Precision ID Panels with the Ion PGM™ System

APPLICATION GUIDE

for use with:
Precision ID mtDNA panels
Precision ID SNP panels
Precision ID GlobalFiler™ NGS STR Panel

for use with:
Precision ID Library Kit
Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate
Precision ID DL8 Kit
Ion PGM™ Hi-Q™ OT2 Kit
Ion PGM™ Hi-Q™ Chef Kit
Ion PGM™ Hi-Q™ STR Chef Kit
Ion PGM™ Hi-Q™ Sequencing Kit
Ion PGM™ Hi-Q™ STR Sequencing Kit
Ion 314™ Chip v2 BC
Ion 316™ Chip v2 BC
Ion 318™ Chip v2 BC

Catalog Numbers A30938, A31443, A25642, A25643, A30939, A26435, A30941, A33586, A33212, A27739, A25948, A32743, A25592, A32745, 4488144, 4483324, 4488150

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Manufacturer: Multiple Life Technologies Corporation manufacturing sites are responsible for manufacturing the products associated with the workflow covered in this guide.

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

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Validation Notice: The following Applied Biosystems™ panels have been internally tested but have not been validated under SWGDAM guidelines: Precision ID Ancestry Panel, Precision ID Identity Panel, Precision ID mtDNA Control Region Panel, Precision ID mtDNA Whole Genome Panel, and Precision ID GlobalFiler™ NGS STR Panel.

Revision history: Pub. No. MAN0015830

Revision	Date	Description
B.0	19 July 2017	• Updated for use of the Precision ID DL8 Kit, and the Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate. • Support added for new kits for sequencing Precision ID STR libraries: – Ion PGM™ Hi-Q™ STR Chef Kit – Ion PGM™ Hi-Q™ STR Sequencing Kit • Optional library amplification procedure added to Chapter 2, "Prepare libraries manually". • "Load the Ion Chef™ System" and "Clean the Ion Chef™ Instrument" topics reorganized for ease of use. • Minor changes and corrections.
A.0	13 May 2016	New document.

Contents

1 Product information

Products covered in this guide

This guide covers the following products:

Item	Cat. No.
Panels	
Precision ID Ancestry Panel	A25642
Precision ID Identity Panel	A25643
Precision ID mtDNA Whole Genome Panel	A30938
Precision ID mtDNA Control Region Panel	A31443
Precision ID GlobalFiler™ NGS STR Panel	A30939
Library preparation kits	
Precision ID Library Kit	A26435 (96 reactions) A30941 (384 reactions)
Precision ID DL8 Kit	A33212
Library preparation bundles	
Precision ID Ancestry Panel and Library Kit Bundle	A26807
Precision ID Identity Panel and Library Kit Bundle	A26808
Barcode adapters	
Precision ID IonCode™ 1–96 Kit in 96 Well PCR Plate	A33586
Template preparation kits	
Ion PGM™ Hi-Q™ Chef Kit [1]	A25948
Ion PGM™ Hi-Q™ STR Chef Kit[2]	A32743
Ion PGM™ Hi-Q™ OT2 Kit	A27739
Sequencing chips	
Ion 314™ Chip v2 BC	4488144
Ion 316™ Chip v2 BC	4483188 4483324
Ion 318™ Chip v2 BC	4488146 4488150
Library and template preparation system	
Ion Chef™ Instrument	A30070
Ion OneTouch™ 2 System	4474779

Item	Cat. No.
Sequencer	
Ion PGM™ Sequencer	4476115
Sequencing kits	
Ion PGM™ Hi-Q™ Sequencing Kit[3]	A25592
Ion PGM™ Hi-Q™ STR Sequencing Kit[4]	A32745
Ion PGM™ Hi-Q™ Chef Kit [5,3]	A25948
Ion PGM™ Hi-Q™ STR Chef Kit[5,4]	A32743
Ion PGM™ Wash 2 Bottle Kit[6]	A25591

[1] This kit also contains sequencing reagents for the Ion PGM™ Sequencer.

[2] This kit is similar to A25948 (Ion PGM™ Hi-Q™ Chef Kit) except that it contains A32745 (Ion PGM™ Hi-Q™ STR Sequencing Kit). This kit is only compatible with A30939 (Precision ID GlobalFiler™ NGS STR Panel).

[3] Used for sequencing of libraries prepared with Precision ID SNP and mtDNA panels.

[4] Used for sequencing of libraries prepared with the Precision ID GlobalFiler™ NGS STR Panel.

[5] This kit also contains template preparation reagents for the Ion Chef™ Instrument.

[6] This kit is required for sequencing on the Ion Chef™ Instrument or the Ion OneTouch™ 2 Instrument.

Precision ID panel overview

Use any the following panels for preparing libraries on the Ion Chef™ Instrument or preparing libraries manually.

IMPORTANT! For the Precision ID GlobalFiler™ NGS STR Panel, we strongly recommend that you prepare libraries manually.

Panel[1]	Cat. No.	Average amplicon size[2]	Amount	No. of primer pairs	Storage
Precision ID Ancestry Panel	A25642	127 bp	1 tube[3]	165	–30°C to –10°C
Precision ID Identity Panel	A25643	138 bp	1 tube[3]	124	
Precision ID mtDNA Whole Genome Panel	A30938	163 bp	2 tubes[3]	81[4]	
Precision ID mtDNA Control Region Panel	A31443	153 bp	2 tubes[3]	7[5]	
Precision ID GlobalFiler™ NGS STR Panel[6]	A30939	—	1 tube[3]	32	

[1] For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures. For licensing and limited use restrictions visit thermofisher.com/HIDlicensing.

[2] Libraries have an additional ~80 bp due to barcode adapters.

[3] Sufficient for 96 reactions if preparing libraries manually, or 32 reactions if preparing libraries using the Ion Chef™ Instrument.

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

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

[6] Do not confuse the Precision ID GlobalFiler™ NGS STR Panel with the Precision ID GlobalFiler™ NGS STR Panel v2 (Cat. No. A33114). These are different panels.

SNP panels

The Precision ID Ancestry Panel (Cat. No. A25642) and the Precision ID Identity Panel (Cat. No. A25643) contain pools of PCR primers for amplification of forensically relevant genomic target regions. The primers contain proprietary modifications that enable removal of primer sequences during library preparation, resulting in efficient target assessment during sequencing.

Mitochondrial 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 AmpliSeq™-designed PCR primers for preparing libraries from mitochondrial DNA (mtDNA). Both kits are a dual-panel pool system with small amplicon overlaps to cover the mtDNA genome.

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.

Product requirement

This panel is 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.

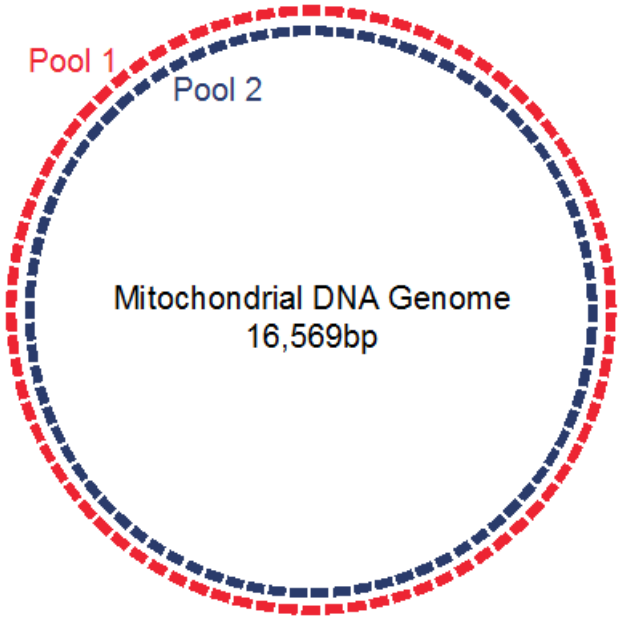

Figure 1 Visual representation of the dual panel system. It does not accurately display the actual number of amplicons.

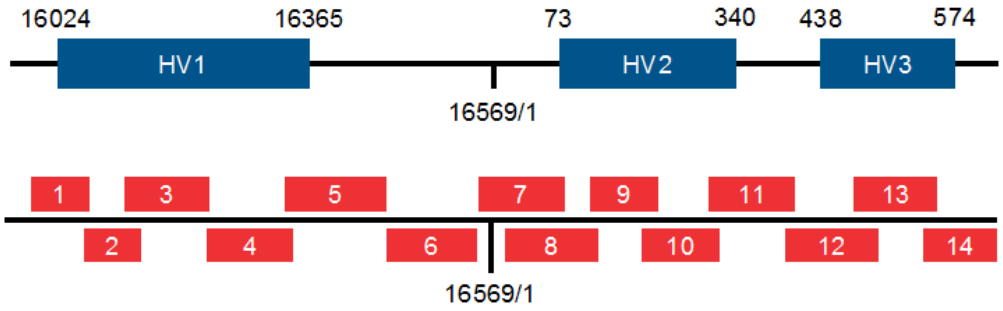

Figure 2 The Hypervariable Regions of the mtDNA Control Region and insert locations of the Precision ID mtDNA Control Region Panel.

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

Three 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. However, no one system has all these optimizations. The procedure that uses these methods is found in "Prepare the mtDNA amplification reaction" on page 24.

Method	Sample type	Reagent use	Amplification and ligation reactions
Full	Very low copy number samples	2X	Amplify each pool separately. In the barcode ligation reaction, use the same barcode adapter for BOTH pools.
2-in-1	Low copy number samples	2X	Amplify each pool separately, then pool 10 µL of each sample to create a new pool. Place 20 µL 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 (*for example, buccal*)	1X	Amplify each pool in a 10-µL 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. **Note:** 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.

Number of degenerate primers for each pool

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

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

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

Precision ID GlobalFiler™ NGS STR Panel

Overview

The Precision ID GlobalFiler™ NGS STR Panel (Cat. No. A30939) is a pool of unlabeled AmpliSeq™-designed primers for preparing libraries of forensically relevant Short Tandem Repeats (STRs) from genomic DNA. The panel targets 33 markers:

- 20 autosomal STR CODIS and Expanded CODIS loci
- 1 Y-chromosome STR locus
- 1 autosomal NC02 locus
- 3 autosomal Low Probability of Identity (PI) (0.09) STR loci
- 5 autosomal Next Generation Sequencing (NGS) STR loci
- 1 indel polymorphic marker on the Y chromosome (Y indel)
- X and Y amelogenin, the sex determining marker

Note: Do not confuse the Precision ID GlobalFiler™ NGS STR Panel with the Precision ID GlobalFiler™ NGS STR Panel v2 (Cat. No. A33114). These are different panels.

Product requirement

The Precision ID GlobalFiler™ NGS STR Panel is designed for use with the Precision ID Library Kits (Cat. Nos. A26435, A30941).

About the primers

The STR primers were designed through the AmpliSeq™ Designer pipeline. The primers are different from primers in Applied Biosystems™ Capillary Electrophoresis (CE) kits, that is, GlobalFiler™ PCR kits, and the Identifiler™ Plus PCR Amplification Kits, for example.

20 autosomal and 1 Y-chromosome STR loci

The 20 autosomal and 1 Y-chromosome STR loci target the same loci as the GlobalFiler™ kit, except for SE33. The following table describes the loci amplified, repeat type, repeat structure, source, chromosome location, and position of the loci.

Note: Chromosome position is based on bioinformatics nomenclature using human reference genome hg19. It is not listed as traditional locus nomenclature.

Note: Due to the Next Generation Sequencing (NGS) chemistries, an allelic ladder is not needed during sequencing. However, a virtual allelic ladder is used during sequence analysis.

Locus	Repeat type	Repeat structure	Source	Chromosome	Position (hg19)
Amelogenin-X	indel	N/A	Sex determination	X	11,315,017
Amelogenin-Y	indel	N/A	Sex determination	Y	6,737,908
CSF1PO	simple	AGAT	CODIS	5	149,455,887
D1S1656	compound	TAGA	Expanded CODIS	1	230,905,362
D2S441	compound	TCTA/TCAA	Expanded CODIS	2	68,239,079
D2S1338	compound	TGCC/TTCC	Expanded CODIS	2	218,879,582
D3S1358	compound	TCTA/TCTG	CODIS	3	45,582,231

Locus	Repeat type	Repeat structure	Source	Chromosome	Position (hg19)
D5S818	simple	AGAT	CODIS	5	123,111,250
D7S820	simple	GATA	CODIS	7	83,789,542
D8S1179	compound	TCTA/TCTG	CODIS	8	125,907,107
D10S1248	simple	GGAA	Expanded CODIS	10	131,092,508
D12S391	compound	AGAT/AGAC	Expanded CODIS	12	12,449,954
D13S317	simple	TATC	CODIS	13	82,722,160
D16S539	simple	GATA	CODIS	16	86,386,308
D18S51	simple	AGAA	CODIS	18	60,948,900
D19S433	compound	AAGG/TAGG	Expanded CODIS	19	30,417,142
D21S11	complex	TCTA/TCTG	CODIS	21	20,554,291
D22S1045	simple	ATT	Expanded CODIS	22	37,536,327
DYS391	simple	TCTA	Expanded CODIS	Y	14,102,795
FGA	compound	CTTT/TTCC	CODIS	4	155,508,888
TH01	simple	TCAT	CODIS	11	2,192,319
TPOX	simple	AATG	CODIS	2	1,493,425
vWA	compound	TCTA/TCTG	CODIS	12	6,093,143

Y-indel

The following table describes the amplicon size and position of the Y-indel:

Locus	Type	Amplicon size	Chromosome	Position (hg19)
rs2032678	Y-indel	93 bp	Y	15,508,706 – 15,508,710

NC02, low PI, and NGS loci

The NC02, low PI, and NGS loci were selected because of their high probability of identity or high sequence variance. The following table describes the loci amplified, the repeat type, repeat structure, source, chromosome location, and position.

Locus	Repeat type	Repeat structure	Source	Chromosome	Position (hg19)
D1S1677	simple	TTCC	NC02	1	163,559,816
D2S1776	simple	AGAT	PI < 0.09	2	169,645,403
D3S4529	simple	ATCT	PI < 0.09	3	85,852,633
D4S2408	simple	ATCT	PI < 0.09	4	31,304,420
D5S2800	compound	GATA/GATT	NGS	5	58,698,958
D6S474	complex	GATA/GACA	NGS	6	112,879,153
D6S1043	compound	AGAT/AGAC	NGS	6	92,449,943
D12ATA63	compound	TAA/CAA	NGS	12	108,322,367
D14S1434	complex	CTGT/CTAT	NGS	14	95,308,391

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 panels, this kit requires 1 ng of DNA 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. A26435 (96 reactions)	Cat. No. A30941 (384 reactions)[1]	
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] 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) • Ion AmpliSeq™ Tip Cartridge L8 • Framed PCR Foil Seal • Enrichment Cartridge	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: • Precision ID IonCode™ Barcode Adapters 1–8 for Chef DL8 in 96 Well PCR Plate (red) • Precision ID IonCode™ Barcode Adapters 9–16 for Chef DL8 in 96 Well PCR Plate (yellow) • Precision ID IonCode™ Barcode Adapters 17–24 for Chef DL8 in 96 Well PCR Plate (green) • Precision ID IonCode™ Barcode Adapters 25–32 for Chef DL8 in 96 Well PCR Plate (blue)	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 and sequencing kits

For the template preparation kit for the Ion Chef™ System, see Chapter 4, "Prepare the template on the Ion Chef™ Instrument" and refer to "Required materials" on page 56.

For the template preparation kit for the Ion OneTouch™ 2 System, see Chapter 5, "Prepare the template on the Ion OneTouch™ 2 Instrument", and refer to "Ion PGM™ Hi-Q™ OT2 Kit components" on page 84.

For sequencing kits for the Ion PGM™ Sequencer, see Chapter 6, "Sequence on the Ion PGM™ System" and refer to "Materials required" on page 88.

Workflow

IMPORTANT! For the Precision ID GlobalFiler™ NGS STR Panel, we strongly recommend that you prepare the libraries manually.

	Prepare the library manually:	**Prepare the library using the Ion Chef™ Instrument:**
Library preparation	Chapter 2, "Prepare libraries manually"	Chapter 3, "Prepare libraries using the Ion Chef™ Instrument"
	▼	▼
Template preparation	Chapter 4, "Prepare the template on the Ion Chef™ Instrument"	Chapter 4, "Prepare the template on the Ion Chef™ Instrument"
	▼	
Sequencing	Chapter 6, "Sequence on the Ion PGM™ System"	

Note: Before starting, ensure that

- You have updated your Ion PGM™ System to Torrent Suite™ Software 5.2.2.
- You have installed appropriate BED and reference files into Torrent Suite™ Software.

2 Prepare libraries manually

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

IMPORTANT! If you are using the Precision ID GlobalFiler™ NGS STR Panel, we strongly recommend that you prepare libraries manually.

