

# Ion PGM™ Hi-Q™ Chef Kit

Catalog Number A25948

Pub. No. MAN0010920 Rev. C.0

**Note:** For safety and biohazard guidelines, see the “Safety” appendix in the *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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## Create a Planned Run

Create a Planned Run in the following software programs for use on the following sequencing systems. In the software, be sure to specify the appropriate library, template, and sequencing kits.

Software	Instrument System
Torrent Suite™ Software, v5.0 or later	Ion PGM™ System
Torrent Suite™ Assay Development Software <sup>[1]</sup> , v5.0 or later	Ion PGM™ Dx System

<sup>[1]</sup> Torrent Suite™ Assay Development Software is For Research Use Only on the Ion PGM™ Dx System. Not for use in diagnostic procedures.

For more information, see the software user documentation, or see:

- *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919) or
- *Ion PGM™ Hi-Q™ Chef Kit – Assay Development Mode User Guide* (Pub. No. MAN0015833)

## Dilute the sample libraries

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

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

<sup>[1]</sup> Library length is average insert length plus adapter length.

## Dilute Ion AmpliSeq™ for Chef combined libraries

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

## Prepare the libraries and consumables

1. At least 45 minutes before use, unbox the Ion PGM™ Hi-Q™ Chef Reagents cartridge and allow it to warm to room temperature.

**IMPORTANT!** The Ion PGM™ Hi-Q™ Chef Reagents cartridge must sit at room temperature for at least 45 minutes before use.

2. Pipet 25 µL of each diluted library to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).

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

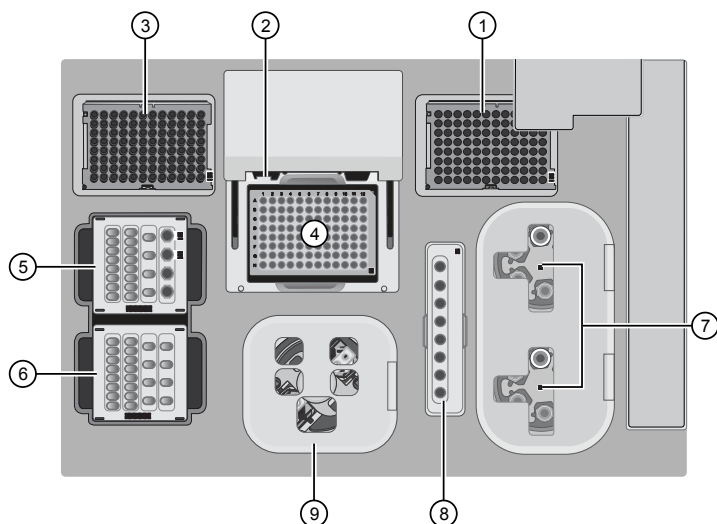
3. Cap, then store the two Library Sample Tubes on ice until you are ready to load them onto the Ion Chef™ Instrument.
4. Remove all cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

## Load the Ion Chef™ System

### IMPORTANT!


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

Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure:



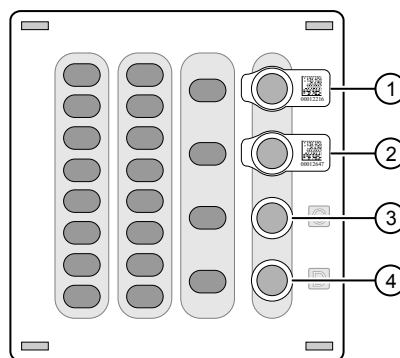
- Empty tip rack (move from new Tip Cartridge position)
- Frame Seal v2
- New Tip Cartridge v2
- PCR plate
- Ion PGM™ Hi-Q™ Chef Reagents cartridge
- Ion PGM™ Hi-Q™ Chef Solutions cartridge
- Recovery Tubes and Recovery Station Disposable Lid v2
- Enrichment Cartridge v2
- Chip Adapter/Chip assemblies

## Load the pipette tip racks and PCR plate

- Touch  (Open Door) in the instrument touchscreen to open the instrument door, then wait for the latch to open.
- Lift the instrument door to the top of the travel until the latch mechanism engages.
- Load an empty pipette tip rack to the *Used (Waste) Pipette Tip Position*, then change gloves.
- Unwrap a new Tip Cartridge v2 and remove the cover to expose the pipette tips, then load it in the *New Pipette Tip Position*.
- Pull the catch forward, then pivot the locking bracket upwards. Load the assembled Tip Cartridge v2 into the *New Pipette Tip Position*, then pull the bracket downwards, then push the catch backwards to lock the cartridge in place.
- Load a new PCR plate into the thermal cycler sample block, then slide a new Frame Seal v2 under the automated heated cover.

