iontorrent

Ion 520™ & Ion 530™ Kit – Chef

USER GUIDE

Instructions for automated template preparation, chip loading, and sequencing

for use with:
Ion Chef™ System
Ion S5™ System
Ion S5™ XL System

Catalog Numbers A27757, A30010
Publication Number MAN0010846
Revision D.0

ThermoFisher
SCIENTIFIC

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

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

Revision history: Pub. No. MAN0010846

Revision	Date	Description

D.0	20 March 2017	• Controls kit updated to the Ion S5™ Controls Kit Plus
		• Recommended dilution of the Human CEPH Control 200 Library changed to 1:25
		• "Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge" topic added to Supplemental procedures
		• Designation of Chip-loading centrifuge positions changed from "A" and "B" to "1" and "2"
		• ISP quality control sections updated and moved to new user guides:
		  – *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387)
		  – *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388)
		• Web links updated

C.0	31 July 2016	• Updated to support new Ion S5™ instrument user interface in Torrent Suite™ Software 5.2. • Graphics enhanced and workflows reorganized for ease of use.
B.0	13 November 2015	Update to user guide for 2 sequencing runs per initialization that accompany new SKU (A30010).
A.0	26 August 2015	New user guide, which includes instructions for using the Ion 520™ & Ion 530™ Kit – Chef to set up and operate Ion Chef™ Instrument for use with the Ion S5™ and Ion S5™ XL Systems.

Contents

                                                     Ion 520™ & Ion 530™ Kit – Chef User Guide

About this guide

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

Purpose of this guide

This user guide describes how to use the Ion Torrent™ Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A27757, A30010) and the Ion Chef™ System to prepare enriched, template-positive Ion Sphere™ Particles (ISPs) for up to 400 base-read sequencing of libraries on the Ion S5™ Sequencer or the Ion S5™ XL Sequencer. The kits include all reagents and materials sufficient for performing four dual-sample template-preparation runs on the Ion Chef™ System for subsequent sequencing on an Ion S5™ 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.

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

Product description

The Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A27757, A30010) include the reagents and materials required to use the Ion Chef™ Instrument to prepare template-positive Ion Sphere™ Particles and load Ion 520™ or Ion 530™ Chips. The kit also includes reagents and materials to sequence the loaded chips on the Ion S5™ or Ion S5™ XL Sequencer.

Note: Ion 520™ & Ion 530™ Kit – Chef (Cat. No. A30010), for use when performing two sequencing runs per initialization, only supports 200 base-read sequencing.

Library compatibility

The Ion 520™ & Ion 530™ Kit – Chef can be used with up to 400-base-read libraries of any type prepared using any available Ion library kit.

Software compatibility

The Ion 520™ & Ion 530™ Kit – Chef is compatible with Torrent Suite™ Software 5.2 and later. Be sure to update your Torrent Server to the latest available version of Torrent Suite™ Software before using this kit.

Ion 520™ & Ion 530™ Kit – Chef

> **IMPORTANT!** Do not substitute 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. Do NOT store the Ion S5™ Sequencing Reagents (Cat. No. A27768) on dry ice or in a closed environment where dry ice is present.

Each Ion 520™ & Ion 530™ Kit – Chef contains all materials that are required to prepare and sequence 8 Ion 520™ or Ion 530™ Chips.

- Catalog No. A27757 supports 8 initializations
- Catalog No. A30010 supports 4 initializations (2 × 200 base-read sequencing runs per initialization)

On arrival, inspect all consumables and contact Technical Support if any of the products have been damaged during shipping.

Kit summary

Component	Part No.	Quantity per kit	
		A30010	**A27757**
Ion S5™ Chef Supplies	A27755	4 boxes	4 boxes
Ion S5™ Chef Solutions	A27754	1 box	1 box
Ion 520™ & Ion 530™ Chef Reagents	A27756	1 box	1 box
Ion S5™ Sequencing Solutions	A27767	1 box	2 boxes
Ion S5™ Sequencing Reagents	A27768	1 box	2 boxes

Kit contents and storage

Contents	Amount / box	Storage
Ion S5™ Chef Supplies (Part No. A27755)		
Chip Adapter	2	15°C to 30°C
Enrichment Cartridge v2	1	
Tip Cartridge	1	
PCR Plate and Frame Seal v2	1 each	
Recovery Station Disposable Lid v2	2	
Recovery Tube v2	12	
Ion S5™ Chef Solutions (Part No. A27754)		
Ion S5™ Chef Solutions	4 cartridges	15°C to 30°C

Contents	Amount / box	Storage
Ion 520™ & Ion 530™ Chef Reagents (Part No. A27756)		
Ion 520™ & Ion 530™ Chef Reagents	4 cartridges	−30°C to −10°C
Ion S5™ Sequencing Solutions (Part No. A27767)		
Ion S5™ Wash Solution	4 × 1.5 L	15°C to 30°C
Ion S5™ Cleaning Solution	250 mL	
Ion S5™ Sequencing Reagents (Part No. A27768)		
Ion S5™ Sequencing Reagents (Cartridges ship at 2°C to 8°C. Store as indicated, do not store on dry ice.)	4 cartridges	−30°C to −10°C

IMPORTANT! Do not store the Ion S5™ Sequencing Reagents (Part No. A27768) on dry ice or in a closed environment containing dry ice.

Compatible Ion Chip™ kits

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

Ion S5™ Calibration Standard

For *de novo* sequencing applications, the Ion S5™ Calibration Standard can be added to diluted libraries to increase base-calling accuracy. The Ion S5™ Calibration Standard (Cat. No. A27988) is ordered separately.

Contents	Amount	Storage
Ion S5™ Calibration Standard	80 µL	−30°C to −10°C

Ion S5™ Controls Kit Plus

Human CEPH Genomic DNA Control is sufficient for 3 library preparations (1 µg each). The Ion S5™ Controls Kit Plus (Cat. No. A30729) is ordered separately.

Contents	Amount	Storage
Human CEPH Genomic DNA Control (red cap)	30 µL	−30°C to −10°C
Human CEPH Control 200 Library (yellow cap)	12 µL	
E. coli DH10B Control 400 Library (orange cap)	16 µL	

Required materials not supplied

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

Item	Source
Non-interruptible Power Supply (UPS) [1]	MLS
Thermal cycler with a heated lid	MLS
Microcentrifuge[2]	MLS
P2, P10, P20, P200, P1000 pipettor set and filtered tips	MLS
0.2-mL PCR tubes, flat cap (Axygen™ MAXYMum Recovery™ PCR Tube	Fisher Scientific 14-222-283 *or* MLS
Microcentrifuge tubes, 1.5-mL or 1.7-mL	MLS
Vortex mixer with a rubber platform	MLS
Gloves, powder-free nitrile	MLS
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	MLS
Isopropyl alcohol, 70% solution	MLS
Wipes, disposable lint-free	MLS

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

[2] Must fit standard 0.2- and 1.5-mL microcentrifuge tubes and generate 21,000 × g. To convert the RPMs of your centrifuge to RCF in units of gravity, see **tools.thermofisher.com/content/sfs/brochures/ TR0040-Centrifuge-speed.pdf**.

About the Ion Chef™ System

The Ion Chef™ System provides automated, high-throughput template preparation and chip loading for use with the Ion S5™ or Ion S5™ XL Sequencer. The system includes a complete set of cartridge-based consumables and reagents that enable a user to load two Ion 520™ or Ion 530™ Chips in approximately 13 hours with less than 15 minutes of hands-on time. The Ion Chef™ System features network integration with the Torrent Suite™ Software to enable sample and reagent traceability throughout the chip preparation workflow.

Ion Chef™ Instrument components

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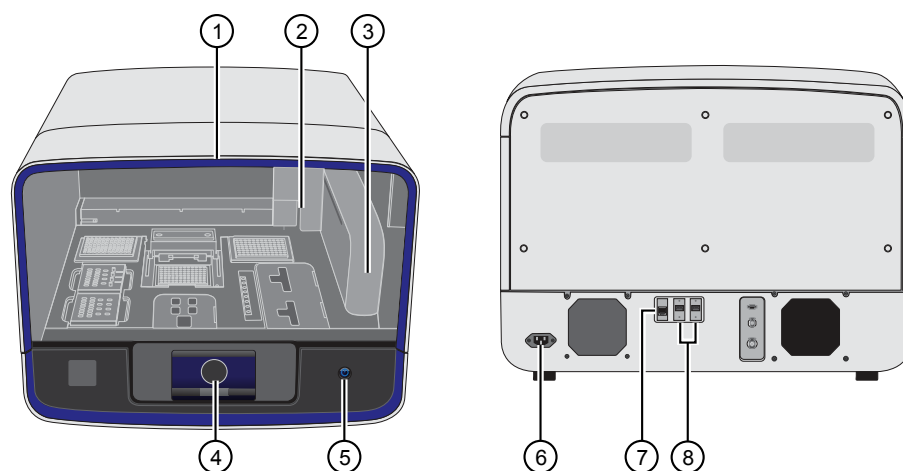
① **Door** – Provides access to the interior of the instrument. The door is locked in the closed position during operation.

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

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

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

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

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

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

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

Interior hardware and consumables

The following figure illustrates the interior of the Ion Chef™ Instrument and describes the stations involved in the preparation of chips for sequencing on the Ion S5™ or Ion S5™ XL System.
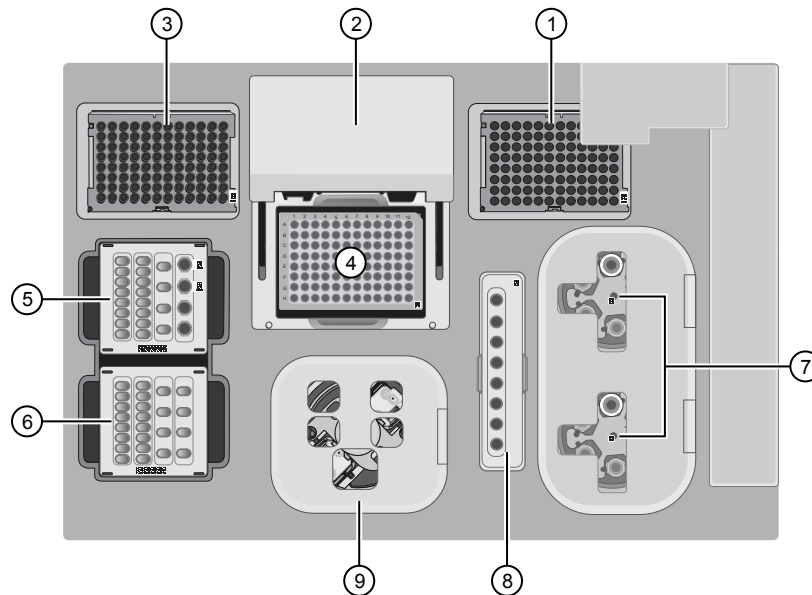
① **Waste pipette tip rack** – The position of the rack containing waste (used) pipette tips.

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

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

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

⑤ **Reagents station** – The position on the instrument deck of the diluted libraries, NaOH, and the Ion 520™ & Ion 530™ Chef Reagents cartridge.

⑥ **Solutions station** – The position on the instrument deck of the Ion S5™ Chef Solutions cartridge, which is maintained at room temperature.

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

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

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

About the Ion Chef™ System touchscreen interface

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

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

② **Open Door** button.

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

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

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

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

Precautions

Avoid nucleic acid contamination

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

Avoid chip damage

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

Guidelines for using Ion Chef™ reagents and consumables

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

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

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

- Use only Ion Torrent™ kits and supplies with the Ion Chef™ Instrument. The use of third-party reagents and supplies can adversely affect the performance of the Ion Chef™ Instrument and 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.
- Remove and sequence chips within 1 hour after the Ion Chef™ System finishes loading them. If you cannot sequence a loaded chip immediately, store it in a chip storage container at 4°C until you are ready to run it (up to 6–8 hours maximum).

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

Workflow

The following workflow illustrates how to prepare and load samples using the Ion 520™ & Ion 530™ Kit – Chef, and Ion Chef™ System for sequencing on the Ion S5™ or Ion S5™ XL Sequencer.

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

▼

Dilute the library samples, and thaw the reagents.

▼

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

▼

Start the Ion Chef™ Instrument

▼

Unload the Ion Chef™ Instrument.

