

# Ion PI™ Hi-Q™ Chef Kit

Catalog Number A27198

Pub. No. MAN0010968 Rev. B.0

**Note:** For safety and biohazard guidelines, see the “Safety” appendix in the *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. Open the Torrent Browser on a computer connected to your Ion Chef™ System.
2. Select the **Plan** tab, click **Templates**, choose the application that you want to run (such as Whole Genome) then select either **Plan Run** or **Plan New Run**.
3. In the Planned Run wizard, review each screen, then edit if needed. In the **Kits** tab, 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, then enter or scan the barcodes of the Ion Chef™ Library Sample Tubes into the appropriate Sample Tube Label fields of the **Plan** tab.
4. When you have completed your selections, click **Plan Run** at the bottom right of the **Plan** tab 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 plans are automatically used by the Ion Chef™ System when the associated samples are loaded.

## Dilute the sample libraries

Dilute the 2 stock libraries 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 TargetSeq™ Exome	50 pM
Ion AmpliSeq™ Exome	50 pM
Ion AmpliSeq™ Exome RDY	50–100 pM
Ion AmpliSeq™ Transcriptome Human Gene Expression	50–100 pM
Ion AmpliSeq™ Comprehensive Cancer Panel	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 libraries and 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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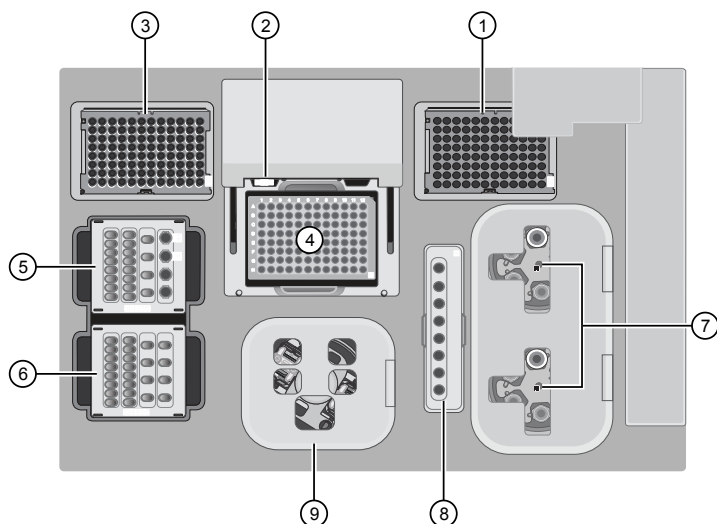
2. Pipet 25 µL of each diluted library to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).
3. Cap, then store the two Library Sample Tubes on ice until you are ready to load them onto the Ion Chef™ Instrument.
4. Remove all cartridges and consumables from their packaging, then place them on the bench next to the Ion Chef™ Instrument.

## Load the Ion Chef™ System

### IMPORTANT!


- Rated centrifuge speeds are only intended 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 has been cleaned following the last use.
- Ensure all components are clean and dry before loading them onto the Ion Chef™ Instrument.
- Ensure the Reagents and Solutions station compartments are free of condensate before loading components.

Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure:



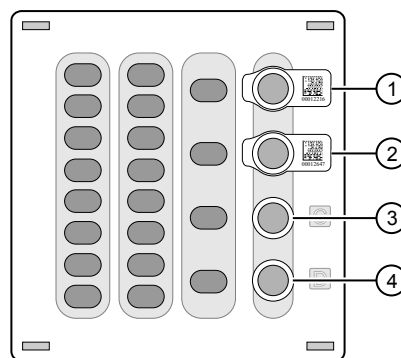
- Empty tip rack (move from new Tip Cartridge position)
- Frame Seal v2
- New Tip Cartridge v2
- PCR plate
- Ion PI™ Hi-Q™ Chef Reagents cartridge
- Ion PI™ Hi-Q™ Chef Solutions cartridge
- Recovery Tubes and Recovery Station Disposable Lid v2
- Enrichment Cartridge v2
- Chip Adapter/Chip assemblies

## Load the pipette tip racks and PCR plate

- Touch  (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 to 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*.
- 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, then slide a new Frame Seal v2 under the automated heated cover.

## Load the Reagents and Solutions cartridges

- Gently tap the Ion PI™ Hi-Q™ Chef Reagents cartridge on the bench to force the reagents to the bottoms of the tubes.
- Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.
- Uncap, then load the two Library Sample Tubes, each containing 25  $\mu$ L of diluted library, into Positions A and B on the Reagents cartridge.



