Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems

Catalog Numbers A34899, A34900, A34901

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide* (Pub. No. MAN0016712). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Extract and amplify the gDNA

Extract genomic DNA

1. Prepare, by FACS or micro-manipulation, 1–10 cells per sample in up to 2.5 µL 1X PBS or Low TE, then pipet the cells into wells of a 96-well optical reaction plate.

2. *(Optional)* Set up one or more gDNA control reactions.
   a. Add 2 µL of stock solution of Control DNA (CEPH 1347-02) to 198 µL of Low TE. Vortex to mix, then centrifuge briefly.
   b. Add 2 µL of this solution to 131 µL of Low TE. Vortex to mix, then centrifuge briefly.
   c. For each reaction, add 2 µL of user-supplied control human gDNA at a concentration of 7.5 pg/µL with 3 µL Cell Extraction Buffer.

3. Add Cell Extraction Buffer (green cap) to each sample well to bring the total volume to 5 µL.

4. (Optional) Prepare a Non-Template Control (NTC) by adding 2.5 µL 1X PBS to 2.5 µL Cell Extraction Buffer.

5. Prepare an Extraction Enzyme master mix in a 1.5-mL tube on ice according to the following table. Scale the volume according to the number of samples (N) and NTCs you have. Vortex briefly, then centrifuge to collect liquid at the bottom of the tube.

   Note: Do not let the pipette tip touch the bottom of the well.

Component	Volume per reaction	Volume for N reactions[1]
Extraction Enzyme Dilution Buffer (violet cap)	4.8 µL	N × 4.8 µL × 1.1
Cell Extraction Enzyme (yellow cap)	0.2 µL	N × 0.2 µL × 1.1

[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

6. Add 5 µL Extraction Enzyme master mix to each cell or gDNA sample (10-µL final volume).

7. Seal the plate with MicroAmp™ Clear Adhesive Film, then centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells.

8. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

Temperature	Time
75°C	10 minutes
95°C	4 minutes
22°C	Hold

9. Remove the plate, centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells, then place on ice or a cold block.

Pre-amplify the gDNA

1. Prepare a preamplification master mix in a 1.5-mL tube on ice according to the following table.

Component	Volume per reaction	Volume for N reactions [1]
Preamplification Buffer (red cap)	4.8 µL	N × 4.8 µL × 1.1
Preamplification Enzyme (white cap)	0.2 µL	N × 0.2 µL × 1.1

[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

Note: To dispense Preamplification master mix with an 8-channel multichannel pipettor, aliquot the master mix to 8 wells of a 96-well plate to serve as a reservoir. For example, aliquot 65 µL/well if you are processing 96 samples.

2. Carefully remove the film seal from the plate, then add 5-µL Preamplification master mix to each sample well (15-µL final volume).

3. Apply a new adhesive film, then centrifuge 1,000 × *g* for 30 seconds.

4. Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program.

Stage	Step	Temperature	Time[1]
Hold	Activate the enzyme	95°C	2 minutes
Cycle (12 cycles)	Denature	95°C	15 seconds
	Anneal	15°C	50 seconds
		25°C	40 seconds
		35°C	30 seconds
	Extend	65°C	40 seconds
		75°C	40 seconds
Hold	—	4°C	Hold

[1] Cycling time is approximately 1 hour.

5. Remove the plate, centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells, then place on ice or a cold block.

Amplify the libraries

1. If you are performing real-time PCR, prepare SYBR™ Green I/ROX dye mix. If you are performing standard endpoint PCR, proceed to step 2, then use Nuclease-free Water instead of the dye mix, as indicated in Step 3.
   a. Dilute the stock SYBR™ Green I reagent 1:1000 with Low TE buffer to make a 10X working solution.
   b. Combine 10X SYBR™ Green I working solution and 25 µM ROX Reference Dye for the number of reactions (N) according to the following table:

Component	Volume per reaction	Volume for N reactions[1]
10X SYBR™ Green I working solution	0.5 µL	N × 0.5 µL × 1.1
25 µM ROX Reference Dye (or 2.5 µM, see below)	1.0 µL	N × 1.0 µL × 1.1
Nuclease-free Water (clear cap)	1.0 µL	N × 1.0 µL × 1.1

[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.