▼

Load the prepared chip into the Ion S5™ or Ion S5™ XL Sequencer and
begin the sequencing run.

Before you begin

Create a Planned Run

About Planned Runs
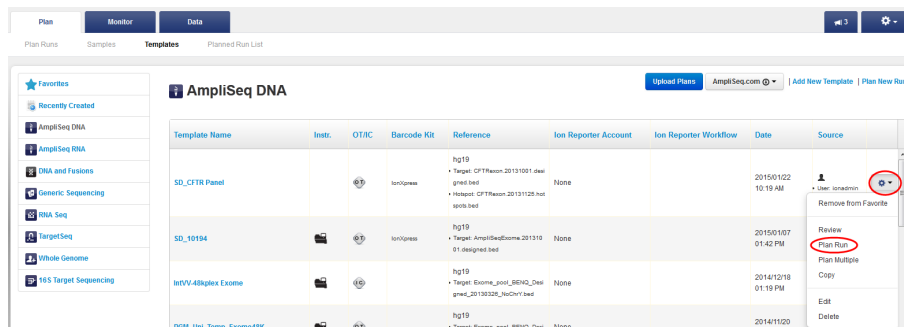
Planned Runs contain all the settings used in a sequencing run, including number of flows, kit types, barcodes, sample information, and reference files (if any). They are used to track samples, chips, and reagents throughout the sequencing workflow, from template preparation on the Ion Chef™ Instrument through sequencing on the Ion S5™ or Ion S5™ XL Sequencer and subsequent data analysis. Each of the two chips prepared in an Ion Chef™ run requires its own Planned Run.

Create a Planned Run

1. Log in to the Torrent Server via the Torrent Browser.

2. Select the **Plan** tab, click **Templates**, select the application that you want to run (such as AmpliSeq™ DNA), then click either:
   • **Plan New Run** to plan a new run using the generic template for the selected application.
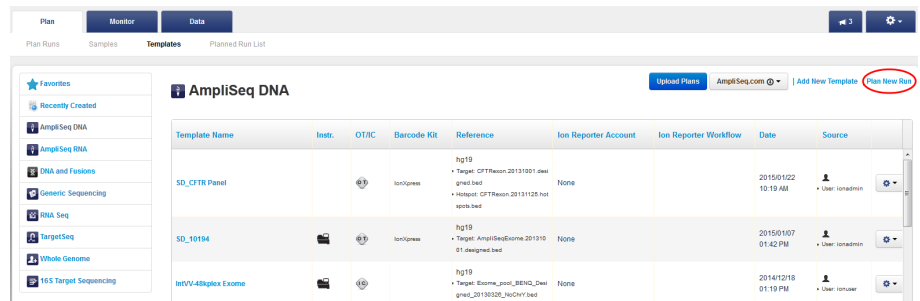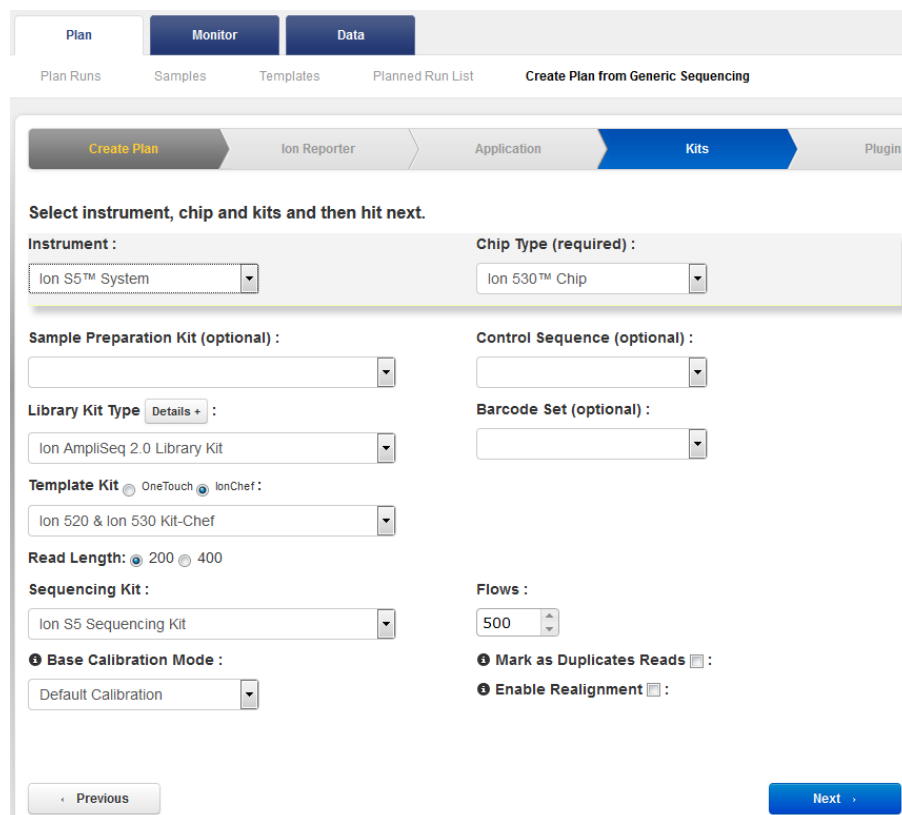
- **Plan Run** in the dropdown menu under the Settings ⚙ tab to the right of the existing template you select from the template list.

3. In the Planned Run wizard, review the **IonReporter** and **Application** tabs and make selections appropriate to your run. In the **Kits** tab, make the following selections:

   a. Select **Ion S5™ System** from the **Instrument** dropdown list.

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

   c. Select **Ion Chef** for Template Kit, and select **Ion 520 & Ion 530 Kit-Chef** from the **Template Kit** dropdown list.

   Note: Use the 200 setting for libraries with <300-base average read length. Use the 400 setting for libraries with ≥300 base average read length.

d. Select **200** or **400** for **Read Length**.

e. Select **Ion S5 Sequencing Kit** from the **Sequencing Kit** dropdown list.

f. Enter the appropriate number of flows: enter 500 flows for 200-base-read sequencing, and 850 flows for 400-base-read sequencing.

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

> **IMPORTANT!** For *de novo* sequencing applications that do not include a reference BAM file, select **Enable Calibration Standard** from the **Base Calibration Mode** dropdown list. Choose this option only if you will add Ion S5™ Calibration Standard to the Ion Chef™ Library Sample Tubes. Selecting this option and adding the Ion S5™ Calibration Standard to your library will allow greater accuracy of base-calling in libraries for which a reference BAM file does not exist.

h. Click **Next**.

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

5. In the **Plan** tab, enter or make the following selections:

a. Enter a Planned Run name and select Reference and BED files appropriate to your run.

b. Enter the number of chips you are creating Planned Runs for. 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.

c. Enter a sample name for each plan in the appropriate **Sample (required)** fields.

    d. Enter or scan the barcodes of the Ion Chef™ Library Sample Tubes into the appropriate **Sample Tube Label** fields.

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

> **Note:** For a complete description of the fields of the Torrent Browser application, see the *Torrent Suite™ Software Help*, available at **thermofisher.com/ ion-s5-docs.html**.

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.

Planned Run wizard key fields

Field name	Description
Ion Reporter	If Ion Reporter™ Software is installed and enabled, and you want to analyze the run data using the software, select the account and workflow.
Application	Select the sequencing application you are performing.
Instrument	Select the sequencing system you are using.
Chip Type	Select the chip type you are using.
Library Kit Type	Select the kit used to prepare the library.
Template Kit	Select **Ion 520 & Ion 530 Kit-Chef**.
Sequencing Kit	Select **Ion S5 Sequencing Kit**.
Barcode Set (optional)	If you are using barcodes with: • **DNA libraries**: Select the appropriate barcode set used. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2**: Select the **IonXpressRNA** barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01–16 Kit. If you are *not* using barcodes with: • **DNA libraries**: Leave the Barcode Set field blank. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2**: Select **RNA_Barcode_None** from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.
Flows	Enter the appropriate number of flows for the read length (for example, 500 flows for 200-base read sequencing and 850 flows for 400-base read sequencing).
Plugins	Select the appropriate plugins for your application.
Projects	Select or add a project within which to group your run data. You can include runs in multiple projects, and remove runs from a project at any time.
Run Plan Name	Enter a name for the run.
Reference Library	Select a reference library uploaded to the Torrent Server, if any.
BED files	Select the **Target Regions** or **HotSpot Regions** BED file on the Ion Torrent Server, if any.
Sample Name (Required)	Is Ion Reporter™ Uploader enabled? • **Yes** – Enter each sample name and select the appropriate values for workflow, relation, relation role, and set ID. • **No** – Scan the barcode of the library tube into the "Sample Name" and "Sample Tube Label" fields for the specific sample.

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

Dilute the libraries

Guidelines for library dilution

- The recommendations for diluting your sample libraries represent optimal input concentrations for control libraries.
- Recommendations are based on qPCR quantification. If libraries are quantified with an Agilent™ 2100 Bioanalyzer™ instrument, a higher calculated concentration may need to be used for equivalent input.
- Prepare a fresh dilution of each library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.

Dilute the sample libraries

IMPORTANT! Before proceeding, dilute the 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 200-bp and 400-bp stock libraries according to the following table. Then use polyclonality and low quality filter results from a sequencing run performed with ISPs templated at the starting concentration and titrate up or down to achieve optimal concentrations, if needed.

Note: The A30010 configuration of the Ion 520™ & Ion 530™ Kit – Chef, when performing two sequencing runs per initialization, supports only 200 base-read sequencing.

Library length[1]	Recommended concentration	Molecules per 25-μL input volume	Templating size in Planned Run setup
<300 bp	40–60 pM	$600–900 \times 10^6$	200
≥300 bp	80–120 pM	$1.2–1.8 \times 10^9$	400

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

Dilute the control library (if used)

If you are running a control library for troubleshooting purposes, dilute the Human CEPH Control 200 Library or *E. coli* DH10B Control 400 Library, obtained from the Ion S5™ Controls Kit Plus (Cat. No. A30729), as follows:

Control library	Volume of library	Volume of Nuclease-free Water
Human CEPH Control 200 Library	1 µL	24 µL
E. coli DH10B Control 400 Library	1 µL	84 µL

Prepare the Ion Chef™ System for use

Before you use the Ion Chef™ Instrument:

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

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

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

- Thaw the Ion 520™ & Ion 530™ Chef Reagents cartridge at room temperature for 45 minutes before use.

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

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

3 Run the Ion Chef™ System

This chapter describes:

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

- Starting an Ion Chef™ run

- Unloading the Ion Chef™ Instrument

Materials required

- Ion 520™ & Ion 530™ Kit – Chef (Cat. Nos. A27757, A30010)
- Ion 520™ or Ion 530™ Chip (2)
- Molecular-biology grade nuclease-free water
- P200 pipettor and filtered tips

Prepare the libraries and consumables

1. Unbox the Ion 520™ & Ion 530™ Chef Reagents cartridge 45 minutes before use and allow it to warm to room temperature.

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

2. Pipet 25 µL of each diluted library (see "Dilute the sample libraries" on page 22) to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).

   IMPORTANT! If you are performing a *de novo* sequencing experiment that does not include a reference BAM file, add 4 µL of the Ion S5™ Calibration Standard to your diluted libraries in the Ion Chef™ Library Sample Tubes. Cap the tubes, vortex to mix, then centrifuge briefly to collect contents at the bottom of the tube.

   Note: If running the Human CEPH Control 200 Library or *E. coli* DH10B Control 400 Library, obtained from the Ion S5™ Controls Kit Plus (Cat. No. A30729), prepare the control library for use by diluting 1 µL of stock library as described in "Dilute the control library (if used)" on page 23, then add 25 µL of diluted control library to the appropriate Ion Chef™ Library Sample Tube.

3. Cap, then store the Library Sample Tubes on ice until you are ready to load them onto the Ion Chef™ Instrument.

4. Remove all cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.
   Prepare the following:
   - Chip Adapter (2)
   - Enrichment Cartridge v2
   - Tip Cartridge
   - PCR Plate and Frame Seal v2
   - Recovery Station Disposable Lid v2 (2)
   - Recovery Tube v2 (12)
   - Ion 520™ & Ion 530™ Chef Reagents cartridge (from Step 1)
   - Ion S5™ Chef Solutions cartridge

   IMPORTANT!
   1. Before use, gently tap the Reagents and Solutions cartridges on the bench to force the reagents to the bottoms of the tubes.
   2. Briefly centrifuge the tube of NaOH (Reagents cartridge, position C) to collect the contents.

