USER GUIDE

Ion Sequencing Kit v2.0 User Guide

For use with the Personal Genome Machine™ (PGM™) System

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Preface

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

IMPORTANT! If the Personal Genome Machine™ (PGM™) System is used or installed in a manner not specified in the installation guide, the protection provided by the equipment may be impaired.

Safety alert words

Four safety alert words appear in Life Technologies user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—IMPORTANT, CAUTION, WARNING, DANGER—implies a particular level of observation or action, as defined below:

IMPORTANT! 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.

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. This signal word is to be limited to the most extreme situations.

Except for IMPORTANT notes, each safety alert word in a Life Technologies document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Life Technologies instruments*.

SDSs

The SDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "Obtaining SDSs" on page 41.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Equipment intended use

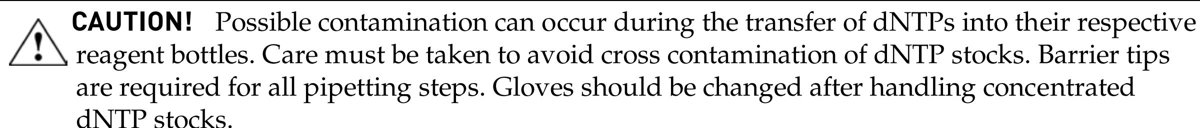
The Personal Genome Machine™ (PGM™) System is intended for performing genomic sequencing of amplified DNA, and should only be used for life science research applications. The PGM™ System should only be used by professionals trained in laboratory techniques and who have studied the instructions for use 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.

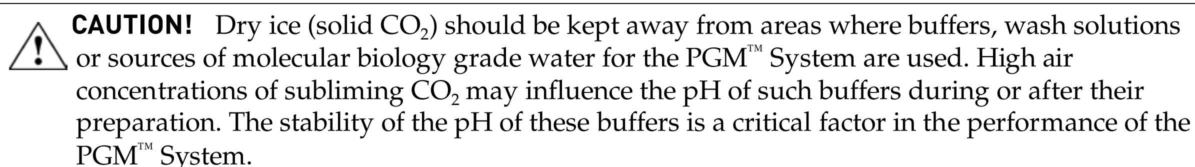
The Personal Genome Machine™ System is For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Contamination

CAUTION! 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.

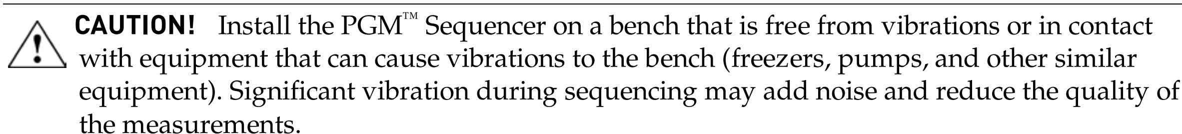


CAUTION! Possible contamination can occur during the transfer of dNTPs into their respective reagent bottles. Care must be taken to avoid cross contamination of dNTP stocks. Barrier tips are required for all pipetting steps. Gloves should be changed after handling concentrated dNTP stocks.



CAUTION! Dry ice (solid CO_2) should be kept away from areas where buffers, wash solutions or sources of molecular biology grade water for the 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 PGM™ System.

Instrument vibration and clearances



CAUTION! Install the PGM™ Sequencer 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). Significant vibration during sequencing may add noise and reduce the quality of the measurements.

Ion PGM™ Sequencer Space Requirements and Clearances: Position the Ion PGM™ sequencer so that the front bezel of the sequencer is a minimum of 12 in. (30.5 cm) and the tubes containing nucleotides are a minimum of 8 in. (20.3 cm) from the front of the laboratory bench. Place the sequencer 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. Placing the chip on other non-grounded surfaces like a bench can result in damage due to static electric discharge.

Components and Storage Conditions

The Ion Sequencing Kit (Part no. 4468997) provides reagents and materials for performing sequencing runs on the PGM™ System. The kit includes reagents and materials for performing eight runs and four initializations. (This provides two runs per initialization if you are performing 65-cycle runs. Some applications allow more runs per initialization; reagents are provided for up to eight runs for all applications.)

Ion PGM™ Supplies Kit (Part no. 4468996)

Component	Part Number	# Per Kit	Storage
Wash Bottle Sippers (gray)	601-1001-01	8 for 250-mL bottle and 4 for 2-L bottle	Room temperature (15–25°C)
Conical Tube Sippers (blue)	601-1002-01	16	
Conical Tubes and labels	601-1003-01	25	
Wash 1 Bottle (250 mL) and label	601-1004-01	1	
Wash 2 Bottle (2 L) and label	601-1005-01	1	
Wash 3 Bottle (250 mL) and label	601-1004-01	1	

Ion Sequencing Reagents Kit (Part no. 4468995)

Component	Cap Color	Part Number	# Per Kit	Volume	Storage
dGTP	Black	602-1015-03	1	100 µL	–20°C
dCTP	Blue	602-1016-03	1	100 µL	
dATP	Green	602-1017-03	1	100 µL	
dTTP	Red	602-1018-03	1	100 µL	
Sequencing Polymerase	Yellow	602-1074-01	1	32 µL	
Sequencing Primer	White	602-1073-03	1	96 µL	
Control Ion Spheres™	Clear	603-1088-01	1	40 µL	

Ion PGM™ Reagents Kit (Part no. 4468994)

Component	Part Number	# Per Kit	Volume	Storage
W2 Solution (round black label)	603-1031-02	1	88 mL	4°C
Annealing Buffer	603-1048-01	1	80 mL	
PGM™ Cleaning Tablet	603-1051-01	4	—	
10X W3 Solution	603-1062-01	1	20 mL	

Description

The Ion Sequencing Kit provides reagents and materials for performing sequencing runs on the PGM™ System. It also includes reagents and materials for cleaning and initializing the instrument. This kit is designed to sequence templates that have been amplified on Ion Sphere™ Particles (ISPs) using the Ion Xpress™ Template Kit.

The kit includes reagents and materials for performing eight runs and four initializations. (This provides two runs per initialization if you are performing 65-cycle runs. Some applications allow more runs per initialization; reagents are provided for up to eight runs for all applications.)

The Control Ion Spheres™ provided in this kit are spike-in controls used to assess the quality of a sequencing run.

Materials and Equipment Required

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

The following table lists required materials and equipment:

✓	Description	Supplier	Part number	Quantity
	Ion 314™ Chip Kit *or*	Life Technologies	4462923	8 pack
	Ion 316™ Chip Kit	Life Technologies	4466616 4469496	4 pack 8 pack
	Ion Torrent PGM™ Sequencer and all included accessories	Life Technologies	4462917	1
	Torrent Server	Life Technologies	4462918	1
	Ion Control Materials Kit	Life Technologies	4466465	1
	Tank of Compressed Argon (99.9% industrial grade or better)	MLS	N/A	N/A
	Multistage (dual-stage) gas regulator (0-50 PSI, 2–3 Bar output)	VWR International	55850-422	1 or 2
	1/8" x 1/4" stem reducing coupler (only required if using a separate tank for the wash station)	McMaster	5779K699	1 per wash
	Microcentrifuge (capable of >15,500 × g, fits 1.5-mL and 0.2-mL microcentrifuge tubes)	VWR International	93000-196	1
	Uninterruptable Power Supply (UPS) (see note below)	MLS (Major Laboratory Supplier; for example, APCC)	N/A	1
	18 MΩ water supplied fresh (**not boxed**) from an appropriate system (i.e., MilliQ or equivalent)	MLS	N/A	1

✓	Description	Supplier	Part number	Quantity
	NaOH (10 M) molecular biology grade	MLS	N/A	Varies
	Isopropanol (100%)	MLS	N/A	Varies
	Magnetic stirrer (must hold 2-L bottle)	MLS	N/A	1
	Magnetic stir bar (4 cm)	MLS	N/A	1
	P2, P20, P200, P1000 µL pipette set and filtered tips	MLS	N/A	1 set
	Barrier pipette tips	MLS	N/A	Varies
	Serological pipettor	MLS	N/A	1
	25 mL serological pipettes	MLS	N/A	Varies
	Rainin® Pipet-Lite® LTS L-20 (2–20 µL)	Rainin	L-20	1
	Rainin® Pipet-Lite® LTS L-100 (10–100 µL)	Rainin	L-100	1
	Rainin® SR-L20F pipette tips	Rainin	SR-L20F	
	Rainin® SR-L200F pipette tips	Rainin	SR-L200F	
	0.2-mL MAXYMum Recovery® Thin Wall PCR Tubes, Flat Cap (do not use polystyrene tubes)	Axygen	PCR-02-L-C	Varies
	1.5-mL or 1.7-mL microcentrifuge tubes	MLS	N/A	Varies
	Graduated cylinders (1 L or 2 L volume)	MLS	N/A	
	Glass bottles (1 L)	MLS	N/A	
	15-mL conical tubes	MLS	N/A	
	Ice buckets and ice	N/A	N/A	
	Sonicating bath (2–3 L tank; 80–160 W, 40 kHz transducer; no heater necessary) with floating PCR tube holder	MLS (Branson or Fisher supported)	N/A (Branson part no. 952-118; Fisher part no. 15-335-20)	1
	Squirt bottle	—	N/A	1
	Vortex mixer with a rubber platform	MLS	N/A	1
	Thermal cycler with a heated lid	MLS	N/A	1