IMPORTANT! Use ROX Reference Dye at 25 µM for the following real-time PCR instruments: Applied Biosystems™ 7300, 7900HT, StepOne™, StepOnePlus™, ABI PRISM™ 7000, and 7700 instruments.

Dilute ROX Reference Dye to 2.5 µM with Nuclease-free Water and use 1.0 µL per reaction for the following instruments: Applied Biosystems™ 7500 and ViiA™ 7 instruments, and Agilent™ Mx3000P™, Mx3005P™, and Mx4000™ instruments.

2. Prepare the Ion SingleSeq™ Barcodes 1–24 or 1–96 plate:
   a. Thaw the plate for 10 minutes at room temperature.
   b. Centrifuge the plate briefly to collect contents at the bottom of the wells.
   c. Wipe the foil seal with 70% ethanol, then allow it to dry.

3. Prepare an Amplification master mix in a 5-mL tube on ice according to the following table.

Component	Volume per reaction	Volume for N reactions[1]
Amplification Buffer (orange cap)	27 µL	N × 27 µL × 1.1
Amplification Enzyme (blue cap)	0.5 µL	N × 0.5 µL × 1.1
SYBR™ Green I/ROX dye mix[2]	2.5 µL	N × 2.5 µL × 1.1

[1] 10% overage added to compensate for pipetting loss. Round to nearest 0.1 µL.
[2] Replace with Nuclease-free Water if performing endpoint PCR.

Note: To dispense Amplification master mix with an 8-channel multichannel pipettor, aliquot the master mix to eight wells of a 96-well plate to serve as a reservoir. For example, aliquot 396 µL/well if you are processing 96 samples.

4. Remove the film seal from the sample plate, then add 30 µL Amplification master mix to each well (45-µL final volume).

5. Pierce the foil above the desired well of the Barcode Plate with a pipette tip. With a new tip, withdraw 5 µL of the Ion SingleSeq™ Barcode Adapter, then add to the appropriate sample (50-µL final volume). Repeat for each sample. Each Barcode Adapter is single-use only.

6. Adjust a pipettor to 30 µL, then mix the samples by pipetting up and down, using a new tip for each sample.

7. Apply a new film seal to the plate and briefly centrifuge to collect liquid at the bottom of the wells.

8. Cycle the samples in the thermal cycler using the following program:

Step	Temperature	Time[1]	Number of cycles
1	95°C	3 minutes	1
2	95°C	20 seconds	4
	50°C	25 seconds	
	72°C	40 seconds	
3[2]	95°C	20 seconds	12
	72°C	55 seconds	
4	4°C	Hold	1

[1] Cycling time is approximately 30 minutes.
[2] Acquire fluorescence data at this step if monitoring amplification in real-time.

9. Remove the plate, centrifuge at 1,000 × *g* for 30 seconds to collect liquid at the bottom of the wells, and place on ice or in a cold block.

Proceed to "Pool, purify, and quantify the libraries".

STOPPING POINT Sample libraries can be stored at –30°C to –10°C before proceeding to the next step.

Pool, purify, and quantify the libraries

Note: We recommend that you perform library pooling, purification, and quantification in one session. Do not store library pools before quantification.

Pool the libraries

1. Pool the libraries according to the PCR method you used to amplify the libraries. Before pooling, vortex the amplification reactions to mix, then centrifuge briefly to collect contents at the bottom of the wells or tubes.