- Position A (Library)
- Position B (Library)
- Position C (NaOH)
- Position D (Empty tube)

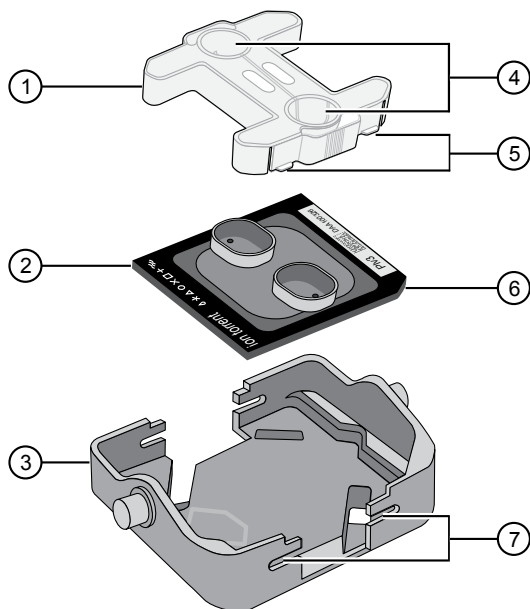
- Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.
- Gently tap the Ion PI™ Hi-Q™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of the tubes.
- Load the Solutions cartridge into the Solutions station until it snaps into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

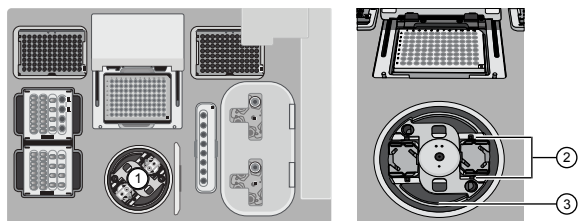
## Load the Chip-loading centrifuge

1. Load 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 groove
- ③ Chip-loading centrifuge

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

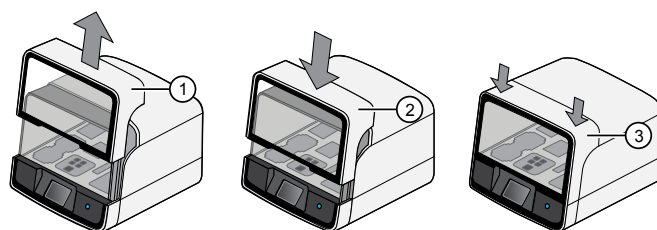
## Confirm that consumables are correctly installed

Before continuing:

- 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

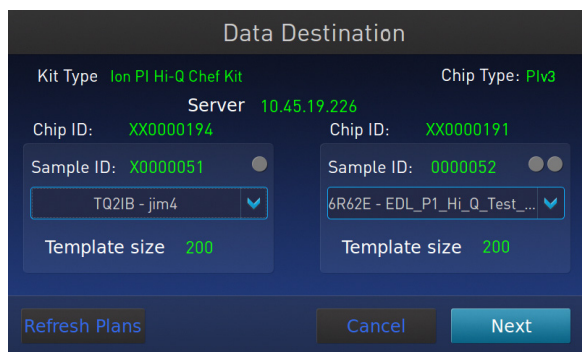
1. Ensure that you have loaded the instrument with all kits and consumables.
2. On the Ion Chef™ Instrument home touchscreen, touch **Set up run**.
3. Touch **Step by Step** to have the instrument lead you through the instrument setup, or touch **Quick Start** to skip the instrument setup screens.
4. 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.



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

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

7. Confirm that the instrument displays the correct kit name, chip types, chip barcodes, and Planned Runs. If the correct Planned Runs do not display, touch the dropdown menu  to select the Planned Run for each chip, then touch **Next**.




8. On the Run Options screen, touch the appropriate option to complete the run, then enter the desired time of run completion, if needed.
9. On the Run Options screen, touch **Start run** to start the run.
10. Clean and initialize the Ion Proton™ Sequencer approximately 1.5 hours before the Ion Chef™ System finishes chip loading.
11. 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 touch **Continue** to complete the run.
12. 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.

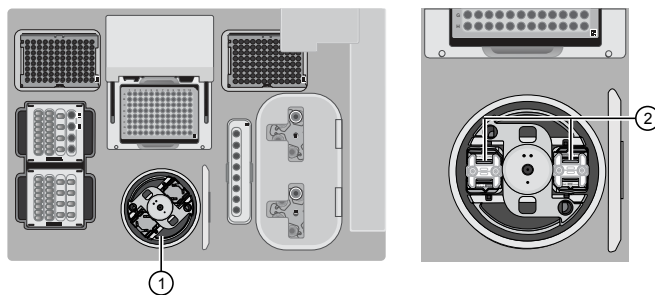
## Clean and initialize the Ion Proton™ Sequencer

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).

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

## Unload the chips for sequencing

1. Open the instrument door:
  - a. In the instrument touchscreen, touch  (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 chip/bucket assemblies from the Ion Chef™ Instrument.

