USER GUIDE

Ion PGM™ Sequencing 400 Kit — Technology Access

For use with:

Ion PGM™ Template IA 500 Kit

Ion Personal Genome Machine® (PGM™) System and the following Ion PGM™ Chips:

Ion 314™ Chip v2

Ion 316™ Chip v2

Ion 318™ Chip v2

Catalog Number 4482002

Publication Number MAN0007242
Revision A.0

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About this guide

IMPORTANT! Before using this kit with the Ion Personal Genome Machine® (PGM™) System, read and understand the information in **Appendix F. Safety** starting on page 63.

Revision history

Revision	Date	Description
A.0	6 Dec. 2013	• Supports 500-base-read sequencing from template prepared using the Ion PGM™ Template IA 500 Kit • Version numbering changed to alphanumeric format and reset to A.0 in conformance with internal document control procedures.
2.0	16 May 2013	• Supports the Ion 316™ Chip v2 (Cat. nos. 4483188 and 4483324) and Ion 318™ Chip v2 (Cat. nos. 4484354 and 4484355) • Includes updated references to most recent version of Torrent Suite™ software: 4.0
1.0	1 March 2013	• Supports the new Ion PGM™ Sequencing 400 Kit (Cat. no. 4482002) and 400 base-read sequencing • Supports the Ion 314™ Chip v2 (Cat. no. 4482261) • Supports the Ion PGM™ Template OT2 400 Kit (Cat. no. 4479878) • Includes images of the redesigned Ion PGM™ Sequencer • Includes updated references to most recent version of Torrent Suite™ software: 3.4.2 • Includes new instrument operation appendix (page 61) and revised safety appendix (page 63) • Includes revised troubleshooting for the Initialization error message, "Added too much W1 to W2," on page 55 • Includes additional warnings to make sure that sippers are firmly attached to all ports

Purpose of the guide

This user guide provides protocols and reference information for using the Ion PGM™ Sequencing 400 Kit (Cat. no. 4482002) with the Ion 314™ Chip v2 (Cat. no. 4482261), Ion 316™ Chip v2 (Cat. nos. 4483188 and 4483324) and Ion 318™ Chip v2 (Cat. nos. 4484354 and 4484355).

1 Product information

Product description

The Ion PGM™ Sequencing 400 Kit (Cat. no. 4482002) includes reagents and materials for sequencing enriched, template-positive Ion Sphere™ Particles (ISPs) using the Ion 314™ Chip v2, Ion 316™ Chip v2 and Ion 318™ Chip v2 on the Ion PGM™ System. It also includes components for cleaning and initializing the instrument.

This kit supports the following sequencing modes, depending on your template kit:

Sequencing type	Number of flows	Template Kit
500-base-read	1100	Ion PGM™ Template IA 500 Kit (Cat. no. A24622)
400-base-read	850	Ion PGM™ Template IA 500 Kit (Cat. no. A24622) *or* Ion PGM™ Template OT2 400 Kit (Cat. no. 4479878)
300-base-read	640	

Note: For additional information regarding number of flows and run times, see **Appendix D. Sequencing run times** on page 60.

Intended use

The Ion PGM™ Sequencer performs real-time measurements of hydrogen ions produced during DNA replication.

Library kit compatibility

Ion AmpliSeq™ libraries

When sequencing Ion AmpliSeq™ libraries, we recommend using the Ion PGM™ Sequencing 200 Kit v2 (Cat. no. 4482006) for optimal accuracy. See the *Ion PGM™ Sequencing 200 Kit v2 User Guide* (Pub. no. MAN0007273) for information about this kit.

All other libraries

The Ion PGM™ Sequencing 400 Kit is recommended for all other library types, e.g., ≥ 250 base-read gDNA and RNA libraries.

Template kit compatibility

Compatible kits

This sequencing kit can be used with the following template preparation kits:

- Ion PGM™ Template IA 500 Kit (Cat. no. A24622), for preparation of enriched, template-positive ISPs using isothermal amplification technology (up to 500-base-read libraries)
- Ion PGM™ Template OT2 400 Kit (Cat. no. 4479878), for preparation of enriched, template-positive ISPs using the Ion OneTouch™ System (300–400 base-read libraries)

Incompatible kits

IMPORTANT! The following template kits are **not recommended** for use with this kit and may not provide optimal results:

- Ion PGM™ Template OT2 200 Kit (Cat. no. 4480974)
- Ion OneTouch™ 200 Template Kit v2 DL (Cat. no. 4480285)

Software compatibility

This sequencing kit is compatible with version 4.0.2 or later of the Torrent Suite™ software and Ion PGM™ System software. Be sure to update your software before using this kit.

Ion PGM™ Chip compatibility

This sequencing kit is compatible with the Ion 314™ Chip v2 (Cat. no. 4482261), Ion 316™ Chip v2 (Cat. nos. 4483188 and 4483324) and Ion 318™ Chip v2 (Cat. nos. 4484354 and 4484355)

Kit contents and storage

IMPORTANT! Do not mix components from the Ion PGM™ Sequencing 400 Kit (Cat. no. 4482002) with components from previous sequencing kits, because some formulations have changed.

The Ion PGM™ Sequencing 400 Kit (Cat. no. 4482002) includes the following components[1]:

Ion PGM™ Sequencing Supplies 400 Kit (Part no. 4482003)				
Component	**Color**	**Quantity**	**Volume**	**Storage**
Wash Bottle Sipper Tubes	Gray	8 tubes for 250-mL bottles 4 tubes for 2-L bottles	—	15°C to 30°C
Reagent Bottle Sipper Tubes	Blue	16 tubes	—	
Reagent Bottles w/ labels (50 mL)	—	25 bottles	—	
Wash 1 Bottle w/ label (250 mL)	Green	1 bottle	—	
Wash 2 Bottle w/ label (2 L)	Green	1 bottle	—	
Wash 3 Bottle w/ label (250 mL)	Green	1 bottle	—	
Ion PGM™ Sequencing Reagents 400 Kit (Part no. 4482004)				
Component	**Cap color**	**Quantity**	**Volume**	**Storage**
Ion PGM™ Sequencing 400 dGTP	Black	1 tube	100 µL	–30°C to –10°C
Ion PGM™ Sequencing 400 dCTP	Blue	1 tube	100 µL	
Ion PGM™ Sequencing 400 dATP	Green	1 tube	100 µL	
Ion PGM™ Sequencing 400 dTTP	Red	1 tube	100 µL	
Ion PGM™ Sequencing 400 Polymerase	Yellow	1 tube	12 µL	
Sequencing Primer	White	1 tube	48 µL	
Control Ion Sphere™ Particles	Clear	1 tube	20 µL	
Ion PGM™ Sequencing Solutions 400 Kit (Part no. 4482005)				
Component	**Label color**	**Quantity**	**Volume**	**Storage**
Ion PGM™ Sequencing 400 W2 Solution	Black	4 bottles	126.25 mL each	2°C to 8°C (store W2 Solution protected from light)
Ion PGM™ Cleaning Tablet	—	4	—	
Annealing Buffer	—	1 bottle	12 mL	
Ion PGM™ Sequencing 400 1X W3 Solution	—	2 bottles	100 mL each	

[1] Reagents provided are only sufficient for a maximum of 4 runs per kit.

Required materials and equipment

The Ion PGM™ Sequencing 400 Kit uses common molecular biology equipment, supplies, and reagents[2]. Where noted, supplies are available from Major Laboratory Suppliers (MLS).

The following table lists required materials and equipment:[3]

✓	Description	Supplier	Catalog number	Quantity
	Ion 314™ Chip Kit v2 ***or***	Life Technologies	4482261	8-pack
	Ion 316™ Chip Kit v2 ***or***		4483188 4483324	4-pack 8-pack
	Ion 318™ Chip Kit v2		4484354 4484355	4-pack 8-pack
	Ion Personal Genome Machine® (PGM™) System and all included accessories	Life Technologies	4482296 ***or*** 4462917	1
	Torrent Server	Life Technologies	4462918	1
	Tank of compressed nitrogen (grade 4.5, 99.995% or better)	Major Laboratory Supplier (MLS)	N/A	N/A
	Multistage (dual-stage) gas regulator (0-50 PSI, 2–3 Bar output)	VWR International	55850-422	1
	ELGA® PURELAB® Flex 3 Water Purification System ***or*** Equivalent 18 MΩ water system	Life Technologies ***or*** MLS	4474524	1
	Ion Chip™ MiniFuge, 120 VAC	Life Technologies	4479672	1
	Microcentrifuge (capable of >15,500 × g, fits 1.5-mL and 0.2-mL microcentrifuge tubes)	MLS	—	1
	0.22-µm or 0.45-µm vacuum filtration system and filters (nylon or PVDF filters, 1 L volume)	MLS	N/A	1
	Rainin® Pipet-Lite® XLS with RFID LTS 10 to 100 µL[4] (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	L-100XLS F21025 022470205	1
	Rainin® pipette tips (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	SR-L200F F167203 022493030	1 case

[2] An Ion PGM™ 2.5 L Waste Bottle (Part no. 4482565) is required for 1100-flow runs.

[3] Life Technologies has validated this protocol using these specific materials. Substitution may adversely affect system performance.

[4] Ensure tips from any vendors are low binding tips. Rainin® pipettes or an alternative product are required in addition to the standard set of 1- to 1000-µL pipettes. These are required for loading samples onto the Ion PGM™ Chips.

✓	Description	Supplier	Catalog number	Quantity
	Rainin® Pipet-Lite® XLS with RFID LTS 2 to 20 µL[5] (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	L-20XLS F21023 022470159	1
	Rainin® barrier pipette tips[6] (Alternatives from Gilson and Eppendorf may be used)	Rainin Gilson Eppendorf	SR-L10F F171303 022493028	1 case
	PCR tubes, Flat Cap, 0.2-mL (do not use polystyrene tubes)	VWR	10011-780	1 box
	Vortexer with a rubber platform	MLS	N/A	1
	Thermal cycler with a heated lid	MLS	N/A	1
	Graduated cylinders (1 L or 2 L volume)	MLS	N/A	1
	Glass bottle (1 L)	MLS	N/A	1
	15-mL conical tubes	MLS	N/A	Varies
	NaOH (10 M) molecular biology grade	MLS	N/A	Varies
	Pipette set and filtered tips, P2, P20, P200, and P1000 µL	MLS	N/A	1 set
	Microcentrifuge tubes, 1.5-mL or 1.7-mL	MLS	N/A	Varies
Optional materials				
	Ion PGM™ Weighted Chip Bucket	Life Technologies	4480283	1
	Ion PGM™ Controls Kit v2 [7]	Life Technologies	4482010	1
	Ion PGM™ Sequencing Sippers Kit[8]	Life Technologies	4478682	1
	Uninterruptable Power Supply (UPS)[9]	MLS	N/A	1
Materials needed if manual adjustment of W2 Solution pH is required (see Appendix C. Manually adjust the W2 Solution pH)				
	Orion® 3-Star Plus pH Benchtop Meter Kit with electrode, electrode stand, and calibration buffers	Thermo Scientific	1112003	1
	Magnetic stirrer (must hold 2-L bottle)	MLS	N/A	1
	Magnetic stir bar (4 cm)	MLS	N/A	1
	1 N HCl	MLS	N/A	Varies

Ion PGM™ System with Reagent and Wash Bottles attached

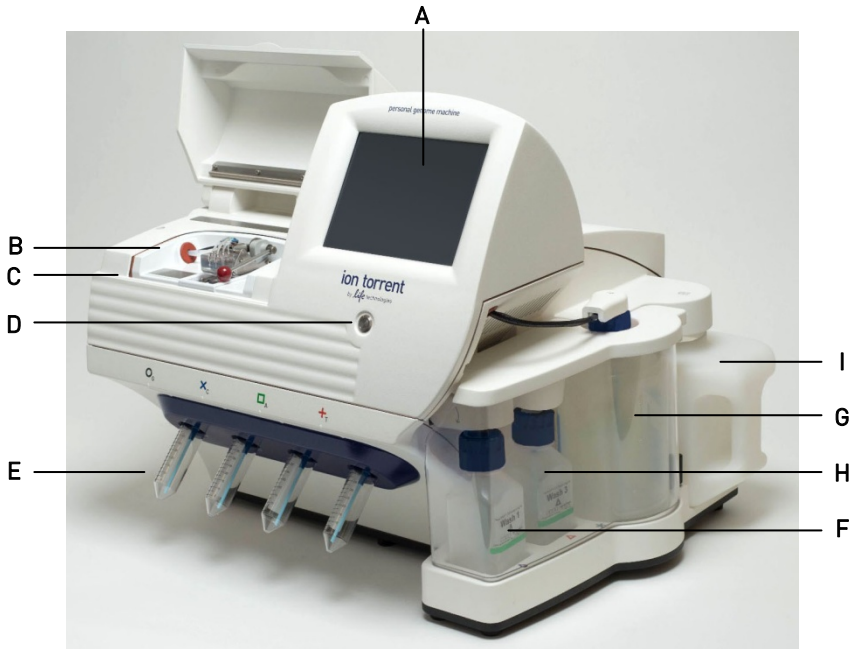
[5] Ensure tips from any vendors are low binding tips. Rainin® pipettes or an alternative product are required in addition to the standard set of 1- to 1000-µL pipettes. These are required for loading samples onto the Ion PGM™ Chips.