   Note: When stored under normal conditions, a precipitate can form in some tubes of the Ion 520™ & Ion 530™ Chef 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 the provided buckets and approved consumable chips, tubes, and sample preparation reagents.
- The Chip-loading centrifuge is rated to operate at the listed rotational frequencies with the chip buckets, chips, and adapters. The centrifuge must be load-balanced. Proper care must be taken to load the buckets properly. If excessive vibrations arise, check that items are installed properly and rotors are load-balanced.
- Use only the materials supplied in the Ion 520™ & Ion 530™ Kit – Chef to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets before each use to assure normal operation.
- Confirm that the instrument is powered ON and has been cleaned following the last use.
- Ensure all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure the Reagents and Solutions station compartments are free of condensate before loading components.

Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure:
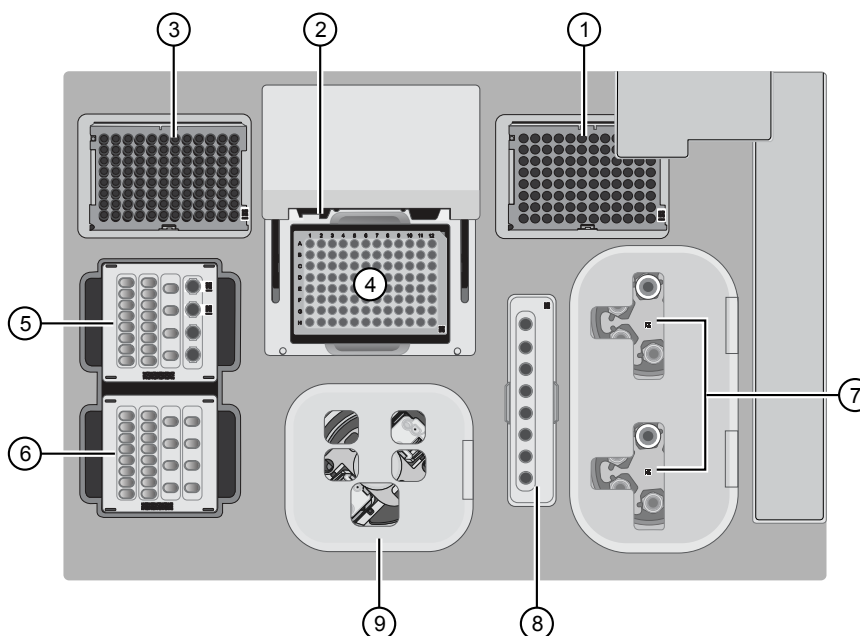
1. Empty tip rack (move from new Tip Cartridge position)
2. Frame Seal v2
3. New Tip Cartridge
4. PCR plate
5. Ion 520™ & Ion 530™ Chef Reagents cartridge
6. Ion S5™ Chef Solutions cartridge
7. Recovery Tubes and Recovery Station Disposable Lid v2
8. Enrichment Cartridge v2
9. Chip Adapter/Chip assemblies

Load the pipette tip racks and PCR plate

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

2. Lift the instrument door to the top of the travel until the latch mechanism engages.
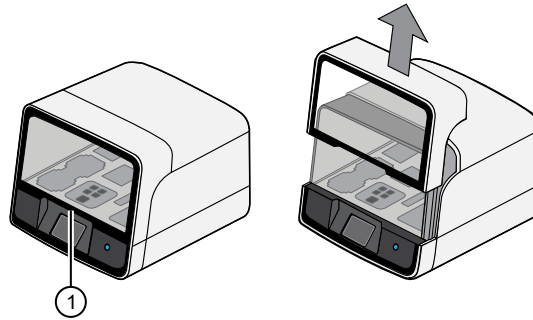
① Hold here, then lift

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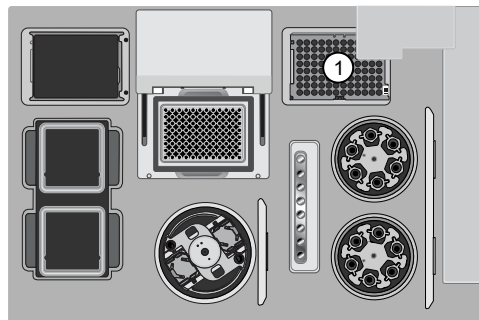
① Used Pipette Tip Position

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

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

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

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

5. Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge into the *New* Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the bracket and cartridge in place.
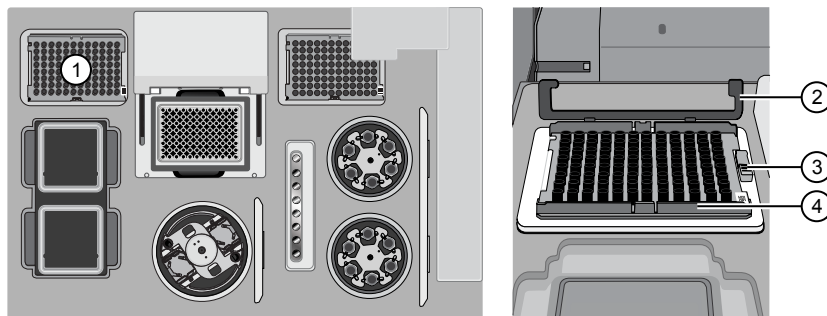
① New Pipette Tip Position
② Bracket
③ Catch
④ New Tip Cartridge

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

   IMPORTANT! When the Frame Seal v2 is positioned correctly, its tabs project upward and contact the heated cover.
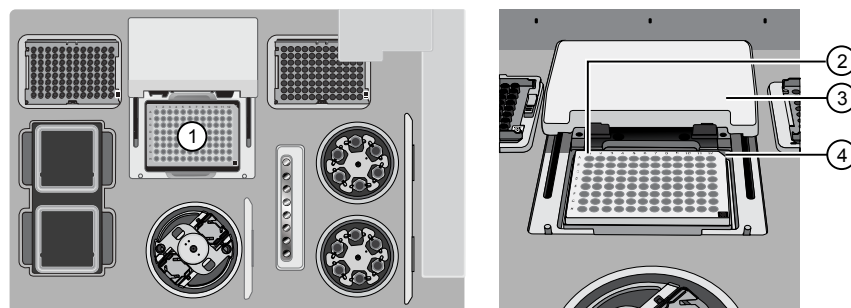
① Thermal cycler sample block
② Well A1
③ Cover
④ Keyed corner

Load the Reagents and Solutions cartridges

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

1. Gently tap the Ion 520™ & Ion 530™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.

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

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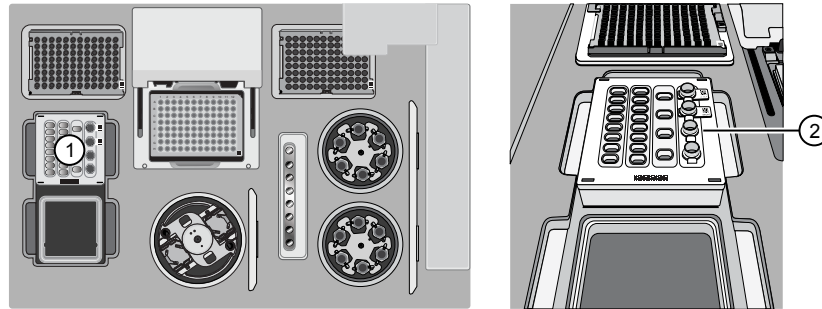

① Reagents station (4°C)
② Ion 520™ & Ion 530™ Chef Reagents cartridge

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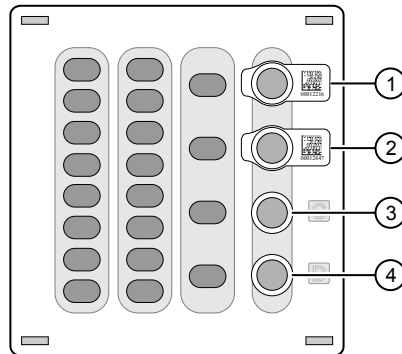
① Position A (Library)
② Position B (Library)
③ Position C (NaOH)
④ Position D (Empty tube)

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

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

   IMPORTANT! When the Reagents cartridge is loaded:

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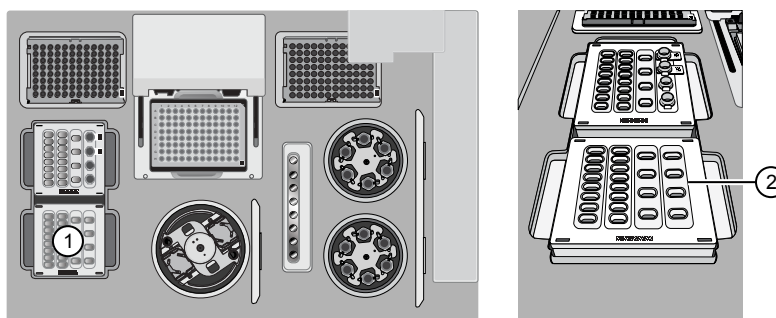   · Confirm that *all* tubes are uncapped, including the tube at Position D.

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

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

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

Load the Recovery Tubes and Enrichment Cartridge v2

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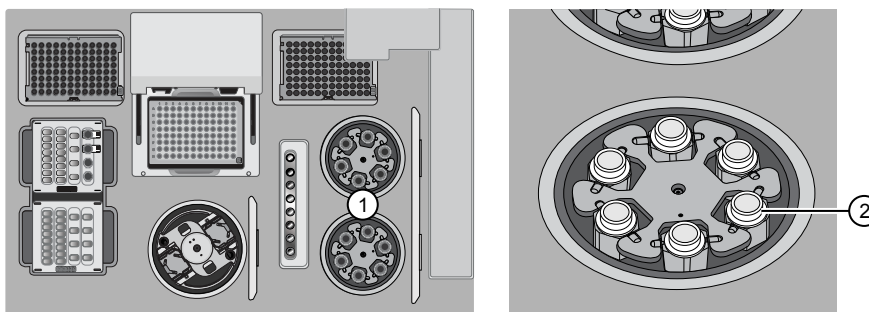
① Recovery centrifuges
② Recovery Tube

Before sealing each centrifuge, confirm that:

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

   IMPORTANT! The centrifuge must be load-balanced.

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

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

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

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

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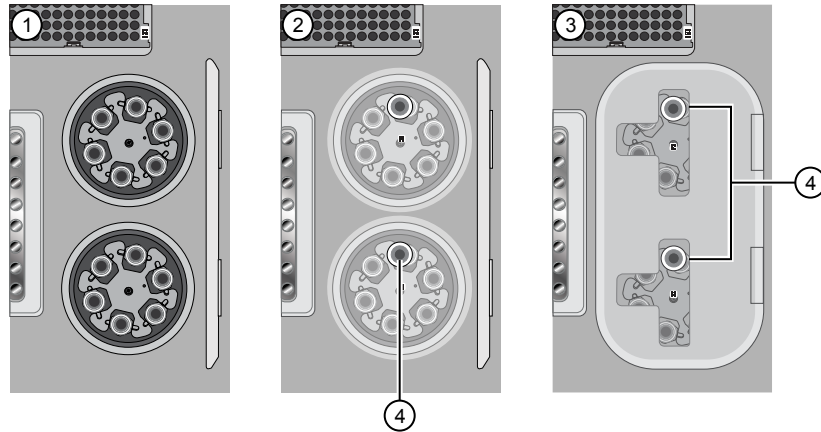- Use only the supplied materials, including buckets and disposables, to run the centrifuges at the rated speeds. Do not remove or change the rotors. Inspect the buckets to assure normal operation before each use.

