

Ion AmpliSeq™ Library Preparation on the Ion Chef™ System

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Note: For safety and biohazard guidelines, see the “Safety” appendix in the *Ion AmpliSeq™ Kit for Chef User Guide* (Pub. No. MAN0013432). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference is intended as a benchtop reference for experienced users of the Ion AmpliSeq™ Kit for Chef DL8. For detailed instructions, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432).

If you are using Torrent Suite™ Software version 5.16 or later, you must create a sample set. For detailed instructions, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432).

Thaw the reagents and prepare the instrument

- Before the run, thaw one Ion AmpliSeq™ Chef Reagents DL8 cartridge at room temperature for 20 minutes.
- Thaw the Ion AmpliSeq™ primer pools.
- If not performed after a previous run, unload and clean the Ion Chef™ Instrument.
- Confirm that the Ion Chef™ Instrument has a connection to the Torrent Server. On the Ion Chef™ home touchscreen, touch **Settings ▶ Torrent Server** to view the connection status of your instrument.

Note: If the instrument is not connected, see the *Ion Chef™ and Torrent Server Network Setup User Guide* (Pub. No. MAN0013444) for instructions on how to configure a direct or indirect network connection of the Ion Chef™ Instrument to a Torrent Server.

Prepare gDNA

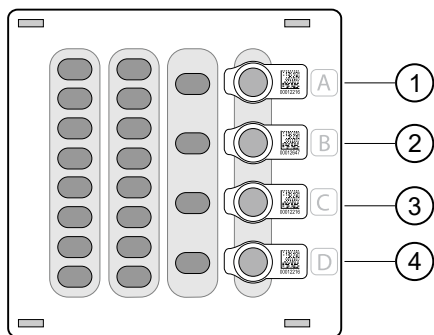
Dilute 8 samples to 0.67 ng/μL with Nuclease-free Water. Prepare 15 μL of each diluted sample (10 ng) for an Ion AmpliSeq™ Chef run.

Note: If you are preparing libraries from RNA, see the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for further information.

Add Ion AmpliSeq™ 2X Primer Pools to Positions A and B of the Reagents cartridge

1. Uncap all 4 tubes in Positions A, B, C, and D in the Ion AmpliSeq™ Chef Reagents DL8 cartridge. Save the caps.

- Standard panels



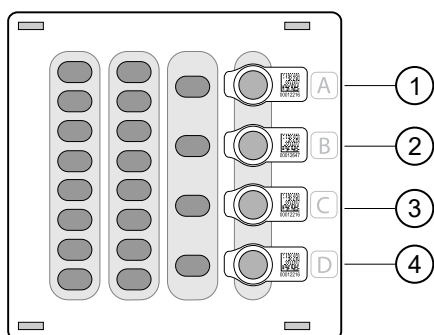
① Position A (150 µL Primer Pool 1 at 2X concentration)

② Position B (150 µL Primer Pool 1 or 2 at 2X concentration)

③ Position C (Empty tube)

④ Position D (Output tube)

- Oncomine™ Comprehensive Assay Plus, DNA and RNA panels



① Position A (150 µL Primer Pool 1 at 2X concentration)

② Position B (150 µL Primer Pool 1 or 2 at 2X concentration)

③ Position C (Output tube)

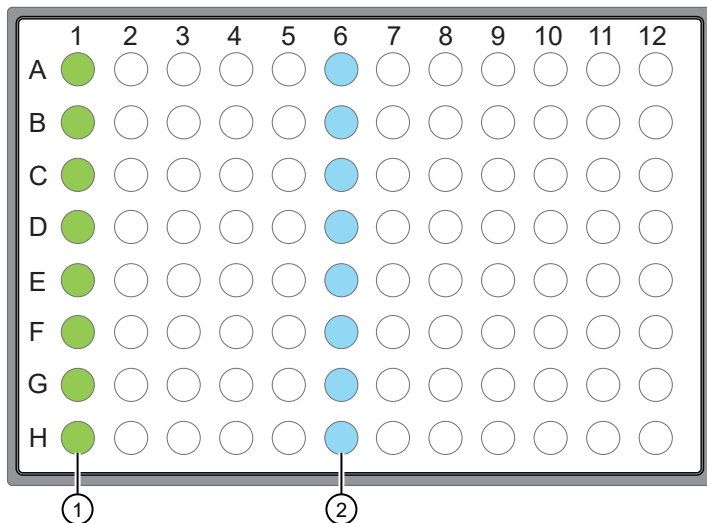
④ Position D (Output tube)