If you used	Action
Standard endpoint PCR amplification	Add 5 µL of each library to a new 1.5-mL tube to create an equi-volume pool. Vortex the tube to mix and pulse-centrifuge to collect contents at the bottom of the tube.
Real-time PCR amplification	Calculate the median C_t value of the libraries you want to pool and adjust the volumes of libraries deviating from the median C_t following these guidelines: • Libraries that fall within 1 C_t of the median should be added at the normal volume (5 µL). • Libraries that fall >3 C_ts later than the median should not be included in the pool. It is likely that the input material was not present in the tube or well or was absorbed to the tube side. • Libraries that fall ≥1 but ≤3 C_ts later than the median should be added at 2X the normal volume (10 µL). • Do not adjust more than 25% of the libraries in a pool (excluding libraries > 3 C_ts later than median). If more than 25% of libraries fall 1 or more C_ts away from the median, adjust libraries with the most extreme C_t deviations until the 25% threshold is reached.

2. Transfer 40 µL of the library pool to a 0.2-µL tube for purification and quantification.

Note:
- When pooling fewer than 8 libraries, the pool volume drops below 40 µL. Add Nuclease-free Water to bring the final volume to 40 µL before library pool purification.
- When pooling more than 8 libraries, remove 40 µL from the pool for purification and quantification. If the remaining volume is ≥40 µL, store at –30°C to –10°C to use if a repeat purification is needed.

Purify the library pool

1. Transfer 40 µL of the library pool to a fresh PCR tube.
2. Heat the 40-µL aliquot of library pool in a thermal cycler using the following program.

Step	Temperature	Time	Number of cycles
1	70°C	2 minutes	1
2	22°C	Hold	1

3. Briefly pulse-centrifuge the tube to collect the contents, then transfer to a new 1.5-mL Eppendorf LoBind™ tube.
4. Add 40 µL (1X volume) of room temperature AMPure™ XP beads to the library pool.
5. Vortex briefly, pulse-centrifuge to collect contents, then incubate for 5 minutes at room temperature.
6. Place the tube in the DynaMag™-2 Magnet, then wait 5 minutes for beads to aggregate to the side of the tube or well of the plate.
7. Aspirate the supernatant carefully, then discard.
8. Wash beads with 250 µL of freshly prepared 70% ethanol while the tube or plate is still on the magnet.
9. Incubate for 30 seconds.
10. Aspirate the wash solution carefully, then discard.
11. Repeat step 8 through step 10, thoroughly removing all ethanol after the second wash.
12. Allow the beads to dry at room temperature for 3–4 minutes with the tube on the magnet.
13. Remove the tube from the magnet, add 40 µL of Low TE, then resuspend the beads by pipetting up and down.
14. Incubate the tube at room temperature for 1 minute.
15. Place the tube in the DynaMag™-2 Magnet, then wait 2–3 minutes for the beads to aggregate to the side of the tube.
16. Transfer 35 µL of the supernatant containing the purified library pool to a new 1.5-mL Eppendorf LoBind™ tube and place on ice. Avoid carryover of beads.

Quantify the library pool

Quantify the Ion SingleSeq™ library pool with the Qubit™ dsDNA HS (High Sensitivity) Assay Kit.

For details instructions, see the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326).

1. Prepare a Qubit™ working solution by diluting the Qubit™ dsDNA HS Reagent (Component A) 1:200 in Qubit™ dsDNA HS Buffer (Component B) in a plastic tube.
2. Prepare the standards.
   a. Add 190 µL Qubit™ working solution to two labeled Qubit™ Assay Tubes used for standards.
   b. Add 10 µL of Qubit™ standard (Components C) to the one tube and 10 µL of Qubit™ standard (Components D) to the other tube.
   c. Mix by vortexing 2–3 seconds. Do not create bubbles.
3. Prepare the unknown.
   a. Aliquot 198 µL Qubit™ working solution to labeled Qubit™ Assay Tubes used for samples.
   b. Add 2 µL of the library pool to the appropriate sample tube.
   c. Mix by vortexing 2–3 seconds. Do not create bubbles.
4. Incubate the tubes in the dark at room temperature for 2 minutes.
5. Measure standards first to generate a standard curve, then measure the library pool.
6. Convert ng/µL to nM by multiplying the ng/µL values obtained in step 5 by 6.06 nmol/mg.
7. Dilute the library pool to 1 nM.

