

# Ion PI™ Hi-Q™ Chef Kit

Catalog Number A27198

Pub. No. MAN0010968 Rev. D.0

**Note:** For safety and biohazard guidelines, see the “Safety” appendix in the following product documentation: *Ion PI™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010967). 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

1. Sign in to Torrent Suite™ Software on the Ion Torrent™ Server.
2. Select the **Plan** tab, click **Templates**, choose the application that you want to run (such as AmpliSeq DNA) then select either **Plan Run** or **Plan New Run**.
3. In the Planned Run wizard, review each screen, then edit if needed.
  - a. In the **Kits** step:
    - Select **Ion Chef**.
    - Select the **Ion PI™ Hi-Q™ Chef Kit** from the **Template Kit** dropdown list.
    - Select **Ion PI™ Hi-Q™ Sequencing 200 Kit** from the **Sequencing Kit** dropdown list.
    - Select the barcode set, if barcodes are used.
    - Select the number of flows.
  - b. In the **Plan** step:
    - Enter a Planned Run name.
    - Select reference files appropriate to your run.
    - Enter the number of barcodes used in your combined library.

- Enter or scan the barcode of the Ion Chef™ Library Sample Tube into the **Sample Tube Label** field.
- Enter sample names for each sample barcode.

4. When you have completed your selections, click **Plan Run** at the bottom right of the **Plan** step screen to save the run. A Planned Run is created for each chip and appears on the Planned Runs page with the name that you specified, appended with sample name. The plan is automatically used by the Ion Chef™ System when the associated samples are loaded.

## Dilute the sample libraries

Dilute the two libraries or combined library pools with nuclease-free water according to the following table. Prepare a fresh dilution of each library before use with the Ion Chef™ System, and use the library dilutions within 48 hours.

Library	Recommended concentration <sup>[1]</sup>
Ion Total RNA-Seq	50–100 pM
Ion AmpliSeq™ Exome RDY	50–100 pM
Ion AmpliSeq™ Transcriptome Human Gene Expression	50–100 pM
Ion AmpliSeq™ Transcriptome Mouse Gene Expression	70 pM
Ion AmpliSeq™ Comprehensive Cancer Panel	50 pM
Oncomine™ cfDNA Assays	50 pM
Human CEPH Control 200 library <sup>[2]</sup>	Dilute 1 µL into 24 µL nuclease-free water

<sup>[1]</sup> Recommendations are based on qPCR quantification. If libraries are quantified with an Agilent™ 2100 Bioanalyzer™ instrument, a higher calculated concentration may need to be used for equivalent input.

<sup>[2]</sup> Obtained from the Ion PI™ Controls 200 Kit (Cat. No. 4488985).

## Prepare the consumables

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

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

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

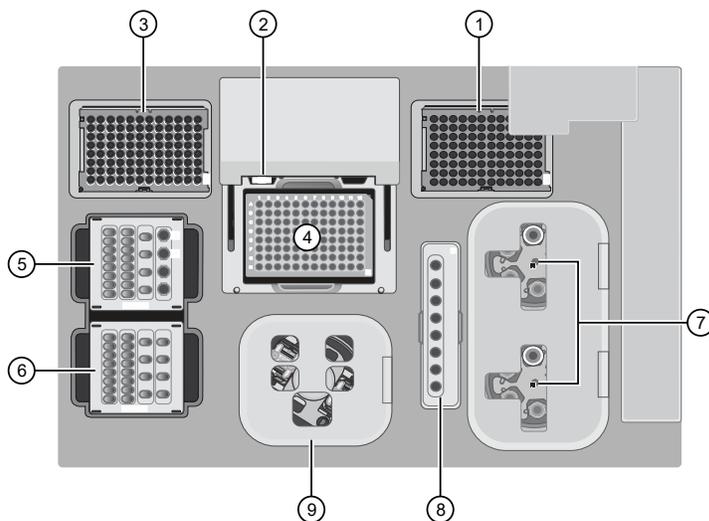
## Load the Ion Chef™ System

### IMPORTANT!

- Rated centrifuge speeds are 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 PI™ 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 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.