[6] Ensure tips from any vendors are low binding tips. Rainin® pipettes or an alternative product are required in addition to the standard set of 1- to 1000-µL pipettes. These are required for loading samples onto the Ion PGM™ Chips.

[7] Not commonly needed, but available for troubleshooting.

[8] Contains additional sipper tubes; not commonly needed.

[9] We recommend using an uninterruptable power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be compatible with 1500 W output or higher. The 1500 VA unit from APCC provides approximately 11 minutes of backup power for an Ion PGM™ System.



Label	Component
A	Touchscreen
B	Chip clamp
C	Grounding plate
D	Power button
E	Reagent bottles
F	Wash 1 Bottle (W1 position)
G	Wash 2 Bottle (W2 position)
H	Wash 3 Bottle (W3 position)
I	Waste Bottle

Precautions before using the Ion PGM™ System

For additional safety information, see in **Appendix F. Safety** starting on page 63.

Update the software

IMPORTANT! Before proceeding, make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.0.2 or later. See **Update the Ion PGM™ Sequencer software** on page 62.

Instrument should only be moved by trained personnel

IMPORTANT! The Ion PGM™ System is installed by trained Life Technologies service personnel and should not be moved.

Nucleic acid contamination

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

IMPORTANT! Possible contamination can occur during the transfer of dNTPs into Reagent Bottles. Be careful to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Change gloves after handling concentrated dNTP stocks.

CO_2 contamination

IMPORTANT! Dry ice (solid CO_2) should be kept away from areas where buffers, wash solutions or sources of molecular biology grade water for the Ion PGM™ System are used. High air concentrations of subliming CO_2 may influence the pH of such buffers during or after their preparation. The stability of the pH of these buffers is a critical factor in the performance of the Ion PGM™ System.

Instrument vibration and clearances

IMPORTANT! Significant vibration during sequencing may add noise and reduce the quality of the measurements. Ion PGM™ System must be installed on a bench that is free from vibrations or in contact with equipment that can cause vibrations to the bench (freezers, pumps, and other similar equipment).

IMPORTANT! The Ion PGM™ System must be positioned so that the front bezel is a minimum of 12 in. (30.5 cm) and the Reagent Bottles containing dNTPs are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. The instrument should be at least 40 in. (1 meter) away from major sources of electronic noise such as refrigerators or microwaves.

Static electricity

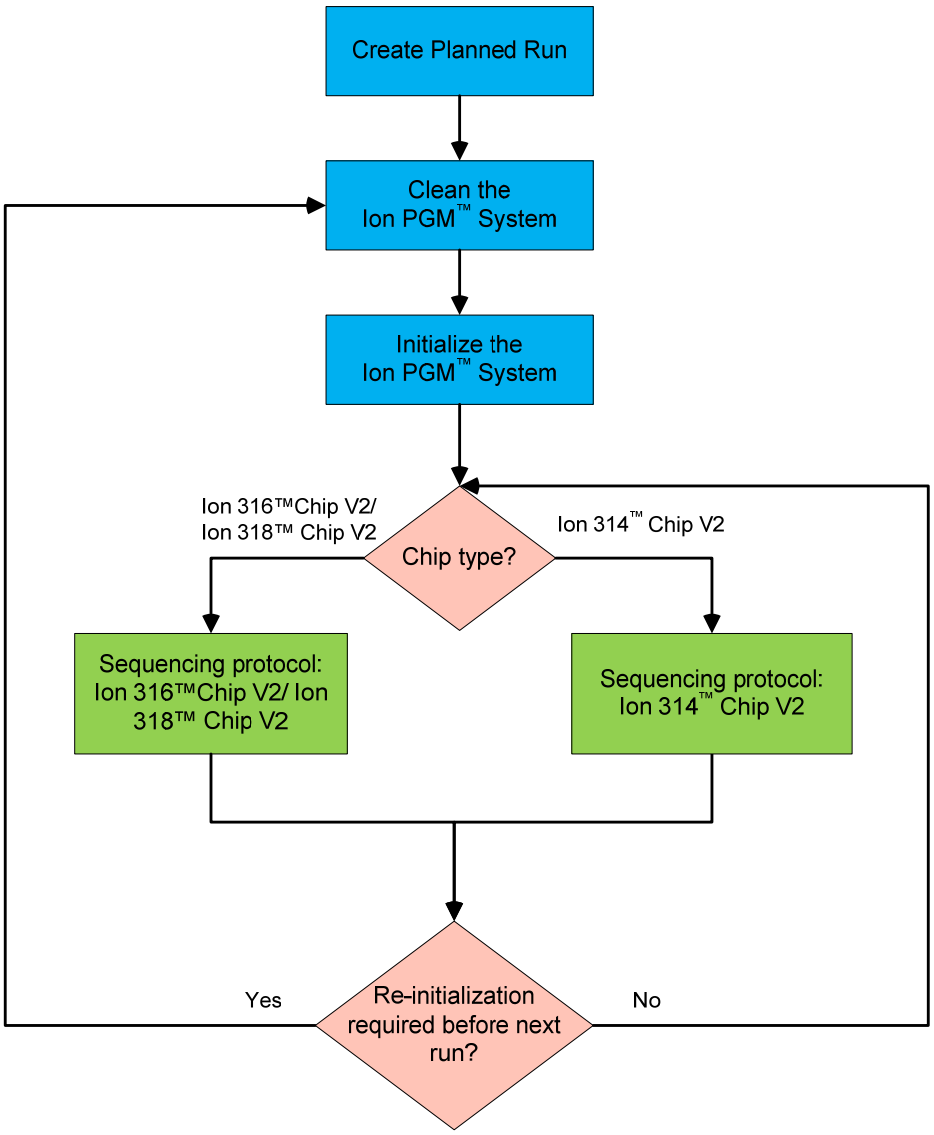
IMPORTANT! Always ground yourself on the touch plate (located next to the chip clamp) prior to handling chips to avoid possible damage from static electricity. Always use the touch plate to hold chips when they are not installed in the chip clamp or chip rotor bucket. Placing the chip on other non-grounded surfaces like a bench can result in damage due to static electric discharge.

Gas safety

WARNING! Ion instrumentation should be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the Ion instruments will be installed and operated in an environment with sufficient ventilation.

Protocol workflow

The Ion PGM™ System uses the following workflow when performing sequencing runs:



2 Create a Planned Run (required)

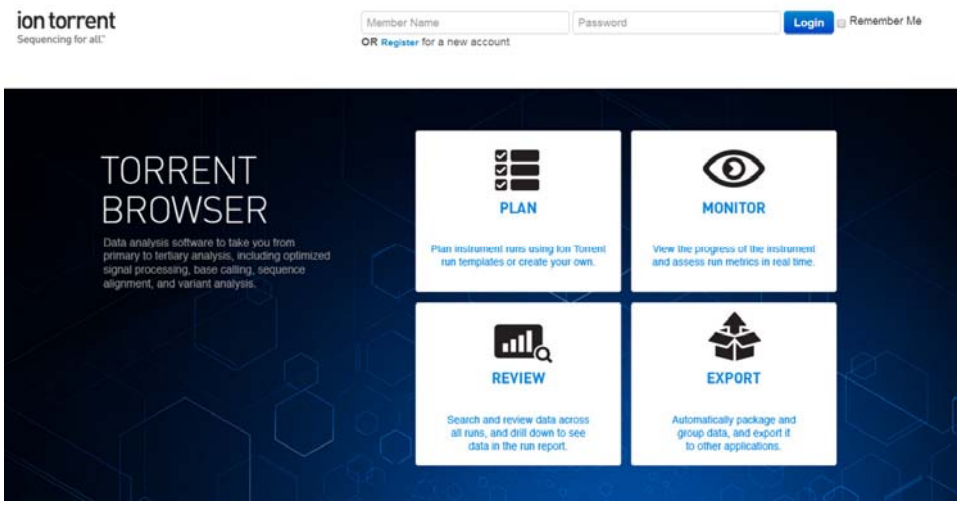
Planned Runs contain all the settings used in a sequencing run, including application type (gDNA, RNA, Ion AmpliSeq™, etc.), kits types, number of flows, barcodes (if any), and reference file.

IMPORTANT! Planned Runs are required if you are using the Ion PGM™ Template IA 500 Kit.

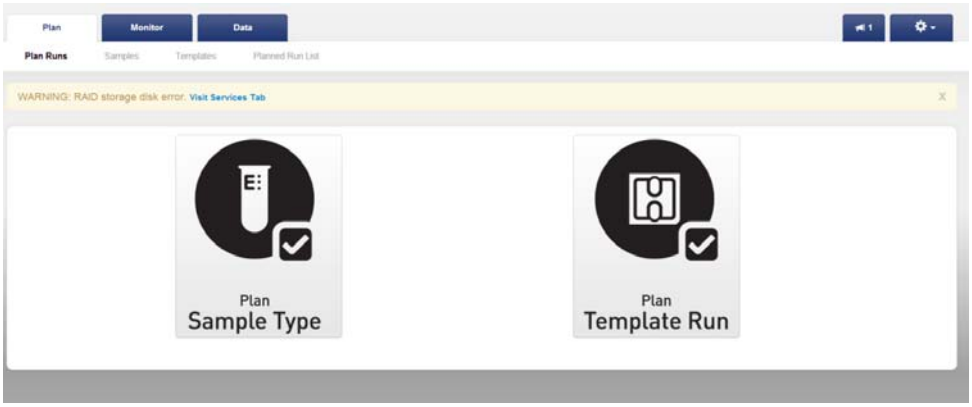
You create and save Planned Runs in the Torrent Browser, and then select the plan in the Ion PGM™ Sequencer touchscreen as part of the Run workflow (see **Select the Planned Run and perform the run** on page 33).

Note: For additional information, see the *Torrent Browser User Interface Guide*, available on the Ion Community at **http://ioncommunity.lifetechnologies.com**.

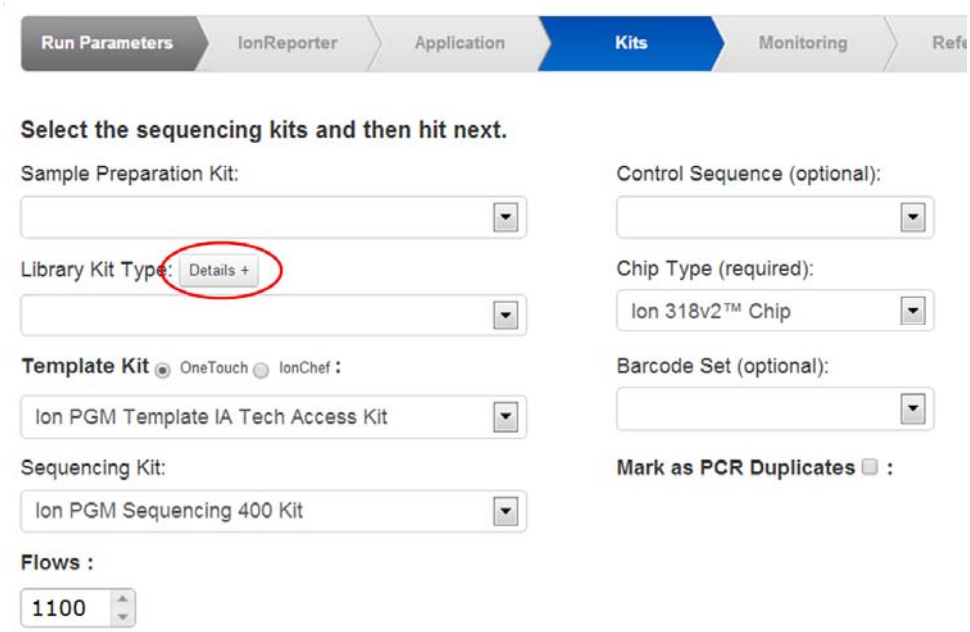
1. Before proceeding, make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.0.2 or later. This version includes important updates for using the Ion PGM™ Sequencing 400 Kit.
2. To create a Planned Run, log into the Torrent Browser for the Torrent Server connected to your Ion PGM™ System.



