USER GUIDE

ion torrent
by Thermo Fisher Scientific

Ion PGM™ IC 200 Kit

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

Catalog Number 4484080
Publication Number MAN0007661
Revision C.0

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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.

Revision history

Revision	Date	Description
C.0	1 April 2015	• Updated for new Graphical User Interface in instrument firmware downloaded with Torrent Suite™ Software v4.4. • Updated for use of the Qubit® 3.0 Fluorometer for quality control of templated ISPs (see "Quality control using the Qubit® 3.0 Fluorometer" on page 98). • New Ion Chef™ and Torrent Server networking appendix added (see Appendix D, "Ion Chef™ and Torrent Server network setup").
B.0	23 September 2014	• Updated for use of the new universal chip-loading centrifuge bucket. • Updated with new library input recommendation. • Ion Chef™ pre-run checklist added (page 38). • Minor corrections and edits.
A.0	1 March 2014	Initial release, which includes instructions to set up and operate the Ion Chef™ System with the Ion PGM™ IC 200 Kit (Cat. no. 4484080).

Purpose

This user guide describes how to use the Ion Chef™ System to prepare enriched, template-positive Ion Sphere™ Particles (ISPs) for up to 200 base-read sequencing of libraries on the Ion PGM™ Sequencer. The Ion PGM™ IC 200 Kit (Cat. no. 4484080) 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 (Life Technologies).

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

1 Product information

About the kit and related products

About the Ion PGM™ IC 200 Kit

The Ion PGM™ IC 200 Kit (Cat. no. 4484080) includes the reagents and materials required to use the Ion Chef™ System to prepare template-positive Ion Sphere™ Particles for sequencing. The kit also includes reagents and materials for loading on Ion 314™ v2 BC, Ion 316™ v2 BC, or Ion 318™ v2 BC Chips for sequencing the prepared template using the Ion PGM™ Sequencer.

Library compatibility

The Ion PGM™ IC 200 Kit can be used with up to 200-base-read libraries of any type prepared using any available Ion library kit.

Software compatibility

The Ion PGM™ IC 200 Kit is compatible with Torrent Suite™ 4.4.2 and later. Be sure to update your Torrent Server to the latest available version of Torrent Suite™ before using this kit.

Note: If you are currently using version 4.2.1 of the Ion Reporter™ Software with an earlier version of the Torrent Suite™, contact Technical Support for assistance with upgrading to Torrent Suite™ 4.4.2.

Kit contents and storage

IMPORTANT! Do not mix components with components from any other Ion sequencing kits. We have 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™ IC 200 Kit (Cat. no. 4484080) contains all materials required for sequencing preparation in the boxes shown below. Upon arrival, inspect all consumables and contact Technical Support if any of the products have been damaged during shipping.

Ion PGM™ IC 200 Kit summary

Ion PGM™ IC 200 Kit (Cat. no. 4484080)		
Component	**Part number**	**Quantity per kit**
Ion PGM™ IC Supplies 200	4484083	4 boxes
Ion PGM™ IC Solutions 200	4484084	4 cartridges
Ion PGM™ IC Reagents 200	4484085	4 cartridges
Ion PGM™ Sequencing Supplies	4488376	1 box
Ion PGM™ IC Sequencing Reagents	4484271	1 box
Ion PGM™ IC Sequencing Solutions	4488375	1 box
W2 Bottle Conditioning Solution	A25043	1 bottle

Ion Chef™ reagents and materials

Ion PGM™ IC Supplies 200 (Part no. 4484083; 4 boxes supplied per kit)		
Component	**Quantity per box**	**Shipping and storage**
Chip Adapter	2 adapters	15° to 30°C
Enrichment Cartridge	1 cartridge	
Ion Chef™ IC 200 Tip Cartridge	1 cartridge	
PCR plate	1 plate	
PCR plate seal	1 plate seal	
Recovery Station Lid	2 lids	
Recovery Tube	12 tubes	
IC Piercing Tips	2 tips	

Ion PGM™ IC Solutions 200 (Part no. 4484084)		
Component	**Quantity**	**Shipping and storage**
Ion PGM™ IC Solutions 200 Cartridge	4 cartridges	15° to 30°C

Ion PGM™ IC Reagents 200 (Part no. 4484085)		
Component	**Quantity**	**Shipping and storage**
Ion PGM™ IC Reagents 200 Cartridge	4 cartridges	-30° to -10°C

Ion PGM™ Sequencer reagents and materials

Ion PGM™ Sequencing Supplies Kit (Part no. 4488376)		
Component	**Quantity**	**Shipping and storage**
Wash 1 Bottle w/ label (250 mL)	1 bottle	15° to 30°C
Wash 2 Bottle[1] w/ label (2 L)	1 bottle	
Wash 3 Bottle w/ label (250 mL)	1 bottle	
Wash Bottle Sipper Tubes	8 tubes for 250-mL bottles 4 tubes for 2-L bottle	
Reagent Bottles w/ labels (50 mL)	25 bottles	
Reagent Bottle Sipper Tubes	16 tubes	

[1] Must be conditioned at least 8 hours before use as described in "Condition the Wash 2 Bottle for first use" on page 48.

Ion PGM™ IC Sequencing Reagents Kit (Part no. 4484271)				
Component	**Cap color**	**Quantity**	**Volume**	**Shipping and storage**
Ion PGM™ IC Sequencing dGTP	Black	1 tube	80 µL	-30° to -10°C
Ion PGM™ IC Sequencing dCTP	Blue	1 tube	80 µL	
Ion PGM™ IC Sequencing dATP	Green	1 tube	80 µL	
Ion PGM™ IC Sequencing dTTP	Red	1 tube	80 µL	

Ion PGM™ IC Sequencing Solutions Kit (Part no. 4488375)			
Component	**Quantity**	**Volume**	**Shipping and storage**
Ion PGM™ IC Sequencing W2 Solution	2 bottles	126.25 mL	2°C to 8°C
Ion PGM™ IC Sequencing 1X W3 Solution	2 bottles	100 mL	
Ion PGM™ Cleaning Tablet	4 tablets	—	

W2 Bottle Conditioning Solution (Part no. A25043)			
Component	**Quantity**	**Volume**	**Shipping and storage**
W2 Bottle Conditioning Solution	1 bottle	125 mL	15° to 30°C

Note: The *Ion Chef™ System Support Information Product Information Sheet* (Pub. no. 4483647) is available from **ioncommunity.lifetechnologies.com**.

Compatible Ion Chip Kits

Contents	Quantity	Catalog no.	Shipping and storage
Ion 318™ Chip Kit v2 BC	4 pack	4488146	15°C to 30°C
	8 pack	4488150	
Ion 316™ Chip Kit v2 BC	4 chips	4488145	
	8 chips	4488149	
Ion 314™ Chip Kit v2 BC	8 chips	4488144	

Required materials and equipment

The following tables list required materials and equipment that are not provided with the Ion PGM™ IC 200 Kit. Where noted, supplies are available from Major Laboratory Suppliers (MLS).

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

Required materials and equipment for sequencing

✓	Description	Supplier	Catalog no.	Quantity
	Ion Personal Genome Machine® (PGM™) System (instrument and server) and included accessories	Life Technologies	4462921	1
	Ion PGM™ Controls Kit v2[1]	Life Technologies	4482010	1
	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)	VWR International	55850-422	1 or 2
	(Optional) 1/8" x 1/4" stem reducing coupler (only required if using a separate tank for the wash station)	McMaster	5779K699	1 per wash
	Uninterruptable Power Supply (UPS) [2]	MLS	—	1
	ELGA® PURELAB® Flex 3 Water Purification System *or* Equivalent 18 MΩ water system	Life Technologies	4474524 MLS	1
	NaOH (10 M) molecular biology grade	MLS	—	—
	Isopropanol (100%)	MLS	—	—
	Nuclease-free water molecular biology grade	MLS	—	—
	0.22-µm or 0.45-µm vacuum filtration system and filters	MLS	—	—
	Microcentrifuge[3]	MLS	—	1
	P2, P10, P20, P200, P1000 µL pipette set and filtered tips	MLS	—	1 set
	25-mL or 50-mL serological pipettes *or* 100-mL graduated cylinder	MLS	—	Varies
	Pipet-Aid® pipettor (If using serological pipettes)	Drummond Scientific	4-000-110	1
	Rainin® Pipet-Lite® XLS pipettor with RFID LTS 2 µL to 20 µL	Rainin	L-20XLS	1

✓	Description	Supplier	Catalog no.	Quantity
	Rainin® Pipet-Lite® XLS pipettor with RFID LTS 10 µL to 100 µL	Rainin	L-100XLS	1
	Rainin® StableRak™ LTS tips 2-20 µL	Rainin	SR-L20F	—
	Rainin® StableRak™ LTS tips 200 µL	Rainin	SR-L200F	—
	0.2-mL MAXYMum Recovery® Thin Wall PCR Tubes, Flat Cap (do not use polystyrene tubes)	Axygen	PCR-02-L-C	Varies
	1.5-mL or 1.7-mL microcentrifuge tubes	MLS	—	Varies
	Glass bottles (1 L)	MLS	—	1
	Ice buckets and ice	—	—	1
	Vortex mixer with a rubber platform	MLS	—	1
	Thermal cycler with a heated lid	MLS	—	1
	Standard laboratory vacuum line or vacuum pump	MLS	—	1
	Liquid trap	MLS	—	1
	Tygon® tubing [4]	MLS	—	1

[1] For installation and troubleshooting.

[2] 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.

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

[4] As needed to connect laboratory vacuum to liquid trap and liquid trap to P200 pipette tip.

Optional materials

The materials in the following table are optional and required only to verify and adjust the pH of the W2 Solution prior to sequencing the chips.

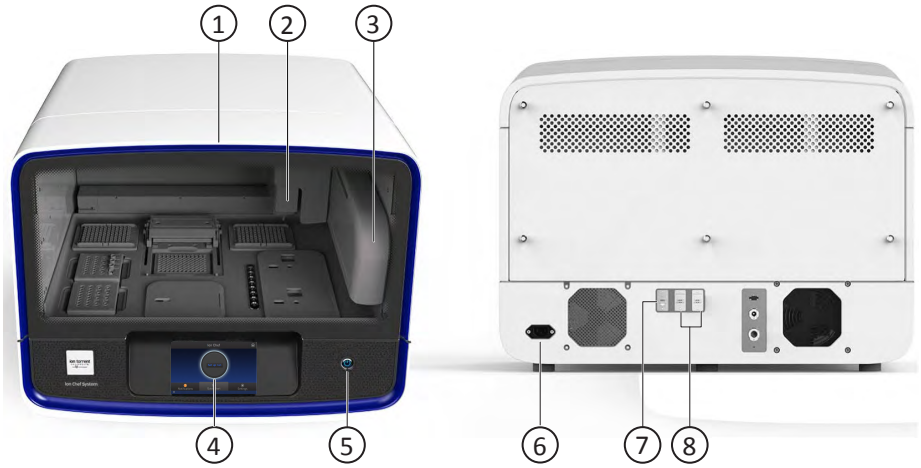
✓	Description	Supplier	Catalog no.	Quantity
	Orion 3-Star Plus pH Benchtop Meter Kit with electrode, electrode stand, and calibration buffers (or equivalent)	Thermo Fisher Scientific	1112003	1
	1 N HCl	MLS	—	Varies
	Magnetic stirrer (must hold 2-L bottle)	MLS	—	1
	Magnetic stir bar (4 cm)	MLS	—	1
	Squirt bottle	MLS	—	1

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 Ion 314™ v2 BC, Ion 316™ v2 BC, or Ion 318™ v2 BC Chips in approximately 10 hours with less than 15 minutes of hands-on time. The Ion Chef™ System features network integration with the Torrent Suite™ 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.

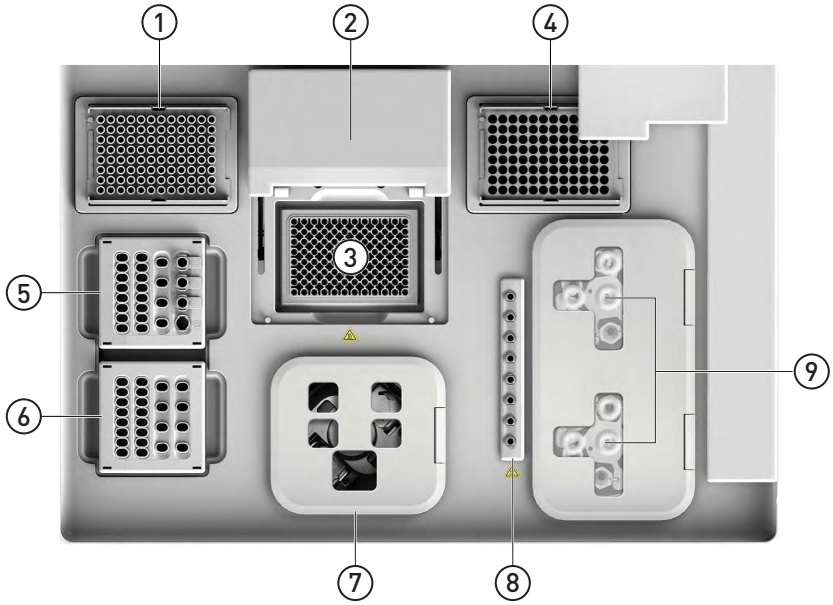
⑥ **Power port** – A 100–240 VAC port that provides power to the Ion Chef™ Instrument.

⑦ **Ethernet port** – An RJ45 port that provides Ethernet (100Mbit) 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 chips.



① **New pipette tips** – The position of the rack containing unused 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 **Thermal cycler sample block** – Performs thermal cycling of the sequencing reactions on a 96-well PCR reaction plate.

④ **Waste pipette tips** – The position of the rack containing waste (used) pipette tips.

⑤ **Reagents station** – The position on the instrument deck of the diluted libraries, NaOH, and the Ion PGM™ IC Reagents 200.

⑥ **Solutions station** – The position on the instrument deck of the Ion PGM™ IC Solutions 200, which is maintained at room temperature.

⑦ **Chip-loading Centrifuge** – Performs centrifugation of Ion chips that have been mounted to chip-loading adapters and loaded with template-positive ISPs.

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

⑨ **Recovery Centrifuges** – Twin stations that perform centrifugation of the ISPs during the recovery phase of template preparation.

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 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 - Read before using the Ion Chef™ System

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 frame of the bench before inserting or removing a chip from a Chip Adapter.

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 upon 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 Ion Chef™ IC 200 Tip Cartridge, do not reuse any of the Ion Chef™ System consumables or reagents. After each run, the empty Pipette Ion Chef™ IC 200 Tip Cartridge is transferred to the waste tip station.

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

- Use only Life Technologies 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 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.
- Unload the Ion Chef™ Instrument and use an Ion Chip™ Minifuge to remove any residual liquid from the chips within 5 minutes after each run ends.
- Sequence chips within 1 hour after the Ion Chef™ System finishes loading them. If you cannot sequence a loaded chip immediately, centrifuge the chip to remove any residual liquid, then store it within a chip storage container at 4°C until you are ready to run it (up to 4.5 hours maximum).

  Note: If you choose to store a loaded chip, remove the chip from storage at least 20 minutes prior to running it, allowing the chip to warm to room temperature.

2 Before you begin

Workflow

The following workflow illustrates how to prepare and load samples using the Ion PGM™ IC 200 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, prepare the Ion chips, and thaw the reagents.

▼

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

▼

Start the Ion Chef™ Instrument.

▼

Clean and initialize the Ion PGM™ Sequencer.

▼

Unload the Ion Chef™ Instrument.

▼

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

Create a Planned Run

IMPORTANT! This sequencing kit is compatible with Torrent Suite™ 4.4.2 and later. Before proceeding, check for updates to the Torrent Suite™ and Ion Chef™ System software, and install the updates if available.

Note: If you are currently using Ion Reporter™ Software v4.2.1 with an earlier version of the Torrent Suite™, contact Technical Support for assistance with upgrading to Torrent Suite™ 4.4.2.

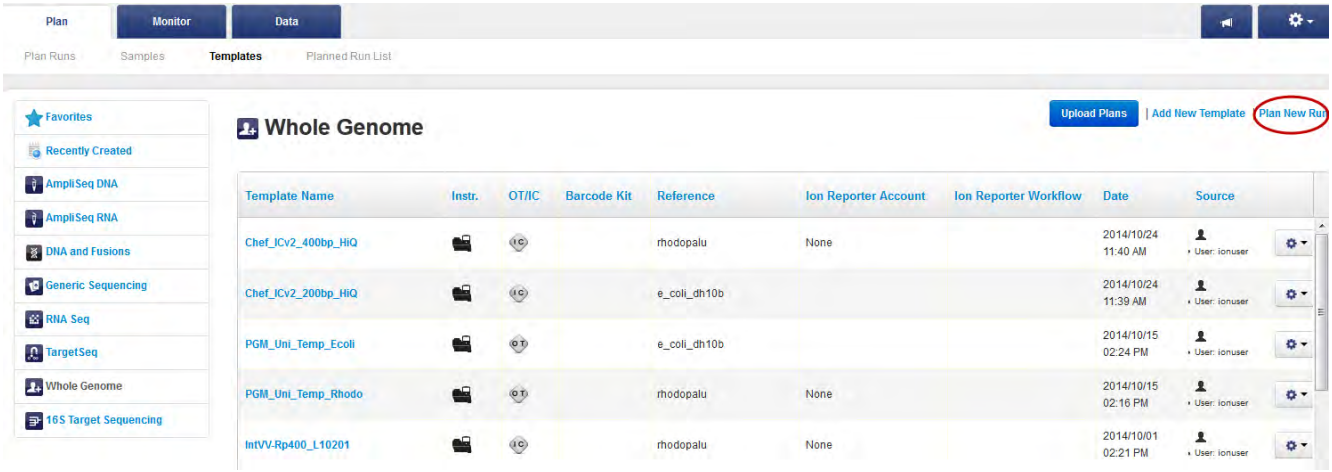
About Planned Runs

Planned Runs contain all of the settings used in a sequencing run, including the number of flows, kit types, barcodes used, run type (such as DNA, RNA, or amplicons), and reference file (if any). They provide a convenient way to set up your runs. You create Planned Runs in the Torrent Browser and assign them to barcoded Library Sample Tubes. The Planned Runs are retrieved by the Ion Chef™ Instrument when it scans the Library Sample Tubes, and by the Ion PGM™ Sequencer when you scan the barcode of the Ion Chef™-prepared sequencing chip you are loading. Each of the two chips prepared in an Ion Chef™ run needs a Planned Run created for it.

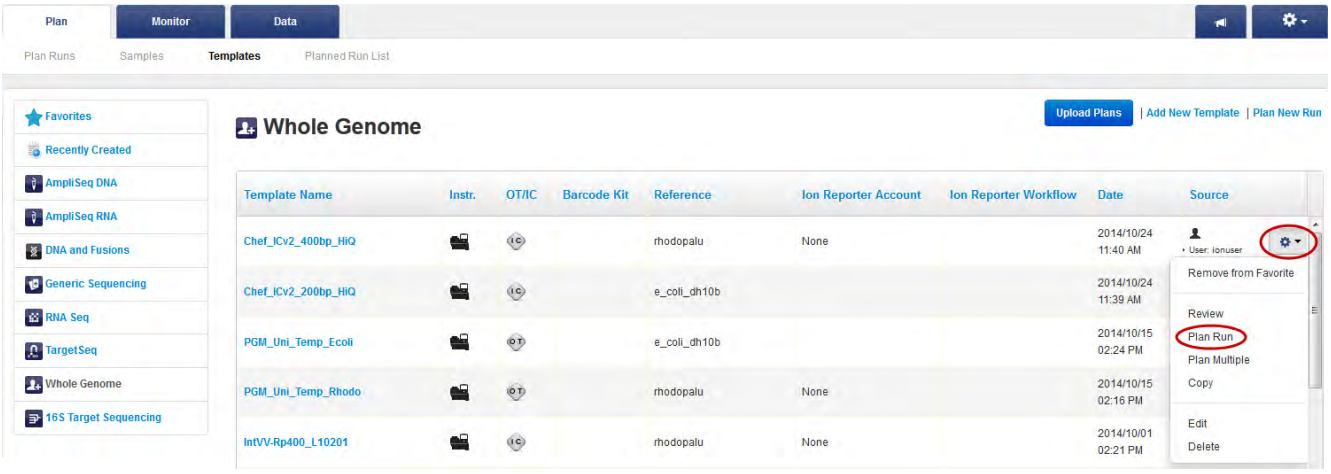
Note: For more information, see *Torrent Suite™ Software Documentation (v4.4)*, available from the Ion Community (**http://ioncommunity.lifetechnologies.com/community/products/torrent_suite**).

Create a Planned Run

1. Open the Torrent Browser for the Torrent Server connected to your Ion Chef™ System.
2. Select the **Plan** tab, click **Templates**, locate 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.