Optional Materials and Equipment

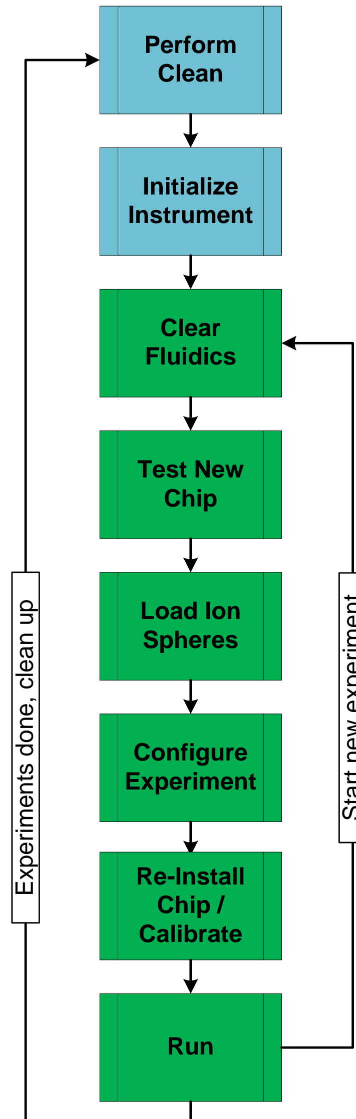
The following may be required to verify and adjust the pH of the Wash 2 solution during Initialization if prompted by the PGM™ Sequencer.

✓	Description	Supplier	Part number	Quantity
	Orion 3-Star Plus pH Benchtop Meter Kit with electrode, electrode stand, and calibration buffers (or equivalent)	Thermo Scientific	1112003	1
	1 N HCl	MLS	N/A	Varies

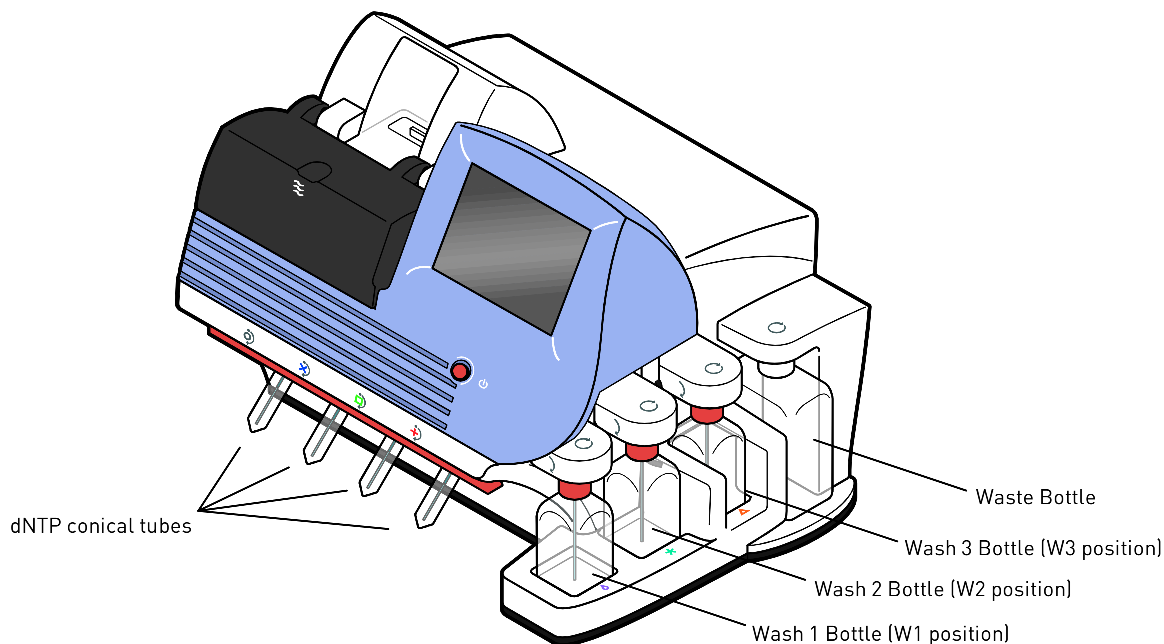
Note on Power Supply: We recommend using an uninterruptable power supply (UPS) for laboratories that experience frequent power outages or line voltage fluctuations. The UPS must be compatible with 500 W output or higher. The 1500 VA unit from APCC provides approximately 11 minutes of backup power for an Ion PGM™ Sequencer.

Protocol Overview

The following protocol describes the steps necessary to prepare a sequencing run or control run. The PGM™ System uses the following workflow when performing sequencing runs:



PGM™ Sequencer with Bottles and Conical Tubes Attached



Cleaning and Initialization

Before Starting

Always use freshly prepared NaOH solution.

Weekly: Prepare a stock of 1 M NaOH by diluting 10 M NaOH with fresh 18 MΩ water on a weekly basis.

Daily: Prepare fresh 100 mM NaOH by diluting the 1 M stock in fresh 18 MΩ water.

Cleaning the PGM™ System

Materials provided in the kit

- **Chlorite cleaning:** PGM™ Cleaning Tablet

Other materials and equipment

- 18 MΩ water
- Cleaning bottles and collection trays (provided with the PGM™ System)
- Used chip (leave on the instrument during cleaning)
- Used sippers (from previous run or provided with the instrument)
- Squirt bottle
- **Chlorite cleaning:** 1 M NaOH
- **Chlorite cleaning:** 0.22-µm or 0.45-µm vacuum filtration system and filters

Cleaning schedule

In general, clean the PGM™ System with 18 MΩ water on a daily basis or after 130 cycles, and with a chlorite solution once a week.

- The instrument should be cleaned with 18 MΩ water and initialized after every 130 cycles (e.g., two 65-cycle runs). Cleaning must be run every time the PGM™ Sequencer is initialized.
- If more than 10 hours have elapsed since the previous cleaning/initialization, re-clean with 18 MΩ water and initialize before starting a run.
- Perform a chlorite cleaning once a week or if the instrument has been left with reagents for more than 48 hours (e.g., over the weekend).

Cleaning setup

- Remove any bottles that may be attached to the PGM™ Sequencer.
- **Do not remove old sippers before performing a cleaning.** The sippers are used as part of the procedure.
- Separate cleaning bottles are provided with the instrument. This will be attached to the W1, W2, and W3 positions on the instrument when prompted. (After you have used the Wash bottles provided with the Sequencing Kit for the specified number of sequencing runs, you can then use them as cleaning bottles; be sure to mark them as for cleaning use only.)
- Make sure 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. (For example, an Ion 314™ chip can be used for cleaning/initialization prior to running an Ion 316™ Chip.)

18 MΩ water cleaning

In general, perform an 18 MΩ-water cleaning on a daily basis, after every 130 cycles, or if the system has been left with reagents for more than 10 hours.

1. Empty any remaining solution from each cleaning bottle and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Select **Clean PGM** on the touch screen.
3. Add 250 mL of 18 MΩ water to a 250-mL cleaning bottle and attach the bottle to the W1 position on the PGM™ Sequencer.
4. Follow the instructions on the touch screen to perform the cleaning procedure.
5. When prompted, remove the W1 cleaning bottle, rinse the outside of the sipper with a squirt bottle containing fresh 18 MΩ water, then rinse and reinstall the 250-mL cleaning bottle filled with 250 mL of fresh 18 MΩ water.
6. When cleaning is finished, press **Next** to return to the Main Menu and proceed to initialization.

Chlorite cleaning

Clean with a chlorite solution once a week or if the system has been left with reagents for more than 48 hours (e.g., over the weekend).

Note: On a weekly basis, prepare a fresh stock of 1 M NaOH by diluting 10 M NaOH with fresh 18 MΩ water.

1. Fill a container with 1 L of 18 MΩ water and add a PGM™ Cleaning Tablet (chlorite tablet). Wait 10 minutes for the tablet to completely dissolve.
2. After 10 minutes, 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).