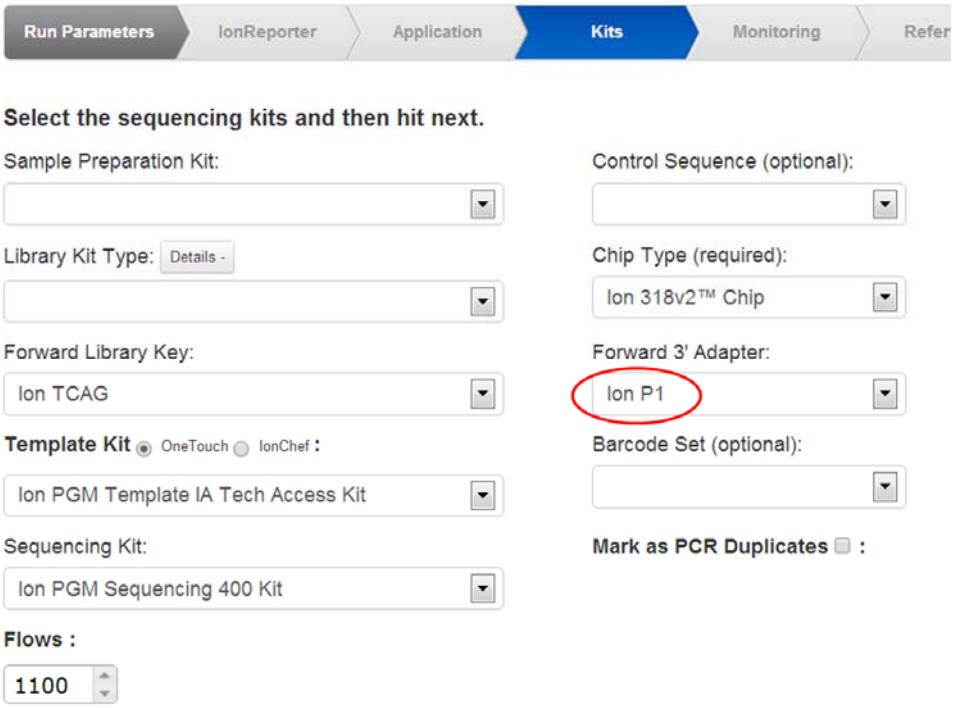
3. Select the **Plan** tab, select **Plan Template Run**, go to the sequencing template you need, for example, **Whole-Genome Seq**, and select **Plan New Run** next to this template.



4. In the wizard, review each screen and make your selections. In the screen for **Kits**, click on the **Details** button next to the Library Kit Type.



5. The Forward 3′ Adapter option will appear. Select **Ion P1** from the drop down menu.



6. Select other fields appropriately according to your application and sequencing kit used.

Field name	Description
Sample Preparation Kit	Select the Sample Preparation Kit you used.
Library Kit Type	Select the Library Kit type you used.
Template Kit	Select the Ion PGM™ Template IA Tech Access Kit *or* Ion PGM™ Template OT2 400 Kit
Sequencing Kit	Select the Ion PGM™ Sequencing 400 Kit.
Flows	Enter the appropriate number of flows for the read length: • 1100 flows for 500-base-read sequencing (only if you are using the Ion PGM™ Template IA 500 Kit) • 850 flows for 400-base-read sequencing • 500 flows for 200-base-read sequencing See **Appendix D. Sequencing run times** on page 60 for a table listing the number of flows for different read lengths.

Field name	Description
Barcode Set (optional)	See Appendix A. Barcoded libraries on page 45 for more information. If you are using barcodes with: • **DNA libraries:** Select the "IonXpress" barcode set, which includes all barcodes in the Ion Xpress™ Barcode Adapters 1–96 Kits. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select the "IonXpressRNA" barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01–16 Kit (Cat. no. 4475485). If you are *not* using barcodes with: • **DNA libraries:** Leave the Barcode field blank. • **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select "RNA_Barcode_None" from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.
Monitoring	Set thresholds for **Bead Loading**, **Usable Sequence**, and **Key Signal**. During a run, in the Torrent Browser under the Monitor tab, the Runs in Progress screen displays an alert if the values for a run fall below the selected thresholds.
Reference	Select the reference library on the Torrent Server, if any.
Plugins	Select the appropriate plugins for your application. See your Ion library kit guide for recommended plugins based on library type.
Output	Projects can be used to group your run data. Select or add a project for the current run, if desired. You can include runs in multiple projects, and remove runs from a project at any time.
Plan	Enter a name for the Run Plan. Enter Sample Name: • If Ion Reporter Uploader is enabled, enter each sample name and select the appropriate values for workflow, relation, relation role, and set ID. • If Ion Reporter Uploader is not enabled, enter the sample name or names separated by commas.

7. When you have completed your selections, click on **Plan Run** at the end of the workflow. The plan will appear listed on the **Planned Runs** page under the name you specified.

   Note: You can then select the plan when you are setting up the run on the Ion PGM™ Sequencer (see **Select the Planned Run and perform the run** on page 33).

3 Clean and initialize the Ion PGM™ System

Before starting

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

Clean the Ion PGM™ System

Materials required

- 18 MΩ water (e.g., ELGA® PURELAB® Flex Water Purification System)
- Cleaning bottles and collection trays (provided with the Ion PGM™ System)
- Used chip (leave chip on the instrument during cleaning)
- Used sipper tubes (from previous run or provided with the instrument)
- Squirt bottle
- **Chlorite cleaning:** Ion PGM™ Cleaning Tablet (provided in the kit)
- **Chlorite cleaning:** 1 M NaOH
- **Chlorite cleaning:** Glass bottle (1 L)
- **Chlorite cleaning:** 0.22-µm or 0.45-µm vacuum filtration system and filters

Cleaning schedule

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

Clean with:	Schedule:
18 MΩ water	• Daily, when instrument is in use (e.g., not necessary on weekends) • After ≤1100 flows (e.g., a single 500-base-read run or 2 × 200-base-read runs) • If more than 27 hours but less than 48 hours have elapsed between the last cleaning/initialization and the start of a run • If you cleaned with chlorite a week ago and have not used the instrument since then
Chlorite solution	• Once a week, unless the instrument has not been used since the last chlorite cleaning (in which case, clean with 18 MΩ water before using) • If the instrument has been left with reagents for more than 48 hours (for example, over the weekend)

Cleaning setup

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

- Remove all bottles and conical tubes that are attached to the Ion PGM™ System.
- Do not remove old sipper tubes before cleaning. The sipper tubes are used as part of the cleaning procedure.
- Separate cleaning bottles are provided with the instrument. After you have used the Wash Bottles provided with the kit for the specified number of runs, you can use them as extra cleaning bottles. Mark them for cleaning use only.
- Ensure that an old chip is in position on the instrument before cleaning.

Note: The chip type used for cleaning/initialization can be different than the chip type used for sequencing.

18 MΩ water cleaning

See **Cleaning schedule** on page 18.

1. Empty any remaining solution from each cleaning bottle (two 250–mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of fresh 18 MΩ water.
2. Press **Clean** on the touchscreen, and select the **18-MOhm water cleaning** checkbox.
3. Add 250 mL of 18 MΩ water to a 250-mL cleaning bottle.
4. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water, and attach the bottle to the W1 position.
5. Following the touchscreen instructions, place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position. Place collection trays below the sipper tubes in the dNTP positions. Press **Next** to begin cleaning.
6. When cleaning is complete, remove all bottles and sipper tubes from the W1, W2, and W3 positions. (Leave the reagent sipper tubes and collection tray(s) in place.) Press **Next** to return to the Main Menu and proceed to initialization.

Chlorite cleaning

See **Cleaning schedule** on page 18.

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

1. Empty any remaining solution from each cleaning bottle (two 250–mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water and add an Ion PGM™ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~10 minutes).
3. When the tablet has dissolved, add 1 mL of 1 M NaOH and filter the solution using a 0.22-µm or 0.45-µm filter. Use the chlorite solution within 2–3 hours. Discard any unused solution after this time.

4. Press **Clean** on the touchscreen, and select the **Chlorite cleaning** checkbox.
5. Add 250 mL of filtered chlorite solution to a 250-mL cleaning bottle.
6. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water, and attach the bottle to the W1 position.
7. Following the touchscreen instructions, place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position. Place collection trays below the sipper tubes in the dNTP positions. Press **Next** to begin cleaning.
8. When prompted, remove the W1 cleaning bottle with chlorite solution, rinse the outside of the sipper with a squirt bottle containing 18 MΩ water, then install a clean 250-mL cleaning bottle filled with 250 mL of 18 MΩ water in the W1 position.

   Note: The second cleaning bottle is different than the one used for chlorite solution.

9. When cleaning is complete, remove all bottles and sipper tubes from the W1, W2, and W3 positions. (Leave the reagent sipper tubes and collection tray(s) in place.) Press **Next** to return to the Main Menu and proceed to initialization.

Initialize the Ion PGM™ System

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

Materials required

Materials provided in the kit

- Wash 1, 2, and 3 Bottles and sipper tubes
- Reagent Bottles and sipper tubes
- Ion PGM™ Sequencing 400 dGTP
- Ion PGM™ Sequencing 400 dCTP
- Ion PGM™ Sequencing 400 dATP
- Ion PGM™ Sequencing 400 dTTP
- Ion PGM™ Sequencing 400 W2 Solution
- Ion PGM™ Sequencing 400 1X W3 Solution

Other materials and equipment

- Used chip (leave chip on the instrument during initialization)
- 18 MΩ water
- 100 mM NaOH (prepared daily)
- Ice
- Serological pipette and 5-mL and 25-mL pipettes
- Filtered and unfiltered pipette tips and pipettes
- Vortex mixer
- Microcentrifuge
- **Optional:** Ion PGM™ Sequencing Sippers Kit (Cat. no. 4478682)

IMPORTANT!

- For each initialization, the run should be started within 1 hour after initialization. Handle nucleotides carefully to avoid cross-contamination.
- Always change gloves after removing used sipper tubes from the Ion PGM™ System to avoid cross contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.
- After four initializations, do not use the Wash 1, 2, and 3 Bottles for initialization or sequencing to avoid breakage or leaking. (You can reuse the bottles in the cleaning procedure.)
- Replace the Reagent Bottles and sipper tubes every time you initialize.
- Make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.0.2 or later.

Before initialization

1. Remove the dNTP stock solutions from the freezer and begin thawing on ice.
2. Check the tank pressure for the nitrogen or argon gas. When the tank pressure drops below 500 psi, change the tank.
3. Note the mold line on the Wash 2 bottle. If there are two mold lines on the bottle, mark the lower line to indicate that it is the correct one.

Prepare the Wash 2 Bottle

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

1. Rinse the Wash 2 Bottle (2 L) three times with 200 mL of 18 MΩ water.
2. If your 18 MΩ water system has a spigot, extend the water spigot into **but not below** the neck of the Wash 2 Bottle.
3. Fill the bottle to the mold line. Volume of water will be ~2 liters.

4. Add the entire bottle of Ion PGM™ Sequencing 400 W2 Solution to the Wash 2 Bottle.

Note: Keep the W2 Solution bottle to scan the barcode during the initialization procedure.

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

   Note: Different sites may require adding different volumes of 100 mM NaOH. Some sites, for example, may require doubling the volume to 140 µL. See **Error message: Added too much W1 to W2** on page 55 for information on determining the volume of 100 mM NaOH to add.

6. Cap the bottle and invert five times to mix, and immediately proceed through the rest of the initialization procedure.

   IMPORTANT! Do not store the mixed Wash 2 Bottle.

Prepare the Wash 1 and Wash 3 Bottles

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

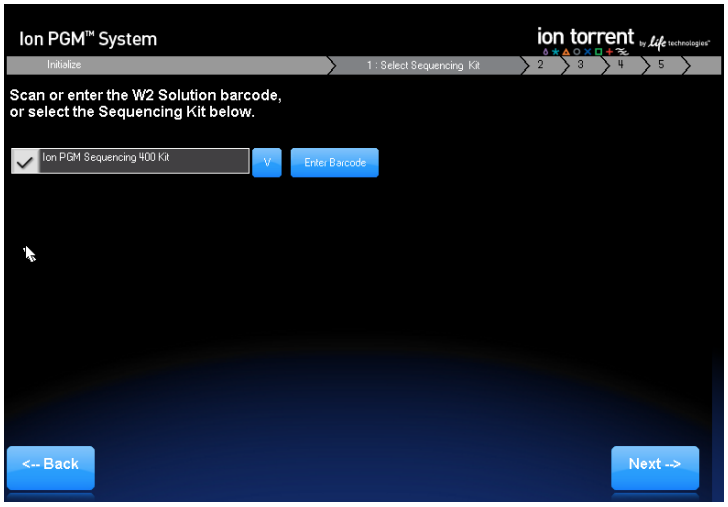
1. Rinse the Wash 1 and Wash 3 Bottles three times with 50 mL of 18 MΩ water.
2. **Wash 1 Bottle:** Add 350 µL of freshly prepared 100 mM NaOH to the Wash 1 Bottle and cap the bottle.
3. **Wash 3 Bottle:** Add Ion PGM™ Sequencing 400 1X W3 Solution to the 50-mL line marked on the Wash 3 Bottle and cap the bottle.

Begin the initialization

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

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

1. Confirm that the chip used to the clean the Ion PGM™ System is still in place on the instrument.
2. On the Main Menu, press **Initialize**.
3. In the next screen, scan or enter the barcode on the Ion PGM™ Sequencing 400 W2 Solution bottle (from Step 4 under **Prepare the Wash 2 Bottle** on page 21). Alternatively, select **Ion PGM™ Sequencing 400 Kit** from the dropdown list.



IMPORTANT! Be careful to scan the correct bottle or select the correct kit type, to ensure proper pH adjustment.

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

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

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

7. Immediately attach the prepared Wash 2 Bottle in the W2 position and tighten the cap. Press **Next**.
8. Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.

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

9. Immediately attach the prepared Wash 1 and 3 Bottles and tighten the caps. Press **Next**.
10. The Ion PGM™ System will test the bottles for leaks, fill the Wash 1 Bottle, and then adjust the pH of the W2 Solution. This procedure takes ~30 minutes.