DNA extraction and quantification kits

Genomic DNA extraction kits

We recommend the PrepFiler *Express*™and PrepFiler *Express* BTA™ Forensic DNA Extraction Kits for extracting, then purifying DNA from various forensic sample types:

- PrepFiler *Express*™ Forensic DNA Extraction Kit (Cat. No. 4441352) is designed for common forensic sample types, including body fluid stains and swabs of body fluids.
- PrepFiler *Express* BTA™ Forensic DNA Extraction Kit (Cat. No. 4441351) is designed for challenging forensic sample types such as bone, teeth, and adhesive-containing substrates including cigarette butts, chewing gum, and tape lifts.

The kits are appropriate for use with samples containing potential PCR inhibitors.

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" on page 23.

Genomic DNA quantification kits

Several commercially available kits are appropriate for quantifying human DNA. We recommend one of the following kits for quantifying DNA from forensic samples:

- Quantifiler™ Duo DNA Quantification Kit (Cat. No. 4387746)
- Quantifiler™ Trio DNA Quantification Kit (Cat. No. 4482910)
- Quantifiler™ HP DNA Quantification Kit (Cat. No. 4482911)
- Quantifiler™ Human DNA Quantification Kit (Cat. No. 4343895)

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.

Mitochondrial DNA quantification kits

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

Required materials for manual library preparation, not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments and equipment	
One of the following HID-approved PCR instruments and software: • 7500 Real-Time PCR Instrument • GeneAmp™ PCR System 9700 with silver or gold block. • Veriti™ 96-Well Thermal Cycler • ProFlex™ 96-well PCR System	See web product pages
DynaMag™-96 Side Magnet, or other plate magnet	12331D
Pipettors (2–200 µL)	MLS
Plates, tubes, and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate	N8010560 4306737 (with barcode)
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes (0.5 mL and 1.5 mL)	MLS
Low-retention, filtered pipette tips	MLS
Accessories	
(*Optional*) Eppendorf™ MixMate™ tool with 96 Tube holder (*for library preparation and elution*)	Eppendorf 5353 000.014
Reagents	
Agencourt™ AMPure™ XP Reagent	Fisher Scientific NC9959336, NC9933872, or MLS
Ion Library TaqMan® Quantitation Kit	4468802
Nuclease-free Water	AM9932
Absolute ethanol	MLS

Workflow: Prepare libraries manually

"Extract, then quantify input DNA" on page 23

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"Prepare DNA target amplification reactions" on page 24

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"Amplify the targets" on page 28

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"Ligate adapters to the amplicons, then purify" on page 30

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"Quantify the libraries by qPCR" on page 32

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"Dilute, pool, and store the libraries" on page 36

Extract, then quantify input DNA

Guidelines for genomic DNA input per reaction

- See "Genomic DNA extraction kits" on page 21 for a list of recommended genomic DNA extraction kits.
- See "Genomic DNA quantification kits" on page 21 for a list of recommended genomic DNA quantification kits.
- Use 1 ng of input genomic DNA per target amplification reaction for all Precision ID panels.

Guidelines for mitochondrial DNA input per reaction

- 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 21 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 DNA target amplification reactions

Refer to one of the following sections depending on the panel that you are using:

- "Prepare the SNP amplification reaction" on page 24
- "Prepare the mtDNA amplification reaction" on page 24
- "Prepare the STR amplification reaction" on page 27

Prepare the SNP amplification reaction

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

1. Add the following components to each well of a 96-well PCR plate.

   Note: Prepare a master mix for multiple reactions.

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Precision ID Identity Panel or Precision ID Ancestry Panel	10 µL
gDNA, 1 ng[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] Less than 1 ng of gDNA can be used, but appropriately adjust the number of PCR cycles in "Amplify the targets" on page 28.

[2] ⩽6 µL

2. Seal the plate with a MicroAmp™ Clear Adhesive Film. To prevent evaporation, create a tight seal by applying pressure with an applicator.

3. Vortex the plate thoroughly, then centrifuge to collect droplets. Place a MicroAmp™ Compression Pad on the plate, then go to "Amplify the targets" on page 28.

Prepare the mtDNA amplification reaction

IMPORTANT! 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. For descriptions of the methods, see "PCR methodologies for manual library preparation" on page 13.

Sample type	Method	Go to
Very low copy number samples	Full	step 2
Low copy number samples	2-in-1	step 3
Non-degraded samples (*for example, buccal*)	Conservative	step 4

2. For the Full method, prepare two master mixes, one for each pool:
   a. 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[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] 0.1 ng ≈ 2900 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 28.

[2] ⩽6 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)	4 µL
Precision ID mtDNA panel Pool 2	10 µL
gDNA, 0.1 ng[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] 0.1 ng ≈ 2900 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 28.

[2] ⩽6 µL

   c. Continue the library preparation as if you are processing two samples. Go to step 5.

3. For the 2-in-1 method, prepare two master mixes, one for each pool:
   a. 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[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] 0.1 ng ≈ 2900 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 28.

[2] ⩽6 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)	4 µL
Precision ID mtDNA panel Pool 2	10 µL
gDNA, 0.1 ng[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] 0.1 ng ≈ 2900 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 28.

[2] ⩽6 µL

c. Go to step 5.

4. For the Conservative method, prepare two master mixes, one for each pool:
   a. 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[1]	X µL[2]
Nuclease-free Water	3– X µL
Total	**10 µL**

[1] 0.1 ng ≈ 2900 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 28.

[2] ⩽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[1]	X µL[2]
Nuclease-free Water	3 – X µL
Total	**10 µL**

[1] 0.1 ng ≈ 2900 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 28.

[2] ⩽3 µL

   c. Go to step 5.

5. Seal the plate with a MicroAmp™ Clear Adhesive Film. To prevent evaporation, create a tight seal by applying pressure with an applicator.

6. Vortex the plate thoroughly, then centrifuge to collect droplets. Place a MicroAmp™ Compression Pad on the plate, then go to "Amplify the targets" on page 28.

Prepare the STR amplification reaction

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

1. Add the following components to each well of a 96-well PCR plate.

   Note: For multiple reactions, prepare a master mix.

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Precision ID STR panel	10 µL
gDNA, 1 ng[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] Less than 1 ng of gDNA can be used, but appropriately adjust the number of PCR cycles in "Amplify the targets" on page 28.

[2] ≤6 µL

2. Seal the plate with a MicroAmp™ Clear Adhesive Film. To prevent evaporation, create a tight seal by applying pressure with an applicator.

3. Vortex the plate thoroughly, then centrifuge to collect droplets. Place a MicroAmp™ Compression Pad on the plate, then go to "Amplify the targets" on page 28.

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 SNP panels	1 ng (300 copies)	21 cycles
	<1 ng (<300 copies)	21 cycles + 1 to 5 cycles
Precision ID mtDNA panels	0.1 ng (~2900 mtDNA copies)[1]	21 cycles
	<0.1 ng	21 cycles + 1 to 5 cycles
Precision ID STR panel	1 ng (300 copies)	23 cycles
	0.5–1 ng	23 cycles
	0.5 ng	24 cycles
	0.250 ng	25 cycles
	0.125 ng	26 cycles

[1] 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.).

1. 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 [1]

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

2. If you are performing an SNP or STR amplification reaction, then this is a stopping point. If you are performing a mtDNA amplification reaction, go to step 3.

STOPPING POINT For SNP or STR amplification methods, the target amplification reactions can be stored at 10°C overnight on the thermal cycler. For longer-term storage, store at –20°C for up to one month.

3. If you are performing a mtDNA amplification, see the following table:

Method	Action
Full method	Proceed as if processing two samples. Use the same barcode adaptor for BOTH pools.
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 the Full, 2-in-1, or Conservative amplification methods, 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

1. Remove the plate seal, then **add 2 µL of FuPa Reagent** (brown cap) to each amplified sample. The total volume is ~22 µL.

2. 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.

3. 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

Precision ID IonCode™ Barcode Adapters are provided at the appropriate concentration, and include forward and reverse adapters in a single well. No further handling is necessary.

IMPORTANT! When handling barcoded adapters, avoid cross-contamination. After use, reseal the Precision ID IonCode™ Barcode Adapter plate with adhesive film and store at –30°C to –5°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 µL
2	Precision ID IonCode™ Barcode Adapters	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume** (including ~22 µL of digested amplicon)	**~30 µL**

   Note: If preparing libraries using the Precision ID mtDNA panels, and using the
   - Full method: use the same barcode for both pools
   - 2-in-1 method: use one barcode
   - Conservative method: use one barcode

2. Seal the plate with a new MicroAmp™ 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.

3. Load the plate in the thermal cycler, then run the following thermal cycling conditions depending on the panel:

Panel	Temperature	Time
Precision ID SNP panels or Precision ID mtDNA panels	22°C	30 minutes
	72°C	10 minutes
	10°C	Hold (for up to 1 hour)
Precision ID STR 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.

1. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library.

2. 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.

3. 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.

4. Carefully remove, then discard the supernatant without disturbing the pellet.

5. 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.

6. 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 µL 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 × 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 µL of supernatant, then combine with 198 µL 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-µL reactions.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Ion 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 reaction mixtures. For each sample, negative control, and standard, combine 10 µL of 2X TaqMan® qPCR Mix and 1 µL of 20X Ion TaqMan® Assay 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 well of a PCR plate.

4. Add 9 µL of the diluted (1:100) library, or 9 µL of each control library dilution, to each well (two wells per sample), for a total reaction volume 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 v1.2	Hold	50°C	2 minutes
	Hold	95°C	20 seconds

Real-time PCR System	Stage	Temperature	Time
7500 Real-Time PCR Instrument with HID Real-Time PCR Analysis Software v1.1 or v1.2	40 Cycles	95°C	3 seconds
		60°C	30 seconds

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

See "Dilute, pool, and store the libraries" on page 36 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, pool, and store the libraries" on page 36.
- If insufficient library was prepared, continue to "(Optional) Amplify and purify the libraries".
- Continue with less than optimal library concentration. 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 µL of each unamplified library (total undiluted library is ~50 µL, from "Elute the libraries" on page 32) with 72 µL of Platinum™ PCR SuperMix HiFi and 3 µL 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 and 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°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 µL of ethanol with 100 µL of Nuclease-free Water per sample.
- Do **NOT** substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

First-round purification

1. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.
2. **Add 25 µL (0.5X sample volume) of Agencourt™ AMPure™ XP Reagent** to each plate well containing ~50 µL of sample. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.
3. Incubate the mixture for 5 minutes at room temperature.
4. Place the plate in a magnet such as the DynaMag™ 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 step 4 above, **add 60 µL (1.2X original sample volume) of Agencourt™ AMPure™ XP Reagent**. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the plate in the magnet for 3 minutes or until the solution is clear. Carefully remove, then discard the supernatant without disturbing the pellet.

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

4. **Add 150 µL of freshly prepared 70% ethanol** to each well, then move the plate side to side in the magnet to wash the beads. Remove 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 pipet up and down 5 times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.

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 µL 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 32.

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 quantity mean) × (library dilution)

   For example:

   - qPCR quantities mean: 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.

   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.

   Recommended library dilutions based on template preparation instrument and panel

Template preparation instrument	Panel	Dilute to	Minimum volume	Templating size in Planned Run setup
Ion Chef™ System	Precision ID SNP panels	30 pM	25 µL	200 bp
	Precision ID mtDNA panels	30 pM	25 µL	200 bp
	Precision ID STR panel	30 pM	25 µL	200 bp
Ion OneTouch™ 2 Instrument [1]	Precision ID SNP panels	8 pM	25 µL	200 bp
	Precision ID mtDNA panels	8 pM	25 µL	200 bp
	Precision ID STR panel	12 pM	25 µL	200 bp

[1] When preparing template with the Ion OneTouch™ 2 Instrument, add the library at the concentration and volume specified in this table to the amplification solution (see the *Ion PGM™ Hi-Q™ OT2 Kit User Guide* Pub. No. MAN0010902) without further dilution.

(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 OneTouch™ 2 Instrument or Ion Chef™ Instrument.

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 Ion Chef™ Instrument

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 PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919).

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

IMPORTANT! If you are using a Precision ID STR panel to prepare libraries, use the manual library preparation method, see Chapter 2, "Prepare libraries manually". We do not recommend using the Precision ID STR panel on the Ion Chef™ Instrument to prepare libraries.

Software version requirements for library preparation

Panel	Software version required
Precision ID Ancestry Panel	Torrent Suite™ Software 5.2.2
Precision ID Identity Panel	
Precision ID mtDNA Control Region Panel	
Precision ID mtDNA Whole Genome Panel	

Required materials for library preparation on the Ion Chef™ System, not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments and equipment	
Non-interruptible Power Supply (UPS) [1]	MLS
Microcentrifuge[2]	MLS
Vortex mixer with a rubber platform	MLS
Pipettors (2–1000 µL)	MLS
Tubes, plates, and other consumables	
Microcentrifuge tubes (1.5 mL or 1.7 mL)	MLS
Filtered pipette tips	MLS
Wipes, disposable lint-free	MLS
Gloves, powder-free nitrile	MLS
Reagents	
Nuclease-free water, molecular biology grade	MLS
Isopropyl alcohol, 70% solution	MLS

[1] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend using a non-interruptible power supply that is compatible with 2500 W output or higher.

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

Workflow: Prepare libraries using the Ion Chef™ Instrument

IMPORTANT! If you are using a Precision ID STR panel to prepare libraries, use the manual library preparation method, see Chapter 2, "Prepare libraries manually". We do not recommend using the Precision ID STR panel on the Ion Chef™ Instrument to prepare libraries.

"Extract, then quantify input DNA" on page 23

▼

"Dilute the gDNA samples" on page 41

▼

"Thaw the reagents, then prepare the instrument" on page 41

▼

"(Optional) Create a sample set" on page 42

▼

"Add Precision ID primer pools to Positions A and B of the Reagents cartridge" on page 42

▼

"Add the DNA to the Precision ID DL8 IonCode™ Barcode Adapters" on page 43

▼

"Load the Ion Chef™ Instrument for library preparation" on page 44

▼

"Run the Ion Chef™ Instrument" on page 46

▼

"Unload the Ion Chef™ Instrument" on page 50

▼

"Dilute the libraries" on page 52

Extract, then quantify input DNA

Guidelines for genomic DNA input per reaction

- See "Genomic DNA extraction kits" on page 21 for a list of recommended genomic DNA extraction kits.
- See "Genomic DNA quantification kits" on page 21 for a list of recommended genomic DNA quantification kits.
- Use 1 ng of input genomic DNA per target amplification reaction for all Precision ID panels.

Guidelines for mitochondrial DNA input per reaction

- 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 21 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 0.067 ng/µL with Nuclease-free Water. Prepare 15 µL of each diluted sample (1 ng) 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™ System" on page 78).
4. Verify that the Ion Chef™ Instrument has a connection to the Torrent Server. On the Ion Chef™ home touchscreen, touch **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.

(*Optional*) Create a sample set

It is not necessary to create a sample set. Leave the sample set blank.

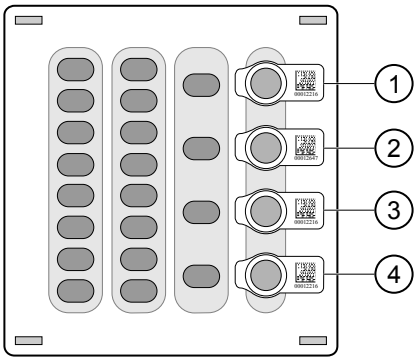
Add Precision ID primer pools to Positions A and B of the Reagents cartridge

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 panels to the Precision ID DL8 Reagents cartridge using the following guidelines (even if processing fewer than 8 samples):

If you are using	Action
Precision ID SNP panels (Identity and Ancestry)	Pipet 150 µL of the Pool into the Position A tube and 150 µL into the Position B tube.
Precision ID mtDNA panels[1]	Pipet 150 µL of Pool 1 into the Position A tube, and 150 µL of Pool 2 into the Position B tube.

[1] The Ion Chef™ Instrument performs similarly to the 2-in-1 method, as described on page 13.



① Position A (150 µL Pool 1)
② Position B (150 µL Pool 1 or 2)
③ Position C (Empty tube)
④ Position D (Output tube)

Note: When the run is complete, the tube in Position D contains 700 µL of combined barcoded libraries at a concentration of approximately 100 pM with 1 ng of input DNA.

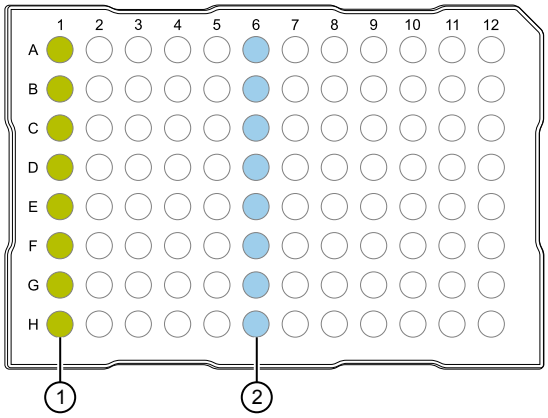
Note: If input DNA is <1 ng, then the library concentration is <100 pM and library quantification with qPCR is required.

