

Precision ID mtDNA Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System: Template Preparation and Sequencing

Using the Ion S5™ Precision ID Chef & Sequencing Kit

Catalog Numbers A30938, A31443, A33208

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the following product documentation: *Precision ID mtDNA Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017770). 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

For complete details for creating a Planned Run in Torrent Suite™ Software, see the *Precision ID mtDNA Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017770).

Note: You must create a Planned Run for each chip in an Ion Chef™ templating run.

1. Sign in to Torrent Suite™ Software.
2. Select **Plan**, then in the **Favorites** list on the **Templates** screen, select **Human Identification**.
3. Select the S5 Planned Run template appropriate to your Precision ID panel.
The wizard launches and displays the **Plan** screen.
4. On the **Plan** screen, select the reference and BED files, enter the Sample names and Sample Tube Label, confirm the default settings, then enter a plan name.

5. To change or verify kit information, click the **Kits** tab. Specify the appropriate library, template and sequencing kits, and flow number and barcode set.
6. After you have completed your selections, click **Plan Run** at the bottom right of the **Plan** screen to save the run. The run is listed on the Planned Runs screen under the name that you specified, and is automatically used by the Ion Chef™ System when the associated sample is loaded.

Dilute the libraries for template preparation on the Ion Chef™ Instrument

Ensure that each pool of sample libraries (or super-pool of Chef-prepared libraries) has been diluted to the recommended concentration. For manually prepared libraries, pool equal volumes of quantified and diluted libraries, then use the pooled library in template preparation reactions on the Ion Chef™ Instrument. For Chef-prepared libraries, pool equal volumes of quantified and diluted combined library.

Recommended library dilutions for Precision ID mtDNA panels.

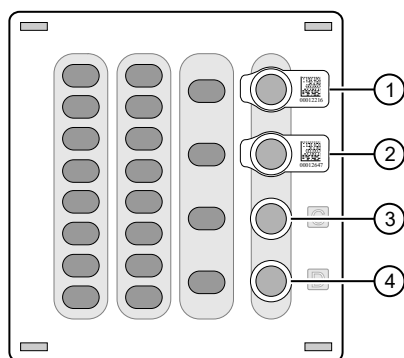
| Library preparation method | Dilute to | Minimum volume |
|----------------------------|-----------|----------------|
| Manual | 30 pM | 25 µL |
| Ion Chef™ Instrument | 30 pM | 25 µL |

Prepare the libraries and consumables

1. Unpack the Ion S5™ Precision ID 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.

- Pipet 25 µL of diluted library pool to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes in the figure below).



Ion S5™ Precision ID Chef Reagents cartridge

- Position A (DNA library pool)
- Position B (DNA library pool, or empty if using single chip loading workflow)
- Position C (NaOH)
- Position D (Empty tube)

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

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

Load the Ion Chef™ System

IMPORTANT!

- Rated centrifuge speeds are intended only 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 S5™ Precision ID Chef & Sequencing 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 was 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 free of condensate before loading components.

Follow the procedures in this section to load the Ion Chef™ Instrument.