2. Add primer panels to the Reagents cartridge using the following guidelines:

If you are using	Action
Chef-Ready panels	
1 or 2 primer pools	<ol style="list-style-type: none"> Vortex the Primer Pool Tubes to mix, then centrifuge. Remove the caps, then replace the tubes in Positions A and B of the Ion AmpliSeq™ Chef Reagents DL8 cartridge with the Chef-Ready panel tubes.
Ion AmpliSeq™ 2X panels—Aliquot only.	
1 primer pool	<ol style="list-style-type: none"> Pipet 150 µL of the 2X Primer Pool into each of the Position A and Position B tubes.
2 primer pools	<ol style="list-style-type: none"> Pipet 150 µL of the 2X Primer Pool 1 into the Position A tube. Pipet 150 µL of the 2X Primer Pool 2 into the Position B tube.
Ion AmpliSeq™ 5X panels—Dilute and Aliquot.	
1 primer pool	<ol style="list-style-type: none"> Dilute to 2X by adding 120 µL of the 5X Primer Pool to 180 µL of Nuclease-free Water. Pipet 150 µL of the 2X Primer Pool into each of the Position A and Position B tubes.
2 primer pools	<ol style="list-style-type: none"> Pipet 60 µL of the 5X Primer Pool 1 into the Position A tube. Pipet 60 µL of the 5X Primer Pool 2 into the Position B tube. Pipet 90 µL of Nuclease-free Water into each of the Position A and Position B tubes. Using a new tip for each tube, pipet up and down 5 times to mix.

Add DNA to the IonCode™ PCR plate

1. Remove the plate seal from an IonCode™ 96-well PCR Plate.
2. Pipet 15 µL of each gDNA sample (0.67 ng/µL, 10 ng), or Direct FFPE DNA sample, into wells A1 to H1 of the plate.



① Each column 1 well contains 15 µL of diluted gDNA sample (0.67 ng/µL, 10 ng total), Direct FFPE DNA, or Nuclease-free Water as non-template control.

② Each column 6 well contains a dried-down IonCode™ barcode. The lowest barcode number is in A6, and the highest barcode number is in H6. All appear light blue in the actual plates.

Note:


- If you are processing fewer than 8 samples, it is preferable to add replicates or positive control samples to the run. Otherwise, pipet 15 µL of Nuclease-free Water as non-template control into column 1 wells that do not contain a DNA sample.
 - If processing 5 or fewer samples, we recommend that you quantify the output combined library by qPCR to ensure that an optimal concentration is used in templating reactions.
3. Carefully inspect each well for air bubbles. Remove any air bubbles by gentle pipetting. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film, then centrifuge the plate briefly in a plate centrifuge. Carefully remove the plate seal.

IMPORTANT!

- If you are sealing the plate, offset the film to the left so that the adhesive does not cover the barcode label. If the barcode label becomes damaged, you can override the error during the Deck Scan.
- If using the Ion AmpliSeq™ Direct FFPE DNA Kit, start the Ion Chef™ run within 10 minutes of transferring the last sample slurry to the IonCode™ 96-well PCR plate.
- If ≥10 minutes has elapsed, pipet each sample slurry up and down at least 5 times to mix, load the IonCode™ 96-well PCR plate onto the Ion Chef™ Instrument, then start the run.

