

Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef

Catalog Numbers A34461, A34019

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion 510™ & Ion 520™ & Ion 530™ Chef Kit User Guide* (Pub. No. MAN0016854). 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

IMPORTANT! If you are using the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef with libraries that were prepared using Oncomine™ panels, see the corresponding Oncomine™ Assay User Guide for assay-specific instructions on creating a Planned Run in Torrent Suite™ Software.

For more information on creating a Planned Run in Torrent Suite™ Software, including a complete description of each field in the **Create Plan** workflow bar, see the *Torrent Suite™ Software Help*, available by clicking the **Help** button in the software.

1. Sign in to the Torrent Suite™ Software.
2. In the **Plan** tab, in the **Templates** screen, select the application that you want to run (such as AmpliSeq™ DNA) from the left navigation menu, then click either **Plan New Run** or **⚙ (Actions) ▶ Plan Run** in the row of the specific Planned Run template.
3. In the **Create Plan** workflow bar, review the **IonReporter** and **Research Application** steps, then make selections appropriate to your run. Click **Next**.
4. In the **Kits** step, make the following selections:
 - a. Select **Ion GeneStudio™ S5 System** from the **Instrument** dropdown list.
 - b. Select the appropriate chip type from the **Chip Type** dropdown list.
 - c. Select the library kit used to prepare your libraries from the **Library Kit Type** dropdown list.
 - d. (Optional) For barcoded libraries, select the barcode set used during library preparation from the **Barcode Set** dropdown list.
 - e. Select **IonChef** for **Template Kit**, then select **Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef** from the **Template Kit** dropdown list.
 - f. Select **Ion S5 Sequencing Kit** from the **Sequencing Kit** dropdown list.
 - g. Enter the appropriate number of flows in the **Flows** field.
 - h. In the **Advanced Settings** pane, select **Customize**, then select the templating protocol from the **Templating Protocol** dropdown list.
 - i. Select or edit the remaining optional information fields appropriately for your run.
 - j. Click **Next**.
5. Review the **Plugins** and **Projects** steps, then make selections appropriate to your run.
6. In the **Plan** step, enter or make the following selections:
 - a. Enter a Run Plan Name, then select Reference and BED files appropriate to your run.
 - b. Enter the number of barcodes you are using in your combined library in the **Number of barcodes** field, then click to the right of this field. Edit the auto-populated list of barcodes that appears, if needed.
 - c. Scan or enter the barcode of the Library Sample Tube into the **Sample Tube Label** field.

- d. Scan or enter the chip barcode into the **Chip Barcode** field.
 - e. Enter a sample name for each barcode in the appropriate **Sample Name (required)** fields.
7. After you have completed your selections, click **Plan Run** at the bottom right of the **Plan** step screen to save the run.

The run is listed in 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 sample libraries

Dilute the individual or combined 200-bp or 400-bp stock libraries according to the following table. If needed, use polyclonality and low-quality filter results from a sequencing run performed with ISPs templated at the starting concentration, then titrate up or down to achieve optimal concentrations.

IMPORTANT! Do NOT mix template sizes (for example, 200-base and 400-base template run lengths) in a single templating run.

Library length ^[1]	Recommended concentration	Molecules per 25-µL input volume	Templating size in Planned Run setup
<300 bp	25–60 pM	600–900 × 10 ⁶	200
≥300 bp	25–60 pM	600–900 × 10 ⁶	400

^[1] Library length is average insert length plus adapter length.

Dilute the control library (if used)

If you are running a control library for troubleshooting purposes, dilute the Human CEPH Control 200 Library or *E. coli* DH10B Control 400 Library, obtained from the Ion S5™ Controls Kit Plus (Cat. No. A30729), as follows:

Control library	Volume of library	Volume of nuclease-free water
Human CEPH Control 200 Library	1 µL	49 µL
<i>E. coli</i> DH10B Control 400 Library	1 µL	124 µL

Prepare the consumables

1. Unbox the Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge 45 minutes before use and allow it to warm to room temperature.