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

IMPORTANT! Confirm that the Enrichment Cartridge v2 is loaded so that the lettering on the cartridge is right-side-up.
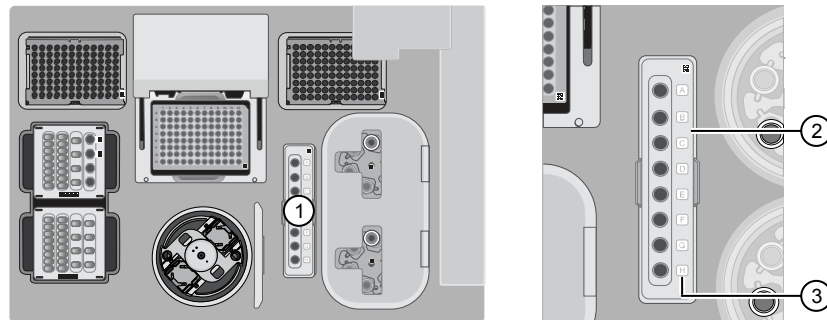
① Enrichment station
② Enrichment Cartridge v2
③ Lettering

3

Load the Chip-loading centrifuge

1. Load each prepared sequencing chip into a centrifuge bucket, then attach a Chip Adapter to the assembly.

   a. Place the chip in the chip-loading bucket with the keyed corners of the chip and bucket aligned, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.

   b. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.

   c. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.

   d. Confirm that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket. Loading can fail if the adapter is not attached securely.

   Note: If desired, you can label the tops of chips to distinguish them. Do not obstruct or overwrite the chip barcode with your label.
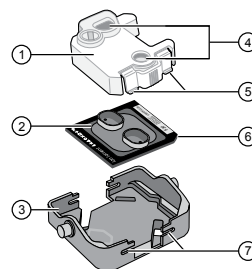
① Chip Adapter
② Ion chip
③ Bucket
④ Ports (align with chip)
⑤ Flexible tabs
⑥ Keyed corner (align with bucket)
⑦ Slots

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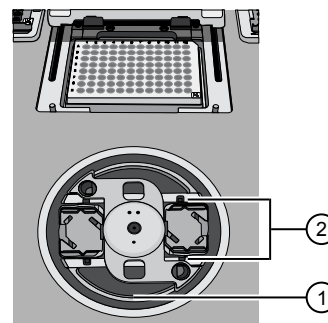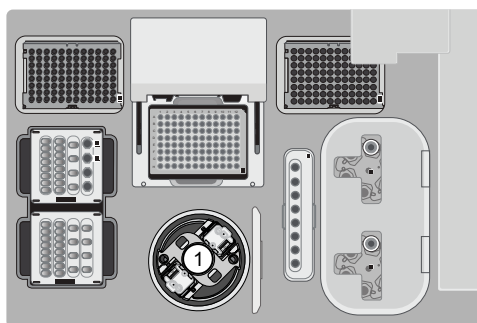
① Chip-loading centrifuge
② Mounting grooves

IMPORTANT! When the Chip-loading centrifuge is loaded, confirm that each Chip Adapter is firmly attached to a bucket, and that the buckets are securely seated in the centrifuge rotors.
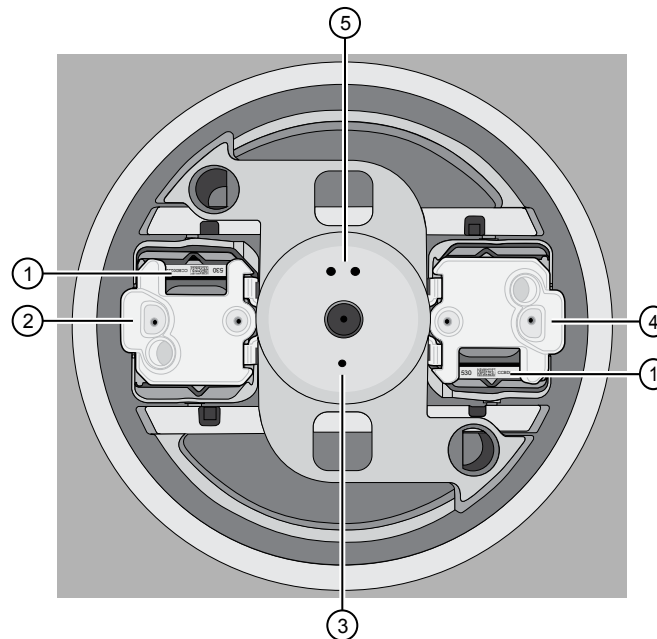
① Chip barcode
② Chip position 1
③ Position 1 marker hole
④ Chip position 2
⑤ Position 2 marker hole

Note: Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest. The chip loaded in Position 1 will be loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position A of the Reagents cartridge. The chip loaded in Position 2 of the centrifuge will be loaded with ISPs prepared from the DNA library in the Library Sample Tube loaded in Position B of the Reagents cartridge.

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

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

Confirm that consumables are correctly installed

Before continuing:

- Confirm that each cartridge is at the correct location and in the correct orientation.
- Press down on all cartridges to confirm that they are firmly pressed into place.
- Confirm that all tubes in the Ion 520™ & Ion 530™ Chef Reagents cartridge, including the tube of NaOH in Position C, are uncapped and firmly pressed into place.
- Confirm that the centrifuge lids are installed correctly so that the port is oriented toward the rear of the instrument.
- Confirm that the tube and chip buckets are seated securely in the rotor arms of the Chip-loading and Recovery centrifuges, and that the consumables they contain are correctly installed.

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

Single chip loading workflow

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

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

Ion Chef™ S5 Series Chip Balance

1. Add the single diluted library to an Ion Chef™ Library Sample Tube, then load the tube into Position A of the Reagents cartridge.

2. Load an empty Ion Chef™ Library Sample Tube into Position B of the Reagents cartridge. Uncap both tubes.

3. Load the chip in Position 1 and the Ion Chef™ S5 Series Chip Balance in Position 2 of the Chip-loading centrifuge.

   IMPORTANT! Do not use Ion Chef™ 314, 316/318, or P-Series versions of the chip balance with the Ion 520™ or Ion 530™ Chip. Each chip balance is weight-matched to the chip (and corresponding chip adapter) specified on the chip balance label.

Note: Position 1 of the Chip-loading centrifuge is the position 90° clockwise from the single hole in the rotor bucket cover at rest.
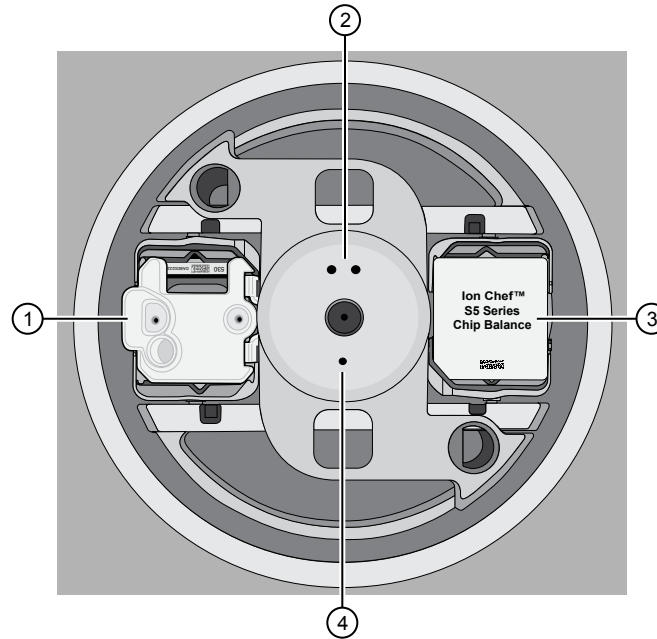
① Position 1 (chip)
② Position 2 marker holes
③ Position 2 (Chip Balance)
④ Position 1 marker holes

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

Ion Chef™ pre-run checklist

1) A new PCR Plate is loaded in the thermal cycler sample block.
2) A Frame Seal v2 is in place and oriented under the heated cover.

1) A new Ion Chef™ Tip Cartridge v2 is loaded in the New Pipette Tip position.
2) Bracket is pulled downward to lock the tip cartridge in place.

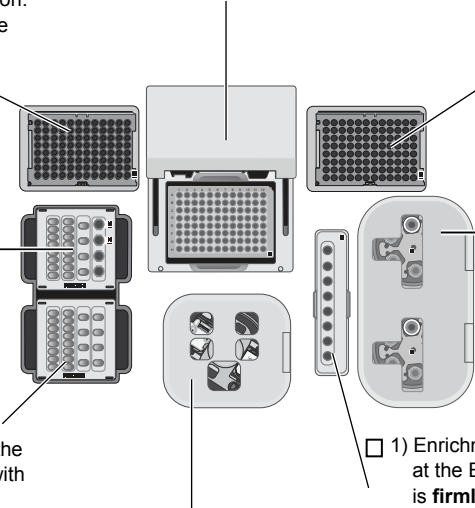1) The tip rack from previous run is transferred from the New Pipette Tip position to the Used Pipette Tip position.

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

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

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

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

1) Adapter/chip assembly is correctly seated in the Chip-loading centrifuge buckets, with the adaptor clips inserted into the bucket slots.
2) Centrifuge buckets containing the adapter/chip assemblies are securely seated in the centrifuge rotor, and freely pivot 90° outwards.
3) Chip-loading centrifuge is load-balanced.

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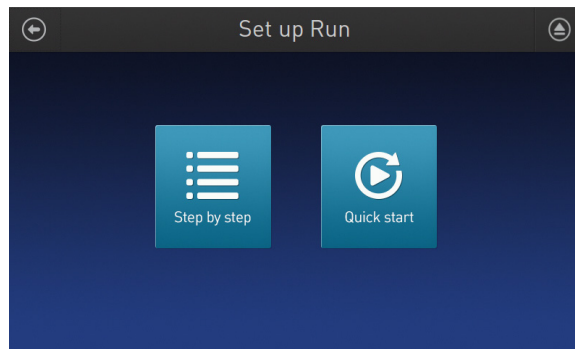
4. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage. After the door closes, the instrument vision system activates.

   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. Ensure both sides of the door are locked after closing it.
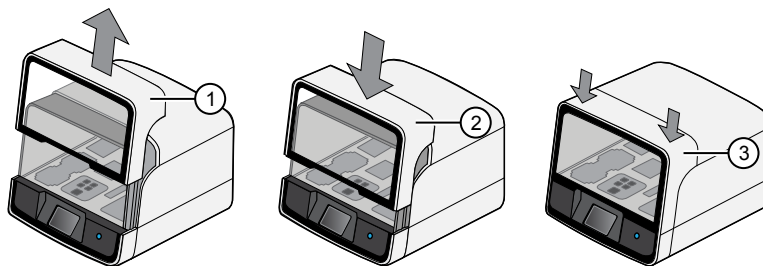
   ① Lift door first
   ② Lower
   ③ Press down to lock

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

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

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

6. When Deck Scan is complete, touch **Next** to display the Data Destination screen.
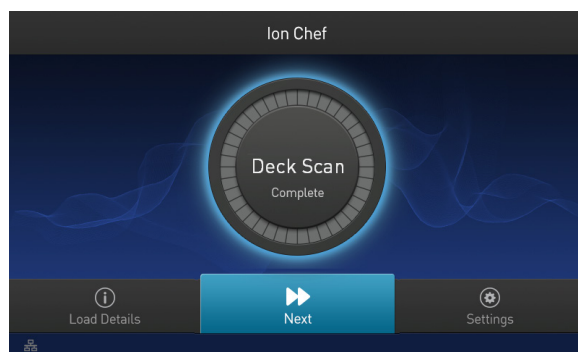
7. Confirm that the instrument displays the correct kit type, chip type, chip barcodes, and Planned Run. If the correct Planned Runs do not display, touch the dropdown menu ⌄ to select the Planned Run for each chip, then touch **Next.**

> **Data Destination**
>
> Kit Type   Ion Chef S530 V1   Chip Type: 530
>   Server   10.45.19.226
> Chip ID: XX0000194   Chip ID: XX0000191
> Sample ID: X0000051   Sample ID: 0000052
>
> ITK4W - TC1_FER-200... ⌄   MIYV8 - mt22_fr_prom... ⌄
> Template size   200   Template size   200
>
> Refresh Plans   Cancel   Next
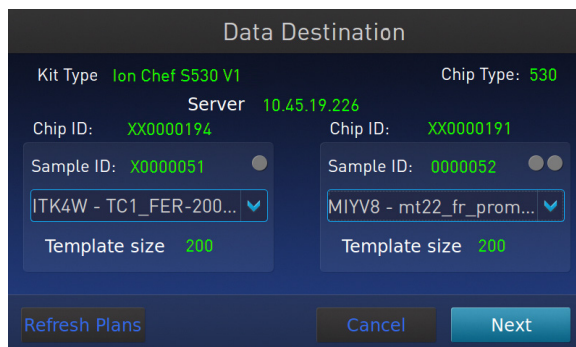
IMPORTANT! If the kit and chip type are not correct, confirm that you are using the correct kit and chip. If you are using the correct kit and chip, and an incorrect kit or chip type is displayed on the screen, contact Technical Support.