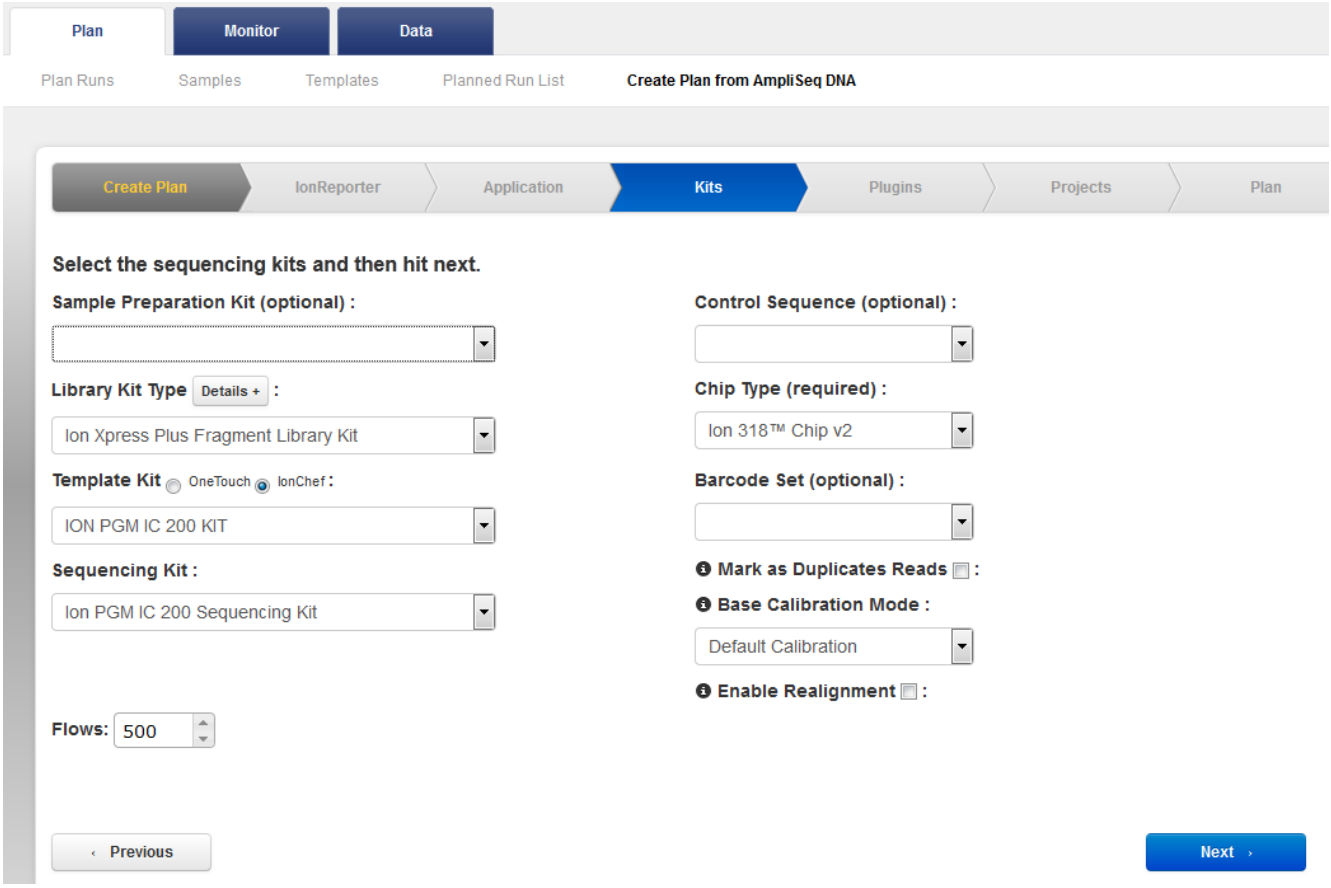


- **Plan Run** in the drop-down menu under the "Gear" tab to the right of an existing template you select from the template list.



3. In the Planned Run wizard, review the **IonReporter** and **Application** tabs and make selections appropriate to your run. In the **Kits** tab, make the following selections:
   a. Select **Ion Chef**, then select the **Ion PGM™ IC 200 Kit** from the **Template Kit** drop-down list.
   b. Select **Ion PGM™ IC 200 Sequencing Kit** from the **Sequencing Kit** drop-down list.
   c. Select the appropriate chip type in the **Chip Type** drop-down list.
   d. Select or edit the optional information fields appropriately for your run.

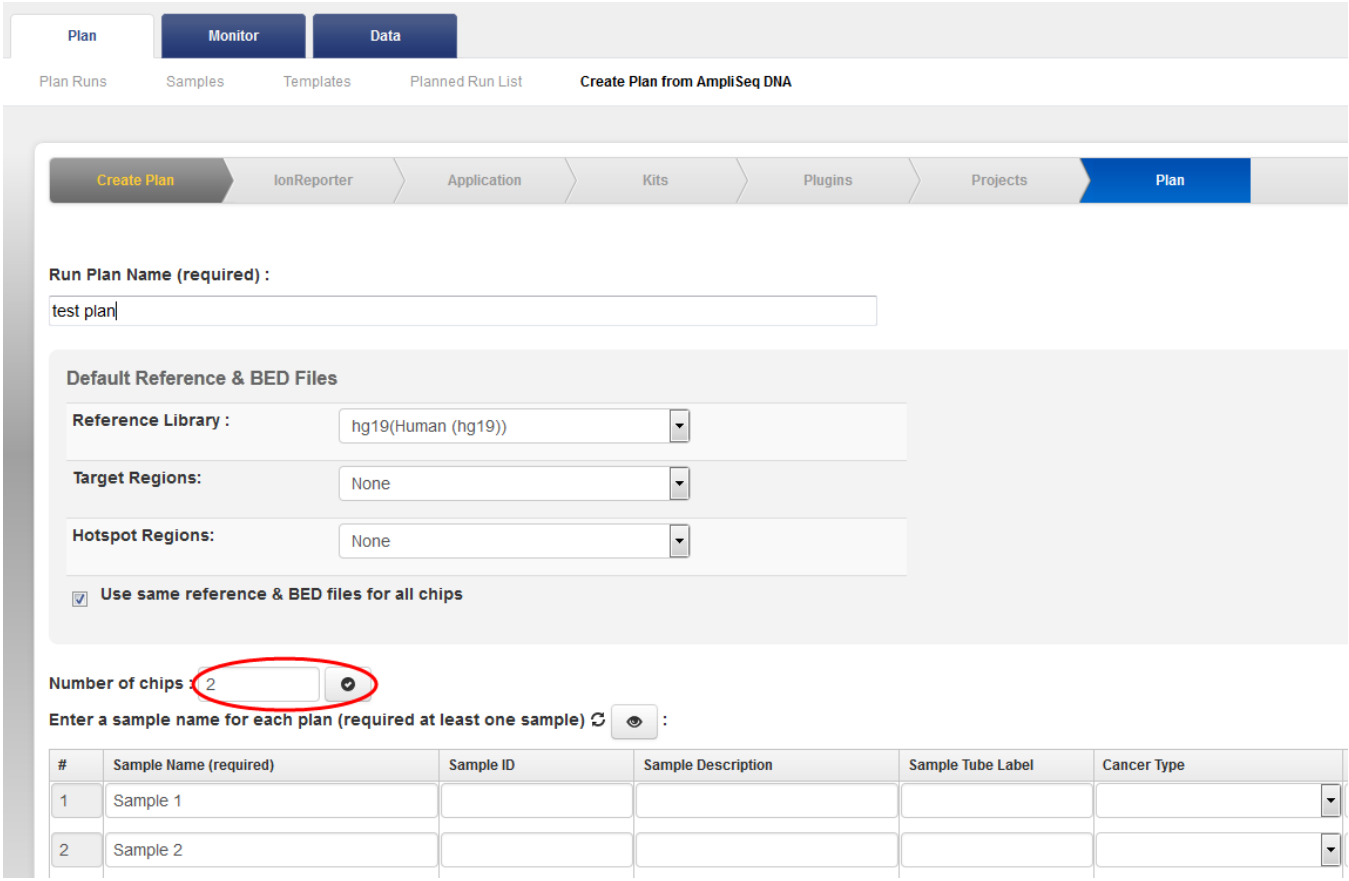
e. Click **Next**.



Note: For a complete description of the fields of the Torrent Browser application, see the *Torrent Browser User Interface Guide*, available from the Ion Community (**http://ioncommunity.lifetechnologies.com/community/products/torrent_suite**).

4. Review the **Plugins** and **Projects** tabs and 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 and then click the check mark button to the right of this field.



Note: For a complete description of the fields of the Torrent Browser application, see the *Torrent Browser User Interface Guide*, available from the Ion Community **http://ioncommunity.lifetechnologies.com/community/products/ torrent_suite**.

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

Planned Run wizard key fields

Field name	Description
Application	Select the sequencing application you are performing.
Barcode Set (optional)	If you are using barcodes with: • **DNA libraries**: Select the **IonXpress** barcode set, which includes all barcodes in the Ion Xpress™ Barcode Adapters 1-96 Kits. • **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 (Cat. no. 4475485). If you are *not* using barcodes with: • **DNA libraries**: Leave the Barcode field blank. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2**: Select **RNA_Barcode_None** from the drop-down list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.
BED files	Select the **Target Regions** or **HotSpot Regions** BED file on the Ion Torrent Server, if any.
Export	If installed and enabled, and you want to upload data to the Ion Reporter™ Software, select the Ion Reporter™ Uploader.
Flows	Enter the appropriate number of flows for the read length (for example, 500 flows for 200-base-read sequencing).
Monitor	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.
Planned Run Name	Enter a name for the run.
Plugins	Select the appropriate plugins for your application.
Project	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.
Reference Library	Select a reference library uploaded to the 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. **Note:** The "Sample Tube Label" field can be in two different locations in the wizard depending on adaptors or library you choose.
Sequencing Kit	Select **Ion PGM IC 200 Sequencing Kit**.
Template Kit	Select **ION PGM IC 200 KIT**.

Dilute the libraries

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

Dilute the two (2) stock libraries to a starting concentration of 50 pM with Nuclease-free Water (approximately 2.1 billion molecules per 70-µL input volume). Then use polyclonality and low quality filter results from a sequencing run performed with ISPs templated at this starting concentration and titrate downward to achieve optimal concentrations, if necessary. Prepare a fresh dilution of each library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.

Note: Library input recommendation is based on qPCR quantification. If libraries are quantified with a 2100 Bioanalyzer® instrument, a higher calculated concentration may need to be used for equivalent input.

Note: If running a control library, prepare the E. coli DH10B Control 200 Library, obtained from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010), for use by diluting 1 µL into 105 µL Nuclease-free Water.

Prepare the Ion Chef™ System for use

Before you use the Ion Chef™ Instrument:

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

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

- Condensate may collect in the empty compartments of the Reagents and Solutions stations, depending on ambient temperature and humidity conditions. Before loading consumables into the instrument for a run, inspect these compartments for condensation. If necessary, wipe them dry with a Kimwipes® wipe or absorbent cloth.
- Thaw the Ion PGM™ IC Reagents 200 Cartridge at room temperature for 45 minutes prior to use.

3 Run the Ion Chef™ System

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™ IC 200 Kit (Cat. no. 4484080)
- Ion 314™ v2 BC, Ion 316™ v2 BC, or Ion 318™ v2 BC Chips (2)
- Molecular-biology grade Nuclease-free Water
- P200 pipette and filtered tips
- Waste container

Prepare the libraries and consumables

1. At least 45 minutes prior to use, unbox the Ion PGM™ IC Reagents 200 Cartridge and allow it to thaw at room temperature.

   IMPORTANT! The IC Reagents Cartridge must warm to room temperature for at least 45 minutes before use.

2. Pipet 70 µL of each diluted library (see "Dilute the libraries" on page 26) to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).

   Note: If running the *E. coli* DH10B Control 200 Library, obtained from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010), prepare the library for use by diluting 1 µL of stock library into 105 µL Nuclease-free Water.

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

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

   Prepare the following:

   - Chip Adapters (2)
   - Enrichment Cartridge
   - Ion Chef™ IC 200 Tip Cartridge
   - PCR plate
   - PCR Plate Seal

- Recovery Station Lids (2)
- Recovery Tubes (12)
- Ion PGM™ IC Solutions 200 Cartridge
- Ion PGM™ IC Reagents 200 Cartridge (from Step 1)
- IC Piercing Tips (2)

IMPORTANT! Prior to use, gently tap the IC Reagents and the IC 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 IC Reagents Cartridge. If present, load the cartridge as directed – the precipitate will dissolve when the reagents are mixed during instrument operation.

Load the Ion Chef™ System

IMPORTANT! Rated centrifuge speeds are only intended for operation with provided buckets and approved consumable chips, tubes, and sample preparation reagents.

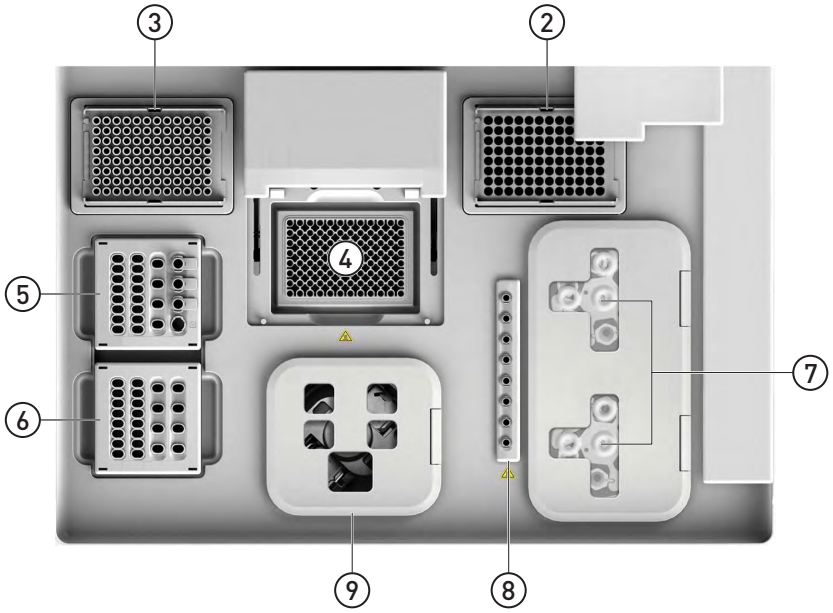
IMPORTANT! The Chip-loading Centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load balanced. Proper care must be taken to load the bucket properly. If excessive vibrations arise, check to ensure that items are installed properly and rotors are equally balanced on each side.

IMPORTANT! Confirm that the instrument is powered ON and has been cleaned recently following the last use.

IMPORTANT! Confirm that all components are clean and dry before loading them onto the Ion Chef™ Instrument.

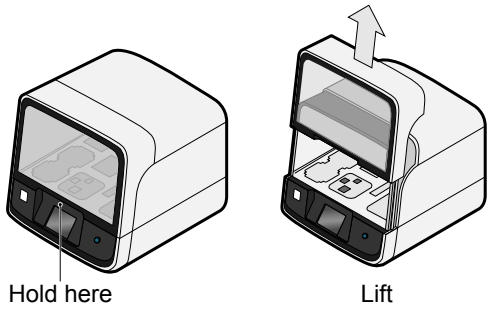
IMPORTANT! Confirm that the Reagents and Solutions station compartments are dry and free of condensate before loading components.

Follow the procedure below to load the Ion Chef™ Instrument, where each step corresponds to a numbered area in the following diagram.

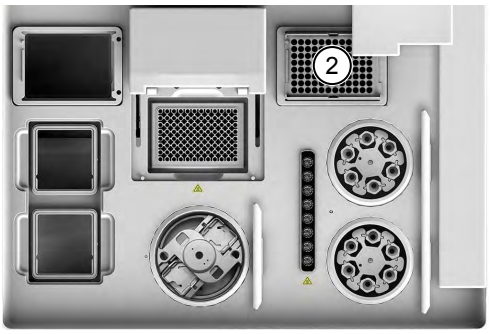


1. Open the instrument door:
   a. In the instrument touchscreen, touch ⏏ (Open Door) then wait for the latch to open.

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



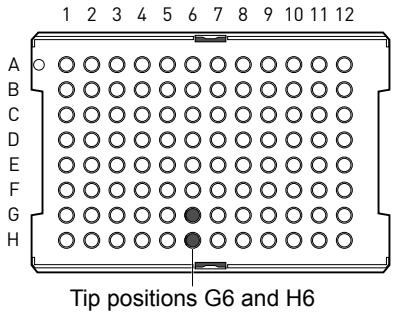
2. Load an empty pipette tip rack to the *Used* (Waste) Pipette Tip Position, then change gloves.



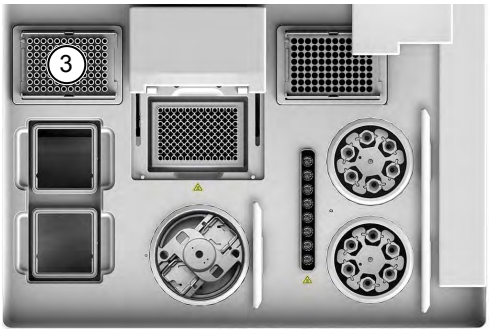
IMPORTANT! Confirm that the pipette tip rack in the *Used* (Waste) Pipette Tip Position does not contain any tips. The instrument will abort the run if tips are present in the *used* position.

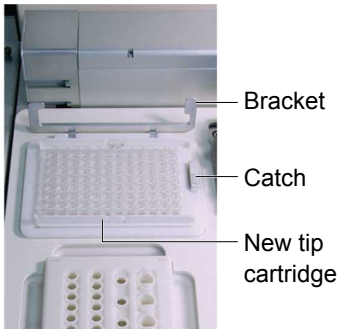
IMPORTANT! To prevent contamination, change gloves immediately after moving the empty pipette tip rack to the *Used* (Waste) Pipette Tip Position.

3. Load a Ion Chef™ IC 200 Tip Cartridge to the *New* Pipette Tip Position:
   a. Unwrap the Ion Chef™ IC 200 Tip Cartridge and remove the cover to expose the pipette tips. Load two new Ion Chef™ IC Piercing Tips into tip positions G6 and H6 on the Ion Chef™ IC 200 Tip Cartridge (numbering the positions from left to right and lettering from top to bottom).



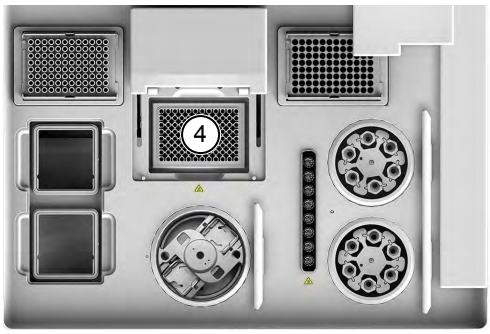
   b. Pull the catch forward, then pivot the locking bracket upwards. Load the assembled Ion Chef™ IC 200 Tip Cartridge into the *New* Pipette Tip Position, then pull the bracket downwards to lock the cartridge in place.

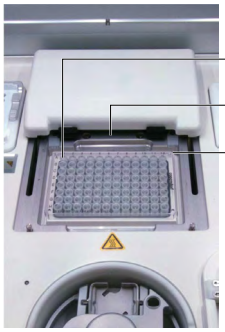




4. Load a new PCR plate into the thermal cycler sample block, then slide a new PCR Plate Seal underneath the automated heated cover.

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





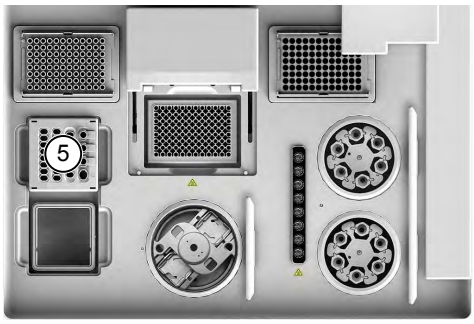
5. Load the Ion PGM™ IC Reagents 200 Cartridge and diluted libraries into the Reagents station:

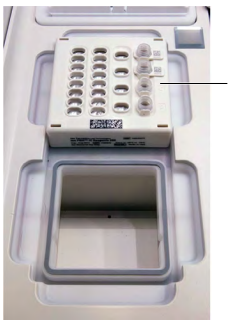
IMPORTANT! Thaw the IC Reagents Cartridge at room temperature for 45 minutes prior to use.

a. Gently tap the Ion PGM™ IC Reagents 200 Cartridge on the bench to force the reagents to the bottoms of the tubes.

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

IMPORTANT! Do not force the 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.



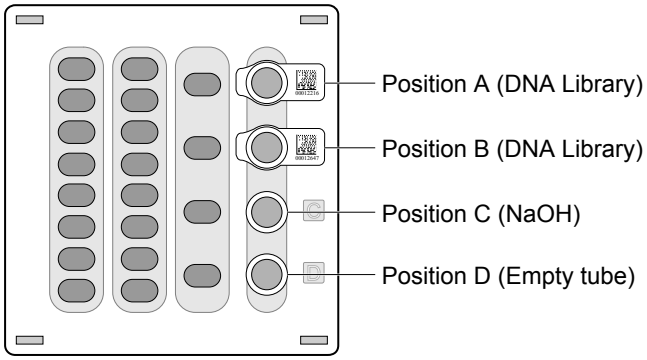


c. Uncap and load the two Library Sample Tubes, each containing 70 µL of diluted library, into Positions A and B on the IC Reagents Cartridge.

IMPORTANT! Make sure to orient the sample tubes so that the barcodes are visible and oriented to the right (see below).

IMPORTANT! Make sure to remove the caps to the Library Sample Tubes before proceeding.

d. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the IC Reagents Cartridge.