Load the Ion Chef™ Instrument

IMPORTANT! When loading the instrument, do not force a cartridge into place. Each cartridge fits only one location on the deck and in one orientation. If a cartridge does not fit, verify that you are loading the correct cartridge in the correct orientation.

1. Touch  (Open Door) in the upper right corner of the touchscreen, wait for the door latch to open, then lift the door to the top of the travel until the latch engages.
2. Gently tap the Ion AmpliSeq™ Chef Solutions DL8 cartridge on the bench to force the reagents to the bottoms of the tubes, then load it into the front Solutions station so that it snaps into place.
3. Gently tap the Ion AmpliSeq™ Chef Reagents DL8 cartridge on the bench to force the reagents to the bottom of the tubes, then load the cartridge into the Reagents station so that it snaps into place.
4. Load an empty Tip Cartridge L8 from a previous run into the Used Pipette Tip station.
5. Load a new Ion AmpliSeq™ Tip Cartridge L8 into the New Pipette Tip station (left side of deck).
 - a. Unwrap the Ion AmpliSeq™ Tip Cartridge L8, then remove the cover to expose the pipette tips.

- b. Slide the catch forward to allow the locking bracket to pivot upward. Load the Ion AmpliSeq™ Tip Cartridge L8 into position, pull the bracket downward, then push the catch backward to lock the cartridge in place.
6. Load the IonCode™ 96 Well PCR Plate containing gDNA onto the thermal cycler sample block, with position A1 in the upper left corner, then press down to seat it.
7. Slide a new PCR Frame Seal underneath the automated heated cover.
8. Load the Enrichment Cartridge into the Enrichment station.
9. Close the instrument™ door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door so that the lower locks engage.

Start the Ion Chef™ run

1. On the Ion Chef™ home touchscreen, touch **Set up run**.
2. Touch **Step by step**, then touch **AmpliSeq** on the **Run Options** screen.
Note: To bypass the step by step deck loading guide, touch **Quick start**.
3. Ensure that you have completely loaded the Ion Chef™ deck with Ion AmpliSeq™ Kit for Chef DL8 consumables by advancing through the Step by Step deck loading steps on the instrument touch screen.
4. Touch **Start check** on the **Close Door** screen. The Ion Chef™ Instrument performs a Deck Scan.
5. After Deck Scan completes (~3 minutes), touch **Next**.
6. On the Data Destination screen, ensure that the Server and Sample set information is correct, then touch **Next**.
Note: If the PCR plate is not recognized, select the appropriate plate when prompted. If no sample set was selected or planned in the Torrent Server, the following warning appears: "No sample Set detected. Do you want to continue?" You must create and select a sample set. (See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide*.)
7. Enter the appropriate number of primer pools, target amplification cycles, and an anneal/extension time for your run.

Recommended number of amplification cycles for Ion AmpliSeq™ panels

Primer pairs per pool	Recommended number of amplification cycles (10 ng DNA, 3,000 copies)		Anneal/extension time ^[1]
	High quality DNA/RNA	Low quality DNA or RNA (FFPE DNA/RNA, or cfDNA/RNA)	
12–24	22	25	4 minutes
25–48	21	24	4 minutes
49–96	20	23	4 minutes
97–192	19	22	4 minutes
193–384	18	21	4 minutes
385–768	17	20	4 minutes
769–1,536	16	19	8 minutes
1,537–3,072	15	18	8 minutes
3,073–6,144	14	17	16 minutes
6,145–24,576	13	16	16 minutes

^[1] For Ion AmpliSeq™ panels using a 375-bp amplicon design, use 8 minutes.

