Ion PGM™ Sequencing 200 Kit v2

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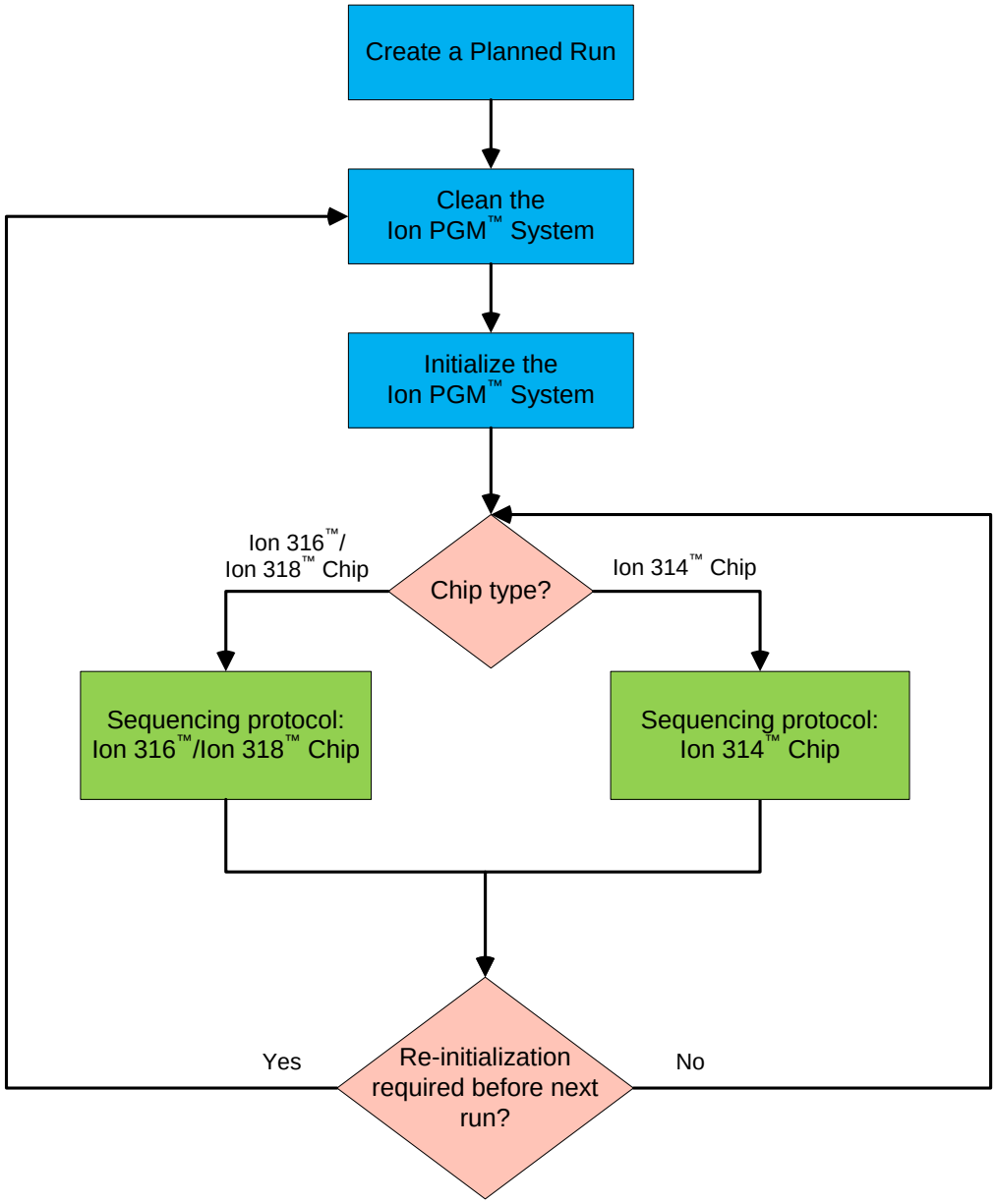
The *Ion PGM™ Sequencing 200 Kit v2 Quick Reference* provides a summary of the workflow and steps for using the Ion PGM™ Sequencing 200 Kit v2 (Catalog no. 4482006) with the Ion Personal Genome Machine® (PGM™) System and Ion 314™, Ion 316™, and Ion 318™ Chips.