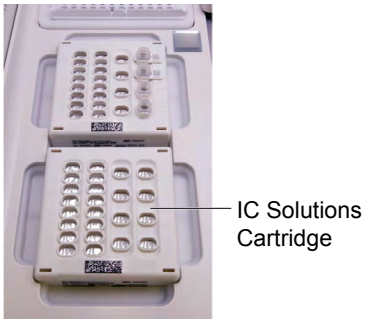
IMPORTANT! When the IC Reagents Cartridge is loaded:

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

6. Load the Ion PGM™ IC Solutions 200 Cartridge to the Solutions station:

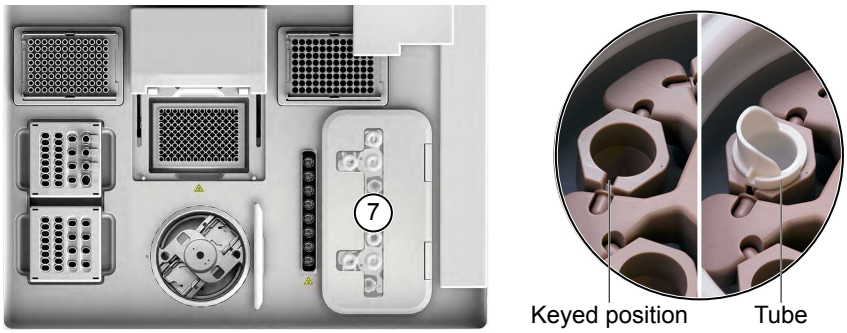
a. Gently tap the Ion PGM™ IC Solutions 200 Cartridge on the bench to force the reagents to the bottoms of the tubes.

b. Load the IC Solutions Cartridge into the Solutions station until it snaps into place and is level on the deck.



7. Load the consumables into the Recovery centrifuges:
   a. Load six Recovery Tubes into each Recovery Centrifuge.

   IMPORTANT! Confirm that the Recovery Tubes are seated correctly within the centrifuge buckets. The buckets are keyed to ensure that the tubes fit in a specific orientation.



   b. Place a Recovery Station Lid over each centrifuge. Orient the lids so that the ports are located as shown in the figure below.

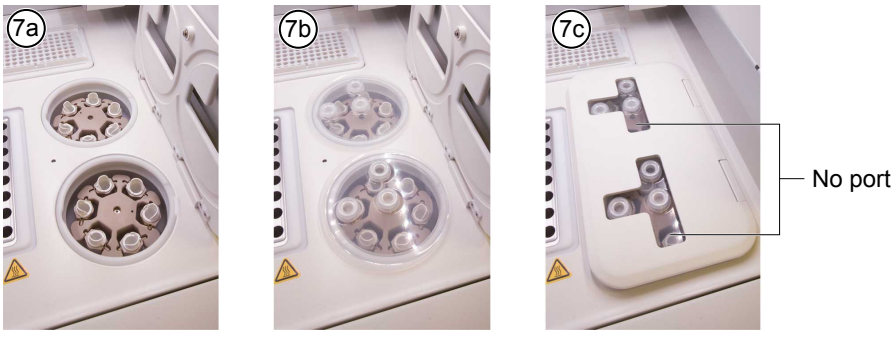
      Before sealing each centrifuge, confirm that:

      - The centrifuge is 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 within the centrifuge so that they pivot outwards.

   c. Close the lid of the Recovery Centrifuges. Confirm that no ports are located in the positions facing the front of the instrument.

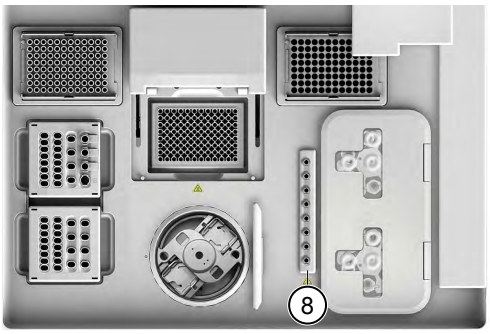


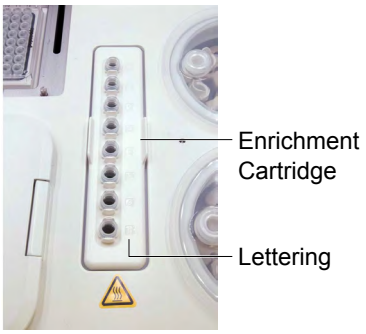
IMPORTANT! Do not obstruct or place any object on top of the lid.

IMPORTANT! 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.

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

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

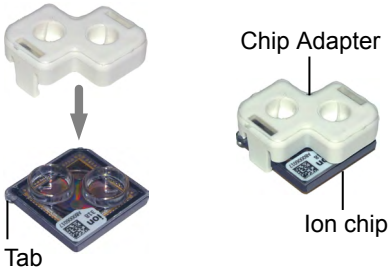




9. Load the Chip-loading Centrifuge:
   a. Attach Chip Adapters to the chips.

IMPORTANT! When attaching a Chip Adapter:

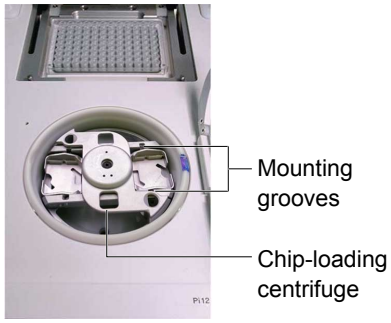
- On the underside of each Chip Adapter, confirm that the rubber washers are in place. If a washer has become detached but can be found, return the washer to the empty port before you use the adapter. Otherwise, discard the Chip Adapter and use a replacement.
- Align the wells of the Chip Adapter to the wells of the chip, then gently push the adapter onto the chip until the clips lock into place.
- Listen for an audible 'snap', which indicates that the Chip Adapter is attached. Loading can fail if the adapter is not attached securely.



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.

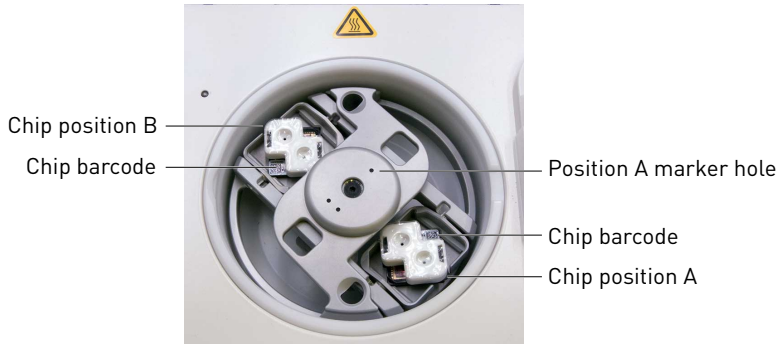
b. Place the adapter/chip assemblies into centrifuge buckets so that the chip barcode aligns above the white outline imprinted on the floor of the bucket.

c. Load the adapter/chip/bucket assemblies into the Chip-loading Centrifuge.



IMPORTANT! When loading the coupled chips, confirm that:

- The Chip Adapter is firmly attached to each chip before loading it into the centrifuge bucket.
- The tabs of the chips are oriented away from the center of the centrifuge.
- The barcodes of the chips are oriented as shown below.
- The clips of the coupled chips are firmly seated within 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 A marker hole. The chip loaded in this position will be loaded with ISPs prepared from the library loaded in Position A of the IC Reagents Cartridge. The chip loaded in

Position B will be loaded with ISPs prepared from the library loaded in Position B of the IC Reagents Cartridge.

d. Close the lid of the Chip-loading Centrifuge.

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

Before closing the centrifuge, confirm that:

- The centrifuge is balanced.

  IMPORTANT! The centrifuge must be load balanced.

- The pins on the sides of the chip buckets are securely seated within the centrifuge.
- The chip buckets are oriented correctly within the centrifuge so that they pivot 90° outwards when touched.

  IMPORTANT! The chip buckets must be correctly seated within the centrifuge.

IMPORTANT! 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 bucket properly. If excessive vibrations arise, check to ensure that items are installed properly and rotors are equally balanced on each side.

IMPORTANT! Use only the materials supplied in the Ion PGM™ IC 200 Kit, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets prior to each use to assure normal operation.

10. Confirm that all cartridges and reagents are installed correctly 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 locked in place.
    - Confirm that all tubes on the Ion PGM™ IC Reagents 200 Cartridge are uncapped and firmly locked in place.
    - Confirm that the centrifuge lids are installed correctly so that the ports are oriented toward the rear of the instrument.
    - Confirm that the tube and chip buckets are seated securely within the rotor arms of the Chip-loading and Recovery Centrifuges, and that the consumables they contain are correctly installed.

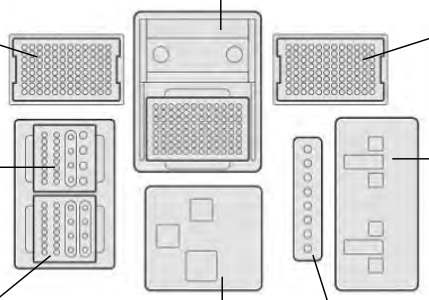
CAUTION! To ensure correct and safe instrument operation, you must confirm that all consumables are installed correctly to the deck before you start a run. The Ion Chef™ Instrument does not verify all aspects of the consumable setup prior to beginning each run.

Ion Chef™ pre-run checklist

☐ 1) A PCR Plate Seal is in place and correctly oriented under the heated cover.
☐ 2) A new PCR Plate is loaded in the thermal cycler sample block.

☐ 1) A new Ion Chef™ IC 200 Tip Cartridge is loaded in the New Pipette Tip position.
☐ 2) Two new Ion Chef™ IC Piercing Tips are loaded in positions G6 and H6 of the Tip Cartridge.
☐ 3) 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) IC Reagents 200 Cartridge contents are thawed (leave at RT for 45 minutes).
☐ 2) IC Reagents 200 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 are securely seated in the correct orientation in the Recovery Centrifuge buckets.
☐ 2) Recovery Centrifuges are load-balanced.
☐ 3) Recovery station lids are positioned so that the ports are correctly oriented: ports should not be located in the positions facing the front of the instrument.

☐ 1) IC Solutions 200 Cartridge is pressed into place at the Solutions station so that the cartridge is **firmly seated** and **level** with the deck.

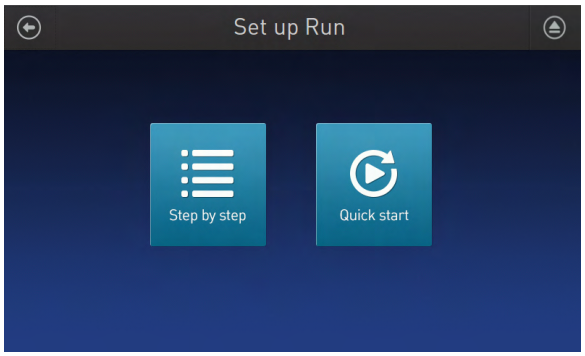
☐ 1) Enrichment Cartridge is pressed into place at the Enrichment station so that the cartridge is **firmly seated** and **level** with the deck.
☐ 2) Lettering on the cartridge is right-side up and positioned to the right of the enrichment tubes.

☐ 1) Chip Adapters 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 run

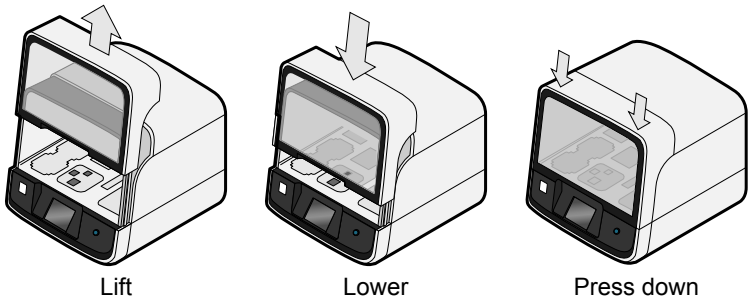
1. Confirm that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, touch **Set up run**.

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. Confirm that both sides of the door are locked after closing it.



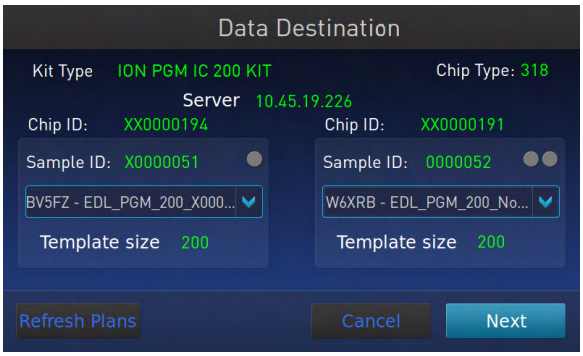
5. When prompted, touch **Start check** to begin Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility.

   During Deck Scan, the touchscreen may display warnings if the Ion Chef™ Instrument detects missing or incompatible consumables. You must address all warnings before the run can begin. 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 prior to starting a run. To ensure proper and safe instrument operation, confirm that all consumables are installed correctly before you continue.

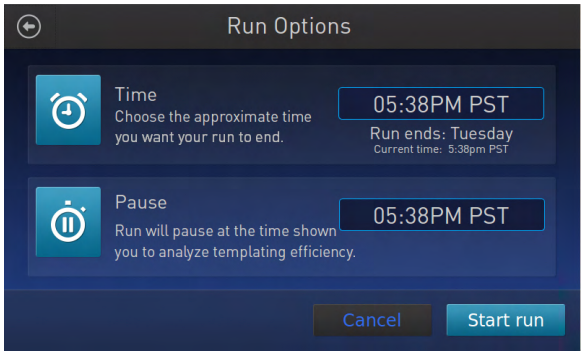
6. When Deck Scan is complete, touch **Next** to display the Data Destination screen.

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



   IMPORTANT! If the kit name and chip type are incorrect, confirm that you are using the correct kit and chip. If you are using the correct kit and chip, contact Technical Support.

8. On the Run Options screen, touch the appropriate option to complete the run and enter the desired time of run completion, if necessary.



The Ion Chef™ Instrument provides two options for obtaining quality control (QC) samples that can be used to assess 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™ IC Reagents 200 Cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge.

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).
Pause	when the instrument pauses operation prior to the chip loading step (9.5 hours into the run).

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

9. On the Run Options screen, touch **Start run** to begin 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 will abort the run and display the error on the instrument touchscreen. If a run fails:

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

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

10. If you intend to 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 48).

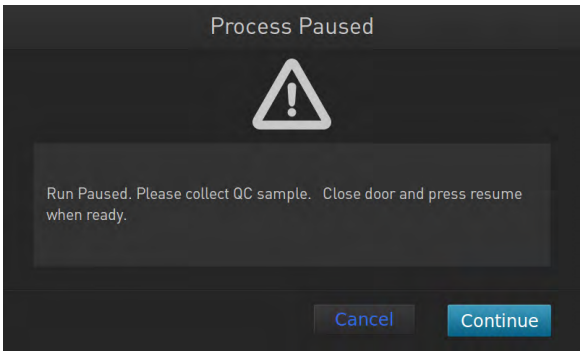
    Wash 2 Bottles must be conditioned with Wash 2 Bottle Conditioning Solution prior to using them for the first time. If filled immediately after you begin the run, the Wash 2 Bottle will be conditioned and ready for use just in time to perform the initialization.

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 after the start of the run).

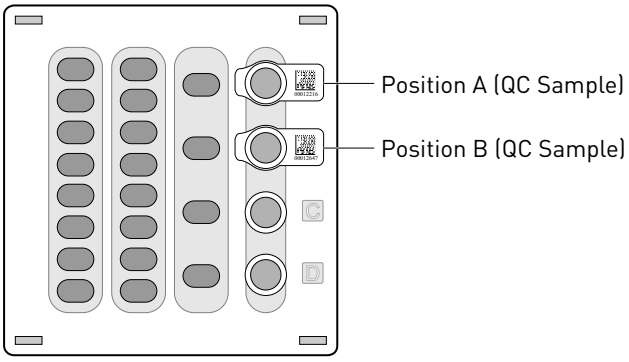
    a. When prompted to remove the QC sample, open the instrument door.



    b. Transfer the unenriched QC samples (entire volume) from Positions A and B of the Ion PGM™ IC Reagents 200 Cartridge on the instrument deck to two new labeled microcentrifuge tubes.

IMPORTANT! Do not remove the Library Sample Tubes from the Ion PGM™ IC Reagents 200 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 once it has been closed.



c. Analyze the QC samples.

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 (see "Unload and prepare the chips for sequencing" on page 56).

Note: If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge 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 84.

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.

Before starting

- **Weekly:** Prepare a stock of 1 M NaOH by diluting 10 M NaOH with 18 MΩ water.
- **Daily:** Prepare 100 mM NaOH by diluting the 1 M stock in 18 MΩ water. You will need 500 µL of 100 mM NaOH per initialization.

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 ⩽1100 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) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water and add an Ion PGM™ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~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, and select the **Chlorite cleaning** checkbox. Press **Next**.
5. 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.

6. Remove all wash and reagent bottles 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. Make sure 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, and 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 begin 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 and attach the bottle in the W1 position. Make sure the cap is tight. Press **Next** to begin 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 and 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. 4488376)
  - Wash Bottle Sipper Tubes
  - Reagent Bottle Sipper Tubes
  - Non-irradiated PP Reagent Bottles
  - Wash 1 Bottle
  - Wash 2 Bottle
  - Wash 3 Bottle
- Wash 2 Bottle Conditioning Solution (Part no. A25043)
- Ion PGM™ IC Sequencing Reagents (Part no. 4484271)
  - Ion PGM™ IC Sequencing dGTP
  - Ion PGM™ IC Sequencing dCTP
  - Ion PGM™ IC Sequencing dATP
  - Ion PGM™ IC Sequencing dTTP
- Ion PGM™ IC Sequencing Solutions (Part no. 4488375)
  - Ion PGM™ IC Sequencing W2 Solution
  - Ion PGM™ Cleaning Tablet
  - Ion PGM™ IC Sequencing 1X 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

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 five 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.

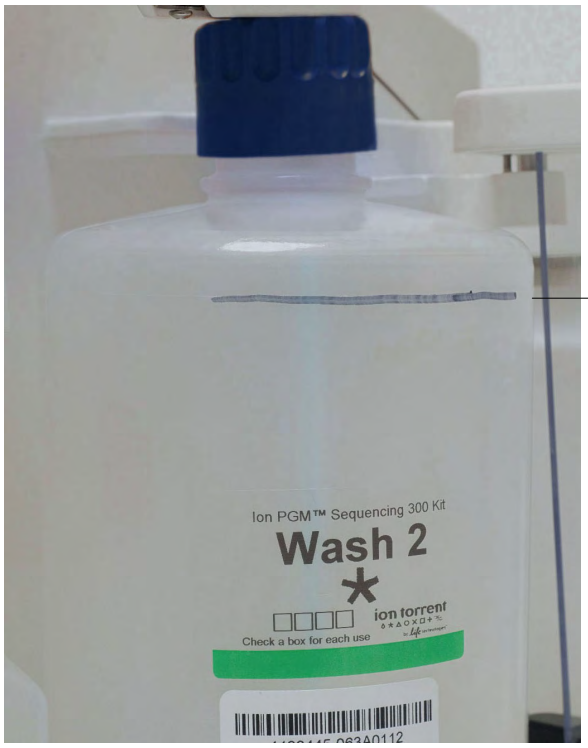
Initialization guidelines

- For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.
- 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.
- After four initializations, do not use the Wash 1 or 3 Bottles for initialization or sequencing to avoid breakage or leaking. (You can reuse the bottles in the cleaning procedure.)

  Note: W2 Bottles can be used up to 40 times before they must be discarded.
- Replace the Reagent Bottles and sipper tubes every time you initialize.
- Make sure that you have updated to Torrent Suite™ 4.4.2 or later.

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.
3. Note the mold line on the conditioned Wash 2 bottle. If there are two mold lines on the bottle, mark the lower line to indicate that it is the correct one.



Prepare the Wash 2 Bottle

IMPORTANT! For all the following steps, pour the 18 MΩ water directly from the purification system into the conditioned Wash 2 Bottle. Do not use water that has been collected or measured in any other containers.

1. Rinse the conditioned Wash 2 Bottle (2 L) three times with 200 mL of 18 MΩ water.
2. If your 18 MΩ water system has a spigot, extend the water spigot into **but not below** the neck of the Wash 2 Bottle.

3. Fill the bottle to the mold line. Volume of water will be ~2 liters.

4. Add the entire bottle of Ion PGM™ IC Sequencing W2 Solution to the Wash 2 Bottle.

Note: Keep the Ion PGM™ IC Sequencing bottle to scan the barcode during the initialization procedure.

5. Add 70 µL of freshly prepared 100 mM NaOH solution (*not* 1 M NaOH) to the Wash 2 Bottle.

   Note: Different sites may require adding different volumes of 100 mM NaOH. Some sites, for example, may require doubling the volume to 140 µL. See "Error message: Added too much W1 to W2" on page 79 for information on determining the volume of 100 mM NaOH to add.

6. Cap the bottle and invert five times to mix, and immediately proceed through the rest 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 three 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 and cap the bottle.
3. **Wash 3 Bottle:** Add Ion PGM™ IC Sequencing W3 Solution to the 50-mL line marked on the Wash 3 Bottle and cap the bottle.