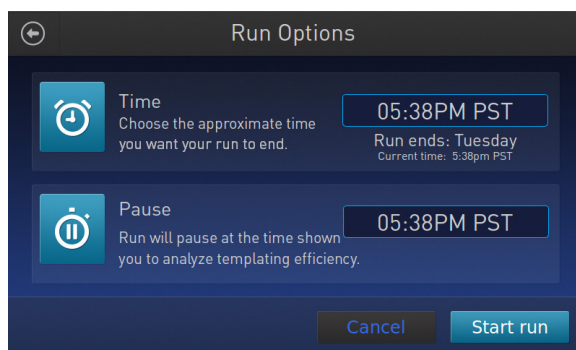8. On the Run Options screen, touch the appropriate option to complete the run, then enter the desired time of run completion, if needed.

> **Run Options**
>
> Time
> Choose the approximate time   **05:38PM PST**
> you want your run to end.   Run ends: Tuesday
>   Current time: 5:38pm PST
>
> Pause
> Run will pause at the time shown   **05:38PM PST**
> you to analyze templating efficiency.
>
>   Cancel   Start run

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

By selecting	You can obtain the QC samples
Time	immediately after the run ends, at the time you specify (12 hours, 45 minutes after run start).
Pause	when the instrument pauses operation before the chip loading step (about 11 hours into the run).

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

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

9. On the Run Options screen, touch **Start run** to 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 aborts the run and displays the error on the instrument touchscreen. If a run fails:

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

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

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

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

11. 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 11 hours after the start of the run).

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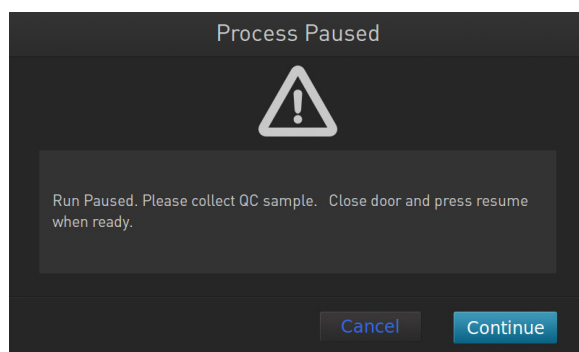
    IMPORTANT! If you unintentionally close the instrument door before you obtain the QC samples, you must wait until the end of the run before you can collect them. You cannot pause the run or open the door after it has been closed.

b. Transfer the QC samples (entire volume) from Positions A and B of the Ion 520™ & Ion 530™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.

IMPORTANT! Do not remove the Library Sample Tubes from the Ion 520™ & Ion 530™ Chef Reagents cartridge.
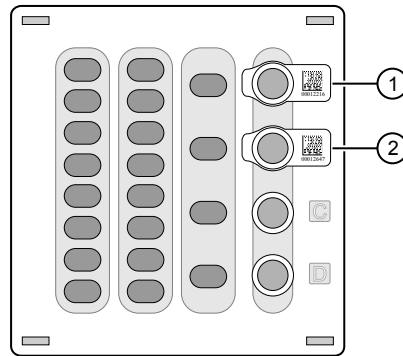
① Position A (QC Sample)
② Position B (QC Sample)

c. Analyze the QC samples.

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

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

d. If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes. See "Quality control ofIon 520™ & Ion 530™ ISPs" on page 68.

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

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

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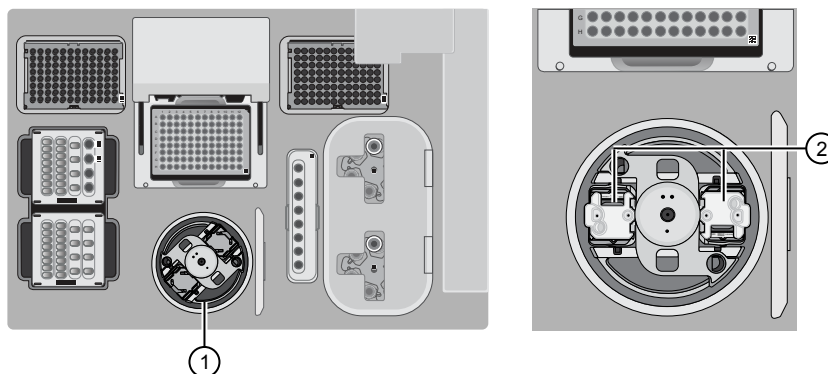
Unload the chips for sequencing

1. Open the instrument door:

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

   b. Lift the instrument door to the top of the travel until the latch mechanism engages.
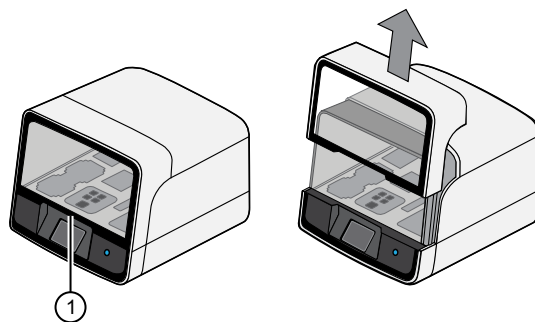
① Hold here, then lift

2. Open the lid of the Chip-loading centrifuge, then unload both chip/bucket assemblies from the Ion Chef™ Instrument.


① Chip-loading centrifuge
② Remove

To unload each chip:

   a. Remove the chip/bucket assembly from the Chip-loading centrifuge.

   b. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.

c. Grasp the chip by its edges, carefully lift the chip out of the bucket, then set it aside on a clean, static-free surface. Return the bucket to the Chip-loading centrifuge.
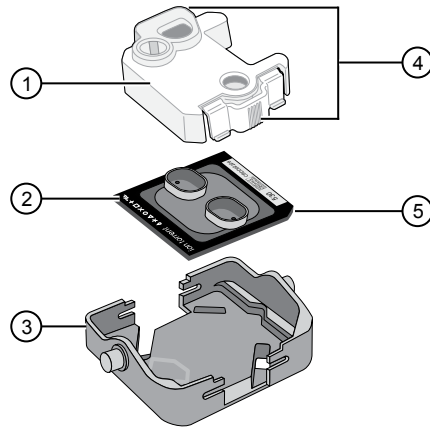
① Chip Adapter
② Ion chip
③ Bucket
④ Press and remove adapter
⑤ Remove from bucket carefully

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

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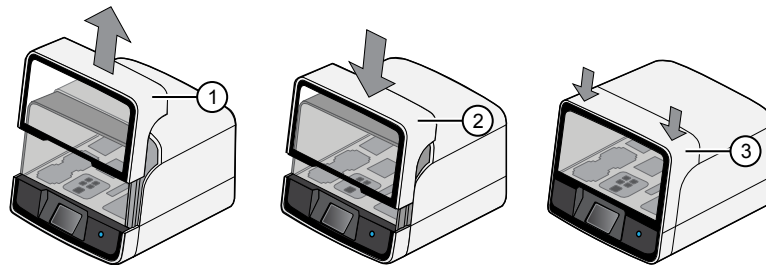
① Lift door first
② Lower
③ Press down to lock

4. Load one or both chips into Ion S5™ Sequencers and promptly start the sequencing runs.

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

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

4

Initialize the sequencer

Initialization takes ~40 minutes.

Ion S5™ System component positions

① Touchscreen
② Power button
③ Ion S5™ Sequencing Reagents cartridge
④ Chip clamp

⑤ Ion S5™ Wash Solution bottle. Waste reservoir located behind the Wash Solution bottle (shown on the right).
⑥ Ion S5™ Cleaning Solution bottle
⑦ Waste reservoir

Note: The system uses RFID technology to verify that the proper reagents are loaded in positions 3, 5, and 6. Reagents that exceed their expiration date or usage count generate an error message prompting the user to replace the reagent before performing the run.

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

Before you begin

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

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

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

- Unbox the Ion S5™ Wash Solution bottle. Invert the bottle 5 times, then swirl at an angle to mix thoroughly.

- Remove the red cap from the Ion S5™ Wash Solution and Ion S5™ Cleaning Solution bottles immediately before installing on the instrument.

When a manual cleaning of the sequencer is required

The Ion S5™ Sequencer and Ion S5™ XL Sequencer require that a cleaning be performed before initialization. This is normally performed automatically at the completion of the previous sequencing run. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run. However, if the "Enable post-run clean" checkbox (see "Start the sequencing run") is unchecked to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. See "Perform a manual cleaning of the sequencer" on page 66 for more information on how to perform a manual cleaning.

If an Ion S5™ Sequencer or an Ion S5™ XL Sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. See "Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge" on page 67 for more information.

Initialize the sequencer

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

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

3. Reinstall the empty waste reservoir.

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

5. Ensure the new Ion S5™ Wash Solution bottle is thoroughly mixed. Then remove the red cap and install.
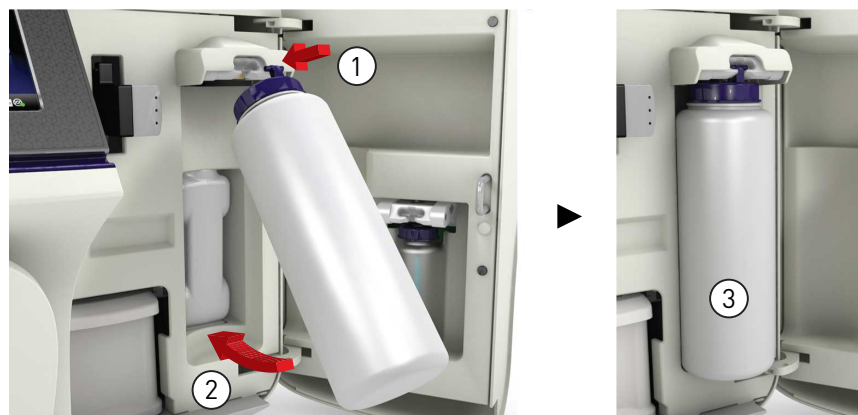
6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.

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

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

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

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

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

   Note: See "Bottle leak check fails" on page 64 if a Checking Reagent: Failed warning appears.

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

The instrument is now ready for a sequencing run.

Reagent consumables disposal

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

CO_2 scrubber removal and disposal

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

1. Invert the Ion S5™ Sequencing Reagents cartridge over an appropriate receptacle to drain all residual liquid.

2. Wearing gloves, insert the Ion S5™ Cartridge Tool firmly into the CO_2 scrubber until the flange stops on the top of the cartridge.
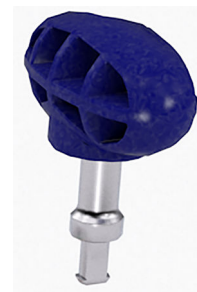
Ion S5™ Cartridge Tool

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

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

Recycle Ion S5™ Wash and Cleaning solution bottles

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

1. Open the expended bottle by unscrewing the cap.

2. Remove the cap, sipper, and filter, then pour any residual liquid into an appropriate receptacle.

3. Rinse the empty bottle with water. Pour out the rinse water into the same liquid waste receptacle.

4. Recycle or dispose of the clean bottle, cap, sipper, and filter according to applicable regulations.

5 Start the sequencing run

Chip handling guidelines

To place a chip in the chip clamp:
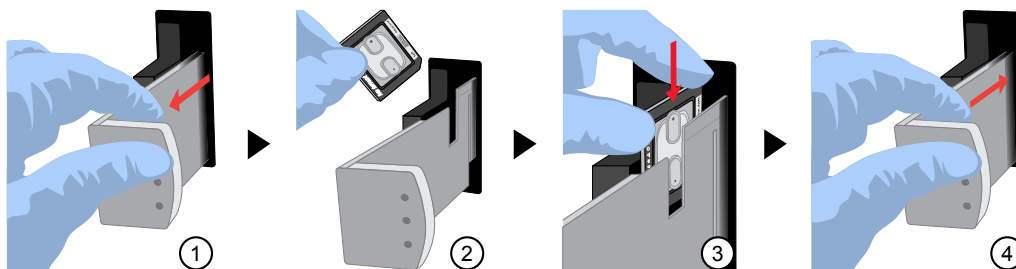
① Slide the chip clamp out.