For complete kit and protocol information, including precautions and safety information, refer to the *Ion PGM™ Sequencing 200 Kit v2 User Guide* (Pub. no. MAN0007273).

Workflow



Create a Planned Run

1. Log into the Torrent Browser for the Torrent Server connected to your Ion PGM™ System.
2. Select the **Plan** tab, select **Templates**, locate the type of experiment you want to run (for example, AmpliSeq™), then select:
   - **Plan Run** to plan a new run using an existing template.
   - **Plan New Run** to plan a new run without using a template.
3. In the wizard, review each screen and make your selections. For more information about specific fields, see the *Ion PGM™ Sequencing 200 Kit v2 User Guide*.
4. When you have completed your selections, click on **Plan Run** at the end of the workflow. The plan will appear listed on the **Planned Runs** page under the name you specified, and be available on the Ion PGM™ Sequencer when you set up the run.

Clean the Ion PGM™ System

Before Starting

- **Weekly:** Prepare a stock of 1 M NaOH by diluting 10 M NaOH with 18 MΩ water.
- **Daily:** Prepare 100 mM NaOH by diluting the 1 M stock in 18 MΩ water.

Cleaning schedule

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

Clean with:	Schedule:
18 MΩ water	• Daily, when instrument is in use (e.g., not necessary on weekends) • After ≤1000 flows (e.g., 2 × 200-base-read runs) • If more than 27 hours but less than 48 hours have elapsed between the last cleaning/initialization and the start of a run • If you cleaned with chlorite a week ago and have not used the instrument since then
Chlorite solution	• Once a week, unless the instrument has not been used since the last chlorite cleaning (in which case, clean with 18 MΩ water before using) • If the instrument has been left with reagents for more than 48 hours (for example, over the weekend)

Cleaning setup

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

- Remove any bottles that are attached to the Ion PGM™ System. Separate cleaning bottles are provided with the instrument.
- Do not remove old sipper tubes before cleaning. The sipper tubes are used as part of the cleaning procedure.
- Ensure that an old chip is in position on the instrument before cleaning. The chip type used for cleaning/initialization can be different than the chip type used for sequencing (e.g., you can clean with a used Ion 314™ chip for an Ion 318™ Chip run).

18 MΩ water cleaning

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

Chlorite cleaning

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

1. Empty any remaining solution from each cleaning bottle (two 250–mL bottles and one 2-L bottle) and rinse each bottle twice with ~100 mL of 18 MΩ water.
2. Fill a glass bottle with 1 L of 18 MΩ water and add a PGM™ Cleaning Tablet (chlorite tablet). Allow the tablet to completely dissolve (~10 minutes).
3. When the tablet has dissolved, add 1 mL of 1 M NaOH and filter the solution using a 0.22-µm or 0.45-µm filter. Use the chlorite solution within 2–3 hours. Discard any unused solution after this time.
4. Press **Clean** on the touchscreen, and select the **Chlorite cleaning** checkbox.
5. Add 250 mL of filtered chlorite solution to a 250-mL cleaning bottle.
6. Rinse the outside of the W1 sipper tube on the instrument with a squirt bottle containing 18 MΩ water, and attach the bottle to the W1 position.
7. Following the touchscreen instructions, place the empty 2-L cleaning bottle in the W2 position and the empty 250-mL bottle in the W3 position. Place collection trays below the sipper tubes in the dNTP positions. Press **Next** to begin cleaning.
8. When prompted, remove the W1 cleaning bottle with chlorite solution, rinse the outside of the sipper with a squirt bottle containing 18 MΩ water, then install a clean 250-mL cleaning bottle filled with 250 mL of 18 MΩ water in the W1 position. (The second cleaning bottle is different than the one used for chlorite solution.)
9. When cleaning is complete, remove all bottles and sipper tubes from the W1, W2, and W3 positions. Press **Next** to return to the Main Menu and proceed to initialization.