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



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

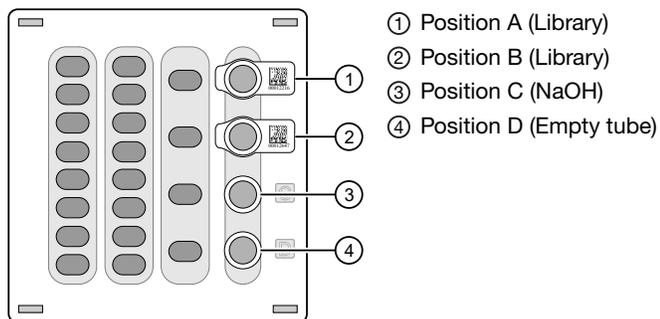
- |  |   |
|--|---|
| ① Empty tip rack (move from new Tip Cartridge v2 position) | ⑥ Ion PI™ Hi-Q™ Chef Solutions cartridge                |
| ② Frame Seal v2  | ⑦ Recovery Tubes and Recovery Station Disposable Lid v2 |
| ③ New Tip Cartridge v2                                     | ⑧ Enrichment Cartridge v2                               |
| ④ PCR Plate and PCR Plate Frame                            | ⑨ Chip Adapter/Chip assemblies                          |
| ⑤ Ion PI™ Hi-Q™ Chef Reagents cartridge                    |   |

### Load the pipette 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 in the *Used (Waste)* Pipette Tip Position, then change gloves.
4. 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.
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.
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 PI™ Hi-Q™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
2. If bubbles are present below the surface of the liquid, repeat step 1.
3. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.
4. Uncap, then load the two Library Sample Tubes, each containing 25 µL of diluted library or combined library pool, into Positions A and B on the Reagents cartridge.



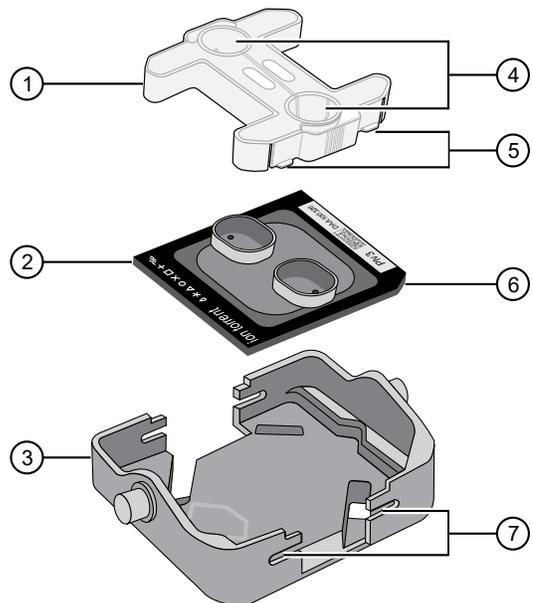
5. Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.
6. Gently tap the Ion PI™ Hi-Q™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.
7. Load the Solutions cartridge into the Solutions station until it snaps into 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.

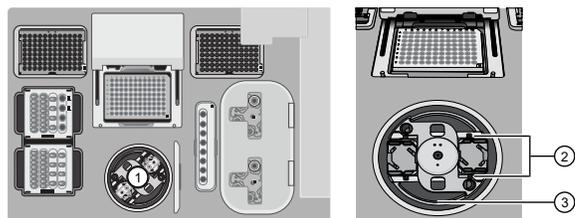
## Load the Chip-loading centrifuge

1. Load each chip into a centrifuge bucket, then attach a Chip Adapter to the assembly.



- ① Chip adapter
- ② PI Chip v3
- ③ Bucket
- ④ Ports (align with chip)
- ⑤ Tabs
- ⑥ Keyed corner (align with bucket)
- ⑦ Slots

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



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

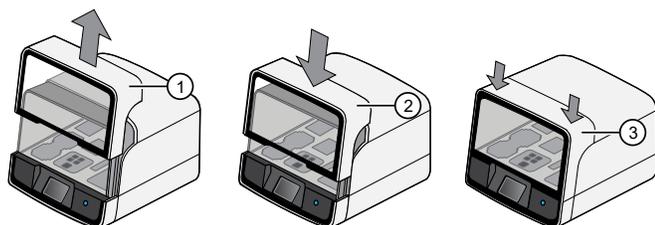
3. Close the lid of the Chip-loading centrifuge.