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

2. 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 Reagents and Solutions cartridges on the bench to force the reagents to the bottoms of the tubes.
- Briefly centrifuge the tube of NaOH (Reagents cartridge, position C) to collect the contents.

Add the diluted libraries to the Ion Chef™ Library Sample Tube

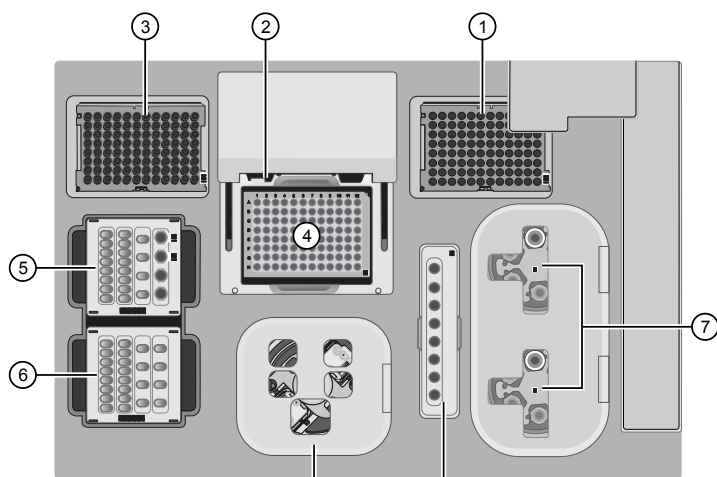
1. Pipet 25 µL of each diluted library or combined library (see “Dilute the sample libraries” on page 2) to the bottom of the appropriate Library Sample Tube (barcoded tubes). Load one individual library or combined library per tube.
2. Cap, then store the sample tubes on ice until you are ready to load them onto 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 510™ & Ion 520™ & Ion 530™ 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:



- ① Empty tip rack (move from new Tip Cartridge position)
- ② Frame Seal v2
- ③ New Tip Cartridge
- ④ PCR Plate
- ⑤ Ion 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge
- ⑥ Ion S5™ Chef Solutions cartridge
- ⑦ Recovery Tubes and Recovery Station Disposable Lid v2
- ⑧ Enrichment Cartridge v2
- ⑨ Chip Adapter/Chip assemblies

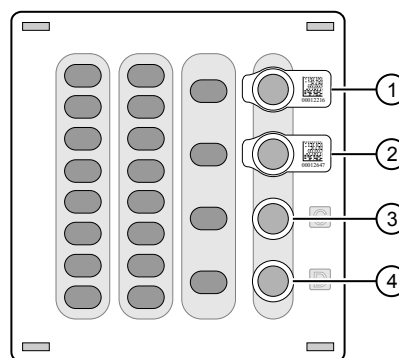
Load the pipette tip racks and PCR Plate

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, then slide a new Frame Seal v2 under the automated heated cover.

Load the Reagents and Solutions cartridges

1. Gently tap the Ion 510™ & Ion 520™ & Ion 530™ 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, 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)

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 S5™ 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.

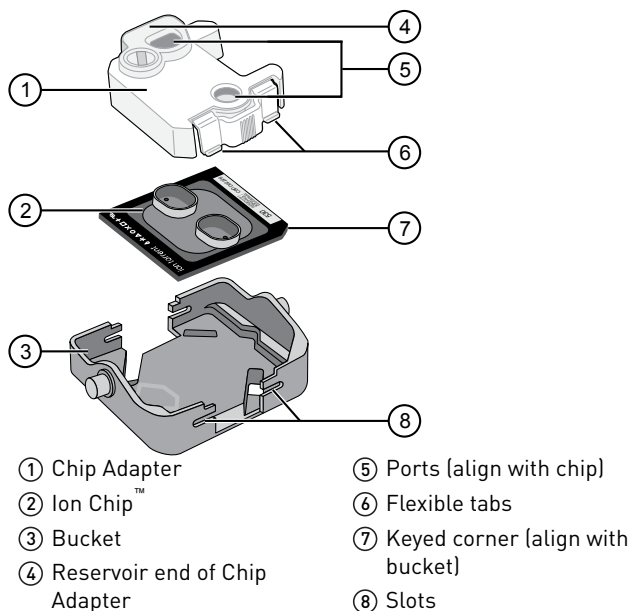
Load the Recovery Tubes and Enrichment Cartridge v2

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 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.