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 µL of each DNA sample (0.067 ng/µL, 1 ng) 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.



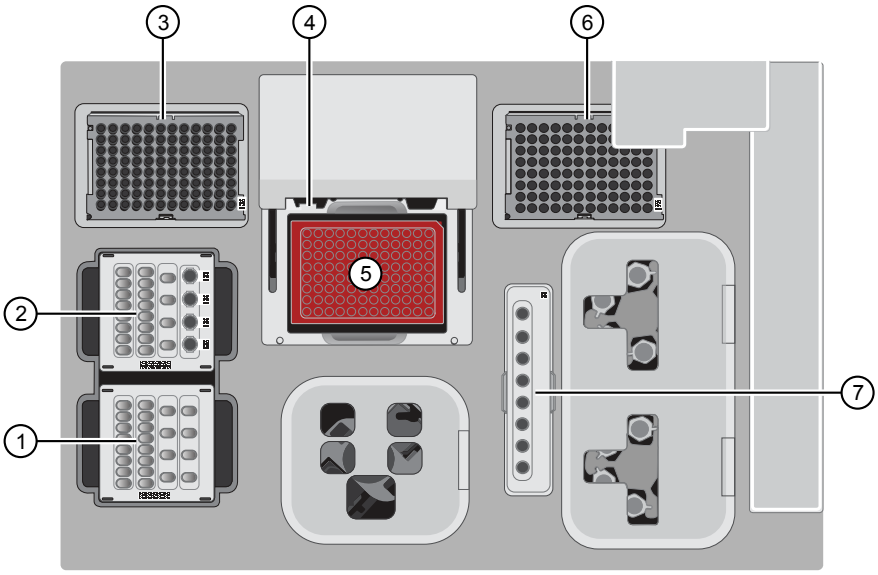
① Column 1 wells containing 15 µL of each diluted DNA sample (0.067 ng/µL).
② Column 6 wells containing 8 dried-down IonCode™ barcodes. Lowest number is in A6 and highest is in H6. All appear light blue in the actual plates.

Note: If processing fewer than 8 samples, it is preferable to add replicates or positive control samples to the run. Otherwise, pipet 15 µL 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

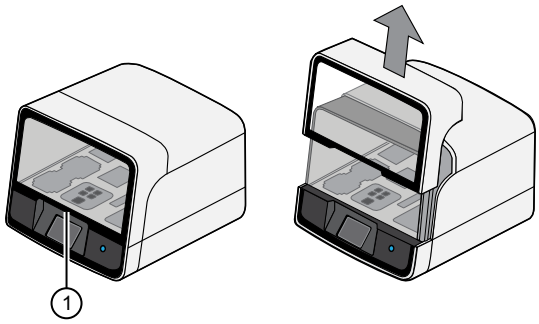
Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure:



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 PCR Plate
⑥ Empty Tip Cartridge L8
⑦ Enrichment Cartridge

1. Open the instrument door:
   a. On the instrument touchscreen, touch ⏏ (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 IonCode™ 96 Well PCR Plate containing gDNA onto the thermal cycler block and press down to seat it.
7. Slide a new Framed PCR Foil Seal underneath the automated heated cover.

   IMPORTANT! When the Framed PCR Foil Seal 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.

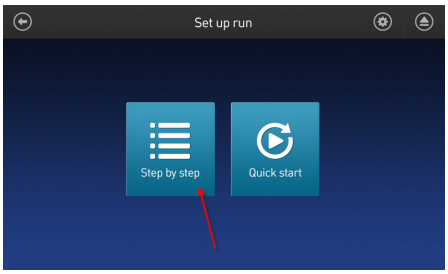
Run the Ion Chef™ Instrument

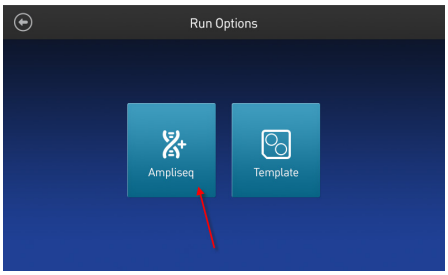
Perform the following steps to start an Ion AmpliSeq™ run on the Ion Chef™ Instrument.

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

2. Touch **Step by step**, then touch **AmpliSeq** on the **Run Options** screen.

   Note: To bypass the step by step deck loading guide, touch **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 touch screen.

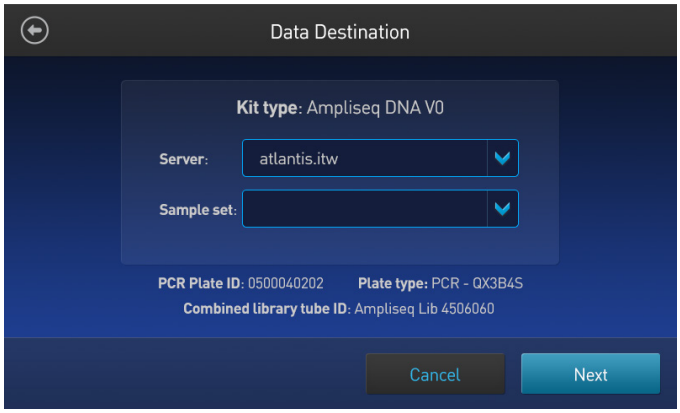
4. Touch **Start check** on the **Close Door** screen. The Ion Chef™ Instrument performs a Deck Scan.

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?" Touch **OK**.

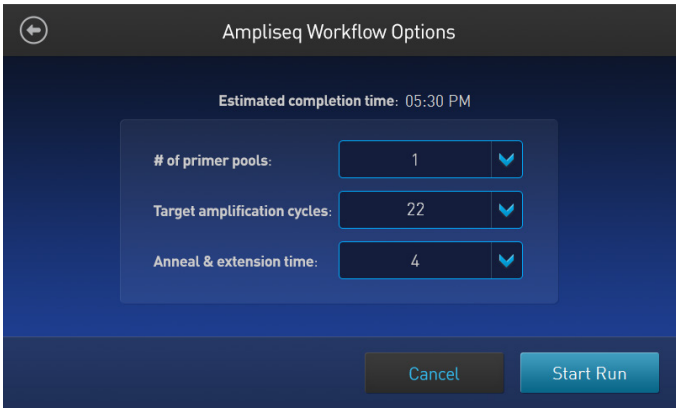
5. After Deck Scan completes (~3 minutes), touch **Next**.

6. In the Data Destination screen, verify the Server information, then touch **Next**.

Note: Sample set creation is not needed. Leave the Sample set blank.



7. Enter the appropriate number of primer pools, target amplification cycles, and an anneal/extension time for your run.



Panel recommendations for AmpliSeq™ Workflow Options shown above.

Panel	Amount of input gDNA added	# of primer pools	Cycle number	Anneal & extension time
Precision ID SNP panels (Identity and Ancestry)	1 ng (300 copies)	1	22 cycles	4
	<1 ng (<300 copies)	1	22 cycles + 1 to 5 cycles	4
Precision ID mtDNA panels	0.1 ng (~2900 copies)[1]	2	22 cycles	4
	<0.1 ng	2	22 cycles + 1 to 5 cycles	4

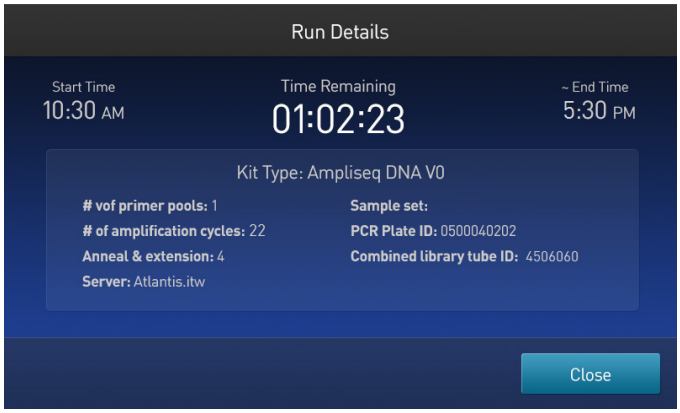
[1] gDNA quantifications were used to extrapolate the copy number of mtDNA. The actual number of mtDNA copies varies from sample source (for example; bone, blood, saliva, hair, etc.).

8. Touch **Start Run**.

9. After about 7 hours, return to the Ion Chef™ Instrument. On the Run Complete screen, touch **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.

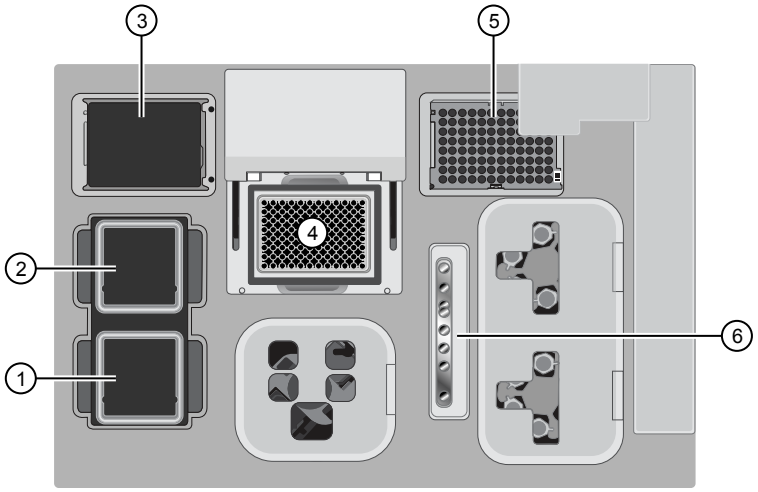
Note: View the run information in the Run Details screen.



You can also monitor your run on the Torrent Browser by navigating to **Monitor** ▸ **Ion Chef** and viewing the **Library Prep Progress** bar and **Library Prep Status** associated with your Sample Set.

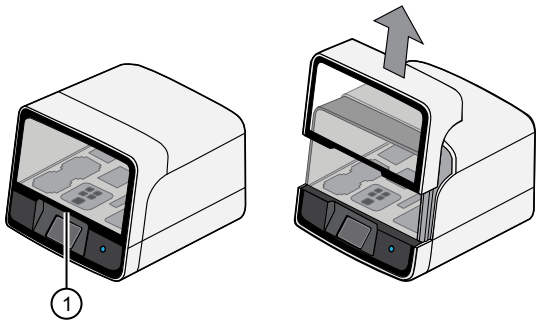
Unload the Ion Chef™ Instrument

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, touch ⏏ (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.



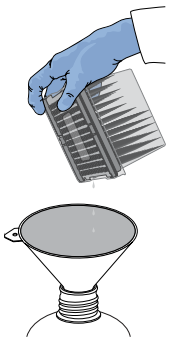
① Hold here and lift

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 Plate and foil seal from the PCR sample block.

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.

Dilute the libraries

IMPORTANT! If input DNA used in library preparation was less than 1 ng, we strongly recommend that you quantify the library pool by qPCR before proceeding. Go to "Quantify the libraries by qPCR" on page 32 for detailed instructions. If libraries are not quantified accurately, lower loading and read number can result.

Dilute the Ion library pool to the optimal input concentration before proceeding to templating. 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.

Recommended library dilutions based on template preparation, instrument, and panel

Template preparation instrument	Panel	Dilute to	Minimum volume	Templating size in Planned Run setup
Ion Chef™ System	Precision ID SNP panels	30 pM	25 µL	200 bp
	Precision ID mtDNA panels	30 pM	25 µL	200 bp
Ion OneTouch™ 2 Instrument [1]	Precision ID SNP panels	8 pM	25 µL	200 bp
	Precision ID mtDNA panels	8 pM	25 µL	200 bp
	Precision ID STR panel	12 pM	25 µL	200 bp

[1] When preparing template with the Ion OneTouch™ 2 Instrument, add the library at the concentration and volume specified in this table to the amplification solution (see the *Ion PGM™ Hi-Q™ OT2 Kit User Guide* (Pub. No. MAN0010902) without further dilution.

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

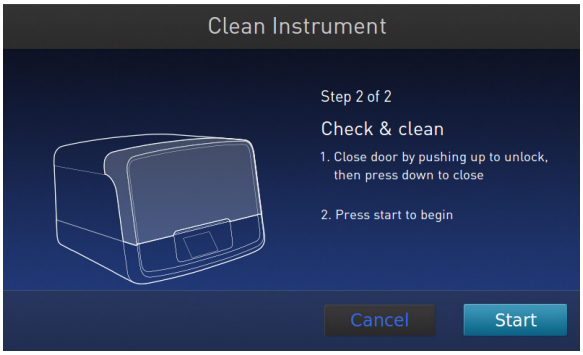
IMPORTANT! Clean the Ion Chef™ Instrument after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.

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, touch **Next**.

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

3. Ensure that you have removed all consumables from the Ion Chef™ Instrument, then touch **Next**.

4. With the door closed, touch **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.

Prepare the template on the Ion Chef™ Instrument

This chapter contains brief procedures for the HID workflow. For complete instrument procedures, troubleshooting, and maintenance information, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432).

Software version requirements for template preparation

Panel	Software version required
Precision ID Ancestry Panel	Torrent Suite™ Software 5.2.2
Precision ID Identity Panel	
Precision ID GlobalFiler™ NGS STR Panel	
Precision ID mtDNA Control Region Panel	
Precision ID mtDNA Whole Genome Panel	

Required materials

Ion PGM™ Hi-Q™ Chef Kit components

IMPORTANT! Use the Ion PGM™ Hi-Q™ Chef Kit for Precision ID SNP and mtDNA libraries.

Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948) contains reagents for preparing templates and sequencing.

Component	Part number	Quantity per kit
Ion PGM™ Hi-Q™ Chef Supplies	A25957	4 boxes
Ion PGM™ Hi-Q™ Chef Solutions	A25956	4 cartridges
Ion PGM™ Hi-Q™ Chef Reagents	A25955	4 cartridges
Ion PGM™ Sequencing Supplies	A25587	1 box
Ion PGM™ Hi-Q™ Sequencing dNTPs	A25590	1 box
Ion PGM™ Hi-Q™ Sequencing Solutions	A25589	1 box

Ion PGM™ Hi-Q™ STR Chef Kit components

IMPORTANT! Use the Ion PGM™ Hi-Q™ STR Chef Kit for Precision ID STR libraries only.

Ion PGM™ Hi-Q™ STR Chef Kit (Cat. No. A32743) contains reagents for preparing templates, up to 8 chips, and up to 8 sequencing runs.

Component	Part No.	Quantity per kit
Ion PGM™ Hi-Q™ Chef Supplies	A25957	4 boxes
Ion PGM™ Hi-Q™ Chef Solutions	A25956	4 cartridges
Ion PGM™ Hi-Q™ Chef Reagents	A25955	4 cartridges
Ion PGM™ Sequencing Supplies	A25587	2 boxes
Ion PGM™ Hi-Q™ Sequencing dNTPs	A25590	2 boxes
Precision ID STR Hi-Q™ Sequencing Solutions	A31864	2 boxes

Workflow: Prepare the template on the Ion Chef™ Instrument

"Create a Planned Run" on page 58

▼

"Dilute the libraries for template preparation on the Ion Chef™ Instrument" on page 61

▼

"Prepare the libraries and consumables" on page 61

▼

"Prepare the libraries and consumables" on page 61

▼

"Load the Ion Chef™ System" on page 63

▼

"Start the Ion Chef™ Instrument run" on page 72

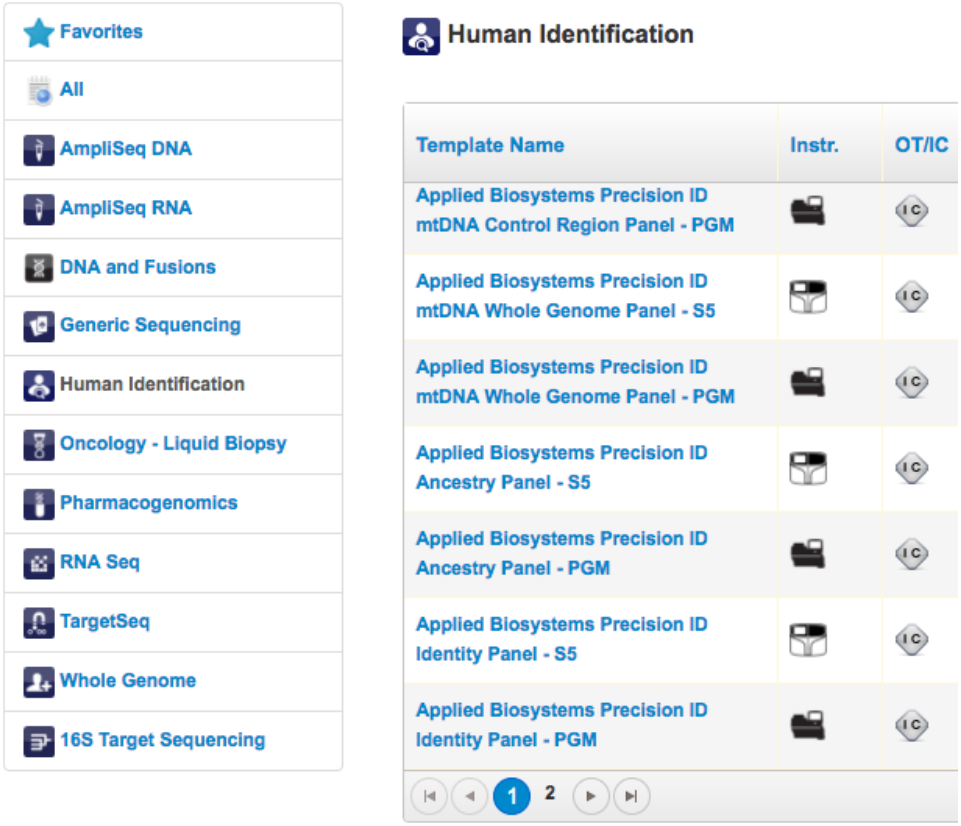
▼

"Clean the Ion Chef™ System" on page 78

Create a Planned Run

Human Identification templates can be used to create Planned Runs for various Applied Biosystems™ Precision ID panels. The templates pre-populate your Planned Run with recommended parameters. You can then select additional settings to plan your run.

