QUICK REFERENCE

Ion PGM™ Checklist — Sequencing

Pub. no. MAN0009127 **Rev.** 1.0

Follow the steps of this checklist if you are an experienced user of the Ion PGM™ Sequencing 200 Kit v2 (Catalog no. 4482006) with the Ion Personal Genome Machine® (PGM™) System.

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), available on the **Ion Community** website.

Before starting

☐ Thaw Sequencing Primer on ice.

Add Control Ion Sphere Particles (IPSs) to the enriched, template-positive ISPs

☐ 1. Ensure that enriched, template-positive ISPs (prepared using the Ion PGM™ Template OT2 200 Kit) are in a 0.2-mL non-polystyrene PCR tube.

☐ 2. Vortex Control ISPs and centrifuge for 2 seconds.

☐ 3. Add 5 µL of Control ISPs to the appropriate volume of enriched, template-positive ISPs for your chip type:

Ion 316™ Chip v2/Ion 318™ Chip v2	Ion 314™ Chip v2
Entire volume of prepared ISPs	*Half the volume* of prepared ISPs (transfer and store half of the volume)

Anneal the Sequencing Primer

☐ 1. Mix the tube containing ISPs and controls by thoroughly pipetting up and down. Centrifuge for 2 minutes at 15,500 × *g*.

☐ 2. Carefully remove the supernatant, leaving the following volume in the tube:

Volume	Ion 316™ Chip v2/Ion 318™ Chip v2	Ion 314™ Chip v2
Remaining in tube	15 µL	3 µL

☐ 3. Add Sequencing Primer and confirm the total volume (adjust the volume with Annealing Buffer, if necessary):

Volume	Ion 316™ Chip v2/Ion 318™ Chip v2	Ion 314™ Chip v2
Sequencing Primer	12 µL	3 µL
Total volume	**27 µL**	**6 µL**

☐ 4. Pipet the sample up and down thoroughly to disrupt the pellet.

☐ 5. Program a thermal cycler (95ºC for 2 minutes, then 37ºC for 2 minutes), insert tube in cycler, and run the program.

Chip Check

☐ 1. Remove a new chip from its package and label it. Press **Run** on the Ion PGM™ Sequencer touchscreen.

☐ 2. When prompted, ground yourself. Replace the old chip in the chip clamp with the new chip. (*Do not wear gloves.*)

☐ 3. When prompted, scan the new chip package barcode or press **Change** to manually enter barcode. Press **Chip Check**.

☐ 4. Check for leaks on the chip.

- If there is a leak, press **Abort** and proceed to Troubleshooting in the User Guide.
- If the chip passes, press **Next**.
- If the chip fails, re-seat the chip, and press **Calibrate**. If the chip still fails, restart with a new chip.

☐ 5. Remove the new chip, re-insert the old chip in the socket, close the clamp, and empty the waste bottle.

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Bind Sequencing Polymerase to the ISPs

☐ 1. Remove ISPs from the thermal cycler and add polymerase according to the following table:

Volume	Ion 316™ Chip v2/Ion 318™ Chip v2	Ion 314™ Chip v2
Ion PGM™ Sequencing 200 v2 Polymerase	3 µL	1 µL
Total volume	**30 µL**	**7 µL**

☐ 2. Pipet the ISPs up and down, and incubate at room temperature for 5 minutes.

Load the chip

☐ 1. Tilt the new chip 45 degrees. Insert pipette tip into the loading port; remove and discard as much liquid as possible.
☐ 2. Place the chip *upside-down* in the Ion MiniFuge bucket; transfer the bucket to the MiniFuge (*chip tab pointing in*).
☐ 3. Centrifuge for 5 seconds. Remove the chip from the bucket and wipe off any liquid on the bucket.
☐ 4. Put the chip back in the bucket and place the bucket on a flat, stable surface.
☐ 5. Following polymerase incubation, collect the entire sample into the pipette tip and insert the tip into chip loading port.
☐ 6. *Dial down the pipette* to gently and slowly deposit the ISPs, leaving ~0.5 µL of sample in the pipette tip.
☐ 7. Remove and discard any displaced liquid from the other port of the chip.
☐ 8. Transfer the chip in the bucket to the MiniFuge (*chip tab in*). Centrifuge for 30 seconds.
☐ 9. Set the pipette volume according to the following table:

Volume	Ion 316™ Chip v2/Ion 318™ Chip v2	Ion 314™ Chip v2
Pipette setting	30 µL	5 µL

☐ 10. Tilt the chip 45 degrees, and slowly pipet the sample in and out of the chip *three times*.
☐ 11. Centrifuge the chip in the Minifuge (*with the chip tab out*) for 30 seconds.
☐ 12. Repeat step 10 one more time, then centrifuge for 30 seconds (*chip tab in*).
☐ 13. Tilt the chip and slowly remove liquid by dialing the pipette.
☐ 14. Discard the liquid. If some liquid remains in the chip, perform a 5-second quick spin (*chip tab out*).
☐ 15. If necessary, lightly tap the point of the chip tab against the benchtop, and remove and discard any collected liquid.

Select the Planned Run and perform the run

☐ 1. Press **Browse** on the sequencer touchscreen and select the name of the plan you created. Press **Next.**
☐ 2. Confirm that the run settings are correct or make any necessary changes. Press **Next**.
☐ 3. Load and clamp the chip, then press **Next**.
☐ 4. Visually inspect the chip in the clamp for leaks before closing the cover.

- If the chip passes, press **Next.**
- If it fails, press **Abort**, re-seat chip, and press **Calibrate**.
- If the chip continues to fail calibration, contact Technical Support.

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Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit iontorrent.com/support or email ionsupport@lifetech.com

lifetechnologies.com

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