

Ion 540™ Kit – Chef

Catalog Numbers A27759, A30011

Pub. No. MAN0014119 Rev. D.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851). 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**, locate 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 and edit as needed. In the **Kits** tab, select **Ion Chef**, select the **Ion 540™ Kit – Chef** from the **Template Kit** dropdown list, select **Ion S5™ Sequencing 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. The run is listed on the Planned Runs page under the name that you specified and is automatically used by the Ion Chef™ System when the associated sample is loaded.

Dilute the libraries

Dilute the two (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 ^[1]
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
Oncomine™ cfDNA Assays	50 pM
Human CEPH Control 200 Library ^[2]	Dilute 1 µL into 24 µL nuclease-free water

^[1] 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.

^[2] Obtained from the Ion S5™ Controls Kit (Cat. No. A27760).

Prepare the libraries and consumables

1. Unbox the Ion 540™ Chef Reagents cartridge 45 minutes prior to use and allow it to warm to room temperature.

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

2. Pipet 25 µL of each diluted library (see “Dilute the libraries” on page 1) to the bottom of the appropriate Ion Chef™ Library Sample Tube (flagged tubes).
3. Cap and store the two diluted DNA libraries on ice until you are ready to load them onto the Ion Chef™ Instrument.
4. Remove all cartridges and consumables from their wrappings and boxes, then place them on the bench next to the Ion Chef™ Instrument.

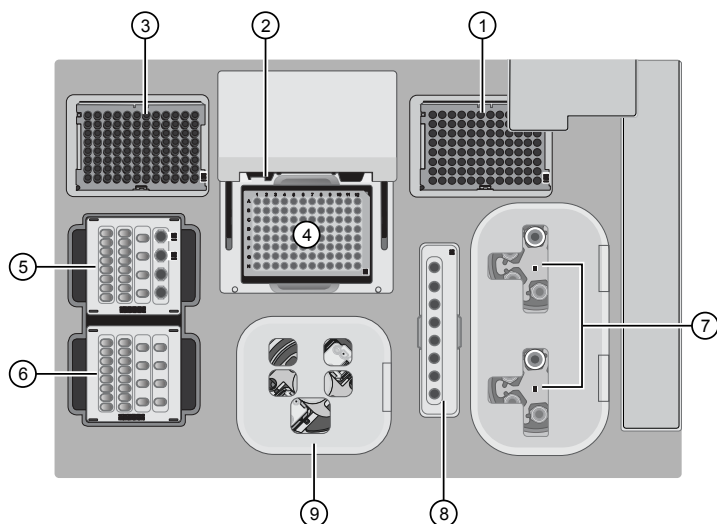
IMPORTANT! Prior to use, gently tap the Reagents and Solutions cartridges on the bench to force the reagents to the bottoms of the tubes.

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 540™ Kit – Chef 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:



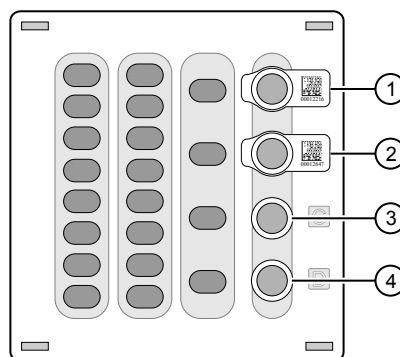
- Empty tip rack (move from new Tip Cartridge position)
- Frame Seal v2
- New Tip Cartridge
- PCR plate
- Ion 540™ Chef Reagents cartridge
- Ion S5™ Chef Solutions cartridge
- Recovery Tubes and Recovery Station Disposable Lid v2
- Enrichment Cartridge v2
- Chip Adapter/Chip assemblies

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 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 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 540™ 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 µL of diluted library, into Positions A and B on the Reagents cartridge.



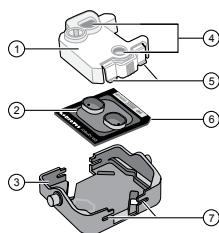
- Position A (Library)
 - Position B (Library)
 - Position C (NaOH)
 - Position D (Empty tube)
- Uncap both the tube of NaOH in Position C and the empty tube in Position D on the Reagents cartridge.
 - Gently tap the Ion S5™ Chef Solutions cartridge on the bench to force the reagents to the bottoms of 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 prepared sequencing chip 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. 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 Ports (align with chip)
- ⑤ Flexible tabs
- ⑥ Keyed corner (align with bucket)
- ⑦ Slots


2. Load the adapter/chip/bucket assemblies into the Chip-loading centrifuge.
3. 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.

