Ion PGM™ Hi-Q™ Chef Kit

USER GUIDE

for use with:
Ion Chef™ System
Ion Personal Genome Machine™ (PGM™) System

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Thermo Fisher
S C I E N T I F I C

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Revision history: MAN0010919

Revision	Date	Description
C.0	8 January 2017	- Ion PGM™ Calibration Standard removed from kit components list. It is now ordered separately (Cat. No. A27832). - Recommendation for dilution of *E. coli* DH10B Control 400 Library added. "Load the Ion Chef™ System" and "Clean the Ion Chef™ Instrument" topics reorganized for ease of use. "Create a Planned Run" on page 20 updated for Torrent Suite™ Software v5.2 - Designation of Chip-loading centrifuge positions changed from "A" and "B" to "1" and "2". - ISP quality control sections updated and moved to new user guides: – *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387) – *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388) - Web links updated.
B.0	28 January 2016	- Guidance added for use with Torrent Suite™ Assay Development Software - Library input recommendations added for Ion AmpliSeq™ for Chef DL8 Kit users. - Updated for Torrent Suite™ Software v5.0 - Instructions added to override warning for no Torrent Server or network connection - Clarifications made in the sequencing workflow, including use of the Line Clear feature in initialization.
A.0	6 February 2015	New user guide, which includes instructions to set up and operate the Ion Chef™ System with the new Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948).

Contents

About this guide

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

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

Purpose

This user guide describes how to use the Ion Chef™ System to prepare enriched, template-positive Ion Sphere™ Particles (ISPs) for up to 400 base-read sequencing of libraries on the Ion PGM™ Sequencer. The Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948) includes reagents and materials sufficient for performing 4 dual-sample, template-preparation runs on the Ion Chef™ System and subsequent sequencing on an Ion PGM™ System.

> ⚠ **WARNING!** The protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer (Thermo Fisher Scientific).

Prerequisites

This guide also assumes that you have:

- A general understanding of Ion Torrent™ sequencing chemistry and workflow
- Knowledge of techniques for handling and preparing DNA libraries

Product information

Product description

The Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948) includes the reagents and materials that are required to use the Ion Chef™ System to prepare template-positive Ion Sphere™ Particles from up to 400-base read libraries and load 8 sequencing chips of the following type:

- Ion 314™ Chip v2 BC
- Ion 316™ Chip v2 BC
- Ion 318™ Chip v2 BC

Note: The kit and chips can also be used for Research Use Only experiments on the Ion PGM™ Dx System running Torrent Suite™ Assay Development Software[1].

The kit also includes reagents and materials for cleaning and initializing the Ion PGM™ Sequencer, sufficient for performing 4 initializations. The number of sequencing runs per kit depends on the length of the base reads, as shown in the following table:

Sequencing type	Number of flows	Maximum number of runs per initialization[1]	Number of sequencing runs per kit	Number of sequencing runs per kit when combined with the Ion PGM™ Hi-Q™ 400 Supplies Kit[2]
400-base read	850	1	4	8
200-base read	500	2	8	N/A

[1] For best results, start the sequencing run within 1 hour after initialization.

[2] Users sequencing 400-base read libraries must obtain additional sequencing reagents and materials for 4 additional sequencing initializations and runs by ordering the Ion PGM™ Hi-Q™ 400 Supplies Kit (Cat. No. A27293), which is sold separately.

[1] Ion PGM™ Dx System with Torrent Suite™ Assay Development Software is For Research Use Only. Not for use in diagnostic procedures.

| Library compatibility | The Ion PGM™ Hi-Q™ Chef Kit can be used with up to 400-base-read libraries of any type prepared using any available Ion library kit. |

Software compatibility

The Ion PGM™ Hi-Q™ View Chef Kits are compatible with the following software and sequencing instrument systems:

- Torrent Suite™ Software v5.0 or later on the Ion PGM™ System.
- Torrent Suite™ Assay Development Software v5.0.2 or later on the Ion PGM™ Dx System[2]. To sequence chips prepared with this kit on the Ion PGM™ Dx System with Torrent Suite™ Assay Development Software, see *Ion PGM™ Hi-Q™ Chef Kit —Assay Development Mode User Guide* (Pub. No. MAN0015833).

Be sure to update your Torrent Server to the latest available version of the software before using this kit.

Kit contents and storage

IMPORTANT! Do not mix components with components from any other Ion sequencing kits. Thermo Fisher Scientific has verified this protocol using these specific materials. Substitution may adversely affect system performance.

IMPORTANT! Store all consumables and cartridges under the recommended conditions and in an upright position.

The Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948) contains all materials required for sequencing preparation. Upon arrival, inspect all consumables and contact Thermo Fisher Scientific if any of the products have been damaged during shipping.

Ion PGM™ Hi-Q™ Chef Kit component summary

Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948)

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	1 box
Ion PGM™ Hi-Q™ Sequencing dNTPs	A25590	1 box
Ion PGM™ Hi-Q™ Sequencing Solutions	A25589	1 box

[2] The Ion PGM™ Dx System with Torrent Suite™ Assay Development Software is For Research Use Only. Not for use in diagnostic procedures.

Ion Chef™ reagents and materials

Part No.	Component	Amount per kit	Storage
A25957	Ion PGM™ Hi-Q™ Chef Supplies • Chip Adapter (2) • Enrichment Cartridge v2 (1) • Tip Cartridge v2 (1) • PCR Plate and Frame Seal v2 (1 each) • Recovery Station Disposable Lid v2 (2) • Recovery Tube v2 (12)	4 boxes	15° to 30°C
A25956	Ion PGM™ Hi-Q™ Chef Solutions	4 cartridges	15° to 30°C
A25955	Ion PGM™ Hi-Q™ Chef Reagents	4 cartridges	-30° to -10°C

Ion PGM™ sequencing reagents and materials

Component	Amount	Storage
Ion PGM™ Sequencing Supplies (Part No. A25587)		
Wash 1 Bottle w/ label (250 mL)	1 bottle	15° to 30°C
Wash 3 Bottle w/ label (250 mL)	1 bottle	
Reagent Bottle Sipper Tubes (blue)	16 tubes	
Wash Bottle Sipper Tubes (gray)	8 tubes for 250-mL bottles 4 tubes for 2-L bottle	
Reagent Bottles w/ labels (50 mL)	25 bottles	
Ion PGM™ Hi-Q™ Sequencing Solutions[1] (Part No. A25589)		
Ion PGM™ Hi-Q™ Sequencing W2 Solution	2 × 126.25 mL	2°C to 8°C
Ion PGM™ Sequencing W3 Solution	2 × 100 mL	
Ion Cleaning Tablet	4 tablets	
Ion PGM™ Hi-Q™ Sequencing dNTPs (Part No. A25590)		
Ion PGM™ Hi-Q™ Sequencing dGTP (black cap)	80 µL	-30° to -10°C
Ion PGM™ Hi-Q™ Sequencing dCTP (blue cap)	80 µL	
Ion PGM™ Hi-Q™ Sequencing dATP (green cap)	80 µL	
Ion PGM™ Hi-Q™ Sequencing dTTP (red cap)	80 µL	

[1] Includes 1 bottle of Annealing Buffer, which is not used in the Ion Chef™ protocol.

Ion PGM™ Calibration Standard

If you are performing a *de novo* sequencing experiment, add the Ion PGM™ Calibration Standard (Cat. No. A27832) to your amplification solution to enable greater accuracy of base-calling in libraries for which a reference BAM file does not exist. The Ion PGM™ Calibration Standard is ordered separately.

Description	Amount	Storage
Ion PGM™ Calibration Standard	80 μL	−30° to −10°C

Ion PGM™ Wash 2 Bottle Kit

The Ion PGM™ Wash 2 Bottle Kit (Cat. No. A25591) is sold separately, 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 47	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	
	8 chips	4488150	
Ion 316™ Chip Kit v2 BC	4 chips	4488145	15°C to 30°C
	8 chips	4488149	
Ion 314™ Chip Kit v2 BC	8 chips	4488144	

Ion PGM™ Hi-Q™ Chef 400 Supplies Kit

The Ion PGM™ Hi-Q™ Chef 400 Supplies Kit (Cat. No. A27293) is sold separately, and provides additional sequencing supplies and reagents for sequencing 400-base-read libraries. The materials in this supplemental kit, together with the materials in the Ion PGM™ Hi-Q™ Chef Kit, enable 8 initializations of the Ion PGM™ Sequencer for eight 400 bp sequencing runs.

Component	Quantity	Part No.	Shipping and storage
Ion PGM™ Sequencing Supplies	1	A25587	15° to 30°C
Ion PGM™ Hi-Q™ Sequencing dNTPs	1	A25590	-30° to -10°C
Ion PGM™ Hi-Q™ Sequencing Solutions	1	A25589	2°C to 8°C

Required materials not supplied

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

Note: This protocol has been verified using these specific materials. Substitution may adversely affect system performance.

✓	Item	Source
	Ion Personal Genome Machine™ (PGM™) System (instrument and server) and included accessories	4462921
	Ion PGM™ Controls Kit v2 [1]	4482010
	2.5-L Ion PGM™ Waste Bottle (required for 400-base-read sequencing)[2]	4482565
	Syringe, 10 CC, with Luer-Lok™ fitting (used for clearing lines)	MLS
	Tank of compressed nitrogen (grade 4.5, 99.995% pure or better)	MLS
	Multistage (dual-stage) gas regulator (0–50 psi, 2–3 Bar output)	Fisher Scientific NC0393866 or MLS
	(Optional) 1/8" x 1/4" stem reducing coupler (only required if using a separate tank for the wash station)	McMaster 5779K699
	(Optional) Uninterruptable Power Supply (UPS) [3]	MLS
	ELGA™ PURELAB™ Flex 3 Water Purification System *or* Equivalent 18 MΩ water system	4474524 MLS
	NaOH (10 M) molecular biology grade	MLS
	Isopropanol (100%)	MLS
	Nuclease-free water molecular biology grade	MLS

✓	Item	Source
	0.22-µm or 0.45-µm vacuum filtration system and filters	MLS
	Microcentrifuge[4]	MLS
	25-mL or 50-mL serological pipettes *or* 100-mL graduated cylinder	MLS
	Pipet-Aid™ Pipet Controller (If using serological pipettes)	Fisher Scientific 13-681-15
	P2, P10, P20, P200, P1000 µL pipette set and filtered tips	MLS
	0.2-mL Thin Wall PCR Tubes, Flat Cap, Axygen (do not use polystyrene tubes), or equivalent	Fisher Scientific 14-222-283
	1.5-mL or 1.7-mL microcentrifuge tubes	MLS
	Glass bottles (1 L)	MLS
	Ice buckets and ice	—
	Vortex mixer with a rubber platform	MLS
	Gloves, powder-free nitrile	MLS
	Thermal cycler with a heated lid	MLS

[1] For installation and troubleshooting.

[2] Order by phone to upgrade from the smaller waste bottle supplied with the original "orange" Ion PGM™ Sequencer.

[3] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use an uninterruptable power supply that is compatible with 2500 W output or higher.

[4] Must fit standard 1.5- and 0.2-mL microcentrifuge tubes and generate 15,500 × *g*.

About the Ion Chef™ System

The Ion Chef™ System provides automated, high-throughput template preparation and chip loading for use with the Ion PGM™ Sequencer. The system includes a complete set of cartridge-based consumables and reagents that enable a user to load two Ion 314™ v2 BC, Ion 316™ v2 BC, or Ion 318™ v2 BC Chips in approximately 10.5 hours for 200-base read libraries and 14 hours for 400-base read libraries, with less than 15 minutes of hands-on time. The Ion Chef™ System features network integration with the Torrent Suite™ Software to enable sample and reagent traceability throughout the chip preparation workflow.

Ion Chef™ Instrument components

The following figure illustrates the major external and internal features of the Ion Chef™ Instrument.
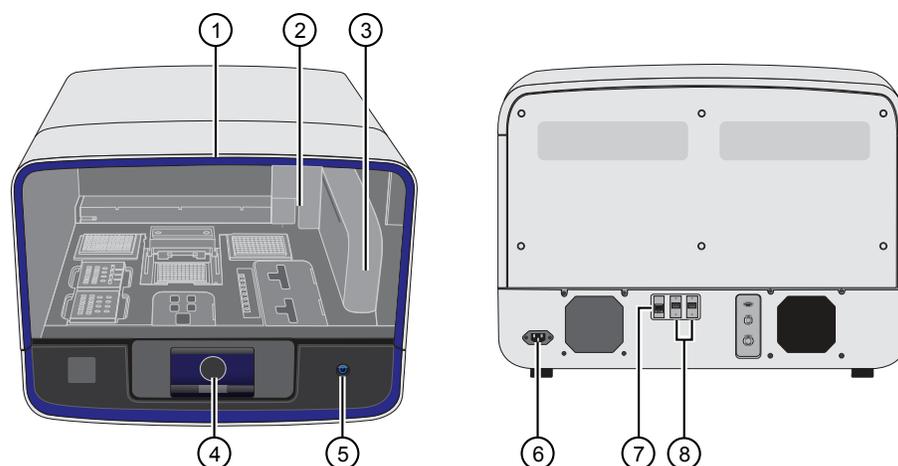
① **Door** – Provides access to the interior of the instrument. The door is locked in the closed position during operation.

② **Micropipettor** – A mechanical positive-displacement pipettor that performs all fluid transfers during sample and chip preparation.

③ **Robotic arm** – Performs fluid transfer during chip loading. The arm also contains an optical sensor that reads the barcodes of instrument reagents and consumables.

④ **Touchscreen** – Provides access to all instrument functions for operation, maintenance, and troubleshooting.

⑤ **Power button** – Power switch for the Ion Chef™ Instrument, where the states are on (illuminated) and off.

⑥ **Power port** – A 100–240 VAC port that provides power to the Ion Chef™ Instrument.

⑦ **Ethernet port** – An RJ45 port that provides Ethernet (100 Mbit) communication with the Ion Chef™ Instrument.

⑧ **USB port** – Provides USB communication with the Ion Chef™ Instrument. Used to update the instrument firmware and to transfer data during service or maintenance.

Interior hardware and consumables

The following figure illustrates the interior of the Ion Chef™ Instrument and describes the stations involved in the preparation of Ion sequencing chips.
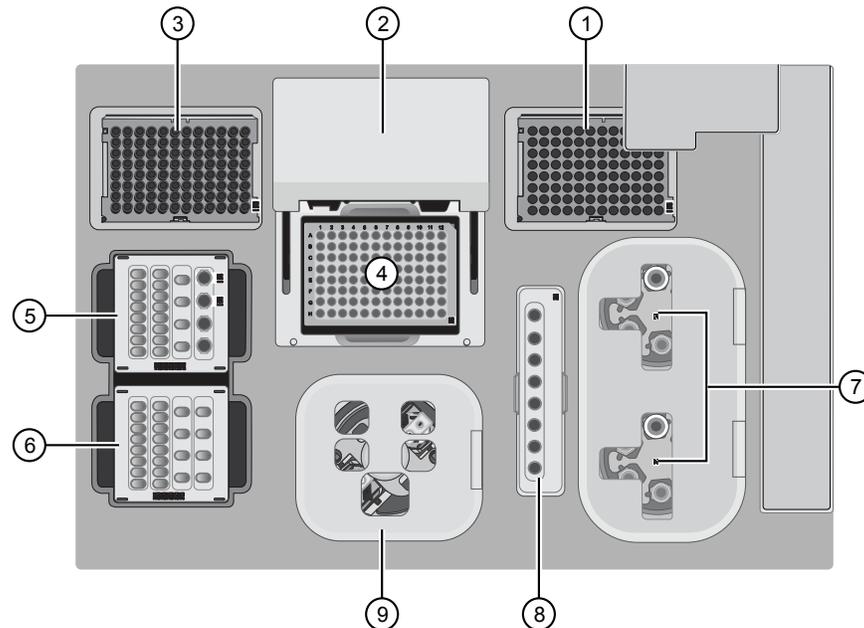
① **Waste pipette tip rack** – The position of the rack containing waste (used) pipette tips.

② **Automated heated cover** – Transfers the plate cover to the PCR reaction plate within the sample block. During thermal cycling, the heated cover applies compression to seal the reaction plate and heats the cover to prevent condensation.

③ **New pipette tips** – The position of the rack containing unused pipette tips.

④ **Thermal cycler sample block** – Performs thermal cycling of the sequencing reactions on a 96-well PCR reaction plate.

⑤ **Reagents station** – The position on the instrument deck of the diluted libraries, NaOH, and the Ion PGM™ Hi-Q™ Chef Reagents.

⑥ **Solutions station** – The position on the instrument deck of the Ion PGM™ Hi-Q™ Chef Solutions, which is maintained at room temperature.

⑦ **Recovery centrifuges** – Twin stations that perform centrifugation of the ISPs during the recovery phase of template preparation.

⑧ **Enrichment station** – The position of the rack containing consumables for the enrichment of the template-positive ISPs.