3. Add 250 mL of filtered chlorite solution to a 250-mL cleaning bottle and attach the bottle to the W1 position on the PGM™ Sequencer.
4. Follow the instructions on the touch screen to perform the cleaning procedure.
5. When prompted, remove the W1 cleaning bottle with chlorite solution, rinse the outside of the sipper with a squirt bottle containing fresh 18 MΩ water, then install a fresh 250-mL cleaning bottle filled with 250 mL of fresh 18 MΩ water.

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

6. When cleaning is finished, press **Next** to return to the Main Menu and proceed to initialization.

Initializing the PGM™ Sequencer

Initialization takes ~1 hour. As part of the initialization process, prepare the wash and dNTP solutions as described in this section.

IMPORTANT! **Sequencing kits are designed for four PGM™ initializations.** After four initializations, the Wash 1, 2, and 3 bottles should not be used for sequencing to avoid breakage or leaking. (Save the bottles for use in the cleaning procedure, if desired.)

IMPORTANT! The dNTP Conical Tubes and sippers must be replaced after every run.

Materials provided in the kit

- Wash 1, 2, and 3 bottles and sippers
- dNTP conical tubes and sippers
- dGTP, dCTP, dATP, and dTTP
- W2 Solution
- W3 Solution

Other materials and equipment

- Used chip (leave on the instrument during initialization)
- 18 MΩ water
- 100 mM NaOH (prepared fresh daily)
- Ice
- Argon gas tank, tube, and flow meter
- Magnetic stirrer
- Magnetic stir bar
- Serological pipette and 25-mL pipettes
- Filtered and unfiltered pipette tips and pipettors
- Vortex mixer
- Microcentrifuge
- **Optional** pH meter, multipoint pH calibration reagents, pH probe, and probe stand

Before initialization

- Remove the dNTP stock solutions from the freezer and begin thawing on ice.

CAUTION! Handle nucleotides carefully to avoid cross-contamination. Always discard gloves after removing used sippers from the PGM™ Sequencer in order to avoid cross contamination of the nucleotides. Gloves should also be changed after handling concentrated dNTP stocks.

- Check the argon tank pressure. When the tank pressure drops below 500 psi, change the tank.

Prepare the Wash 2 bottle

1. Rinse the Wash 2 bottle (2 L) **three times** with 200 mL of 18 MΩ water.

   Note: Ensure the 18 MΩ water is fresh from a purification system and has not been stored for more than 3–4 hours in a container.

2. For the first use of the bottle, add 1,978 mL of 18 MΩ water to the Wash 2 bottle, mark the fill line, and empty the bottle. (This prevents having to measure the water separately each time.)
3. Place a clean magnetic stir bar into the empty Wash 2 bottle.
4. Insert the argon gas tube into the bottle, set the argon flow meter to 0.5 liters per minute (LPM), and flow argon into the Wash 2 bottle for 5 minutes. This will purge carbon dioxide from the container.

   Note: For the next steps, if your 18 MΩ water source is not next to your argon source, cap the bottle before moving it between locations.

5. Remove the argon gas tube and add 18 MΩ water to the fill line marked on the Wash 2 bottle (1,978 mL). Be careful to extend the water spigot into the bottle to minimize air exchange.
6. Place the Wash 2 bottle on a stir plate. Secure the argon gas tube so that it extends inside the mouth of the bottle but not below the surface of the water.
7. Set the argon flow to 0.5 LPM. Start mixing the water fast enough for a small whirlpool to form.

   Note: For the following steps, be careful to distinguish the W2 and W3 solution bottles to avoid confusion.

8. Add 22 mL of **W2 Solution to the Wash 2 bottle**. Stir until the solution is fully dissolved, usually in less than 1 minute. While the solution is mixing, proceed to preparing the Wash 1 and 3 bottles.

Prepare the Wash 1 and Wash 3 bottles

1. Rinse the Wash 1 and Wash 3 bottles **three times** with 50 mL of 18 MΩ water.
2. Add 350 µL of **freshly prepared 100 mM NaOH** solution (*not* 1 M NaOH) to the Wash 1 bottle. Cap the bottle.
3. Add 5 mL of the 10X W3 Solution from the kit to the Wash 3 bottle, then add 45 mL of 18 MΩ water, measured using 50-mL conical tube or serological pipette. Cap the bottle.

Begin the initialization

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

IMPORTANT! Load the bottles as quickly as possible to prevent atmospheric carbon dioxide from reducing the pH of the Wash 2 bottle solution.

1. Confirm that a chip is in place on the PGM™ Sequencer. This should be the same chip used to clean the instrument.

   Note: The chip type used for cleaning/initialization can be different than the chip type used for sequencing. (For example, an Ion 314™ chip can be used for cleaning/initialization prior to running an Ion 316™ Chip.)

2. From the main menu, press **Init PGM**. 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.
3. Wearing clean gloves, follow the on-screen prompts to install a new sipper (long gray) in the cap on the W2 position on the PGM™ Sequencer. **Do not let the sipper touch any surfaces.**

Note: Use two hands to attach the sipper, as shown below. Be careful to avoid cross-threading, and make sure the sipper is sealed properly.

4. Immediately attach the prepared Wash 2 bottle. Press **Next**.
5. Install new sippers (short gray) in the caps on the W1 and W3 positions, following the same procedure used for the W2 sipper.
6. Attach the prepared Wash 1 and 3 bottles and tighten the caps on the bottles. Press **Next**.
7. The PGM™ Sequencer will test the bottles for leaks, fill the Wash 1 bottle, and then adjust the pH of the Wash 2 solution. This procedure takes approximately 30 minutes. Meanwhile, proceed to preparing the dNTP Conical Tubes.

If a wash bottle leaks or if an error occurs during the automatic pH process, see "Appendix B. Troubleshooting."

Prepare the 50-mL dNTP Conical Tubes

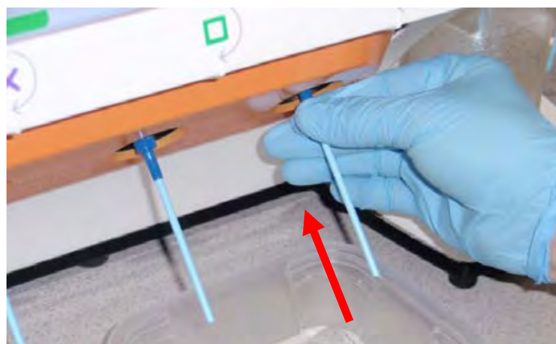
CAUTION! In the following steps, handle the nucleotides carefully to avoid cross-contamination and ensure that the correct dNTP tubes are installed in each position on the PGM™ Sequencer.

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 Ion PGM™ Supplies Kit to label four new Conical Tubes 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 Conical Tube.

Attach the dNTP sippers and Conical Tubes

1. After the Wash solutions have initialized, follow the on-screen prompts to remove the four used dNTP sippers and collection trays from the PGM™ Sequencer. Change gloves.
2. Using fresh gloves, attach new sippers (blue) to each of the dNTP ports. **Do not let the sippers touch any surfaces.**

   Note: The dNTP sippers do not lock—they only need to be pushed on securely.



3. Attach each Conical Tube to the PGM™ Sequencer and tighten firmly until snug. The correct order of the Conical Tubes on the PGM™ Sequencer is dGTP, dCTP, dATP, and dTTP (left to right when facing the instrument).

   Note: The PGM™ Sequencer will check the pressure of the dNTP tubes and Wash bottles. If a bottle leaks, you will be prompted to check that it is tightly attached to the instrument. If it continues to leak, there may be a crack and it should be replaced. If you replace the bottle but the instrument still does not pass the leak check, contact Technical Support.

4. Follow the on-screen prompts to complete initialization. The PGM™ Sequencer will fill each Conical Tube with 40 mL of Wash 2 solution.

   Note: To conserve time, the following ISP preparation procedure can be started while the PGM™ System is initializing. When initialization is complete, the experiment can begin.

At the end of initialization, the PGM™ Sequencer will take pH measurements of all 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."

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

Ion 314™ Chip Sequencing Protocol

Note: For a protocol using the Ion 316™ Chip, see page 21.

Ion 314™ Chip — Prepare the template-positive ISPs for sequencing

Materials provided in the kit

- Sequencing Primer (thawed on ice)
- Control Ion Spheres™
- Annealing Buffer

Other materials and equipment

- Enriched template-positive ISPs, prepared as described in the *Ion Xpress Template Kit User Guide v2.0* (Part no. 4469004)
- 0.2-mL PCR tube (non-polystyrene)
- Pipette tips and pipettor
- Vortex mixer
- Microcentrifuge
- Thermal cycler with heated lid

Before starting

- Use the enriched, template-positive ISPs that were enriched using the protocol provided in the *Ion Xpress™ Template Kit User Guide v2.0*.
- Thaw the Sequencing Primer on ice.