Create a Planned Run

1. Sign in to the Torrent Server in Torrent Suite™ Software.
2. Click the **Plan** tab, then click **Reproductive** from the list of applications on the left side of the screen.
3. Select **Low-pass Whole-genome Aneuploidy** from the list under **Template Name**.
4. In the **Plan** step, enter or make the following selections:
   a. Enter a new **Run Plan Name**.
   b. Select **hg19(Homo sapiens)** from the **Reference Library** dropdown list.
   c. Select **None** from the **Target Regions** and **Hotspot Regions** dropdown lists.
   d. Enter the number of barcodes in your Sample Set.
   e. Select a Barcode, then enter a unique Sample Name for each sample.

5. In the **Kits** step, verify the selections, or make changes appropriate for your run:
   a. Select **Ion GeneStudio™ S5 System** from the **Instrument** dropdown list, if it is not auto-populated.

      Note: Use this setting if you are using an Ion S5™ System or Ion S5™ XL System.

   b. Select the chip type that you are using.
   c. Select **Ion SingleSeq Kit** from the **Library Kit Type** dropdown list.
   d. Select **Ion ReproSeq PGS Kits-Chef-GPR** from the **Template Kit** dropdown list.
   e. Select **Ion S5 ExT Sequencing Kit-GPR** from the **Sequencing Kit** dropdown list.
   f. Select the appropriate Ion SingleSeq™ Barcode set:
      - **Ion SingleSeq Barcode Set 1–96** (default)
      - **Ion SingleSeq Barcode Set 1–24**
   g. Enter **250** flows.
   h. Click **Next** twice to proceed to the **Projects** step.

6. Return to the **Plan** step, then click **Plan Run** in the lower right corner to save the Planned Run. The run is listed on the Planned Runs screen under the name you entered.

   Note: For details about creating a Planned Run, and enabling Ion Reporter™ auto-analysis, see the *Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide* (Pub. No. MAN0016712).

Run the Ion Chef™ System

Prepare the consumables

1. Unbox the Ion S5™ ExT Chef Reagents cartridge 45 minutes before use, then allow it to warm to room temperature.

   IMPORTANT! The Reagents cartridge must sit at room temperature for 45 minutes before use.

2. Remove the other cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

   IMPORTANT! Before use, gently tap the Reagents cartridge and Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.

Add the library to the Library Sample Tube

1. Pipet 2–4 µL of the 1 nM pooled library into the Library Sample Tube (barcoded tube) from Position A of the Reagents cartridge. Add Nuclease-free Water to 50 µL (final concentration of 40–80 pM). Pipet up and down 5 times to mix.

2. Cap the Library Sample Tube and store on ice until you are ready to load the tube into the Reagents cartridge and the Ion Chef™ Instrument.

Load the Ion Chef™ System

IMPORTANT!

- Rated centrifuge speeds are intended for operation only 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, chip, adapter, and chip balance. The centrifuge must be load-balanced. Proper care must be taken to load the bucket properly. If excessive vibrations arise, check to ensure that items are installed properly and rotors are load-balanced.
- Use only the materials supplied in the Ion ReproSeq™ PGS 510, 520, and 530 Kits 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.
- Ensure that the instrument is powered on and has been cleaned following the last use.
- Ensure that all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure that the Reagents and Solutions station compartments are dry and free of condensate before loading components.

Follow the procedure described in the following sections to load the Ion Chef™ Instrument.