Initialize the Ion PGM™ System

IMPORTANT!

- The first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.
- Handle nucleotides carefully to avoid cross-contamination. Always change gloves after removing used sipper tubes and after handling concentrated dNTP stocks.
- After four initializations, do not use the Wash 1, 2, and 3 Bottles for initialization or sequencing to avoid breakage or leaking.
- Replace the Reagent Bottles and sipper tubes every time you initialize.
- Make sure that you have updated the Torrent Suite and Ion PGM™ System software to Version 3.2.1 or later.

Before initialization

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

Prepare the Wash 2 Bottle

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

1. Rinse the Wash 2 bottle three times with 200 mL of 18 MΩ water.
2. If your 18 MΩ water system has a spigot, extend the water spigot into **but not below** the neck of the Wash 2 Bottle.
3. Fill the bottle to the mold line. Volume of water will be ~2 liters.
4. Add the entire bottle of Ion PGM™ Sequencing 200 v2 W2 Solution to the Wash 2 bottle.
5. Add 70 µL of freshly prepared 100 mM NaOH solution (*not* 1 M NaOH) to the Wash 2 bottle.
6. Cap the bottle and invert five times to mix, and immediately proceed through the rest of the initialization procedure.

IMPORTANT! Do not store the mixed Wash 2 bottle.

Prepare the Wash 1 and Wash 3 Bottles

1. Rinse the Wash 1 and Wash 3 Bottles three times with 50 mL of 18 MΩ water.
2. **Wash 1 Bottle:** Add 350 µL of freshly prepared 100 mM NaOH to the Wash 1 Bottle and cap the bottle.
3. **Wash 3 Bottle:** Add Ion PGM™ Sequencing 200 v2 1X W3 Solution to the 50-mL line marked on the Wash 3 Bottle and cap the bottle.

Begin the initialization

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

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

1. Confirm that the chip used to the clean the Ion PGM™ System is still in place on the instrument.
2. On the main menu, press **Initialize**.
3. In the next screen, scan or enter the barcode on the Ion PGM™ Sequencing 200 v2 W2 Solution bottle *or* select the **Ion PGM™ Sequencing 200 Kit v2** from the dropdown list.
4. Press **Next** and confirm that the cleaning chip is on the instrument and the Reagent Bottle sipper tubes and collection trays are in place. Press **Next** again.
5. The system will verify the gas pressure. If the gas pressure is sufficient, press **Next**. If the pressure is low, press **Yes** to re-verify. If the pressure remains low, contact Technical Support.
6. Wearing clean gloves, insert a new sipper tube (long gray) into the cap in the W2 position. **Do not let the sipper tube touch any surfaces.**
7. Immediately attach the prepared Wash 2 Bottle and tighten the cap. Press **Next.**
8. Change gloves and install new sipper tubes (short gray) in the caps in the W1 and W3 positions.
9. Immediately attach the prepared Wash 1 and 3 Bottles and tighten the caps. Press **Next** to begin initialization (~30 minutes).

Prepare the 50-mL Reagent Bottles with dNTP solutions

IMPORTANT! In the following steps, handle the dNTPs carefully to avoid cross-contamination and keep the dNTP vials on ice.

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

Attach the sipper tubes and Reagent Bottles

1. After the wash solutions have initialized, remove the used sipper tubes and collection trays from the dNTP ports.
2. Using new gloves, attach a new sipper tube (blue) to each dNTP port.
3. Attach each Reagent Bottle to the correct dNTP port and tighten until snug.
4. Follow the touchscreen prompts to complete initialization.
5. At the end of initialization, the Ion PGM™ System will measure the pH of the reagents. If there is a problem with pH, see Troubleshooting in th*e Ion PGM™ Sequencing 200 Kit v2 User Guide*.
6. Press **Next** to finish initialization and return to the main menu.
7. Proceed to the appropriate sequencing protocol for your chip type.