Using Ion Sphere™ Test Fragments for installation or troubleshooting

If you are performing an installation or troubleshooting sequencing run, skip directly to step 3 below and substitute **5 µL of Ion Sphere™ Test Fragments** from the Ion Control Material Kit (Part no. 4466465) for the 5 µL of Control Ion Spheres™ in the procedure.

Add Control Ion Spheres™ and anneal Sequencing Primer to the enriched ISPs.

1. Transfer **half of the volume** of enriched template-positive ISPs (prepared as described in the *Ion Xpress™ Template Kit User Guide v2.0*) to a new 0.2-mL PCR tube. Do not use polystyrene tubes.

   Note: Store the remaining unused, enriched ISPs at 4°C for up to 1 week. They may be used for another sequencing run.

2. If the transferred volume of ISPs in step 1 was :
   - ≤50 µL: Proceed to step 3.
   - >50 µL: Collect the ISPs by centrifuging the tube for 2 minutes at 15,500 × g. Remove the supernatant, leaving ~ 50 µL at the bottom of the tube, then proceed to step 3.
3. Vortex the Control Ion Spheres™ and centrifuge for 2 seconds before taking aliquots.
4. Add 5 µL of Control Ion Spheres™ to the PCR tube containing the enriched particles. (**Note:** For installation or troubleshooting, add the 5 µL of Ion Sphere™ Test Fragments directly to the Annealing Buffer in the next step.)

   Note: 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.

5. Add 150 µL of Annealing Buffer. Mix by vortexing or pipetting up and down.
6. Collect the ISPs by centrifuging the tube for 2 minutes at 15,500 × g.

7. Carefully remove the supernatant with a pipette tip, leaving slightly less than 9 µL of supernatant in the bottom of the tube. (Visually compare the volume to a separate tube containing 9 µL of liquid.)
8. Measure the volume of supernatant. If needed, add Annealing Buffer to increase the volume to 9 µL.
9. Add 5 µL of Sequencing Primer to the ISP sample, then pipet the sample up and down.

IMPORTANT! Pipette thoroughly to make sure the pellet is completely disrupted before proceeding.

10. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
11. 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.

Ion 314™ Chip — Test a new Ion Chip (Chip Check)

Materials provided in the kit

- Annealing Buffer

Other materials and equipment

- New Ion 314™ Chip
- 100% isopropanol
- Rainin® SR-L200F pipette tips
- 1.5-mL microcentrifuge tube
- Barcode scanner (included with the PGM™ System)
- Ion centrifuge adapter/rotor (included with the PGM™ System)

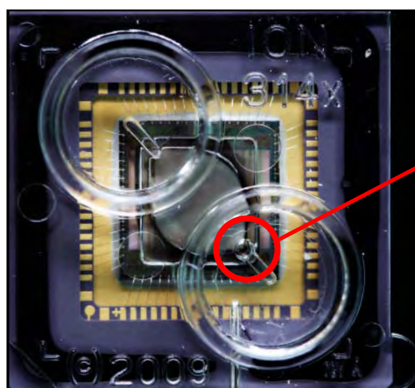
Handling Ion Chips

- 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 PGM™ Sequencer or in the custom Ion centrifuge adapter/rotor bucket.
- To avoid ESD damage, **do not wear gloves** when transferring chips onto and off the instrument.

Wash the chip

IMPORTANT! Use Rainin® SR-L200F pipette tips to enable slow, steady washing of the chip. Do not let the chip dry out.

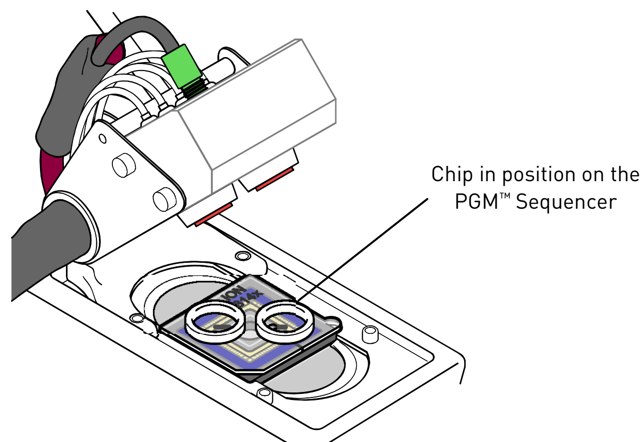
IMPORTANT! When loading liquid into the chip, press the pipette tip firmly into the circular **loading port** on the chip (not into the slit), and keep the pipette tip at a **90° angle** to the chip. **Do not introduce bubbles into the chip.**



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 PGM™ Sequencer grounding plate or in the Ion centrifuge adapter/rotor bucket.
3. Using a Rainin® SR-L200F pipette tip, slowly and steadily pipet 50 µL of 100% isopropanol into the large port of the chip.
4. Aspirate any displaced solution from the other port. **Proceed immediately to the next step.**
5. Wash the chip **two times** with Annealing Buffer as follows:
   a. Slowly and steadily pipet 50 µL of Annealing Buffer into the large port on the chip.
   b. Aspirate any displaced solution from the other port.

Chip check on the instrument

1. Press **Experiment** on the main menu and follow the prompts to prepare the PGM™ Sequencer to test a new Ion Chip.
2. When prompted, ground yourself by touching the grounding pad next to the chip clamp on the instrument and replace the old chip with the new one for the experiment. **Do not wear gloves when transferring the chips on and off the instrument.**



3. Use the barcode scanner to scan the chip barcode located on the chip package.

   Note: To enter the barcode manually, press the **Change** button next to the barcode field. A chip cannot be run without scanning or entering the barcode.

4. After the barcode is entered, press the **Chip Check** button. Chip Check tests the chip and ensures that it is functioning properly prior to loading the sample.
5. After Chip Check is complete, press **Next** to proceed to calibration. The chip will be flushed with the solution from the Wash 2 bottle prior to calibration.
6. Check for leaks on the chip. If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Then proceed to “Appendix B. Troubleshooting.”

CAUTION! **The chip socket can be damaged by rubbing or wiping its surface.** When cleaning up spills, always gently dab the socket without rubbing.

7. After calibration is complete, the system indicates if the chip passed or failed:
   - If the chip passed, press **Next** and follow the prompts to remove the Ion Chip.
   - If the chip failed, open the chip clamp, re-seat the chip in the socket, close the clamp and repeat the Chip Check. 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.

8. After removing the chip from the socket, use a Rainin® SR-L200F pipette tip to slowly and steadily pipet 50 µL of Annealing Buffer into the large port on the chip.
9. With the same pipette tip, aspirate any displaced solution from the other port.

Ion 314™ Chip — Load the ISPs and configure the experiment

Materials provided in the kit

- Sequencing Polymerase
- Annealing Buffer

Other materials and equipment

- 1.5-mL microcentrifuge tube
- Rainin® SR-L20F pipette tips
- Sonication bath (40 KHz)
- Vortex mixer
- Microcentrifuge

Bind Sequencing Polymerase to the ISPs

1. After annealing the Sequencing Primer to the ISPs, remove the ISPs from the thermal cycler and add 1 µL of Sequencing Polymerase. Pipet the sample up and down to mix.
2. Incubate the ISPs with polymerase at room temperature for 5 minutes.
3. Sonicate the ISPs for 10 seconds using a standard 40 KHz sonication bath.

   Note: Make sure the water level in the sonicator is filled to the appropriate mark.

Load the ISPs on the chip

IMPORTANT! To ensure successful loading of the ISPs on the chip:
- Press the pipette tip firmly into the circular loading port on the chip
- Keep the pipette tip at a 90° angle to the chip
- Load at a consistent speed and apply gentle pressure between the pipette tip and chip
- Do not suction the mixture back into the pipette tip

1. Place the chip on the PGM™ Sequencer grounding plate or in the bucket of the Ion centrifuge adapter/rotor.
2. Use a Rainin® SR-L20F pipette tip to carefully deposit 7 µL of the sample (~half of the sample) into the large port of the chip. Avoid introducing bubbles into the chip by leaving a small amount of sample in the pipette (~0.5 µL).
3. Remove the displaced liquid from the other port of the chip using the same pipette tip.
4. Transfer the Ion Chip to the centrifuge (with the custom Ion centrifuge adapter/rotor). If preparing one Ion Chip at a time, always balance the centrifuge adapter with a used chip. Mark the used chip with a laboratory marker to differentiate it from the new chip for the current experiment.