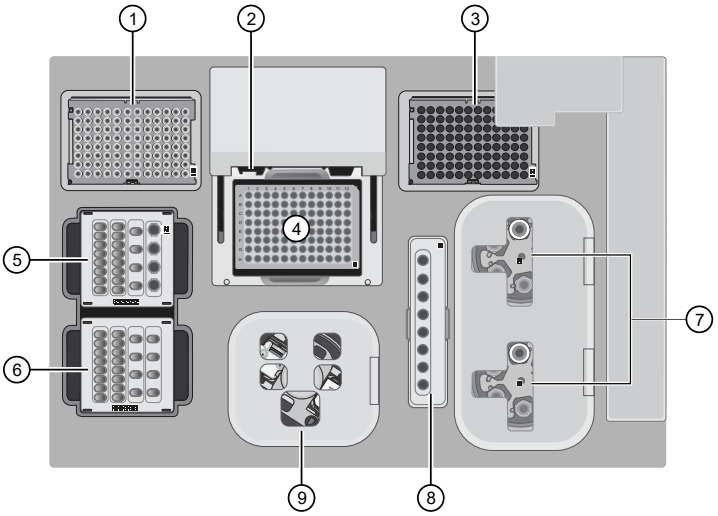


Figure 1 A schematic of a loaded Ion Chef™ Instrument.

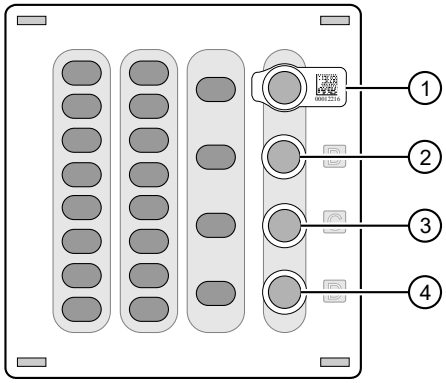
① New Ion S5™ ExT Tip Cartridge
② Frame Seal v2 (inserted)
③ Empty tip rack (move from new Tip cartridge position)
④ PCR Plate and PCR Plate Frame
⑤ Ion S5™ ExT Chef Reagents cartridge
⑥ Ion S5™ Chef Solutions cartridge
⑦ Recovery centrifuges: Recovery Tubes and Recovery Station Disposable Lid v2
⑧ Enrichment Cartridge v2
⑨ Chip-loading centrifuge: Chip Adapter/Chip assembly and Chip Balance

Load the tip racks and PCR Plate

1. Tap ⏏ (Open Door) in the instrument touchscreen to open the instrument door, then wait for the latch to open.
2. Lift the instrument door to the top of the travel until the latch mechanism engages.
3. Load an empty pipette tip rack to the *Used* (Waste) Pipette Tip Position, then change gloves.
4. Unwrap a new Ion S5™ ExT Tip Cartridge and remove the cover to expose the pipette tips, then load it in the *New* Pipette Tip Position.
5. Slide the catch forward to allow the locking bracket to pivot upward. Load the Ion S5™ ExT Tip Cartridge into the *New* Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the cartridge in place.
6. Load a new PCR plate into the thermal cycler sample block.
7. With the white dot on the PCR Plate Frame facing upward, load the PCR Plate Frame into the thermal cycler sample block pressing down firmly on each corner, then insert a new Frame Seal v2 underneath the automated heated cover. Ensure that the PCR Plate Frame is pressed completely down onto the thermal cycler block and that the PCR Plate Frame sits lower than the PCR Plate.

Load the Reagents and Solutions cartridges

1. Gently tap the Ion S5™ ExT Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
2. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.
3. Uncap, then load the Library Sample Tube containing 50 µL of diluted library into Position A on the Reagents cartridge.



① Position A (Library Sample Tube)
② Position B (2M NaOH)
③ Position C (Ion S5™ ExT Pellet)
④ Position D (Empty tube)

4. Uncap the tube of 2M NaOH in Position B, the tube containing the Ion S5™ ExT Pellet in Position C, and the empty tube in Position D.
5. Gently tap the Ion S5™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.
6. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.