    Note: If a wash bottle leaks or if an error occurs during the automatic pH process, see **Appendix B. Troubleshooting**. If an error message indicates problems adjusting the pH of the prepared W2 Solution, see **Initialization: Auto pH errors** on page 52 in the Troubleshooting section.

Prepare the 50-mL Reagent Bottles with dNTP solutions

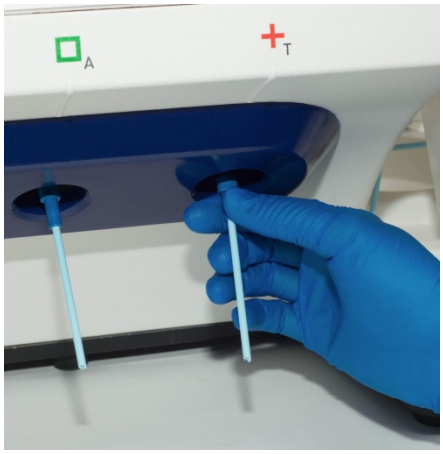
IMPORTANT! In the following steps, handle the nucleotides carefully to avoid cross-contamination and ensure that the correct dNTP solution is installed in each position on the Ion PGM™ System.

1. After each dNTP stock solution has thawed, vortex to mix and centrifuge to collect the contents. Keep dNTP stock solutions on ice throughout this procedure.
2. Use the labels provided with the kit to label four new Reagent Bottles as dGTP, dCTP, dATP, and dTTP.
3. Using filtered pipette tips and clean gloves, carefully transfer 20 µL of each dNTP stock solution into its respective Reagent Bottle.

Attach the sipper tubes and Reagent Bottles

1. After the wash solutions have initialized, follow the touchscreen prompts to remove the used sipper tubes and collection trays from the dNTP ports. Change gloves.
2. Using new gloves, firmly insert a new sipper tube (blue) into each dNTP port. Do not let the sipper touch any surfaces.

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



3. Attach each Reagent Bottle to the correct dNTP port and tighten firmly until snug. The correct order of the Reagent Bottles on the Ion PGM™ System is dGTP, dCTP, dATP, and dTTP (left to right when facing the instrument).

   Note: The Ion PGM™ System checks the pressure of the Reagent Bottles and Wash Bottles. If a bottle leaks, you are prompted to check that it is tightly attached to the instrument. If it continues to leak, it should be replaced. If the instrument still does not pass the leak check, contact Technical Support.

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

   Note: You can create Planned Runs and/or prepare the ISPs while the Ion PGM™ System is initializing. See the following sections.

5. At the end of initialization, the Ion PGM™ System will measure the pH of the reagents:
   - If every reagent is in the target pH range, a green **Passed** screen will be displayed.
   - If a red failure screen appears, see **Appendix B. Troubleshooting**.
6. Press **Next** to finish the initialization process and return to the Main Menu.
7. Proceed to the appropriate sequencing protocol for your chip type.

4 Sequencing protocol— Ion 316™ Chip v2 or Ion 318™ Chip v2

Use the following sequencing protocol with the Ion 316™ Chip v2 or Ion 318™ Chip v2.

Materials required

Materials provided in the kit

- Sequencing Primer
- Control Ion Sphere™ Particles
- Annealing Buffer
- Ion PGM™ Sequencing 400 Polymerase

Materials provided in the Ion PGM™ Controls Kit v2

- (*Optional*) Ion Sphere™ Test Fragments

Other materials and equipment

- Ion 316™ Chip v2 or Ion 318™ Chip v2
- Enriched template-positive ISPs
- 0.2-mL PCR tube (non-polystyrene)
- Rainin® SR-L200F pipette and tips
- Vortex mixer
- MiniFuge
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Barcode scanner (included with the Ion PGM™ System)

Before starting

- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.0.2 or later.

IMPORTANT! For each initialization, run should be started within 1 hour after initialization. The ISPs are difficult to see. To avoid aspirating the particles in the following steps, orient the PCR tube the same way each time when centrifuging so that it is easy to know where the pellet has formed, and remove the supernatant from the top down.

Optional: Prepare Ion Sphere™ Test Fragments

If you are performing an installation or troubleshooting sequencing run:

1. Vortex the Ion Sphere™ Test Fragments from the Ion PGM™ Controls Kit v2 (Cat. no. 4482010) and centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of Ion Sphere™ Test Fragments to 100 µL of Annealing Buffer in a 0.2-mL non-polystyrene PCR tube.
3. Skip directly to **Anneal the Sequencing Primer**.

Prepare enriched, template-positive ISPs

1. Vortex the Control Ion Sphere™ Particles and centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of Control Ion Sphere™ Particles directly to the entire volume of enriched, template-positive ISPs in a 0.2-mL non-polystyrene PCR tube.
3. Proceed to **Anneal the Sequencing Primer**.

Anneal the Sequencing Primer

1. Mix the contents of the tube by thoroughly pipetting up and down. Centrifuge for 2 minutes at 15,500 × g.
2. Carefully remove the supernatant without disturbing the pellet, leaving 15 µL in the tube (visually compare to 15 µL of liquid in a separate tube).
3. Add 12 µL of the Sequencing Primer and confirm that the total volume is 27 µL (add Annealing Buffer if necessary).
4. Pipet the sample up and down thoroughly to disrupt the pellet.
5. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
6. Place the tube in the thermal cycler and run the program. After cycling, the reaction can remain in the cycler at room temperature while you proceed with Chip Check.

Chip Check

Chip Check tests the chip and ensures that it is functioning properly prior to loading the sample.

IMPORTANT! To avoid damage due to electrostatic discharge (ESD), **do not place the chip directly on the bench or any other surface.** Always place the chip either on the grounding plate on the Ion PGM™ System or in the custom Ion centrifuge adapter/rotor bucket.

IMPORTANT! To avoid ESD damage, **do not wear gloves** when transferring chips on and off the instrument.

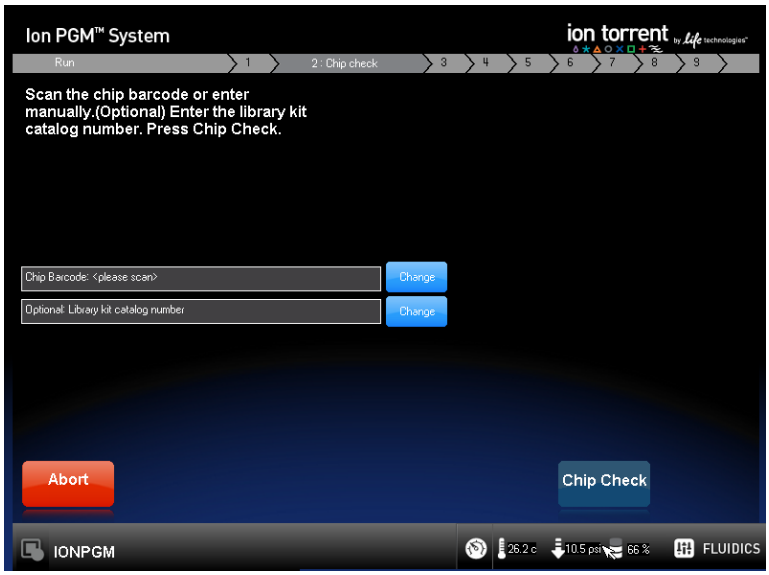
1. Remove a new chip from its packaging and label it to identify the experiment. Save the chip package to scan the barcode later.
2. Place the chip on the Ion PGM™ Sequencer grounding plate or in the Ion centrifuge adapter/rotor bucket.
3. Press **Run** on the main menu and follow the touchscreen prompts to prepare the Ion PGM™ System to test a new Ion PGM™ Chip.

   Note: When prompted to insert a cleaning chip, you can use the same used chip that was used for initialization.

4. When prompted, ground yourself by touching the grounding pad next to the chip clamp on the instrument and replace the old chip in the chip socket with the new one for the experiment. **Do not wear gloves when transferring the chips on and off the instrument.** Close the chip clamp.

5. When prompted, use the barcode scanner to scan the barcode located on the chip package, or press **Change** to enter the barcode manually.

   Note: A chip cannot be run without scanning or entering the barcode.



6. Press **Chip Check** on the touchscreen.

7. During the initial part of Chip Check, visually inspect the chip in the clamp for leaks. If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Then proceed to **Appendix B. Troubleshooting**.

   IMPORTANT! The chip socket can be damaged by rubbing or wiping its surface. If there is a spill or leak onto the chip socket, contact technical support (see **Ion contact information** on page 70).

8. When Chip Check is complete:
   - If the chip passes, press **Next**.
   - If the chip fails, open the chip clamp, re-seat the chip in the socket, close the clamp, and press **Calibrate** to repeat the procedure. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart the experiment with a new chip. See also **Appendix B. Troubleshooting**.

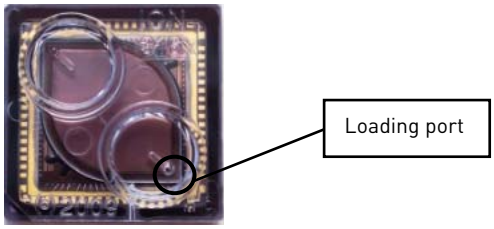
   Note: To return *damaged* chips, contact Life Technologies Technical Support.

9. Following a successful Chip Check, remove the new chip and place it on the grounding plate. Insert a used chip in the socket and close the clamp.
10. Completely empty the waste bottle as instructed in the touchscreen. Note that this should be a 2.5-L waste bottle; older models of the Ion PGM™ Sequencer may have a smaller waste bottle, which is not suitable for longer-read sequencing.
11. Proceed immediately through the following steps to load the chip.

Bind Sequencing Polymerase to the ISPs

1. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 3 µL of Ion PGM™ Sequencing 400 Polymerase to the ISPs, for a total final volume of 30 µL.
2. Pipet the sample up and down to mix, and incubate at room temperature for 5 minutes.

Load the chip



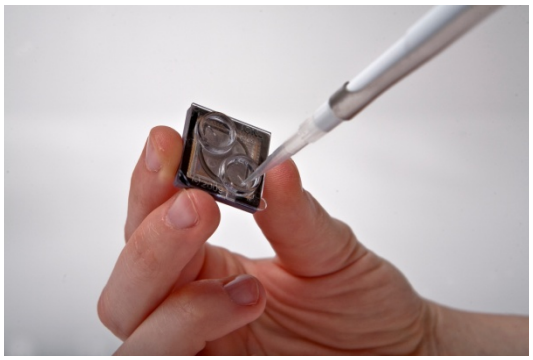
Alternate chip loading method: Ion PGM™ Chip loading with the Ion PGM™ Weighted Chip Bucket

For an alternate chip loading method with fewer handling steps, see the *Ion PGM™ Chip Loading with the Ion PGM™ Weighted Chip Bucket User Bulletin* (Pub. no. MAN0007517), which is available for download from the Ion Community at **ioncommunity.lifetechnologies.com**.

This protocol requires the use of the Ion PGM™ Weighted Chip Bucket (Cat. no. 4480283), which can be ordered by contacting Life Technologies customer service as described on page 70.

Remove liquid from the chip

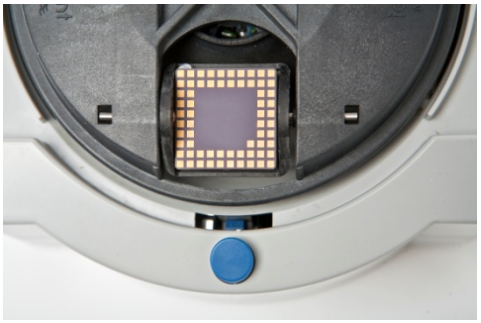
1. Tilt the chip 45 degrees so that the loading port is the lower port, as shown below.



2. Insert the pipette tip firmly into the loading port and remove as much liquid as possible from the loading port. Discard the liquid.

IMPORTANT! For the next steps, balance the centrifuge adapter with a used chip of the same chip type and orientation if you are preparing one Ion PGM™ Chip at a time. Be careful to balance an upside-down chip with another upside-down chip. Mark the used chip with a laboratory marker to differentiate it from the new chip containing the sample.

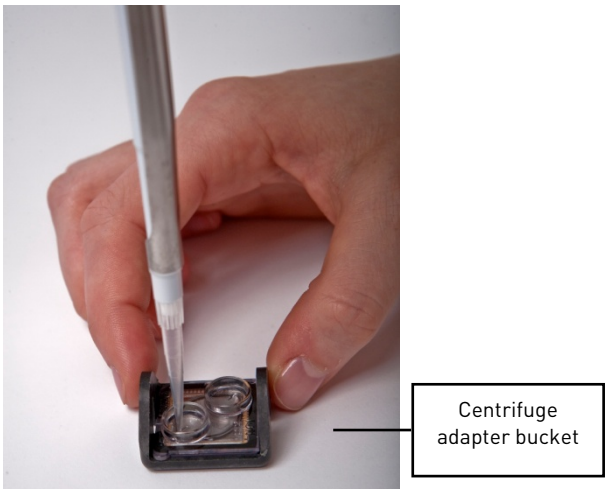
3. Place the chip **upside-down** in the centrifuge adapter bucket and transfer the bucket to the MiniFuge **with the chip tab pointing in** (toward the center of the MiniFuge), as shown below.