Sequencing protocol—Ion 316™/Ion 318™ Chip

Use the following protocol with Ion 316™ or Ion 318™ Chips. For Ion 314™ Chips, see page 7.

Before starting

- Prepare the template-positive ISPs.
- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Torrent Suite and Ion PGM™ System software to Version 3.2.1 or later.

IMPORTANT! For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.

Optional: Prepare Ion Sphere™ Test Fragments

If you are performing an installation or troubleshooting sequencing run:

1. Vortex the Ion Sphere™ Test Fragments from the Ion Controls Materials 200 Kit and centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of Ion Sphere™ Test Fragments to 100 µL of Annealing Buffer in a 0.2-mL non-polystyrene PCR tube.
3. Skip directly to **Anneal the Sequencing Primer**.

Prepare enriched, template-positive ISPs

Select one of the following options, depending on your template preparation method.

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

Anneal the Sequencing Primer

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

Chip Check

IMPORTANT! Do not wear gloves when transferring chips onto and off the instrument and do not place the chip directly on the bench. Place the chip either on the grounding plate on the Ion PGM™ System or in the bucket from the Ion centrifuge adapter/rotor.

1. Remove a new chip from its packaging and label it. Save the chip package to scan the barcode.
2. Place the chip on the Ion PGM™ Sequencer grounding plate or in the Ion centrifuge adapter/rotor bucket.
3. Press **Run** on the main menu and follow the touchscreen prompts.
4. 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.)
5. When prompted, scan the barcode located on the chip package or manually enter the barcode number.
6. Press **Chip Check** on the touchscreen.
7. Check for leaks on the chip. If there is a leak, press the **Abort** button and proceed to Troubleshooting in th*e Ion PGM™ Sequencing 200 Kit v2 User Guide.*
8. When Chip Check is complete, if the chip passes press **Next**. If the chip fails, open the chip clamp, re-seat the chip, and press **Calibrate** to retest. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart with a new chip.

9. Following a successful Chip Check, remove the new chip and insert a used chip in the socket and close the clamp.
10. Completely empty the waste bottle as instructed in the touchscreen.

Bind Sequencing Polymerase to the ISPs

1. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 3 µL of Ion PGM™ Sequencing 200 v2 Polymerase to the ISPs, for a total final volume of 30 µL.
2. Pipet the sample up and down to mix.
3. Incubate at room temperature for 5 minutes.

Load the chip

Remove liquid from the chip

1. Tilt the chip 45 degrees so that the loading port is the lower port.
2. Insert the pipette tip firmly into the loading port and remove as much liquid as possible. Discard the liquid.
3. Place the chip **upside-down** in the centrifuge adapter bucket and transfer the bucket to the MiniFuge **with the chip tab pointing in** (toward the center of the MiniFuge). Be careful to balance the upside-down chip in the MiniFuge with another upside-down chip.
4. Centrifuge for 5 seconds to completely empty the chip. Remove the chip from the bucket and wipe off any liquid on the bucket.

Load the sample on the chip

1. Put the chip back in the bucket and place it on a flat, stable surface.
2. Following polymerase incubation, collect the entire sample (~30 µL) into a Rainin® SR-L200F pipette tip and insert the tip firmly into the chip loading port.
3. Dial down the pipette to gently and slowly deposit the ISPs at a rate of ~1 µL per second. To avoid introducing bubbles, leave a small amount of sample in the pipette tip (~0.5 µL).
4. Remove and discard any displaced liquid from the other port of the chip.
5. Transfer the chip in the bucket to the MiniFuge **with the chip tab pointing in.**
6. Centrifuge for 30 seconds, then remove the chip from the centrifuge bucket.
7. Mix the sample in the chip:
   a. Set the pipette volume to 30 µL.
   b. Tilt the chip 45 degrees so that the loading port is the lower port, and insert the pipette tip into the loading port.
   c. Without removing the tip, slowly pipet the sample in and out of the chip three times. **Pipet slowly to avoid creating bubbles.**
8. Centrifuge the chip for 30 seconds **with the chip tab pointing out** (away from the center of the MiniFuge).
9. Repeat the chip mixing in step 7 one more time, then spin for 30 seconds **with the chip tab pointing in**.
10. Tilt the chip at a 45-degree angle and slowly remove as much liquid as possible from the loading port by dialing the pipette. Discard the liquid.
11. If some liquid remains in the chip, perform a 5-second quick spin with the chip tab pointing out and remove and discard any additional liquid.
12. If some liquid remains in the chip after the quick spin, lightly and rapidly tap the point of the chip tab against the benchtop a few times, and remove and discard any collected liquid. Do not flush the chip.
13. Immediately proceed to **Select the Planned Run and perform the run**.