## Load the Reagents and Solutions cartridges

- Gently tap the Ion PGM™ Hi-Q™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
- Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.
- Uncap, then load the two Library Sample Tubes, each containing 25  $\mu$ L of diluted library, into Positions A and B on the Reagents cartridge.



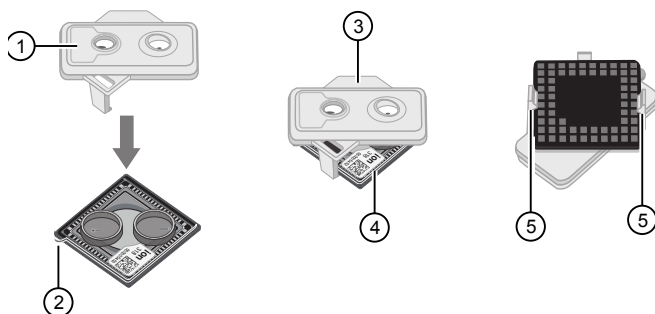
- Position A (Library)
  - Position B (Library)
  - Position C (NaOH)
  - Position D (Empty tube)
- Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.
  - Gently tap the Ion PGM™ Hi-Q™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.
  - Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.

## Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes (v2) into each Recovery centrifuge.
2. Place a Recovery Station Disposable Lid v2 over each centrifuge with the port oriented toward the rear of the instrument, then press down. Make certain the lids snap completely into place by applying firm downward pressure along the lid perimeter.
3. Close the hinged cover of the Recovery centrifuges.
4. Load the Enrichment Cartridge v2, then press down on the cartridge to ensure that it is level with the instrument deck.

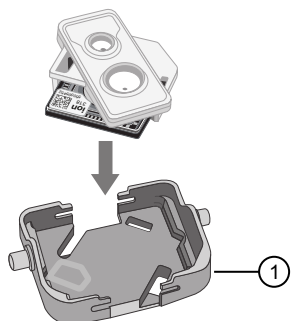
## Load the Chip-loading centrifuge

1. Attach a Chip Adapter v2 to each chip.



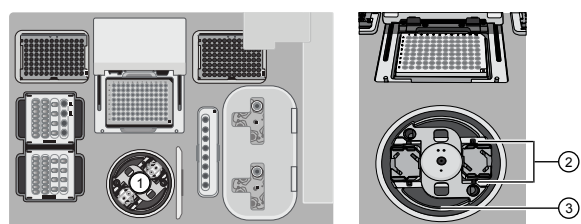
- ① Reservoir
- ② Tab
- ③ Chip Adapter
- ④ Ion chip
- ⑤ Clip

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



- ① Chip-loading centrifuge bucket

3. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.



- ① Chip-loading centrifuge
- ② Mounting grooves
- ③ Chip-loading centrifuge

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

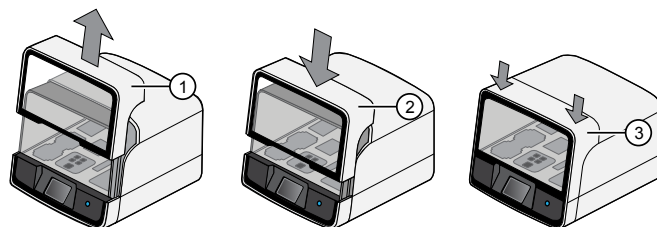
## Confirm that consumables are correctly installed

Before continuing:

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


## Start the Ion Chef™ run

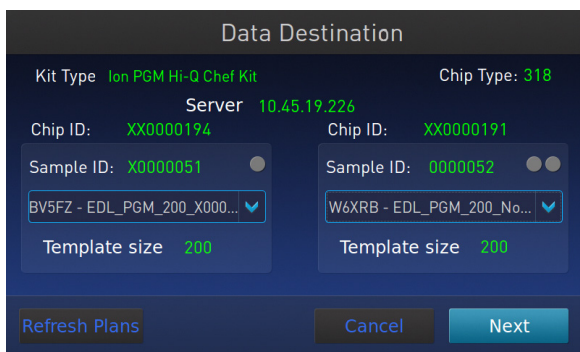
1. Ensure that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, touch **Set up run**.
3. Touch **Step by Step** to have the instrument lead you through the instrument setup, or touch **Quick Start** to skip the instrument setup screens.
4. Follow the on-screen instructions. When prompted, close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage. After the door closes, the instrument vision system activates.



- ① Lift door first
- ② Lower
- ③ Press down to lock

5. When prompted, touch **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility.
6. When Deck Scan is complete, touch **Next** to display the Data Destination screen.

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



- On the Run Options screen, touch the appropriate option to complete the run, then enter the desired time of run completion, if needed.
- On the Run Options screen, touch **Start run** to start the run.
- Clean and initialize the Ion PGM™ Sequencer approximately 1.5 hours before the Ion Chef™ System finishes chip loading.
- If you chose to pause the run to analyze the templating efficiency, remove the samples for testing when prompted to do so by the Ion Chef™ Instrument (approximately 9.5 hours and 13 hours for 200- and 400-bp runs, respectively, after the start of the run).
  - When prompted to remove the QC sample, open the instrument door.
  - Transfer the QC samples (entire volume) from Positions A and B of the Ion PGM™ Hi-Q™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.
  - Analyze the QC samples.
  - Close the instrument door, then touch **Continue** to complete the run.
- When the run is complete, unload the Ion Chef™ Instrument and sequence the chips immediately.

**Note:** If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes. See Appendix B, "Supplemental procedures", of the *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919) for more information.

## Clean and initialize the Ion PGM™ Sequencer

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

**IMPORTANT!** Use only the specified materials and follow the protocols found in this document. The Ion Chef™ Instrument cleaning and initialization procedures described here are similar to that of other Ion sequencing kits, but the materials and protocols are not identical. Do not substitute reagents from other kits.

## Condition the Wash 2 Bottle for first use

New Wash 2 Bottles must be conditioned with Wash 2 Bottle Conditioning Solution for at least 8 hours before first use.

To condition the Wash 2 Bottle:

- Fill the bottle to the mold line with 18 MΩ water, add the entire container of Wash 2 Bottle Conditioning Solution, then cap the bottle and invert it 5 times to mix.
- Allow the bottle to sit at room temperature for at least 8 hours and preferably overnight, then dispose of the contents. The bottle is now ready for use.

## Clean the Ion PGM™ System

Cleaning schedule

Clean with	Schedule
18 MΩ water	<ul style="list-style-type: none"> <li>Daily, when instrument is in use</li> <li>After one or more runs totaling ≤1,100 flows</li> <li>If more than 27 hours but less than 48 hours have elapsed between the last cleaning/initialization and the start of a run</li> <li>If you cleaned with chlorite a week ago and have not used the instrument since then</li> </ul>
Chlorite solution	<ul style="list-style-type: none"> <li>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)</li> <li>If the instrument has been left with reagents for more than 48 hours</li> </ul>

Cleaning setup

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

- Remove any wash and reagent bottles that are attached to the Ion PGM™ System before cleaning.
- Do not remove old sippers before cleaning. The sippers are used as part of the cleaning procedure.
- Old chips that have been used for sequencing can be marked and used in the cleaning procedure.
- Wash bottles (250 mL and 2 L) provided as part of instrument installation can be marked and used for cleaning. After you have used the wash bottles provided with the sequencing kit for the specified number of runs, you can use them as extra cleaning bottles. Mark them for cleaning use only.