4. Centrifuge for 5 seconds to completely empty the chip. Remove the chip from the bucket and wipe off any liquid on the bucket.

Load the sample on the chip

IMPORTANT! When loading liquid into the chip, keep the pipette tip at a 90° angle to the chip, press the tip firmly into the circular loading port, and apply gentle pressure between the pipette tip and chip.



1. Place the Ion PGM™ Chip back in the centrifuge adapter bucket and place the bucket on a flat, stable surface such as a benchtop.
2. Following polymerase incubation, collect the entire sample (~30 µL) into a Rainin® SR-L200F pipette tip and insert the tip firmly into the loading port of the chip.
3. Dial down the pipette as shown below to gently and slowly deposit the ISPs at a rate of ~1 µL per second. To avoid introducing bubbles into the chip, leave a small amount of sample in the pipette tip (~0.5 µL).

4. Remove and discard any displaced liquid from the other port of the chip.
5. Transfer the chip to the MiniFuge **with the chip tab pointing in** (toward the center of the MiniFuge).

6. Centrifuge for 30 seconds, then remove the chip from the centrifuge bucket.

7. Mix the sample in the chip:
   a. Set the pipette volume to 25 µL.
   b. Tilt the chip 45 degrees so that the loading port is the lower port, and insert the pipette tip into the loading port.
   c. Without removing the tip, slowly pipet the sample in and out of the chip three times. **Pipet slowly to avoid creating bubbles.**
8. Centrifuge the chip for 30 seconds **with the chip tab pointing out** (away from the center of the MiniFuge).

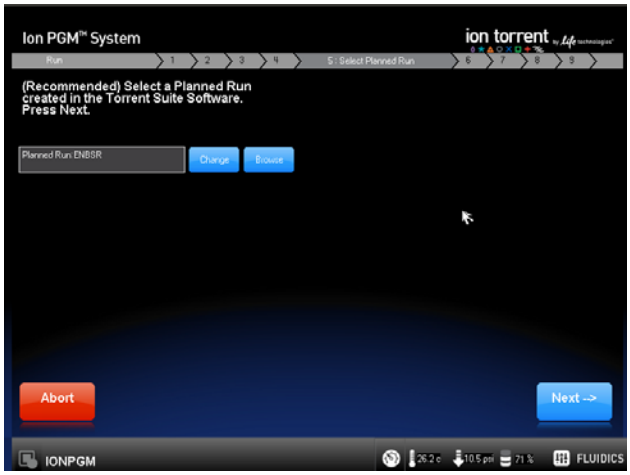
9. Repeat the chip mixing in step 7 one more time, then spin for 30 seconds **with the chip tab pointing in** (toward the center of the MiniFuge).
10. Repeat the chip mixing in step 7, this time pipetting the sample in and out of the chip ***five times***.
11. Tilt the chip at a 45-degree angle and slowly remove as much liquid as possible from the loading port by dialing the pipette. Discard the liquid.
12. If some liquid remains in the chip, perform a 5-second quick spin with the chip tab pointing out and remove and discard any additional liquid. **Do not spin the chip upside down.**
13. If some liquid remains in the chip after the quick spin, lightly and rapidly tap the point of the chip tab against the benchtop a few times, and remove and discard any collected liquid. Do not flush the chip.
14. When chip loading is complete, press **Next** on the touchscreen and proceed immediately to **Select the Planned Run and perform the run**.

Select the Planned Run and perform the run

Select the Planned Run

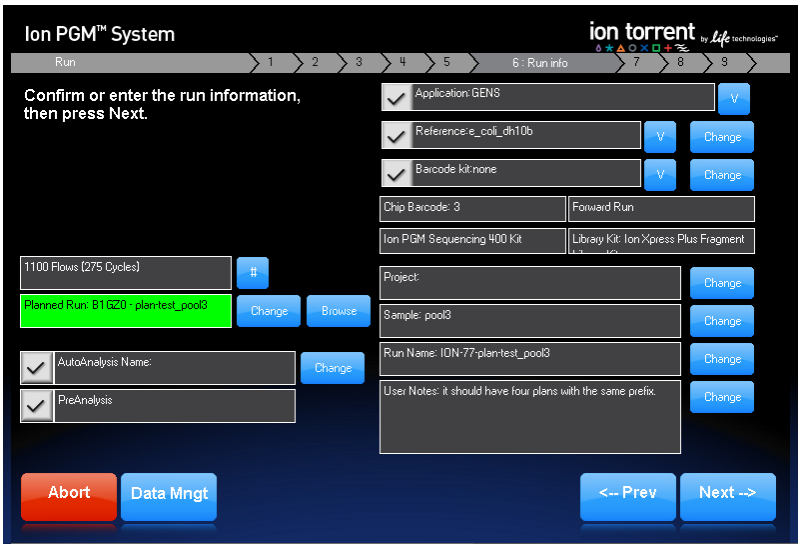
To create a Planned Run, see **Create a Planned Run** on page 14.

1. In the touchscreen, press the **Change** or **Browse** button next to the **Planned Run** field and enter or select the name of the plan you created, then press **Next**.



2. The Planned Run settings will be displayed on the touchscreen.

IMPORTANT! Do not edit these settings. If you need to change the settings, go back to Torrent Browser and edit the Planned Run.



Note: If you need to free up disk space on the Ion Torrent Server to perform the run, press the **Data Mngt** button (this can also be accessed from the Tools Menu) and delete old runs.

Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup. Press **OK** to confirm the settings or press **Cancel** to return to the previous screen to adjust the settings.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. At the beginning of the run, visually inspect the chip in the clamp for leaks before closing the cover. The instrument will flush any loose ISPs from the chip and begin calibrating the chip.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful:
   - If the chip passes calibration, press **Next** to proceed with the sequencing run.
   - If the chip fails calibration, press **Abort**, reseat the chip, then press **Calibrate** to re-calibrate.
   - If the chip still fails calibration, proceed with the run anyway and contact Technical Support after the run is complete. See also **Appendix B. Troubleshooting**.
5. After 90 seconds, the run will automatically begin, or press **Next** to begin the run immediately.

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

6. When the run is complete, the touchscreen will return to the Main Menu. You can then proceed with another run or perform a cleaning/initializing if required.

   Note: See **Cleaning schedule** on page 18 to determine whether cleaning is required after the run.

5 Sequencing protocol—Ion 314™ Chip v2

Materials required

Materials provided in the kit

- Sequencing Primer
- Control Ion Sphere™ Particles
- Annealing Buffer
- Ion PGM™ Sequencing 400 Polymerase

Materials provided in the Ion PGM™ Controls Kit

- (*Optional*) Ion Sphere™ Test Fragments

Other materials and equipment

- Ion 314™ Chip v2
- Enriched, template-positive ISPs
- 0.2-mL PCR tube (non-polystyrene)
- Rainin® SR-L10F and SR-L200F pipette and tips
- Vortex mixer
- MiniFuge
- Thermal cycler with heated lid (programmed at 95°C for 2 minutes and 37°C for 2 minutes)
- Barcode scanner (included with the Ion PGM™ System)

Before starting

- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Torrent Suite™ and Ion PGM™ System software to Version 4.0.2 or later.

IMPORTANT! For each initialization, run should be started within 1 hour after initialization. The ISPs are difficult to see. To avoid aspirating the particles in the following steps, orient the PCR tube the same way each time when centrifuging so that it is easy to know where the pellet has formed, and remove the supernatant from the top down.

Optional: Prepare Ion Sphere™ Test Fragments

If you are performing an installation or troubleshooting sequencing run:

1. Vortex the Ion Sphere™ Test Fragments from the Ion PGM™ Controls Kit (Cat. no. 4480449) and centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of Ion Sphere™ Test Fragments to 100 µL of Annealing Buffer in a 0.2-mL non-polystyrene PCR tube.
3. Skip directly to **Anneal the Sequencing Primer**.

Prepare enriched, template-positive ISPs

Note: The Ion 314™ Chip v2 uses only half the volume of enriched, template-positive ISPs prepared using the template kit.

1. Transfer **half the volume** of enriched, template-positive ISPs to a new 0.2-mL non-polystyrene PCR tube and store at 2–8°C for up to 1 week. They may be used for another sequencing run.
2. Vortex the Control Ion Sphere™ Particles and centrifuge for 2 seconds before taking aliquots.
3. Add 5 µL of Control Ion Sphere™ Particles directly to the half-volume of enriched, template-positive ISPs in a 0.2-mL non-polystyrene PCR tube.
4. Proceed to **Anneal the Sequencing Primer**.

Anneal the Sequencing Primer

1. Mix the contents of the tube by thoroughly pipetting up and down. Centrifuge for 2 minutes at 15,500 × g.
2. Carefully remove the supernatant without disturbing the pellet, leaving 3 µL in the tube (visually compare to 3 µL of liquid in a separate tube).
3. Add 3 µL of Sequencing Primer and confirm that the total volume is 6 µL (add Annealing Buffer if necessary).
4. Pipet the sample up and down thoroughly to disrupt the pellet.
5. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
6. Place the tube in the thermal cycler and run the program. After cycling, the reaction can remain in the cycler at room temperature while you proceed with Chip Check.

Chip Check

Chip Check tests the chip and ensures that it is functioning properly prior to loading the sample.

IMPORTANT! To avoid damage due to electrostatic discharge (ESD), **do not place the chip directly on the bench or any other surface.** Always place the chip either on the grounding plate on the Ion PGM™ System or in the custom Ion centrifuge adapter/rotor bucket.

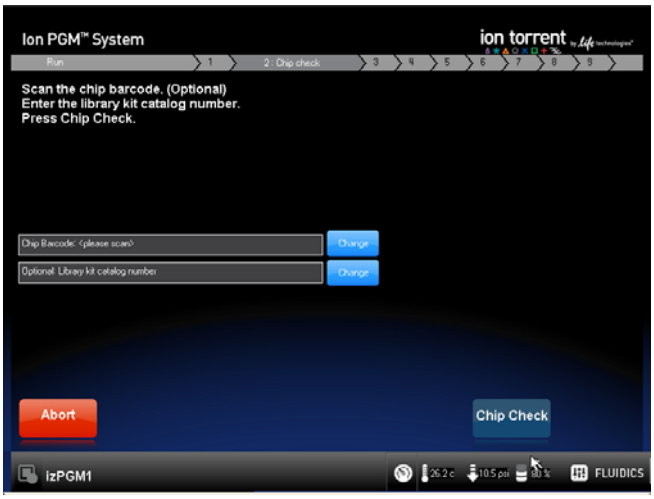
IMPORTANT! To avoid ESD damage, **do not wear gloves** when transferring chips on and off the instrument.

1. Remove a new chip from its packaging and label it to identify the experiment. Save the chip package to scan the barcode later.
2. Place the chip on the Ion PGM™ Sequencer grounding plate or in the Ion centrifuge adapter/rotor bucket.
3. Press **Run** on the Main Menu and follow the touchscreen prompts to prepare the Ion PGM™ System to test a new Ion PGM™ Chip.

   Note: When prompted to insert a cleaning chip, you can use the same used chip that was used for initialization.

4. When prompted, ground yourself by touching the grounding pad next to the chip clamp on the instrument and replace the old chip in the chip socket with the new one for the experiment. **Do not wear gloves when transferring the chips on and off the instrument.** Close the chip clamp.

5. When prompted, use the barcode scanner to scan the barcode located on the chip package, or press **Change** to enter the barcode manually.



Note: A chip cannot be run without scanning or entering the barcode.

6. Press **Chip Check** on the touchscreen.
7. During the initial part of Chip Check, visually inspect the chip in the clamp for leaks. If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Then proceed to **Appendix B. Troubleshooting**.

IMPORTANT! **The chip socket can be damaged by rubbing or wiping its surface.** If there is a spill or leak onto the chip socket, contact technical support (see **Ion contact information** on page 70).

8. When Chip Check is complete:
   - If the chip passes, press **Next**.
   - If the chip fails, open the chip clamp, re-seat the chip in the socket, close the clamp, and press **Calibrate** to repeat the procedure. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart the experiment with a new chip. See also **Appendix B. Troubleshooting**.

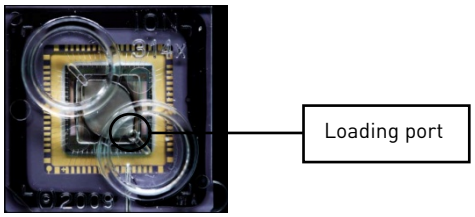
   Note: To return *damaged* chips, contact Life Technologies Technical Support.
9. Following a successful Chip Check, remove the new chip and place it on the grounding plate. Insert a used chip in the socket and close the clamp.
10. Completely empty the waste bottle as instructed in the touchscreen.

    IMPORTANT! Ion PGM™ System—Blue requires use of the Ion PGM™ 2.5 L Waste Bottle. Be sure to completely empty and return the waste container before each run.
11. Proceed immediately through the following steps to load the chip.