② Remove the chip currently in the clamp.

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

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

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

Ion 520™ & Ion 530™ Kit – Chef User Guide

Start the sequencing run

We recommend that you sequence loaded chips on the Ion S5™ or Ion S5™ XL Sequencer as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

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

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

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

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

4. Confirm that the correct Planned Run has auto-populated, then press **Review**.

5. *(Optional)* If this is to be the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then press **Review**.

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

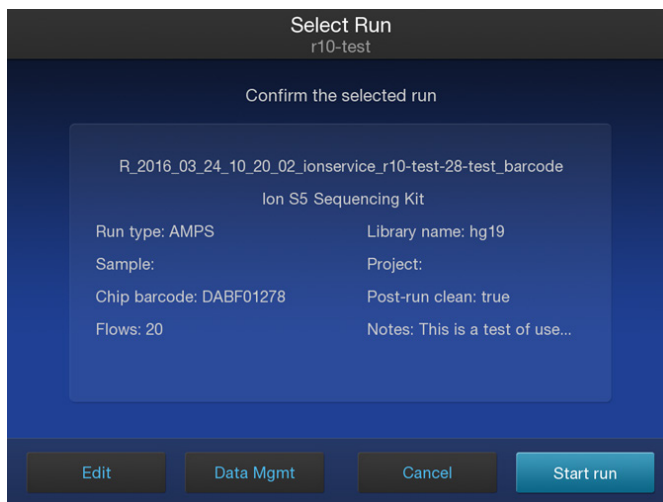6. Confirm the remaining pre-populated settings are correct, or make changes using the buttons and dropdown lists if necessary.

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

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

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

Maintain the sequencer

Required materials

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

Clean or decontaminate the sequencer

In the event of a spill or leak on or inside the instrument, perform the following steps.

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

1. Remove the Ion S5™ Wash Solution bottle, then remove and empty the waste reservoir.

2. Remove the Ion S5™ Sequencing Reagents cartridge.

3. Inspect the waste and nucleotide reagent bays for liquid.

4. Using absorbent paper soak up as much liquid as possible, then wash the affected area with 10% bleach solution.

5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

6 Clean the Ion Chef™ System

About the cleaning protocol

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


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

Materials required

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

Clean the Ion Chef™ Instrument

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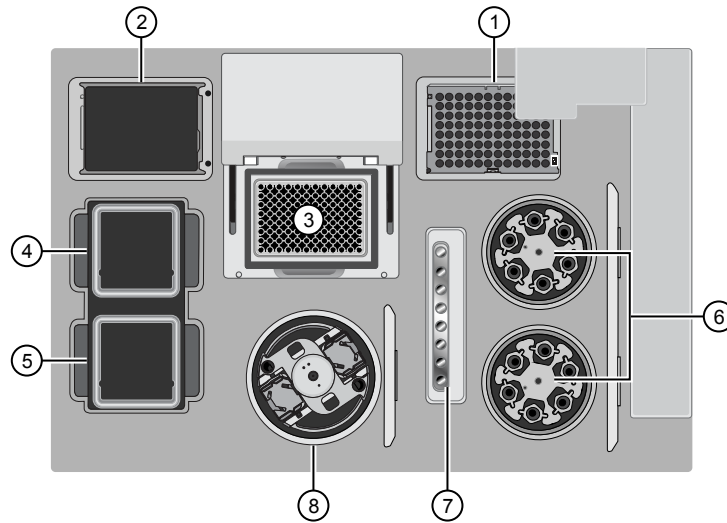

Ion Chef™ Instrument stations

① Waste pipette tip position
② Empty Tip Cartridge: move to waste pipette tip station
③ Thermal cycler sample block
④ Reagents station

⑤ Solutions station
⑥ Recovery centrifuges
⑦ Enrichment station
⑧ Chip-loading centrifuge

Remove and dispose of used consumables

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

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

2. Lift the instrument door to the top of the travel until the latch mechanism engages.
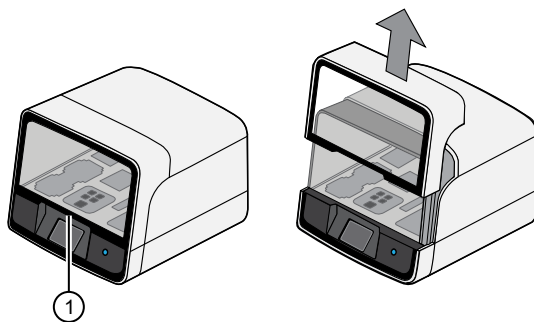
① Hold here, then lift

3. Remove, then discard the PCR plate from the thermal cycler sample block.

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

IMPORTANT! Handle the disposable reservoir in the waste tip position with care. During the run, liquid waste collects in the reservoir. Dispose of the liquid waste by tipping the reservoir on one corner and pouring the waste into an appropriate waste container:
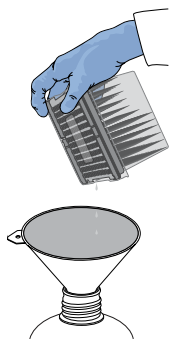
IMPORTANT! Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge from the new tip position to the waste tip position.

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

6. Remove, then discard the
   - Ion 520™ & Ion 530™ Chef Reagents cartridge
   - Ion S5™ Chef Solutions cartridge
   - Enrichment Cartridge v2

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

8. Close the Chip-loading centrifuge cover.

Inspect and clean the Recovery centrifuges and buckets

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

| Is liquid present?	Action
No	Go to "Start the cleaning" on page 58.
Yes	Clean the centrifuge bowl and buckets as described below. **IMPORTANT!** Clean the Recovery centrifuges occasionally, only when excess liquid is noticeable in the bowl and/or buckets. You do *not* need to clean the centrifuge after every run.

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

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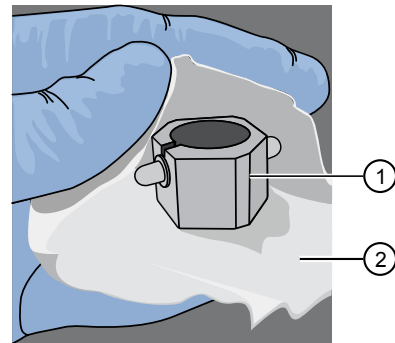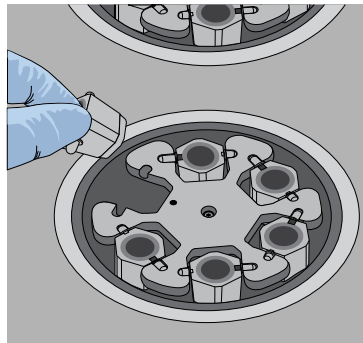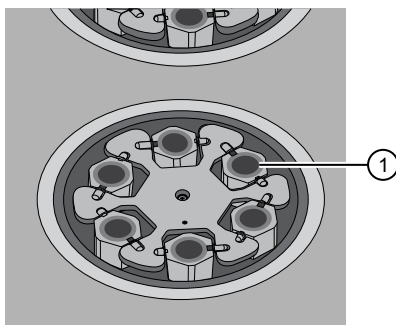

① Bucket
② Lint-free wipe

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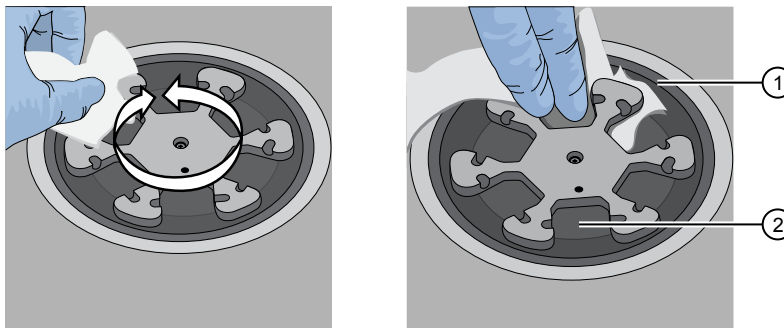
① Inside rim of the centrifuge
② Bottom of the centrifuge bowls

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

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

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

① Buckets (cleaned and installed)

Start the cleaning

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

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

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


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

3. Confirm that you have removed all consumables from the Ion Chef™ Instrument, except the empty pipette tip rack in the waste tip position, then touch **Next**.
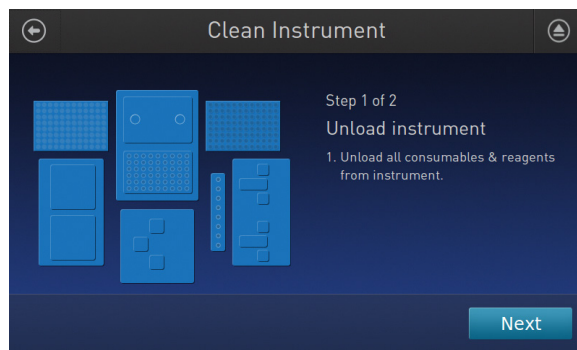
4. With the door closed, touch **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.
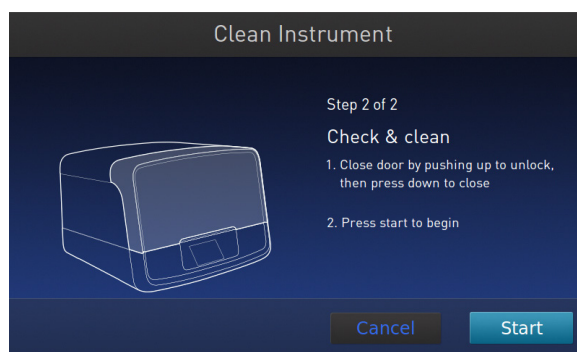
CAUTION! The Ion Chef™ Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.

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 are possible.	1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.
Cannot open the instrument door	An obstruction is present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error has occurred.	Contact Technical Support to report the problem and for further assistance.
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.
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.
Liquid residue is present in the Recovery centrifuges following a run	During normal instrument operation, a noticeable coating of liquid can collect on the bowl and buckets of the Recovery centrifuges following repeated runs.	Remove the residue as instructed in "Inspect and clean the Recovery centrifuges and buckets" on page 57.

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 520™ & Ion 530™ Chef Reagents (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.

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.

Ion S5™ System setup and operation

Ion S5™
Sequencer alarms
and events

Observation	Possible cause	Recommended action
Red "Alarms" and/or "Events" message in Main Menu Ion S5 Initialize Alarms Settings -0.12 psi 11.89 psi 27.00 °C 28.22 °C 8% 19%	• Software updates available • Connectivity issues • Instrument not detecting required files or hardware	Click on the red pop-up to see detailed messages. • If a message states "Newer Software Available": **IMPORTANT!** After updates are installed, the instrument must be restarted. a. On the home screen, select **Settings ▸ Check for Updates**. b. Select the **Released Updates** checkbox, then press **Update**. c. When installation is complete, follow the onscreen prompts to restart the instrument. **Note:** In some cases, the instrument restarts automatically after software installation. • If a message states "No Connectivity to Torrent Server", "No Connectivity to ftp server", or "Network Manager not connected", disconnect and re-connect the ethernet cable, confirm that the router is operational, and verify that the network is up and running. • For any other messages: a. Power off the instrument: On the home screen, select **Settings ▸ System Tools ▸ Shut Down ▸ Shut Down**. b. Wait 30 seconds, then press the button on the front of instrument to power on the instrument. • If the red "Alarms" and/or "Events" message still appears in the main menu, contact Technical Support.

Initialization—
General errors

Observation	Possible cause	Recommended action
Bottle leak check fails	• Bottle seal 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 Sphere™ Assay troubleshooting table

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

Qubit™ Fluorometer observation	Ion S5™ System observation	Possible cause	Recommended action
<10% Templated ISPs	• Lower loading • Lower % enriched • Lower key signal • Lower throughput	Too little library input into template preparation	• Increase library input to target 20–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>30% Templated ISPs, but <70%	Increased number of filtered reads	Too much library input into template preparation	• Decrease library input to target 20–25% templated ISPs. *or* • Continue with sequencing; expect lower throughput.
>70% Templated ISPs	• Increased % primer dimer filtered reads • Lower throughput	Adapter dimer contaminating library, more likely in short amplicon, Ion AmpliSeq™ or miRNA libraries	• Check Bioanalyzer™ traces for adapter dimer peak (Amplicon library or Ion AmpliSeq™ library peak around 70 bp; miRNA library peak around 60bp). • Re-purify library using Agencourt™ AMPure™ XP Kit clean-up steps as outlined in the appropriate user guides.
	• Low loading • Low % enriched • Lower throughput • High % filtered reads	Ion Chef™ System under performance	Troubleshoot with Technical Support or a Field Application Scientist.