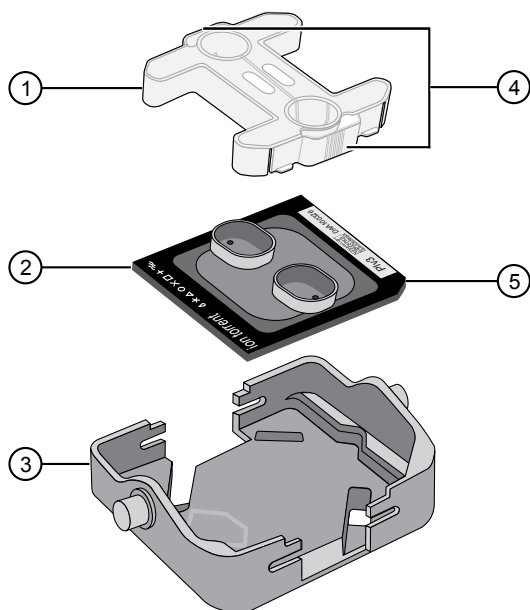


- ① Chip-loading centrifuge
- ② Remove

To unload each chip:

- a. Remove the chip/bucket assembly from the Chip-loading centrifuge.
- b. Apply pressure to both ends of the Chip Adapter, then remove and discard the Chip Adapter.

- c. 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.



- ① Chip Adapter
- ② Ion chip
- ③ Bucket
- ④ Press and remove adapter
- ⑤ Remove from bucket carefully

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 Ion Proton™ Sequencers and promptly start the sequencing 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).

**IMPORTANT!** If you chose to store a loaded chip, remove the chip from the container and place it on a clean surface in the dark to warm to room temperature at least 20 minutes before running it.

## Sequence the chip on the Ion Proton™ Sequencer

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

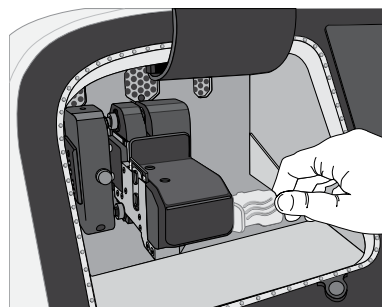
- The Ion Proton™ Sequencer must be cleaned and initialized before sequencing the chips.
- Empty the waste container *before* you start the sequencing run.
- Do not use reagents from other sequencing kits for sequencing chips prepared by the Ion Chef™ System.
- To avoid damage due to electrostatic discharge (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, store them under the recommended conditions.

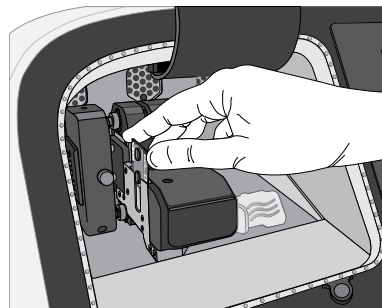
**IMPORTANT!** Do not start the sequencing run with the loaded chip. Use a used chip for the line cleaning at the start of the run.

1. With the used chip from initialization still in the chip clamp, press **Run** on the Main Menu, then touch **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, touch **Next**.
3. When prompted, enter or scan the barcode of the chip that you intend to sequence, then touch **Next**.
4. Confirm that the pre-populated settings are correct. Make changes using the buttons and drop-down lists, if needed.
5. Load the chip:
  - a. Pull the metal tab forward to release the chip clamp.

**IMPORTANT!** Do not wear gloves when transferring the chips on and off the instrument.



- b. Remove the chip currently in the clamp.

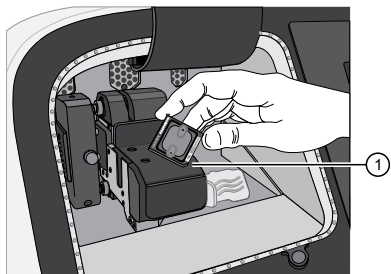


- c. Place the chip to be sequenced in the chip clamp with the chip notch in the bottom-front corner.

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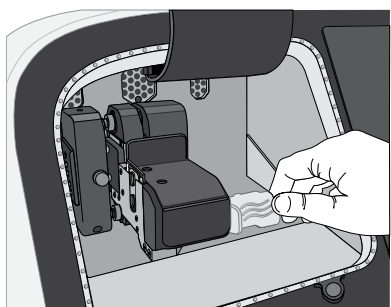
**IMPORTANT!** Do not force the chip into the clamp. If the chip does not fit easily in the clamp, ensure that the notch is oriented as shown:


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① Notch

- d. Push the metal tab back until it clicks to engage the clamp.



- e. Wait until the Chip Status icon in the lower left corner of the screen indicates "Ready" , then touch **Next**.

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

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**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.

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6. If you are sequencing a loaded chip that you have stored, remove the stored chip from the chip container in the refrigerator 20 minutes before the end of the first run, then place it on a clean surface in the dark 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.

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**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 initiate the second run. Load the second chip when prompted.
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When the run is complete, the touchscreen returns to the Main Menu. Use the Torrent Browser to review the results.


## Clean the Ion Chef™ Instrument

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

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### Remove and dispose of used consumables

1. Touch  (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 from the thermal cycler sample block.
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 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 Lids 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, touch **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 touch **Next**.

4. With the door closed, touch **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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**Revision history:** MAN0010968

Revision	Date	Description
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

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