Bind Sequencing Polymerase to the ISPs

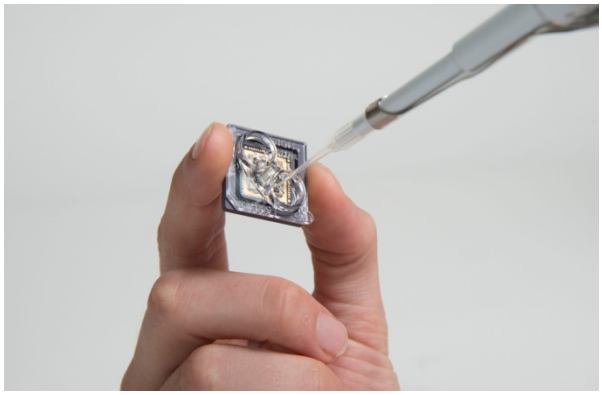
1. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 1 µL of Ion PGM™ Sequencing 400 Polymerase to the ISPs, for a total final volume of 7 µL.
2. Pipet the sample up and down to mix, and incubate at room temperature for 5 minutes.

Load the chip



Remove liquid from the chip

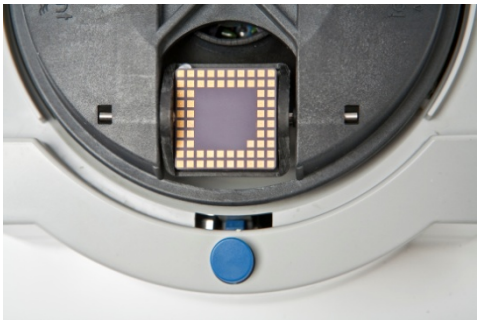
1. Tilt the chip 45 degrees so that the loading port is the lower port, as shown below.



2. Insert the pipette tip firmly into the loading port and remove as much liquid as possible from the loading port. Discard the liquid.

IMPORTANT! For the next steps, balance the centrifuge adapter with a used chip of the same chip type and orientation if you are preparing one Ion PGM™ Chip at a time. Be careful to balance an upside-down chip with another upside-down chip. Mark the used chip with a laboratory marker to differentiate it from the new chip containing the sample.

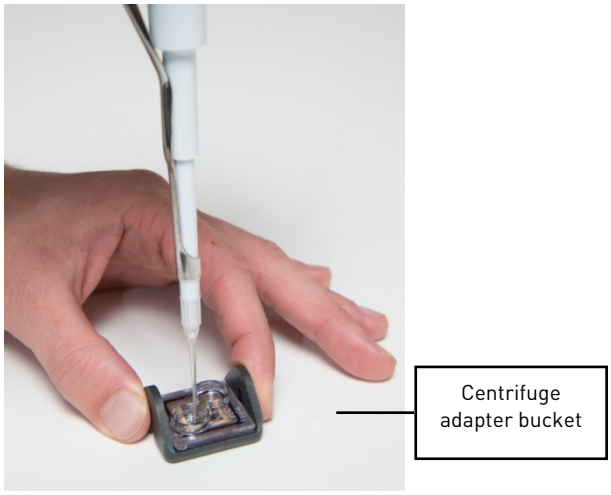
3. Place the chip **upside-down** in the centrifuge adapter bucket as shown below, and transfer the bucket to the MiniFuge **with the chip tab pointing in** (toward the center of the MiniFuge).



4. Centrifuge for 5 seconds to completely empty the chip. Remove the chip from the bucket and wipe off any liquid on the bucket.

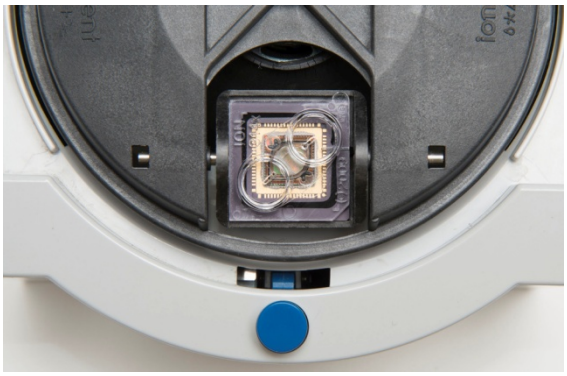
Load the sample on the chip

IMPORTANT! When loading liquid into the chip, keep the pipette tip at a 90° angle to the chip, press the tip firmly into the circular loading port, and apply gentle pressure between the pipette tip and chip.

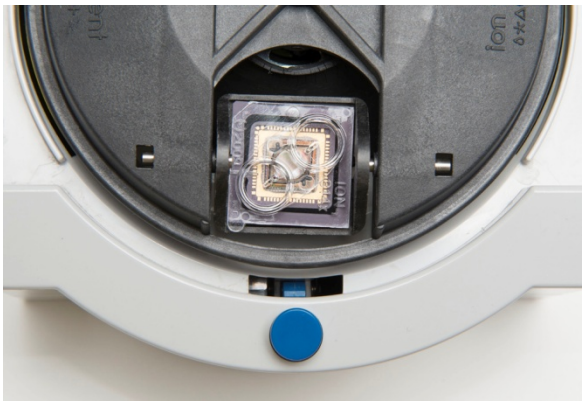


1. Place the Ion PGM™ Chip back in the centrifuge adapter bucket and place the bucket on a flat, stable surface such as a benchtop.
2. Following polymerase incubation, collect the entire sample (~7 µL) into a Rainin® SR-L10F pipette tip and insert the tip firmly into the loading port of the chip.
3. **Dial down the pipette as shown below to gently and slowly deposit the ISPs at a rate of ~1 µL per second**. To avoid introducing bubbles into the chip, leave a small amount of sample in the pipette tip (~0.5 µL).

4. Remove and discard any displaced liquid from the other port of the chip.
5. Transfer the chip to the MiniFuge **with the chip tab pointing in** (toward the center of the MiniFuge).



6. Centrifuge for 30 seconds, then remove the chip from the centrifuge bucket.
7. Mix the sample in the chip:
   a. Set the pipette volume to 5 µL.
   b. Tilt the chip 45 degrees so that the loading port is the lower port, and insert the pipette tip into the loading port.
   c. Without removing the tip, slowly pipet the sample in and out of the chip *three times*. **Pipet slowly to avoid creating small bubbles.**
8. Centrifuge the chip for 30 seconds **with the chip tab pointing out** (away from the center of the MiniFuge).



9. Repeat the chip mixing in step 7, then spin for 30 seconds **with the chip tab pointing in** (toward the center of the MiniFuge).
10. Repeat the chip mixing in step 7, this time pipetting the sample in and out of the chip ***five times***.
11. Tilt the chip at a 45-degree angle and slowly remove as much liquid as possible from the loading port by dialing the pipette. Discard the liquid.
12. If some liquid remains in the chip, perform a 5-second quick spin with the chip tab pointing out and remove and discard any additional liquid. **Do not spin the chip upside down.**
13. If some liquid remains in the chip after the quick spin, lightly and rapidly tap the point of the chip tab against the benchtop a few times, and remove and discard any collected liquid. **Do not flush the chip.**

    Note: Not all the liquid can be removed from the Ion 314™ Chip v2. Remove as much liquid as possible using the methods above, and then proceed with the run.

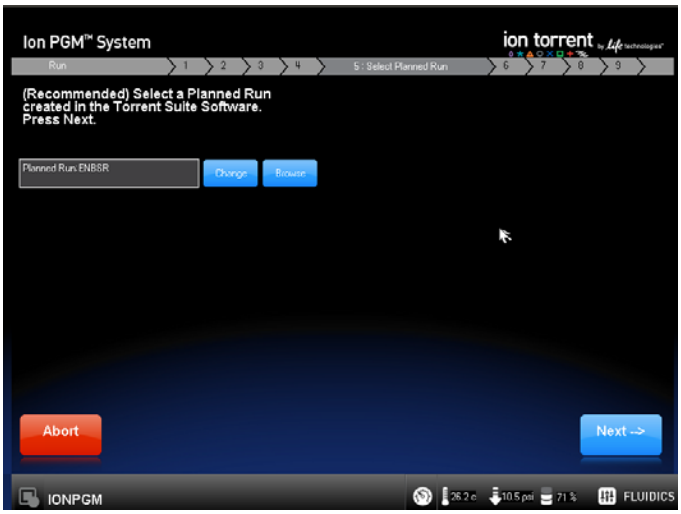
14. Immediately proceed to **Select the Planned Run and perform the run**.

Select the Planned Run and perform the run

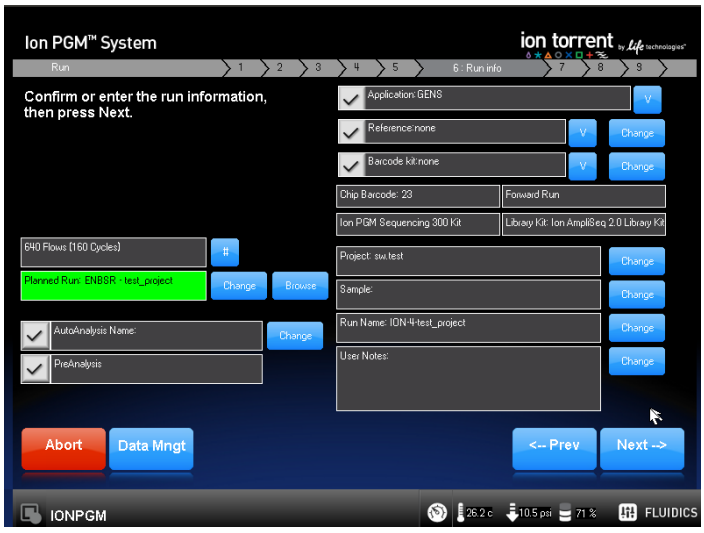
Select the Planned Run or enter settings manually

In the touchscreen, we recommend that you select a Planned Run (see **Create a Planned Run** on page 14). Alternatively, you can make the run selections manually on the following screen.

1. Press the **Browse** button next to the **Planned Run** field and select the name of the plan you created, then press **Next**.



2. The run settings will be automatically populated based on the Planned Run. Confirm that these settings are correct. Make any changes using the buttons and dropdown lists if necessary.



Note: If the number of flows to be run cannot be selected, there may not be enough disk space to store the experiment data. Press the **Data Mngt** button to start the Data Management application (this can also be accessed from the Tools Menu) and delete old runs from the Ion PGM™ System.

Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup. Press **OK** to confirm the settings or press **Cancel** to return to the touchscreen to adjust the settings.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. At the beginning of the run, visually inspect the chip in the clamp for leaks before closing the cover. The instrument will flush any loose ISPs from the chip and begin calibrating the chip.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful:
   - If the chip passes calibration, press **Next** to proceed with the sequencing run.
   - If the chip fails calibration, press **Abort**, reseat the chip, then press **Calibrate** to re-calibrate.
   - If the chip still fails calibration, proceed with the run anyway and contact Technical Support after the run is complete. See also **Appendix B. Troubleshooting**.
5. After 90 seconds, the run will automatically begin, or press **Next** to begin the run immediately.

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

6. When the run is complete, the touchscreen will return to the Main Menu. You can then proceed with another run or perform a cleaning/initializing if required.

   Note: See **Cleaning schedule** on page 18 to determine whether cleaning is required after the run.

A Appendix A. Barcoded libraries

This appendix describes how to select and create barcode sets on the Ion PGM™ System for sequencing barcoded libraries.

Pre-installed barcode sets

The Torrent Suite™ software includes the pre-installed barcode sets "IonXpress" and "IonXpressRNA."

When setting up a Planned Run or performing a run, select the appropriate barcode set for your library type as follows:

- **DNA libraries:** Select the "IonXpress" barcode set, which includes all barcodes in the Ion Xpress™ Barcode Adapters 1–96 Kits.
- **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select the "IonXpressRNA" barcode set, which contains all 16 barcodes in the Ion Xpress™ RNA BC01–16 Kit (Cat. no. 4475485).

If you are **not** using barcodes:

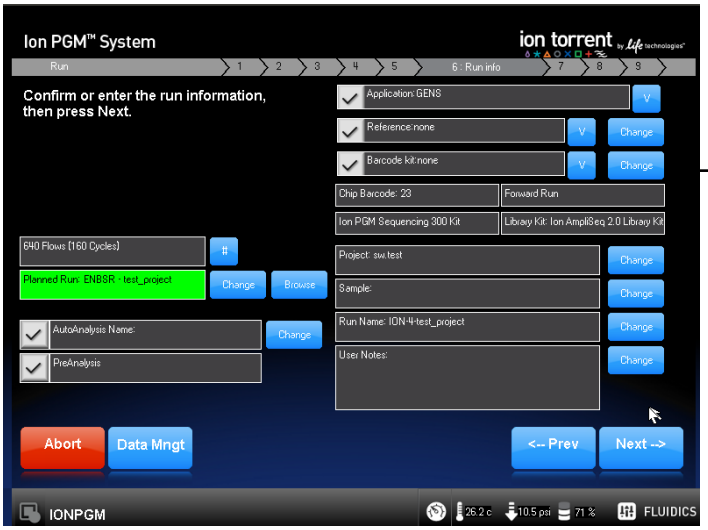
- **DNA libraries:** Leave the Barcode field blank
- **RNA libraries prepared using the Ion Total RNA-Seq Kit v2:** Select "RNA_Barcode_None" from the dropdown list. This will ensure that the proper trimming is performed on the resulting sequence when the RNA library does not have a barcode.