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 510™ & Ion 520™ & Ion 530™ 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.

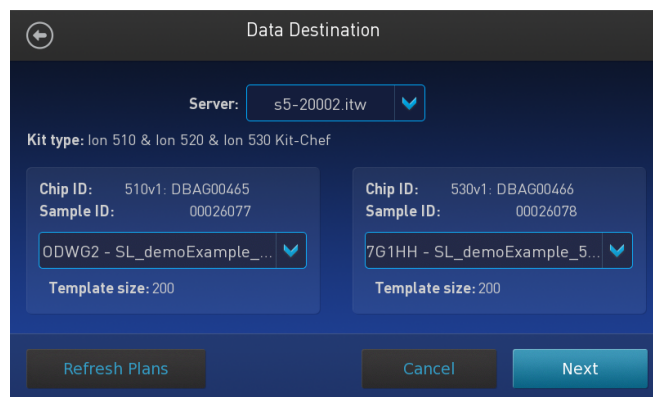
Start the Ion Chef™ run

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. 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 confirm their presence and compatibility.
6. When Deck Scan is complete, tap **Next** to display the **Data Destination** screen.
7. Ensure that the instrument displays the correct kit type(s), chip positions (mixed-chip run), 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**.



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 sequencer at least 50 minutes before the Ion Chef™ System finishes chip loading. For more information, see “Initialize the sequencer” on page 5.
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).


Note: For the Ion 510™ Chips, the time varies for different libraries.

- 200–bp libraries: 12 hours 50 minutes.
- 400–bp libraries: 11 hours 50 minutes.

- 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 510™ & Ion 520™ & Ion 530™ Chef Reagents cartridge on the instrument deck to two new labeled microcentrifuge tubes.
- c. 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.

- d. Analyze the QC samples. For more information, see "Quality control of Ion 510™ ISPs, Ion 520™ ISPs, or Ion 530™ ISPs" in Appendix B "Supplementary procedures" of the *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854).
 - e. Close the instrument door, then tap **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, 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.
3. Unload each 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 one or both chips into a sequencer and promptly start the sequencing run(s).

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, Ion S5™ XL Sequencer, and Ion GeneStudio™ S5 Series 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 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 on how to perform a manual cleaning, see the *Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef User Guide* (Pub. No. MAN0016854).

If the 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 510™ & Ion 520™ & Ion 530™ Kit – Chef 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™ 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 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 correct Planned Run has auto-populated. If this run is the first of two sequencing runs on this initialization, **deselect** the **Enable post-run clean** checkbox, then tap **Review**.

IMPORTANT!

- 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 that the **Enable post-run clean** checkbox is selected so that the post-run cleaning is performed automatically.

5. Confirm that the remaining pre-populated settings are correct, or tap **Edit** to make changes if needed.
6. 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 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 510™ & Ion 520™ & Ion 530™ 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. 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 510™ & Ion 520™ & Ion 530™ Chef Kit User Guide* (Pub. No. MAN0016854).
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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Revision history: Pub. No. MAN0016855

Revision	Date	Description
D.0	05 December 2018	<ul style="list-style-type: none">• Updated for Ion GeneStudio™ S5 Systems.• Clarification made to dilute barcoded library pools, if used.• Updated for Torrent Suite™ Software 5.10; Planned Run creation topics reorganized for ease of use.• Ion S5™ Controls Kit replaced with Ion S5™ Controls Kit Plus.• Updated reference to ISP quality control procedure.
C.0	20 October 2017	<ul style="list-style-type: none">• Updated for Torrent Suite™ Software 5.6.• Support added for performing mixed chip runs on the Ion Chef™ Instrument.
B.0	21 August 2017	<ul style="list-style-type: none">• Correction of product skus
A.0	12 June 2017	<ul style="list-style-type: none">• New document

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