1. Sign in to the Torrent Server via the Torrent Browser.
2. Select **Plan** ▸ **Templates**, then in the Favorites list, select **Human Identification**.



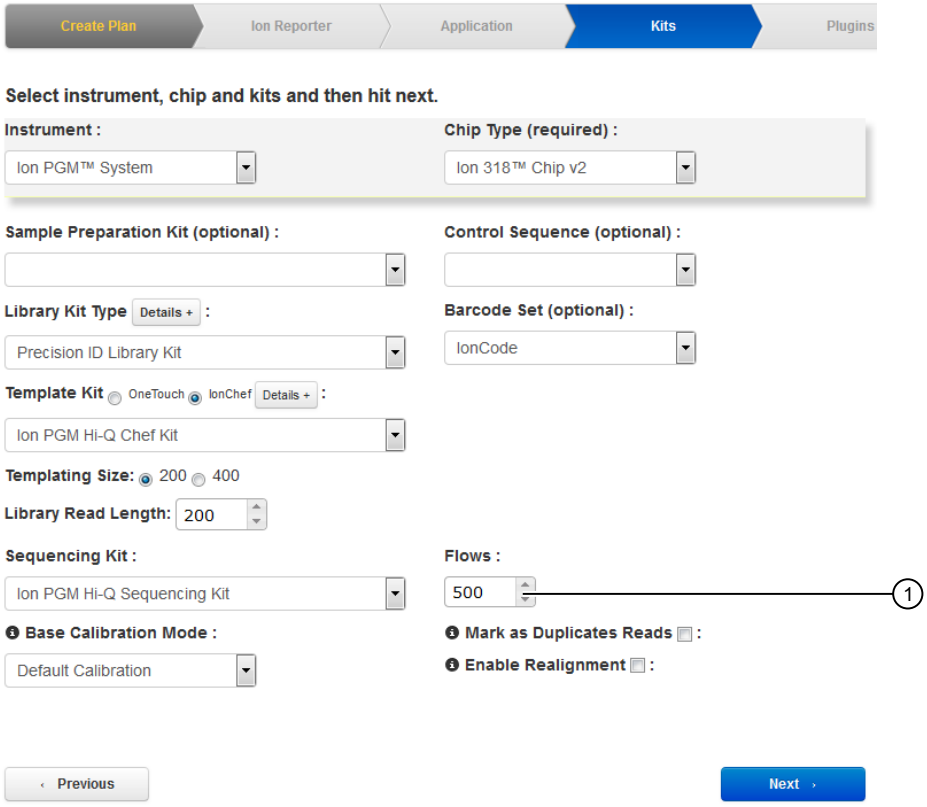
3. Select the Planned Run template appropriate to your panel and sequencer. The wizard launches and displays the **Plan** page.

   Note: All templates default to the Ion Chef™ Instrument.

4. On the **Plan** page, select the reference and BED files, enter the Sample names and Sample Tube Label, confirm the default settings, then enter a plan name.

5. To verify or change kit information, click the **Kits** tab. Specify the appropriate library, template and sequencing kits, and flow number and Barcode Set.

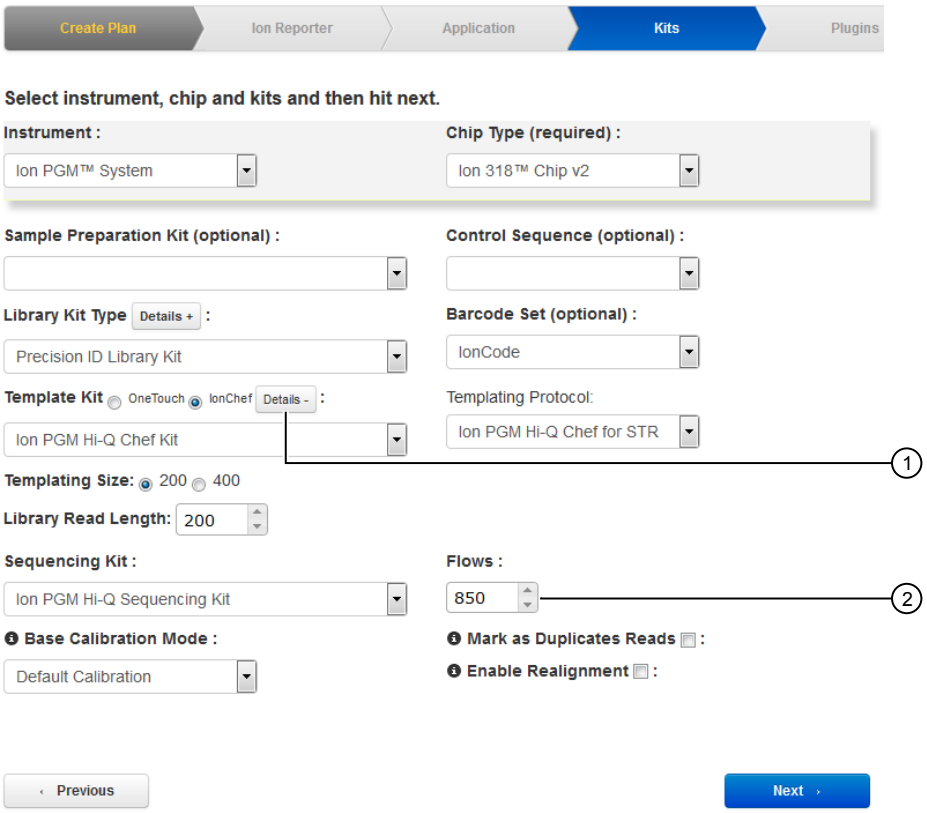
   - For the Precision ID SNP panels and the Precision ID mtDNA panels, enter **500** flows.



① Enter 500 flows

- For the Precision ID GlobalFiler™ NGS STR Panel,
  - Click the **Details +** button to display the **Templating Protocol** dropdown field, then select **Ion PGM Hi-Q Chef for STR**.
  - Enter **850** flows.

  Note: Users must select the **Applied Biosystems Precision ID GlobalFiler NGS STR Panel - PGM** Planned Run Template for the **Templating Protocol** option to be auto-selected.



① **Details +** button
② Enter 850 flows

6. When you have completed your selections, click **Plan Run** at the bottom right of the **Plan** screen to save the run. The run is listed on the Planned Runs page under the name that you specified and is automatically used by the Ion Chef™ System when the associated sample is loaded.

   Note: For more information, see the software user documentation, the *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919), the *HID STR Genotyper Plugin User Guide* (Pub. No. MAN0015879), or the *HID SNP Genotyper Plugin User Guide* (Pub. No. MAN0010641).

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 page 36. If you prepared the libraries using the Ion Chef™ Instrument, see page 52.

Prepare the libraries and consumables

1. At least 45 minutes before use, unpack the Ion PGM™Hi-Q™ Chef Reagents cartridge, then allow it to warm to room temperature.

   IMPORTANT! The Ion PGM™Hi-Q™ Chef Reagents cartridge must sit at room temperature for at least 45 minutes before use.

2. Pipet 25 µL of diluted library pool (for manually prepared libraries, see "Dilute the libraries" on page 36; for libraries prepared using the Ion Chef™ Instrument see "Dilute the libraries" on page 52.) to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes in the following figure).

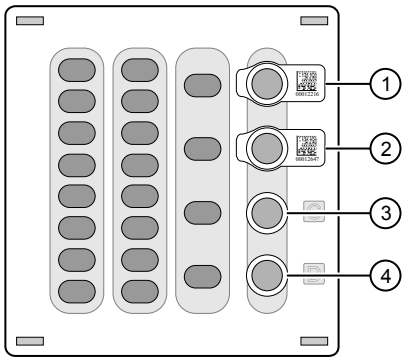


Figure 3 Ion PGM™Hi-Q™ Chef Reagents cartridge

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

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 v2 (2)
   - Enrichment Cartridge v2
   - Tip Cartridge v2
   - PCR Plate and Frame Seal v2
   - Recovery Station Disposable Lid v2 (2)

- Recovery Tube v2 (12)
- Ion PGM™ Hi-Q™ Chef Solutions
- Ion PGM™ Hi-Q™ Chef Reagents (from Step 1)

IMPORTANT! Before use, gently tap the Hi-Q™ Chef Reagents and the Hi-Q™ Chef Solutions cartridges on the bench to force the reagents to the bottoms of the tubes.

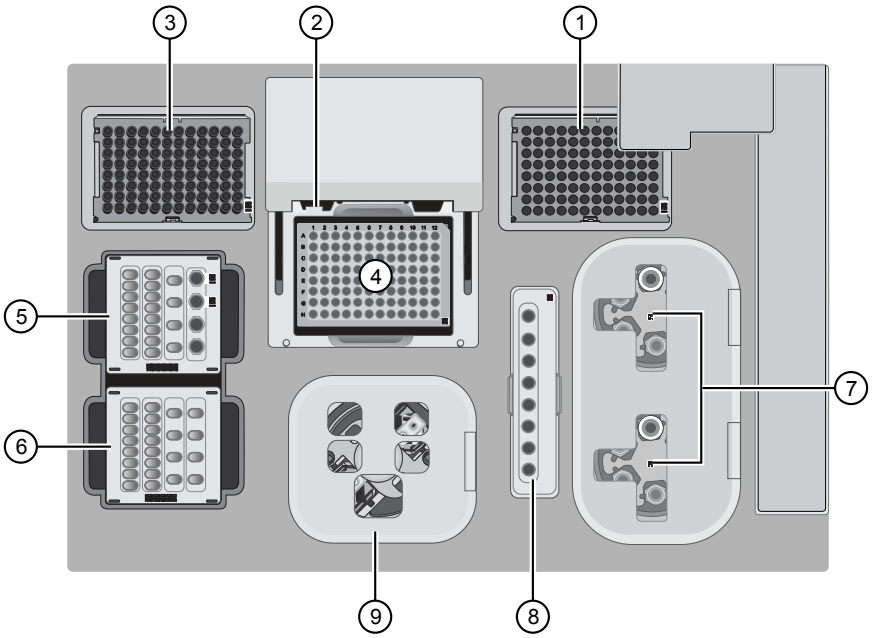
Note: When stored under normal conditions, a precipitate can form in some tubes of the Ion PGM™Hi-Q™ Chef Reagents cartridge. If present, load the cartridge as directed. The precipitate dissolves when the reagents are mixed during instrument operation.

Load the Ion Chef™ System

IMPORTANT!

- Rated centrifuge speeds are only intended 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 PGM™ Hi-Q™ Chef 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 has been cleaned following the last use.
- Ensure all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure the Reagents and Solutions station compartments are free of condensate before loading components.

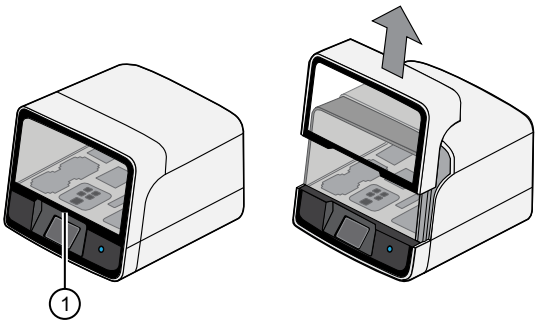
Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure:



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

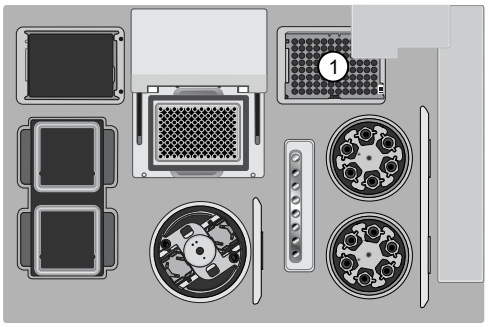
Load the pipette tip racks and PCR plate

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



① Hold here, then lift

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



① Used Pipette Tip Position

IMPORTANT!

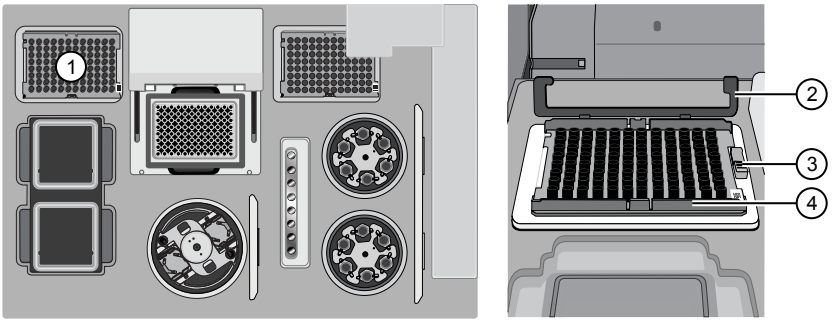
- Confirm that the pipette tip rack in the *Used* (Waste) Pipette Tip Position does not contain any tips. The instrument will abort the run if tips are present in the *used* position.
- To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used* (Waste) Pipette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

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

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.

   Note: Two Ion Chef™ Piercing Tips are pre-loaded into tip positions G7 and H7 on the Tip Cartridge v2.

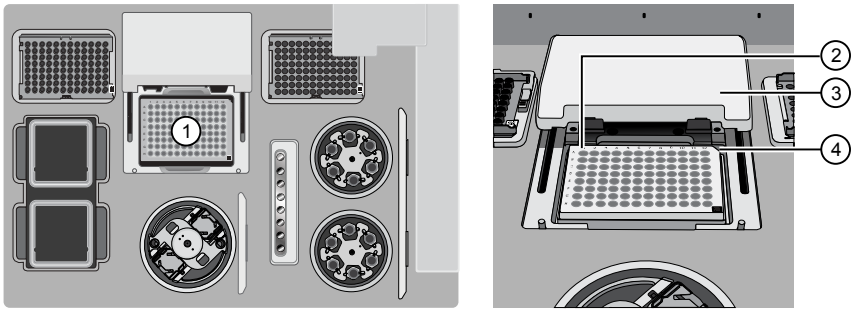
5. Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge v2 into the *New* Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the bracket and cartridge in place.



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

6. Load a new PCR plate into the thermal cycler sample block, then slide a new Frame Seal v2 under the automated heated cover.

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



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

Load the Reagents and Solutions cartridges

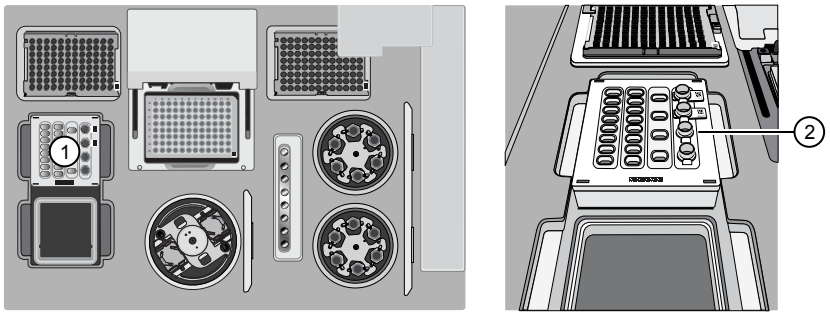
IMPORTANT! Thaw the Reagents cartridge at room temperature for 45 minutes before use.

1. Gently tap the Ion PGM™ Hi-Q™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.

2. If bubbles are present below the surface of the liquid, repeat step 1.

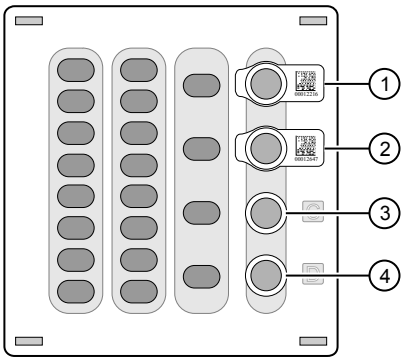
3. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

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 PGM™ Hi-Q™ Chef Reagents cartridge

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



① Position A (Library)
② Position B (Library)
③ Position C (NaOH)
④ Position D (Empty tube)

IMPORTANT!