   Note: For the following steps, ignore the 10-minute timer on the PGM™ System screen. Centrifuge for **4 minutes**.

5. Centrifuge the chip for 4 minutes using the custom centrifuge adapter/rotor.
6. Use a fresh Rainin® SR-L20F pipette tip to mix the remaining sample by pipetting up and down, being careful to not introduce bubbles.
7. Use the same pipette tip to carefully deposit the remainder of the sample into the loading port on the chip. Avoid introducing bubbles into the chip.
8. Remove the displaced liquid from the other port of the chip using the same pipette tip.
9. Centrifuge the chip for 4 minutes using the custom centrifuge adapter/rotor.
10. While the chip is spinning, enter the experimental configuration on the PGM™ screen.

11. Using the table below, determine the number of cycles you want to run based on the average base-pair read length. Press # to specify the number of cycles if it is different than the default (default = 65 cycles).

Application	Number of cycles	Average read length	Number of Runs per Initialization*	Run time
Small RNA (miRNA)	20 cycles	25 bp	5	~45 minutes
All other supported applications	65 cycles	100 bp	2	~2 hours
*Within a 10-hour time frame				

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

12. Ensure that **Autoanalysis** and **Pre Analysis** are selected.

Note: For barcoded libraries, select the appropriate barcode set on this screen. See "Appendix A. Sequencing barcoded libraries" for more information.

Load the chip on the PGM™ Sequencer

1. When the final spin is complete, press **Next** to verify the experimental setup. (You do not have to wait for the timer to finish counting down.) Press **OK** to confirm the settings or press **Cancel** to return to the Experimental Configuration Screen to adjust the configuration.
2. When prompted by the instrument, load and clamp the chip on the PGM™ Sequencer.

Ion 314™ Chip — Begin the experiment

1. Follow the touch screen prompts to begin calibrating the chip.
2. The instrument will automatically flush any loose ISPs from the chip and begin calibrating the chip. Be sure to check the chip for leaks before closing the lid.
3. When the calibration is complete (~1 minute), the PGM™ Sequencer will indicate whether calibration was successful.

   Note: If calibration fails, press **Abort**, reseat the chip, then press **Calibrate**. If the chip then passes calibration, press **Next** to proceed with the sequencing run. 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."

4. After 90 seconds, the run will automatically begin, or press **Next** to begin the run immediately.

CAUTION! During a run, avoid touching the dNTP Conical Tubes and wash bottles or lifting the chip tray lid. Touching these components while the instrument is sequencing may reduce the quality of the measurements.

5. When the run is complete, the PGM™ Sequencer will return to the Main Menu. You can then proceed with another run or perform a cleaning/initialization if required.

   Note: The instrument should be cleaned and initialized after 130 cycles (two runs for the standard 65-cycle workflow) or if the reagents have been on the instrument >10 hours.

Ion 316™ Chip Sequencing Protocol

Note: For a protocol using the Ion 314™ Chip, see page 15.

Ion 316™ Chip — Prepare the template-positive ISPs for sequencing

Materials provided in the kit

- Sequencing Primer (thawed on ice)
- Control Ion Spheres™
- Annealing Buffer

Other materials and equipment

- Enriched template-positive ISPs, prepared as described in the *Ion Template Kit User Guide v2.0* (Part no. 4469004)
- 0.2-mL PCR tube (non-polystyrene)
- Pipette tips and pipettor
- Vortex mixer
- Microcentrifuge
- Thermal cycler with heated lid, programmed at 95ºC for 2 minutes and 37ºC for 2 minutes

Before starting

- Use the template-positive ISPs that were enriched using the protocol provided in the *Ion Xpress™ Template Kit User Guide v2.0*.
- Thaw the Sequencing Primer on ice.

Using Ion Sphere™ Test Fragments for installation or troubleshooting

If you are performing an installation or troubleshooting sequencing run, skip directly to step 2 below and substitute **5 µL of Ion Sphere™ Test Fragments** from the Ion Control Material Kit (Part no. 4466465) for the 5 µL of Control Ion Spheres™ in the procedure.

Add Control Ion Spheres™ and anneal Sequencing Primer to the enriched ISPs.

1. Transfer **the entire volume** of enriched ISPs (prepared as described in the *Ion Xpress™ Template Kit User Guide v2.0*) to a new 0.2-mL PCR tube. Do not use polystyrene tubes.
2. Vortex the Control Ion Spheres™ and centrifuge for 2 seconds before taking aliquots.
3. Add 5 µL of Control Ion Spheres™ to the PCR tube containing the enriched particles. (**Note:** If you are using Ion Sphere™ Test Fragments for installation or troubleshooting, add the 5 µL of Test Fragments directly to the Annealing Buffer in the next step.)

   Note: 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.
4. Add 100 µL of Annealing Buffer. Mix by vortexing or pipetting up and down.
5. Centrifuge the tube for 2 minutes at 15,500 × g.
6. Carefully remove the supernatant with a pipette tip, leaving ~20 µL of supernatant in the bottom of the tube.
7. Add 150 µL of Annealing Buffer. Mix by vortexing or pipetting up and down.
8. Collect the ISPs by centrifuging the tube for 2 minutes at 15,500 × g.

9. Carefully remove the supernatant with a pipette tip, leaving slightly less than 15 µL of supernatant in the bottom of the tube. (Visually compare the volume to a separate tube containing 15 µL of liquid.)
10. Measure the volume of supernatant. If needed, use Annealing Buffer to adjust the volume up to 15 µL
11. Add 12 µL of Sequencing Primer to the ISP sample, then pipet the sample up and down.

IMPORTANT! Pipette thoroughly to make sure the pellet is completely disrupted before proceeding.

12. Program a thermal cycler for 95°C for 2 minutes and then 37°C for 2 minutes, using the heated lid option.
13. 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.

Ion 316™ Chip — Test a new Ion Chip (Chip Check)

Materials provided in the kit

- Annealing Buffer

Other materials and equipment

- New Ion 316™ Chip
- 100% isopropanol
- Rainin® SR-L200F pipette tips
- 1.5-mL microcentrifuge tube
- Barcode scanner (included with the PGM™ Sequencer)

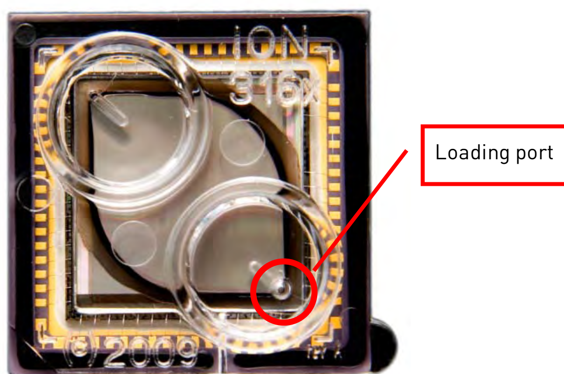
Handling Ion Chips

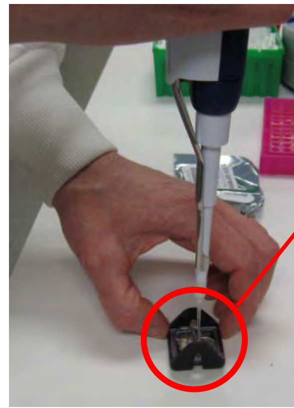
- To avoid damage due to electrostatic discharge (ESD), **do not place the chip directly on the bench or any other surface.** Always place the chip either on the grounding plate on the PGM™ Sequencer or in the custom Ion centrifuge adapter/rotor bucket.
- To avoid ESD damage, **do not wear gloves** when transferring chips onto and off the instrument.

Wash the chip

IMPORTANT! Use Rainin® SR-L200F pipette tips to enable slow, steady washing of the chip. Do not let the chip dry out.