## 18 MΩ water cleaning

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Press **Clean** on the touchscreen, and select the **18-MO $\Omega$  water cleaning** checkbox. Press **Next**.
3. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

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**IMPORTANT!** Always make sure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.

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4. Remove all wash and reagent bottles attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
5. Add 250 mL of 18 MΩ water to an empty 250-mL cleaning bottle.
6. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
7. Attach the 250-mL bottle containing 18 MΩ water to the W1 position, ensuring that the W1 cap is screwed on tightly. Press **Next**.
8. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, and insert the sippers into the bottles. Do not screw on the caps.
9. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to begin cleaning.
10. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu and proceed to initialization.

## Chlorite cleaning

1. Empty any remaining solution from each cleaning bottle (two 250-mL bottles and one 2-L bottle), then rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water, then add an Ion Cleaning tablet (chlorite tablet). Allow the tablet to dissolve completely (~10 minutes).
3. When the tablet has dissolved, add 1 mL of 1 M NaOH and filter the solution using a 0.22- $\mu$ m or 0.45- $\mu$ m filter. Use the chlorite solution within 2–3 hours. Discard any unused solution after this time.
4. Press **Clean** on the touchscreen, then select the **Chlorite cleaning** checkbox. Press **Next**.

5. Using ungloved hands, secure a used chip designated for cleaning in the chip clamp.

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**IMPORTANT!** Always ensure that both red rubber gasket port fittings are securely in place when securing chips with the chip clamp. Failure to do so can result in a spill hazard and instrument damage.

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6. Remove all wash and reagent bottles that are attached to the instrument. Keep the sippers in place at all positions. Press **Next**.
7. Add 250 mL of the filtered chlorite solution to an empty 250-mL cleaning bottle.
8. Rinse the outside of the sipper tube in the W1 position on the instrument with a squirt bottle containing 18 MΩ water.
9. Attach the 250-mL bottle with the filtered chlorite solution to the W1 position. Ensure that the W1 cap is tight. Press **Next**.
10. Place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position, then insert the sippers into the bottles. Do not screw on the caps.
11. Place collection trays below the reagent sippers in the dNTP positions. Press **Next** to start cleaning.
12. When prompted, remove the bottle containing the chlorite solution from the W1 position.
13. Rinse the outside of the W1 sipper tube with a squirt bottle containing 18 MΩ water.
14. Fill a clean 250-mL bottle with 250 mL of 18 MΩ water, then attach the bottle in the W1 position. Ensure the cap is tight. Press **Next** to start the water rinse.
15. When cleaning is complete, remove the bottles and sippers from the W1, W2 and W3 positions. Leave the reagent sippers and collection trays in place. Press **Next** to return to the main menu, then proceed to initialization.

## Initialize the Ion PGM™ System

### Initialization guidelines

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**IMPORTANT!** Handle nucleotides carefully to avoid cross-contamination. Always change gloves after removing used sipper tubes from the Ion PGM™ System to avoid cross contamination of the nucleotides. Also change gloves after handling concentrated dNTP stocks.

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For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 24 hours after initialization.

### Bottle usage

- Wash 2 Bottles can be used for up to 40 initializations, after which you can use them in the cleaning procedure.
- Wash 1 and Wash 3 Bottles can be used for up to 4 initializations, after which you can reuse them in the cleaning procedure.

- Replace the Reagent Bottles and sipper tubes every time you initialize.

#### Before initialization

1. Remove the dNTP stock solutions from the freezer and begin thawing on ice.
2. Check the tank pressure for the nitrogen gas. When the tank pressure drops below 500 psi, change the tank.

#### Prepare the Wash 2 Bottle

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**IMPORTANT!** Do not let the new sippers touch any surfaces.

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1. Rinse the Wash 2 Bottle (2 L) 3 times with 200 mL of 18 MΩ water.
2. Prepare 500 μL of 100 mM NaOH by diluting 50 μL of 1 M NaOH in 450 μL of nuclease-free water.
3. If your 18 MΩ water system has a spigot, extend it into **but not below** the neck of the Wash 2 Bottle. Otherwise, position the nozzle as close to the mouth of the bottle as possible.
4. Fill the bottle to the mold line with 18 MΩ water. The volume of water is ~2 liters. (You can mark the mold line on the bottle for clarity.)
5. Add the entire bottle of Ion PGM™ Hi-Q™ Sequencing W2 Solution to the Wash 2 Bottle.
6. Using a P200 pipette, add 70 μL of 100 mM NaOH to the Wash 2 Bottle.
7. Cap the bottle and invert 5 times to mix, and immediately proceed through the remainder of the initialization procedure.