## 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 PI™ Hi-Q™ 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

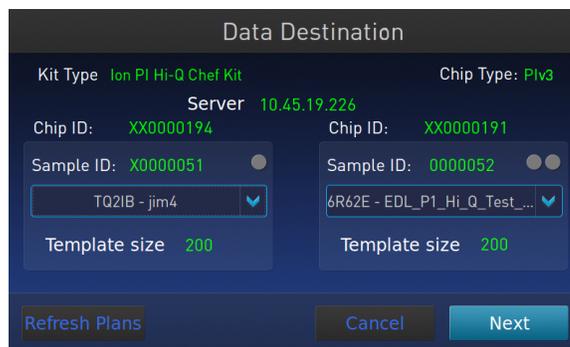
1. Ensure that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, tap **Set up run**.
3. Tap **Step by step** to have the instrument lead you through the instrument setup, or tap **Quick Start** to skip the instrument setup screens.
4. If you selected **Step by step**, the **Run Options** screen opens. 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.



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

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

8. Confirm that the instrument displays the correct kit name, chip types, chip barcodes, and Planned Runs. If the correct Planned Runs do not display, tap the dropdown menu  to select the Planned Run for each chip, 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.

The Ion Chef™ Instrument provides two options for obtaining quality control (QC) samples that can be used to evaluate templating efficiency. Depending on your selection, the QC samples will be made available either during or after the run. In either case, you can obtain unenriched samples from the corresponding Library Sample Tubes at Positions A and B on the Ion PI™ Hi-Q™ Chef Reagents cartridge, or enriched samples from Positions A and E on the Enrichment Cartridge v2.

By selecting	You can obtain the QC samples
Time	immediately after the run ends, at the time you specify (12.75 hours after run start).
Pause	when the instrument pauses operation before the chip loading step (10.8 hours into the run).

**Note:** The DNA library in the Library Sample Tube loaded in Position A is templated onto ISPs that can be sampled in Position E of Enrichment Cartridge v2 after a run. The DNA library in the Library Sample Tube loaded in Position B is templated onto ISPs that can be sampled in Position A of Enrichment Cartridge v2.

**Note:** Select **Pause** if you are uncertain of library quality and want to evaluate templating efficiency before chips are loaded. If you do not pause the run, collect QC samples after the run and save until sequence analysis is complete to have available for troubleshooting.

10. On the **Run Options** screen, tap **Start run** to start the run.
11. Clean and initialize the Ion Proton™ Sequencer approximately 1.5 hours before the Ion Chef™ Instrument finishes chip loading.
12. 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 10.8 hours after the start of the run).
  - a. When prompted to remove the QC sample, open the instrument door.

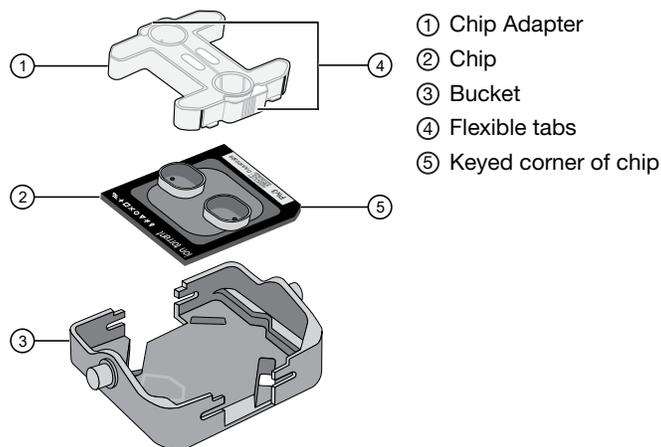
- b. Transfer the QC samples (entire volume) from Positions A and B of the Ion PI™ Hi-Q™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.
- c. Analyze the QC samples.
- d. If you are performing quality assessment of enriched samples, transfer QC samples from positions A and E of the Enrichment Cartridge v2 to two new labeled microcentrifuge tubes. See Appendix B, "Supplemental procedures", of the *Ion PI™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010967) for more information.
- 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 chips immediately. You can collect QC samples from the Reagents and/or Enrichment cartridges if you have not done so already.

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

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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 one or both chips into a sequencer, then promptly start the sequencing run or runs.

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

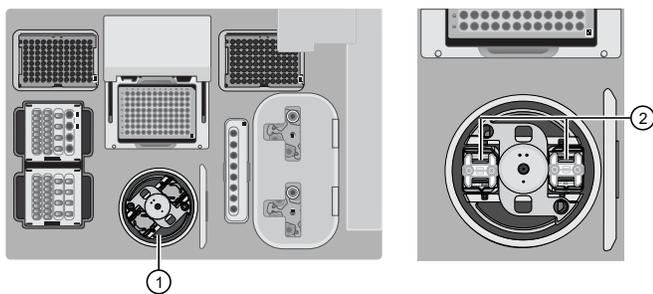
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**IMPORTANT!**

- 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.
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## 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. Open the lid of the Chip-loading centrifuge, then unload both adapter/chip/bucket assemblies from the instrument.