IMPORTANT! When loading liquid into the chip, press the pipette tip firmly into the circular **loading port** on the chip (not into the slit), and keep the pipette tip at a **90° angle** to the chip. **Do not introduce bubbles into the chip.**

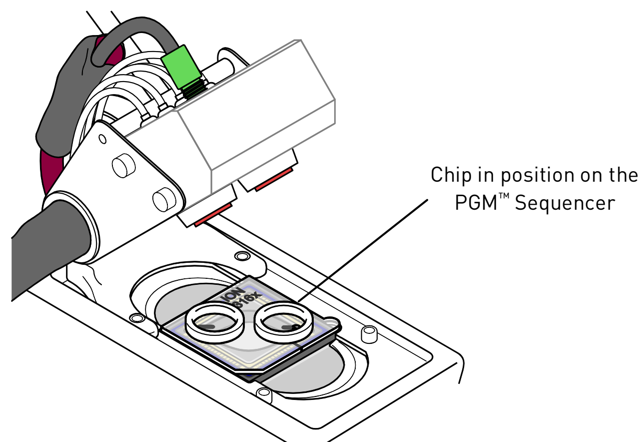




1. Remove a new chip from its packaging and label it to identify the experiment. Save the chip package to scan the barcode later.
2. Place the chip on the PGM™ Sequencer grounding plate or in the bucket of the Ion centrifuge adapter/rotor.
3. Using a Rainin® SR-L200F pipette tip, slowly and steadily pipet 100 µL of 100% isopropanol into the large port of the chip.
4. Aspirate any displaced solution from the other port. **Proceed immediately to the next step.**
5. Wash the chip **two times** with Annealing Buffer as follows:
   a. Slowly and steadily pipet 100 µL of Annealing Buffer into the large port on the chip.
   b. Aspirate any displaced solution from the other port.

Chip check on the instrument

1. Press **Experiment** on the main menu and follow the prompts to prepare the PGM™ Sequencer to test a new Ion Chip.
2. When prompted, ground yourself by touching the grounding pad next to the chip clamp on the instrument and replace the old chip with the new one for the experiment. **Do not wear gloves when transferring the chips on and off the instrument.**



3. Use the barcode scanner to scan the chip barcode located on the chip package.

   Note: To enter the barcode manually, press the **Change** button next to the barcode field. A chip cannot be run without scanning or entering the barcode.

4. After the barcode is entered, press the **Chip Check** button. Chip Check tests the chip and ensures that it is functioning properly prior to loading the sample.

5. After Chip Check is complete, press **Next** to proceed to calibration. The chip will be flushed with the solution from the Wash 2 bottle prior to calibration.
6. Check for leaks on the chip. If there is a leak, press the **Abort** button immediately to stop the flow to the chip. Then proceed to “Appendix B. Troubleshooting.”
7.

CAUTION! The chip socket can be damaged by rubbing or wiping its surface. When cleaning up spills, always gently dab the socket without rubbing.

8. After calibration is complete, the system indicates if the chip passed or failed:
   - If the chip passed, press **Next** and follow the prompts to remove the Ion Chip.
   - If the chip failed, open the chip clamp, re-seat the chip in the socket, close the clamp and repeat the Chip Check. 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.”
9. After removing the chip from the socket, use a Rainin® SR-L200F pipette tip to slowly and steadily pipet 100 µL of Annealing Buffer into the large port on the chip.
10. With the same pipette tip, aspirate any displaced solution from the other port.

Ion 316™ Chip — Load the ISPs on the chip and configure the experiment

Materials provided in the kit

- Sequencing Polymerase
- Annealing Buffer

Other materials and equipment

- 18-MΩ water
- 0.2-mL PCR tube (non-polystyrene)
- 1.5-mL microcentrifuge tube
- Rainin® SR-L200F pipette tips
- Sonication bath (40 KHz)
- Custom centrifuge adapter/rotor with chip-spinning bucket (included with the PGM™ Sequencer)
- Vortex mixer
- Microcentrifuge

Bind the Sequencing Polymerase to the ISPs

1. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 3 µL of Sequencing Polymerase to the ISPs. Pipet the sample up and down to mix.
2. Incubate the sample at room temperature for 5 minutes.
3. Add 30 µL of 50% Annealing Buffer/ 50% water mixture to sample, bringing the total to 60 µL.
4. Sonicate the ISPs for 10 seconds using a standard 40 KHz sonication bath.

   Note: Make sure the water level in the sonicator is filled to the appropriate mark.

Load the ISPs on the chip

IMPORTANT! To ensure successful loading of the ISPs on the chip:
- Press the pipette tip firmly into the circular loading port on the chip
- Keep the pipette tip at a 90º angle to the chip
- Load at a consistent speed by dialing down the pipettor
- Apply gentle pressure between the pipette tip and chip
- Do not suction the mixture back into the pipette tip

1. When loading the chip, place the chip on the grounding plate on the PGM™ Sequencer or remove the custom Ion centrifuge adapter/rotor bucket and place the chip in it.
2. Use a Rainin® SR-L200F pipette tip to carefully deposit 30 µL of the sample (~half of the sample) into the large port of the chip, *dialing down the pipettor to gently and slowly deposit the ISPs*. A good loading speed is ~1 µL/second. Stop the flow at the 3-µL setting. Avoid introducing bubbles into the chip.
3. Remove the displaced liquid from the other port of the chip using the same pipette tip.
4. Transfer the Ion Chip to the centrifuge (with the custom Ion centrifuge adapter/rotor). If preparing one Ion Chip at a time, always balance the centrifuge adapter with a used chip. Mark the used chip with a laboratory marker to differentiate it from the new chip for the current experiment.

   Note: For the following steps, ignore the 10-minute timer on the PGM™ System screen. Centrifuge for **4 minutes**.
5. Centrifuge the chip for 4 minutes using the custom centrifuge adapter/rotor.
6. After the first 4-minute spin, use a Rainin® SR-L200F pipette tip to mix the remaining sample by pipetting up and down, being careful to not introduce bubbles.
7. Use the same pipette tip to carefully deposit the remainder of the sample into the large port of the chip, dialing down the pipettor to gently and slowly deposit the ISPs. A good loading speed is ~1 µL/second. Stop the flow at the 3-µL setting. Avoid introducing bubbles into the chip.
8. Remove the displaced liquid from the other port of the chip using the same pipette tip
9. Centrifuge the chip for 4 minutes using the custom centrifuge adapter/rotor.
10. While the chip is spinning, enter the experimental configuration on the PGM™ screen.
11. Using the table below, determine the number of cycles you want to run based on the average base-pair read length. Press # to specify the number of cycles if it is different than the default (default = 65 cycles).

Application	Number of cycles	Average read length	Number of Runs per Initialization*	Run time
Small RNA (miRNA)	20 cycles	25 bp	5	~45 minutes
All other supported applications	65 cycles	100 bp	2	~2 hours

*Within a 10-hour time frame

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

12. Ensure that **Autoanalysis** and **Pre Analysis** are selected.

   Note: For barcoded libraries, select the appropriate barcode set on this screen. See "Appendix A. Sequencing barcoded libraries" for more information.

Load the chip on the PGM™ Sequencer

1. When the final spin is complete, press **Next** to verify the experimental setup. (You do not have to wait for the timer to finish counting down.) Press **OK** to confirm the settings or press **Cancel** to return to the Experimental Configuration Screen to adjust the configuration.
2. When prompted by the instrument, load and clamp the chip on the PGM™ Sequencer.

Ion 316™ Chip — Begin the experiment

1. Follow the touch screen prompts to begin calibrating the chip.
2. The instrument will automatically flush any loose ISPs from the chip and begin calibrating the chip. Be sure to check the chip for leaks before closing the lid.
3. When the calibration is complete (~1 minute), the PGM™ Sequencer will indicate whether calibration was successful.

   Note: If calibration fails, press **Abort**, reseat the chip, then press **Calibrate**. If the chip then passes calibration, press **Next** to proceed with the sequencing run. If the chip still fails, proceed with the run anyway and contact Technical Support after the run is complete. See also ""Appendix B. Troubleshooting."

4. After 90 seconds, the run automatically begins, or press **Next** to begin the run immediately.

   CAUTION! During a run, avoid touching the dNTP Conical Tubes and wash bottles or lifting the chip tray lid. Touching these components while the instrument is sequencing may reduce the quality of the measurements.

5. When the run is complete, the PGM™ Sequencer will return to the Main Menu. You can then proceed with another run or perform a cleaning/initialization if required.

   Note: The instrument should be cleaned and initialized after 130 cycles (two runs for the standard 65-cycle workflow) or if the reagents have been on the instrument >10 hours.

Appendix A. Sequencing barcoded libraries

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

Pre-installed barcode sets

Ion Torrent provides customers with the pre-installed barcode set **IonSet1** on the Torrent Server (Release 1.4 or later). This set contains all 16 barcodes in the Ion DNA Barcoding 1–16 Kit (Part no. 4468654), and is viewable using the Torrent Browser:

Barcodes in ionSet1
Sequence
CGTGTCGCAC
GATGATTGCC
CGATAATCTT
CTTACACCAC
AGCCAAGTAC
GACATTACTT
GCCTTACCGC
ACCGAGGCAC
GCAAGCCTTC
ACATTACATC
CAAGCACCGC
AGCTTACCGC
CATGATCAAC
GACCGCATCC
GGTGTAGCAC
ACTCACGATA

Custom barcode sets

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

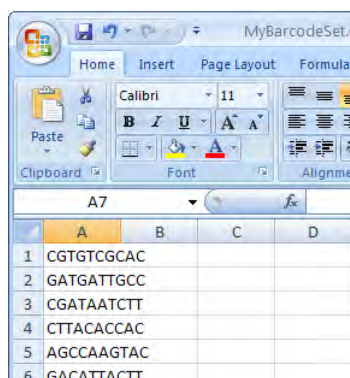
Note: To access the Torrent Server, you must have a username and password.