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**IMPORTANT!** Do not store the mixed Wash 2 Bottle.

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#### Prepare the Wash 1 and Wash 3 Bottles

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

#### Begin the initialization

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**IMPORTANT!** Do not let the new sipper tubes touch any surfaces.

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1. On the main menu, press **Initialize**.
2. Make the following selections in the next screen, then press **Next**:
  - Click **Enter barcode** to scan or enter the barcode on the Ion PGM™ Hi-Q™ Sequencing W2 Solution bottle, or the 2D barcode on the Ion PGM™ Hi-Q™ Sequencing Solutions box.
  - Alternatively, select the checkbox for the **Ion PGM™ Hi-Q™ Sequencing dNTPs** from the dropdown list.
  - In the same screen, if you routinely experience clogging during initialization, select the **Line Clear** checkbox to clear any blockage in the fluid lines before initialization. This is optional.

After you press **Next**, the system will check the gas pressure.

3. Following the gas pressure check:

Result	Action
<b>If the pressure is sufficient</b>	Ensure that the cleaning chip, reagent sipper tubes, and collection trays are in place, and press <b>Next</b> to start the initialization.
<b>If the pressure is low</b>	Press <b>Yes</b> to re-check the pressure. If the pressure remains low, contact Technical Support.

4. Wearing clean gloves, firmly attach a new, long gray sipper to the cap in the W2 position.
5. Immediately attach the prepared Wash 2 Bottle in the W2 position, then tighten the cap. Press **Next**.
6. Change gloves and firmly install new sipper tubes (short gray) in the caps in the W1 and W3 positions.
7. Immediately attach the prepared Wash 1 and 3 Bottles, then tighten the caps. Press **Next**.
8. Following line clear, or if you did not select that option, the sequencer begins adjusting the pH of the W2 Solution, which takes ~30 minutes. After 15 minutes, check the instrument touchscreen to confirm that initialization is proceeding normally.

#### Prepare the 50-mL Reagent Bottles with dNTPs

1. Use the labels provided with the kit to label four new Reagent Bottles as dGTP, dCTP, dATP, and dTTP.
2. Confirm that no ice crystals are visible in each thawed dNTP stock solution. Vortex each tube to mix, and centrifuge to collect the contents. Keep the dNTP stock solutions on ice throughout this procedure.

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**IMPORTANT!** To avoid cross-contamination in the next step, open only one dNTP stock tube at a time and use a fresh pipette tip for each aliquot.

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
- Using separate filtered pipette tips and clean gloves, carefully transfer 20  $\mu$ L of each dNTP stock solution into its respective Reagent Bottle.
- Cap each Reagent Bottle and store on ice until you are ready to attach it to the instrument. Place the remaining dNTP stocks back into  $-20^{\circ}\text{C}$  for storage.

#### Attach the sipper tubes and Reagent Bottles

- 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, then firmly insert a new sipper tube (blue) into each dNTP port. Do not let the sipper touch any surfaces.
- Attach each prepared Reagent Bottle to the correct dNTP port (e.g., the dGTP tube on the port marked "G") and tighten firmly by hand until snug. Press **Next**.
- Follow the touchscreen prompts to complete initialization. The instrument will fill each Reagent Bottle with 40 mL of W2 Solution.
- At the end of initialization, Ion PGM™ System will measure the pH of the reagents:
  - If every reagent is in the target pH range, a green **Passed** screen will be displayed.
  - If a red failure screen appears, see the troubleshooting section of the user guide.
- Press **Next** to finish the initialization process and return to the main menu.
- Proceed to the appropriate sequencing protocol for your chip type.