- Make sure to orient the sample tubes so that the barcodes are visible and oriented to the right.
- Make sure to remove the caps to the Library Sample Tubes before proceeding.
- Because 200- and 400-base-read libraries require different run lengths, do not load a 200-base-read library and 400-base-read library in a single Ion Chef™ run. Both libraries loaded in a run must have a similar read length.

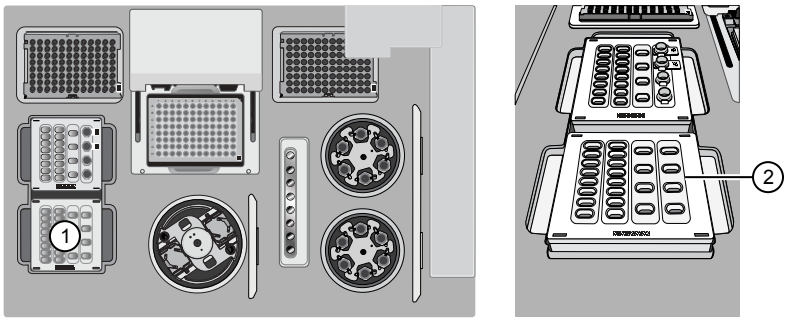
5. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.

IMPORTANT! When the Reagents cartridge is loaded:

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

6. Gently tap the Ion PGM™ Hi-Q™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.

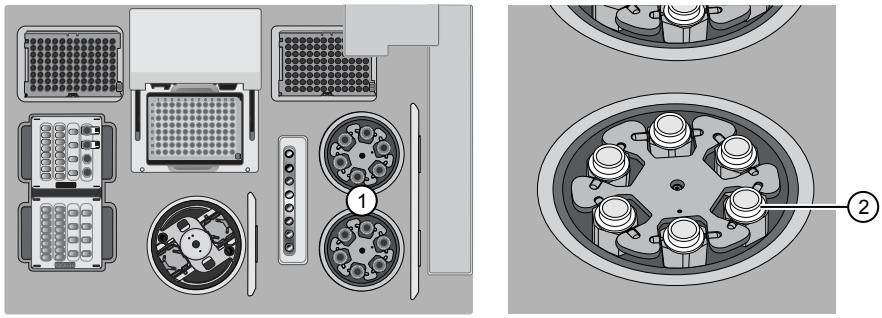
7. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.



① Solutions station (room temperature)
② Ion PGM™ Hi-Q™ Chef Solutions cartridge

Load the Recovery Tubes and Enrichment Cartridge v2

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



① Recovery centrifuges
② Recovery Tube

Before sealing each centrifuge, confirm that:

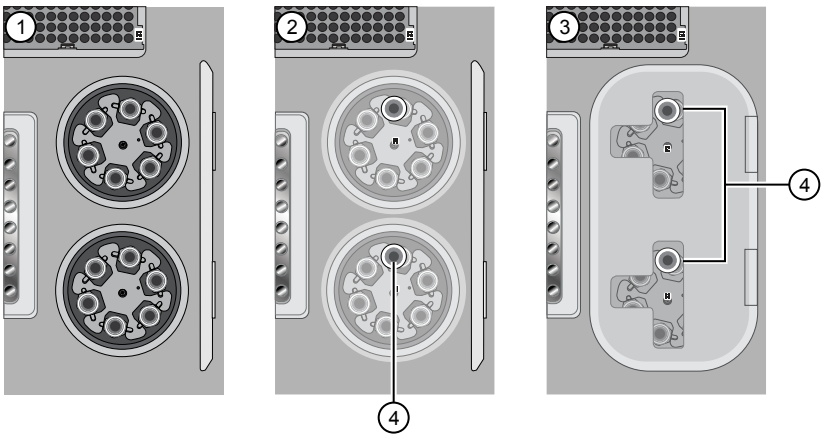
- The centrifuge is load-balanced with all required consumables.

  IMPORTANT! The centrifuge must be load-balanced.

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

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

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



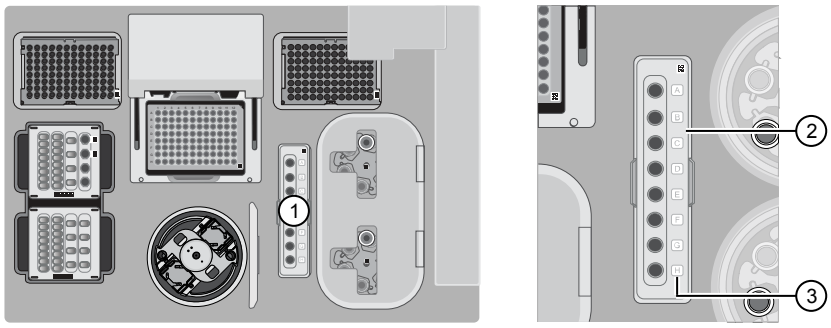
① Recovery Tubes installed
② Recovery Station Disposable Lids installed
③ Recovery centrifuge cover closed
④ Port

IMPORTANT!

- Do not obstruct or place any object on top of the Recovery centrifuge cover.
- Use only the supplied materials, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets to assure normal operation 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.



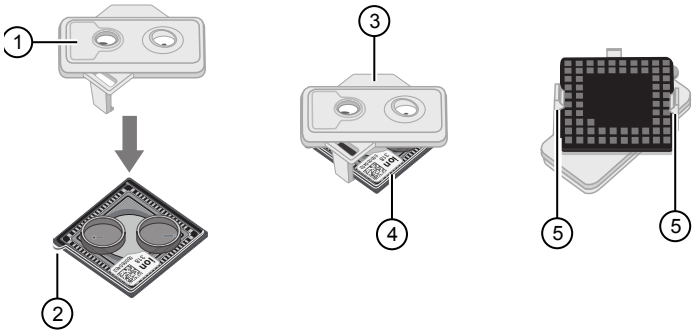
① Enrichment station
② Enrichment Cartridge v2
③ Lettering

Load the Chip-loading centrifuge

1. Attach a Chip Adapter to each chip.

IMPORTANT! When attaching a Chip Adapter:

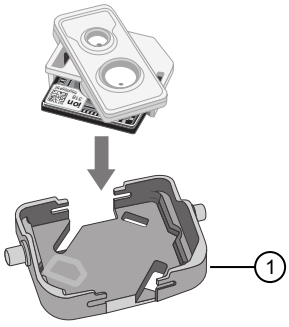
- Align the wells of the Chip Adapter to the wells of the chip, then gently push the adapter onto the chip until the clips lock into place. Attach the adapter so that the end of the adapter housing the reservoir is above the tab of the chip, and the barcode of the chip is visible.
- Listen for an audible 'snap', which indicates that the Chip Adapter is attached. Loading can fail if the adapter is not attached securely.



① Reservoir
② Tab
③ Chip Adapter
④ Ion chip
⑤ Clip

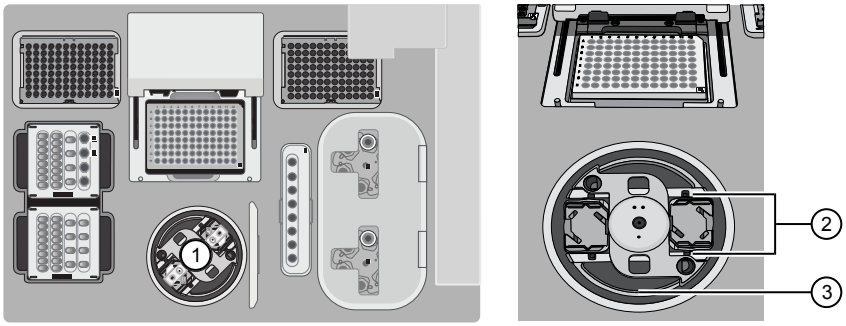
Note: If desired, you can label the back of chips to distinguish them. Mark only the centers of the chips. Do not mark the gold contacts or the chip barcode.

2. Place the adapter/chip assemblies into centrifuge buckets so that the chip barcode aligns above the white outline imprinted on the floor of the bucket.



① Chip-loading centrifuge bucket

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

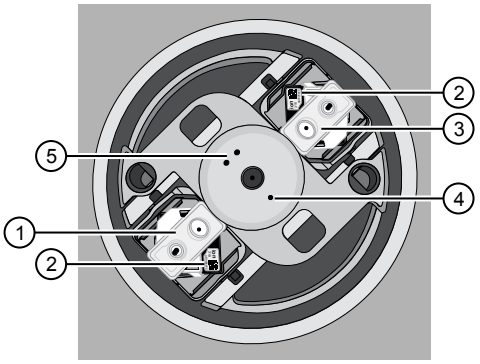


① Chip-loading centrifuge
② Mounting grooves
③ Chip-loading centrifuge

IMPORTANT! When loading the coupled chips, confirm that:

- The Chip Adapter is firmly attached to each chip before loading it into the centrifuge bucket.
- The tabs of the chips are oriented away from the center of the centrifuge.
- The barcodes of the chips are oriented as shown in the following figure.
- The clips of the coupled chips are firmly seated in the slots of the centrifuge buckets.
- The buckets are securely seated in the centrifuge rotors.

Note: Chip position A is 90° clockwise from the Position 1 marker hole. The chip that is loaded in this position is loaded with ISPs prepared from the library loaded in Position A of the Reagents cartridge. The chip that is loaded in Position 2 is loaded with ISPs prepared from the library loaded in Position B.



① Chip position 1
② Chip barcode
③ Chip position 2
④ Position 1 marker hole
⑤ Position 2 marker holes

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

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

Confirm that consumables are correctly installed

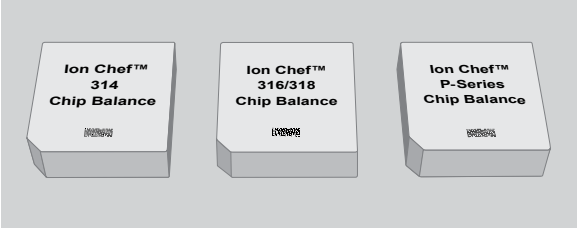
Before continuing:

- 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 PGM™ Hi-Q™ 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 prior to beginning each run.

Single chip loading workflow

You can set up an Ion Chef™ Hi-Q™ run to load a single chip instead of two, using the appropriate Ion Chef™ Chip Balance loaded opposite to the chip in the Chip-loading centrifuge. Contact Customer Service to obtain an Ion Chef™ Chip Balance Pack. The pack contains a set of barcoded Chip Balances for use with singly loaded Ion PGM™ chips, and P-Series chips.



Ion Chef™ Chip Balance Pack

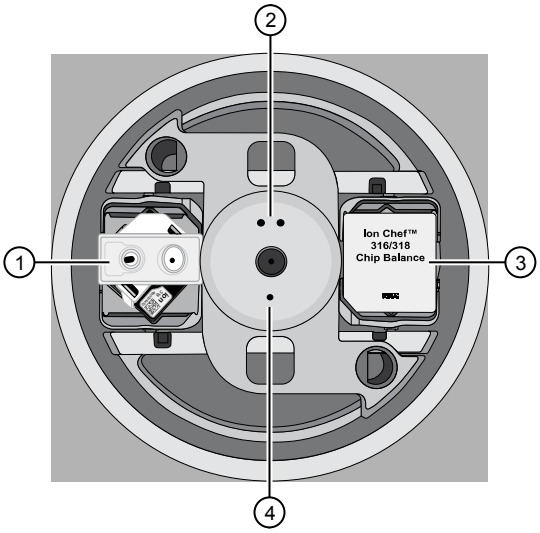
Load the Ion Chef™ Instrument as you would normally load the system. For single chip loading, perform the following steps:

1. Add the single diluted DNA 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 a chip in Position 1 and the appropriate Ion Chef™ Chip Balance in Position 2 of the Chip-loading centrifuge.

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

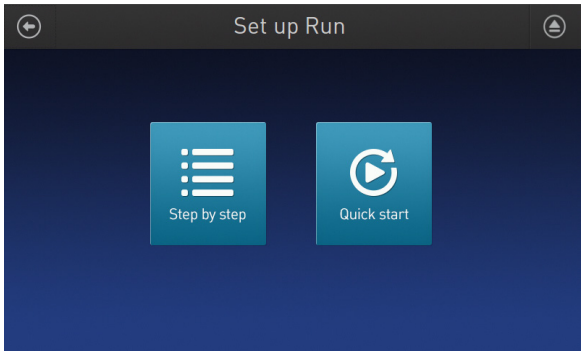
IMPORTANT! Use the Chip Balance appropriate for the sequencing chip you have loaded. Each Chip Balance is weight-matched to the chip and chip adapter specified.

4. Resume the normal workflow in "Load the Chip-loading centrifuge" on page 69 at step 4. The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.

Start the Ion Chef™ Instrument run

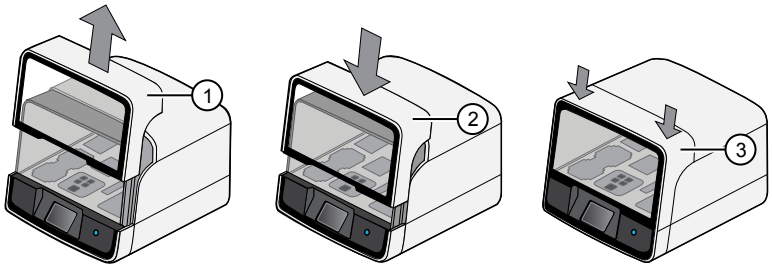
1. Verify that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, touch **Set up run**.

3. Touch **Step by Step** to have the instrument guide you through the instrument setup, or touch **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.



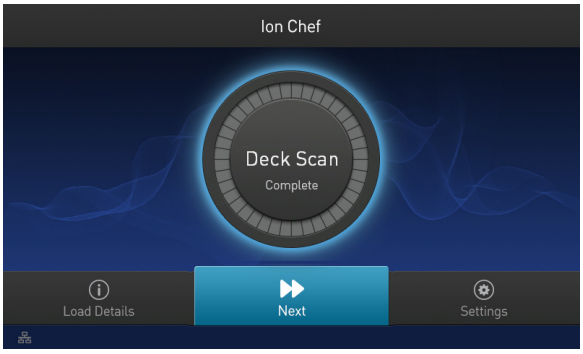
① Lift
② Lower
③ Press down

5. When prompted, touch **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to ensure their presence and compatibility.

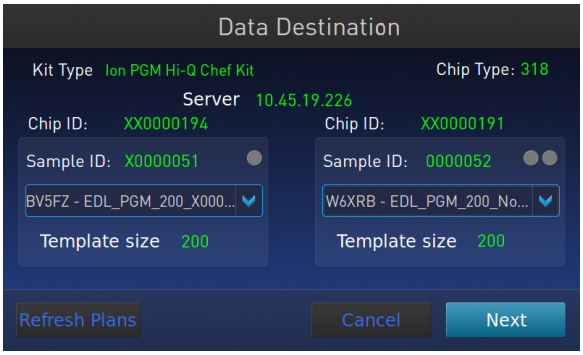
Note: During Deck Scan, the touchscreen can show warnings if the instrument detects missing or incompatible consumables. Address all warnings before the run can start. After you address each condition, touch **Yes** to continue.

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

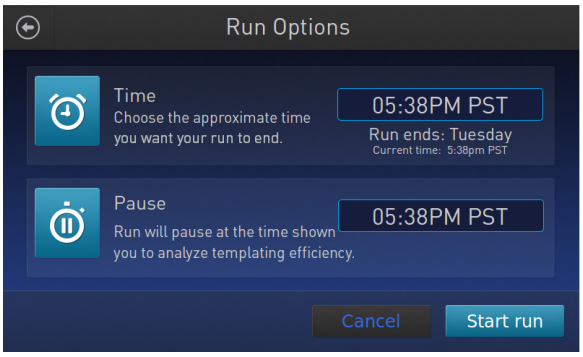
6. When Deck Scan is complete, touch **Next** to display the Data Destination screen.



7. Verify that the instrument displays the correct kit name, chip types, chip barcodes, and Planned Runs. If the correct Planned Runs do not display, touch the drop-down menu to select the Planned Run for each chip, then touch **Next.**



8. On the Run Options screen, touch 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 that can be used to evaluate templating efficiency. Depending on your selection, the QC samples will be made available either during or after the run. In either case, 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.

By selecting	You can obtain the QC samples
Time	immediately after the run ends, at the time you specify (10.5 hours after run start).
Pause	when the instrument pauses operation before the chip loading step (about 9.5 hours into the run).

Note: The DNA library in the Library Sample Tube loaded in Position A will be templated onto ISPs that can be sampled in Position E of Enrichment Cartridge v2 after a run. The DNA library in the Library Sample Tube loaded in Position B is templated onto ISPs that can be sampled in Position A of Enrichment Cartridge v2.