Select the Planned Run and perform the run

Select the Planned Run or enter settings manually

1. Press the **Browse** button next to the **Planned Run** field and select the name of the plan you created, then press **Next**.
2. The run settings will be automatically populated based on the Planned Run. Confirm that these settings are correct. Make any changes using the buttons and dropdown lists if necessary.

Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. Visually inspect the chip in the clamp for leaks before closing the cover.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful. If calibration fails, press **Abort**, reseat the chip, then press **Calibrate** to re-calibrate. If it fails again, continue with the run and contact Technical Support after the run is complete.
5. After 90 seconds, the run will automatically begin, or press **Next** to begin the run immediately. Avoid touching the instrument and any of the attached bottles or tubes during a run, as it may reduce the quality of the measurements.
6. When the run is complete, the touchscreen will return to the Main Menu. You can then proceed with another run or perform a cleaning/initialization if required (see Cleaning schedule on page 2).

Sequencing Protocol—Ion 314™ Chip

Use the following protocol with Ion 314™ Chips. For Ion 316™ or Ion 318™ Chips, see page 5.

Before starting

- Prepare the template-positive ISPs.
- Thaw the Sequencing Primer on ice.
- Make sure that you have updated the Torrent Suite and Ion PGM™ System software to Version 3.2.1 or later.

IMPORTANT! For each initialization, the first run should be started within 1 hour after initialization, and the last run must be started within 27 hours after initialization.

Optional: Prepare Ion Sphere™ Test Fragments

If you are performing an installation or troubleshooting sequencing run:

1. Vortex the Ion Sphere™ Test Fragments from the Ion Controls Materials 200 Kit and centrifuge for 2 seconds before taking aliquots.
2. Add 5 µL of Ion Sphere™ Test Fragments to 100 µL of Annealing Buffer in a 0.2-mL non-polystyrene PCR tube.
3. Skip directly to **Anneal the Sequencing Primer**.

Prepare enriched, template-positive ISPs

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

Anneal the Sequencing Primer

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

Chip Check

IMPORTANT! Do not wear gloves when transferring chips onto and off the instrument and do not place the chip directly on the bench. Place the chip either on the grounding plate on the Ion PGM™ System or in the bucket from the Ion centrifuge adapter/rotor.

1. Remove a new chip from its packaging and label it. Save the chip package to scan the barcode.
2. Place the chip on the Ion PGM™ Sequencer grounding plate or in the Ion centrifuge adapter/rotor bucket.
3. Press **Run** on the main menu and follow the touchscreen prompts.
4. 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.)
5. When prompted, scan the barcode located on the chip package or manually enter the barcode number.
6. Press **Chip Check** on the touchscreen.
7. Check for leaks on the chip. If there is a leak, press the **Abort** button and proceed to Troubleshooting in th*e Ion PGM™ Sequencing 200 Kit v2 User Guide.*
8. When Chip Check is complete, if the chip passes press **Next**. If the chip fails, open the chip clamp, re-seat the chip, and press **Calibrate** to retest. If the chip passes, press **Next**. If the chip still fails, press **Main Menu** and restart with a new chip.
9. Following a successful Chip Check, remove the new chip and insert a used chip in the socket and close the clamp.
10. Completely empty the waste bottle as instructed in the touchscreen.