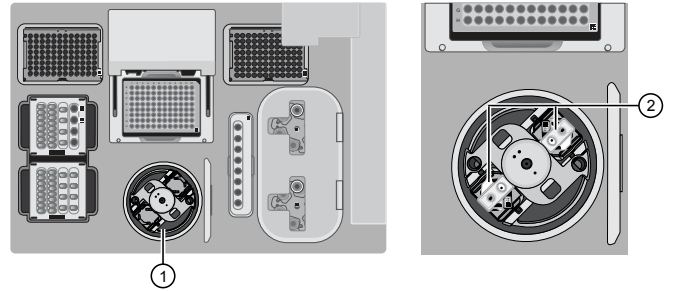
## Start the sequencing run

### Unload and prepare the chips for sequencing

- Open the instrument door:
  - In the instrument touchscreen, touch  (Open Door) then wait for the latch to open.
  - Lift the instrument door to the top of the travel until the latch mechanism engages.

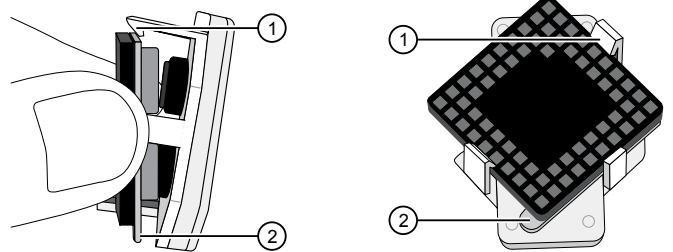
- Open the lid of the Chip-loading centrifuge, then gently remove the chip/adapter assemblies from the instrument.

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



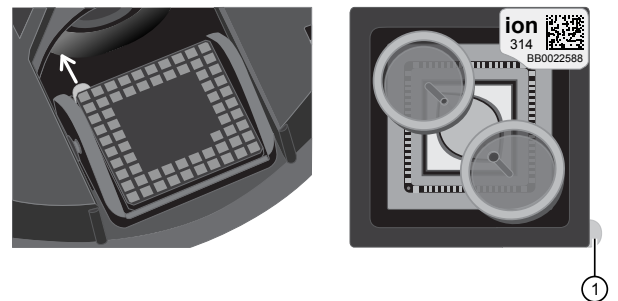
- Chip-loading centrifuge
- Remove chip/adapter assemblies

- Remove the Chip Adapters from the chips, then discard the Chip Adapters. To remove an adapter, hold the chip with the tab facing downward, then remove the Chip Adapters starting with the top clip first.



- Upper clip (remove first)
- Tab (orient down)

- For Ion 314™ v2 BC Chips only: use an Ion Chip™ Minifuge to remove the remaining liquid from the Ion 314™ v2 BC Chips. This step is not required for Ion 316™ or Ion 318™ v2 BC Chips.
  - Load both chips into the Ion Chip™ Minifuge. Place each chip upside-down in a bucket so that the tab faces inwards, toward the center.



- Tab (orient inward)

- Close the lid, then centrifuge the chips for 5 seconds.
  - When the centrifuge stops, remove the chips, then use a lint-free wipe to remove any liquid from the buckets.
- Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.

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

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

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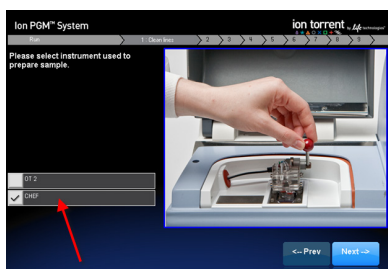
**IMPORTANT!** If you chose to store a loaded chip, remove the chip from the container and place it on a clean surface in the dark to warm to room temperature at least 20 minutes before running it.

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## Sequence the Ion chips on the Ion PGM™ Sequencer

Sequence the loaded Ion chips on the Ion PGM™ Sequencer as soon as possible after unloading the Ion Chef™ Instrument.

- Touch **Run** on the main menu, then follow the on-screen instructions to empty the waste bottle, load the cleaning chip, and clean the Ion PGM™ Sequencer fluid lines.
- When the following screen appears, touch **CHEF** to select the instrument used to prepare the sample and initiate the Chef sequencing workflow. Then touch **Next**.