IMPORTANT! Do not edit, delete, or modify the pre-installed barcode sets.

Select a barcode set for a sequencing run

To select a barcode set:

- **Recommended:** Select the barcode set in the Torrent Browser when planning the run. See **Create a Planned Run** on page 14.
- **Optional:** Select the barcode set in the Ion PGM™ Sequencer touchscreen when setting up the sequencing run. See **Select the Planned Run and perform the run** on page 43, depending on your chip type.



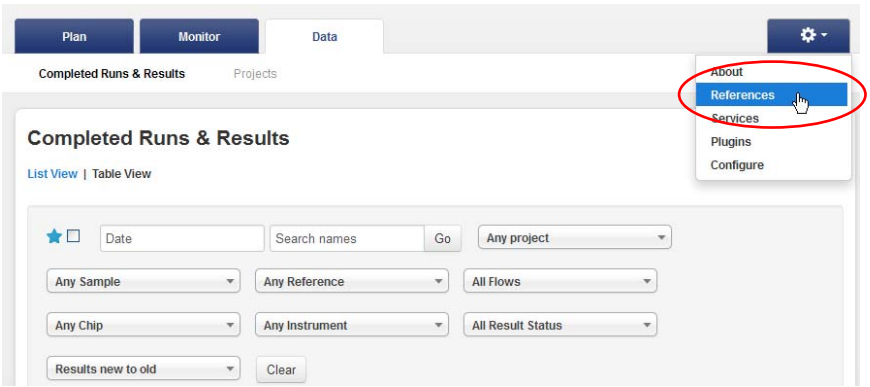
Custom barcode sets

You can create custom sets of barcodes as **comma-separated value (.csv) files**, then load these sets onto the Torrent Server for use during sequencing runs.

To access the Torrent Server, you must have a username and password. For more information on working with custom barcode sets, refer to the *Torrent Browser User Interface Guide*.

Create and add a custom barcode set on the Torrent Server

1. Create the Comma-Separated Variable (.csv) text file of the custom barcode set. The .csv text file can contain up to 384 barcodes.
2. To add the custom barcode set to the Torrent Server, go to the Torrent Browser and click the Settings button on the right side of the window, then select **References**.



3. Scroll down to the **DNA Barcodes** section.
4. Click Add new DNA Barcodes.
5. Click the **Download the example file** link to download an example file to your computer.
6. On your computer, edit the .csv example file directly, or use Microsoft® Excel®, Notepad, or similar software to create a custom barcode set in the same format, with each barcode sequence listed on a separate line. The

barcode list can contain up to 384 barcodes. Save the .csv file on your computer.

Note: You can run fewer than 384 barcodes in a sequencing run; the Ion PGM™ System automatically detects and selects the barcodes used in the run from the selected set.

7. Back in the Torrent Browser, enter a name for the barcode set and click **Browse** to select the .csv file that you created.
8. Click **Upload & Save**. The barcode set file name is displayed in the Barcode panel.

Other barcode set operations

View a barcode set

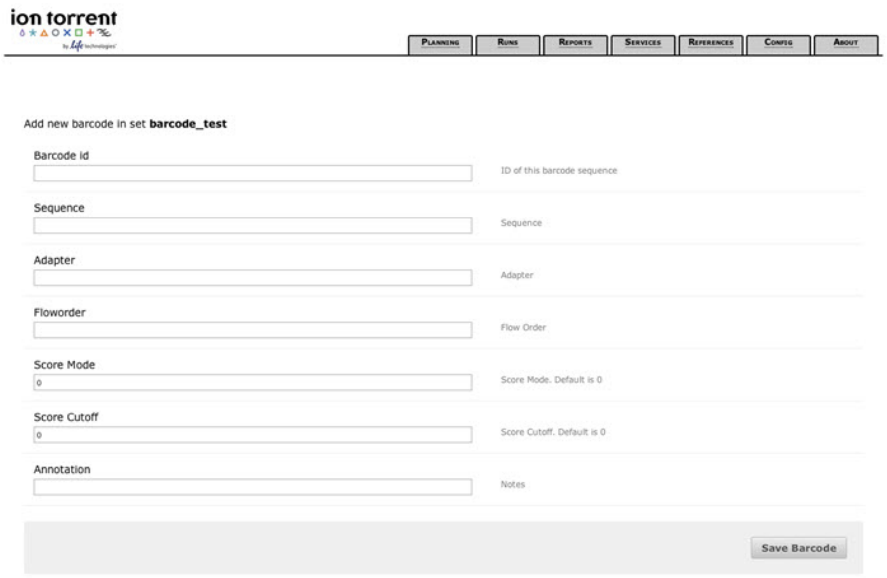
1. To view a barcode set, go to the Torrent Browser and click the **References** tab.
2. Scroll down to the Barcodes section and click on the barcode set name to display the list of barcodes in the set.

Delete a custom barcode set from the Torrent Server

1. To view the barcode set names, click the **References** tab in the Torrent Browser.
2. Scroll down to the Barcodes section and click the name of the barcode set that you want to delete.
3. In the barcode set page, click **+ Delete Barcode Set** then click **Yes** to confirm the deletion.

Add a barcode to a custom barcode set

1. Open the Torrent Browser and click the **References** tab.
2. Scroll down to the Barcodes section and click the name of the barcode set to be edited.
3. Click **+ Add Barcode**. You see the new barcode window:



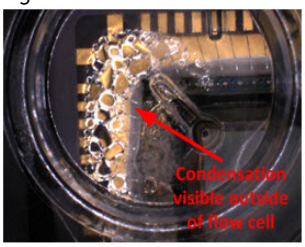
4. Complete the fields, then click **Save Barcode.**

Edit or delete a barcode from a set

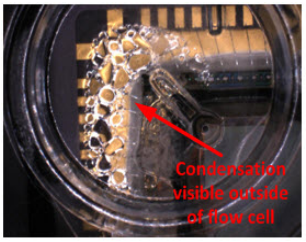
1. Open the Torrent Browser and click the Settings button on the right side of the window, then select **References**.
2. In the Barcodes panel, click the file name of the barcode set to be edited.
3. Click the button under Action to edit or delete the panel.
   - To *edit* a barcode, change the barcode in the edit window, then click **Save Barcode**.
   - To *delete* a barcode from a set, click **Delete Barcode,** then click **Yes** to confirm the deletion.

B Appendix B. Troubleshooting

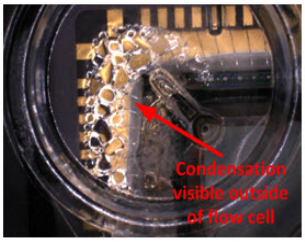
Chip Check

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

Chip calibration

Chip calibration (before loading sample)		
Observation	**Possible cause**	**Recommended action**
Leak of unknown origin	• Chip leak • Chip clamp not closed properly • Problem with the chip clamp or socket	1. Press Main Menu. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. **Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.** 3. Rinse the socket with 18 MΩ water and gently absorb most of the water with the lab wipe. 4. Repeat the rinse, then gently dab the chip socket until dry. 5. Place a lab wipe on the grounding plate and dampen it with 18 MΩ water. Wipe the bottom of the chip on this wipe to remove salts from the chip contacts. 6. Remove the wipe, dry the grounding plate, and place chip on grounding plate. Confirm that there is no condensation outside the flow cell:  7. Replace the chip with a new (unused) one if needed. (**Note:** The new chip can be used for sequencing after initialization completes.) 8. Press **Run** to restart the experiment 9. When prompted to install the new chip, make certain that the chip clamp is fully closed. 10. If the chip leaks again, clean the chip socket as described above. Continued leaking, even with new chips, may indicate a chip clamp or socket problem. Contact Technical support.
Error message: Calibration FAILED	• Chip not seated in socket correctly • Chip is damaged	1. Remove the chip and confirm that there is no leakage or debris on the chip socket. If leaking or debris is seen, follow the procedure for inspecting the chip and clearing debris as described under "Chip Check fails" and/or "Leak of unknown origin" above. If no leaking or debris is seen, reseat the chip in the socket. 2. Press **Calibrate** to repeat the calibration. 3. If the chip passes, press **Next**. If the chip still fails return to the Main Menu and restart the experiment with a new chip. 4. If you continue to have chip calibration issues, there may be an issue with the chip socket. Contact Technical Support.

Chip calibration (with sample already loaded)

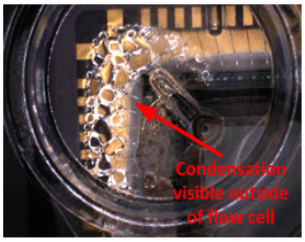
Observation	Possible cause	Recommended action
Leak of unknown origin	• Chip leak • Chip clamp not closed properly	1. Press the **Abort** button. 2. Open the chip clamp, remove the chip, and gently dab the chip socket with a lab wipe tissue to absorb any fluid. Do not rub or wipe the chip socket. 3. Rinse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

Initialization

General Initialization errors

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

Initialization: Auto pH errors

Observation	Possible cause	Recommended action
Error message: Please insert a chip and press Start	Instrument cannot detect the chip in chip socket	1. Open the chip clamp and remove the chip. 2. Check for debris under the chip or in the chip socket. Remove any debris by rinsing with 18 MΩ water and gently dabbing the socket with a lab wipe tissue. **Never rub or wipe the socket. Rubbing the socket can damage it and cause it to fail.** 3. Look for liquid outside the flow cell of the chip:  4. If you see liquid, replace the chip with a new (unused) one. (**Note:** The new chip can be used for sequencing after initialization completes.) 5. Close the clamp, then press **Start** to restart the process. 6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support.
Error message: Chip calibration failed	• Chip not seated in socket correctly • Damaged chip • Loose Sipper	Follow the procedure for "Error message: Please insert a chip and press **Start**." Follow the procedure for "Error message. Wash 2 average not stable."

Initialization: Auto pH errors, continued

Observation	Possible cause	Recommended action
Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again.	• The waste lines may be blocked • Wash 1 or Wash 2 sipper may be loose • Chip does not detect large enough pH difference between the NaOH and W2 Solution • Damaged chip	**Check for blockage** 1. Remove the waste bottle. 2. Place lab wipes under the waste arm. 3. Gently wipe the waste arm with a lab wipe to clear liquid from around the waste line.  4. Press **Flow check** one or more times to observe the flow rates from both lines. One line should drip slightly faster than the other. If one or both lines are blocked (no flow) or the drip rates are significantly different, go to the next step. If the flow rates are normal, see "Check for a damaged chip" below. 5. Press **Line Clear**. Follow the prompts and use the syringe supplied with the Ion PGM™ System. 6. After Line Clear, press **Flow check** and check for normal flow rates from the waste lines. 7. If the flow rates are still not normal, perform **Line Clear** one more time. 8. If the line(s) remain blocked, contact Technical Support. Otherwise, press **Start** to restart auto-pH. **Check Wash 1 and Wash 2 Bottles for loose sipper tubes** 1. Loosen the Wash 1 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the NaOH solution and is not a hazard.) 2. Loosen the Wash 2 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 3. Press **Start** to re-start the auto-pH process.

Initialization: Auto pH errors, continued

Observation	Possible cause	Recommended action
Continued...	Continued...	**Check for a damaged chip or chip clamp/pariposer** 1. Replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start**. (**Note:** The new chip can be used for sequencing after initialization completes.) 2. If the error persists, there could be a problem with the chip clamp. Contact Technical Support. **Forgot to add NaOH to the Wash 1 Bottle** 1. If there is no NaOH in the Wash 1 Bottle, loosen the cap and add 350 µL of 100 mM NaOH to the Wash 1 Bottle. (The flowing gas is not harmful to the NaOH solution and is not a hazard.) 2. Recap the bottle and shake gently to mix. 3. Press **Start** to restart auto-pH.
Error message: W2 average not stable. Try reseating/ replacing chip	Reading for W2 Solution is not stabilizing quickly enough	1. Remove the waste bottle and gently wipe excess fluid from the waste lines with a lab wipe.  2. Check for leaks and reseat the chip (see troubleshooting for "Chip Check" and "Chip calibration" above). Replace the chip with a new (unused) one if needed. (**Note:** The new chip can be used for sequencing after initialization completes.) 3. Loosen the Wash 2 cap and re-tighten the sipper. Since the gas flows when the cap is loose, tighten the sipper as quickly as possible. (The gas is not harmful to the W2 Solution and is not a hazard.) 4. After performing one or more above steps, press **Start** to re-start auto-pH. If auto-pH fails even after replacing the chip, contact Technical Support and manually adjust the pH of the W2 Solution as described in **Appendix C. Manually adjust the pH of the W2 Solution**.
Error message: W2 out of range	• Chip measurements very unstable • Chip is damaged	See troubleshooting tips for "W2 average not stable" above.