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

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

   Note: To stop the run, touch **Cancel**, then touch **Yes**.
   If the instrument encounters a problem during the run, it aborts the run, then displays the error on the instrument touchscreen. If a run fails:

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

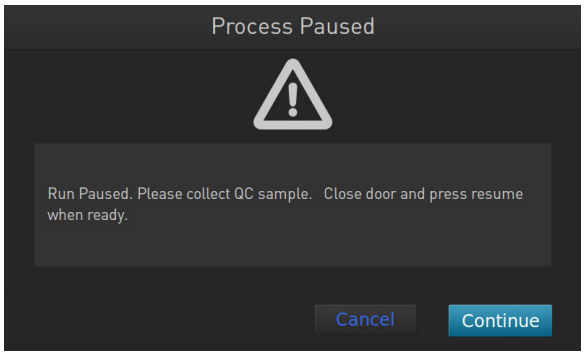
   b. Reset, then reattempt the run. If the run fails again, contact Technical Support to troubleshoot the problem.

10. Clean, then initialize the Ion PGM™ Sequencer approximately 1.5 hours before the Ion Chef™ System finishes chip loading.

    By preparing the sequencer during the last stages of chip loading, you ensure that the chips can be sequenced as soon as possible after loading is complete.

11. If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the Ion Chef™ System (approximately after the start of the run).

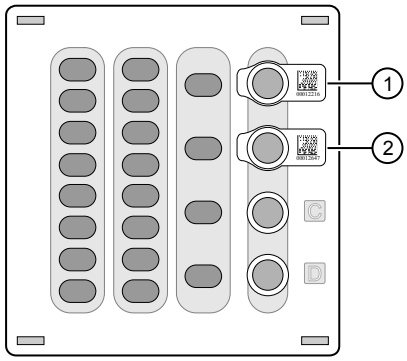
    a. When prompted to remove the QC sample, open the instrument door.



b. Transfer the QC samples (entire volume) from Positions A and B of the Ion PGM™ Hi-Q™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.

IMPORTANT! Do not remove the Library Sample Tubes from the Ion PGM™ Hi-Q™ Chef Reagents cartridge.

IMPORTANT! If you unintentionally close the instrument door before you obtain the QC samples, you must wait until the end of the run before you can collect them. You cannot pause the run or open the door after it has been closed.



① Position A; Pre-enriched QC Sample
② Position B; Pre-enriched QC Sample

c. Analyze the QC samples.

Note: For detailed protocols, see the
- *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387) or
- *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388)

available at **thermofisher.com/order/catalog/product/4468656**.

d. If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes. See Appendix B, "Supplementary procedures," in the *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919).

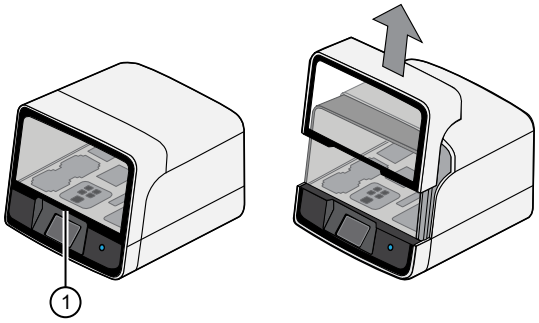
e. Close the instrument door, then touch **Continue** to complete the run.

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

Unload the chips for sequencing

1. Open the instrument door:
   a. In the instrument touchscreen, touch ⏏ (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.

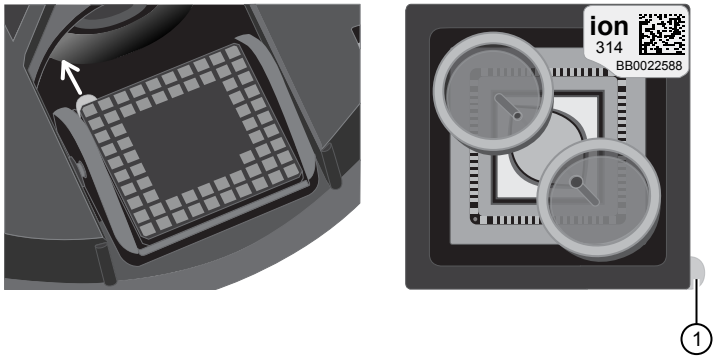


① Hold here and lift

2. Remove the chip/adapter assemblies from the Chip-loading centrifuge. Carefully remove the Chip Adapter from the chip, then discard the adapter. Set the chips aside on a clean, static-free surface.

3. For Ion 314™ v2 BC Chips only: use an Ion Chip™ Minifuge to remove the remaining liquid from the Ion 314™ v2 BC Chips. This step is not required for Ion 316™ or Ion 318™ v2 BC Chips.
   a. Load both chips into the Ion Chip™ Minifuge. Place each chip upside-down in a bucket so that the tab faces inwards, toward the center.

   IMPORTANT! Before closing the centrifuge lid, ensure that the chips are centered in the buckets to ensure that the centrifuge is balanced.



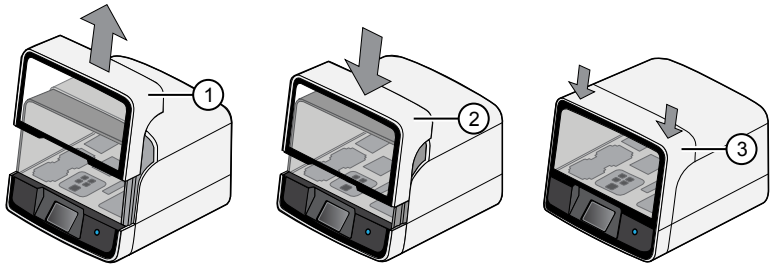
① Tab (orient inward)

   b. Close the lid, then centrifuge the chips for 5 seconds.

   c. When the centrifuge stops, remove the chips, then use a lint-free wipe to remove any liquid from the buckets.

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

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



① Lift door first
② Lower
③ Press down to lock

5. Load one or both chips into Ion PGM™ Sequencers and promptly start the sequencing runs.

   Note: Start sequencer cleaning and initialization ahead of time so that the Ion PGM™ 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.

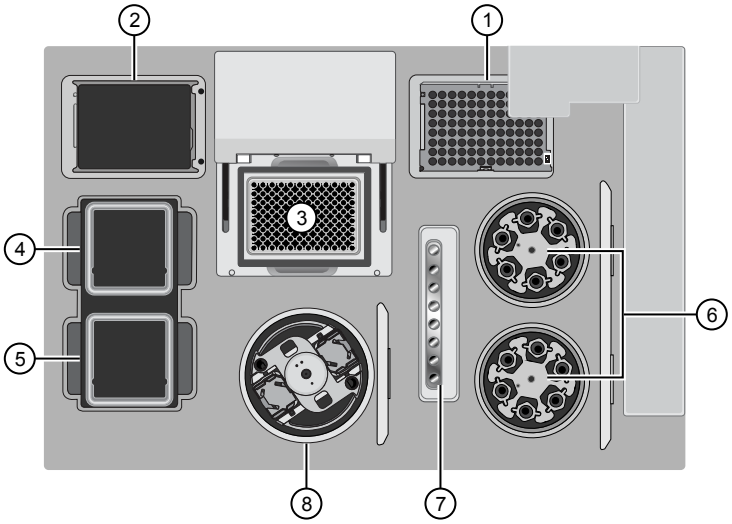
IMPORTANT! Although the Ion Chef™ Instrument cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries for use or when preparing the Ion Chef™ Instrument, make certain to observe sterile laboratory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



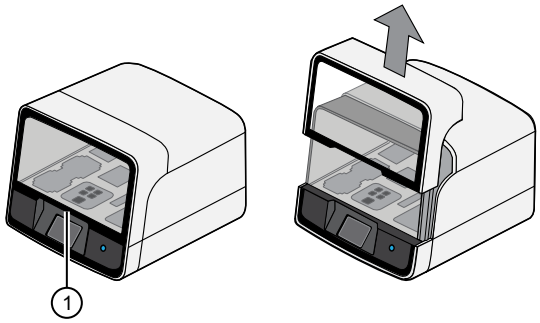
Ion Chef™ Instrument stations

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

Remove and dispose of used consumables

IMPORTANT!
- Do not discard the empty Tip Cartridge v2.
- Make sure to transfer the QC samples before you remove and discard the Reagents cartridge.

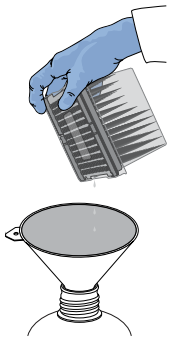
1. Touch ⏏ (Open Door) in the instrument touchscreen, then wait for the latch to open.
2. Lift the instrument door to the top of the travel until the latch mechanism engages.



① Hold here, then lift

3. Remove, then discard the PCR plate from the thermal cycler sample block.
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 PGM™ Hi-Q™ Chef Reagents cartridge
   - Ion PGM™ Hi-Q™ Chef Solutions cartridge
   - Enrichment Cartridge v2
7. Remove, then discard the consumables from the Recovery centrifuges, including the:
   - Recovery Station Disposable Lids v2
   - Recovery Tubes v2
8. Close the Chip-loading centrifuge cover.

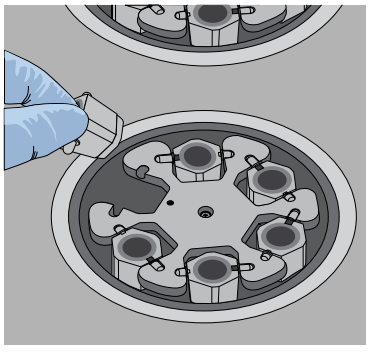
Inspect and clean the Recovery centrifuges and buckets

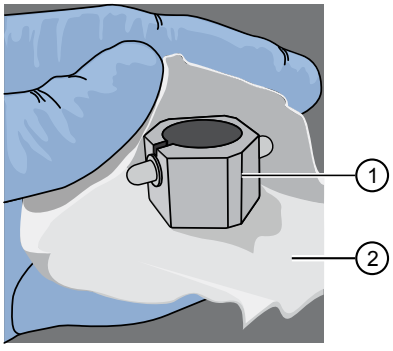
1. Inspect the Recovery centrifuges, then clean the components if excess liquid is present.

Is liquid present?	Action
No	Go to "Start the cleaning" on page 82.
Yes	Clean the centrifuge bowl and buckets as described below. **IMPORTANT!** Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do *not* need to clean the centrifuge after every run.

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

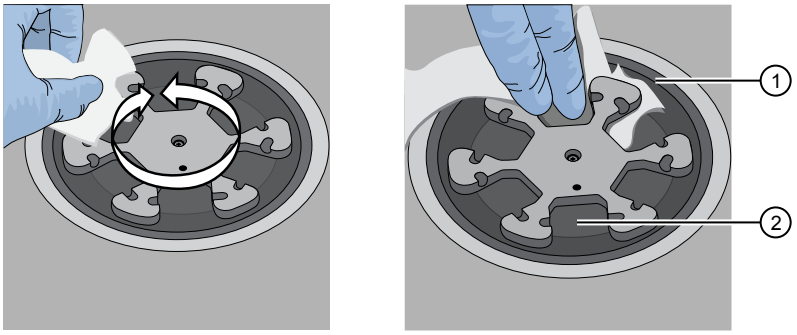
2. Remove the buckets from the Recovery centrifuges. Clean the inside and outside of each bucket using a lint-free wipe, then place the buckets on a clean, dry surface while you clean the centrifuge.





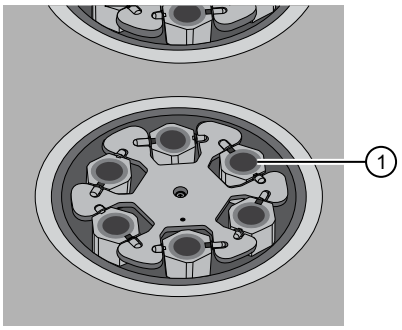
① Bucket
② Lint-free wipe

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:
   - 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.

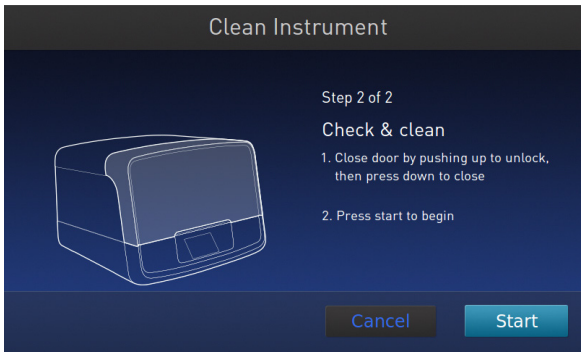
IMPORTANT! Before closing the door, ensure the covers of the Chip-loading and Recovery centrifuges are closed.

2. To start the cleaning, touch **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. Touch **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 touch **Next**.

4. With the door closed, touch **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.

5 Prepare the template on the Ion OneTouch™ 2 Instrument

IMPORTANT! This chapter contains brief procedures for the HID workflow. For complete instrument procedures, troubleshooting, and maintenance information, see the *Ion PGM™ Hi-Q™ OT2 Kit User Guide* (Pub. No. MAN0010902).

Software version requirements for template preparation

Panel	Software version required
Precision ID Ancestry Panel	Torrent Suite™ Software 5.2.2
Precision ID Identity Panel	
Precision ID GlobalFiler™ NGS STR Panel	
Precision ID mtDNA Control Region Panel	
Precision ID mtDNA Whole Genome Panel	

Required materials

Ion PGM™ Hi-Q™ OT2 Kit components

The Ion PGM™ Hi-Q™ OT2 Kit (Cat. No. A27739) contains reagents for manually preparing templates for Precision ID SNP, mtDNA, and STR libraries with the Ion OneTouch™ 2 Instrument.

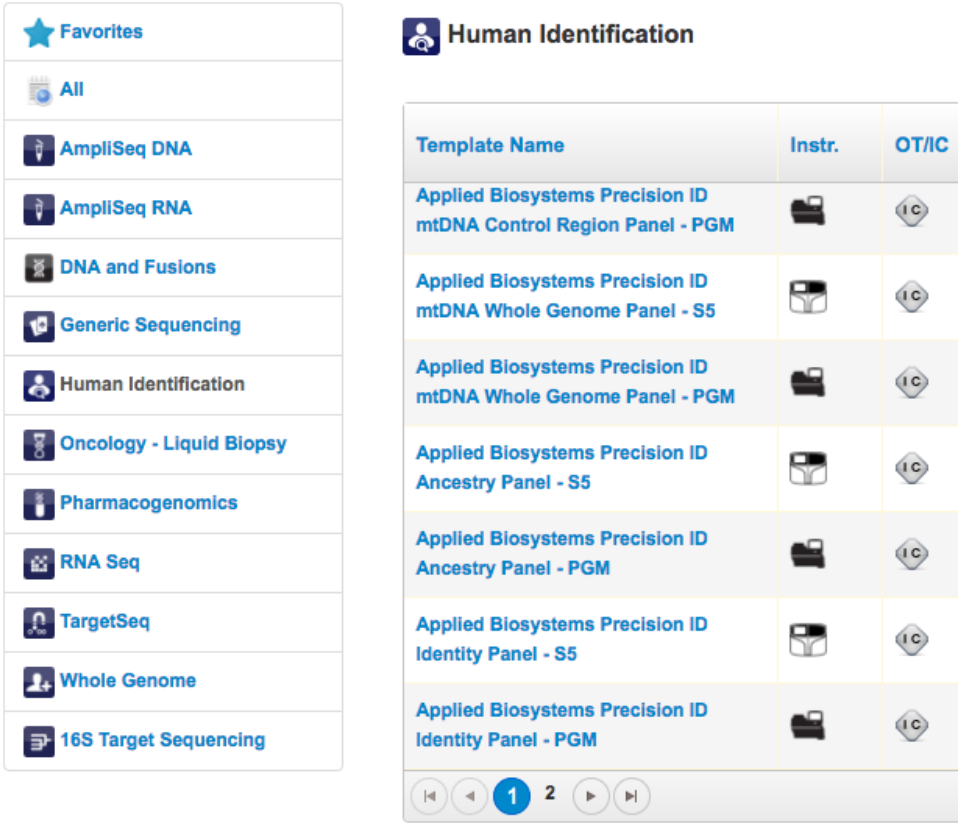
Component	Part No.	Quantity per kit
Ion PGM™ OT2 Supplies	A27744	1 box
Ion PGM™ Hi-Q™ OT2 Reagents	A27743	1 box
Ion PGM™ Hi-Q™ OT2 Solutions	A27742	1 box

Create a Planned Run

Human Identification templates can be used to create Planned Runs for various Applied Biosystems™ Precision ID panels. The templates pre-populate your Planned Run with recommended parameters. You can then select additional settings to plan your run.

Note: It is not necessary to create a Planned Run before template preparation on the Ion OneTouch™ 2 Instrument, however, you need to create a Planned Run before starting a sequencing run on the Ion PGM™ Sequencer.

1. Sign in to the Torrent Server via the Torrent Browser.
2. Select **Plan** ▸ **Templates**, then in the Favorites list, select **Human Identification**.