Supplemental procedures

Perform a manual cleaning of the sequencer

A cleaning protocol is normally performed automatically at the completion of each sequencing run. In the event that a cleaning is necessary, follow the listed steps:

1. On the home screen, select **Settings ‣ Clean Instrument**.
   The instrument door unlocks allowing access to the consumables.

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

3. Reinstall the empty waste reservoir and a *used* Ion S5™ Wash Solution bottle.

4. Ensure the Ion S5™ Sequencing Reagents cartridge and Ion S5™ Wash Solution bottle are properly installed.

   IMPORTANT! Perform the cleaning with a used reagent cartridge and wash solution bottle installed. The cleaning procedure pumps cleaning solution into the wash solution bottle and reagent cartridge making them unsuitable for sequencing.

   ---

5. Place a used sequencing chip in the chip clamp, then push the chip clamp in all the way to engage.

Appendix B Supplemental procedures
Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge

B

6. Close the instrument door, then press **Next**.

   Cleaning takes ~35 minutes to complete. Upon completion the instrument door automatically unlocks and the chip and cartridge clamps disengage.

7. Proceed to "Initialize the sequencer" on page 47.

Perform an instrument reset run with an initialized, unused Sequencing Reagents cartridge

Cleaning is normally performed at completion of a sequencing run automatically. If an Ion S5™ Sequencer or an Ion S5™ XL Sequencer is initialized and

- a sequencing run is not started within 24 hours after initialization, *or*
- a sequencing run is not completed due to a power failure or an abort, and <200 flows occurred before the stoppage

an instrument reset run is required to ensure proper cleaning before reinitialization. Do NOT perform a manual cleaning with an unused, initialized Ion S5™ Sequencing Reagents cartridge.

Note:
· If a power failure or abort occurs during the second of two runs started after a single initialization, a manual cleaning (page 66) is sufficient.
· If the number of flows that occurred before a power failure or abort is unknown, perform an instrument reset run.

To perform an instrument reset run, use the following procedure before reinitialization:

1. In the instrument touchscreen main menu, press **Run**.
   The instrument door and chip clamp unlocks.

2. Ensure that a used sequencing chip is in the chip clamp, then push the chip clamp in all the way to engage.

3. Close the instrument door, then press **Next**.

4. When prompted, select **Planned Run (none)**. Ensure that the **Enable post-run clean** checkbox is selected, then press **Review**.

5. In the **Select Run** screen, press **Edit**, then in the **Detail** screen set the number of flows to **200** manually. Ensure that the **Post-Run/Clean** checkbox is selected, then press **Close**.

6. Press **Start run**, then press **Accept** to confirm that Post-Run Clean is enabled, and to start the run.

When the instrument reset run completes, the instrument automatically performs the cleaning procedure. After cleaning, the touchscreen returns to the main menu.

Quality control of Ion 520™ & Ion 530™ ISPs

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

The Qubit™ 2.0 Fluorometer or the Qubit™ 3.0 Fluorometer can be used to perform a quality assessment of unenriched Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion S5™ Sequencer. For detailed protocols, see the

- *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387) or
- *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388)

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

Acceptance criteria for unenriched Ion 520™ & Ion 530™ ISPs

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

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

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

Note: If the results are outside the desired Percent Templated ISPs range, then increase or decrease the library input appropriately. See the "Ion Sphere™ Assay troubleshooting table" on page 65 for more information.

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

Quality control using the Guava™ easyCyte™ 5 Flow Cytometer

The Guava™ easyCyte™ 5 Flow Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion S5™ Sequencer or Ion S5™ XL Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Ion 520™ & Ion 530™ Chef Reagents cartridge (positions A and B).
- **Enriched samples** – Obtain sample 1 from position E and sample 2 from position A on the Enrichment Cartridge v2.

For details, see the *Ion PI™ Ion Sphere™ Particles Quality Assessment Using the Guava™ easyCyte™ 5 Flow Cytometer User Bulletin* (Pub. No. MAN0007496), available at **thermofisher.com**.

Quality control using the Attune™ Acoustic Focusing Cytometer

The Applied Biosystems™ Attune™ Acoustic Focusing Cytometer can be used for quality assessment of unenriched and enriched Ion Sphere™ Particles generated for up to 400 base-read sequencing on the Ion S5™ Sequencer or Ion S5™ XL Sequencer.

- **Unenriched samples** – Obtain the QC samples from the corresponding Library Sample Tubes on the Ion 520™ & Ion 530™ Chef Reagents cartridge (positions A and B).

- **Enriched samples** – Obtain sample 1 from position E and sample 2 from position A on the Enrichment Cartridge v2.

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

Maintain the Ion Chef™ System

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

Install a firmware update

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

Check the Ion Chef™ Instrument firmware

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

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

3. On the **Software Update** screen, touch **Release** to search the server for updates to the Ion Chef™ Instrument firmware.
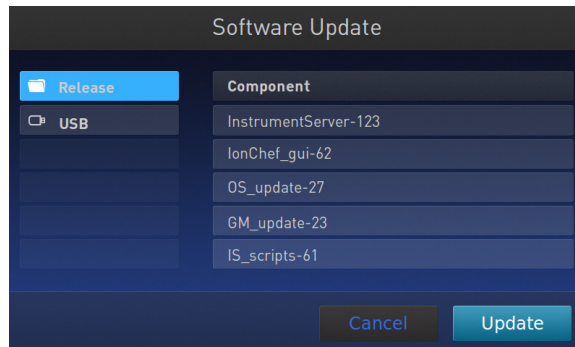
Note: Users are also notified of the availability of firmware updates on the Notifications screen.

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

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

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

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

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

Change the instrument name

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

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

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

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

   Note: Use only alphanumeric characters. Do not use special characters or spaces in the name.
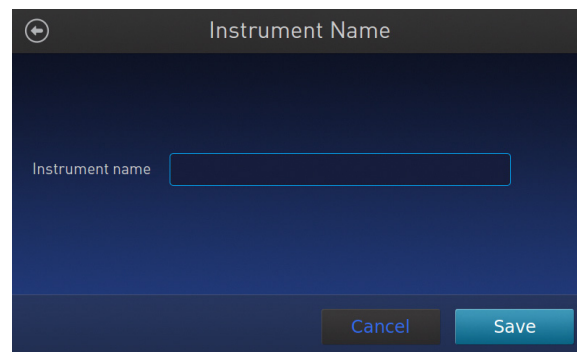
4. In the next screen, touch **Save** again. Power off and on the Ion Chef™ Instrument to effect the name change.

C

Ion Chef™ and Torrent Server network setup

Networking overview

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

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

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

This guide describes how to:

• Set up the Ion Chef™ instrument to talk to the Torrent Server on a local area network (LAN).

• Enable Planned Run sharing.

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

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

About the Torrent Server

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

Networking guidelines and best practices

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

Network setup workflow

<div align="center">

Collect the required network information.

▼

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

▼

Configure the Ion Chef™ instrument with the required information.

▼

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

</div>

Network setup examples

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

Network examples include:

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

Collect the required network information

Obtain the following information from your network administrator:

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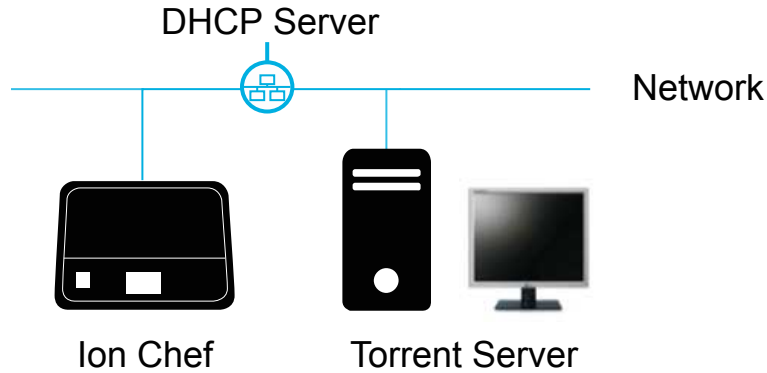
Materials required

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

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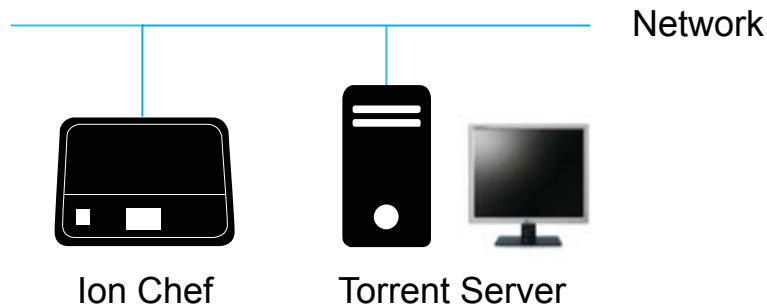
General requirements

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

DHCP IP

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

C

Direct connection

Network

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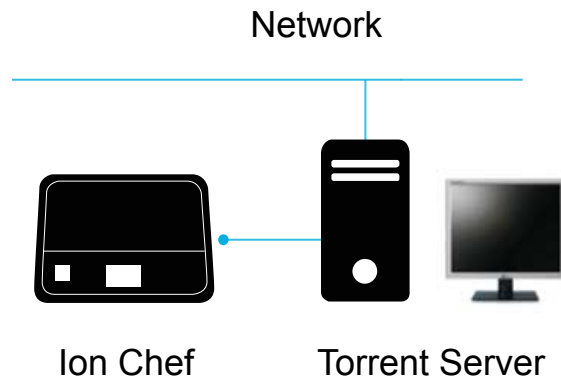
To configure the direct connection between an Ion Chef™ instrument and a Torrent Server, you will need a Category 6 cable of sufficient length.

Another direct connection configuration option consists of one Ion Chef™ instrument, one Torrent Server and two Ion S5™ XL Sequencers. However, this configuration is not recommended due to increased analysis times.

⚠ **WARNING!** The direct configuration shown below is known to result in lengthy analysis times.