Note: For more information on working with custom barcode sets, refer to the *Torrent Browser User Interface Guide.* On the Torrent Browser, click the **References** tab, then click **Working with Barcodes.**

Create a custom barcode set

1. Using Microsoft® Excel®, Notepad, or similar software, create a custom barcode set with each barcode sequence listed on a separate line in a single-column format, as shown below. A barcode set list can contain up to 384 barcodes.

   Note: You can run fewer than 384 barcodes in a sequencing run; the PGM™ Sequencer will automatically detect and select the barcodes used in the run from the selected set.



2. Save the file as a **.csv** file and transfer the file to the Torrent Server as described below.

Add a custom barcode set to the Torrent Server

1. To add the custom barcode set to the Torrent Server, go to the Torrent Browser and click on the **References** tab.
2. In the Barcodes panel, click **Add** to display the **Add new DNA barcodes** window.
3. Enter the barcode set name in the edit window.
4. Click **Browse** to select the file you created.
5. Click **Upload & Save**. The barcode set file name will be displayed in the Barcode panel.

View a barcode set

1. To view a barcode set, go to the Torrent Browser and click on the **References** tab.
2. In the **Barcodes** panel, click on the barcode set name to display it.

Delete a barcode set from the Torrent Server

1. To view the barcode set names, go to the Torrent Browser and click the **References** tab.
2. In the Barcodes panel, click the name of the barcode set that you want to delete. The Torrent Browser will display the barcode set.
3. At the bottom of the page, click **Delete Barcode Set**. A dialog will prompt you to confirm the deletion.

Add a barcode to a set

1. Open the Torrent Browser and click the **References** tab.
2. In the Barcodes panel, click the file name of the barcode set to be edited.
3. Enter the new barcode sequence in the edit window, then click **Add Barcode**. For example:

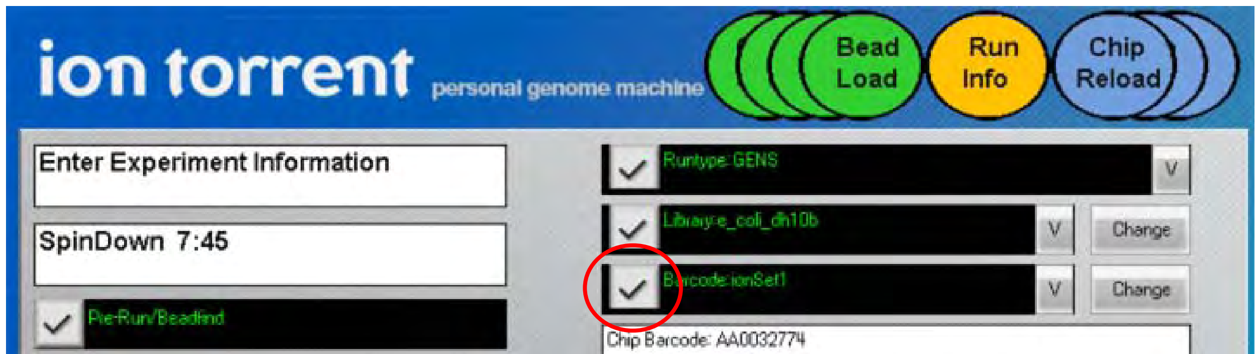
Delete a barcode from a set

1. Open the Torrent Browser and click on the **References** tab.
2. In the Barcodes panel, click the file name of the barcode set to be edited.
3. Click the **Delete** button next to the barcode sequence to be deleted. You will be prompted to confirm the deletion.

Selecting a barcode set on the PGM™ Sequencer

1. During the experimental setup of a sequencing run, follow the touch screen prompts to the **Run Info** screen.
2. On the **Run Info** screen, select the appropriate reference library from the library reference drop-down menu.
3. Click the barcode drop-down menu and select the barcode set containing the barcodes used in the sequencing run, as shown below.

   Note: A barcode set can contain up to 384 barcodes. The barcodes used in the run may be a subset of the selected barcode set, as long as the set contains all of the barcodes used in the run.



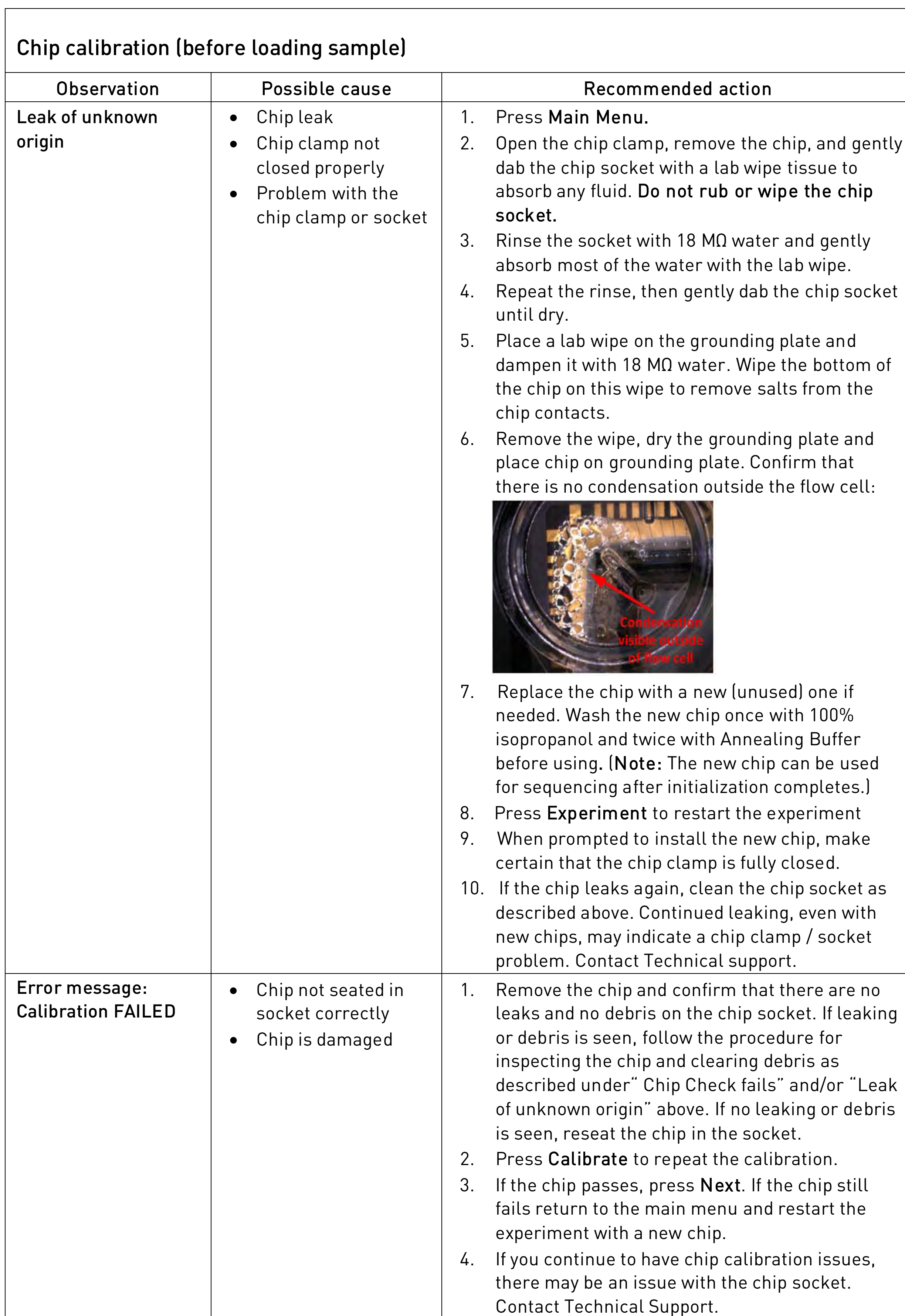
4. Enter the remaining information as needed on the **Run Info** screen.
5. Click **Next** and follow the remaining prompts to start the run.