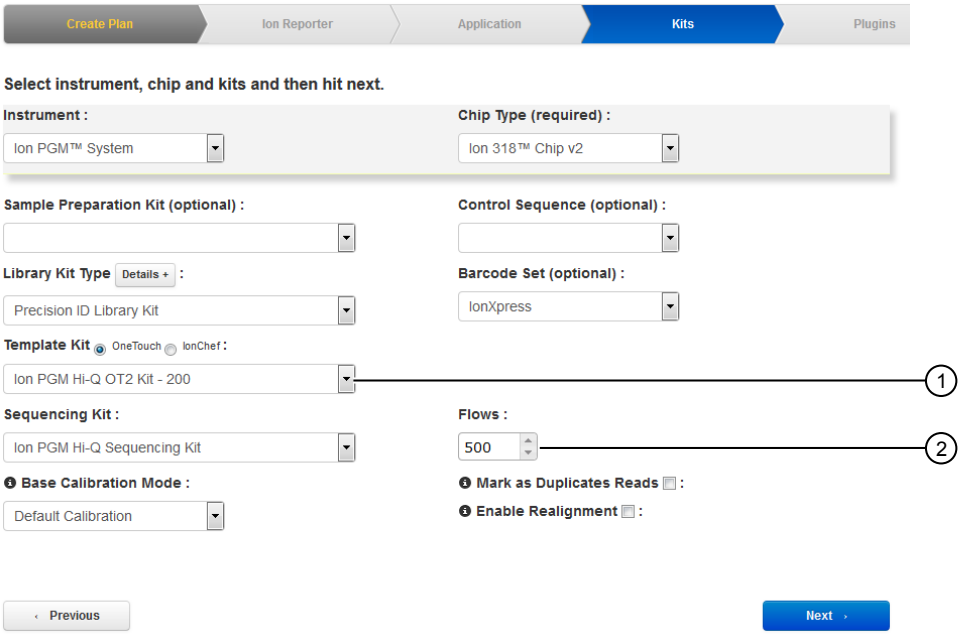


3. Select the Planned Run template appropriate to your panel and sequencer. The wizard launches and displays the **Plan** page.

   Note: All templates default to the Ion Chef™ Instrument.

4. Select the reference and BED files, enter the Sample names, confirm the default settings, then enter a plan name.

5. To verify or change kit information, click the **Kits** tab. Specify the appropriate library, template and sequencing kits, and flow number and Barcode Set.



① For the Precision ID SNP panels, Precision ID mtDNA panels, and Precision ID GlobalFiler™ NGS STR Panel, select **Ion PGM Hi-Q OT2 Kit - 200**.

② For the Precision ID SNP panels and the Precision ID mtDNA panels, choose **500 Flows**. For the Precision ID GlobalFiler™ NGS STR Panel, choose **850 Flows**.

6. When you have completed your selections, click **Plan Run** at the bottom right of the **Plan** screen to save the run. The run is listed on the Planned Runs page under the name that you specified.

Note: For more information see the software user documentation, the *Ion PGM™ Hi-Q™ OT2 Kit User Guide* (Pub. No. MAN0010902), the *HID STR Genotyper Plugin User Guide* (Pub. No. MAN0015879), or the *HID SNP Genotyper Plugin User Guide* (Pub. No. MAN0010641).

Dilute the libraries for Ion OneTouch™ 2 System template preparation

Ensure that sample library (or pooled sample libraries) has been previously diluted. If you manually prepared the library, see page 36. If you prepared the library using the Ion Chef™ Instrument, see page 52.

To continue with the procedure, see the *Ion PGM™ Hi-Q™ OT2 Kit User Guide* (Pub. No. MAN0010902).

6 Sequence on the Ion PGM™ System

This chapter contains brief procedures for the HID workflow. For complete instrument procedures, troubleshooting, and maintenance information, see the *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919), or the *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816).

IMPORTANT! The workflow for sequencing on the Ion PGM™ Sequencer applies to templates prepared manually or prepared on the Ion Chef™ Instrument. If you prepared your template using the Ion OneTouch™ 2 Instrument, see the *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816) for detailed instructions for manually loading Ion PGM™ Chips with template-positive ISPs.

Software version requirements for sequencing

Panel	Software version required
Precision ID Ancestry Panel	Torrent Suite™ Software 5.2.2
Precision ID Identity Panel	
Precision ID GlobalFiler™ NGS STR Panel	
Precision ID mtDNA Control Region Panel	
Precision ID mtDNA Whole Genome Panel	

Materials required

Ion PGM™ Hi-Q™ Sequencing Kit

IMPORTANT! Use the Ion PGM™ Hi-Q™ Sequencing Kit for Precision ID SNP and mtDNA libraries.

The Ion PGM™ Hi-Q™ Sequencing Kit (Cat. No. A25592) contains reagents for sequencing on the Ion PGM™ Sequencer.

Component	Part No.	Storage
Ion PGM™ Sequencing Supplies	A25587	15° to 30°C
Ion PGM™ Hi-Q™ Sequencing Reagents	A25588	–30°C to – 10°C
Ion PGM™ Hi-Q™ Sequencing Solutions	A25589	2 to 8°C
Ion PGM™ Hi-Q™ Sequencing dNTPs	A25590	–30°C to – 10°C

Ion PGM™ Hi-Q™ STR Sequencing Kit

IMPORTANT! Use the Ion PGM™ Hi-Q™ STR Sequencing Kit for Precision ID STR libraries only.

The Ion PGM™ Hi-Q™ STR Sequencing Kit (Cat. No. A32745) contains reagents for 8 sequencing runs on the Ion PGM™ Sequencer.

Component	Part No.	Storage
Ion PGM™ Sequencing Supplies	A25587	15° to 30°C
Ion PGM™ Hi-Q™ Sequencing Reagents	A25588	–30°C to – 10°C
Precision ID STR Hi-Q™ Sequencing Solutions	A31864	2 to 8°C
Ion PGM™ Hi-Q™ Sequencing dNTPs	A25590	–30°C to – 10°C

Wash 2 Bottle kit

The Ion PGM™ Hi-Q™ Wash Bottle 2 Kit (Cat. No. A25591) is for use with the Precision ID mtDNA panels and the Precision ID SNP panels, and includes the following components:

Component	Amount	Storage
Wash 2 Bottle w/ label (2 L) **Note:** Must be conditioned at least 8 hours before use as described in "Condition the Wash 2 Bottle for first use" on page 90	1 bottle	15°C to 30°C
Wash 2 Bottle Conditioning Solution	1 × 125 mL	

Compatible Ion Chip kits

Contents	Quantity	Cat. No.	Storage
Ion 318™ Chip Kit v2 BC	4 chips	4488146	15°C to 30°C
	8 chips	4488150	
Ion 316™ Chip Kit v2 BC	4 chips	4488145	
	8 chips	4488149	
Ion 314™ Chip Kit v2 BC	8 chips	4488144	

Workflow: Sequencing on the Ion PGM™ Sequencer

"Condition the Wash 2 Bottle for first use" on page 90

▼

"Clean the Ion PGM™ System" on page 90

▼

"Initialize the Ion PGM™ System" on page 93

▼

"Start the sequencing run" on page 100

Clean and initialize the Ion PGM™ Sequencer

At least 1.5 hours before the completion of the template instrument run, clean and initialize the Ion PGM™ Sequencer.

IMPORTANT! Use only the specified materials and follow the protocols found in this document. The cleaning and initialization procedures described here are similar to that of other Ion sequencing kits, but the materials and protocols are not identical. Do not substitute reagents from other kits.

Condition the Wash 2 Bottle for first use

New Wash 2 Bottles must be conditioned with Wash 2 Bottle Conditioning Solution for at least 8 hours before first use.

Note: If necessary, you can reuse an existing Wash 2 Bottle while you condition a new bottle. Bottles can be used for sequencing up to 40 times before they must be replaced.

To condition the Wash 2 Bottle:

1. Fill the bottle to the mold line with 18 MΩ water, add the entire container of Wash 2 Bottle Conditioning Solution, then cap the bottle and invert it 5 times to mix.
2. Allow the bottle to sit at room temperature for at least 8 hours and preferably overnight, then dispose of the contents. The bottle is now ready for use.

Clean the Ion PGM™ System

Cleaning schedule

The Ion PGM™ Sequencer requires cleaning with either 18-MΩ water or a chlorite solution every time the instrument is initialized.

Clean with	Schedule
18 MΩ water	• Daily, when instrument is in use (e.g., not necessary on weekends) • After one or more runs totaling ≤1,100 flows • If more than 27 hours but less than 48 hours have elapsed between the last cleaning/initialization and the start of a run • If you cleaned with chlorite a week ago and have not used the instrument since then
Chlorite solution	• Once a week, unless the instrument has not been used since the last chlorite cleaning (in which case, clean with 18 MΩ water before using) • If the instrument has been left with reagents for more than 48 hours (for example, over the weekend)

Cleaning setup

IMPORTANT! For all the following steps, use 18 MΩ water directly from the purification system. Do not use water that has been collected or stored in any other containers.

- Remove any wash and reagent bottles that are attached to the Ion PGM™ System before cleaning.
- Do not remove old sippers before cleaning. The sippers are used as part of the cleaning procedure.
- Old chips that have been used for sequencing can be marked and used in the cleaning procedure.
- Wash bottles (250 mL and 2 L) provided as part of instrument installation can be marked and used for cleaning. After you have used the wash bottles provided with the sequencing kit for the specified number of runs, you can use them as extra cleaning bottles. Mark them for cleaning use only.

18 MΩ water cleaning

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Press **Clean** on the touchscreen, and select the **18-MOhm water cleaning** checkbox. Press **Next**.
3. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

   IMPORTANT! Always make sure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.
4. Remove all wash and reagent bottles attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
5. Add 250 mL of 18 MΩ water to an empty 250-mL cleaning bottle.
6. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
7. Attach the 250-mL bottle containing 18 MΩ water to the W1 position, ensuring that the W1 cap is screwed on tightly. Press **Next**.
8. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, and insert the sippers into the bottles. Do not screw on the caps.
9. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to begin cleaning.
10. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu and proceed to initialization.

Chlorite cleaning

Note: Prepare a stock of 1 M NaOH each week by diluting 10 M NaOH with 18 MΩ water.

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle), then rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water, then add an Ion Cleaning tablet (chlorite tablet). Allow the tablet to dissolve completely (~10 minutes).
3. When the tablet has dissolved, add 1 mL of 1 M NaOH and filter the solution using a 0.22-µm or 0.45-µm filter. Use the chlorite solution within 2–3 hours. Discard any unused solution after this time.
4. Press **Clean** on the touchscreen, then select the **Chlorite cleaning** checkbox. Press **Next**.
5. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

   IMPORTANT! Always ensure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.
6. Remove all wash and reagent bottles that are attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
7. Add 250 mL of the filtered chlorite solution to an empty 250-mL cleaning bottle.
8. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
9. Attach the 250-mL bottle with the filtered chlorite solution to the W1 position. Ensure that the W1 cap is tight. Press **Next**.
10. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, then insert the sippers into the bottles. Do not screw on the caps.
11. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to start cleaning.
12. When prompted, remove the bottle containing the chlorite solution from the W1 position.
13. Rinse the outside of the W1 sipper tube with a squirt bottle containing 18 MΩ water.
14. Fill a clean 250-mL bottle with 250 mL of 18 MΩ water, then attach the bottle in the W1 position. Ensure the cap is tight. Press **Next** to start the water rinse.
15. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu, then proceed to initialization.

Initialize the Ion PGM™ System

Initialization takes ~1 hour. As part of the initialization process, first prepare the Wash and Reagent Bottles as described in this section.

Initialization guidelines

IMPORTANT! Handle nucleotides carefully to avoid cross-contamination. Always change gloves after removing used sipper tubes from the Ion PGM™ System to avoid cross contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.

For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 24 hours after initialization.

Bottle usage

- Wash 2 Bottles can be used for up to 40 initializations, after which you can use them in the cleaning procedure.
- Wash 1 and Wash 3 Bottles can be used for up to 4 initializations, after which you can reuse them in the cleaning procedure.
- Replace the Reagent Bottles and sipper tubes every time you initialize.

Before initialization

1. Remove the dNTP stock solutions from the freezer and begin thawing on ice.
2. Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank.

Prepare the Wash 2 Bottle

Note:

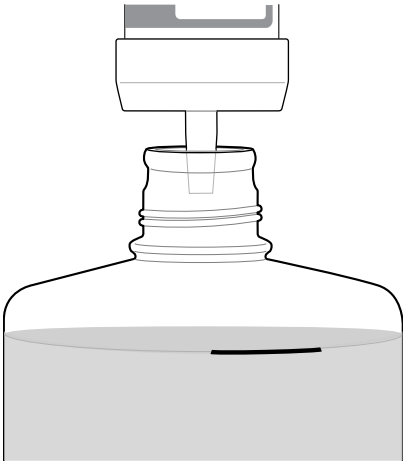
- Do not remove the old sippers from the dNTP ports until instructed to do so.
- Load the bottles as quickly as possible to prevent atmospheric CO_2 from reducing the pH of the Wash solution.
- For all the following steps, pour the 18 MΩ water directly from the purification system into the Wash 2 Bottle. Do not use water that has been collected or measured in any other containers.

IMPORTANT! If you are using the Ion PGM™ Hi-Q™ STR Sequencing Kit, prepare the Wash 2 bottle identically with the Ion PGM™ Hi-Q™ Sequencing Kit, but use the Precision ID STR Hi-Q™ Sequencing W2 Solution concentrate supplied in the kit.

IMPORTANT! Do not let the new sippers touch any surfaces.

1. Rinse the Wash 2 Bottle (2 L) three times with 200 mL of 18 MΩ water.
2. Prepare 500 µL of 100 mM NaOH by diluting 50 µL of 1 M NaOH in 450 µL of Nuclease-free Water.

3. If your 18 MΩ water system has a spigot, extend it into **but not below** the neck of the Wash 2 Bottle. Otherwise, position the nozzle as close to the mouth of the bottle as possible.



Note: If your water system has a digital display, make sure it reads "18 MΩ" throughout filling the bottle. If not, see *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816).

4. Fill the bottle to the mold line with 18 MΩ water. The volume of water will be ~2 liters. (You can mark the mold line on the bottle for clarity.)

Note: If you are preparing bottles for multiple sequencers, cap each bottle immediately after filling, and leave capped until you are ready to add the W2 Solution.

5. Add the entire bottle of Ion PGM™ Hi-Q™ Sequencing W2 Solution, or Precision ID STR Hi-Q™ Sequencing W2 Solution, to the Wash 2 Bottle.

Note: Keep the bottle to scan the barcode during the initialization procedure.

6. Using a P200 pipette, add 70 µL of 100 mM NaOH to the Wash 2 Bottle.

   Note: Different sites may require adding different volumes of 100 mM NaOH. Some sites, for example, may require doubling the volume to 140 µL. See *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816) for information on determining the volume of 100 mM NaOH to add.

7. Cap the bottle and invert 5 times to mix, then immediately proceed through the rest of the initialization procedure.

   IMPORTANT! Do not store the mixed Wash 2 Bottles.

Prepare the Wash 1 and Wash 3 Bottles

Note: For the following steps, label the Wash 1 and Wash 3 Bottles to avoid confusion.

1. Rinse the Wash 1 and Wash 3 Bottles 3 times with 50 mL of 18 MΩ water.

2. **Wash 1 Bottle:** Add 350 µL of freshly prepared 100 mM NaOH to the Wash 1 Bottle, then cap the bottle.

3. **Wash 3 Bottle:** Add Ion PGM™ Hi-Q™ Sequencing W3 Solution, or Precision ID STR Hi-Q™ Sequencing W3 Solution, to the 50-mL line marked on the Wash 3 Bottle, then cap the bottle.

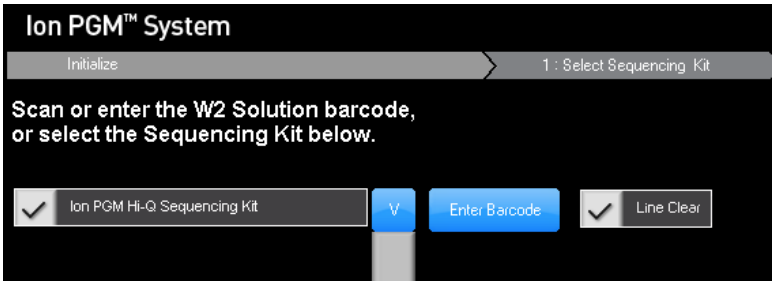
Start initialization

Note:

- Do not remove the old sipper tubes from the dNTP ports until instructed to do so.
- Load the bottles as quickly as possible to prevent atmospheric CO_2 from reducing the pH of the Wash 2 Bottle solution.

IMPORTANT! Do not let the new sipper tubes touch any surfaces.

1. On the main menu, press **Initialize**.
2. Make the following selections in the next screen, then press **Next**:
   - Click **Enter barcode** to scan or enter the barcode on the Ion PGM™ Hi-Q™ Sequencing W2 Solution bottle, or the 2D barcode on the Ion PGM™ Hi-Q™ Sequencing Solutions box.
   - Alternatively, select the checkbox for the **Ion PGM™Hi-Q™ Sequencing Kit** from the dropdown list.
   - In the same screen, if you frequently experience clogging during initialization, select the **Line Clear** checkbox to clear any blockage in the fluid lines before initialization. Line Clear is optional.