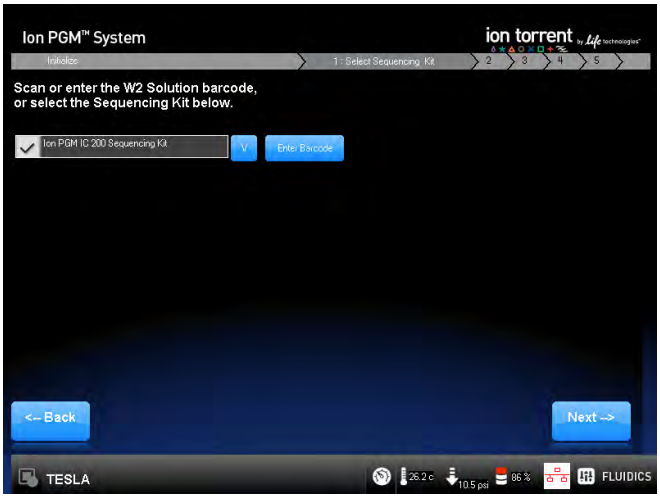
Begin the initialization

IMPORTANT! Do not remove the old sipper tubes from the dNTP ports until instructed to do so. **Do not let the new sipper tubes touch any surfaces.**

IMPORTANT! Load the bottles as quickly as possible to prevent atmospheric CO_2 from reducing the pH of the Wash 2 Bottle solution.

1. Confirm that the chip used to the clean the Ion PGM™ System is still in place on the instrument.
2. On the main menu, press **Initialize**.
3. In the next screen, select **Ion PGM™ IC 200 Sequencing Kit** from the drop-down list, then press **Next**.

   IMPORTANT! Do *not* select any other sequencing kit from the drop-down list. Be careful to select the correct kit type to ensure proper pH adjustment.



4. Press **Next** and confirm that the cleaning chip is on the instrument and the Reagent Bottle sipper tubes and collection trays are in place. Press **Next** again.
5. The system will verify the gas pressure. If the gas pressure is sufficient, press **Next** to begin the initialization. If the gas pressure is low, press **Yes** to retry gas-pressure verification. If the gas pressure remains low, contact Technical Support.

6. Wearing clean gloves, firmly attach a new sipper tube (long gray) to the cap in the W2 position. New sipper attachments are push-on (shown below), whereas older models may be threaded. **Do not let the sipper touch any surfaces.**

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

7. Immediately attach the prepared Wash 2 Bottle in the W2 position and tighten the cap. Press **Next**.

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

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

9. Immediately attach the prepared Wash 1 and 3 Bottles and tighten the caps. Press **Next**.

10. The Ion PGM™ System will test the bottles for leaks, fill the Wash 1 Bottle, and then adjust the pH of the W2 Solution. This procedure takes ~30 minutes.

    Note: If a wash bottle leaks or if an error occurs during the automatic pH process, see Appendix A, "Troubleshooting". If an error message indicates problems adjusting the pH of the prepared W2 Solution, see "Ion PGM™ System Initialization: Auto pH errors" on page 75.

Prepare the 50-mL Reagent Bottles with dNTP solutions

IMPORTANT! In the following steps, handle the nucleotides carefully to avoid cross-contamination and ensure that the correct dNTP solution is installed in each position on the Ion PGM™ System.

1. After each dNTP stock solution has thawed, vortex to mix and centrifuge to collect the contents. Keep dNTP stock solutions on ice throughout this procedure.

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

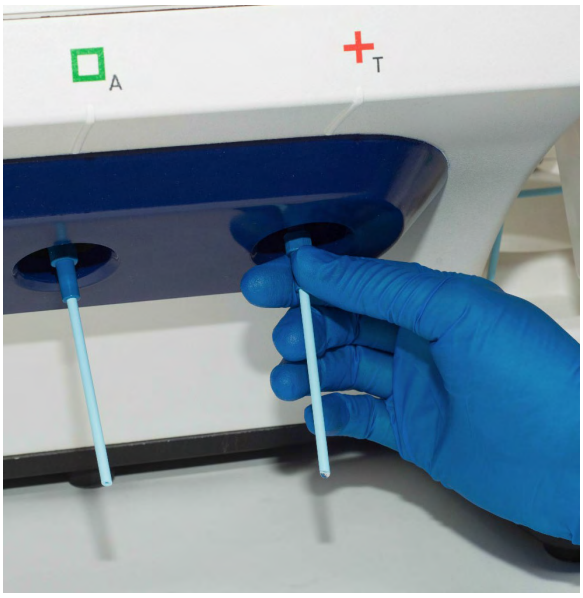
3. Using filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.

Attach the sipper tubes and Reagent Bottles

1. After the wash solutions have initialized (from "Begin the initialization" on page 52), 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," as shown below) 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.

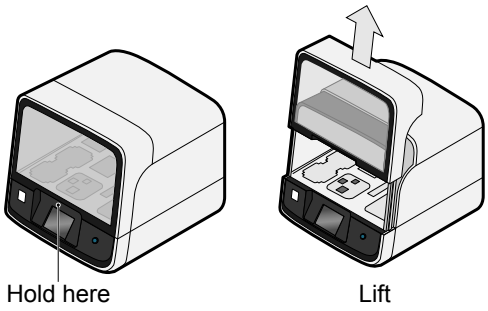
7. Proceed to the appropriate sequencing protocol for your chip type.

5 Start the sequencing run

Unload and prepare the chips for sequencing

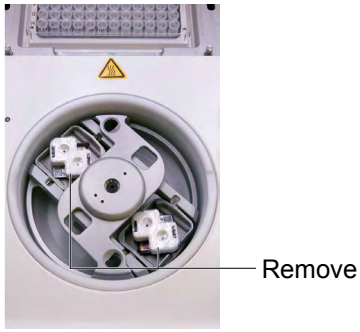
1. Open the instrument door:
   a. In the instrument touchscreen, touch ⏏ (Open Door) then wait for the latch to open.

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

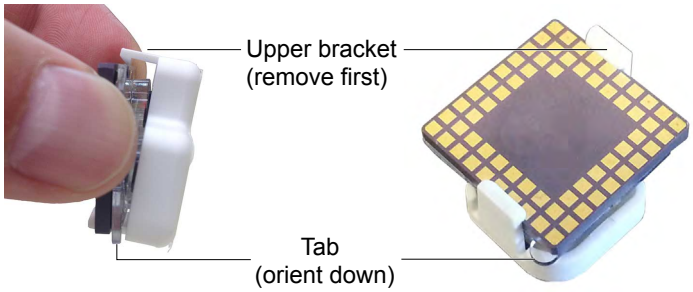


2. Open the lid of the Chip-loading Centrifuge, then gently remove the chip/bucket assemblies from the instrument.

   IMPORTANT! When removing each chip, be careful not to disturb the residual liquid in the outer port of the Chip Adapter.

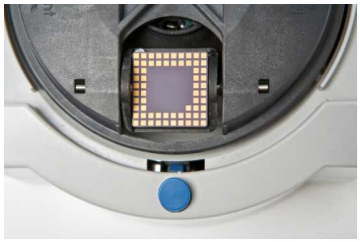


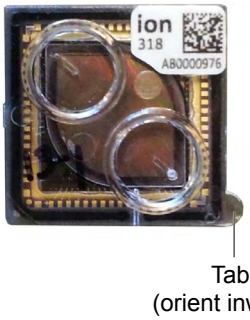
3. Remove the chip from the centrifuge bucket, then remove the Chip Adapters from the chips and discard them. To remove an adapter, hold the chip with the tab facing downward, then remove the Chip Adapter starting with the top hinge first.



4. Use an Ion Chip™ Minifuge to remove the residual liquid from the chips:
   a. Load both chips into the Ion Chip™ Minifuge. Place each chip upside-down within a bucket so that the tab faces inwards, toward the center.

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



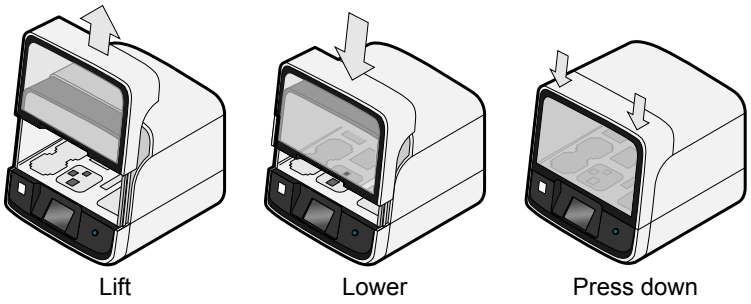


   b. Close the lid and centrifuge the chips for 5 seconds.

   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. Confirm that both sides of the door are locked after closing it.



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

   If you cannot sequence a loaded chip immediately, place the chip into a separate chip storage container *after* centrifugation and store at +4°C until you are ready to run it (up to 4.5 hours maximum).

   IMPORTANT! If you choose to store a loaded chip:

   - Centrifuge the chip *before* you store it.
   - At least 20 minutes before you intend to run the stored chip, remove the chip from the container, and place it on a clean surface in the dark to warm to room temperature.

Sequence the Ion chips on the Ion PGM™ Sequencer

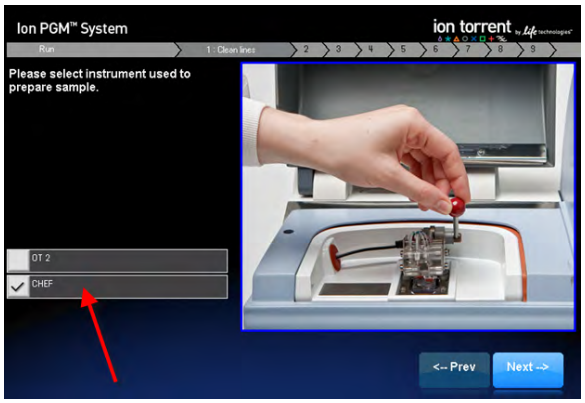
IMPORTANT! Observe the following when performing the chip check and sequencing the chip:

- The Ion PGM™ Sequencer must be cleaned and initialized before sequencing the chips.
- Do not use reagents from other sequencing kits for sequencing 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 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**.

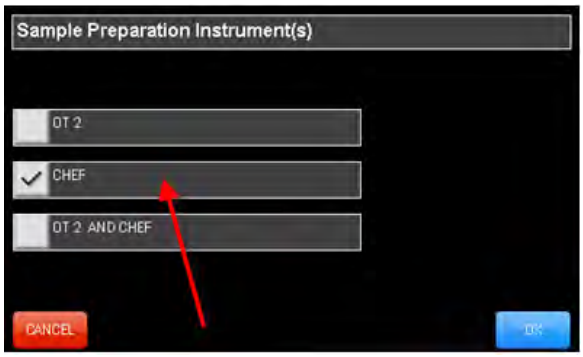


Note: You can preselect the Chef option if you use only the Ion Chef™ Instrument for sample preparation:

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

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

   c. On the next screen, touch **CHEF**, the touch **OK**. The instrument will now automatically select the Chef option.



3. Scan the barcode on the loaded chip, or enter the barcode manually.

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

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

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

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

   IMPORTANT! Ion PGM™ Sequencer—Blue sequencing requires use of the Ion PGM™ 2.5-L Waste Bottle. Be sure to completely empty and return the waste container 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 drop-down list. If the drop-down list does not contain your Planned Run, contact Technical Support.

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

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

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

     Note: To return damaged chips, contact Technical Support.

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

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

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

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

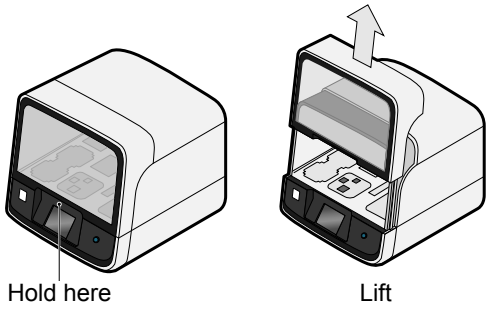
- Gloves, powder-free nitrile
- Isopropyl alcohol, 70% solution
- Wipes, lint-free

Clean the instrument

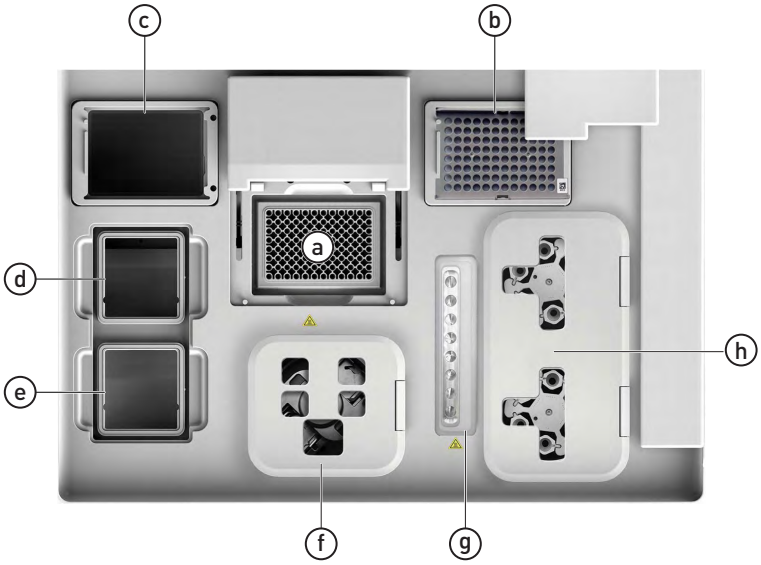
IMPORTANT! Clean the Ion Chef™ Instrument as described below after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.

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.



2. Remove and dispose of any used consumables from the instrument.



a. Remove and discard the PCR plate from the PCR sample block.

b. Remove and 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 may have collected within the container.

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

c. Move the empty Tip Cartridge to the waste tip position.

IMPORTANT! Do not discard the empty Tip Cartridge.

d. Remove and discard the Ion PGM™ IC Reagents 200 Cartridge.

IMPORTANT! Make sure to transfer the QC samples before you remove and discard the IC Reagents Cartridge.

e. Remove and discard the Ion PGM™ IC Solutions 200 Cartridge.

f. Close the lid of the Chip-loading Centrifuge.

g. Remove and discard the Enrichment Cartridge.

h. Remove and discard the consumables from the Recovery Centrifuges, including the:
   - Recovery Station Lids
   - Recovery Tubes

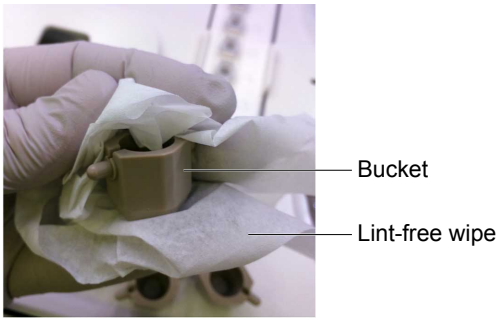
3. Inspect the Recovery Centrifuges and clean the components if excess liquid is present.

Is liquid present?	Then...
No	Go to step 4.
Yes	Clean the centrifuge bowl and buckets as described below. **IMPORTANT!** Clean the Recovery Centrifuge occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do *not* need to clean the centrifuge after every run.

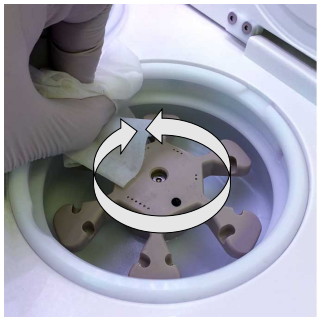
To clean the Recovery Centrifuge bowl and buckets:

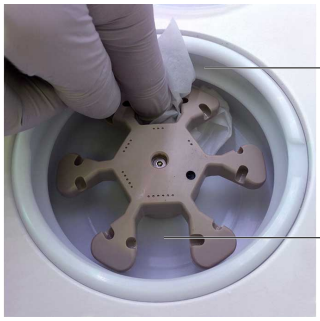
IMPORTANT! Wear powder-free, nitrile gloves when cleaning the Recovery Centrifuge.

a. Remove the buckets from the Recovery Centrifuge. 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.



b. Use lint-free wipes to clean the inside rim of the centrifuge, then remove all fluid from the bottom of centrifuge bowl.



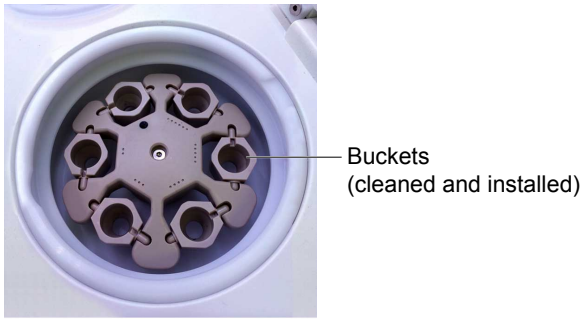


Note: When cleaning the centrifuge bowl, fold the wipe in half to provide extra absorbency.

c. 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.

d. Use lint-free wipes to dry the centrifuge and buckets.

e. Install the centrifuge buckets, then close the centrifuge lid.



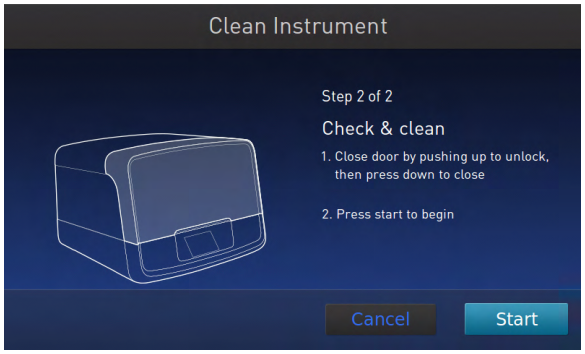
4. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.

5. From the Ion Chef™ Instrument touchscreen, begin the cleaning:
   a. On the screen that appears after run completion, touch **Next**.

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

   b. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except for the empty Tip Cartridge in the waste tip position, then touch **Next**.

c. With the door closed, touch **Start**. The instrument performs a load check before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation and illuminates the ultraviolet (UV) light within the instrument.



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

A Troubleshooting

Ion Chef™ System setup and operation

Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes	1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.

Observation	Possible cause	Recommended action
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.

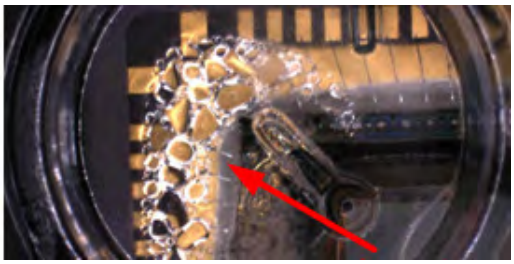
	• Network connection to the server has been interrupted.
	• User name or password is incorrect.
		1. Touch the Instrument status button to view the alert(s).
		2. In the Instrument status screen, confirm that the name of the Torrent Server connection is red.
		3. Contact your network administrator to 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.

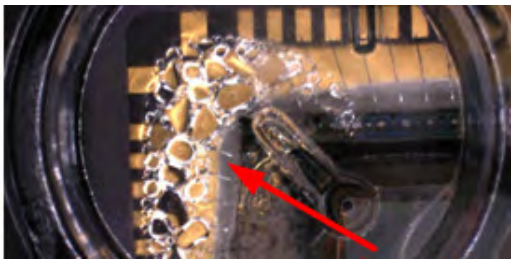
Liquid residue is present in the Recovery Centrifuge following a run	During normal instrument operation, a noticeable coating of liquid can collect on the bowl and buckets of the Recovery Centrifuge following repeated runs.	Remove the residue as instructed in "Clean the instrument" on page 62.

Observation	Possible cause	Recommended action
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™ IC Reagents 200 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.
	Hardware or software error	Contact Technical Support to report the problem and for further assistance.
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.

Observation	Possible cause	Recommended action
Chip-loading centrifuge stopped unexpectedly	Centrifuge obstructed	1. Touch ⏏ **Open Door**, then lift the door of the instrument. 2. Lift the lid of the centrifuge then remove the snap cover. 3. Remove the centrifuge buckets with the Ion Chips. 4. If the centrifuge is obstructed, remove the obstruction. 5. Install the centrifuge buckets and Ion Chips in the centrifuge. 6. Snap the centrifuge cover into place, then lower the lid. 7. Close the instrument door. 8. Resume the planned run. 9. If the centrifuge still does not rotate or it makes excessive or an unusual noise while turning, contact Thermo Fisher Scientific Support. **IMPORTANT!** Do not again attempt to perform the planned run on the instrument. You may damage the instrument.

Ion PGM™ Sequencer chip check and calibration

Observation	Possible cause	Recommended action
Chip Check fails	• Clamp not closed • Chip not properly seated • Debris on the chip socket • Chip damaged	1. Open the chip clamp, remove the chip, and look for signs of water outside the flow cell:  2. 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. 3. Close the clamp and repeat the Chip Check. 4. If the chip passes, click **Next**. If Chip Check continues to fail, there could be a problem with the chip socket. Contact Technical Support.