⑨ **Chip-loading centrifuge** – Performs centrifugation of sequencing chips that have been mounted to chip-loading adapters and loaded with template-positive ISPs.

About the Ion Chef™ System touchscreen interface

The Ion Chef™ System features a simple interface for loading chips, cleaning the instrument, and performing system maintenance and configuration tasks.

① **Set up Run** button – Set up the Ion Chef™ template preparation and chip-loading routine. Choose **Step by Step** to have the instrument lead you stepwise through installation of reagents and consumables, or choose **Quick Start** to proceed if you have already installed the consumables.

② **Open Door** button.

③ **Notifications** button – View notifications about instrument status during and between runs.

④ **Quick Start** button – Proceed directly to the Quick Start instrument setup mode. User verifies the loading of a new pipette tip cartridge and an empty pipette tip rack to hold waste tips generated during the run, before proceeding to Deck Scan.

⑤ **Settings** button – Advance to a screen providing the following options:

- **Notifications**: view notifications about instrument status during and between runs
- **Instrument Settings**: view current settings and network configuration, set instrument name, adjust time zone
- **Service tools**: access screens for service-related maintenance and instrument diagnostics
- **Torrent Server**: add and manage Torrent Server connections
- **Clean Ion Chef**: proceed directly to the instrument cleaning routine
- **Check for updates**: check availability of system software updates

Precautions

Avoid nucleic acid contamination

IMPORTANT! A primary source of contamination is spurious DNA fragments from previous sample processing steps. Do not introduce amplified DNA into the library preparation laboratory or work area.

IMPORTANT! Handle nucleotides carefully to avoid cross-contamination. Always discard gloves after removing used Sippers from the sequencer in order to avoid cross-contamination of the nucleotides. Always discard gloves after handling concentrated dNTP stocks. Barrier tips are required for all dNTP pipetting steps.

Avoid chip damage

IMPORTANT! To avoid possible damage to the chip due to electrostatic discharge, ground yourself before picking up a chip or placing a chip on a surface such as a lab bench. For example, touch the metal trim on the chip compartment before inserting or removing a chip from the chip clamp.

Guidelines for using Ion Chef™ reagents and consumables

To ensure the proper function of the Ion Chef™ System, handle the associated consumables and reagents according to the following guidelines:

- Store all Ion Chef™ System consumables and cartridges under the recommended conditions and in an upright position.
- Inspect all Ion Chef™ System consumables and cartridges for damage on arrival and again before use.
- Hold sequencing chips by gently gripping them by their edges.
- When the Ion Chef™ System is not in use, remove all consumables and reagents from the deck and close the instrument door.
- Except for the *New* Pipette Tip Cartridge v2, do not reuse any of the Ion Chef™ System consumables or reagents. After each run, the empty Pipette Tip Cartridge v2 is transferred to the waste tip station.

  IMPORTANT! All Ion Chef™ Instrument components are single-use only.

- Use only Ion Torrent™ kits and supplies with the Ion Chef™ Instrument. The use of third-party reagents and supplies can adversely affect the performance of the Ion Chef™ Instrument and Ion chips prepared.
- Always load two of the same model of sequencing chips into the Ion Chef™ System. The instrument cannot load different models of sequencing chips during the same run.
- Loaded chips can be stored at 4°C for up to 24 hours, but we recommend that chips be sequenced as soon as possible (within 1 hour) for optimal performance. If you cannot sequence a loaded chip immediately, store it in a chip storage container at 4°C until you are ready to run it.

  Note: If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the chip storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

Workflow

Use the following workflow to prepare and load samples using the Ion PGM™ Hi-Q™ Chef Kit and Ion Chef™ System for sequencing on the Ion PGM™ Sequencer.

Create a Planned Run within the Torrent Suite™ Software
and set the run parameters.

▼

Dilute the library samples.

▼

Load the Ion Chef™ Instrument with Ion sequencing chips,
consumables, reagents, and libraries.

▼

Start the Ion Chef™ Instrument.

▼

Clean and initialize the Ion PGM™ Sequencer.

▼

Unload the Ion Chef™ Instrument.

▼

Load the prepared chip into the Ion PGM™ Sequencer
and begin the sequencing run.

Before you begin

Create a Planned Run

Planned Runs can be created with the following software programs for use on the following systems:

Software	Instrument System
Torrent Suite™ Software, v5.0 or later	Ion PGM™ System
Torrent Suite™ Assay Development Software, v5.0 or later	Ion PGM™ Dx System

IMPORTANT! If you are using the Ion PGM™ Dx System with Torrent Suite™ Assay Development Software[3], see *Ion PGM™ Hi-Q™ Chef Kit — Assay Development Mode User Guide* (Pub. No. MAN0015833).

Planned Run set-up and the Ion PGM™ Dx System workflow differ from those described in this user guide.

About Planned Runs

Planned Runs contain all the settings used in a sequencing run, including number of flows, kit types, barcodes, sample information, and reference files (if any). They are used to track samples, chips, and reagents throughout the sequencing workflow, from template preparation on the Ion Chef™ Instrument through sequencing on the Ion PGM™ Sequencer and subsequent data analysis. Each of the two chips prepared in an Ion Chef™ run requires its own Planned Run.

[3] The Ion PGM™ Dx System with Torrent Suite™ Assay Development Software is For Research Use Only. Not for use in diagnostic procedures.

Create a Planned Run

The following provides a summary of steps for creating a Planned Run in Torrent Suite™ Software. For a complete description of the fields of the Torrent Browser application, see the *Torrent Suite™ Software Help Guide*, available at **thermofisher.com/ion-pgm-docs.html**.

1. Open the Torrent Browser on a computer connected to your Ion Chef™ System.

2. Select the **Plan** tab, click **Templates**, select the application that you want to run (such as Whole Genome), then click either:

   • **Plan New Run** to plan a new run using the generic template for the selected application.
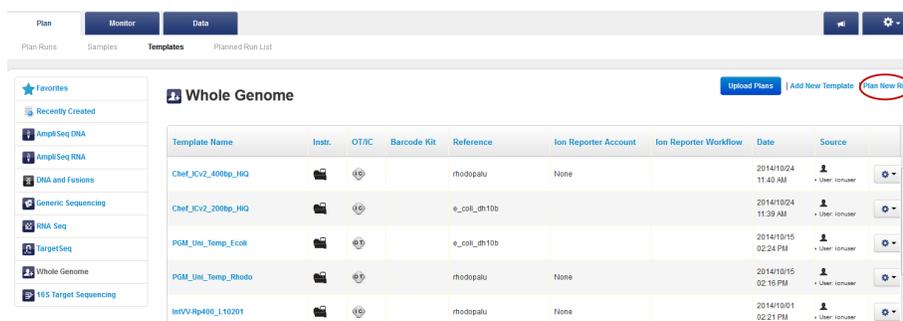
   • **Plan Run** in the dropdown menu under the "Gear" ⚙ tab to the right of the existing template you select from the template list.
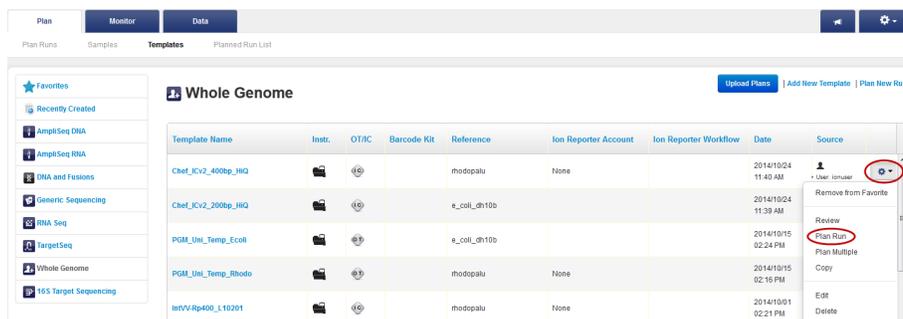
3. In the Planned Run wizard, review the **IonReporter** and **Application** tabs, then make selections appropriate to your run. In the **Kits** tab, make the following selections:

   a. Select **Ion PGM™ System** from the **Instrument** dropdown list.

   b. Select the appropriate chip type in the **Chip Type** dropdown list.

   c. Select **Ion Chef** for Template Kit, then select **Ion PGM™ Hi-Q™ Chef Kit** from the **Template Kit** dropdown list.

d. Select **200** or **400** for **Templating Size**.

> **Note:** Use the 200 setting for libraries with <300-base read average length. Use the 400 setting for libraries with ≥300-base read average length.
>
> ---
>
> **IMPORTANT!** Because 200- and 400-base read libraries require different run lengths, do not load a 200- and 400-base read library in a single Ion Chef™ run.

e. Enter the appropriate **Library Read Length** if the correct read length is not shown.

f. Select **Ion PGM™ Hi-Q™ Sequencing Kit** from the **Sequencing Kit** dropdown list.

Create Plan	Ion Reporter	Application	**Kits**	Plugins
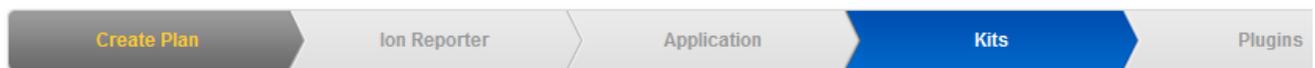
Select instrument, chip and kits and then hit next.

Instrument :

Ion PGM™ System ▾

Chip Type (required) :

Ion 318™ Chip v2 ▾

Sample Preparation Kit (optional) :

▾

Control Sequence (optional) :

▾

Library Kit Type Details + **:**

Ion Xpress Plus Fragment Library Kit ▾

Barcode Set (optional) :

▾

Template Kit ◯ OneTouch ◉ IonChef **:**

Ion PGM Hi-Q Chef Kit ▾

Templating Size: ◯ 200 ◉ 400

Library Read Length: 850 ▲▼

Sequencing Kit :

Ion PGM Hi-Q Sequencing Kit ▾

Flows :

850 ▲▼

ⓘ **Base Calibration Mode :**

Default Calibration ▾

ⓘ **Mark as Duplicates Reads** ☐ **:**

ⓘ **Enable Realignment** ☐ **:**

‹ Previous   Next ›

g. Select or edit the optional information fields appropriately for your run.

> IMPORTANT! For *de novo* sequencing applications that do not include a reference BAM file, select **Enable Calibration Standard** from the **Base Calibration Mode** dropdown list. Select this option only if you add Ion PGM™ Calibration Standard to the Ion Chef™ Library Sample Tubes. Selecting this option and adding the Ion PGM™ Calibration Standard to your library allows greater accuracy of base-calling in libraries for which a reference BAM file does not exist.

h. Click **Next**.

4. Review the **Plugins** and **Projects** tabs, then make selections appropriate to your run.

5. In the **Plan** tab, enter or scan the barcodes of the Ion Chef™ Library Sample Tubes into the appropriate Sample Tube Label fields. To specify two sample names, enter "2" in the **Number of chips** field, then click the check mark button to the right of this field.

Create Plan	Ion Reporter	Application	Kits	Plugins

Run Plan Name (required) :

Ion PGM Hi-Q Chef 400 bp

Analysis Parameters: ⦿ Default (Recommended)   ○ Custom   [Details +]

Default Reference & BED Files   —

Reference Library : hg19(Homo sapiens) ▼

Target Regions: None ▼

Hotspot Regions: None ▼

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

Number of chips : [2] ●   [Save Samples Table] [Load Samples Table]

Enter a sample name for each plan (require at least one sample) 👁 **:**

#	Sample (required)	Sample Description	Sample ID	Sample Tube Label	Chip ID
1	Sample 1				
2	Sample 2				

Note: If you selected a Barcode Set in the Kits tab, fields appear in the Plan tab where you enter the number of barcodes that are used, and the corresponding Sample Tube Label. Then enter a sample name for each barcode used in the Samples table. Return to step 2 and repeat the procedure from the start to create a Planned Run for a second chip. You can copy the first Planned Run in the Planned Run page and edit appropriately.

6. When you have completed your selections, click **Plan Run** at the bottom right of the **Plan** tab screen to save the run. A Planned Run is created for each chip and appears on the Planned Runs page with the name that you specified, appended with sample name. The plans are automatically used by the Ion Chef™ System when the associated samples are loaded.

Planned Run wizard key fields

Field name	Description
Ion Reporter	If Ion Reporter™ Software is installed and enabled, and you want to analyze the run data using the software, select the account and workflow.
Application	Select the sequencing application you are performing.
Instrument	Select the sequencing system you are using.
Chip Type	Select the chip type you are using.
Library Kit Type	Select the kit used to prepare the library.
Template Kit	Select **Ion PGM Hi-Q Chef Kit**.
Sequencing Kit	Select **Ion PGM Hi-Q Sequencing Kit**.
Barcode Set (optional)	If you are using barcodes with: • **DNA libraries**: Select the appropriate barcode set used. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2**: Select the **IonXpressRNA** barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01–16 Kit. If you are *not* using barcodes with: • **DNA libraries**: Leave the Barcode Set field blank. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2**: Select **RNA_Barcode_None** from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.
Flows	Enter the appropriate number of flows for the read length (for example, 500 flows for 200-base read sequencing and 850 flows for 400-base read sequencing).
Plugins	Select the appropriate plugins for your application.
Projects	Select or add a project within which to group your run data. You can include runs in multiple projects, and remove runs from a project at any time.
Run Plan Name	Enter a name for the run.
Reference Library	Select a reference library uploaded to the Torrent Server, if any.
BED files	Select the **Target Regions** or **HotSpot Regions** BED file on the Ion Torrent Server, if any.
Sample Name (Required)	Is Ion Reporter™ Uploader enabled? • **Yes** – Enter each sample name and select the appropriate values for workflow, relation, relation role, and set ID. • **No** – Scan the barcode of the library tube into the "Sample Name" and "Sample Tube Label" fields for the specific sample.

Field name	Description
Sample Tube Label (Required)	Scan or enter the barcodes located on the Ion Chef™ Library Sample Tubes that you intend to use to load samples into the Ion Chef™ Instrument. **IMPORTANT!** You must scan or enter the barcode of each Ion Chef™ Library Sample Tube used on the Ion Chef™ Instrument.
Monitoring Thresholds	Set thresholds for Bead Loading, Usable Sequence, and Key Signal. In the Torrent Browser **Monitor ▸ Runs in Progress** tab, an alert is displayed if the values for a run fall below the selected thresholds.

Guidelines for library dilution

- The library dilution recommendations below represent optimal input concentrations for control libraries. Users who have already optimized a library concentration for the Ion PGM™ IC 200 Kit do not need to re-optimize library concentration for the Ion PGM™ Hi-Q™ Chef Kit. Users only need to reduce the input volume from 70 µL to 25 µL (see "Prepare the libraries and consumables" on page 27).
- Recommendations are based on qPCR quantification. If libraries are quantified with an Agilent™ 2100 Bioanalyzer™ instrument, a higher calculated concentration may need to be used for equivalent input.
- Prepare a fresh dilution of each library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.

Dilute the sample libraries

IMPORTANT! Before proceeding, dilute the 2 Ion libraries to the optimal input concentration. The quality of your sequencing data relies greatly upon achieving the correct concentration of starting library.

Dilute libraries depending on length according to the following table. Then use polyclonality and low quality filter results from a sequencing run performed with ISPs templated at the starting concentration and titrate up or down to achieve optimal concentrations, if necessary.

Library length[1]	Recommended concentration	Molecules per 25-µL input volume	Templating size in Planned Run setup
<300 bases	20–30 pM	$300–450 \times 10^6$	200
≥300 bases	40–60 pM	$600–900 \times 10^6$	400

[1] Library length is average insert length plus adapter length.

Dilute Ion AmpliSeq™ for Chef combined libraries

If you used the Ion AmpliSeq™ for Chef DL8 Kit to prepare libraries, dilute 12.5 µL of each combined library with 12.5 µL of Nuclease-free Water. If you have quantified the combined libraries by qPCR, dilute them to a concentration of 40-60 pM.

Dilute the control library (if used)

If you are running a control library for troubleshooting purposes, dilute the *E. coli* DH10B Control 200 or *E. coli* DH10B Control 400 Library, obtained from the Ion PGM™ Controls Kit v2 (Cat. No. 4482010), as follows:

Control library	Volume of library	Volume of Nuclease-free Water
E. coli DH10B Control 200 Library	1 µL	105 µL
E. coli DH10B Control 400 Library	1 µL	170 µL

Prepare the Ion Chef™ System for use

Before you use the Ion Chef™ Instrument:

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

  Note: For more information on the cleaning procedure, see Chapter 6, "Clean the Ion Chef™ System".

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

- Thaw the Ion PGM™ Hi-Q™ Chef Reagents cartridge at room temperature for 45 minutes before use.

- Ensure that the Ion Chef™ Instrument has a connection to your 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 Appendix C, "Ion Chef™ and Torrent Server network setup" for instructions on how to configure a direct or indirect network connection of the Ion Chef™ Instrument to a Torrent Server.