① Chip-loading centrifuge      ② Adapter/chip/bucket assemblies

3. Unload each chip from the adapter/chip/bucket assembly.
  - a. Apply pressure to the flexible adapter 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.

## Clean and initialize the Ion Proton™ Sequencer

At least one hour before the completion of the Ion Chef™ Instrument run, clean, and initialize the Ion Proton™ Sequencer as described in the *Ion PI™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010967).

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

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## Sequence the chip on the Ion Proton™ Sequencer

**IMPORTANT!** Observe the following when sequencing the chips:

- The Ion Proton™ Sequencer must be cleaned and initialized before sequencing the chips.
- Empty the waste container *before* you start the sequencing run.
- Do not use reagents from other sequencing kits for sequencing chips that are prepared by the Ion Chef™ System.
- To avoid damage due to electrostatic discharge (ESD), do not place the chip directly on the bench or any other surface. Always place the chip either on the Ion Proton™ Sequencer grounding plate or in the custom Ion centrifuge adapter/rotor bucket.
- To avoid ESD damage, do not wear gloves when transferring chips to and from the instrument.

Sequence the loaded chips on the Ion Proton™ Sequencer as soon as possible after unloading the Ion Chef™ Instrument. If not, place the chips into a chip storage container and store at 4°C until you are ready to sequence (up to 6 hours maximum).

**IMPORTANT!** Do not start the sequencing run with the loaded chip. Use a used chip for the line cleaning at the start of the run.

1. With the used chip from initialization still in the chip clamp, tap **Run** on the main menu, then tap **Next**, and confirm that "Cleaning fluid lines" displays on the instrument touchscreen. Observe the chip for leaks. The **Run Setup** screen automatically populates the fields when the Ion Proton™ Sequencer connects to the server.
2. Follow the on-screen instructions to empty the waste bottle, load the cleaning chip, then clean the fluid lines of the Ion Proton™ Sequencer. After line cleaning, tap **Next**.
3. When prompted, enter or scan the barcode of the chip that you intend to sequence, then tap **Next**.
4. Confirm that the pre-populated settings are correct. Make changes using the buttons and drop-down lists, if needed.
5. Remove the used chip from the chip clamp, then secure the chip that is loaded with template-positive ISPs. Close the chip compartment lid, wait until the Chip Status icon in the lower left corner of the screen indicates "Ready" , then tap **Next** to start the sequencing run.

The system calibrates the chip (~1 minute), then starts the sequencing run.

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

6. If you are sequencing a loaded chip that you have stored, remove the chip from 4°C storage (but keep it in the chip container) at least 20 minutes before the end of the first run to warm to room temperature.

7. When the first run is complete, empty the waste container, then sequence the remaining chip as soon as possible.

**IMPORTANT!** After the first sequencing run is complete:

- Empty the waste container *before* you start the next sequencing run.
- Leave the first chip in the instrument while you start the second run. Load the second chip when prompted.

When the run is complete, the touchscreen returns to the main menu. Use Torrent Suite™ Software to review the results.

## 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.
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.

**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.

6. Remove, then discard the
  - Ion PI™ Hi-Q™ Chef Reagents cartridge
  - Ion PI™ Hi-Q™ 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 *Ion PI™ Hi-Q™ Chef Kit User Guide* (Pub. No. MAN0010967).
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.

## Limited product warranty

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

**Revision history:** Pub. No. MAN0010968 D.0

Revision	Date	Description
D.0	18 September 2023	<ul style="list-style-type: none"><li>• Updated instructions to include loading the PCR Plate Frame. See "Load the pipette tip racks and PCR Plate" on page 2.</li><li>• Updated instructions to include removing the PCR Plate Frame. See "Remove and dispose of used consumables" on page 6.</li></ul>
C.0	2 July 2019	<ul style="list-style-type: none"><li>• Updated for Torrent Suite™ Software 5.12</li><li>• Guidance clarified for warming loaded chips that had been stored before sequencing.</li><li>• Clarification made in "Prepare the libraries and consumables" to dilute barcoded library pools, if used.</li></ul>
B.0	23 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>• Graphics enhanced.</li></ul>
A.0	27 March 2015	New quick reference for the Ion PI™ Hi-Q™ Chef Kit.

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

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