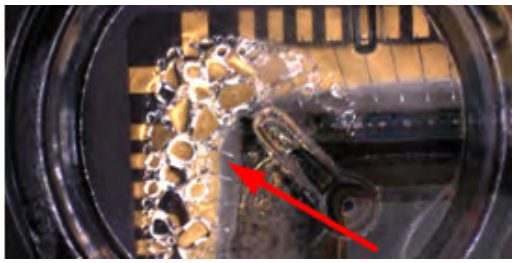
Observation	Possible cause	Recommended action
Leak of unknown origin	• Chip leak • Chip clamp not closed properly	1. Press the **Abort** button. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. Do not rub or wipe the chip socket. 3. Rinse 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 begin the experiment. 10. If the chip leaks again, clean the chip and chip socket as described above. Continued leaking may indicate a chip clamp or socket problem. Contact Technical Support.

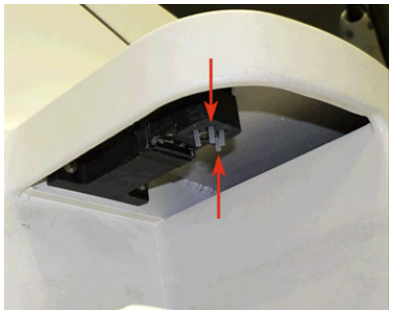
Observation	Possible cause	Recommended action
Error message: Calibration FAILED	• Chip not seated in socket correctly • Chip is damaged	1. Remove the chip and check for leaks and/or debris on the chip socket, following the procedures described in "Chip Check fails" and/or "Leak of unknown origin," above. 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.

Ion PGM™ System 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. 1. 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. 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 not seated in socket correctly • Damaged chip • Loose Sipper	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 may be clogged	1. Press the **Troubleshoot** button. **Note:** You may choose to 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 from around the waste line.  5. Press **Next** to begin 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 are 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.
	Damaged chip	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**.

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. **Note:** The new chip can be used for sequencing after initialization completes. 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 Appendix C, "Manually adjust W2 pH".
Error message: W2 out of range	• Chip measurements 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	• Poor water quality • 18 MΩ water exposed to air for too long • Incorrect solution added to the Wash 2 Bottle • Too little NaOH added to the Wash 1 Bottle • Damaged chip	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 80 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.

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 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. 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	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.

ISP Quality Control assay troubleshooting table

The following table contains a troubleshooting information for the unenriched Ion Sphere™ Quality Control 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 Agencourt® library using 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.

Supplementary procedures

Section: Quality control of Ion PGM™ IC 200 Ion Sphere™ Particles

Quality Control assay

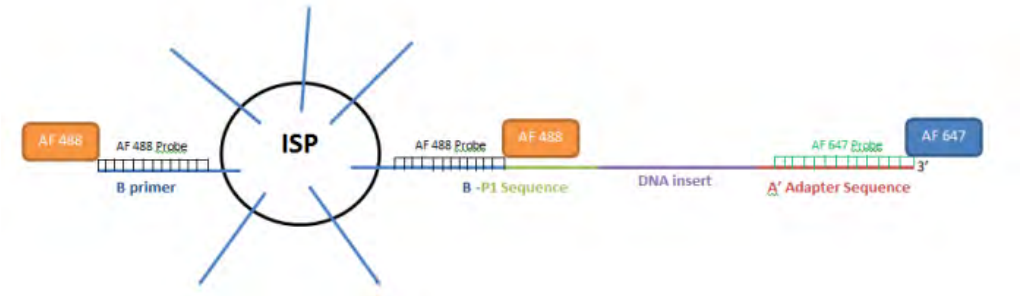
The Ion Sphere™ Quality Control assay on the Qubit® 2.0 or Qubit® 3.0 Fluorometer measures the fluorescence of template-positive Ion Sphere™ Particles (ISPs) labeled with two fluorophores: Alexa Fluor® 488 and Alexa Fluor® 647.

- A probe labeled with Alexa Fluor® 488 anneals to primer B sites, or all of the ISPs present.
- A probe labeled with Alexa Fluor® 647 anneals to primer A sites, or only the ISPs with extended templates.

The ratio of the Alexa Fluor® 647 fluorescence (templated ISPs) to the Alexa Fluor® 488 fluorescence (all ISPs present) yields the % templated ISPs.

The following is the schematic of the probes labeled Alexa Fluor® 488 and Alexa Fluor® 647 annealed to an ISP:

Note: The drawing is not to scale.



Quality control using the Qubit® 2.0 or Qubit® 3.0 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 200 base-read sequencing on the Ion PGM™ Sequencer. For details, see "Quality control using the Qubit® 2.0 Fluorometer" on page 85 or "Quality control using the Qubit® 3.0 Fluorometer" on page 98.

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 200 base-read sequencing on the Ion PGM™ Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Ion PGM™ IC Reagents 200 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 *Ion Sphere™ Particles (ISPs) Quality Assessment Using the Guava® easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. no. 4470082), available on the Ion Community website:

ioncommunity.lifetechnologies.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 200 base-read sequencing on the Ion PGM™ Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the IC 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 on the Ion Community website:

ioncommunity.lifetechnologies.com

IMPORTANT! Life Technologies Demonstrated Protocols have been successfully verified by Life Technologies for research use. There are no technical specifications for Life Technologies Demonstrated Protocols. Users assume all risk when using these protocols, and recognize that support for Life Technologies Demonstrated Protocols occurs through community discussion. All customers are encouraged to discuss and contribute via the Ion Community.

Section: Quality control using the Qubit® 2.0 Fluorometer

IMPORTANT! Effective 15 February 2012, the Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards replace the spectrally similar FAM™ and Cy®5 dyes, respectively, previously used in the kit. To update the existing plugin on the Qubit® 2.0 Fluorometer with the new dye names, install the new plugin file, Ion_PluginV310_AF.qbt.

Both sets of dye-labeled calibration standards can be used in conjunction with firmware version 3.00 and previous versions of plugin files (ion_plugin.qbt or Ion_plugin_AF.qbt).

We highly recommend that you install the new firmware version 3.10 and the new Ion plugin file (Ion_PluginV310_AF) for ease of use as these new versions allow you to operate the Ion plugin on the Qubit® 2.0 Fluorometer without requiring the USB drive Log to be connected into the device at all times.

The selection options on the user interface of the instrument matches the new Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards once the new plugin file is installed.

Materials required

- Qubit® 2.0 Fluorometer (Cat. no. Q32866) with the V3.10 firmware and the Ion Sphere™ Quality Control assay
- Qubit® Assay Tubes (Cat. no. Q32856)
- PCR tubes, 0.2 mL (Axygen Cat. no. PCR-02-L-C or BioExpress Cat. no. T-3035-1)
- Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file containing the instrument specific Calibration Factor
- Ion Sphere™ Quality Control Kit (Cat. no. 4468656)
- Unenriched Ion Sphere™ Particles
- GeneAmp® PCR System 9700 thermal cycler (Cat. no. N8050200) or equivalent

Upgrade the Qubit® 2.0 Fluorometer firmware and software

This section provides information about upgrading the firmware and the software of the Qubit® 2.0 Fluorometer. The following illustration summarizes the upgrade path for each firmware version.

Qubit® 2.0 Fluorometer Firmware v2.00 or v3.00

To a USB drive, download the program and upgrade files (Ion_PluginV310_AF.qbt, V3.10 Qubit_FW_MainCPU.bin, and V3.10 Qubit_FW_UsbHost.bin).

▼

Power on the Qubit® 2.0 Fluorometer and connect the USB drive.

▼

Touch **V2.00** or **V3.00**.

▼

Touch **Update**.

▼

When prompted, touch **Yes** to upload the Ion Sphere™ Quality Control Assay for V3.10.

Qubit® 2.0 Fluorometer Firmware v3.10

To a USB drive, download the program file (Ion_PluginV310_AF.qbt).

▼

Power on the Qubit® 2.0 Fluorometer and connect the USB drive.

▼

When prompted, touch **Yes** to upload the Ion Sphere™ Quality Control Assay for V3.10.

Upgrade for firmware V2.00 or V3.00

Note: The previous program files (.qbt) are not compatible with the new V3.10 firmware. The new program file (Ion_PluginV310_AF.qbt) is not compatible with V3.00 firmware.

Materials required for update

- Qubit® 2.0 Fluorometer (Part no. Q32866)
- USB drive (included with Qubit® 2.0 Fluorometer)
- Program (.qbt) and upgrade (.bin) files:
  - Ion_PluginV310_AF.qbt
  - V3.10 Qubit_FW_MainCPU.bin
  - V3.10 Qubit_FW_UsbHost.bin

Guidelines for downloading files

We recommend that you use a Windows® OS-based computer to download and transfer the Qubit® 2.0 Fluorometer files to the USB drive.

Note: Do not use a Macintosh® computer to transfer the files to the USB drive if the serial number of your Qubit® 2.0 Fluorometer is lower than 1104004846.

We also recommend that you use the USB drive provided with Qubit® 2.0 Fluorometer for file transfer.

Other compatible USB drives may also be used. Visit:**http://ioncommunity.lifetechnologies.com/community/products/pgm/** to see a list of approved USB drives. Logging into the Ion Community is required.

1. Download the program file (.qbt) and two upgrade files (.bin) from**http://ioncommunity.lifetechnologies.com/community/products/pgm/** onto a Windows®-based PC desktop. Logging into the Ion Community is required.
2. Remove the existing program file (.qbt) and two upgrade files (.bin) from the USB drive, if any.
3. Transfer the three downloaded files to the USB drive provided with the Qubit® 2.0 Fluorometer (recommended), or another compatible USB drive.

   Note: Files on the USB drive must be in the root directory and cannot be in a folder.

Upgrade the firmware from the previous versions

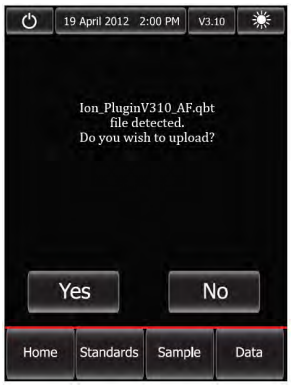
1. Power on the Qubit® 2.0 Fluorometer by plugging in the unit.
2. Insert the USB drive, containing the program (.qbt) and upgrade (.bin) files, into the USB port on the instrument.
3. In the upper-right corner of the main menu, touch **V2.00**, or **V3.00** depending on the version.
4. If the image of the USB drive in the update screen displays a green dot, touch Update to upgrade the instrument firmware.

   The firmware update lasts approximately 2 minutes, during which the Qubit® 2.0 Fluorometer screen flashes:

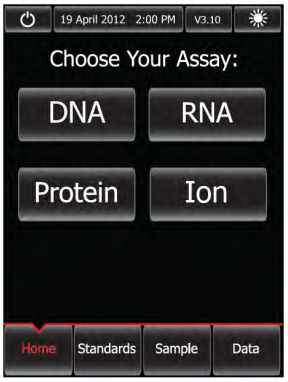
Note: If the image displays a red dot, the Qubit® 2.0 Fluorometer cannot detect the USB drive. Ensure the USB drive is in place securely. If the problem persists, remove the USB drive and reinsert.

5. When the Qubit® 2.0 Fluorometer displays the main screen, confirm that V3.10 is displayed (in the upper-right corner), which confirms that the upgrade was successful.

   After the firmware has been updated, the following screen will be displayed. Confirm that V3.10 is displayed in the upper-right corner. Touch **Yes** to permanently upload the Ion Sphere™ QC program to the instrument:

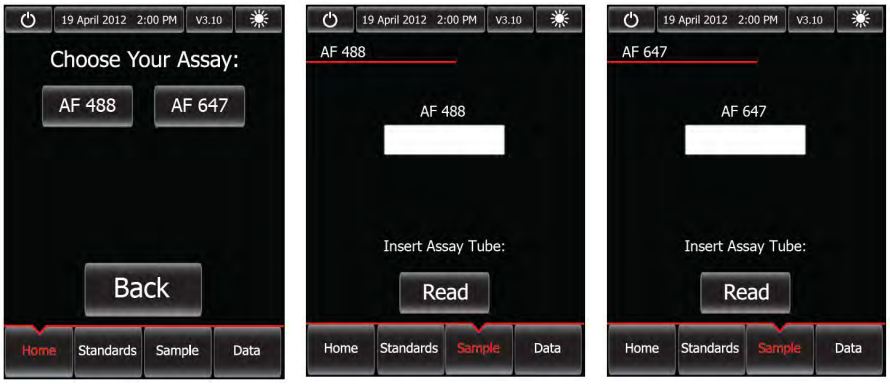


6. Confirm the Ion Sphere™ QC program (.qbt) file is functional by checking the following screens.
   a. In the main menu, the Ion selection option is present:



   b. After touching **Ion** (Ion), **AF 488** (AF 488) and **AF 647** (AF 647) selection options appear on the screen:

      Note: Touching AF 488 or AF 647 enters the respective measurement channels.



7. Proceed to complete the instruction in "Calculate the Qubit® 2.0 Fluorometer Calibration Factor" on page 91.

Upgrade for firmware V3.10

If your Qubit® 2.0 Fluorometer is equipped with V3.10 firmware, you *only* need to upload the Ion Sphere™ Quality Control assay into your fluorometer:

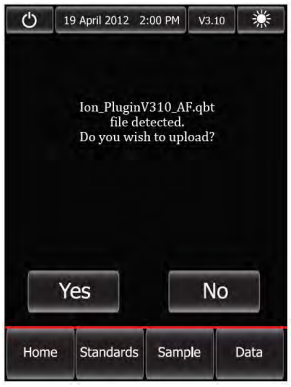
IMPORTANT! Do not re-upgrade the firmware if the instrument is shipped with V3.10 version.

To upload the Ion Sphere™ Quality Control assay into your fluorometer, use the following steps:

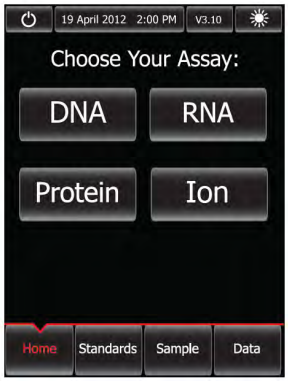
1. Download the new Ion_PluginV310_AF.qbt file (from:**http://ioncommunity.lifetechnologies.com/community/products/pgm/**) to your USB drive to activate the application to accept the new names. Logging into the Ion Community is required.

   Note: We recommend that you use the USB drive provided with Qubit® 2.0 Fluorometer for file transfer; however, other compatible USB drives may also be used. Visit:**http://ioncommunity.lifetechnologies.com/community/products/pgm/** to see a list of approved USB drives. Logging into the Ion Community is required.

2. With the USB drive (containing the Ion_PluginV310_AF.qbt file) inserted into the Qubit® 2.0 Fluorometer, power-cycle the instrument by unplugging and plugging it back in. Touch **Yes** to permanently upload the file to the instrument:

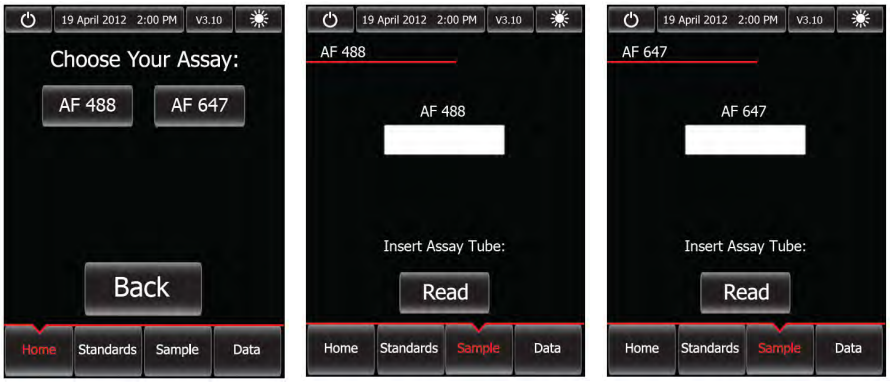


3. Confirm the Ion Sphere™ QC program (.qbt) file is functional by checking the following screens:
   a. In the main menu, the Ion selection option is present.



b. After touching **Ion** (Ion), **AF 488** (AF 488) and **AF 647** (AF 647) selection options appear on the screen:

Note: Touching AF 488 or AF 647 enters the respective measurement channels.



4. Proceed to "Calculate the Qubit® 2.0 Fluorometer Calibration Factor".

Calculate the Qubit® 2.0 Fluorometer Calibration Factor

This section describes the procedure to determine the Qubit® 2.0 Fluorometer instrument-specific Calibration Factor.

IMPORTANT! You must upgrade the Qubit® 2.0 Fluorometer firmware and software prior to performing the following procedure. See "Upgrade the Qubit® 2.0 Fluorometer firmware and software" on page 86 for more information.

Each Qubit® 2.0 Fluorometer has a unique Calibration Factor that must be calculated and applied to all Percent Templated ISPs calculations.

Note: It is only necessary to calculate the Calibration Factor once for a particular instrument, unless a problem is suspected.

Materials required

- Qubit® Easy Calculator Microsoft® Excel® Spreadsheet (Logging into the Ion Community is required.)
- Qubit® 2.0 Fluorometer (Part no. Q32866) with V3.10 firmware
- USB drive containing the ".qbt" file
- Qubit® Assay Tubes (Part no. Q32856)
- Ion Sphere™ Quality Control Kit (Cat. no. 4468656)

Download the Qubit® Easy Calculator

Download the Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file from: **http://ioncommunity.lifetechnologies.com/community/products/pgm/** (Logging into the Ion Community is required), and save the file to the computer used for Qubit® 2.0 Fluorometer data analysis.

Calibration standard preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standard reagents.

   Note: Both the Alexa Fluor® 488 and Alexa Fluor® 647 molecules are photosensitive, so avoid exposure to light for long periods of time and direct sunlight.

2. Vortex well to mix and pulse-spin the tube to remove any liquid trapped in the cap.

3. Transfer 200 µL of each standard into two separate Qubit® assay tubes. Pulse-spin to bring all the liquid to the bottom of the tube.

Calibration standard measurement

IMPORTANT! Prior to using the Qubit® 2.0 Fluorometer, ensure that the instrument is running Firmware V3.10, and the Ion_PluginV310_AF.qbt file has been permanently uploaded to the instrument. See section "Upgrade the Qubit® 2.0 Fluorometer firmware and software" on page 86 for more information about managing Qubit® 2.0 Fluorometer firmware versions.

1. Touch **Ion** to access Alexa Fluor® 488 and Alexa Fluor® 647 measurement options.

2. Touch **AF 488** and insert the Alexa Fluor® 488 Calibration Standard reagent into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   Note: The lettering on the Read (Read) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

3. Record the RFU value and remove sample from Qubit® 2.0 Fluorometer.

4. Touch **Home**, touch **Ion**, and then touch **AF 647**. Insert the Alexa Fluor® 647 Calibration Standard into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   Note: The lettering on the Read (Read) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

5. Record the RFU value and remove sample from Qubit® 2.0 Fluorometer.

Calibration Factor calculation

1. In the Qubit® Easy Calculator, enter each recorded RFU value in the appropriately labeled green cell to display the Calibration Factor specific for the Qubit® 2.0 Fluorometer.

2. Save a copy of the Qubit® Easy Calculator containing the Calibration Factor for use as a template for future Percent Templated ISPs calculations:

   Note: Affix a sticker with the instrument-specific Calibration Factor to the Qubit® 2.0 Fluorometer.

Qubit Calibration Factor Calculation

Calibration Standard	RFU	Calibration Factor
Alexa Fluor® 488 Calibration Standard		#DIV/0!
Alexa Fluor® 647 Calibration Standard		

Percent Templated ISPs

	Raw RFU Value		Background RFU (Negative Control Tube)			
Sample ID	AF 488	AF 647	AF 488	AF 647	Conversion Factor*	Percent Templated ISPs
						#DIV/0!
						#DIV/0!
						#DIV/0!
						#DIV/0!

* Conversion factor can be found on Ion Community website (http://ioncommunity.lifetechnologies.com/docs/DOC-9093) and is Template Kit lot specific.

Green Cells = Raw RFU values of FAM and Cy5 Calibration Standards supplied in the Ion Sphere Quality Control Kit
Red Cells = Raw RFU values measured in "Measure the templated unenriched sample"
Purple Cells = Raw RFU values measured for negative control in "Measure the templated unenriched sample"
Blue Cells= Template kit lot specific conversion factor

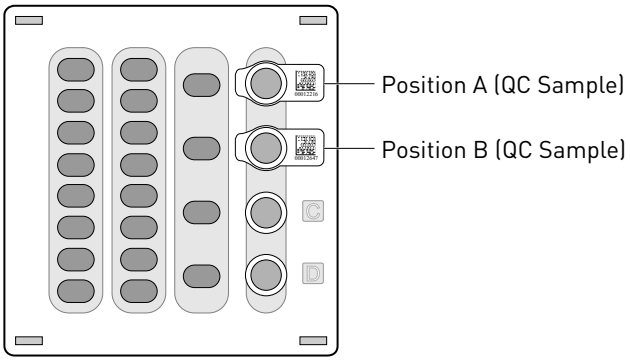
IMPORTANT! For each Qubit® 2.0 Fluorometer used, save a separate Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for that particular instrument.

Measure the templated unenriched sample

This section describes the procedure for determining the percent templated ISPs for unenriched Ion Sphere™ Particles.

Sample preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion Probes tube, Annealing Buffer, and Quality Control Wash Buffer.
2. Centrifuge the samples taken from Positions A and B on the IC Reagents Cartridge at 15000 RCF for 2 minutes to reduce the total volume per sample to approximately 10 µL.