Bind Sequencing Polymerase to the ISPs

1. After annealing the Sequencing Primer, remove the ISPs from the thermal cycler and add 1 µL of Ion PGM™ Sequencing 200 v2 Polymerase to the ISPs, for a total final volume of 7 µL.
2. Pipet the sample up and down to mix.
3. Incubate at room temperature for 5 minutes.

Load the chip

Remove liquid from the chip

1. Tilt the chip 45 degrees so that the loading port is the lower port.
2. Insert the pipette tip firmly into the loading port and remove as much liquid as possible. Discard the liquid.
3. Place the chip **upside-down** in the centrifuge adapter bucket and transfer the bucket to the MiniFuge **with the chip tab pointing in** (toward the center of the MiniFuge). Be careful to balance the upside-down chip in the MiniFuge with another upside-down chip.
4. Centrifuge for 5 seconds to completely empty the chip. Remove the chip from the bucket and wipe off any liquid on the bucket.

Load the sample on the chip

1. Put the chip back in the bucket and place it on a flat, stable surface.
2. Following polymerase incubation, collect the entire sample (~7 µL) into a Rainin® SR-L10F pipette tip and insert the tip firmly into the chip loading port.
3. Dial down the pipette to gently and slowly deposit the ISPs at a rate of ~1 µL per second. To avoid introducing bubbles, leave a small amount of sample in the pipette tip (~0.5 µL).
4. Remove and discard any displaced liquid from the other port of the chip.
5. Transfer the chip in the bucket to the MiniFuge **with the chip tab pointing in.**
6. Centrifuge for 30 seconds, then remove the chip from the centrifuge bucket.
7. Mix the sample in the chip:
   a. Set the pipette volume to 5 µL.
   b. Tilt the chip 45 degrees so that the loading port is the lower port, and insert the pipette tip into the loading port.
   c. Without removing the tip, slowly pipet the sample in and out of the chip three times. **Pipet slowly to avoid creating bubbles.**
8. Centrifuge the chip for 30 seconds **with the chip tab pointing out** (away from the center of the MiniFuge).
9. Repeat the chip mixing in step 7 one more time, then spin for 30 seconds **with the chip tab pointing in**.

10. Tilt the chip at a 45-degree angle and slowly remove as much liquid as possible from the loading port by dialing the pipette. Discard the liquid.
11. If some liquid remains in the chip, perform a 5-second quick spin with the chip tab pointing out and remove and discard any additional liquid.
12. If some liquid remains in the chip after the quick spin, lightly and rapidly tap the point of the chip tab against the benchtop a few times, and remove and discard any collected liquid. Do not flush the chip.

    Note: Not all the liquid can be removed from the Ion 314™ Chip. Remove as much liquid as possible.
13. Immediately proceed to **Select the Planned Run and perform the run**.

Select the Planned Run and perform the run

Select the Planned Run or enter settings manually

1. Press the **Browse** button next to the **Planned Run** field and select the name of the plan you created, then press **Next**.
2. The run settings will be automatically populated based on the Planned Run. Confirm that these settings are correct. Make any changes using the buttons and dropdown lists if necessary.

Perform the run

1. After you enter the Planned Run, press **Next** to verify the experimental setup.
2. When prompted by the instrument, load and clamp the chip, then press **Next**.
3. Visually inspect the chip in the clamp for leaks before closing the cover.
4. When the calibration is complete (~1 minute), the touchscreen will indicate whether calibration was successful. If calibration fails, press **Abort**, reseat the chip, then press **Calibrate** to re-calibrate. If it fails again, continue with the run and contact Technical Support after the run is complete.
5. After 90 seconds, the run will automatically begin, or press **Next** to begin the run immediately. Avoid touching the instrument and any of the attached bottles or tubes during a run, as it may reduce the quality of the measurements.
6. When the run is complete, the touchscreen will return to the Main Menu. You can then proceed with another run or perform a cleaning/initialization if required (see Cleaning schedule on page 2).

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