Run the Ion Chef™ System

This chapter describes:

- Setting up the Ion Chef™ Instrument for use by diluting the two Ion libraries, and loading the instrument with all of the required reagents and consumables
- Starting an Ion Chef™ run
- Unloading the Ion Chef™ Instrument

Set up the Ion Chef™ System

In the following procedure, you will prepare the Ion Chef™ System for use by diluting the two Ion libraries and loading the instrument with all of the required reagents and consumables.

Materials required
- Ion PGM™ Hi-Q™ Chef Kit (Cat. No. A25948)
- Ion 314™ v2 BC, Ion 316™ v2 BC, or Ion 318™ v2 BC Chip (2)
- Molecular-biology grade Nuclease-free Water
- P200 pipette and filtered tips
- Waste container

Prepare the libraries and consumables

1. At least 45 minutes before use, unbox the Ion PGM™ Hi-Q™ Chef Reagents cartridge and 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 each diluted library (see "Dilute the sample libraries" on page 25) to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).

   IMPORTANT! If you are performing a *de novo* sequencing experiment that does not include a reference BAM file, add 10 µL of the Ion PGM™ Calibration Standard (Cat. No. A27832) to your diluted libraries in the Ion Chef™ Library Sample Tubes. Cap the tubes, vortex to mix, then centrifuge for 1–2 seconds to collect contents at the bottom of the tube.

   Note: If running the *E. coli* DH10B Control 200 or 400 Library, obtained from the Ion PGM™ Controls Kit v2 (Cat. No. 4482010), prepare the library for use by diluting 1 µL of stock library as described in "Dilute the control library (if used) " on page 26, then add 25 µL of diluted control library to the appropriate Ion Chef™ Library Sample Tube.

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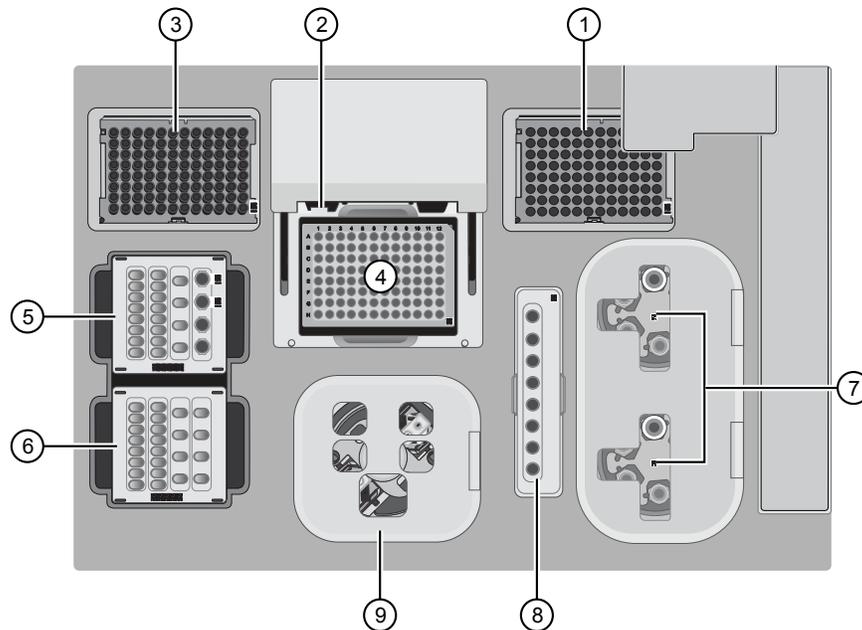
① Empty tip rack (move from new Tip Cartridge position)
② Frame Seal v2
③ New Tip Cartridge v2
④ PCR plate
⑤ Ion PGM™ Hi-Q™ Chef Reagents cartridge
⑥ Ion PGM™ Hi-Q™ Chef Solutions cartridge
⑦ Recovery Tubes and Recovery Station Disposable Lid v2
⑧ Enrichment Cartridge v2
⑨ Chip Adapter/Chip assemblies

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.
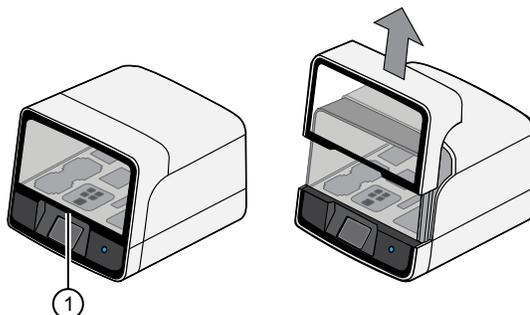
① Hold here, then lift

3. Load an empty pipette tip rack to the *Used* (Waste) Pipette Tip Position, then change gloves.
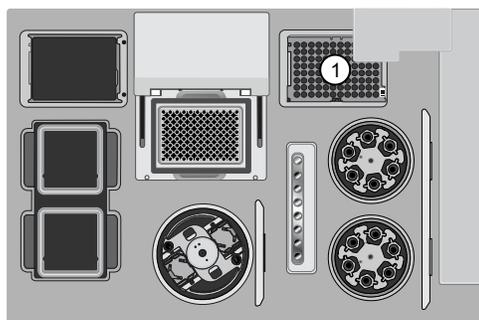
① Used Pipette Tip Position

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. Pull the catch forward, then pivot the locking bracket upwards. Load the assembled Tip Cartridge v2 into the *New* Pipette Tip Position, then pull the bracket downwards, then push the catch backwards to lock the cartridge in place.
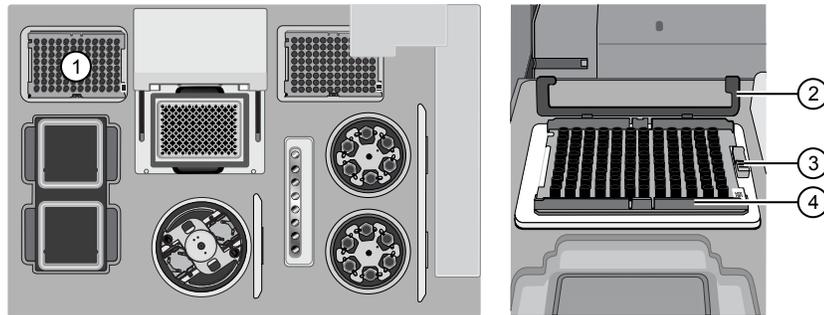
① New Pipette Tip Position
② Bracket
③ Catch
④ New Tip Cartridge

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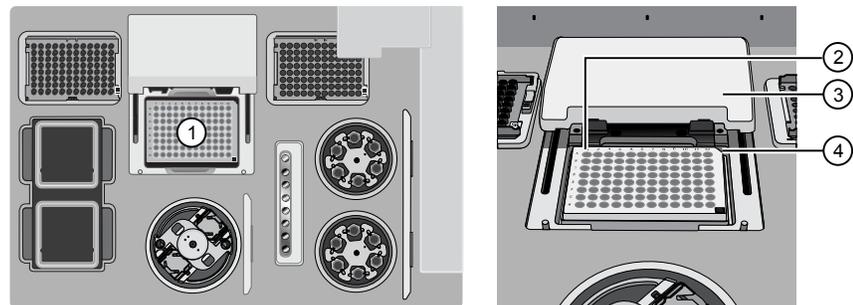
① Thermal cycler sample block
② Well A1
③ Cover
④ Keyed corner

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. 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.
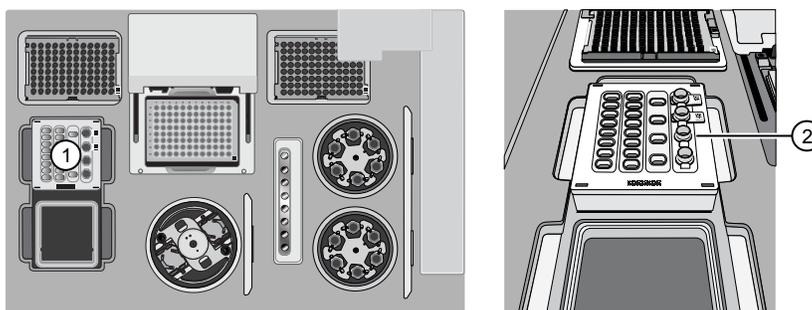
① Reagents station (4°C)
② Ion PGM™ Hi-Q™ Chef Reagents cartridge

3. Uncap, then load the two Library Sample Tubes, each containing 25 μL of diluted library, into Positions A and B on the Reagents cartridge.
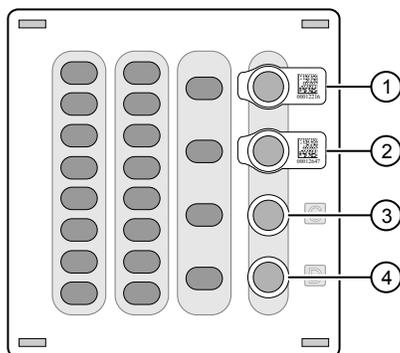
① Position A (Library)
② Position B (Library)
③ Position C (NaOH)
④ Position D (Empty tube)

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.

4. 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.

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

6. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.
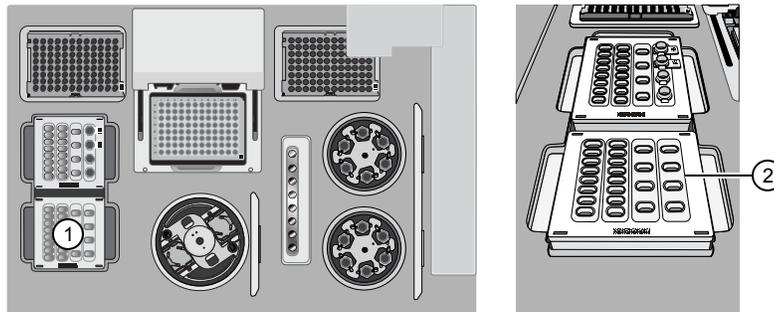
① Solutions station (room temperature)
② Ion PGM™ Hi-Q™ Chef Solutions cartridge

Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes (v2) into each Recovery centrifuge.
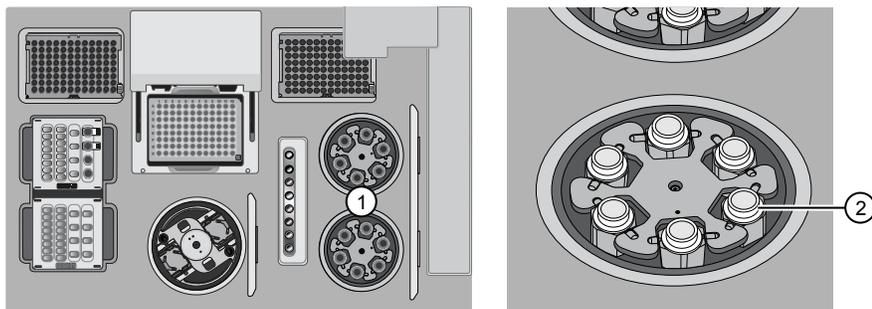
① Recovery centrifuges
② Recovery Tube

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 with the port oriented toward the rear as shown in the figure below, then press down. Make certain 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.
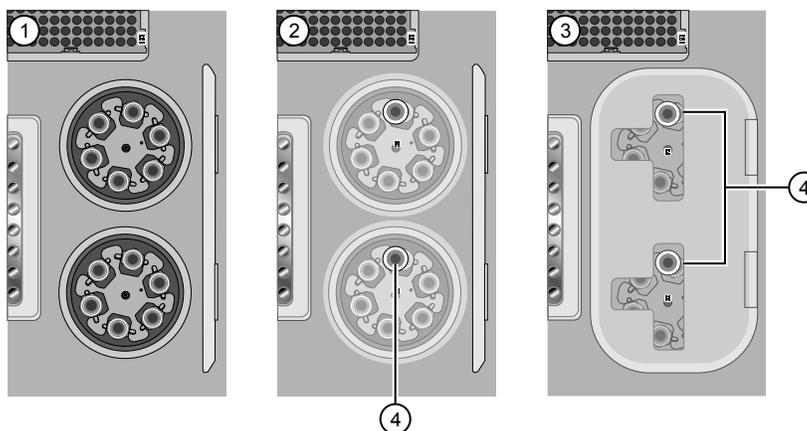
① Recovery Tubes installed
② Recovery Station Disposable Lids installed
③ Recovery centrifuge cover closed
④ Port

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.
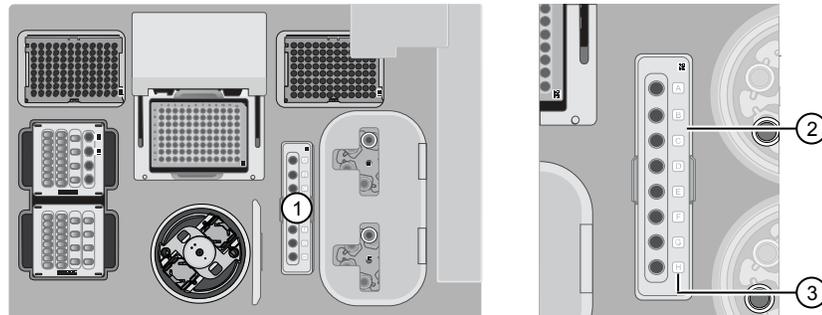
① Enrichment station
② Enrichment Cartridge v2
③ Lettering

Load the Chip-loading centrifuge

1. Attach a Chip Adapter v2 to each chip.

IMPORTANT! When attaching a Chip Adapter v2:

- Align the wells of the Chip Adapter v2 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 v2 is attached. Loading can fail if the adapter is not attached securely.
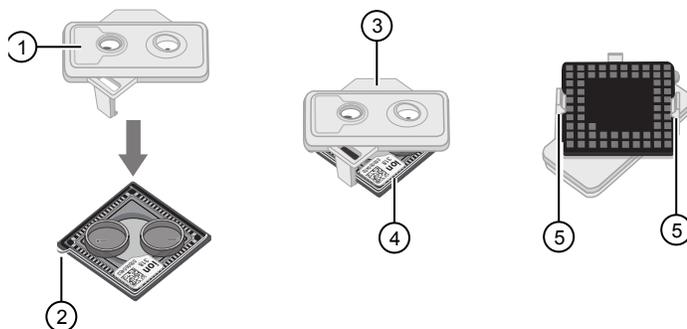
① Reservoir
② Tab
③ Chip Adapter
④ Ion chip
⑤ Clip

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.
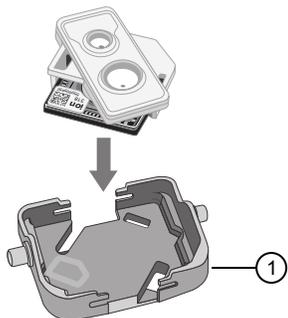
① Chip-loading centrifuge bucket

3. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.
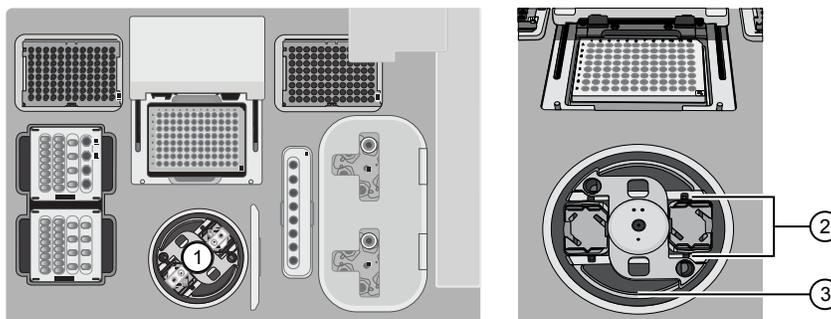
① Chip-loading centrifuge
② Mounting grooves
③ Chip-loading centrifuge

IMPORTANT! When loading the coupled chips, confirm that:

- The Chip Adapter v2 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.
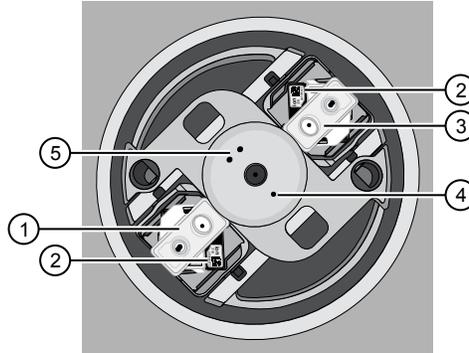
① Chip position 1
② Chip barcode
③ Chip position 2
④ Position 1 marker hole
⑤ Position 2 marker holes

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 on the Ion PGM™ Hi-Q™ Chef Reagents cartridge 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.
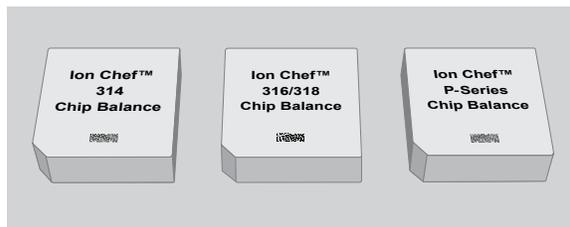
Ion Chef™ Chip Balance Pack

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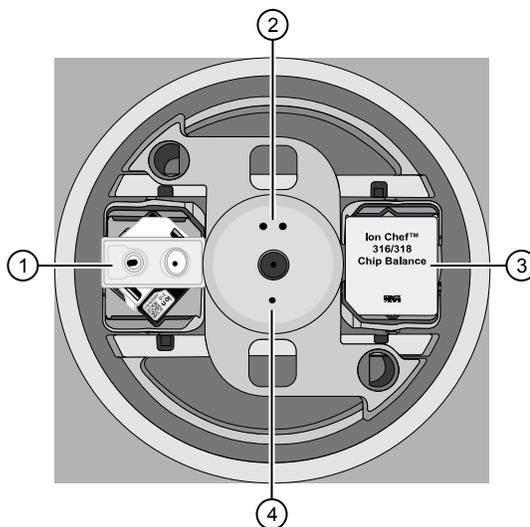
① Position 1 (Chip)
② Position 2 marker hole
③ Position 2 (Chip Balance)
④ Position 1 marker hole

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 35 at step 4. The Ion Chef™ Instrument detects the presence of the single chip during Deck Scan before the run starts.

