

### **uniformity of base coverage**

The percentage of bases in all targeted regions (or whole genome) covered by at least 0.2X the average base coverage depth.

### **usable sequence / usable reads**

Usable reads consist of library ISPs that pass the polyclonal, low quality, and primer dimer filter.

### **VCF file**

A variant call format (VCF) file specifies a variant of interest and its location. This file stores the differences between the BAM file and the reference file.

### **wells with ISPs**

Number of wells that were determined to be positive for the presence of an ISP within the well. Wells containing ISPs have a delayed pH change due to the presence of an ISP slowing the detection of the pH change from the solution.