IMPORTANT! To ensure proper pH adjustment, be careful to select the correct kit. If you are using the Ion PGM™ Hi-Q™ STR Sequencing Kit, select the Ion PGM™ Hi-Q™ Sequencing Kit.

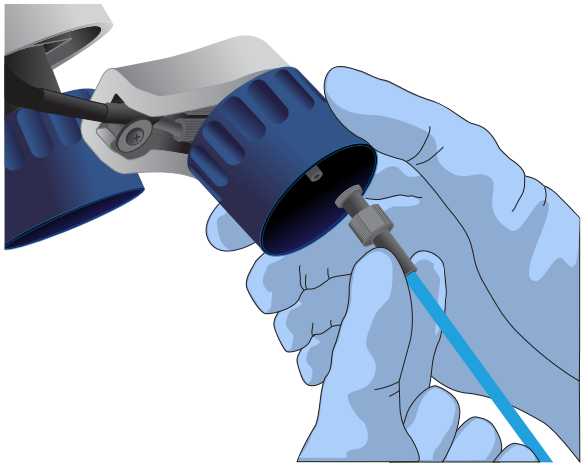
After you press **Next**, the system checks the gas pressure.

3. Following the gas pressure check:

Result	Action
If the pressure is sufficient	Ensure that the cleaning chip, reagent sipper tubes, and collection trays are in place, and press **Next** to start the initialization
If the pressure is low	Press **Yes** to re-check the pressure. If the pressure remains low, contact Technical Support

4. Wearing clean gloves, firmly attach a new, long gray sipper to the cap in the W2 position.

   IMPORTANT! Do not let the sipper touch any surfaces, and firmly attach the sipper to the port. Loosely attached sippers can adversely affect results.



5. Immediately attach the prepared Wash 2 Bottle in the W2 position, then tighten the cap. Press **Next**.

6. Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.

7. Immediately attach the prepared Wash 1 and 3 Bottles, then tighten the caps. Press **Next**.

8. If you selected the **Line Clear** checkbox in the earlier screen, press **Next**, then follow the touchscreen prompts to perform the line clear procedure. At the beginning and end of the procedure, you are be prompted to select one of the following:

Option	Description
Press **Line Clear**	To start a new line clear procedure
Press **Re-flow**	To retest the lines after you have performed a line clear
Press **Auto pH**	If the lines are clear and you are ready to continue with initialization

9. Following line clear, or if you did not select that option, the sequencer starts adjusting the pH of the W2 Solution, which takes ~30 minutes. After 15 minutes, check the instrument touchscreen to confirm that initialization is proceeding normally.

   Note:
   - If an error occurs during the automatic pH process, note the error message and see the *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816).
   - During the process, you can prepare the Reagent Bottles with dNTPs as described in the next section.

Prepare the 50-mL Reagent Bottles with dNTPs

1. Use the labels provided with the kit to label four new Reagent Bottles as dGTP, dCTP, dATP, and dTTP.

2. Confirm that no ice crystals are visible in each thawed dNTP stock solution. Vortex each tube to mix, and centrifuge to collect the contents. Keep the dNTP stock solutions on ice throughout this procedure.

   IMPORTANT! To avoid cross-contamination in the next step, open only one dNTP stock tube at a time and use a fresh pipette tip for each aliquot.

3. Using separate filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.

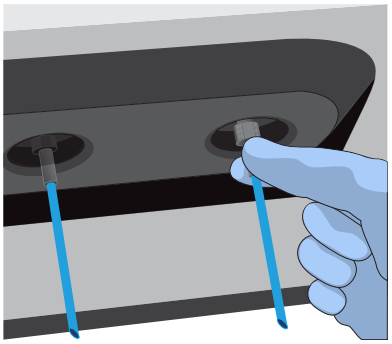
4. Cap each Reagent Bottle and store on ice until you are ready to attach it to the instrument. Place the remaining dNTP stocks back into –20°C for storage.

Attach sipper tubes and Reagent Bottles

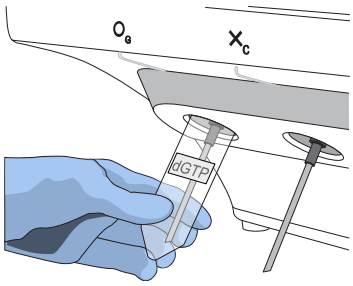
1. After the wash solutions have initialized, follow the touchscreen prompts to remove the used sipper tubes and collection trays from the dNTP ports.

2. Change gloves, then firmly insert a new sipper tube (blue) into each dNTP port. Do not let the sipper touch any surfaces.

   IMPORTANT! Be careful to firmly push each sipper onto the port. Loosely attached sippers may adversely affect results.



3. Attach each prepared Reagent Bottle to the correct dNTP port (e.g., the dGTP tube on the port marked "G") and tighten firmly by hand until snug. Press **Next**.



Note: The instrument checks the pressure of the Reagent Bottles and Wash Bottles. If a bottle leaks, check that it is tightly attached to the instrument. If it continues to leak, replace it. If the instrument still does not pass the leak check, contact Technical Support.

4. Follow the touchscreen prompts to complete initialization. The instrument will fill each Reagent Bottle with 40 mL of W2 Solution.

5. At the end of initialization, Ion PGM™ System will measure the pH of the reagents:
   - If every reagent is in the target pH range, a green **Passed** screen will be displayed.
   - If a red failure screen appears, see Appendix A, "Troubleshooting".

6. Press **Next** to finish the initialization process and return to the main menu.

7. Proceed to the appropriate sequencing protocol for your chip type.

Start the sequencing run

This section describes how to start a run after Ion Chef™ Instrument templating. If the Ion OneTouch™ 2 Instrument was used for template preparation, see the *Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816) for procedures for manually loading an Ion PGM™ chip with template-positive ISPs. After manually loading the chip, return to this section, or continue using the *Ion PGM™ Hi-Q™ Sequencing Kit User Guide*.

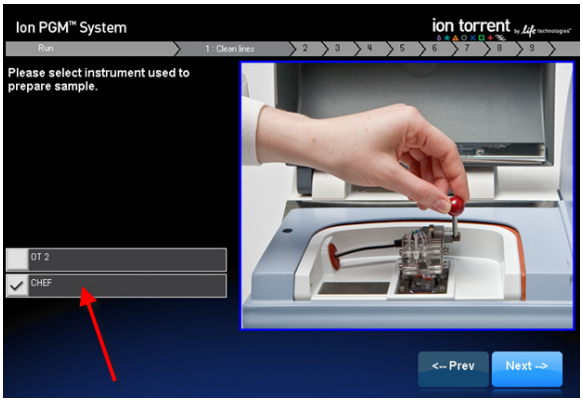
IMPORTANT! Observe the following when performing the chip check and sequencing the chip:

- The Ion PGM™ Sequencer must be cleaned and initialized before sequencing the Ion chips.
- Do not use reagents from other sequencing kits for sequencing Ion chips prepared by the Ion Chef™ System.
- To avoid damage due to electrostatic discharge (ESD), do not place the chip directly on the bench or any other surface. Always place the chip either on the grounding plate on the Ion PGM™ Sequencer or in the custom Ion centrifuge adapter/rotor bucket.
- To avoid ESD damage, do not wear gloves when transferring chips to and from the instrument.

Sequence the loaded Ion chips on the Ion PGM™ Sequencer as soon as possible after unloading the Ion Chef™ Instrument, or manually loading an Ion chip.

1. Touch **Run** on the main menu, then follow the on-screen instructions to empty the waste bottle, load the cleaning chip, and clean the Ion PGM™ Sequencer fluid lines.

2. When the following screen appears, touch **CHEF** or **OT2** to select the instrument used to prepare the sample and initiate the appropriate sequencing workflow. Then touch **Next**.

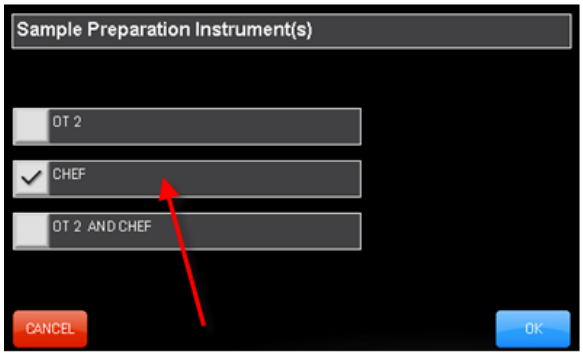


Note: You can preselect the Chef or OT2 option if you use only one instrument for sample preparation.

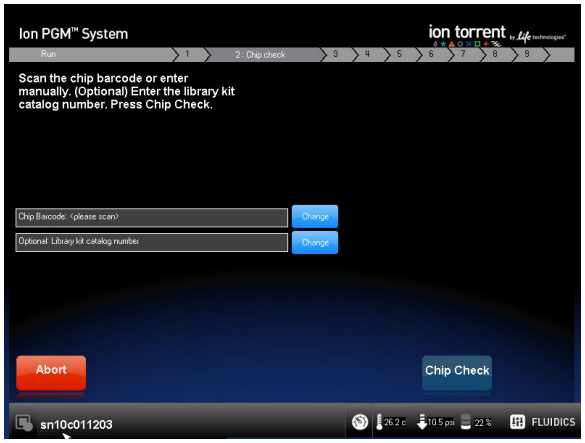
   a. Touch **Options** in the main menu, then touch **Advanced**.

b. Touch the **Change** button to the right of the **Sample Prep: Chef and OT2** option.

c. On the next screen, touch **CHEF**, or **OT2** (depending on your template preparation method), then touch **OK**. The instrument will now automatically select the option you chose.

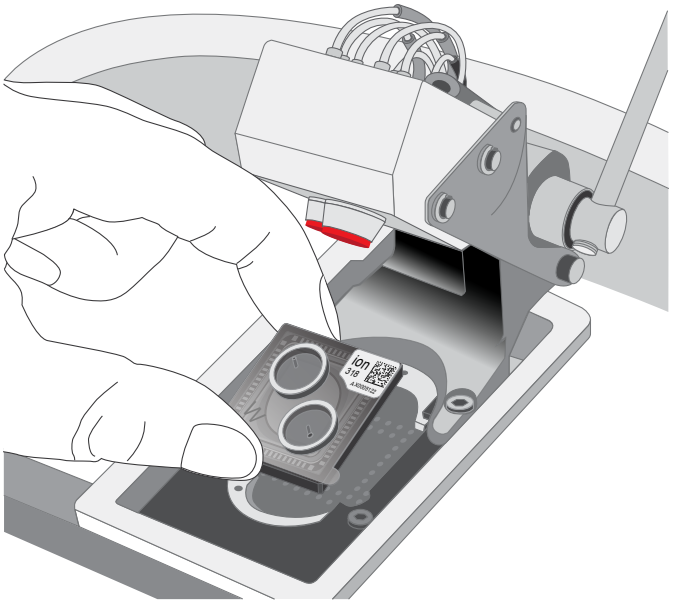


3. Scan the barcode on the loaded chip, or press **Change** to enter the barcode manually.



4. When prompted by the instrument, ground yourself by touching the grounding plate next to the chip clamp on the instrument, replace the cleaning chip in the chip socket with the chip to be sequenced, close the chip clamp, and touch **Next**.

   IMPORTANT! Do not wear gloves when transferring the chips on and off the instrument.



5. Touch **Chip Check** to perform the first chip check.

6. After the instrument successfully completes the chip check, follow the on-screen instructions to empty the waste bottle, then touch **Next**.

7. When prompted to select a Planned Run, confirm that the correct run is displayed, then touch **Next**.

   The Run Setup screen automatically populates the Planned Run field when the Ion PGM™ Sequencer connects to the run. If the correct Planned Run is not displayed, select your run from the drop-down list. If the dropdown list does not contain your Planned Run, contact Technical Support.

8. When run information is displayed, confirm that the run details are correct, then touch **Next**. The instrument will perform a second chip check and calibration.

   During the initial part of Chip Check, visually inspect the chip in the clamp for leaks. If there is a leak, press **Abort** immediately to stop the flow to the chip. When the calibration is complete (~1 minute), the touchscreen indicates the calibration status.

   - If the chip *passes* calibration, touch **Next** to begin the run.
   - If the chip *fails* calibration, touch **Abort**, reseat the chip, then touch **Calibrate** to recalibrate. If the chip fails calibration again, proceed with the run and contact Technical Support after the run is complete.

     Note: To return damaged chips, contact Technical Support.

   IMPORTANT! During a run, avoid touching the instrument and any of the attached bottles or tubes, as this may reduce the quality of the measurements.

9. Twenty minutes before the end of the first run, remove the remaining Ion chip from the chip container in the refrigerator, and place it on a clean surface to warm to room temperature.

10. When first run is complete, sequence the remaining chip as soon as possible. Perform a cleaning and/or initialization if required.

7 Analyze the sequencing results

Related documentation for data analysis

For information about how to analyze	See
Precision ID SNP panels	*HID SNP Genotyper Plugin User Guide* (Pub. No. MAN0010641)
The Precision ID GlobalFiler™ NGS STR Panel	*HID STR Genotyper Plugin User Guide* (Pub. No. MAN0015879)
Precision ID mtDNA panels	*Precision ID mtDNA Panel Analysis User Guide* (Pub. No. MAN0015910)

A

Troubleshooting

This appendix contains brief information for troubleshooting manual library preparation.

For complete troubleshooting information for	See
Library preparation on the Ion Chef™ Instrument	*Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432)
Template preparation on the Ion Chef™ Instrument and sequencing on the Ion PGM™ Sequencer	*Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919)
Template preparation on the Ion OneTouch™ 2 Instrument	*Ion PGM™ Hi-Q™ OT2 Kit User Guide* (Pub. No. MAN0010902) *Ion OneTouch™ 2 System User Guide* (Pub. No. MAN0014388)
Sequencing on the Ion PGM™ Sequencer	*Ion PGM™ Hi-Q™ Sequencing Kit User Guide* (Pub. No. MAN0009816)

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 "Genomic DNA quantification kits" on page 21.
	Residual ethanol in sample DNA inhibited target amplification.	Carefully remove all drops, using an additional centrifugation and removal step, if necessary.
	Less than 1 ng of input DNA was used.	Add more DNA or add up to 4 target amplification cycles.
	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.
Amplicons are lost	The library was poorly purified.	Vortex the AMPure™ XP Reagent thoroughly before use, and dispense the full volume.

Observation	Possible cause	Recommended action
Amplicons are lost		Increase the AMPure™ XP Reagent volume from 1.5X to 1.7X. See "Purify the libraries" on page 31.
	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 29.
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.
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.	• An error occurred during library preparation and/or sequencing. • Barcode adapters did not ligate properly during library preparation, so no key is available to sequence. • Sequencing flow and raw signal acquisition errors occurred.	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%) 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 31.
	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 (>40%)	The library input for template preparation was too high.	Decrease the amount of sample library for templating preparation by 50%.
	The library was inaccurately quantified.	Re-quantify library to ensure accurate quantification.
	The TDF was incorrectly calculated.	Ensure that the TDF is calculated correctly.

Observation	Possible cause	Recommended action
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.
		Use a fresh dilution of library prepared in a low-bind tube.
	Library quality was low.	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.
	The Neutralization solution was omitted during the Ion OneTouch™ ES run, and ISPs remained in melt-off solution for >15 minutes.	Perform a new OT2 template preparation reaction. Ensure that you add Neutralization solution before starting the ES run. Promptly remove samples from ES when the run completes.
Extremely low ISP loading (<30%), then sequencing failure	The sequencing primer step was omitted.	If half the volume of enriched ISPs was saved before the sequencing failure, redo sequencing protocol steps - Ion 314™ Chip v2. If no ISPs were saved, start over at template preparation.
	The sequencing polymerase step was omitted.	If half the volume of enriched ISPs was saved before the sequencing failure, redo sequencing protocol steps - Ion 314™ Chip v2. If no 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.	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).

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

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/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
Data analysis	
HID SNP Genotyper Plugin User Guide	MAN0010641
HID STR Genotyper Plugin User Guide	MAN0015879
Precision ID mtDNA Panel Analysis User Guide	MAN0015910
Manual library preparation	
HID-Ion AmpliSeq™ Library Preparation Quick Reference	MAN0010638
IonCode™ Barcode Adapters 1–384 Kit Product Information Sheet	MAN0014640
Automated library preparation on the Ion Chef™ System	
Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide	MAN0013432
Ion AmpliSeq™ Library Preparation on the Ion Chef™ System Quick Reference	MAN0013433
Template preparation on the Ion Chef™ System for PGM	
Ion PGM™ Hi-Q™ Chef Kit User Guide	MAN0010919
Ion PGM™ Hi-Q™ Chef Kit Quick Reference	MAN0010920
Template preparation on the Ion OneTouch™ 2 Instrument for PGM	
Ion PGM™ Hi-Q™ OT2 Kit User Guide	MAN0010902

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.
- For latest services and support information for all locations, go to **thermofisher.com/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 found on Life Technologies' website at **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**.