Ion Chef™ pre-run checklist
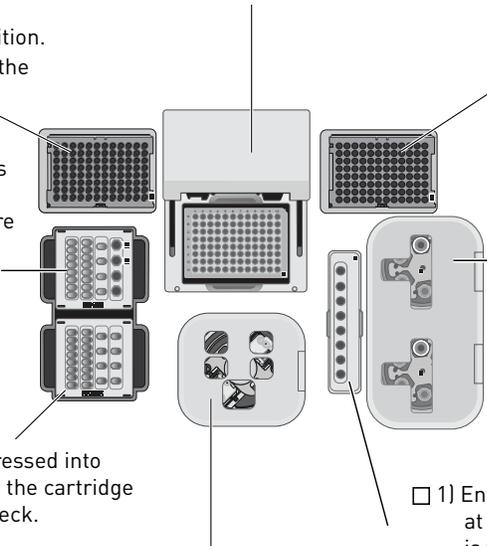
☐ 1) A new PCR Plate is loaded in the thermal cycler sample block.
☐ 2) A Frame Seal v2 is in place and oriented under the heated cover.

☐ 1) A new Hi-Q™ Chef Tip Cartridge v2 is loaded in the New Pipette Tip position.
☐ 2) Bracket is pulled downward to lock the Tip Cartridge in place.

☐ 1) The tip rack from previous run is transferred from the New Pipette Tip position to the Used Pipette Tip position.

☐ 1) Hi-Q™ Chef Reagents cartridge contents are thawed (leave at RT for 45 minutes).
☐ 2) Hi-Q™ Chef Reagents cartridge tubes are uncapped and pressed into place at the Reagents station so that the tubes are **firmly seated** and **level** with the deck.
☐ 3) Library Sample tubes are **firmly seated** and in the correct orientation with barcodes facing the PCR Plate and caps removed.

☐ 1) Recovery Tubes (v2) are securely seated in the Recovery centrifuge buckets.
☐ 2) Recovery centrifuges are load-balanced.
☐ 3) Recovery Station Disposable Lids (v2) are positioned so that the port is correctly oriented: port should not be located in the position facing the front of the instrument.

☐ 1) Hi-Q™ Chef Solutions cartridge is pressed into place at the Solutions station so that the cartridge is **firmly seated** and **level** with the deck.

☐ 1) Enrichment Cartridge v2 is pressed into place at the Enrichment station so that the cartridge is **firmly seated** and **level** with the deck.
☐ 2) Lettering on the cartridge is right-side up and positioned to the right of the enrichment tubes.

☐ 1) Chip Adapters (v2) are firmly attached to the Ion Chips.
☐ 2) Adapter/chip assemblies are correctly seated in the Chip-loading centrifuge buckets, with the adaptor clips inserted into the bucket slots.
☐ 3) Chip tab is facing outward
☐ 4) Centrifuge buckets containing the adapter/chip assemblies are securely seated in the centrifuge rotor, and freely pivot 90º outwards.
☐ 5) Chip-loading centrifuge is load-balanced.

Start the Ion Chef™ run

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

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

3. Touch **Step by Step** to have the instrument lead you through the instrument setup, or touch **Quick Start** to skip the instrument setup screens.
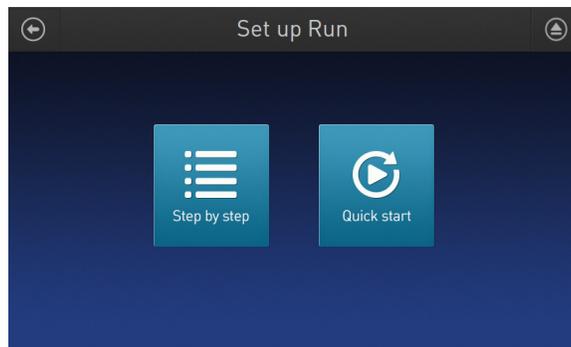
4. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage. After the door closes, the instrument vision system activates.

   IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.
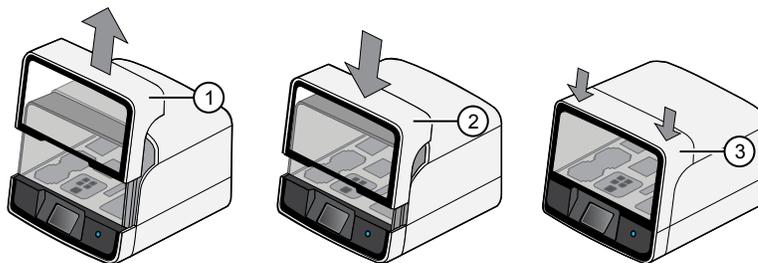
① Lift door first
② Lower
③ Press down to lock

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

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

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

6. When Deck Scan is complete, touch **Next** to display the Data Destination screen.
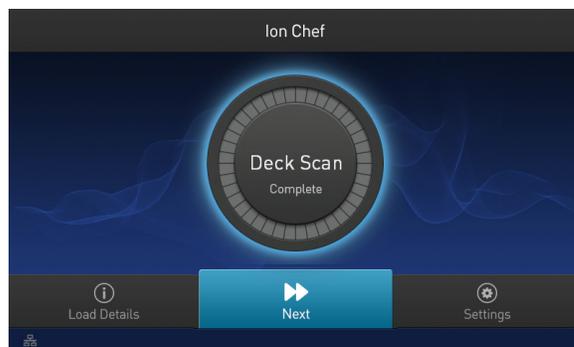
7. Confirm that the instrument displays the correct kit name, chip types, chip barcodes, and Planned Runs. If the correct Planned Runs do not display, touch the dropdown menu ⌄ to select the Planned Run for each chip, then touch **Next.**
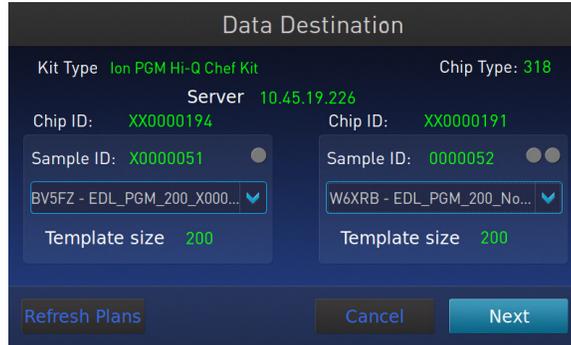
IMPORTANT! If the kit name and chip type are not correct, ensure that you are using the correct kit and chip. If you are using the correct kit and chip, and an incorrect kit or chip type is displayed on the screen, contact Technical Support.

Note: If the Ion Chef™ Instrument is not connected to a Torrent Server, a warning screen appears after touching **Next**:
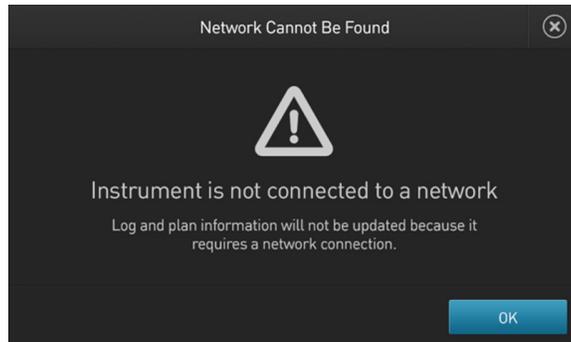
See Appendix C, "Ion Chef™ and Torrent Server network setup" to troubleshoot your network connection. Touch the close symbol ⊗ in the upper right corner to return to the Data Destination screen. To bypass a Torrent Server or network connection and proceed with the run, touch **OK**, then select the template size for your run on the next screen:
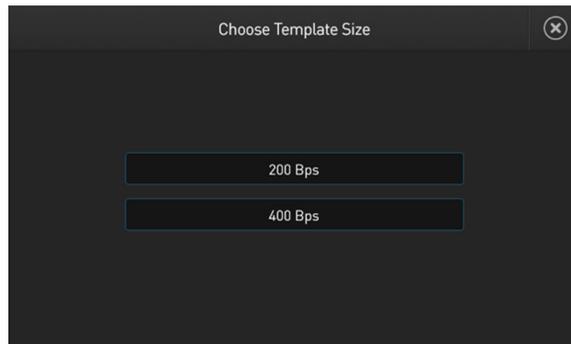
After you make your selection, you advance to the Run Options screen.

8. On the Run Options screen, touch the appropriate option to complete the run, then enter the desired time of run completion, if needed.
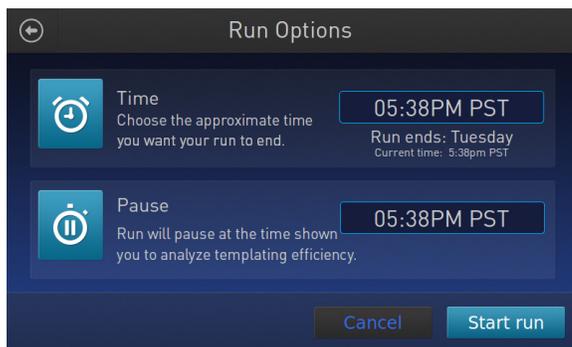
The Ion Chef™ Instrument provides two options for obtaining quality control (QC) samples that can be used to evaluate templating efficiency. Depending on your selection, the QC samples will be made available either during or after the run. In either case, you can obtain unenriched samples from the corresponding Library Sample Tubes at Positions A and B on the Ion PGM™ Hi-Q™ Chef 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 (run length is about 10.5 hours for 200-base-read sequencing and about 14 hours for 400-base-read sequencing).
Pause	when the instrument pauses operation before the chip loading step (9.5 hours into the run for 200-base-read sequencing and about 13 hours into the run for 400-base-read sequencing).

Note: The DNA library in the Library Sample Tube loaded in Position A is templated onto ISPs that can be sampled in Position E of the 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.

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

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

If the Ion Chef™ Instrument encounters a problem during the run, it aborts the run and display 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. If you will use a new Wash 2 Bottle to initialize the Ion PGM™ System, condition the bottle soon after you start the run (see "Condition the Wash 2 Bottle for first use" on page 47).

    Wash 2 Bottles must be conditioned with Wash 2 Bottle Conditioning Solution before using them for the first time. If filled immediately after you start the run, the Wash 2 Bottle will be conditioned and ready for use just in time to perform the initialization.

11. Clean and 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.

12. 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™ Instrument (approximately 9.5 hours and 13 hours for 200- and 400-bp runs, respectively, after the start of the run).

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

    Process Paused

    Run Paused. Please collect QC sample. Close door and press resume when ready.

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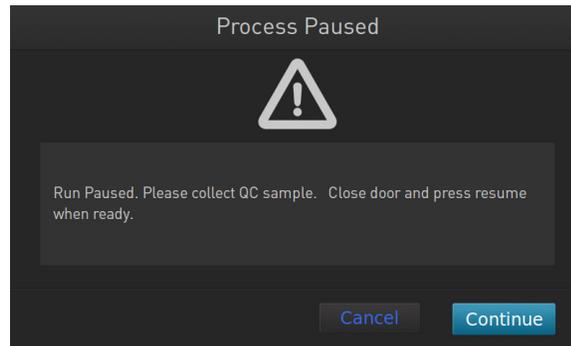
b. Transfer the QC samples (entire volume) from Positions A and B of the Ion PGM™ Hi-Q™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.

> **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.
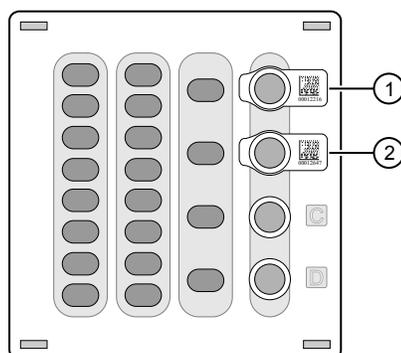
① Position A (QC sample)
② Position B (QC sample)

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

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

> **Note:** 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 "Quality control using the Guava™ easyCyte™ 5 Flow Cytometer" or "Quality control using the Attune™ Cytometer" on page 85.

4 Clean and initialize the Ion PGM™ Sequencer

At least one hour before the completion of the Ion Chef™ Instrument run, clean and initialize the Ion PGM™ Sequencer.

IMPORTANT! Use only the specified materials and follow the protocols found in this document. The Ion Chef™ Instrument 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.

If you begin conditioning a Wash 2 Bottle immediately after starting an Ion Chef™ System run, the bottle will be ready to use in time for sequencer initialization.

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

Materials required

- 18 MΩ water (e.g., the ELGA™ PURELAB™ Flex Water Purification System)
- Cleaning bottles and collection trays (provided with the Ion PGM™ System)
- Old chip that has been used for sequencing, marked for cleaning
- Used sipper tubes (from the previous run)
- Squirt bottle
- **Chlorite cleaning:** Ion PGM™ Cleaning Tablet (provided in the kit)
- **Chlorite cleaning:** 1 M NaOH, diluted fresh each week from 10 M NaOH
- **Chlorite cleaning:** Glass bottle (1 L)
- **Chlorite cleaning:** 0.22-μm or 0.45-μm vacuum filtration system and filters

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.

Materials required

Materials provided in the kits

- Ion PGM™ Sequencing Supplies (Part No. A25587)
    – Wash Bottle Sipper Tubes
    – Reagent Bottle Sipper Tubes
    – Non-irradiated PP Reagent Bottles
    – Wash 1 Bottle
    – Wash 3 Bottle
- Ion PGM™ Wash 2 Bottle Kit (Cat. No. A25591)
    – Wash 2 Bottle
    – Wash 2 Bottle Conditioning Solution
- Ion PGM™ Hi-Q™ Sequencing dNTPs (Part No. A25590)
    – Ion PGM™ Hi-Q™ Sequencing dGTP
    – Ion PGM™ Hi-Q™ Sequencing dCTP
    – Ion PGM™ Hi-Q™ Sequencing dATP
    – Ion PGM™ Hi-Q™ Sequencing dTTP
- Ion PGM™ Hi-Q™ Sequencing Solutions (Part No. A25589)
    – Ion PGM™ Hi-Q™ Sequencing W2 Solution
    – Ion PGM™ Sequencing W3 Solution

Other materials and equipment

- Used chip (leave chip on the instrument during initialization)
- 18 MΩ water
- 100 mM NaOH (prepared daily)
- Ice
- 5-mL and 25-mL pipettes
- Filtered and unfiltered pipette tips and pipettes
- Vortex mixer
- Microcentrifuge

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

1. Rinse the Wash 2 Bottle (2 L) 3 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.
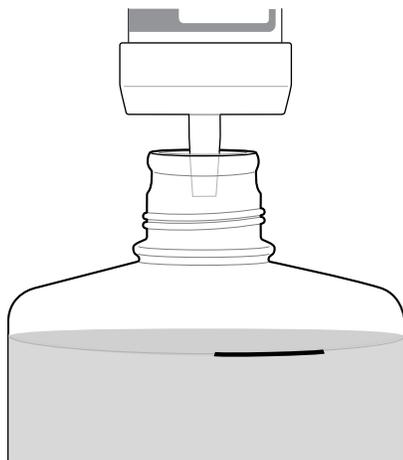
Note: If your water system has a digital display, ensure it reads "18 MΩ" throughout filling the bottle. If not, see Appendix A, "Troubleshooting".

4. Fill the bottle to the mold line with 18 MΩ water. The volume of water is ~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 Ion PGM™ Hi-Q™ Sequencing W2 Solution.

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

Note: Keep the Ion PGM™ Hi-Q™ Sequencing W2 Solution 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 can require adding different volumes of 100 mM NaOH. Some sites, for example, can require doubling the volume to 140 μL. See "Error message: Added too much W1 to W2" on page 80 for information on determining the volume of 100 mM NaOH to add.