View a report of the barcoded library sequencing run

1. To view a report of the barcoded library sequencing run, go to the Torrent Browser and click the **Reports** tab.
2. Click the report with the name of the selected barcode set.

Appendix B. Troubleshooting

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 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 (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. **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. 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. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer before using. (**Note:** The new chip can be used for sequencing after initialization completes.) 8. Press **Experiment** 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 / 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 are no leaks and no 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.

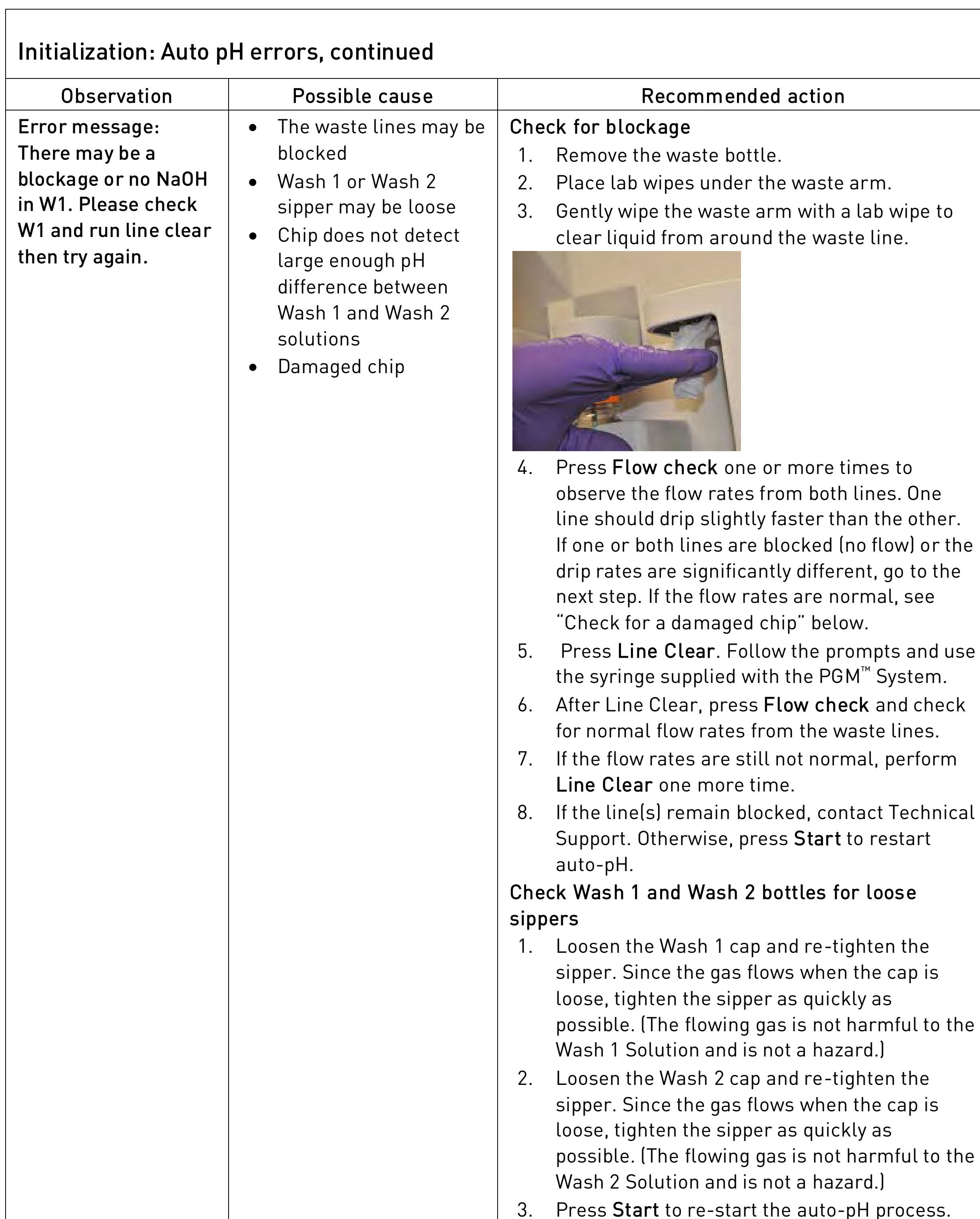
Initialization: General

Observation	Possible cause	Recommended action
Error message: Confirm instrument has gas pressure	Argon cylinder may be turned off or empty	1. Verify that the argon cylinder has at least 500 PSI and 40 PSI at the outlet of the regulator. Confirm that all valves between the cylinder and PGM are open. 2. Once you confirm argon 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. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer before using. (**Note:** The new chip can be used for sequencing after initialization completes.) 5. Close the clamp, then press **Start** to restart the process. 6. If the new chip also fails, there could be a problem with the chip socket. Contact Technical Support.
Error message: Chip calibration failed	• Chip not seated in socket correctly • Damaged chip	Follow the procedure for "Error message: Please insert a chip and press Start"

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 Wash 1 and Wash 2 solutions • 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 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 sippers** 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 flowing gas is not harmful to the Wash 1 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 flowing gas is not harmful to the Wash 2 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. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer, 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 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. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer before using. (**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 flowing gas is not harmful to the Wash 2 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 Wash 2 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 / 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. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer, 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 • Too much W2 concentrate added to prepare the W2 Solution • Incorrect solution added to the W2 Solution • Too little NaOH added to Wash 1 bottle • Damaged chip	1. Confirm high water quality and correct preparation of the 100 mM NaOH and W2 Solution. 2. If solution preparation is incorrect or water quality is poor, correctly prepare the solution(s) and/or use high-quality water. 3. Clean the instrument. 4. Repeat instrument initialization with fresh reagents and a new (unused) chip. (**Note:** The new chip can be used for sequencing after initialization completes.) **Note:** Once the system has added too much NaOH, the only recourse is to clean the PGM and restart initialization or manually pH the Wash 2 solution (see "Appendix C. Manually adjust the pH of the Wash 2 solution").
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.)

Initialization: Auto pH errors, continued		
Observation	**Possible cause**	**Recommended action**
Error message: OVERSHOT TARGET PH: W2 pH = n.nn Failed	Auto-pH added more Wash 1 to the Wash 2 bottle than was needed, and reports the pH value	1. Open the bottle of Wash 2 Solution just enough to pipette ~10 µL of 100 mM HCl for every 0.1 pH unit >7.5 into the bottle. **Note:** Argon will be flowing out of the cap while doing this—this is not a hazard and will not cause any issues. 2. Press **Start** to re-start auto-pH. If the pH is still high, replace the chip with a new (unused) one. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer, 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 Wash 2 Solution (see "Appendix C. Manually adjust the pH of the Wash 2 solution").

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, something likely went wrong with the initialization and you should 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. Wash the new chip once with 100% isopropanol and twice with Annealing Buffer before using. (**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, there may be a problem with the chip socket. Contact Technical Support.

Appendix C. Manually adjust the pH of the Wash 2 solution

Materials and equipment needed

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

Procedure

1. Before proceeding, rinse an empty Wash 2 bottle and have it ready next to the instrument.
2. Remove and cap the Wash 2 bottle attached to the instrument. Gas will be flowing out of the sipper in the W2 position.

   Note: The gas will be flowing out of the Wash 2 cap, so work as quickly as possible (flowing gas will not harm the Wash 2 solution, and is not a hazard).

3. Secure the empty Wash 2 bottle (from step 1) to the instrument—do not remove the sipper. This bottle will keep gas from flowing out of the instrument while you pH the Wash 2 solution and protect the sipper from contamination.
4. Move the Wash 2 solution to the stir plate near the argon gas tube.
5. Secure the argon gas tube so that it extends inside the mouth of the bottle but not below the surface of the water.
6. Set the argon flow to 0.5 LPM. Start mixing the water 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. Using the calibrated pH meter, adjust the pH of the Wash 2 bottle to 7.5 ± 0.1 by adding freshly prepared 100 mM NaOH. Add small aliquots and allow the pH to equilibrate before adding more.

   Note: If the pH rises above 7.6, use 100 mM hydrochloric acid (HCl) to readjust the pH to 7.5 ± 0.1.

9. When the pH is stable, turn off the argon, 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.

Appendix D. Safety

Safety Information

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 the "Documentation and Support" section in this document.

Chemical safety

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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

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

Biological hazard safety

WARNING! **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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the information below:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at:
  www.cdc.gov/biosafety
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Appendix E. Documentation and Support

Obtaining SDSs

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

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

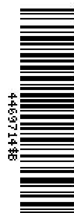
Obtaining support

For the latest services and support information for all locations, go to:

www.iontorrent.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, application notes, and other product support documents



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