Network

Torrent Server

Ion Chef

S5 XL 1   S5 XL 2
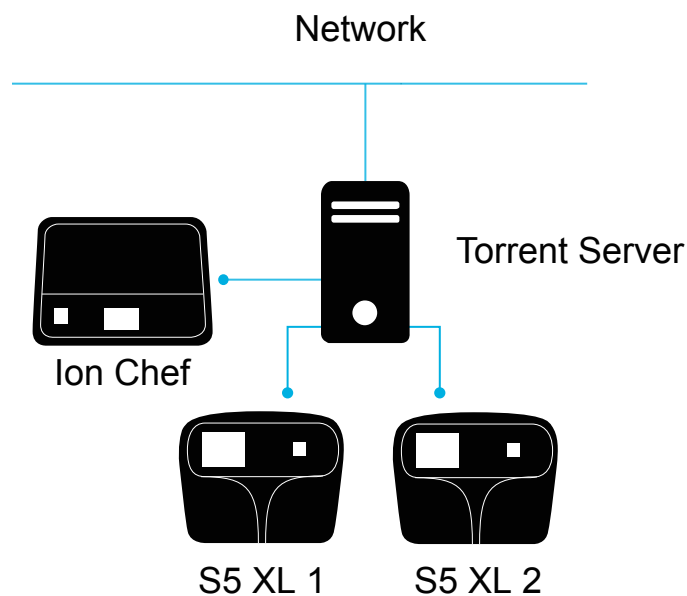
Ion Chef™ Networking Configuration Options

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

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

Target the Torrent Server IP Address

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

1. Log into Torrent Browser as the admin.

2. Click the Gear button and select **Configure**.
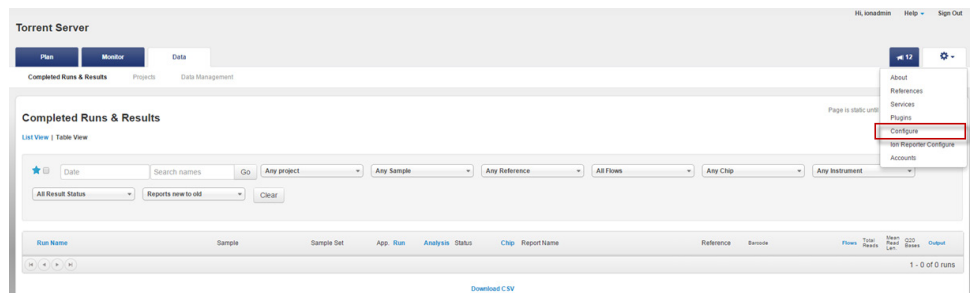
3. Scroll down to the Database Administration section and click the **Admin Interface** link.
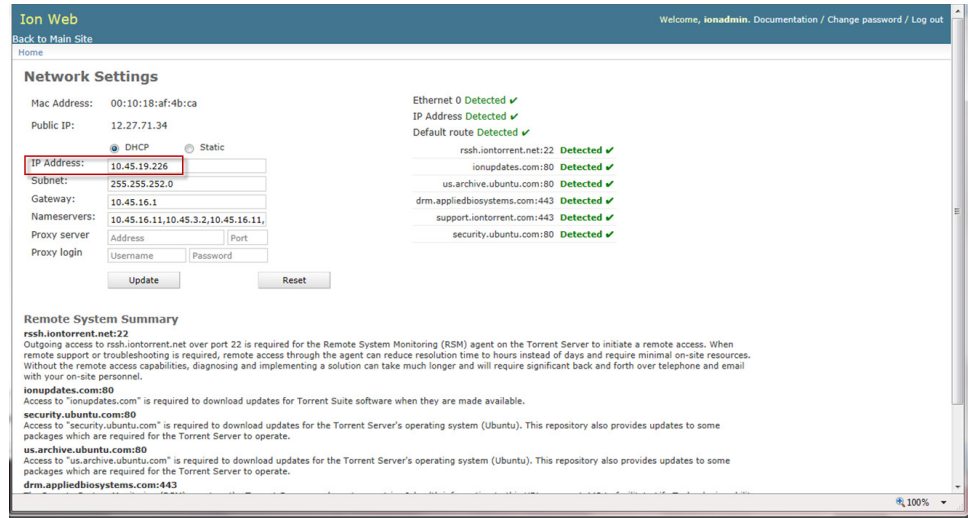
4. The Site Administration screen appears. Scroll to the bottom and click **View Network Settings**.
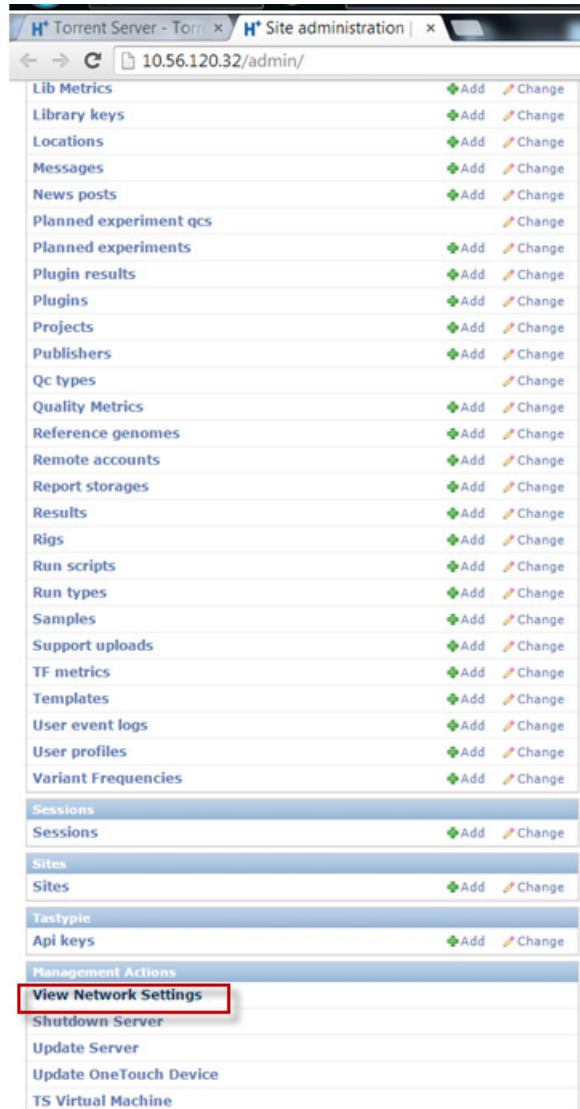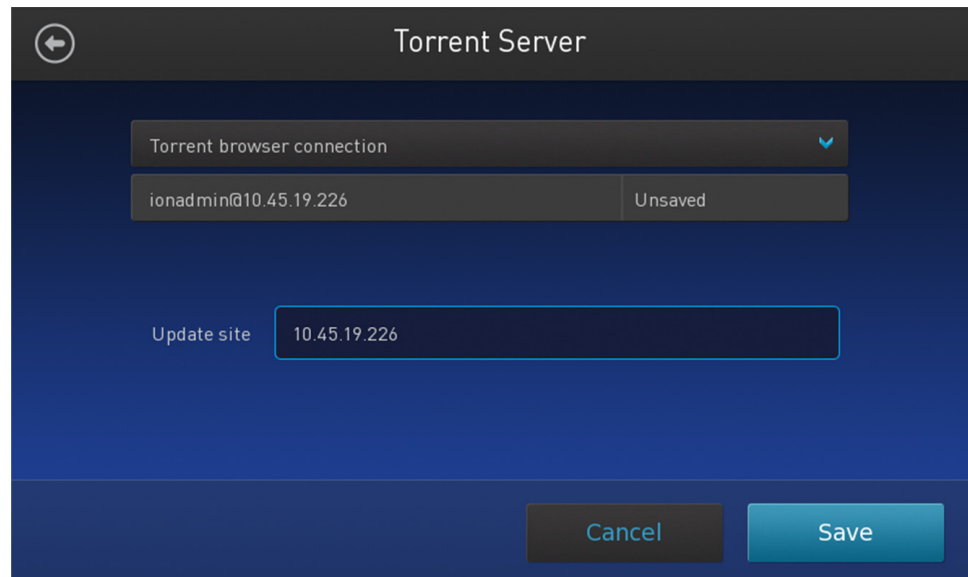
5. Note (copy or write down) the Torrent Server IP address displayed on the Network Settings screen.

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

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

C

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

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

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

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

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

Create an exception

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

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

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

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

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

3. Follow this command by manually applying the change:

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

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

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

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

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

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

5. Reboot the server.

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

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

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

Enable Planned Run sharing among Torrent Servers

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

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

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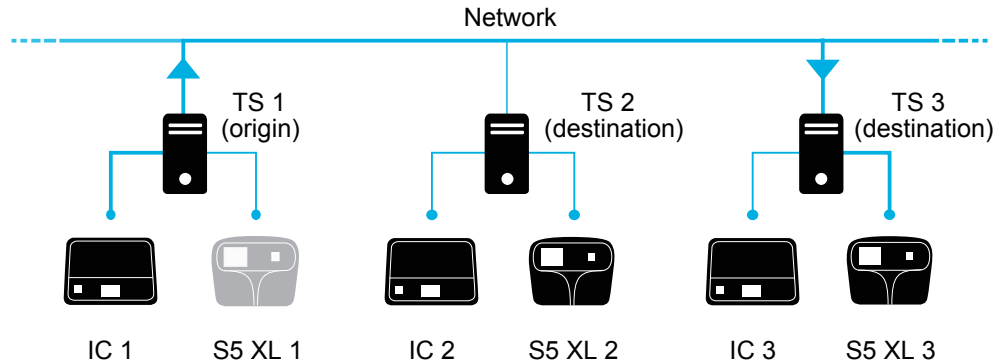
Requirements include:

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

Set up Server Network (admin action)

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

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.

*Ion 520™ & Ion 530™ Kit – Chef User Guide*

Transfer a Planned Run

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

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

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

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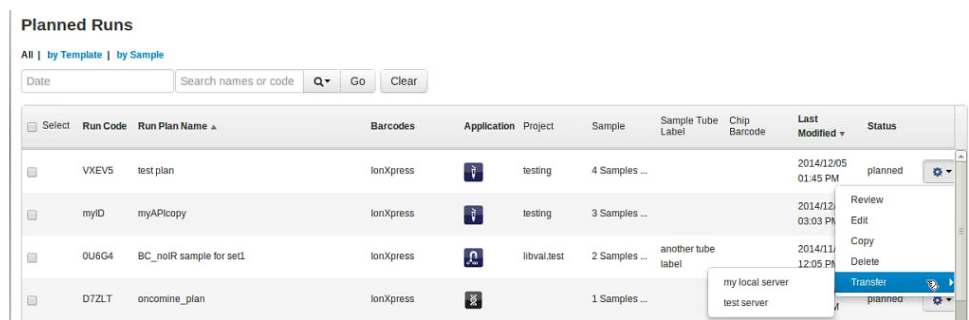
3. A confirmation window appears. Check the information, then click **Transfer**.

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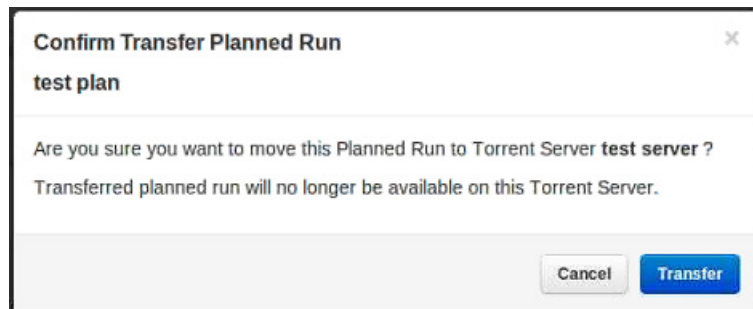
4. A status window displays the results of the transfer:

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

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

test plan

Successfully created test plan on Torrent Server test server

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

....found BED files: target.bed

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

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

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

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

Close

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

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

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

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

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

Undo a Planned Run transfer (administrator)

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

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

Undo a Planned Run transfer (user)

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

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

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

D

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.
CE	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
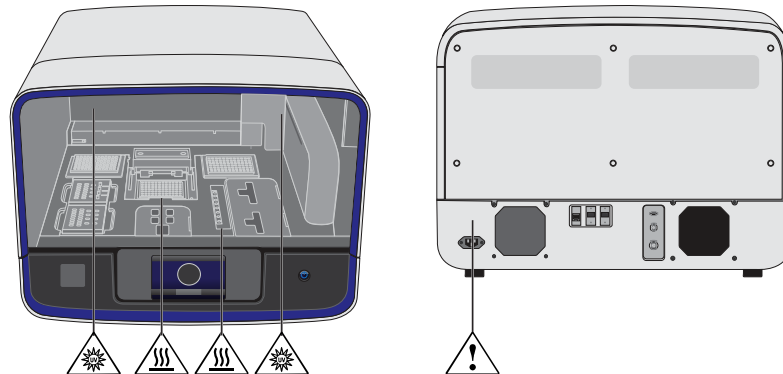
Safety information for instruments not manufactured by Thermo Fisher Scientific

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

Instrument safety

General

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

Physical injury

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

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

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

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

Electrical

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

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

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

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

Cleaning and decontamination

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

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

Ultraviolet (UV) Safety

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

Safety and electromagnetic compatibility (EMC) standards

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

Safety compliance

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

EMC

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

Environmental design

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

Instrument safety — Ion S5™/Ion S5™ XL System

For detailed information on Ion S5™ and Ion S5™ XL instrument safety symbols and alerts, safety and electromagnetic compatibility standards, and general instrument safety, see the Safety appendix of the *Ion S5™ and Ion S5™ XL Instrument User Guide* (Pub. No. MAN0010811), available at **thermofisher.com**.

Chemical safety

⚠ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

· Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.

· Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).

· Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).

· Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.

· Handle chemical wastes in a fume hood.

· Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

· After emptying a waste container, seal it with the cap provided.

· Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

· Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

· **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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

Biological hazard safety

⚠ **WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

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

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

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

ThermoFisher
SCIENTIFIC