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

   IMPORTANT! Do not store the mixed Wash 2 Bottle.

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 to the 50-mL line marked on the Wash 3 Bottle, then cap the bottle.

Begin the 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 dNTPs** from the dropdown list.

   • In the same screen, if you routinely experience clogging during initialization, select the **Line Clear** checkbox to clear any blockage in the fluid lines before initialization. This is optional.
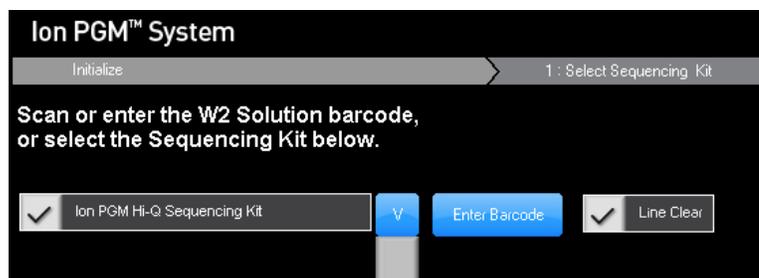
IMPORTANT! Be careful to select the correct kit, to ensure proper pH adjustment.

After you press **Next**, the system will check 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.
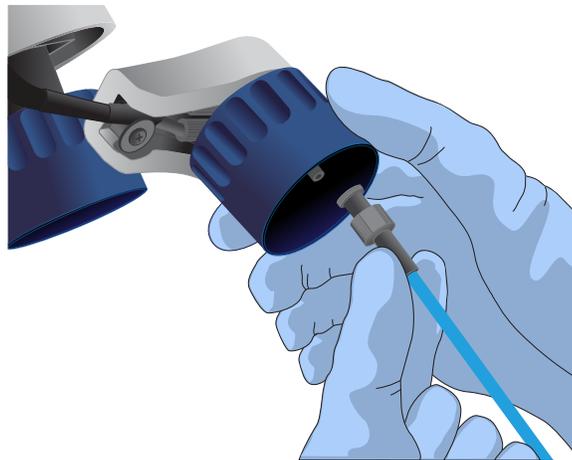
5. Immediately attach the prepared Wash 2 Bottle in the W2 position, then tighten the cap. Press **Next**.

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 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 begins 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 proceed to "Initialization—Auto pH errors" on page 76.
   - During the process, you can start preparing 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 the 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**.
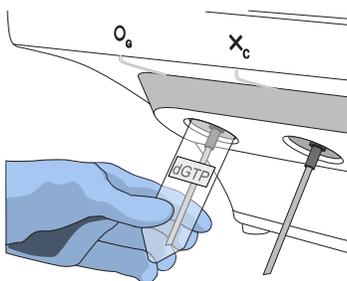
   Note: The instrument checks the pressure of the Reagent Bottles and Wash Bottles. If a bottle leaks, check that it is tightly attached to the instrument. If it continues to leak, replace it. If the instrument still does not pass the leak check, contact Technical Support.

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

Unload and prepare 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.
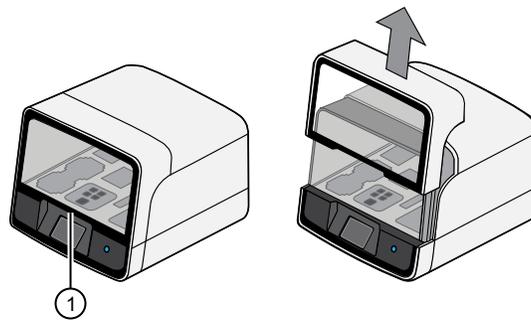
   ① Hold here, then lift

2. Open the lid of the Chip-loading centrifuge, then gently remove the chip/adapter assemblies from the instrument.

   IMPORTANT! When removing each chip, be careful not to disturb the remaining liquid in the outer port of the Chip Adapter.
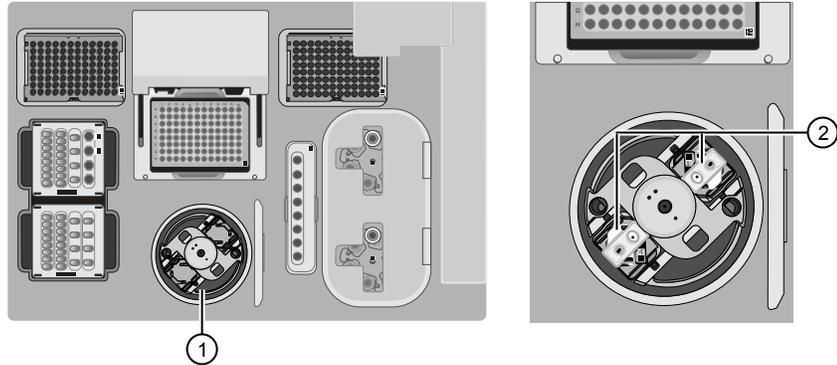
① Chip-loading centrifuge
② Remove chip/adapter assemblies

3. Remove the Chip Adapters from the chips, then discard the Chip Adapters. To remove an adapter, hold the chip with the tab facing downward, then remove the Chip Adapters starting with the top clip first.
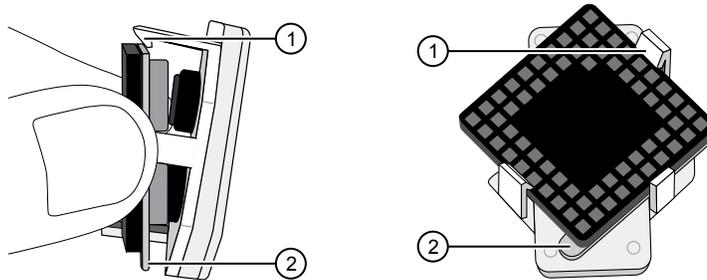
① Upper clip (remove first)
② Tab (orient down)

4. 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 Ion chips are centered in the buckets to ensure that the centrifuge is balanced.
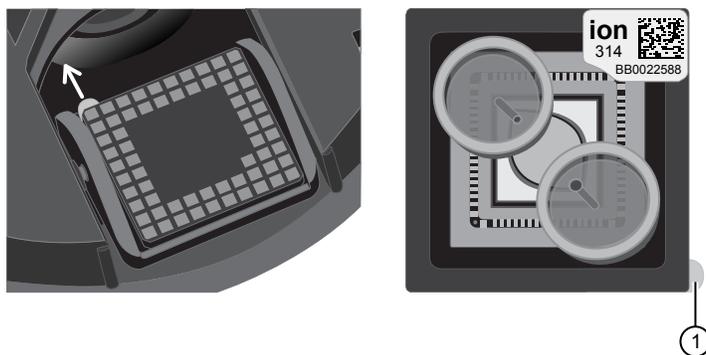
   ① Tab (orient inward)

   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.

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

   IMPORTANT! Do not close the door by pulling it straight down from the open position. You must lift the door slightly before you can close it. Ensure that both sides of the door are locked after closing it.
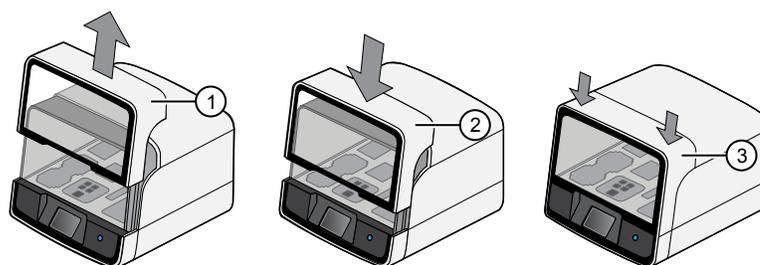
   ① Lift door first
   ② Lower
   ③ Press down to lock

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

   Note: If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container *after* centrifugation and store at 4°C. For users sequencing 200 base-read libraries (500 flows or fewer), sequence the second chip within 8 hours after completion of the Ion Chef™ run for optimal performance. For users sequencing 400 base-read libraries, the chip can be stored for up to 24 hours, but we recommend that chips be sequenced as soon as possible for optimal performance.

   IMPORTANT! If you chose to store a loaded chip, remove the chip from the container and place it on a clean surface in the dark to warm to room temperature at least 20 minutes before running it.

Sequence the Ion chips on the Ion PGM™ Sequencer

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.

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** to select the instrument used to prepare the sample and initiate the Chef sequencing workflow. Then touch **Next**.
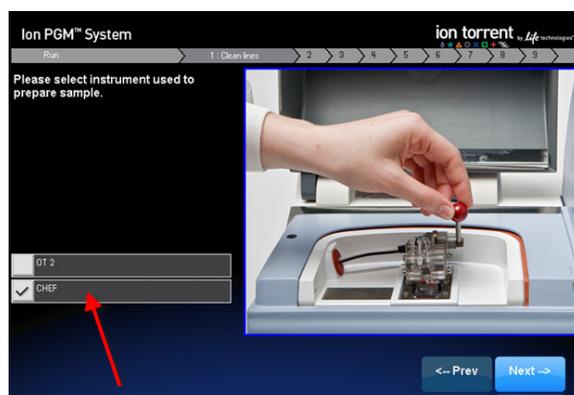
Note: If you use only the Ion Chef™ Instrument for sample preparation, you can preselect the Chef option:

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**, the touch **OK**. The instrument now automatically selects the Chef option.
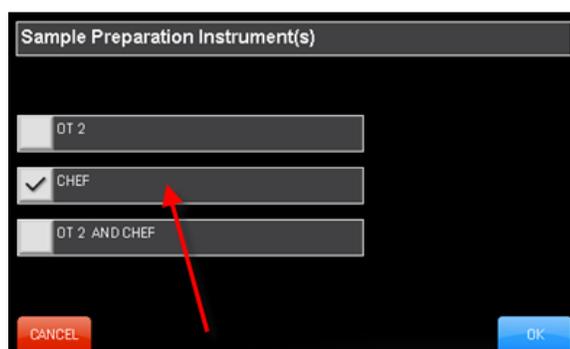
3. Scan the barcode on the loaded chip, or press **Change** to enter the barcode manually.
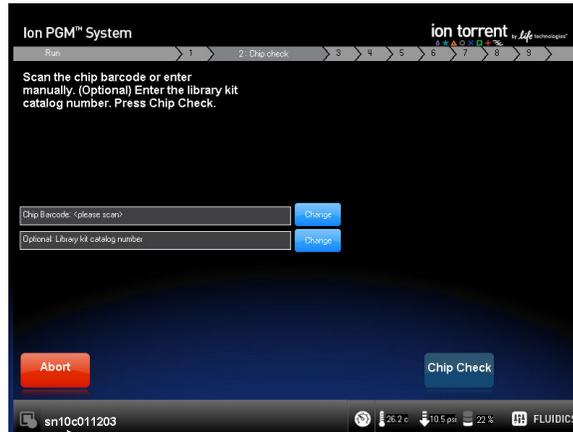
4. When prompted by the instrument, ground yourself by touching the grounding plate next to the chip clamp on the instrument, replace the cleaning chip in the chip socket with the chip to be sequenced, close the chip clamp, then touch **Next**.

   IMPORTANT! Do not wear gloves when transferring the chips on and off the instrument.
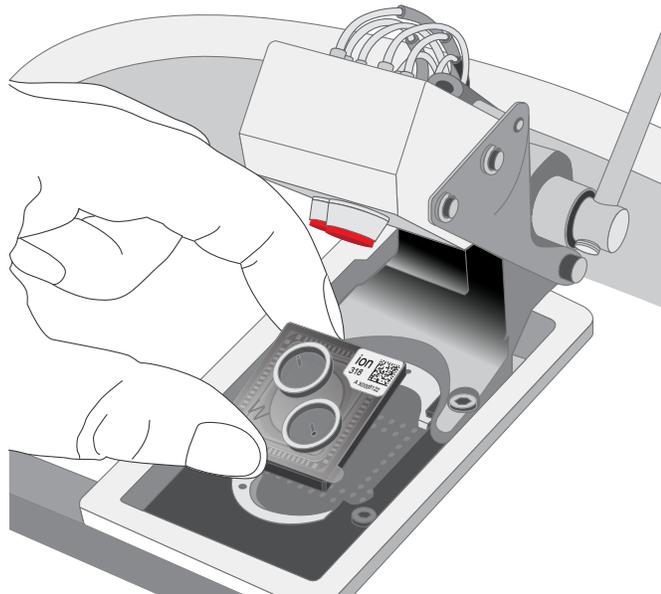
5. Touch **Chip Check** to perform the first chip check.

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

   IMPORTANT! Ion PGM™ Sequencer—Blue and 400-base-read sequencing require use of the Ion PGM™ 2.5-L Waste Bottle. Be sure to empty the waste container completely and return it before each run.

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 dropdown 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 performs 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, because this can reduce the quality of the measurements.

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9. Twenty minutes before the end of the first run, remove the remaining Ion chip from the chip container in the refrigerator, then 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 needed.

6 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.
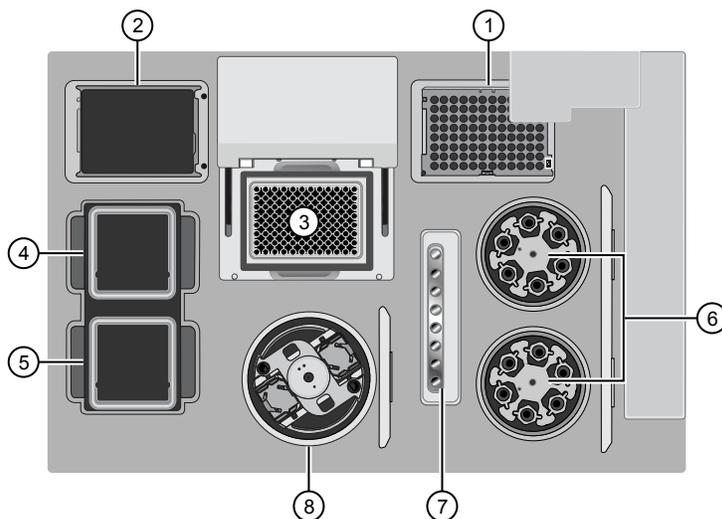
Ion Chef™ Instrument stations
- ① Used pipette tip station
- ② Empty Tip Cartridge v2: move to Used pipette tip station
- ③ Thermal cycler sample block
- ④ Reagents station
- ⑤ Solutions station
- ⑥ Recovery centrifuges
- ⑦ Enrichment station
- ⑧ Chip-loading centrifuge

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.
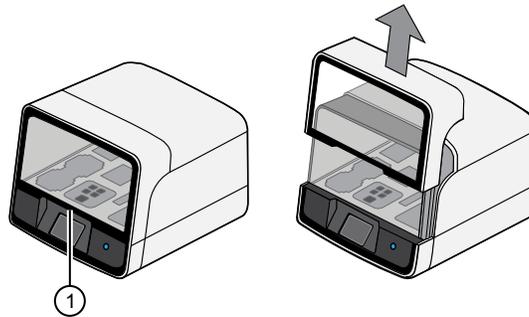
① Hold here, then lift

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 69.
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.
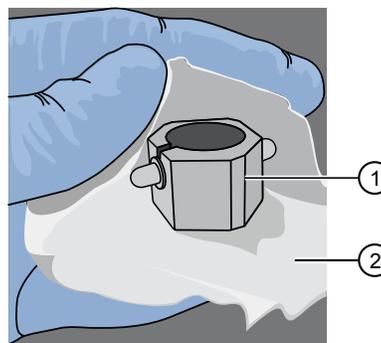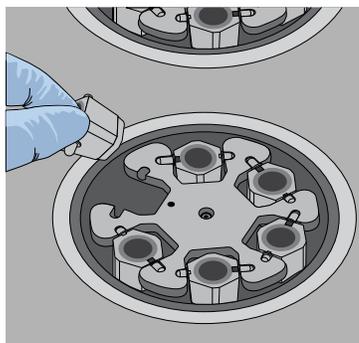
① Bucket
② Lint-free wipe

3. Use lint-free wipes to remove all fluid from inside the centrifuge bowl.
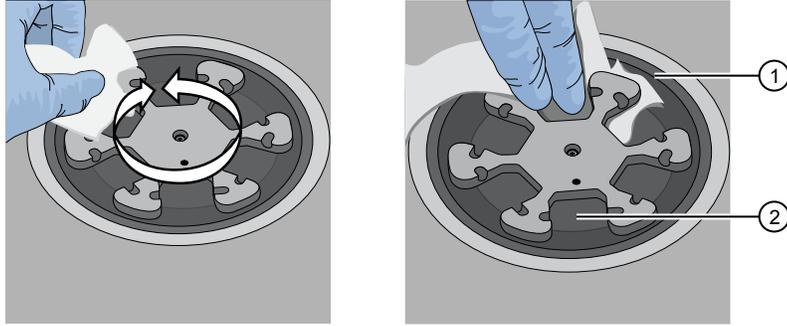
① Inside rim of the centrifuge
② Bottom of the centrifuge bowls

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.
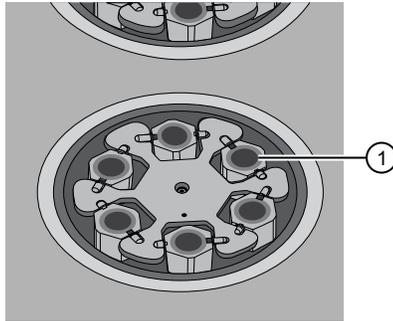
① Buckets (cleaned and installed)

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 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, 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.