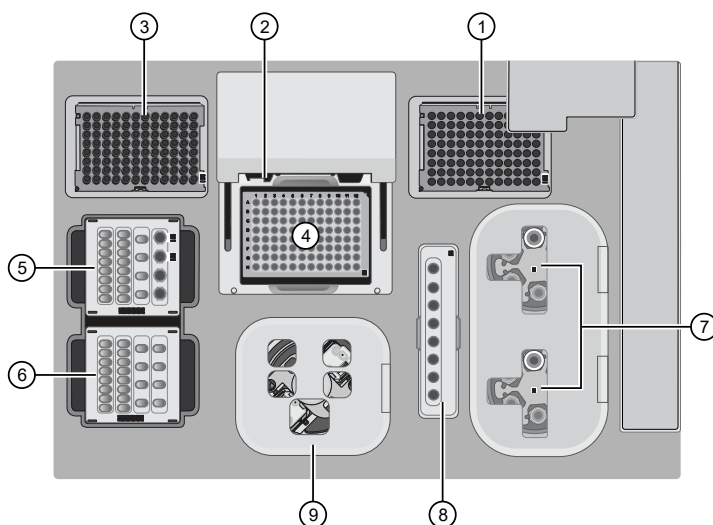



Figure 1 A schematic of a loaded Ion Chef™ Instrument

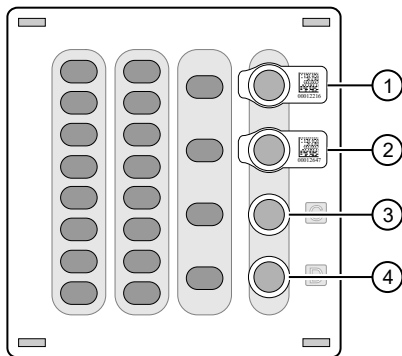
- Empty tip rack (move from new Tip Cartridge position)
- Frame Seal v2
- New Tip Cartridge v2
- PCR Plate and PCR Plate Frame
- Ion S5™ Precision ID Chef Reagents cartridge
- Ion S5™ Precision ID Chef Solutions cartridge
- Recovery Tubes and Recovery Station Disposable Lid v2
- Enrichment Cartridge v2
- Chip Adapter/Chip assemblies

Load the pipette tip racks and PCR Plate

- Tap  (**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 in 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. See the figure in step 5.
- Slide the catch forward to allow the locking bracket to pivot upward. Load the Tip Cartridge v2 into the *New* Pipette Tip Position, pull the bracket downward, then push the catch backward to lock the bracket and cartridge in place.
- Load a new PCR Plate into the thermal cycler sample block.
- 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 Reagent and Solution cartridges

1. Gently tap the Ion S5™ Precision ID 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 two Library Sample Tubes, each containing 25 µL of diluted library, into Positions A and B on the Reagents cartridge.



- ① Position A (Library)
- ② Position B (Library)
- ③ Position C (NaOH)
- ④ Position D (Empty tube)

Note: For single-chip (single library pool) runs, see the *Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™ /HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0016129).

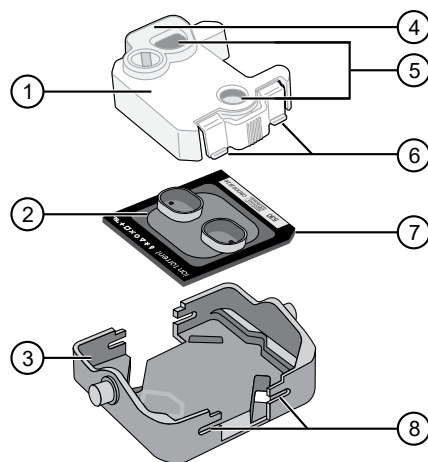
4. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.
5. Gently tap the Ion S5™ Precision ID Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.
6. Load the Solutions cartridge into the Solutions station until it snaps into place and is level on the deck.

Load the Recovery Tubes and Enrichment Cartridge v2

1. Load six Recovery Tubes 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 each 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 with the keyed corners of the chip and bucket aligned, 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. Ensure 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 Flexible tabs
- ⑦ Keyed corner (align with bucket)
- ⑧ Slots

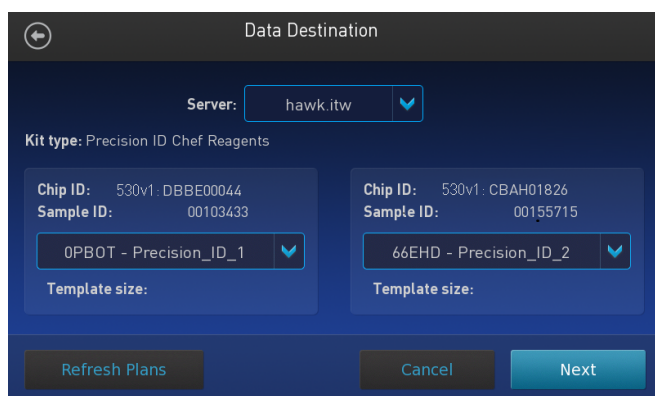
2. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.
3. Ensure that the centrifuge is load-balanced and the chip buckets are securely seated and oriented correctly in the centrifuge so that they pivot 90° outwards when touched. Then close the lid of the Chip-loading centrifuge.

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™ Precision ID 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. Verify 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.
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.
5. When prompted, tap **Start check** to start Deck Scan. Wait while the instrument scans the barcodes of all consumables and reagents to verify their presence and compatibility.
6. When Deck Scan is complete, tap **Next** to display the **Data Destination** screen.
7. Verify that the instrument displays the correct kit type, chip type, chip barcodes, and Planned Run. If the correct Planned Runs do not display, tap the dropdown list  to select the Planned Run for each chip, then tap **Next**.