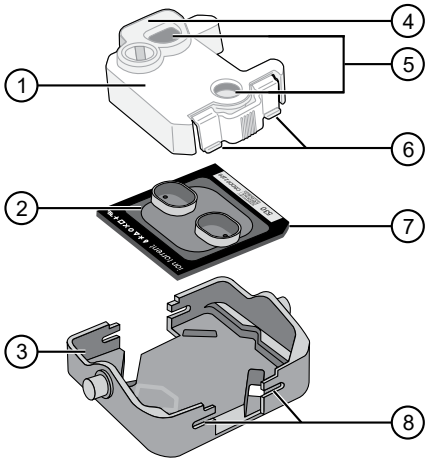
Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes (v2) into each Recovery centrifuge.
2. Place a Recovery Station Disposable Lid v2 over each centrifuge by lining up the tab with the depression on the deck, then snap into place. Ensure that 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. Load the chip that you will use for templating and sequencing into a centrifuge bucket, then attach a Chip Adapter to the assembly.
   a. Place the chip in the chip-loading bucket, then align the wells of the Chip Adapter to the wells of the chip, orienting the adapter onto the chip so that the chip barcode is visible.
   b. Place the adapter onto the chip, then insert the stationary tabs at the reservoir end of the adapter into the slots of the bucket.
   c. Gently squeeze the flexible tabs at the other end of the adapter into the bucket slots until the adapter locks into place.

d. Confirm that the tabs at all four corners of the adapter are fitted into the slots in the centrifuge bucket. Loading can fail if the adapter is not attached securely.



① Chip Adapter
② Ion Chip™
③ Bucket
④ Reservoir end of Chip Adapter
⑤ Ports (align with chip)
⑥ Tabs
⑦ Keyed corner (align with bucket)
⑧ Slots

2. Load the adapter/chip/bucket assembly into Position 1 of the Chip-loading centrifuge.

3. Load an Ion Chef™ S5 Series Chip Balance into Position 2 of the 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 lid of the Chip-loading centrifuge.

Confirm that consumables are correctly installed

- 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 in the Ion S5™ ExT Chef Reagents cartridge, including the tube of NaOH in Position C, 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, tap **Set up run**.

3. Tap **Step by step** to have the instrument lead you through the instrument setup, or tap **Quick Start** to skip the instrument setup screens and proceed to Deck Scan.

4. *(**Step by step** setup only)* In the **Run Options** screen, tap **Prepare Chip** to select the templating run option.

5. 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.

6. When prompted, tap **Start check** to begin Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to confirm their presence and compatibility.

7. When the Deck Scan is complete, tap **Next** to display the **Data Destination** screen.

8. Ensure that the instrument displays the correct kit type, chip type, chip barcode, and Planned Run. If the correct Planned Run does not display, tap the dropdown list to select the appropriate Planned Run, then tap **Next.**

9. On the **Run Options** screen, tap the appropriate option to complete the run, then enter the desired time of run completion if needed.

10. On the **Run Options** screen, tap **Start run** to start the run.

11. Initialize the sequencer at least 50 minutes before the Ion Chef™ System finishes chip loading. See “Initialize the sequencer” on page 8.

12. If you chose to pause the run to analyze the templating efficiency, remove the sample for testing when prompted to do so by the Ion Chef™ Instrument (approximately 4 hours 15 minutes into the run).
    a. When prompted to remove the QC sample, open the instrument door.
    b. Transfer the entire volume of the QC sample from Position A of the Ion S5™ ExT Chef Reagents cartridge on the instrument deck to a new labeled microcentrifuge tube.
    c. If you are performing quality assessment of an enriched sample, transfer the QC sample from Position E of the Enrichment Cartridge v2 to a new labeled microcentrifuge tube.
    d. Analyze the QC sample. For more information, see "*Quality control of ISPs*" in *Appendix B* "*Supplementary procedures*" of the *Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide* (Pub. No. MAN0016712).
    e. Close the instrument door, then tap **Continue** to complete the run.

13. When the run is complete, unload the Ion Chef™ Instrument and sequence the chip immediately. You can collect QC samples from the Reagents and/or Enrichment cartridges if you have not done so already.

IMPORTANT! Liquid may be present in the chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.