Troubleshooting

Ion Chef™ System setup and operation

Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.
Instrument displays one or more alerts during a run	The instrument has detected one or more problems during the run.	After the instrument completes the run, contact Technical Support. **IMPORTANT!** The detected problem might impact the performance of the sequencing run.

	• Network connection to the server has been interrupted.
	• User name or password is incorrect.
		1. Touch the Instrument status button to view the alert(s).
		2. In the Instrument status screen, confirm that the name of the Torrent Server connection is red.
		3. Contact your network administrator to confirm that:
		   • The Torrent Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection.
		   • The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials.
		4. If the alert persists, contact Technical Support for further assistance.

Observation	Possible cause	Recommended action
Liquid residue is present in the Recovery centrifuge following a run	During normal instrument operation, a noticeable coating of liquid can collect on the bowl and buckets of the Recovery centrifuge following repeated runs.	Remove the residue as instructed in "Inspect and clean the Recovery centrifuges and buckets" on page 68.
Instrument will not begin a run	The instrument has encountered a Deck Scan error (one or more consumables are absent or loaded improperly).	1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the error(s) displayed, replace the missing consumable as directed, press **No** when prompted then press **Next** to cancel the run. After returning to the home screen, restart the run. 2. If the error persists, confirm that: • All buckets are seated correctly in the rotors of the Recovery and Chip-Loading centrifuges. • All cartridges are loaded correctly and are level on the instrument deck. • The barcodes of the Ion Chef™ Library Sample Tubes are visible and positioned correctly. • All tubes are both present *and* uncapped on the Ion PGM™ Hi-Q™ Chef Reagents cartridge (Library Sample Tubes, NaOH tube, and the empty tube). 3. If the error persists after you check the consumables on the instrument deck, do one of the following: • If you are confident that the Ion Chef™ Instrument is set up correctly and you are comfortable disregarding the warnings, touch **YES** following Deck Scan to proceed with the run. • If the instrument cannot begin the run, contact Technical Support for further assistance.
	The instrument has encountered an internal error.	1. Record the error displayed on the instrument display, then touch **OK**. 2. Contact Technical Support to report the problem and for further assistance.
Cannot open the instrument door	An obstruction is present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error has occurred.	Contact Technical Support to report the problem and for further assistance.

Observation	Possible cause	Recommended action
Instrument stops during a run	The instrument has encountered an internal error.	1. Record the error displayed on the instrument display, then touch **OK**. 2. Contact Technical Support to report the problem and for further assistance.

Ion PGM™ Sequencer chip check and calibration

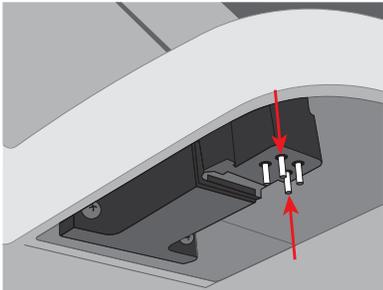| Observation	Possible cause	Recommended action
Chip Check fails	• Clamp is not closed. • Chip is not properly seated. • Debris is present on the chip socket. • Chip is damaged.	1. Open the chip clamp, remove the chip, and look for signs of water outside the flow cell: 2. If the chip appears damaged, replace it with a new one. 3. Look for debris on the chip socket. Remove any debris by rinsing with 18-MΩ water and gently dabbing the socket with a lab wipe tissue. **IMPORTANT!** Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail. 4. Close the clamp and repeat the Chip Check. 5. If the chip passes, click **Next**. If the chip fails, replace it with a new chip, scan the barcode of the new chip, then press **Chip Check**. 6. If Chip Check continues to fail, there could be a problem with the chip socket. Contact Technical Support.

Observation	Possible cause	Recommended action
Leak of unknown origin occurs	• Chip is leaking. • Chip clamp is not closed properly.	1. Press the **Abort** button. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. Do not rub or wipe the chip socket. 3. Rinse the socket with 18 MΩ water and gently absorb most of the water with the lab wipe tissue. 4. Repeat the rinse, then gently dab the chip socket until dry. 5. Place a lab wipe tissue on the grounding plate and dampen it with 18 MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts. 6. Remove the wipe, dry the grounding plate, and place the chip on the grounding plate. Check for condensation outside the flow cell: 7. If there is condensation or fluid, the chip is damaged and cannot be run. 8. If there is no condensation or fluid, press **Calibrate** to restart the calibration procedure. 9. If calibration passes and no leaks are visible, press **Next** to start the experiment. 10. If the chip leaks again, clean the chip and chip socket as described above. Continued leaking can indicate a chip clamp or socket problem. Contact Technical Support.
Error message: Calibration FAILED	• Chip is not seated in socket correctly. • Chip is damaged.	1. Remove the chip and check for leaks and/or debris on the chip socket, following the procedures described in "Chip Check fails" and/or "Leak of unknown origin," above. If no leaks or debris are visible, reseat the chip in the socket. 2. Press **Calibrate**. 3. If the chip passes, press **Next** to start the experiment. If the chip still fails, you can try reseating the chip multiple times and pressing **Calibrate**. If you are still unable to pass calibration, press **Next** to start the run anyhow-you may still get some data on your sample. 4. If you continue to have chip calibration issues, there may be an issue with the chip or chip socket. Contact Technical Support.

Ion PGM™ System Initialization: General errors

Observation	Possible cause	Recommended action
Error message: Confirm instrument has gas pressure	Gas cylinder may be turned off or empty.	1. Verify that the cylinder has at least 500 PSI and 30 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and the Ion PGM™ Sequencer are open. 2. Once you confirm gas pressure leading into the instrument, press **Yes** to retry verification of gas pressure. If the test continues to fail, contact Technical Support.
Bottle leak check fails	• Bottle seal is not tight. • Bottle may be damaged / defective.	1. Finger-tighten the bottles. 2. If the bottle continues to leak, replace the bottle. 3. If leak check continues to fail, contact Technical Support.

Initialization—Auto pH errors

Observation	Possible cause	Recommended action

Error message: Please insert a chip and press **Start**	Instrument cannot detect the chip in chip socket.	1. Open the chip clamp and remove the chip.
		2. Check for debris under the chip or in the chip socket. Remove any debris by rinsing with 18-MΩ water and gently dabbing the socket with a lab wipe tissue.
		
		   IMPORTANT! Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.
		
		3. Look for liquid outside the flow cell of the chip:
		
		4. If you see liquid, replace the chip with a new (unused) one. Wash the new chip once with 100% isopropanol and twice with SEQ Sample Buffer before using.
		
		   Note: The new chip can be used for sequencing after initialization completes.
		5. Close the clamp, then press **Start** to restart the process.
		6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support.

Error message: Chip calibration failed	• Chip is not seated in socket correctly. • Chip is damaged. • Sipper is loose.	Follow the procedure for "Error message: Please insert a chip and press Start." Follow the procedure for "Error message: Wash 2 average not stable."

Observation	Possible cause	Recommended action

Error message: The system did not reach the target W2 pH and/or has a clog	The waste lines can be clogged.	1. Press the **Troubleshoot** button.
		
		Note: You can skip the **Troubleshoot** button and change the chip to restart the Auto-pH routine.
		
		2. Remove the waste bottle.
		3. Place lab wipes under the waste arm.
		4. Gently wipe the waste arm with a lab wipe to clear liquid near the waste line.
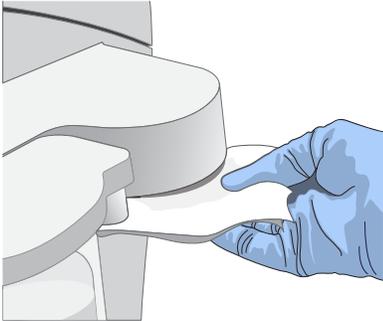		
		5. Press **Next** to start buffer flow. Observe flow rates from both waste lines. One line should drip slightly faster than the other. Following the flow rate check, one of 3 results is possible:
		
		  a. If flow rate appears normal, press **Cancel** and test another chip. If Auto pH failure persists, contact Technical Support.
		  b. If flow is blocked, press **Line Clear** to run the standard Line Clear procedure. If the line is unable to clear, contact Technical Support.
		  c. If the result of the flow rate check is uncertain, press **Re-flow** to re-flow the buffer and re-test the flow.

Observation	Possible cause	Recommended action
Error message: The system did not reach the target W2 pH (*continued*)	Wash 1 or Wash 2 sipper may be loose.	1. Loosen the Wash 1 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.) 2. Loosen the Wash 2 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 3. Press **Start** to re-start the auto-pH process.
	Forgot to add NaOH to the Wash 1 Bottle.	1. If there is no NaOH in the Wash 1 Bottle, loosen the cap and add 350 μL of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.) 2. Recap the bottle and shake gently to mix. 3. Press **Start** to restart auto-pH.
	Chip is damaged.	1. Replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. **Note:** The new chip can be used for sequencing after initialization completes. 2. If the error persists, there could be a problem with the chip clamp. Contact Technical Support.
Error message: Chip reading inconsistent. Please replace chip and try again	• pH response of the chip is not uniform or reliable. • Ran out of W3 Solution or volume too low.	1. Verify that there is enough W3 Solution (>25 mL) in the Wash 3 Bottle and that the sipper is secure. 2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the W3 Solution and is not a hazard.) 3. If there is enough W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. **Note:** The new chip can be used for sequencing after initialization completes.

Observation	Possible cause	Recommended action
Error message: W2 average not stable. Try reseating chip/replacing chip	Reading for W2 solution is not stabilizing quickly enough.	1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe. 2. Check for leaks and reseat the chip (see troubleshooting for "Chip Check" and "Chip calibration" above). Replace the chip with a new (unused) one if needed. 3. Loosen the cap in the W2 position and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful and not a hazard.) 4. After performing one or more above steps, press **Start** to re-start auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support and manually adjust the pH of the W2 Solution as described in "Manually adjust W2 pH" in the *Ion PGM™ System Reference Guide* (Pub. No. MAN0009783).
Error message: W2 out of range	• Chip measurements are very unstable. • Chip is damaged.	See troubleshooting tips for "W2 average not stable" above.
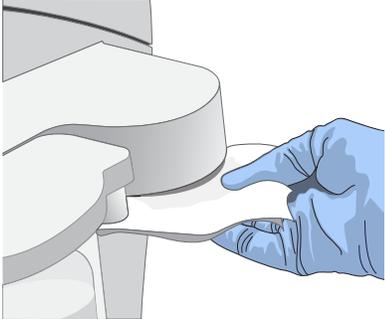
Observation	Possible cause	Recommended action
Error message: Added too much W1 to W2	• Water quality is poor. • 18 MΩ water was exposed to air for too long. • Incorrect solution was added to the Wash 2 Bottle. • Too little NaOH was added to Wash 1 Bottle. • Chip is damaged.	1. Check whether the water meets the 18 MΩ specification and 100 mM NaOH and W2 Solution were added correctly. 2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use high-quality water. Abort the initialization and restart using correct solutions/water. 3. If solutions are correct and water meets specifications, abort the initialization, return to the main menu, and proceed to the next steps. 4. Leave the Wash 2 Bottle on the instrument. 5. Remove the Wash 1 Bottle, leaving the sipper on the W1 port. Empty the bottle, and rinse the bottle twice with 18 MΩ water. 6. Add 350 µL of 100 mM NaOH to the Wash 1 Bottle and reinstall on the instrument. 7. Press **Initialize**, select the kit type, and keep pressing the **Next** button to skip all bottle prep steps until the instrument begins purging air from the bottle. Then proceed through the touchscreens as normal to complete the initialization. 8. The next time you initialize the instrument, add 140 µL of 100 mM NaOH to the Wash 2 Bottle instead of 70 µL. Continue to use this larger volume for subsequent initializations until you receive an "Overshot Target" error message at the first auto-pH iteration, at which point follow the troubleshooting steps in "Error message: The system overshot the target W2 pH." on page 81 on the following page and then return to adding 70 µL of 100 mM NaOH. 9. If you still receive the same initialization error ("Added too much W1 to W2"), contact Technical Support.
Error message: UNDERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH couldn't add enough Wash 1 to the Wash 2 before the maximum iterations, 10, occurred.	1. A blockage may have occurred. Follow the procedure for "Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again." 2. Press **Start** to re-start auto-pH. If you still get the "Undershot target pH" error, try replacing the chip with a new (unused) chip and restarting auto-pH. **Note:** The new chip can be used for sequencing after initialization completes.

Observation	Possible cause	Recommended action
Error message: The system overshot the target W2 pH.	Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed, and reports the pH value.	1. Press the **Overshoot** button to proceed with W2 pH adjustment. 2. Unscrew the cap of the Wash 2 Bottle. Without removing the sipper from the bottle, lift the cap high enough to pipette 15 µL of 100 mM HCl into the Wash 2 Bottle, close and tighten cap. 3. Press **Next** to re-pressurize the Wash 2 Bottle and mix the W2 solution. 4. Press **Start** to retry auto-pH.
W2 pH consistently undershoots target	pH of water is too low before any NaOH is added.	When preparing the Wash 2 Bottle, add more than the recommended 70 µL of 100 mM NaOH. After adding the NaOH, the Wash 2 Bottle should be in the range of pH 6.0–6.5 before you begin initialization.

Ion PGM™ System Initialization: Reagent pH verification

Observation	Possible cause	Recommended action
Red failure screen, reagent pH is displayed	One or more reagents are not within the target pH.	1. Press **Start** to repeat the pH measurements to confirm the measurement. 2. If any reagents still fail, try replacing the chip with a new (unused) chip and repeating. **Note:** The new chip can be used for sequencing after initialization completes. 3. If any reagents still fail, clean and re-initialize the instrument with fresh reagents and a new chip.
	A possible line clog exists which persisted through the Auto pH process.	1. From the **Tools** menu, perform a W1 line clear. 2. Press **Start** to repeat the pH check.
Red failure screen reagent pH *not* displayed	The chip did not calibrate.	1. Replace the chip with a new (unused) one. 2. Press **Start** to restart the pH measurement. 3. If the second test fails, contact Technical Support.

Ion Sphere™ Assay troubleshooting table

The following table contains troubleshooting information for unenriched ISPs tested with the Ion Sphere™ Assay on the Qubit™ 2.0 or Qubit™ 3.0 Fluorometer.

Qubit™ Fluorometer observation	Ion PGM™ System observation	Possible cause	Recommended action
<10% Templated ISPs	• Lower loading • Lower % enriched • Lower key signal • Lower throughput	Too little library input into template preparation	• Increase library input to target 20–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>30% Templated ISPs, but <70%	Increased number of filtered reads	Too much library input into template preparation	• Decrease library input to target 20–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>70% Templated ISPs	• Increased % primer dimer filtered reads • Lower throughput	Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries	• Check Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp). • Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.
	• Low loading • Low % enriched • Lower throughput • High % filtered reads	Ion Chef™ System under performance	Troubleshoot with Technical Support or a Field Application Scientist.

B

Supplemental procedures

Quality control of Ion PGM™ Hi-Q™ ISPs

Quality control using the Ion Sphere™ Assay on the Qubit™ Fluorometer

The Qubit™ 2.0 Fluorometer or the Qubit™ 3.0 Fluorometer can be used to perform a quality assessment of unenriched Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion PGM™ Sequencer. 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**.

Acceptance criteria for unenriched Ion PGM™ Hi-Q™ ISPs

The optimal amount of library corresponds to the library dilution point that gives Percent Templated ISPs between 10–30%.

Samples that fall within the recommended range generally produce the most data; however, samples that fall outside of the recommended range can still meet the throughput specifications on the Ion chips.

The recommended optimal range is not intended to be a pass/fail criteria. The range provides guidance for the quality of the sample.

Note: If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately. See the "Ion Sphere™ Assay troubleshooting table" on page 83 for more information.

Percent Templated ISPs	Description
<10%	Sample contains an insufficient number of templated ISPs to achieve optimal loading density on the Ion Chip.
10–30%	Optimal amount of library.
>30%	Sample will yield multi-templated ISPs (mixed reads).

Quality control using the Guava™ easyCyte™ 5 Flow Cytometer

The Guava™ easyCyte™ 5 Flow Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion PGM™ Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Ion PGM™ Hi-Q™ Chef Reagents Cartridge (Positions A and B).
- **Enriched samples** – Obtain sample 1 from Position E and sample 2 from Position A on the Enrichment Cartridge.

For details, see the *Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0015799), available at **thermofisher.com**.