Data Destination

Server: hawk.itw


Kit type: Precision ID Chef Reagents

| | |
|---|---|
| Chip ID: 530v1:DBBE00044 Sample ID: 00103433 | Chip ID: 530v1:CBAH01826 Sample ID: 00155715 |
| 0PBOT - Precision_ID_1 | 66EHD - Precision_ID_2 |
| Template size: | Template size: |

Refresh Plans Cancel Next

8. On the **Run Options** screen, tap the appropriate option to complete the run, then enter the desired time of run completion, if needed.
9. On the **Run Options** screen, tap **Start run** to start the run.
10. Initialize the Ion S5™, Ion S5™ XL, or Ion GeneStudio™ S5 Series Sequencer or at least 40 minutes before the Ion Chef™ System finishes chip loading.
11. When the run is complete, unload the Ion Chef™ Instrument and sequence the chips immediately.

Unload the chips 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. Remove the chip/bucket assemblies from the Chip-loading centrifuge. Remove the Chip Adapter from the chip, then discard the adapter. Carefully remove the chip from each bucket, then set the chips aside on a clean, static-free surface. Return the buckets to the Chip-loading centrifuge.
3. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then push down on the door until the locks engage.
4. Load one or both chips into sequencers and promptly start the sequencing runs.

Note: Start sequencer initialization ahead of time so that the sequencer is ready to load when the Ion Chef™ Instrument run completes.

Sequence on the Ion S5™ System or Ion GeneStudio™ S5 System

Before you begin

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

Required sequencer cleanings

HID Ion S5™ /Ion S5™ XL Sequencers and HID Ion GeneStudio™ S5 Series Sequencers require cleaning before initialization. Cleaning is normally performed automatically at the completion of the previous sequencing run. When two sequencing runs are performed on a single initialization, the post-run cleaning is performed after the second sequencing run. However, if the "Enable post-run clean" checkbox is deselected 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, see the *Precision ID mtDNA Panels with the HID Ion S5™ /HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017770).

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 *Precision ID mtDNA Panels with the HID Ion S5™ /HID Ion GeneStudio™ S5 System Application 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 Precision ID 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™ Precision ID Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.

Note: Dispose of used reagents appropriately.

5. Ensure that the new Precision ID Wash Solution bottle has been thoroughly mixed. If not, invert the bottle 5 times, then swirl the bottle 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 Precision ID Cleaning Solution bottle.
8. Close the door, then tap **Next**.
9. When initialization is complete (~30–40 minutes), tap **Home**.

The instrument is now ready for a sequencing run.

Start the sequencing run

We recommend that you sequence loaded chips on the sequencer 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.

Note: Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.

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 correct Planned Run has auto-populated, then tap **Review**.
5. If the run is the first of two sequencing runs after an initialization, deselect the **Enable post-run clean** checkbox, then tap **Review**.

Note: When starting the second sequencing run on a single initialization, ensure that the **Enable post-run clean** checkbox is selected, so that the post-run cleaning is performed automatically.

6. Confirm that the pre-populated settings are correct, or make changes using the buttons and dropdown lists if needed.

7. Confirm that the instrument door is closed, then tap **Start run** to start 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.

IMPORTANT! Do NOT reuse the Ion S5™ Sequencing Reagents cartridge after a post-run clean. The cartridge is unusable and should be discarded appropriately.

Review results and analyze in Converge™ Software


Review primary sequencing results in Torrent Suite™ Software, then analyze results in Converge™ Software v2.3.

See the *Precision ID mtDNA Panels with the HID Ion S5™ /HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017770) for example run results, and *Converge™ Software Help* for information on analyzing your data.

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 by the door handle 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.

4. Remove, then discard the box of used pipette tips from the waste tip position.
5. Move the empty Tip Cartridge v2 to the waste tip position.
6. Remove, then discard the
 - Ion S5™ Precision ID Chef Reagents cartridge
 - Ion S5™ Precision ID 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. 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 *Precision ID mtDNA Panels with the HID Ion S5™ /HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017770).
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.

Customer and technical support

For support:

- **In North America**—Send an email to HIDTechSupport@thermofisher.com, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to [thermofisher.com/support](https://www.thermofisher.com/support) to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support

- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

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 at 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.



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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

Revision history: Pub. No. MAN0017934 B.0

| Revision | Date | Description |
|----------|----------------|---|
| B.0 | 3 October 2023 | <ul style="list-style-type: none">• Updated instructions to include loading the PCR Plate Frame. See "Load the pipette tip racks and PCR Plate" on page 2.• Updated instructions to include removing the PCR Plate Frame. See "Remove and dispose of used consumables" on page 6.• Updated the Converge™ Software version to v.2.3. |
| A.0 | 9 October 2018 | New Quick Reference. |

The information in this guide is subject to change without notice.

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