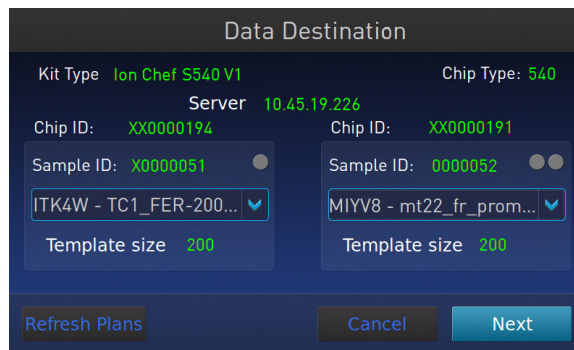
Confirm that consumables are correctly installed

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 540™ 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. Confirm 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.
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 type, chip type, chip barcodes, and Planned Run. 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 begin the run.


10. Initialize the Ion S5™ or Ion S5™ XL Sequencer at least 40 minutes 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 11 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 540™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.
 - c. Analyze the QC samples.

Note: For detailed protocols, see the

 - *Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide* (Pub. No. MAN0016387) or
 - *Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0016388)

available at thermofisher.com/order/catalog/product/4468656.
 - 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 "Supplementary procedures" of the *Ion 540™ Kit – Chef User Guide* (Pub. No. MAN0010851) 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.

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.

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.
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 S5™ Sequencers and promptly start the sequencing runs.

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! 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.

When a manual cleaning of the sequencer is required

The Ion S5™ Sequencer and Ion S5™ XL Sequencer 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 540™ Kit – Chef User Guide* (Pub. No. MAN0010851).

If an Ion S5™ Sequencer or an Ion S5™ XL 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 540™ Kit – Chef User Guide*.

Initialize the sequencer

1. In the instrument touchscreen main menu, touch **Initialize**. The door, chip, and Reagent cartridge clamps unlock.
2. When prompted, remove the Ion S5™ 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™ Sequencing Reagents cartridge with a new cartridge equilibrated to room temperature.
5. Invert a new Ion S5™ 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 touch **Next**.
9. When initialization is complete (~30–40 minutes), touch **Home**.

The instrument is now ready for a sequencing run.

Start the sequencing run

We recommend that you sequence loaded chips on the Ion S5™ or Ion S5™ XL 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 may result in sequencing run failure and loss of sample.

1. After completion of initialization, press **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 press **Next**.
4. Confirm that the correct Planned Run has auto-populated, then press **Review**.
5. (Optional) If this is to be the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then press **Review**.

Note:

- Failure to deselect the checkbox results in a cleaning performed automatically after the first run. A second run is not available.
 - When starting the second sequencing run on a single initialization, ensure the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.
6. Confirm the remaining pre-populated settings are correct, or make changes using the buttons and dropdown lists if necessary.
 7. Confirm that the instrument door is closed, then press **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 may 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 the Torrent Browser 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. 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 to the waste tip position.
6. Remove, then discard the
 - Ion 540™ 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 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 540™ Kit – Chef User Guide* (Pub. No. MAN0010851).
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: Pub. No. MAN0014119

Revision	Date	Description
D.0	20 January 2017	<ul style="list-style-type: none">Users referred to new ISP quality control user guides:<ul style="list-style-type: none"><i>Ion Sphere™ Assay on the Qubit™ 2.0 Fluorometer User Guide</i> (Pub. No. MAN0016387)<i>Ion Sphere™ Assay on the Qubit™ 3.0 Fluorometer User Guide</i> (Pub. No. MAN0016388)Web links updated
C.0	27 July 2016	<ul style="list-style-type: none">Updated to support new Ion S5™ instrument user interface in Torrent Suite™ Software v5.2Graphics enhanced and workflows reorganized for ease of use
B.0	13 November 2015	Update to user guide for 2 sequencing runs per initialization that accompany new SKU (A30011)
A.0	26 August 2015	New quick reference

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