Initialization: Auto pH errors, continued

Observation	Possible cause	Recommended action
Error message: Chip reading inconsistent. Please replace chip and try again	• pH response of the chip is not uniform or reliable • Ran out of W3 Solution or volume too low	1. Verify that there is enough W3 Solution (>25 mL) in the Wash 3 Bottle and that the sipper is secure. 2. If necessary, loosen the Wash 3 Bottle cap, tighten the sipper, and add more W3 Solution to fill to 50 mL. Since the gas flows when the cap is loose, perform these operations as quickly as possible. (The gas is not harmful to the W3 Solution and is not a hazard.) 3. If there is enough W3 Solution, replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start.** (**Note:** The new chip can be used for sequencing after initialization completes.)
Error message: Added too much W1 to W2	• Poor water quality • 18 MΩ water exposed to air for too long • Incorrect solution added to the W2 Solution • Too little NaOH added to Wash 1 Bottle • Damaged chip	1. Check whether the water meets the 18MΩ specification and 100 mM NaOH and W2 Solution were prepared correctly. 2. If solutions are incorrect or water does not meet specifications, correctly prepare the solution(s) and/or use high-quality water. Abort the initialization and restart using correct solutions/water. 3. If solutions are correct and water meets specifications, abort the initialization, return to the Main Menu, and proceed to the next steps. 4. Leave the W2 bottle on the instrument. 5. Remove the W1 bottle, leaving the sipper on the W1 port. Empty the bottle, and rinse the bottle twice with 18MΩ water. 6. Add 350 µL of 100 mM NaOH to the W1 bottle and reinstall on the instrument. 7. Press **Initialize**, select the kit type, and keep pressing the **Next** button to skip all bottle prep steps until the instrument begins purging air from the bottle. Then proceed through the touchscreens as normal to complete the initialization. 8. The next time you initialize the instrument, add 140 µL of 100 mM NaOH to the W2 Solution instead of 70 µL. Continue to use this larger volume for subsequent initializations until you receive an "Overshot Target" error message at the first auto pH iteration, at which point follow the troubleshooting steps on page 55 and then return to adding 70 µL of 100 mM NaOH. 9. If you still receive the same initialization error ("Added too much W1 to W2"), contact Technical Support.

Initialization: Auto pH errors, continued

Observation	Possible cause	Recommended action
Error message: UNDERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH couldn't add enough Wash 1 to the Wash 2 before the maximum iterations, 10, occurred	1. A blockage may have occurred. Follow the procedure for "Error message: There may be a blockage or no NaOH in W1. Please check W1 and run line clear then try again." 2. Press **Start** to re-start auto-pH. If you still get the "Undershot target pH" error, try replacing the chip with a new (unused) chip and restarting auto-pH. (**Note:** The new chip can be used for sequencing after initialization completes.)
Error message: OVERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH added more NaOH from the Wash 1 Bottle to the Wash 2 Bottle than was needed, and reports the pH value	**Note:** If you increased the volume of 100 mM NaOH added to the W2 Solution as described under **Error message: Added too much W1 to W2**, perform the following steps and then return to adding 70 µL of 100 mM NaOH for subsequent initializations. 1. Open the Wash 2 Bottle just enough to pipette ~10 µL of 100 mM HCl for every 0.1 pH unit >7.55 into the bottle. (The flowing gas is not a hazard.) 2. Press **Start** to re-start auto-pH. If the pH is still high, replace the chip with a new (unused) one. Insert the chip in the socket, then press **Start.** (**Note:** The new chip can be used for sequencing after initialization completes.) 3. If the pH is consistent with the pH of the previous chip, add more HCl if needed. If auto-pH fails, manually adjust the pH of the W2 Solution (see **Appendix C. Manually adjust the pH of the W2 Solution**).
W2 pH consistently overshoots target	pH of water is too low before any NaOH is added	When preparing the Wash 2 Bottle (see **Prepare the Wash 2 Bottle** on page 21), add more than the recommended 70 µL of 100 mM NaOH. After adding the NaOH, the Wash 2 Bottle should be in the range of pH 6.0–6.5 before you begin initialization.

Initialization: Reagent pH Verification

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

Control Ion Sphere™ Particles

Troubleshooting using the Control Ion Sphere™ Particles		
Observation	**Possible cause**	**Recommended action**
Ion Sphere™ Test Fragments are not present in the Test Fragment Report section of the run report, and library sequencing is poor.	• Poor chip loading • Control Ion Sphere™ Particles were not added to the sample	1. Confirm that the Control Ion Sphere™ Particles (included in this sequencing kit) were added. 2. If controls were added, contact Technical Support.
Control Ion Sphere™ Particles were added, but sample loading is poor.	Problems with library or template preparation	Verify the quantity and quality of the library and template preparations.

Appendix C. Manually adjust the W2 Solution pH

Materials and equipment needed

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

Procedure

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

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

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

2. Remove the Wash 2 Bottle attached to the instrument, and cap the bottle.
3. Secure the empty Wash 2 Bottle (from step 1) to the instrument—do not remove the sipper. This bottle will contain the gas flowing out of the instrument while you pH the W2 Solution and protect the sipper from contamination.
4. Move the Wash 2 Bottle containing the W2 Solution to the stir plate near the nitrogen or argon gas tube.
5. Secure the gas tube so that it extends inside the mouth of the Wash 2 Bottle but not below the surface of the W2 Solution.
6. Set the gas flow to 0.5 lpm. Start mixing the W2 Solution fast enough for a small whirlpool to form.
7. Calibrate the pH meter using a three-point calibration. Rinse any buffering solution from the pH probe prior to preparing solutions.
8. Adjust the pH of the W2 Solution to 7.65 ± 0.1 by adding a small amount of freshly prepared 100 mM NaOH to the solution, and then measuring the pH using the pH meter. Add small aliquots and allow the pH to equilibrate before adding more.

   Note: If the pH rises above 7.75, use 100 mM hydrochloric acid (HCl) to readjust the pH to 7.65 ± 0.1.

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

10. Move the bottle to the instrument, remove the empty Wash 2 Bottle from the instrument, and place the sipper inside the Wash 2 Bottle whose pH adjusted.
11. Secure the cap firmly. Press **Next** to exit the automated pH check and continue with instrument initialization.

D

Appendix D. Sequencing run times

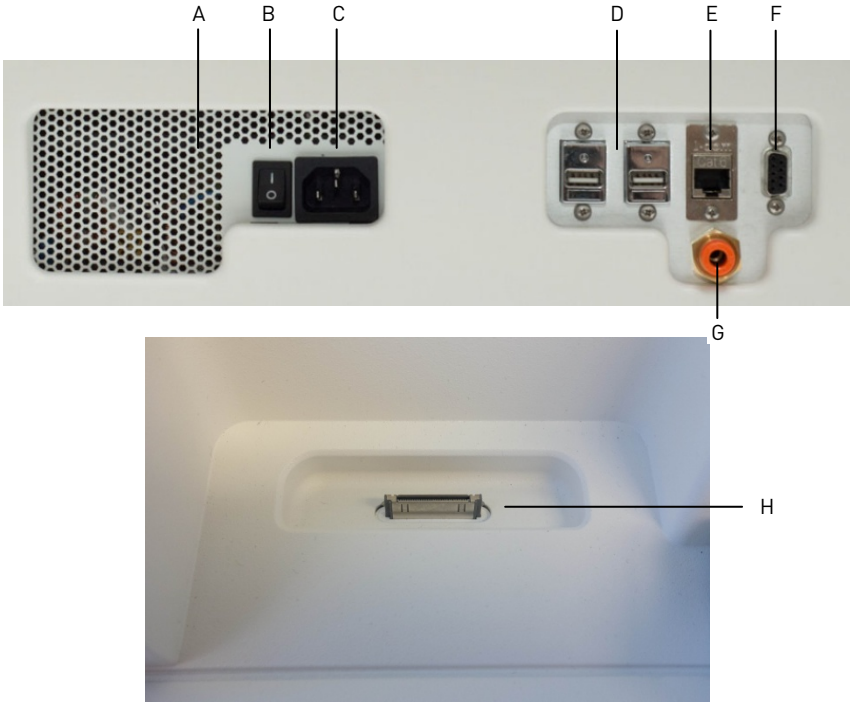
Number of flows	Average read length*	Average run time by chip type: 314v2/316v2/318v2	Single read runs/kit†
1100	500 bp	NA / 5.8 / 8.5 hours	4
850	400 bp	3.7 / 4.9 / 7.25 hours	4
640	300 bp	2.9 /3.8 / 5.5 hours	4

*Read length may vary based on your library size.

† Only 4 runs are supported for any read length. For best results, run should be started within 1 hour after initialization.

Appendix E. Additional instrument information

Ion PGM™ Sequencer input and output connections



Label	Component	Description
A	Instrument fan cover	**IMPORTANT!** The fan cover must be unobstructed to ensure adequate cooling and proper functioning of the Ion PGM™ Sequencer.
B	On/off switch	Power switch, where the states are on (**|**) or off (**O**).
C	Power port	100-240VAC port that provides power to the instrument.
D	USB ports	Connects the barcode reader to the instrument.
E	Ethernet port	An RJ45 port that provides Ethernet (Gigabit) communication with the Ion PGM™ Sequencer.
F	RS232 port	A diagnostic port
G	Gas inlet	For nitrogen gas.
H	iPod® port	A port for docking your iPod® portable media player

Power the Ion PGM™ Sequencer on or off

Power on

Note: If the Ion PGM™ Sequencer is powered on, and the touchscreen is blank, touch the screen to "wake" the touchscreen.

1. Locate the power switch on the back of the instrument and turn to the on (|) position.
2. Press the power button on the front of the instrument. The switch should illuminate. When the instrument touchscreen Main Menu appears, the instrument is ready for use.
3. See **Cleaning schedule** on page 18 for when to perform 18 MΩ water or chlorite solution cleaning after powering on.

Power off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument will not be used for more than 3 days, power off the instrument as follows:

1. In the Main Menu, select **Tools > Shut Down**.
2. If you have not already cleaned the instrument, select 18 MΩ water cleaning, then press **Next** to start the cleaning process.
3. When cleaning is complete, press **Shut Down**.
4. After you exit the main touchscreen, press the **Halt** button, then **OK** when prompted. The instrument will power down.

Update the Ion PGM™ Sequencer software

IMPORTANT! After updates are installed, the instrument must be restarted.

If an update to the Ion PGM™ Sequencer software is available, the red "Alarms and Events" pop-up appears in the touchscreen Main Menu to alert you. Click the red pop-up to see the detailed messages. If a message states **New Software Available**, update the software as follows:

1. In the Main Menu, select **Options > Updates**.
2. Select the **Released Updates** checkbox, then press **Check**.
3. When the message **Press Update** to begin update process appears, press **Update**.

   Note: If the message "All Software Current" appears, press **Back** to return to the Main Menu.

4. When the message "Installing Completed" displays, follow the onscreen prompts to restart the instrument.

   Note: In some cases, the instrument restarts automatically after software installation.

Appendix F. 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. 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 **Appendix G. Documentation and support** on page 70.

Equipment use

Note: The Ion PGM™ Sequencer performs real-time measurements of hydrogen ions produced during DNA replication.

The Ion PGM™ System is for performing sequencing of amplified DNA, and should only be used for life science research applications. The Ion PGM™ System should only be used by professionals trained in laboratory techniques and who have studied the instructions for use of this instrument. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

IMPORTANT! If you use and/or install the Ion PGM™ System in an unspecified manner, you may impair the protection provided by the equipment.

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.
	Caution, hot surface	Attention, surface chaude
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Potential biohazard	Danger biologique potentiel
	Laser radiation	Rayonnement laser
	Caution, piercing hazard	Attention, danger de perforation
	On	On (marche)
	Off	Off (arret)
	On/Off	On/Off (marche/arrêt)
	Earth (ground) terminal	Borne de (mise à la) terre
	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
	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 on this instrument

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

Safety alerts on this instrument

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

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

Safety information for instruments not manufactured by Life Technologies

Some of the accessories provided as part of the instrument system are not designed or built by Life Technologies. 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.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

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 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

Laser

CAUTION! LASER HAZARD, Bar Code Scanner. The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Gas safety

Verify that your installation room can accommodate gas cylinders.

WARNING! Ion instrumentation should be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Assess the need for ventilation or atmospheric monitoring to avoid asphyxiation accidents from inert gases and/or oxygen depletion, and take measures to clearly identify potentially hazardous areas through training or signage. Please contact your Environmental Health and Safety Coordinator to confirm that the Ion instruments will be installed and operated in an environment with sufficient ventilation

WARNING! Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your EHS coordinator for guidance on the proper installation of a gas cylinder.

Safety and electromagnetic compatibility (EMC) standards

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

Safety

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

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	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 2002/96/EC	European Union "WEEE Directive" – Waste electrical and electronic equipment

Chemical safety

WARNING! General chemical handling. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see **Appendix G. Documentation and support** on page 69.
- 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.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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

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

Appendix G. Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**.

For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:
www.lifetechnologies.com/support/

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Ion contact information

Web site: **www.lifetechnologies.com/iontorrent**

Ion community: **ioncommunity.lifetechnologies.com**

Support email: **ionsupport@lifetech.com**

Phone numbers
In North America: 1-87-SEQUENCE (1-877-378-3623)
Outside of North America: +1-203-458-8552

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.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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6 December 2013