Unload the chip for sequencing

1. Open the instrument door.
   a. In the instrument touchscreen, tap ⏏ **(Open Door)**, then wait for the latch to open.
   b. Lift the instrument door to the top of the travel until the latch mechanism engages.
2. Open the lid of the Chip-loading centrifuge, then unload the adapter/chip/bucket assembly from the instrument.
3. Unload the chip from the adapter/chip/bucket assembly.
   a. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.
   b. Grasp the chip by its edges, carefully lift the chip out of the bucket, then set it aside on a clean, static-free surface. Return the bucket to the Chip-loading centrifuge.
4. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.
5. Load the chip into a sequencer, then promptly start the sequencing run.

   If you cannot sequence a loaded chip immediately or plan to sequence two chips per initialization, place the chip into a separate chip storage container and store at 4°C until you are ready to sequence it (up to 6–8 hours maximum).

IMPORTANT!
- Liquid may be present in chip wells after the Ion Chef™ run. Do NOT remove any residual liquid from the wells.
- If you choose to store a loaded chip, remove the chip from 4°C storage (but keep it in the storage container) at least 20 minutes before running it, allowing the chip to warm to room temperature.

Initialize the sequencer

Initialization takes ~50 minutes.

Note: The instructions in this chapter also apply to both Ion S5™ Systems and Ion S5™ XL Systems.

Before you begin

The Ion S5™/Ion S5™ XL and Ion GeneStudio™ S5 Series Sequencers are equipped to verify the compatibility of each chip and consumable that is loaded during initialization and sequencing, and that these components do not exceed their expiration date. To avoid exceptions during initialization, inspect this information for each consumable before installing onto the instrument.

- Unbox the Ion S5™ ExT Sequencing Reagents cartridge 45 minutes before use, then allow it to equilibrate to room temperature.
- Unbox the Ion S5™ ExT Wash Solution bottle. Invert the bottle 5 times within its vacuum-sealed bag, then swirl at an angle to mix thoroughly.
- Remove the Ion S5™ ExT Wash Solution bottle from its vacuum-sealed bag, then remove the red cap from the Ion S5™ ExT Wash Solution and Ion S5™ Cleaning Solution bottles immediately before installing on the instrument.

When a manual cleaning of the sequencer is required

The Ion S5™/Ion S5™ XL and Ion GeneStudio™ S5 Series Sequencers require that a cleaning be performed before initialization. This is normally performed automatically at the completion of the previous sequencing run. However, if the "Enable post-run clean" checkbox is unchecked to allow a second run, and a second run is not performed, the instrument will not allow the subsequent initialization to proceed until a manual cleaning has been performed. For more information on how to perform a manual cleaning, see the *Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide* (Pub. No. MAN0016712).

If a sequencer is initialized and a sequencing run is not started within 24 hours, or a run is not started or completed due to a power failure or an abort, do not perform a manual cleaning. An instrument reset run is required before reinitialization. For more information on how to perform an instrument reset run, see the *Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide*.

Initialize the sequencer

1. In the instrument touchscreen main menu, tap **Initialize**.

   The door, chip, and Reagent cartridge clamps unlock.
2. When prompted, remove the Ion S5™ ExT Wash Solution bottle to access the waste reservoir, then remove and empty the waste reservoir.
3. Reinstall the empty waste reservoir.
4. Replace the expended Ion S5™ ExT Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.

5. Invert a new Ion S5™ ExT Wash Solution bottle 5 times and swirl at an angle to mix thoroughly. Then remove the red cap and install.

6. Ensure that the used sequencing chip from the previous run is properly seated in the chip clamp and the chip clamp is pushed in all the way.

7. If necessary, install a new Ion S5™ Cleaning Solution bottle.

8. Close the door, then tap **Next**.

9. When initialization is complete (~50 minutes), tap **Home**.

The instrument is now ready for a sequencing run.

Start the sequencing run

We recommend that you start a sequencing run as soon as possible after chip loading and instrument initialization are complete. However, successful sequencing runs can be started up to 24 hours after instrument initialization.