Quality control using the Attune™ Cytometer

The Applied Biosystems™ Attune™ Acoustic Focusing Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion PGM™ Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Hi-Q™ Chef Reagents Cartridge (Positions A and B).
- **Enriched samples** – Obtain sample 1 from Position E and sample 2 from Position A on the Enrichment Cartridge.

For details, refer to the *Demonstrated Protocol: Ion Sphere™ Particles (ISPs) Quality Assessment using the Applied Biosystems™ Attune™ Acoustic Focusing Cytometer User Bulletin* (Pub. No. 4477181), available at **thermofisher.com**.

IMPORTANT! Thermo Fisher Scientific Demonstrated Protocols have been successfully verified by Thermo Fisher Scientific for research use. There are no technical specifications for Thermo Fisher Scientific Demonstrated Protocols. Users assume all risk when using these protocols, and recognize that support for Thermo Fisher Scientific Demonstrated Protocols occurs through community discussion.

Maintain the Ion Chef™ System

The Ion Chef™ System supports the user-performed maintenance procedures found in this section.

Install a firmware update

To ensure proper operation of the Ion Chef™ Instrument, we recommend periodically confirming that your instrument is running the most current firmware. We occasionally release updates to the instrument firmware, which can include important changes to the Ion Chef™ System operation. To ensure that your instrument is running the most current firmware, check the firmware version as explained below.

Check the Ion Chef™ Instrument firmware

1. On the Ion Chef™ Instrument touchscreen, touch **Settings**.

2. On the **Settings** screen, touch **Check for updates**.

3. On the **Software Update** screen, touch **Release** to search the server for updates to the Ion Chef™ Instrument firmware.

Note: Users are also notified of the availability of firmware updates on the Notifications screen.

4. Select the available component update, touch **Update**, then wait for the instrument to complete the update.

   Note: If the Ion Chef™ Instrument is operating on an isolated network and cannot connect to our website, you must transfer the firmware update manually. To perform a manual update, transfer the firmware files to a USB drive and insert the drive into the USB port at the rear of the instrument. Touch **USB** on the Software Update screen, select from the list of available software components, then touch **Update**.

   When finished, the Ion Chef™ Instrument displays the update status.

5. Power off and on the Ion Chef™ Instrument to complete the update.

   IMPORTANT! If you are updating the firmware manually, remove the USB drive before powering on the Ion Chef™ Instrument.
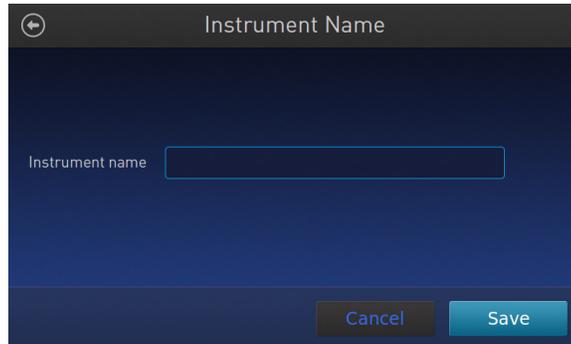
Change the instrument name

The following procedure describes how to change the name used to identify the Ion Chef™ Instrument both on the network and in the data that it generates.

1. In the Ion Chef™ Instrument home screen, touch **Settings**.

2. In the Settings screen, touch **Instrument settings**.

3. In the Instrument settings screen, touch **Set Instrument Name**. Touch the name field and enter a new instrument name using the keypad that appears, then touch **Save**.

   Note: Use only alphanumeric characters. Do not use special characters or spaces in the name.

4. In the next screen, touch **Save** again. Power off and on the Ion Chef™ Instrument to effect the name change.

Manually adjust W2 solution pH

If an error message during the automatic pH process during Ion PGM™ Sequencer initialization indicates that there is a problem adjusting the pH of the W2 Solution, see "Manually adjust W2 pH " in the *Ion PGM™ System Reference Guide* (Pub. No. MAN0009783, available at **http://thermofisher.com/ion-pgm-docs.html**) for a procedure to adjust W2 pH manually.

Ion Chef™ and Torrent Server network setup

Networking overview

If you are preparing a laboratory for use with new Ion instruments, refer to the respective site preparation and safety guides. If you currently have an Ion Chef™ instrument configured in your laboratory to communicate to more than one Torrent Server, Torrent Suite™ Software v4.4 or later requires the configuration of the Ion Chef™ instrument to communicate with one primary Torrent Server. After upgrading to Torrent Suite™ Software v4.4 or later, follow the guidelines in this document to configure the instrument to communicate with a primary Torrent Server. From that Torrent Server, the new Planned Run sharing option can be used to target additional Torrent Servers if needed.

Networking with the Torrent Server running Torrent Suite™ Assay Development Software and the Ion PGM™ Dx System requires v5.0 or later. The Ion Chef™ will not connect to the Ion PGM™ Dx in IVD mode.

If you need to connect a Torrent Server to the network for the first time, see the Torrent Server networking requirements in the site prep and safety guide for the respective sequencer.

This guide describes how to:

- Set up the Ion Chef™ instrument to talk to the Torrent Server on a local area network (LAN).
- Enable Planned Run sharing.

**About the
Ion Chef™
instrument**

The instrument is factory-configured for IPv4 TCP/IP communication and includes a fast Ethernet adapter (1000 Mbps) with a RJ45-type connector for integrating the device into a local area network (LAN). If the instrument will be connected to a LAN, an active network port must be available/assigned in place before the scheduled installation date.

If you are activating a newly installed LAN port for your Ion Chef™ instrument, please ensure the LAN has been configured to permit HTTP-443, SSH-22, and FTP-20/21 traffic. For additional information, please refer to the Networking Requirements section of the *Ion Chef™ Site Preparation Guide* (Pub. no. MAN0007956).

**About the Torrent
Server**

The Torrent Server T620/T430 is an integral part of the system and includes a quad-port gigabit NIC for direct communication with the network or instruments such as Ion Chef™, PGM™, Ion Proton™, Ion S5™, or Ion S5™ XL instruments. When the Torrent Server is connected to the network, Torrent Servers that are on the same software versions and subnet can transfer planned runs from one Torrent Server to another.

**Networking
guidelines and
best practices**

We recommend that you consult a network administrator before connecting the Ion Chef™ instrument to your laboratory network. Please refer to the networking requirements in the *Ion Chef™ System Site Preparation Guide*.

**Network setup
workflow**

<div align="center">

Collect the required network information.

▼

Connect the Ion Chef™ instrument to the configured LAN port.

▼

Configure the Ion Chef™ instrument with the required information.

▼

Monitor the Ion Chef™ instrument to test the network connection.

</div>

**Network setup
examples**

This guide provides a basic description of how to integrate the Torrent Server and the Ion Chef™ instruments into several possible network architectures. Because your network may contain advanced features, such as a firewall or network domains, we recommend that you consult a network administrator before connecting Ion Chef™instrument to your laboratory network.

Network examples include:

- Direct connection
- Static IP (indirect connection)
- DHCP IP (indirect connection)

**Collect the
required network
information**

Obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP IP or static IP).
- If the network requires static IP addresses, obtain the static IP address, subnet mask, gateway address, and DNS server name for the LAN port.
- For the Planned Run sharing and FTP functionality to be successful, the subnet must be the same on all Torrent Servers and Ion Chef™ instruments on the LAN.
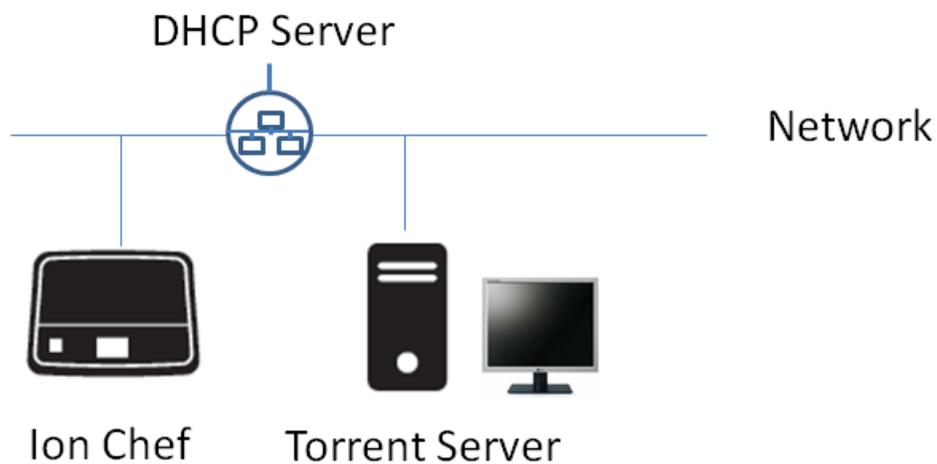
Materials required

An ethernet cable with RJ45 connectors (CAT6 Ethernet cable for a 1000Mbit/s network connection or a CAT5 for 100Mbit/s connection).

See *Ion Chef™ System Site Preparation Guide* (Pub. no. MAN0007956) for additional information.
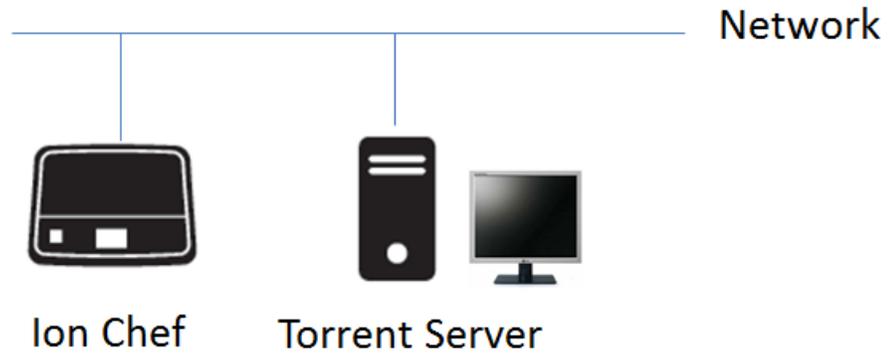
General requirements

See *Ion Chef™ System Site Preparation Guide* (Pub. no. MAN0007956).

DHCP IP

If using DHCP for the LAN port, please make sure the port is set to auto-populate when the Ion Chef™ instrument is connected.
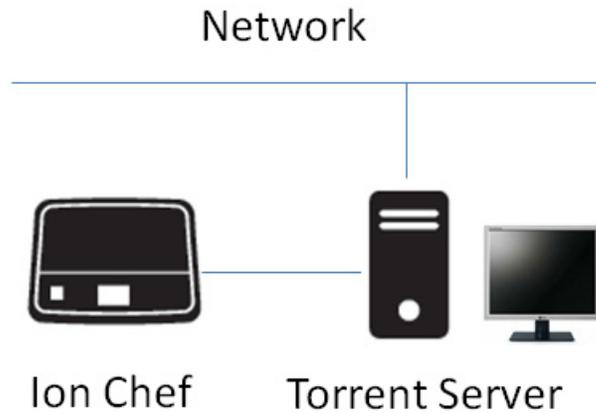
Static IP

If the network requires static IP addresses, obtain the static IP address, subnet mask, gateway address, and DNS server name for the LAN port.

For planned run sharing and FTP functionality to be successful, the subnet must be the same on all Torrent Servers and Ion Chef™ instruments on the LAN.

Direct connection

To configure the direct connection between an Ion Chef™ instrument and a Torrent Server, you will need a Category 6 cable of sufficient length.

Another direct connection configuration option consists of one Ion Chef™ instrument, one Torrent Server and two sequencers. However, this configuration is not recommended due to increased analysis times.

⚠ **WARNING!** The direct configuration shown below is known to result in lengthy analysis times.
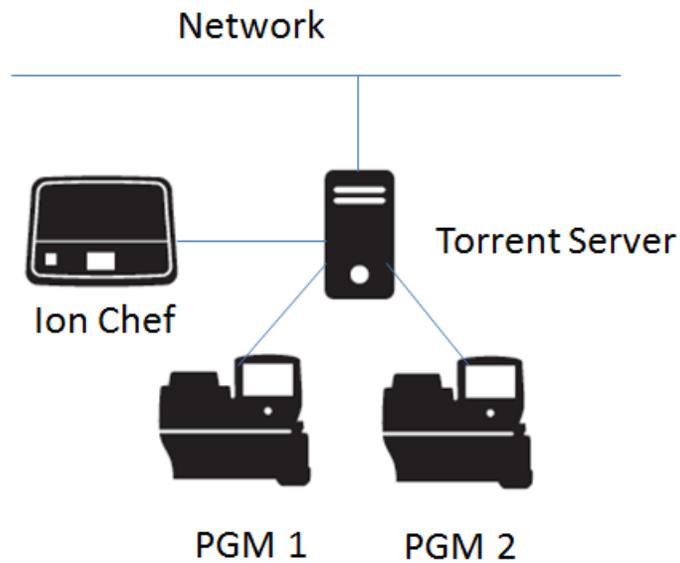
Ion Chef™ Networking Configuration Options

With the Torrent Suite™ software v4.4 release, the Ion Chef™ software will only connect to one Torrent Server. From that server, Planned Run sharing can be configured to access additional servers. When the Ion Chef™ is connected to a Torrent Server, an icon on the lower left side of the screen appears.


Connect the Ion Chef™ Instrument to the Torrent Server in one of three ways: Direct, Static IP (non-direct) or DHCP IP (non-direct). See respective instructions below.

Target the Torrent Server IP Address

To target the Torrent Server IP address from the Ion Chef™ user interface, you will first look up the Torrent Server IP address and then enter it into the Ion Chef™ user interface as the Torrent Browser target and the Update Target.

1. Log into Torrent Browser as the admin.

2. Click the Gear button and select **Configure**.

C
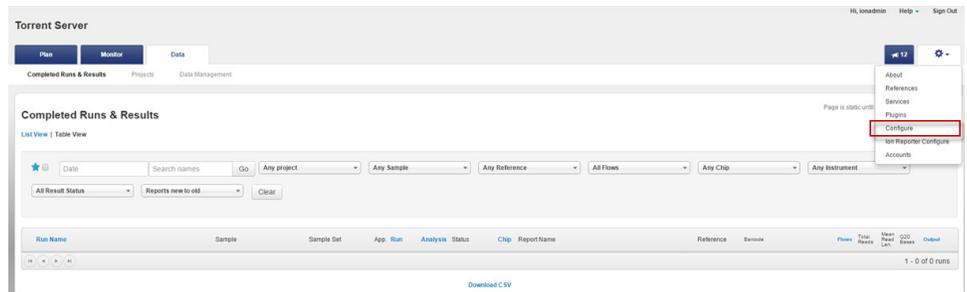
3. Scroll down to the Database Administration section and click the **Admin Interface** link.

4. The Site Administration screen appears. Scroll to the bottom and click **View Network Settings**.
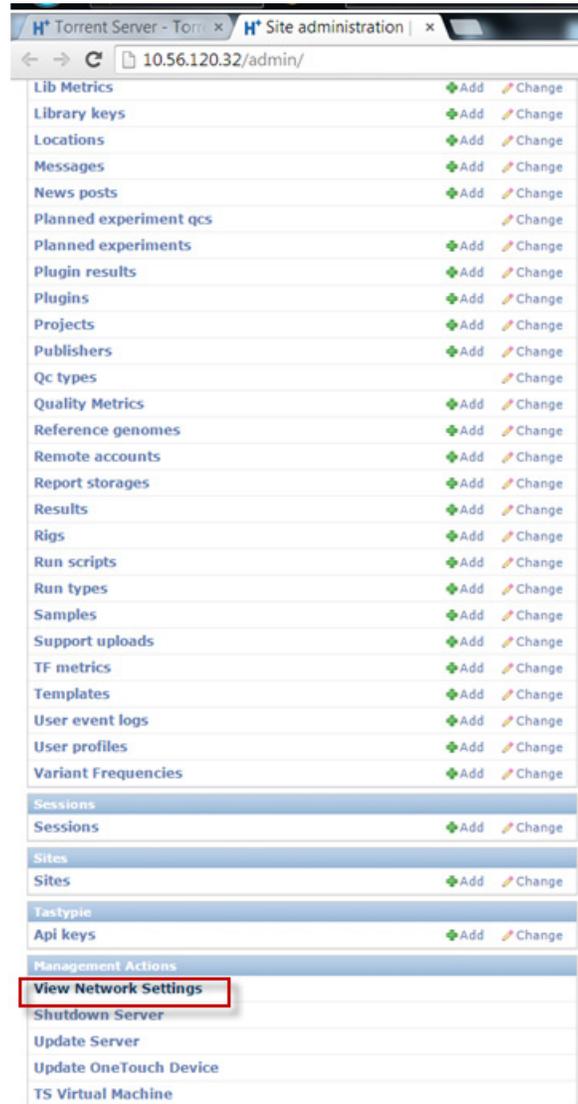
5. Note (copy or write down) the Torrent Server IP address displayed on the Network Settings screen.

6. Go back to the Ion Chef™ user interface and tap on the Torrent Browser IP address. This opens the Manage Connections window.