Note: If you want use both Guava® flow cytometry and Qubit® fluorometry for quality assessment, remove 1 µL of the sample prior to centrifugation for Guava® analysis before processing the remainder for Qubit® analysis.

Note: If you are assessing quality after completion of the Ion Chef™ run, you may remove the Library Sample Tubes from the reagents cartridge and centrifuge the QC samples in these tubes.

3. Pipet each sample up and down to mix and then transfer each sample to a new labeled 0.2-mL PCR tube.
4. Add 10 µL Annealing Buffer and 1 µL Ion Probes directly to each 0.2-mL PCR tube containing the ISPs, then mix well by pipetting up and down.

   Note: If processing multiple samples, generate an Ion Probe Master Mix as follows:
   (10 µL Annealing Buffer × # samples) + (1 µL Ion Probes × # samples) = total volume required
5. Load the tube(s) into a thermal cycler, then perform the following protocol to anneal the Ion Probes:

Stage	Temperature	Time
Hold	95ºC	2 min
Hold	37ºC	2 min

6. Remove unbound probes by washing the sample(s) three times with 200 µL of Quality Control Wash Buffer.
   a. Add 200 µL of Quality Control Wash Buffer to the 0.2-mL tube(s).

b. Vortex properly to mix and centrifuge at 15,500 × *g* for 1.5 minutes.

c. Being careful not to disturb the pelleted ISPs, remove the supernatant and leave behind 10 µL.

   Note: Compare to a 10 µL standard for reference.

d. Repeat substep a through substep c two times for a total of three Quality Control Wash Buffer washes.

7. After the final wash, add 190 µL of Quality Control Wash Buffer for a total volume of 200 µL, mix by pipetting up and down five times and transfer the entire sample to a Qubit® assay tube.

   IMPORTANT! Ensure that you measure the volumes accurately.

8. To generate a negative control, add 200 µL of Quality Control Wash Buffer to a fresh Qubit® assay tube.

9. Read the sample(s) as described below.

Sample measurement

IMPORTANT! Prior to using the Qubit® 2.0 Fluorometer, ensure that the instrument is running Firmware V3.10, and the Ion_PluginV310_AF.qbt file has been permanently uploaded to the instrument. See section "Upgrade the Qubit® 2.0 Fluorometer firmware and software" on page 86 for more information about managing Qubit® 2.0 Fluorometer firmware versions.

1. Power on the Qubit® 2.0 Fluorometer.

2. Touch **Ion** to access Alexa Fluor® 488 and Alexa Fluor® 647 measurement options.

3. Touch **AF 488** and insert the sample into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   Note: The lettering on the Read (Read) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

   Note: If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

4. Record the value.

   Note: The data retained on the Qubit® 2.0 Fluorometer can be transferred to a USB drive. See the "(Optional) Data Transfer to USB Drive" on page 96 for details. If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

5. Touch **Home**, touch **Ion**, and then touch **AF 647**. Insert the sample into the Qubit® 2.0 Fluorometer, close the lid, and touch **Read**.

   Note: The lettering on the Read (Read) selection option changes from white to red when reading a sample. The lettering changes back to white when the reading of the sample is finished in approximately 5 seconds.

6. Record the value.

IMPORTANT! Ensure that you read the negative control (Quality Control Wash Buffer only) in both the Alexa Fluor® 488 and Alexa Fluor® 647 settings and record the RFU values.

(Optional) Data Transfer to USB Drive

1. Ensure that the USB drive is inserted in the instrument.
2. In the main menu, touch Data (at the bottom-right corner of the screen).
3. In the data screen, touch **USB drive**, then wait for the instrument to download the data to the USB drive:

   Note: The download creates a ".csv" file that can be opened on your computer using any spreadsheet software, such as Microsoft® Excel® software.

Templated ISP evaluation

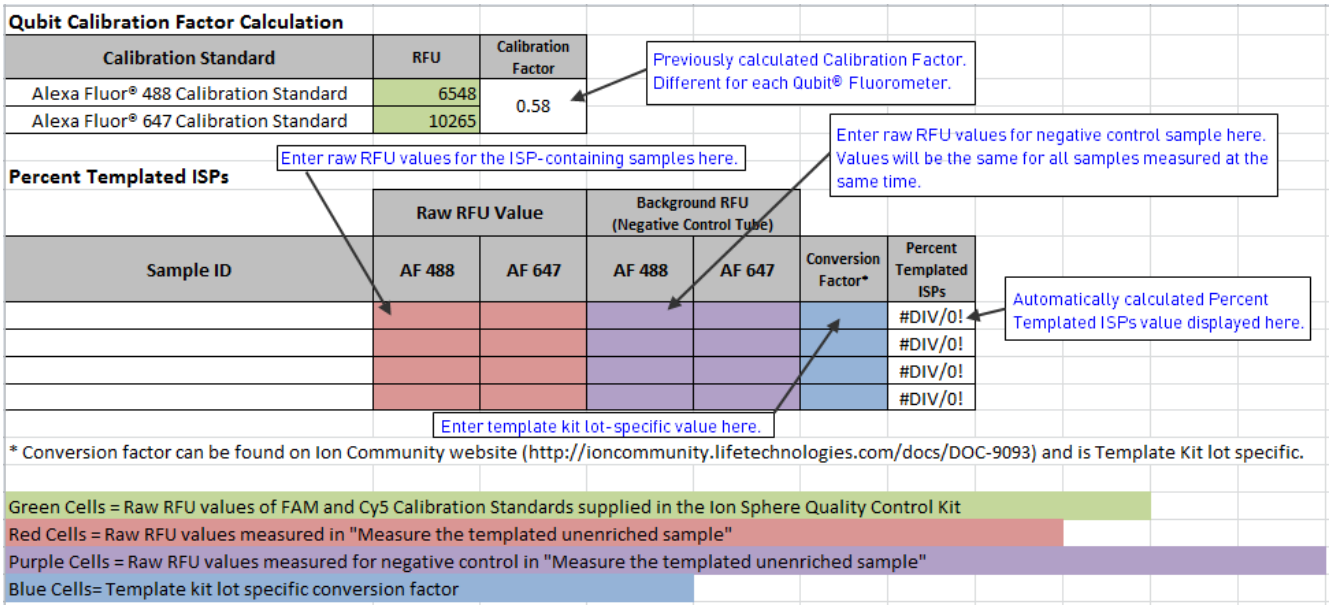
1. Open the saved Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for the Qubit® Fluorometer used.
2. Enter the raw RFU values from Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards measurements in the appropriate fields for both the ISPs containing samples (red cells) and negative control sample (purple cells).

IMPORTANT! The Alexa Fluor® 488 value must be >100 counts to produce a valid % Templated ISPs value. If the Alexa Fluor® 488 value is <100 counts, see the "ISP Quality Control assay troubleshooting table" on page 81.

Fluorophore	Acceptable RFU range
Alexa Fluor® 488	>100 counts; no upper limit Samples with <100 counts usually correlate with no or very few ISPs in the assay.
Alexa Fluor® 647	Any value, with the condition that the Alexa Fluor® 488 RFU value is >100 counts.

3. In the appropriate field (blue cells), enter the lot-specific conversion factor for unenriched Ion Sphere™ Particles, available at: **http://ioncommunity.lifetechnologies.com/docs/DOC-9093**. (Log in is required.)

4. The Percent Templated ISPs calculates automatically and is displayed for each sample:



Acceptance criteria for unenriched Ion Sphere™ Particles

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 "ISP Quality Control assay troubleshooting table" on page 81 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).

Section: Quality control using the Qubit® 3.0 Fluorometer

The Qubit® 3.0 Fluorometer features a pre-loaded Ion Sphere™ quality control assay for determining the percentage of templated ISPs. As with the Qubit® 2.0 Fluorometer, a unique instrument-specific Calibration Factor must be calculated and applied to all percent templated ISP calculations.

Materials required

- Qubit® 3.0 Fluorometer (Cat. no. Q33216)
- Qubit® Assay Tubes (Cat. no. Q32856)
- PCR tubes, 0.2 mL (Axygen Cat. no. PCR-02-L-C or BioExpress Cat. no. T-3035-1)
- Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file containing the instrument-specific Calibration Factor
- Ion Sphere™ Quality Control Kit (Cat. no. 4468656)
- Unenriched Ion Sphere™ Particles
- GeneAmp® PCR System 9700 thermal cycler (Cat. no. N8050200) or equivalent

Calculate the Qubit® 3.0 Fluorometer Calibration Factor

This section describes the procedure to determine the Qubit® 3.0 Fluorometer instrument-specific Calibration Factor.

Note: It is only necessary to calculate the Calibration Factor once for a particular instrument, unless a problem is suspected.

Download the Qubit® Easy Calculator

Download the Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file from: **http://ioncommunity.lifetechnologies.com/community/products/pgm/** (Logging into the Ion Community is required), and save the file to the computer used for Qubit® 3.0 Fluorometer data analysis.

Calibration standard preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standard reagents.

   Note: Both the Alexa Fluor® 488 and Alexa Fluor® 647 molecules are photosensitive, so avoid exposure to light for long periods of time and direct sunlight.

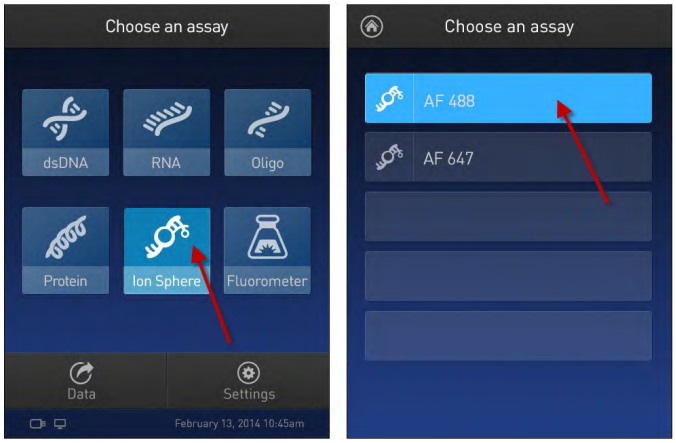
2. Vortex well to mix and pulse-spin the tube to remove any liquid trapped in the cap.

3. Transfer 200 µL of each standard into two separate Qubit® assay tubes. Pulse-spin to bring all the liquid to the bottom of the tube.

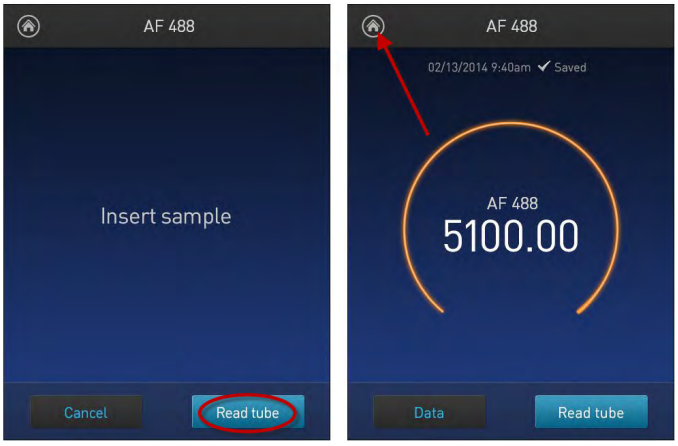
Calibration standard measurement

1. Power on the Qubit® 3.0 Fluorometer.

2. Touch **Ion Sphere** on the Qubit® 3.0 Fluorometer home screen open the Ion Sphere™ Assay. Touch **AF 488**.

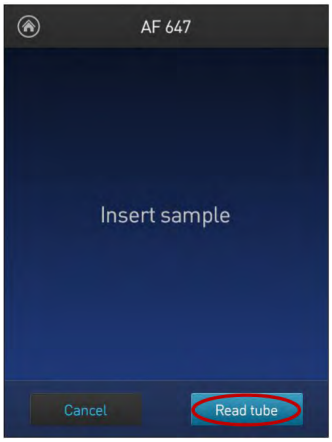


3. Insert the Alexa Fluor® 488 Calibration Standard reagent into the Qubit® 3.0 Fluorometer, close the lid, and touch **Read tube** (Read tube).

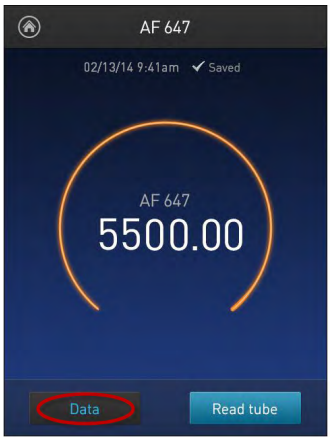


4. Record the RFU value, remove the assay tube from the Qubit® 3.0 Fluorometer and touch the **Home** icon in the upper left corner of the screen.

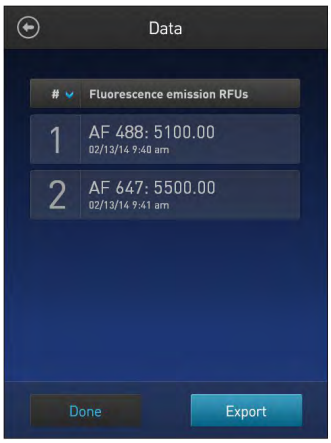
5. On the Home screen, touch **Ion Sphere**, and then touch **AF 647**. Insert the Alexa Fluor® 647 Calibration Standard into the Qubit® 3.0 Fluorometer, close the lid, and touch **Read tube** (Read tube).



6. Record the RFU value, remove the assay tube from the Qubit® 3.0 Fluorometer. Touch **Data** (Data).



7. Touch **Export** (Export) to export data to a USB storage drive or to a USB-connected computer. Touch **Done** (Done) to return to the Home screen.



Calibration Factor calculation

1. In the Qubit® Easy Calculator, enter each recorded RFU value in the appropriately labeled green cell to display the Calibration Factor specific for the Qubit® 3.0 Fluorometer.

2. Save a copy of the Qubit® Easy Calculator containing the Calibration Factor for use as a template for future Percent Templated ISPs calculations:

   Note: Affix a sticker with the instrument-specific Calibration Factor to the Qubit® 3.0 Fluorometer.

Qubit Calibration Factor Calculation

Calibration Standard	RFU	Calibration Factor
Alexa Fluor® 488 Calibration Standard		#DIV/0!
Alexa Fluor® 647 Calibration Standard		

Percent Templated ISPs

	Raw RFU Value		Background RFU (Negative Control Tube)			
Sample ID	AF 488	AF 647	AF 488	AF 647	Conversion Factor*	Percent Templated ISPs
						#DIV/0!
						#DIV/0!
						#DIV/0!
						#DIV/0!

* Conversion factor can be found on Ion Community website (http://ioncommunity.lifetechnologies.com/docs/DOC-9093) and is Template Kit lot specific.

Green Cells = Raw RFU values of FAM and Cy5 Calibration Standards supplied in the Ion Sphere Quality Control Kit
Red Cells = Raw RFU values measured in "Measure the templated unenriched sample"
Purple Cells = Raw RFU values measured for negative control in "Measure the templated unenriched sample"
Blue Cells= Template kit lot specific conversion factor

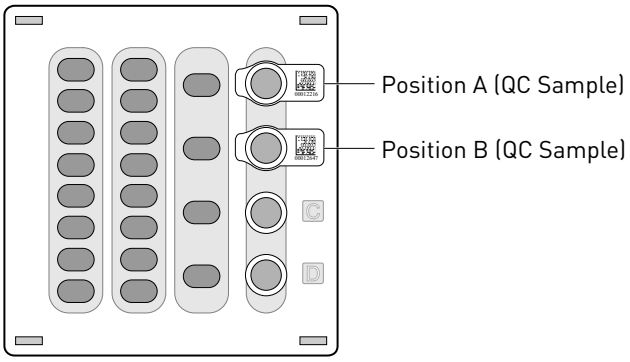
IMPORTANT! For each Qubit® 3.0 Fluorometer used, save a separate Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for that particular instrument.

Measure the templated unenriched sample

This section describes the procedure for determining the percent templated ISPs for unenriched Ion Sphere™ Particles.

Sample preparation

1. From the Ion Sphere™ Quality Control Kit, thaw the Ion Probes tube, Annealing Buffer, and Quality Control Wash Buffer.
2. Centrifuge the samples taken from Positions A and B on the IC Reagents Cartridge at 15000 RCF for 2 minutes to reduce the total volume per sample to approximately 10 µL.



Note: If you want use both Guava® flow cytometry and Qubit® fluorometry for quality assessment, remove 1 µL of the sample prior to centrifugation for Guava® analysis before processing the remainder for Qubit® analysis.

Note: If you are assessing quality after completion of the Ion Chef™ run, you may remove the Library Sample Tubes from the reagents cartridge and centrifuge the QC samples in these tubes.

3. Pipet each sample up and down to mix and then transfer each sample to a new labeled 0.2-mL PCR tube.
4. Add 10 µL Annealing Buffer and 1 µL Ion Probes directly to each 0.2-mL PCR tube containing the ISPs, then mix well by pipetting up and down.

   Note: If processing multiple samples, generate an Ion Probe Master Mix as follows:
   (10 µL Annealing Buffer × # samples) + (1 µL Ion Probes × # samples) = total volume required
5. Load the tube(s) into a thermal cycler, then perform the following protocol to anneal the Ion Probes:

Stage	Temperature	Time
Hold	95ºC	2 min
Hold	37ºC	2 min

6. Remove unbound probes by washing the sample(s) three times with 200 µL of Quality Control Wash Buffer.
   a. Add 200 µL of Quality Control Wash Buffer to the 0.2-mL tube(s).

b. Vortex properly to mix and centrifuge at 15,500 × *g* for 1.5 minutes.

c. Being careful not to disturb the pelleted ISPs, remove the supernatant and leave behind 10 µL.

   Note: Compare to a 10 µL standard for reference.

d. Repeat substep a through substep c two times for a total of three Quality Control Wash Buffer washes.

7. After the final wash, add 190 µL of Quality Control Wash Buffer for a total volume of 200 µL, mix by pipetting up and down five times and transfer the entire sample to a Qubit® assay tube.

   IMPORTANT! Ensure that you measure the volumes accurately.

8. To generate a negative control, add 200 µL of Quality Control Wash Buffer to a fresh Qubit® assay tube.

9. Read the sample(s) as described below.

Sample measurement

1. Power on the Qubit® 3.0 Fluorometer.

2. Touch **Ion Sphere** to access Alexa Fluor® 488 and Alexa Fluor® 647 measurement options.

3. Touch **AF 488** and insert the sample into the Qubit® 3.0 Fluorometer, close the lid, and touch **Read tube**.

   Note: If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

4. Record the value.

   Note: The data retained on the Qubit® 3.0 Fluorometer can be transferred to a USB drive. See the "(Optional) Data transfer to USB drive or computer" for details. If more than one sample is being processed, all samples can be read with the AF 488 setting before moving on to the AF 647 setting.

5. Touch **Home**, touch **Ion Sphere**, and then touch **AF 647**. Insert the sample into the Qubit® 3.0 Fluorometer, close the lid, and touch **Read tube**.

6. Record the value.

   IMPORTANT! Ensure that you read the negative control (Quality Control Wash Buffer only) in both the Alexa Fluor® 488 and Alexa Fluor® 647 settings and record the RFU values.

(Optional) Data transfer to USB drive or computer

1. Ensure that the USB drive is inserted in the instrument, or a computer is connected by USB cable.
2. On the Home screen, touch **Data** (at the bottom-left of the screen).
3. On the Data screen, touch **Export**, then wait for the instrument to download the data to the USB drive or computer.

   Note: The download creates a ".csv" file that can be opened on your computer using any spreadsheet software, such as Microsoft® Excel® software.

Templated ISP evaluation

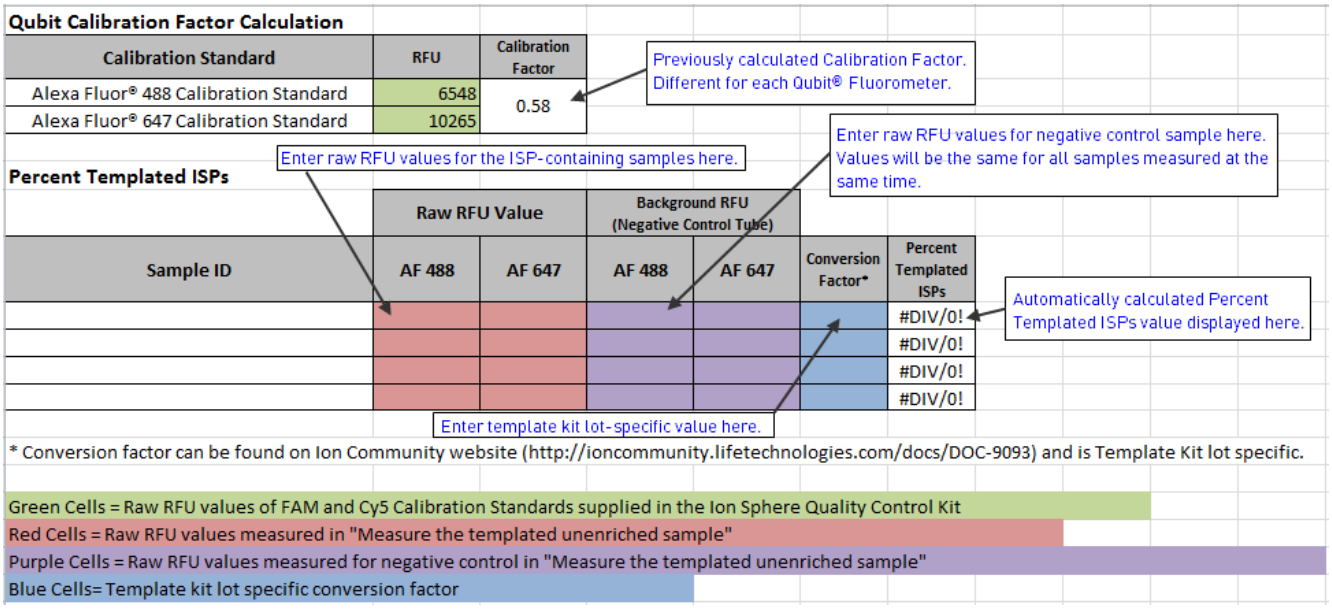
1. Open the saved Qubit® Easy Calculator Microsoft® Excel® Spreadsheet file containing the Calibration Factor specifically calculated for the Qubit® Fluorometer used.
2. Enter the raw RFU values from Alexa Fluor® 488 and Alexa Fluor® 647 Calibration Standards measurements in the appropriate fields for both the ISPs containing samples (red cells) and negative control sample (purple cells).