- Scan the barcode on the loaded chip, or press **Change** to enter the barcode manually.
- When prompted by the instrument, ground yourself by touching the grounding plate next to the chip clamp on the instrument, replace the cleaning chip in the chip socket with the chip to be sequenced, close the chip clamp, then touch **Next**.

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

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- Touch **Chip Check** to perform the first chip check.
- After the instrument successfully completes the chip check, follow the on-screen instructions to empty the waste bottle, then touch **Next**.

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

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- When prompted to select a Planned Run, confirm that the correct run is displayed, then touch **Next**.

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

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

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

- Twenty minutes before the end of the first run, remove the remaining Ion chip from the chip container in the refrigerator, then place it on a clean surface to warm to room temperature.
- When first run is complete, sequence the remaining chip as soon as possible. Perform a cleaning and/or initialization if needed.

## Clean the Ion Chef™ Instrument

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

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### Remove and dispose of used consumables

- Touch **⏏** (Open Door) in the instrument touchscreen, then wait for the latch to open.
- Lift the instrument door to the top of the travel until the latch mechanism engages.
- Remove, then discard the PCR plate from the thermal cycler sample block.
- Remove, then discard the box of used pipette tips from the waste tip position.
- Move the empty Tip Cartridge v2 to the waste tip position.
- Remove, then discard the
  - Ion PGM™ Hi-Q™ Chef Reagents cartridge
  - Ion PGM™ Hi-Q™ Chef Solutions cartridge
  - Enrichment Cartridge v2
- Remove, then discard the consumables from the Recovery centrifuges, including the:
  - Recovery Station Disposable Lids v2
  - Recovery Tubes v2
- Close the Chip-loading centrifuge cover.



## Inspect and clean the Recovery centrifuges and buckets

1. Inspect the Recovery centrifuge for residue. If excessive liquid is present, clean the centrifuge bowl and buckets as described in the *Ion PGM™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010919).
2. Close the Recovery centrifuge cover.

## Start the cleaning

1. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.
2. To start the cleaning, touch **Next** on the Ion Chef™ Instrument touchscreen that appears after run completion.
3. Confirm that you have removed all consumables from the Ion Chef™ Instrument, then touch **Next**.
4. With the door closed, touch **Start**. The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation, then illuminates the ultraviolet (UV) light in the instrument for ~1 minute.



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

## Kits, consumables, and accessories

Catalog or Part No.	Description
A25948	Ion PGM™ Hi-Q™ Chef Kit, 8-reactions
4488374	Ion Chef™ Installation Kit
A27293	Ion PGM™ Hi-Q™ Chef 400 Supplies Kit <sup>[1]</sup>
4482010	Ion PGM™ Controls Kit v2
A27832	Ion PGM™ Calibration Standard
A25591	Ion PGM™ Wash 2 Bottle Kit
4488144	Ion 314™ Chip Kit v2 BC, 8-pack
4488145	Ion 316™ Chip Kit v2 BC, 4-pack
4488149	Ion 316™ Chip Kit v2 BC, 8-pack
4488146	Ion 318™ Chip Kit v2 BC, 4-pack
4488150	Ion 318™ Chip Kit v2 BC, 8-pack

<sup>[1]</sup> Enables eight initializations of the Ion PGM™ Sequencer for eight 400 bp sequencing runs when used with the full Ion PGM™ Hi-Q™ Chef Kit.

## Limited product warranty

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

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**Revision history:** MAN0010920

Revision	Date	Description
C.0	8 January 2017	<ul style="list-style-type: none"><li>• "Load the Ion Chef™ System" and "Clean the Ion Chef™ Instrument" topics reorganized for ease of use.</li><li>• Web links updated.</li></ul>
B.0	28 January 2016	<ul style="list-style-type: none"><li>• Guidance added for use with Torrent Suite™ Assay Development Software</li><li>• Library input recommendations added for Ion AmpliSeq™ for Chef DL8 Kit users.</li><li>• Clarifications made in the sequencing workflow, including use of the Line Clear feature in initialization.</li></ul>
A.0	6 February 2015	New Quick Reference

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