Appendix C Ion Chef™ and Torrent Server network setup
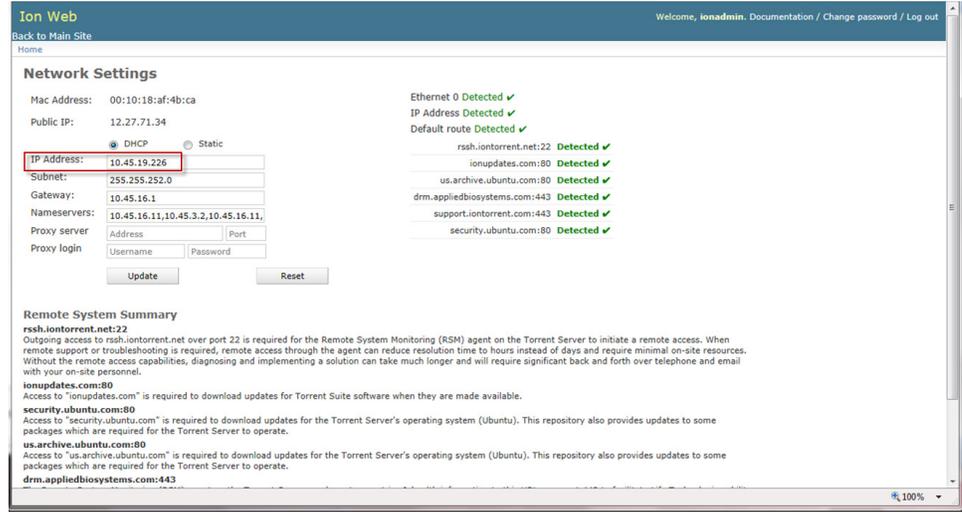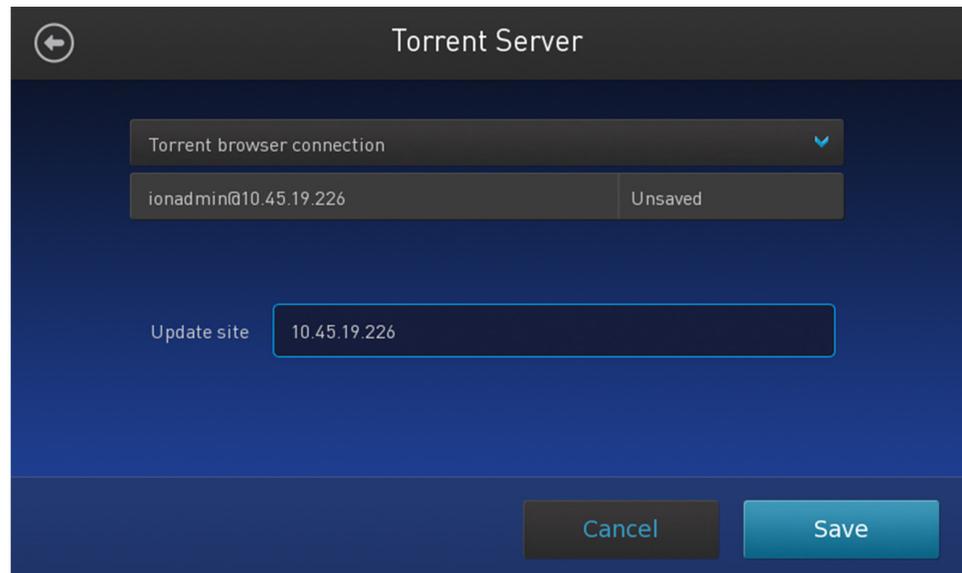*Editing the Torrent Server firewall tables to allow the LAN port IP*

C

7. Enter the server IP address under Server and repeat for Update Site. Click **Save**.

Editing the Torrent Server firewall tables to allow the LAN port IP

In addition, when connecting through a LAN, the Torrent Server firewall settings will need to be modified for both DHCP and Static LAN connections.

Note: While directly connected, instruments are on the 192.168.20x.x subnet from which all traffic is enabled, so this is not required.

Any exception would mean that editing the local firewall tables to include the LAN IP is required. Also, if the Ion Chef™ instrument is getting a DHCP address from your network, you will need to create an exception for the entire DHCP subnet.

Create an exception

1. On the Torrent Server, edit the /etc/iptables.custom file and add a firewall rule to allow all traffic from Ion Chef™ instrument's IP address as shown below. (This is a blank file initially and is intended for custom firewall configuration.)

   IMPORTANT! Only edit the /etc/iptables.custom file and not /etc/iptables.rules, since /etc/iptables.rules is overwritten when software is updated.

2. Enter "sudo^nano^/etc/iptables.custom" and add the three lines below:
   - `*filter`
   - `-A INPUT -s xxx.xxx.xxx.xxx -j ACCEPT`
   - `COMMIT`

Appendix C Ion Chef™ and Torrent Server network setup
Editing the Torrent Server firewall tables to allow the LAN port IP

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Note: `-A INPUT -s xxx.xxx.xxx.xxx -j ACCEPT` adds the rule to the format. Enter the IP address of the Ion Chef™ instrument in place of "`xxx.xxx.xxx.xxx`". When finished editing, save changes by pressing **CTRL + O** and **CTRL + X**.

```
*filter
A INPUT -s xxx.xxx.xxx.xxx -j ACCEPT
COMMIT
```

3. Follow this command by manually applying the change:

   `sudo^iptables-restore^--noflush^<^/etc/iptables.custom`

   Note: You can also list the firewall rules with: `sudo^iptables-L`.

   If this command returns with no output, it means it succeeded.

4. If the Ion Chef™ instrument is getting a DHCP address from your network, please create the exception for the entire DHCP subnet. The example below assumes that the DHCP address for the Ion Chef™ instrument is 172.16.48.0 and the DHCP subnet range is 172.16.48.1 to 172.16.48.254. Add these 3 lines:

   - `*filter`
   - `-A INPUT -s 172.16.48.0/24 -j ACCEPT`
   - `COMMIT`

```
*filter
-A INPUT -s 172.16.48.0/24 -j ACCEPT
COMMIT
```

5. Reboot the server.

6. If you want to repeat this process and completely drop all rules and start fresh, enter:

   - `sudo^iptables-F`
   - `sudo^iptables-restore^<^/etc/iptables.rules`
   - `sudo^iptables-restore--noflush^</^etc/iptables.custom`

   Removing a rule from either of these files would remove it from the active firewall rules, because the `iptables-restore` command does not overwrite rules; it appends them to all active rules.

Enable Planned Run sharing among Torrent Servers

New in Torrent Suite™ v4.4, Planned Runs created on one Torrent Server can now be transferred to another Torrent Server if the sequencer connected directly to the server is offline or busy. Possible configurations include:

Ion Chef™ instrument 1 → Torrent Server 1 ↔ Torrent Server 2 → Sequencer 2

Ion Chef™ instrument 1 → Torrent Server 1 ↔ Torrent Server 3 → Sequencer 3
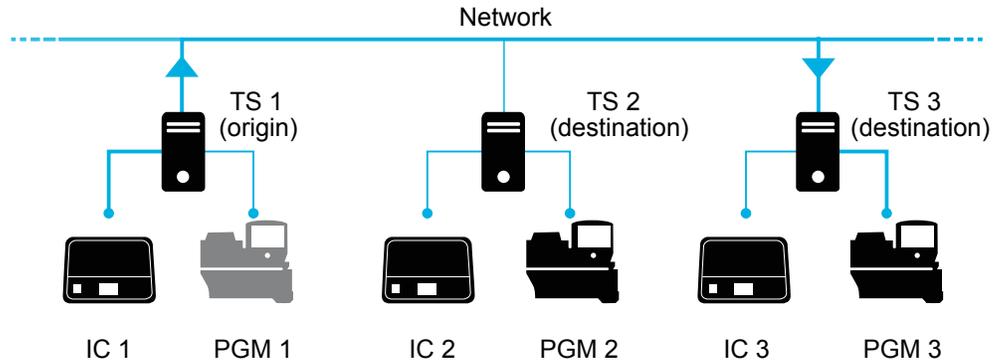
Requirements include:

- All Torrent Servers must be running the same software version.
- All Torrent Servers must have the same genomic reference, barcode set, BED files, Variant Caller config files, etc. All Torrent Servers must be on the same subnet.

Set up Server Network (admin action)

1. On the *origin* server (e.g., TS1) Site administration page, scroll down and select Shared servers. The Select shared server to change window appears.

Site administration

Auth		
Groups	✚Add	✎Change
Users	✚Add	✎Change

Rundb		
3' Adapters	✚Add	✎Change
Analysis Args	✚Add	✎Change
Analysis metrics	✚Add	✎Change
Appl products	✚Add	✎Change
Backup configs		✎Change
Backups		✎Change
Chips	✚Add	✎Change
Content uploads	✚Add	✎Change
Contents	✚Add	✎Change
Crunchers	✚Add	✎Change
DNA Barcodes	✚Add	✎Change
Dm file sets	✚Add	✎Change
Dm file stats	✚Add	✎Change
Shared servers	✚Add	✎Change
Support uploads	✚Add	✎Change
TF metrics	✚Add	✎Change
Templates	✚Add	✎Change
User event logs	✚Add	✎Change
User profiles	✚Add	✎Change
Variant Frequencies	✚Add	✎Change

Recent Actions

My Actions
- ✎test_plan
  Planned experiment
- ✖R_2013_04_02_16_12_55_DM...
  Experiment analysis settings
- ✖R_2013_04_02_16_12_55_DM...
  Experiment analysis settings
- ✖R_2013_04_11_10_32_06_Bar...
  Experiment analysis settings
- ✖NONE_ReportOnly_NONE/1193
  Experiment analysis settings
- ✎SharedServer object
  Shared server
- ✎SharedServer object
  Shared server
- ✎SharedServer object
  Shared server
- ✎SharedServer object
  Shared server
- ✎SharedServer object
  Shared server

2. If you are adding your *destination* server for the first time, click **Add shared server**.

Select shared server to change

Add shared server ✚

Action: --------- ▼ Go   0 of 2 selected

	Name	Address	Username	Active
☐	test server	6wvvpq1.ite	ionadmin	✓
☐	my local server	127.0.0.1	ionuser	✓

2 shared servers

3. Define your *destination* server.
   a. Enter the name, address (can be IP address), user name and password for the *destination* server.
   b. Click **Active** if you want this server enabled for sharing.
   c. *(Optional)* Add a comment.
   d. Click one of the **Save** options.

4. *(Optional)* If you want to configure the origin Torrent Server to also be a destination server, you must go to another server and repeat these steps to set the origin server as a destination server. Once the Torrent Servers are configured, you or a user can now transfer Planned Runs between Torrent Servers.

Transfer a Planned Run

You can execute a Planned Run on the Ion Chef™ instrument connected to the origin server, and then, when the run is complete, transfer the Planned Run to a destination server for sequencing.

Alternatively, you can create the Planned Run on an origin server and then transfer it to a destination server for both the Ion Chef™ run and the sequencing run.

1. Using the Torrent Browser on the *origin* Torrent Server, go to **Plan ▸ Planned Run List**.

2. Open the Gear ⚙ menu of the Planned Run you want to transfer, and select **Transfer**. Then select the *destination* Torrent Server.
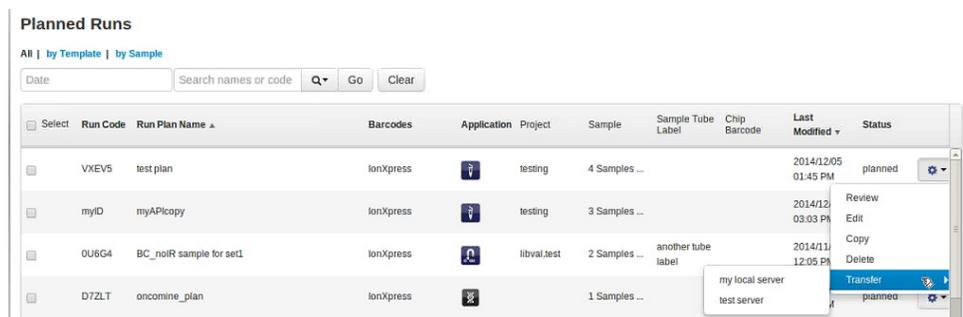
3. A confirmation window appears. Check the information, then click **Transfer**.

Note: You can no longer access this Planned Run on the origin server after it has transferred.
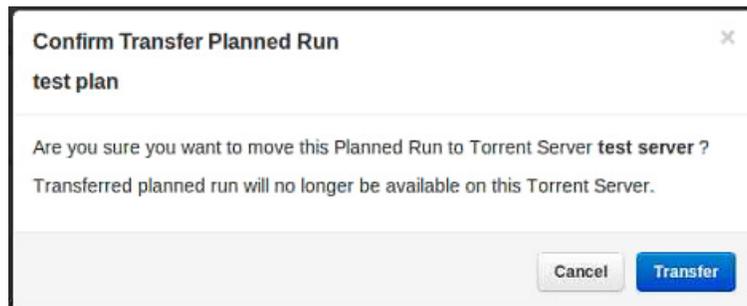
4. A status window displays the results of the transfer:

   - The green box lists the samples successfully processed and the required target BED files found on the destination server.

   - The red box lists any required BED files or plugins that are not present on the destination server. To successfully perform the run, you will need to edit the transferred Planned Run on the destination server and manually add the missing BED files or plugins.

---

test plan

Successfully created test plan on Torrent Server **test server**

....processed Samples: Sample 2, Sample 3, Sample 1

....found BED files: target.bed

....found IR account IonEast IR (Version: 4.2 | User: Ion User | Org: IR Org)

Planned run data is incomplete, please **Edit test plan** to fix the following errors

Unable to find bedfile: HSMv12.1_hotspots.bed for reference: hg19

Unable to find bedfile: atarget.bed for reference: hg19

Close

---

5. To edit the transferred Planned Run and add missing files:

   a. Download required files using the References tab of the Torrent browser of the destination server

   b. Go to the Edit Plan wizard of the transferred Planned Run by selecting Edit on the gear pull-down menu to the right of the Planned Run.

   c. Select the files or plugins as needed, then click **Update Plan**.

   Note: You can also navigate to the Edit Plan wizard by clicking the **Edit test plan** link in the status page above.

Undo a Planned Run transfer (administrator)

1. On the *destination* server, delete the transferred Planned Run from either the Planned Run page or the admin page.

2. On the *origin* server, locate the plan on the /admin/rundb/plannedexperiment/page, uncheck **PlanExecuted** and change **PlanStatus** to **Planned**.

Undo a Planned Run transfer (user)

If you transferred a Planned Run in error, you can transfer it back to the origin server or to another server.

1. On the destination Torrent Server, navigate to **Plan ▸ Planned Run List** and locate the transferred Planned Run.

2. From the Gear ⚙ menu of the Planned Run, select **Transfer**, then select the Torrent Server to which you wish to transfer the run.

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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.

Symbols on this instrument

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

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

- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

| Symbol	English	Français
⚠	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Moving parts	Parties mobiles
	Caution, hot surface	Attention, surface chaude
	Ultraviolet light	Rayonnement ultraviolet
⏻	On/Off	On/Off (marche/arrêt)
⏻	Standby	En attente
⏚	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
~	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif

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

Conformity symbols

The following table applies only to the Ion Chef™ Instrument.

Conformity mark	Description
(TÜV Rheinland logo)	Indicates conformity with safety requirements for Canada and U.S.A.
CE	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
C	Indicates conformity with Australian standards for electromagnetic compatibility.

Location of safety labels on this instrument
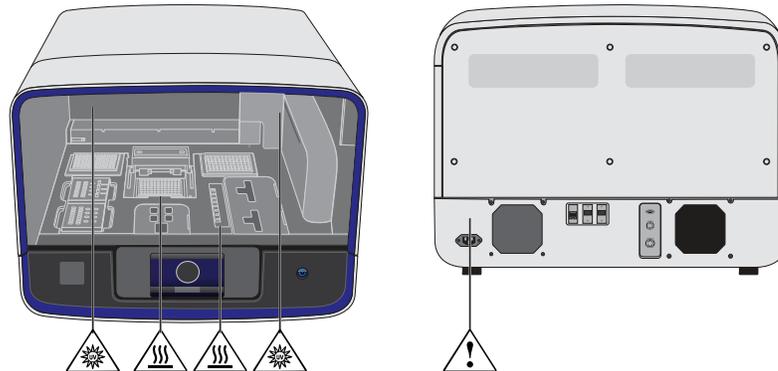
Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving and Lifting Injury. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:
- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.

- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Ultraviolet (UV) Safety

The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to ensure that the UV lamp is not powered when the door is open.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety compliance

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

EMC

Reference	Description
Directive 2004/108/EC	*European Union "EMC Directive"*
FCC Part 15	*U.S. Standard "Industrial, Scientific, and Medical Equipment"*
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

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

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.

⚠ **WARNING! HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

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

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/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**.