   IMPORTANT! The Alexa Fluor® 488 value must be >100 counts to produce a valid % Templated ISPs value. If the Alexa Fluor® 488 value is <100 counts, see the "ISP Quality Control assay troubleshooting table" on page 81.

Fluorophore	Acceptable RFU range
Alexa Fluor® 488	>100 counts; no upper limit Samples with <100 counts usually correlate with no or very few ISPs in the assay.
Alexa Fluor® 647	Any value, with the condition that the Alexa Fluor® 488 RFU value is >100 counts.

3. In the appropriate field (blue cells), enter the lot-specific conversion factor for unenriched Ion Sphere™ Particles, available at: **http://ioncommunity.lifetechnologies.com/docs/DOC-9093**. (Log in is required.)

4. The Percent Templated ISPs calculates automatically and is displayed for each sample:



Acceptance criteria for unenriched Ion Sphere™ Particles

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 "ISP Quality Control assay troubleshooting table" on page 81 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).

Section: Maintain the Ion Chef™ System

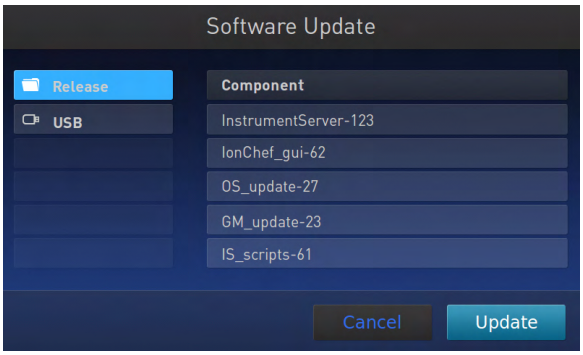
The Ion Chef™ System supports the user-performed maintenance procedures found in this section.

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. Occasionally, Life Technologies may 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 the Life Technologies 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.

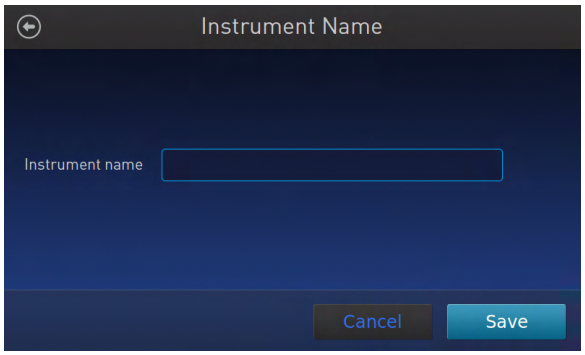
IMPORTANT! If you are updating the firmware manually, remove the USB drive before powering on the Ion Chef™ Instrument.

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.

C

Manually adjust W2 pH

Materials and equipment needed

- Orion® 3-Star Plus pH Benchtop Meter Kit or equivalent
- Nitrogen gas tank, tube, and flow meter
- 100 mM NaOH (prepared fresh daily)
- Pipette tips and pipette
- Magnetic stirrer and stir bar
- 100 mM HCl

Procedure

If an error message during the automatic pH process indicates that there is a problem adjusting the pH of the W2 Solution, use the following procedure to manually adjust the pH of the W2 Solution in the Wash 2 Bottle.

1. Before proceeding, rinse an empty Wash 2 Bottle and have it ready next to the instrument. Also have an additional Wash 2 Bottle cap ready.

   Note: Gas will be flowing out of the Wash 2 cap, so perform the next steps as quickly as possible (flowing gas will not harm the W2 Solution, and is not a hazard).

2. Remove the Wash 2 Bottle attached to the instrument, and cap the bottle.

3. Secure the empty Wash 2 Bottle (from step 1) to the instrument—do not remove the sipper. This bottle will contain the gas flowing out of the instrument while you pH the W2 Solution and protect the sipper from contamination.

4. Move the Wash 2 Bottle containing the W2 Solution to the stir plate near the nitrogen gas tube.

5. Secure the gas tube so that it extends inside the mouth of the Wash 2 Bottle but not below the surface of the W2 Solution.

6. Set the gas flow to 0.5 lpm. Start mixing the W2 Solution fast enough for a small whirlpool to form.

7. Calibrate the pH meter using a three-point calibration. Rinse any buffering solution from the pH probe prior to preparing solutions.

8. Adjust the pH of the W2 Solution to 7.55 ± 0.1 by adding a small amount of freshly prepared 100 mM NaOH to the solution, and then measuring the pH using the pH meter. Add small aliquots and allow the pH to equilibrate before adding more.

   Note: If the pH rises above 7.75, use 100 mM hydrochloric acid (HCl) to readjust the pH to 7.55 ± 0.1.

9. When the pH is stable, turn off the gas, remove the gas line, and cap the Wash 2 Bottle.

10. Move the bottle to the instrument, remove the empty Wash 2 Bottle from the instrument, and place the sipper inside the Wash 2 Bottle whose pH adjusted.

11. Secure the cap firmly. Press **Next** to exit the automated pH check and continue with instrument initialization.

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, the release of Torrent Suite™ v4.4 Software will require the reconfiguration of the Ion Chef™ instrument to communicate with only one primary Torrent Server. After upgrading to Torrent Suite v4.4, 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.

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 LAN.
- Enable Planned Run sharing.

Note: If you added an Ion Chef™ instrument to your LAN and your Torrent Servers have Torrent Suite™ software version 4.2, upgrading your Torrent Servers to software version 4.4 will require Planned Run sharing to see more than one Torrent Server. The Ion Chef™ instrument is now programmed to see only one primary Torrent Server (previously, it could see two). Configuring Planned Run sharing on Torrent Servers will allow your Ion Chef™ instrument to share planned runs with multiple other Torrent Servers (and ultimately, sequencers). This requires the primary Torrent Server to be chosen as the Ion Chef™ server and it will be the starting point (origin server). Once chosen and configured, this Torrent Server can be programmed to share Planned Runs with multiple other Torrent Servers, hence allowing an Ion Chef™ instrument to share Planned Runs with multiple servers.

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 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™, or Ion Proton™ 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

Consult a network administrator.

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

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.

Network setup examples

Note: 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 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.

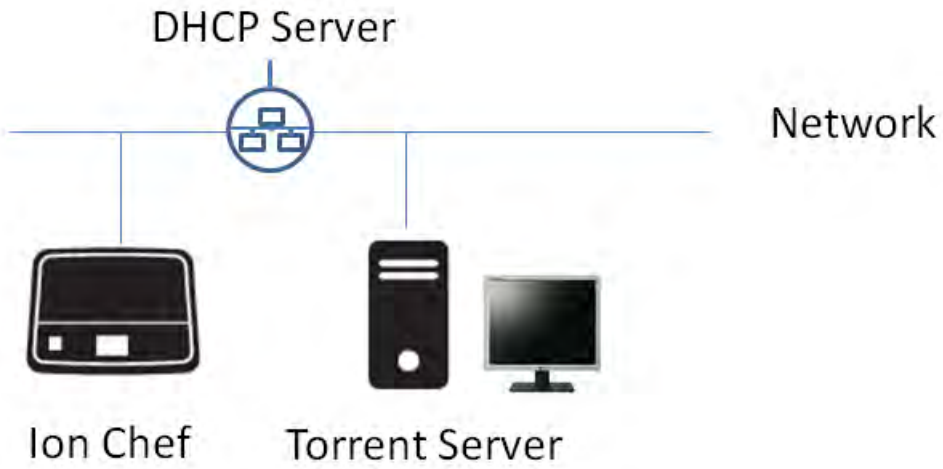
Materials required

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

General requirements

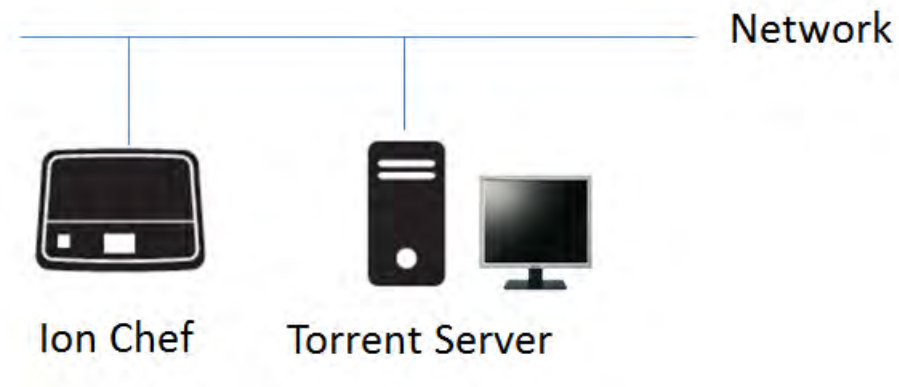
See *Ion Chef™ System Site Preparation Guide* (Pub. no. MAN0007956).

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 and ensure that all the below requirements are met.

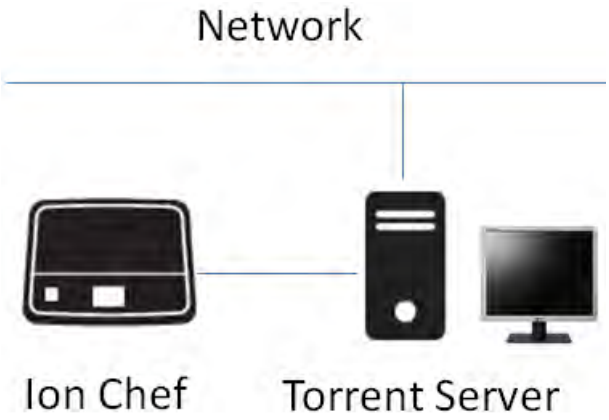
Static IP



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

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

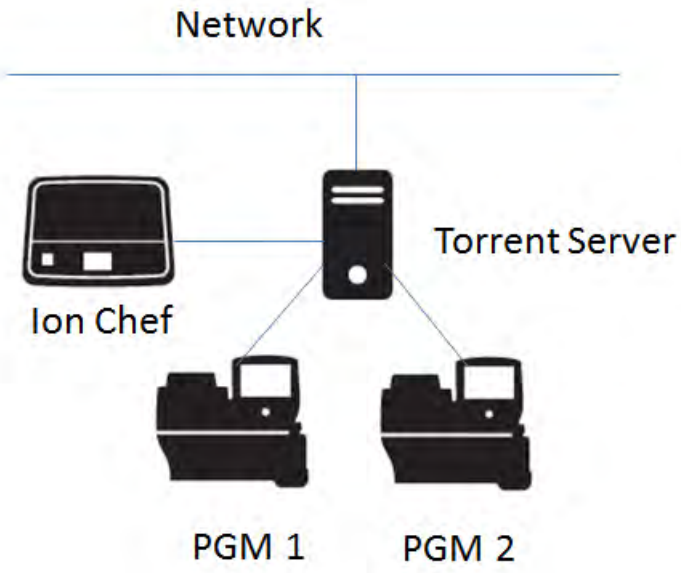
Direct connection



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 Ion PGM™ 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.

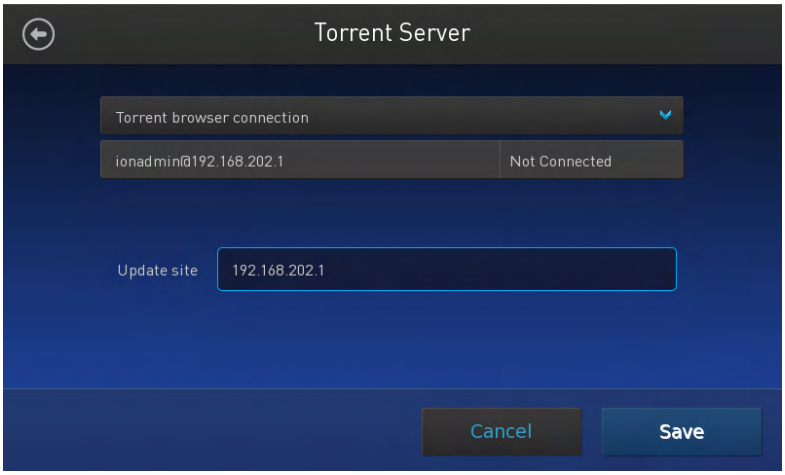
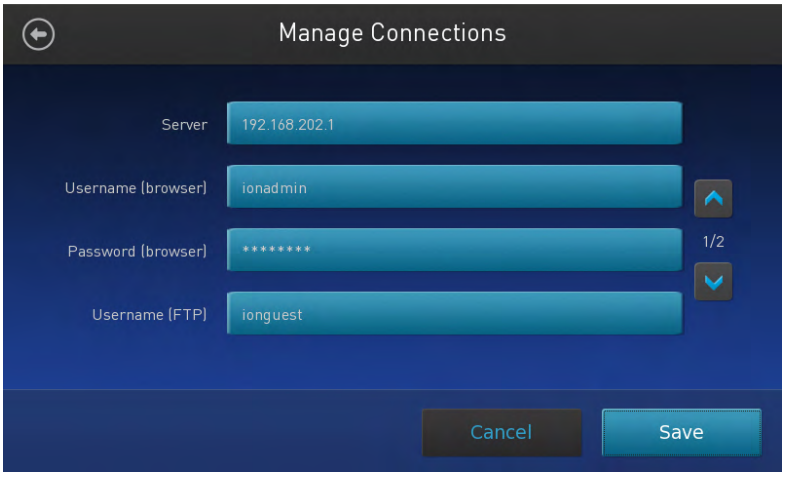


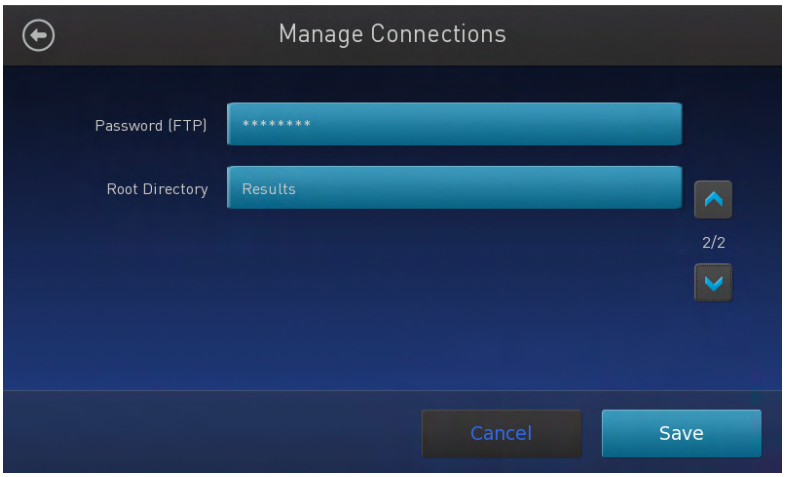
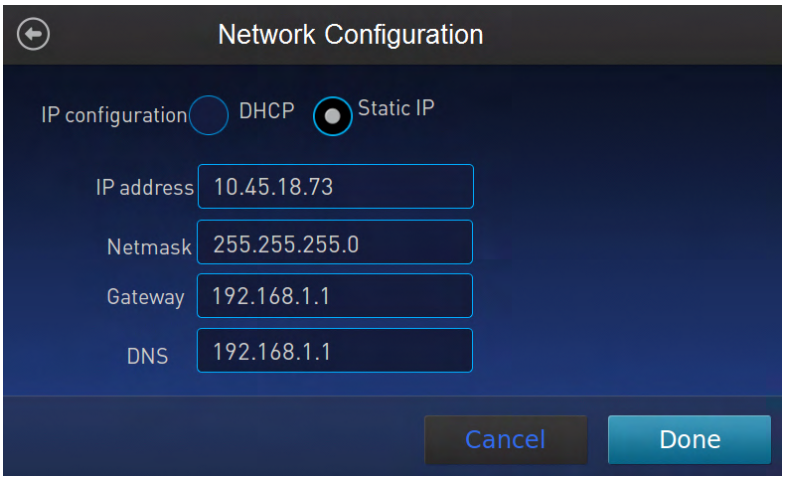
Ion Chef™ Networking Configuration Options

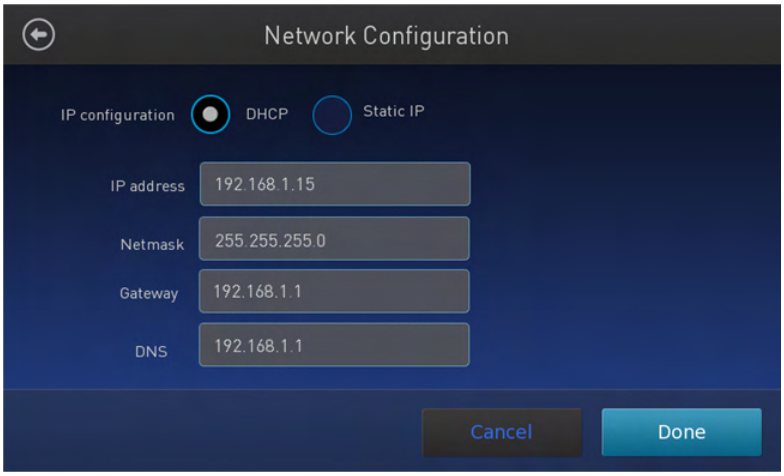
With the Torrent Suite™ software v4.4 release, the Ion Chef™ software will only connect to one Torrent Server. From that server, Planned Run sharing can be configured to access additional servers. When the Ion Chef™ is connected to a Torrent Server, an icon on the lower left side of the screen appears.

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.

Option	Description
Direct connection	1. Verify Torrent Server connection on the Ion Chef™ Instrument. 2. The Ion Chef™ Instrument can plug directly into one of the available ports on the back of the Torrent Server. The ports have the address 192.168.201 (202, 203).1 according to which port you are connected to. 

Option	Description
	3. Configure the Ion Chef™ Instrument. 4. Go to **Settings** > **Torrent Server**. 5. Verify the Torrent Server settings are correct. The Torrent Browser URL will be the IP address of the port you are connected to on the back of the Torrent Server (192.168.201(or .202, or .203).1 and ensure that the browser password is correct.  6. The FTP address is now directly linked to the above address . 7. Set Password to customer's ionadmin account password. 

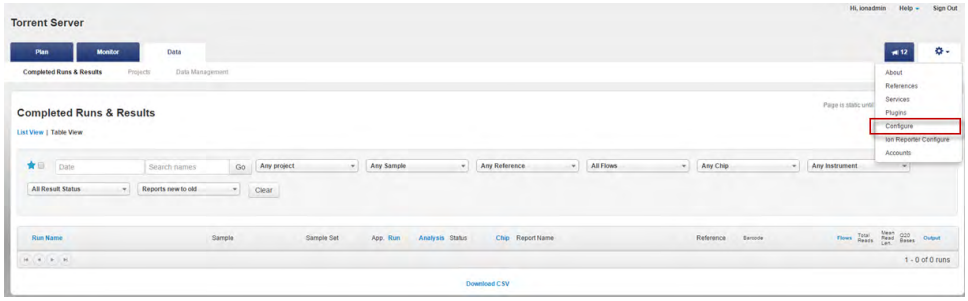
Option	Description
	8. Enter ionguest as the FTP password. 
Static IP	1. If you are going to connect to the Torrent Server through the company LAN configured as a Static IP, then you will need to configure the customer provided Static IP, Gateway, Netmask, and DNS Server Names. (Only one DNS server is required for the Ion Chef™ Instrument.) 2. Go to**Settings** ▸ **Instrument Settings** ▸ **Network** ▸ **Configuration**. 3. Select the **Static IP** radio button. 4. Enter the addresses and press **Done**. 
DHCP IP (non-direct)	1. To connect to the Torrent Server through the company LAN, you will need to target ServerPortIP (Eth1). With LAN set to DHCP, the IP, Gateway, Netmask, and DNS Name Servers should populate automatically. 2. Go to**Settings** ▸ **Instrument Settings** ▸ **Network Configuration** ▸ . 3. Verify the **DHCP** radio button is selected.

Option	Description
	4. Confirm the information is correct. Press **Done**. 