1. After completion of initialization, tap **Run** in the instrument touchscreen. The door and chip clamp unlock.

2. Remove the used sequencing chip, then secure a chip loaded with template-positive Ion Sphere™ Particles in the chip clamp.

3. Push the chip clamp all the way in to engage, close the instrument door, then tap **Next**.

4. Confirm that the instrument door is closed, then tap **Start run** to begin the sequencing run.

IMPORTANT! During a run, do not open the instrument door, and avoid touching the instrument. Touching the instrument during the sequencing run can reduce the quality of the measurements.

When the sequencing run is complete, the instrument automatically performs the cleaning procedure unless the **Enable post-run clean** checkbox was deselected. After cleaning, the touchscreen returns to the main menu. Use Torrent Suite™ Software to review the results.

If you are sequencing a second chip on a single initialization, start the second run within 24 hours of start of initialization.

Clean the Ion Chef™ Instrument

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

Remove and dispose of used consumables

1. Tap ⏏ (Open Door) in the instrument touchscreen, then wait for the latch to open.

2. Lift the instrument door to the top of the travel until the latch mechanism engages.

3. Remove, then discard the PCR Plate with the PCR Plate Frame and Frame Seal v2 from the thermal cycler sample block in unison.

IMPORTANT! Do not attempt to separate the PCR Plate Frame from the PCR Plate and Frame Seal v2, as this may cause PCR product to splash and contaminate the instrument deck.

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4. Remove, then discard the box of used pipette tips from the waste tip position. Dispose of the liquid waste appropriately.

5. Move the empty Ion S5™ ExT Tip Cartridge to the waste tip position.

6. Remove, then discard the
   - Ion S5™ ExT Chef Reagents cartridge
   - Ion S5™ Chef Solutions cartridge
   - Enrichment Cartridge v2

7. Remove, then discard the consumables from the Recovery centrifuges, including the:
   - Recovery Station Disposable Lid v2
   - Recovery Tubes v2

8. Remove the Chip Balance from the Chip-loading centrifuge. Do not discard.

9. Close the Chip-loading centrifuge lid.

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 ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide* (Pub. No. MAN0016712).

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, tap **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, except the empty pipette tip rack in the waste tip position, then tap **Next**.

4. With the door closed, tap **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.

Manually launch an Ion Reporter™ analysis

See the *Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide* (Pub. No. MAN0016712) for detailed instructions for launching an Ion Reporter™ analysis using the Ion ReproSeq™ PGS w1.1 workflows to analyze your samples.

If you planned your run for automatic analysis with Ion Reporter™ Software, proceed to step 6.

1. Import your samples into Ion Reporter™ Software using the Ion Reporter™ Uploader plugin.
2. In the Ion Reporter™ **Home** tab, click **Launch analysis** after the Ion Reporter™ Uploader plugin has completed.
3. In the **Workflow** step, select **Reproductive** from the **Research Category** menu to view the Aneuploidy workflows. Select one of the three ReproSeq w1.1 workflows from the list, then click **Next**.
4. In the **Samples** step, select one or more samples.
5. Click **Next** twice to advance through the **Plugins** step to **Confirm & Launch**.
6. In the **Confirm & Launch** step, to change the default name, enter a name for the analysis, then click **Launch Analysis**.
7. Review your results by selecting from the Analyses list after navigating to **Analysis ▸ Overview**. See *Ion Reporter™ Software Help* for further details.

Limited product warranty

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Revision	Date	Description
D.0	15 August 2023	• Updated dilution recommendation in “Add the library to the Library Sample Tube” on page 5. • Updated instructions to include loading the PCR Plate Frame. See “Load the tip racks and PCR Plate” on page 6. • Updated instructions to include removing the PCR Plate Frame. See “Remove and dispose of used consumables” on page 9.
C.0	7 June 2017	Missing topic added: “Add the library to the Library Sample Tube” on page 5.
B.0	21 March 2018	Updated for the Ion GeneStudio™ S5 Systems.
A.0	7 June 2017	New Quick Reference for the Ion ReproSeq™ PGS Kits – Ion S5™ System.