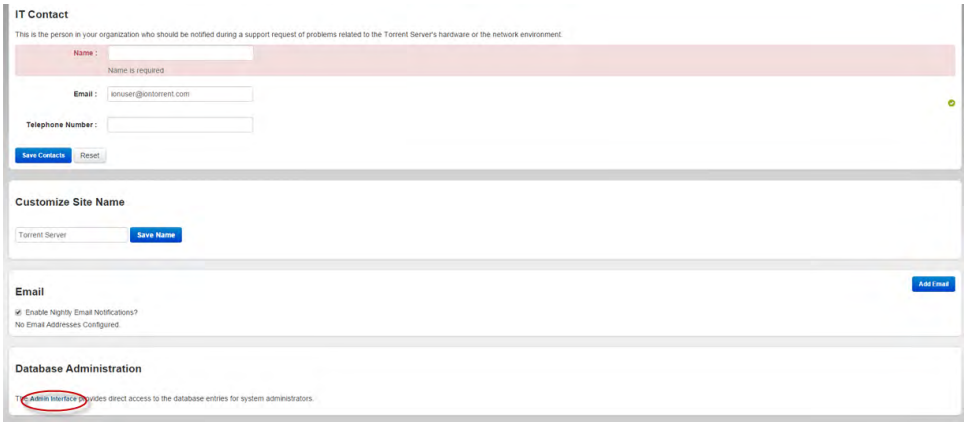
Target the Torrent Server IP Address

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.

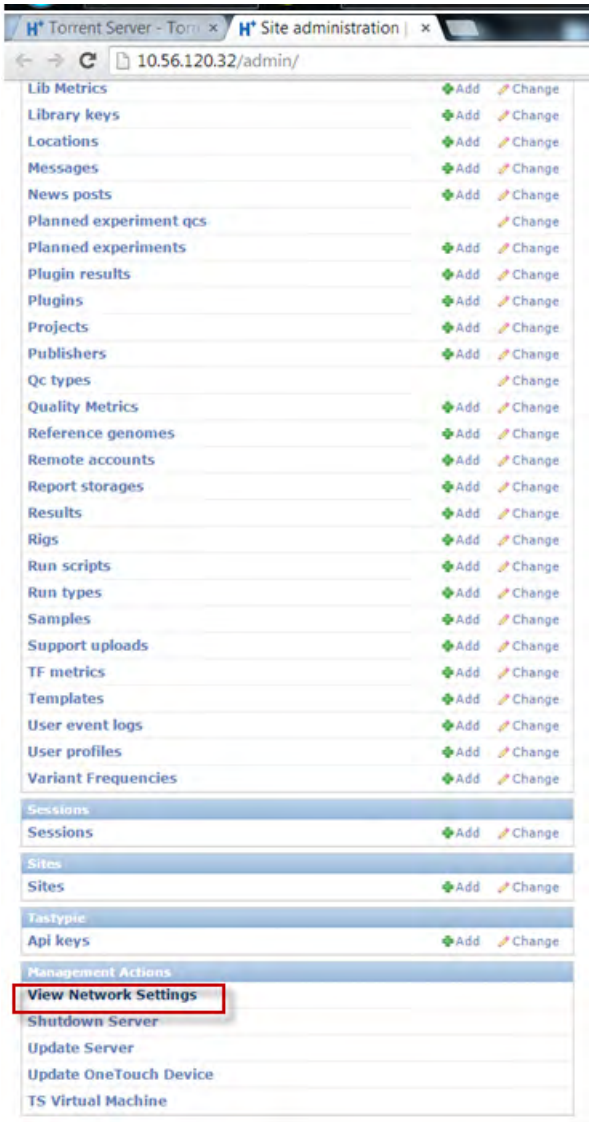
1. Log into Torrent Browser as the admin.
2. Click the Gear button and select **Configure**.



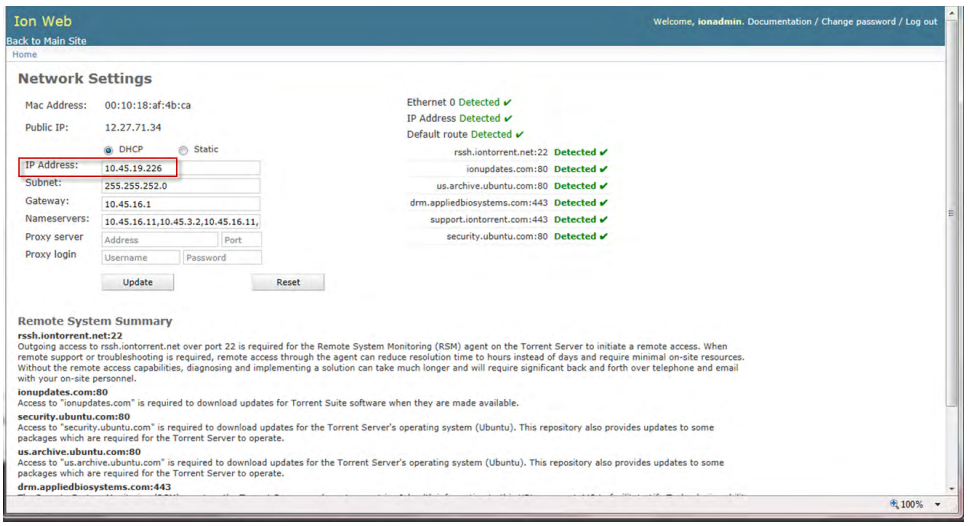
3. Scroll down to the Database Administration section and click the **Admin Interface** link.



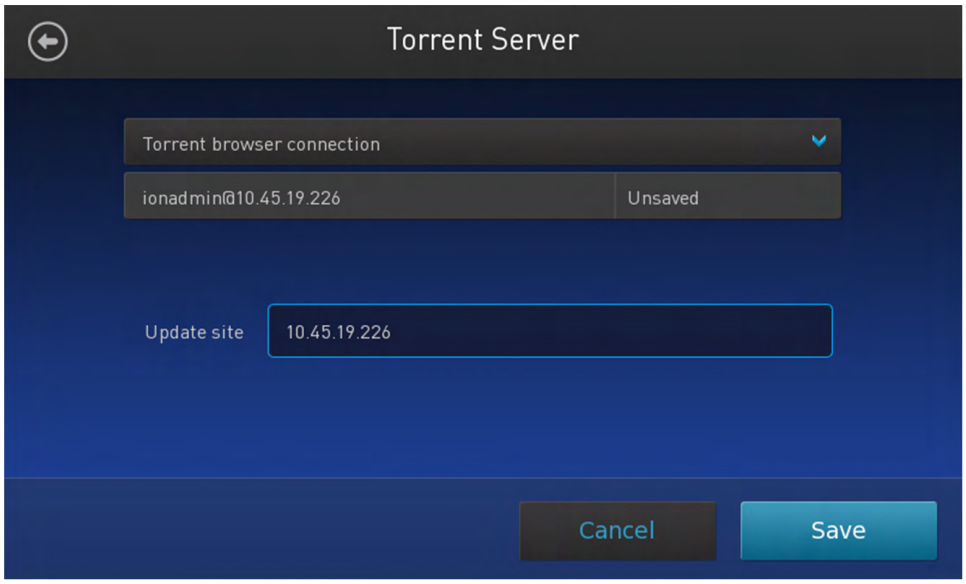
4. The Site Administration screen appears. Scroll to the bottom and click **View Network Settings**.



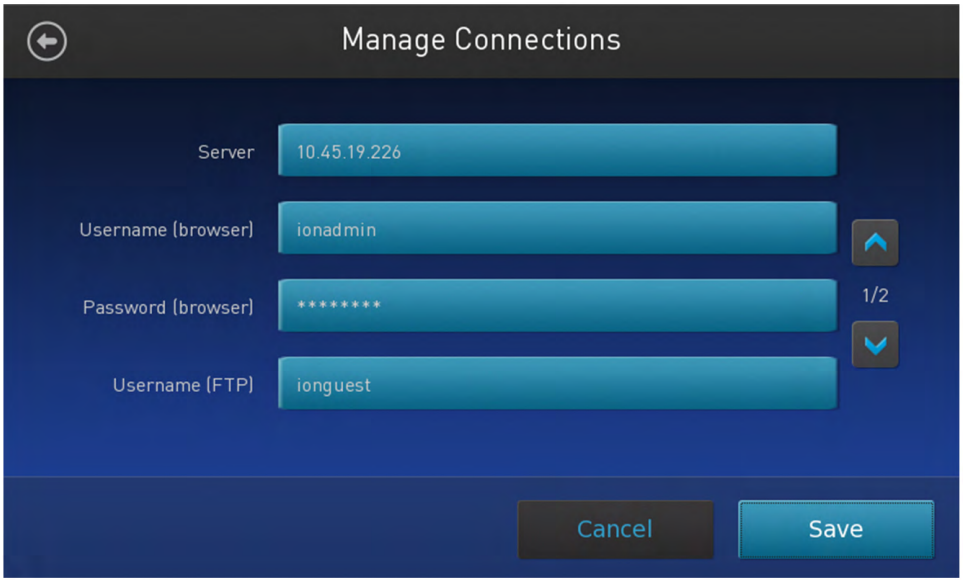
5. Note (copy or write down) the Torrent Server IP address displayed on the Network Settings screen.



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



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.

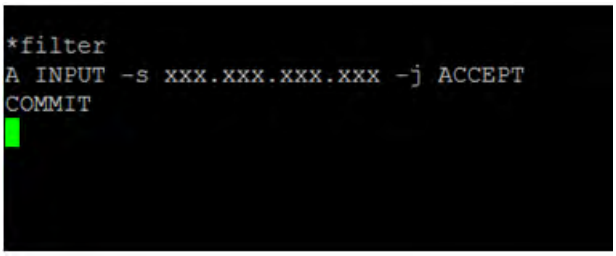
Create an exception

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`

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**.



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 172.16.48.15 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`

5. Restart networking by running this command: `sudo /etc/init.d/networking restart`.

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 ptables-restore--noflush</etc/iptables.custom`

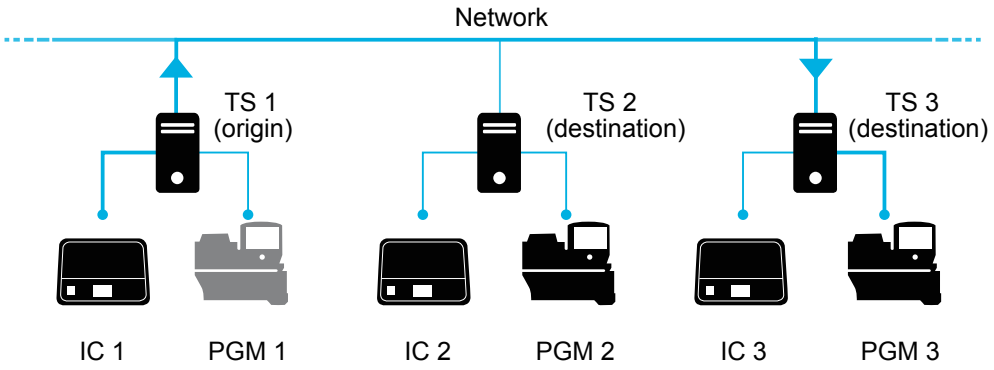
7. If a rule is ever removed from either of these files, this would remove it from the active firewall rules because the `iptables-restore` command does not overwrite rules, it appends them to all active rules.

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

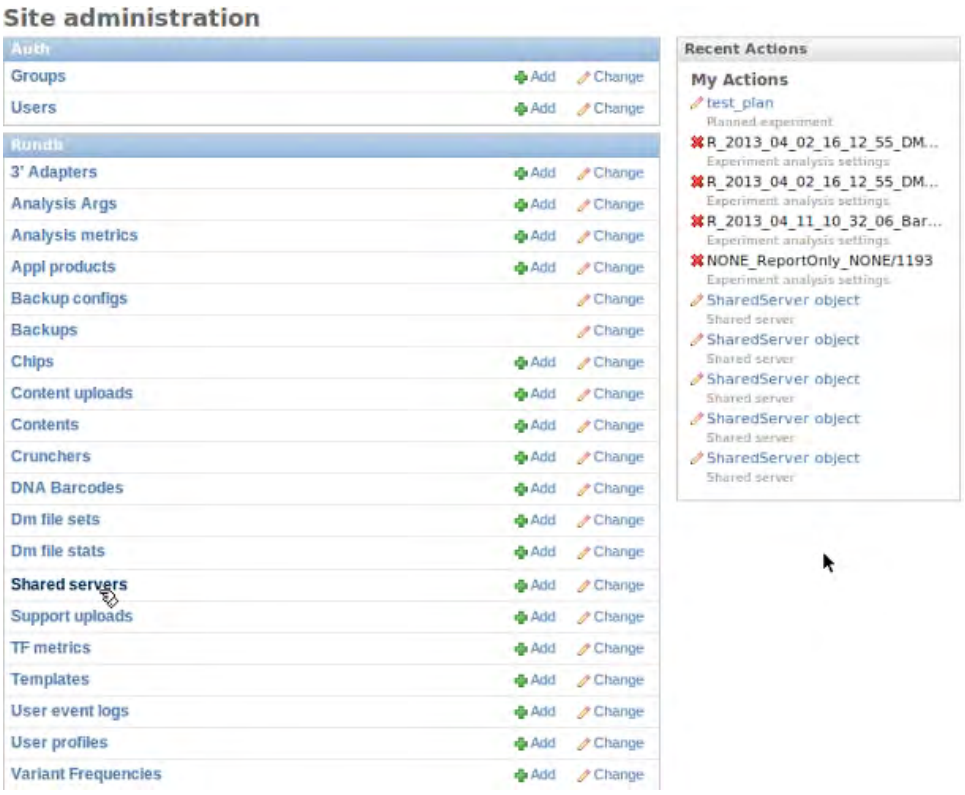


Requirements include:

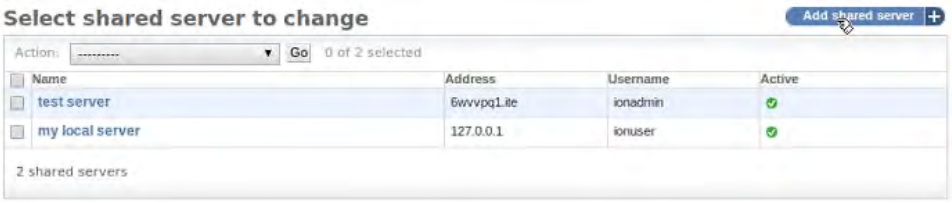
- All Torrent Servers must be running the same software version.
- All Torrent Servers must have the same genomic reference, barcode set, BED files, Variant Caller config files, etc. All Torrent Servers must be on the same subnet.

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.



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



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.

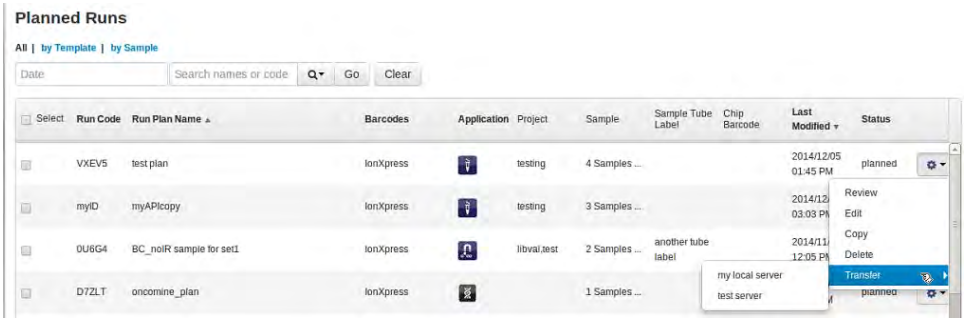
Transfer a Planned Run

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**.

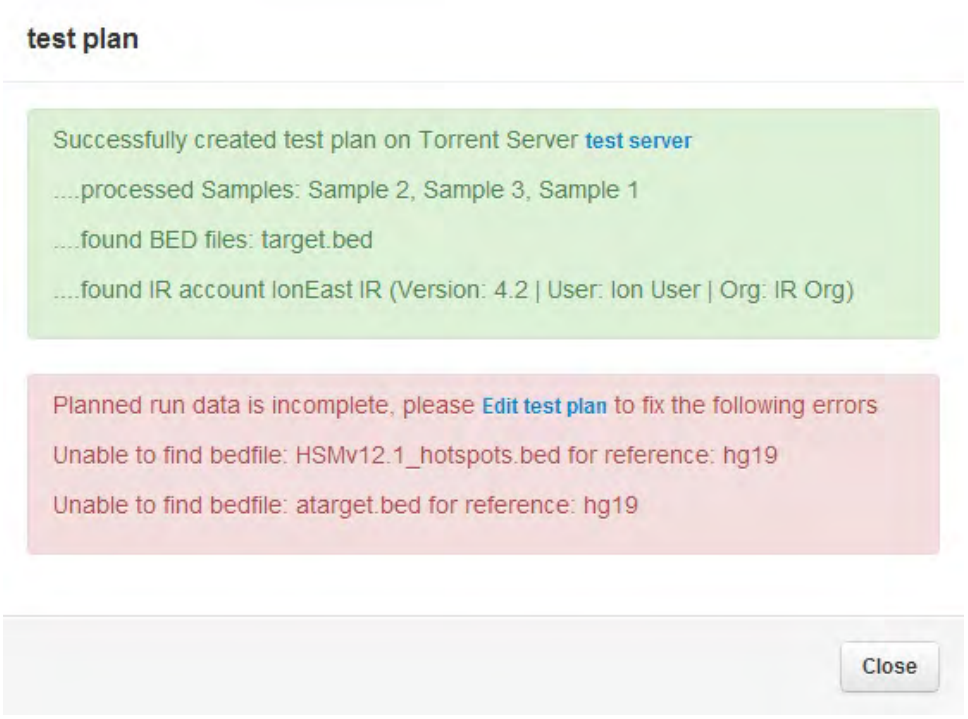
2. Open the Gear ⚙ menu of the Planned Run you want to transfer, and select **Transfer**. Then select the *destination* Torrent Server.



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.

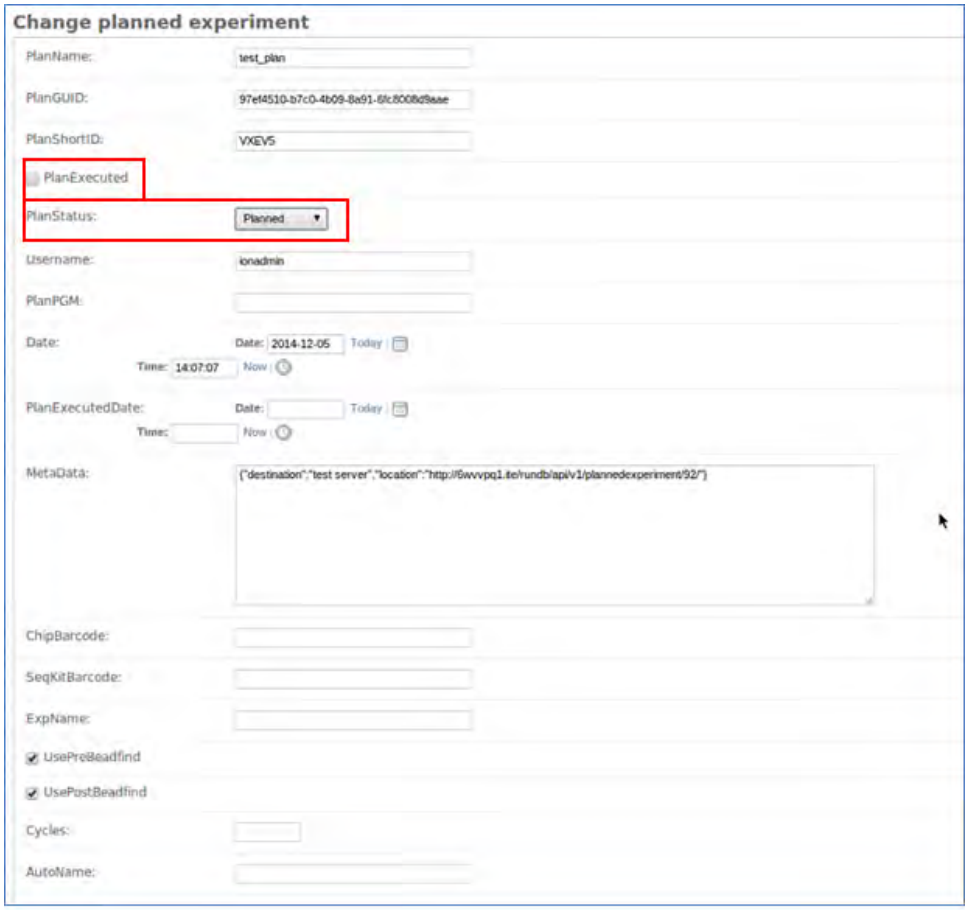


5. To edit the transferred Planned Run and add missing files:
   a. Download required files using the References tab of the Torrent browser of the destination server
   b. Go to the Edit Plan wizard of the transferred Planned Run by selecting Edit on the gear pull-down menu to the right of the Planned 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.

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
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Location of safety labels on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	French translation	Location on Instrument
⚠ **CAUTION! Hazardous chemicals.** Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.	Back panel of the instrument.
⚠ **CAUTION! Hazardous waste.** Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.	

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

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 61010-2-020 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

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, and 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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may 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

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  - 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

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1 April 2015

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