Recommended number of amplification cycles for Ion AmpliSeq™ and OncoPrint™ Research Assay Chef-Ready panels

Assay	Recommended number of amplification cycles (10 ng DNA/RNA, 3,000 copies)		Anneal/ extension time (minutes)
	High quality DNA/RNA	Low quality DNA/RNA ^[1]	
Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-Ready Kit ^[2] (Cat. No. A31446)	13	17	16
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit ^[3] (Cat. No. A36412)	13	17	16
OncoPrint™ Tumor Mutation Load Assay – Chef-Ready Library Preparation Kit (Cat. No. A37910)	15	16	16
OncoPrint™ Immune Response Research Assay – Chef-Ready (Cat. No. A32928)	20	22	4
OncoPrint™ Myeloid Research Assay – Chef-Ready (Cat. No. A36941)	17 (DNA) 25–28 (RNA)	N/A	4
OncoPrint™ Comprehensive Assay v3C (Cat. No. A35806)	15 (DNA) 28 (RNA)	18 (DNA) 31 (RNA)	8 (DNA) 4 (RNA)
OncoPrint™ Comprehensive Assay Plus – Automated Library Preparation (Cat. No. A49667)	13 (DNA) 23 (RNA)	16 (DNA) 29 (RNA)	16 (DNA) 4 (RNA)
OncoPrint™ Childhood Cancer Research Assay – Chef-Ready (Cat. No. A36486)	16 (DNA) 28 (RNA)	19 (DNA) 31 (RNA)	8 (DNA) 4 (RNA)
OncoPrint™ Focus Assay, Chef-Ready (Cat. No. A42008)	16 (DNA) 28 (RNA)	19 (DNA) 31 (RNA)	8 (DNA) 4 (RNA)

^[1] FFPE DNA/RNA or cfDNA/RNA

^[2] 20,800 primer pairs/pool

^[3] 23,930 primer pairs/pool

8. Touch **Start Run**.

9. After approximately 7–11 hours, return to the Ion Chef™ Instrument. On the **Run Complete** screen, touch **Next** to proceed to the unloading and cleaning steps.

IMPORTANT! The Ion Chef™ Instrument holds the barcoded libraries in the tube in Position D of the Reagents cartridge. If you are using the OncoPrint™ Comprehensive Assay Plus, both positions C and D of the Reagents Cartridge contain the barcoded libraries. Remove and cap the tube as soon as possible after run completion. Do not leave the tube in the instrument longer than 24 hours after the start of the run. After 24 hours from the start of the run, the instrument chiller stops actively cooling, and the sample is held at 27°C.

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.


1. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.
2. On the Ion Chef™ Instrument touchscreen that appears after run completion, tap **Next**.
3. Ensure that you have removed all consumables from the Ion Chef™ Instrument, 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 and illuminates the ultraviolet (UV) light in the instrument.



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.

Unload the Ion Chef™ Instrument

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. Remove the Ion AmpliSeq™ Chef Reagents DL8 cartridge. Remove and cap the combined library tube from Position D, then discard the cartridge.

IMPORTANT! If you are using the Oncomine™ Comprehensive Assay Plus – Automated Library Preparation, cap both combined library tubes from Positions C and D. The Oncomine™ Comprehensive Assay Plus – Automated Library Preparation combines 4 libraries per pool, A1–A4 in Position C and A5–A8 in position D.

3. Remove, then discard the Ion AmpliSeq™ Chef Solutions DL8 cartridge.
4. Remove, then discard the IonCode™ 96 Well PCR Plate and seal from the thermal cycler sample block.
5. Remove, then discard the box of used pipette tips from the Used Pipette Tip station. Discard liquid waste in the tip box by pouring the waste into a waste container through the corner slot.
6. Move the empty Tip Cartridge L8 from the New Pipette Tip station to the Used Pipette Tip station.
7. Remove and discard the Enrichment Cartridge.

IMPORTANT! After completion of an Ion Chef™ run, clean the instrument. See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for more information.

The libraries are at ~100 pM (total combined library concentration) and are ready to use in template preparation. Store unused portions of combined libraries at 4°C to 8°C for up to 1 month. For longer-term storage, store at –30°C to –10°C. See the appropriate Ion Chef™ or Ion OneTouch™ 2 template kit user guide for detailed instructions for template preparation.

Limited product warranty

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Revision history: Pub. No. MAN0013433

Revision	Date	Description
F.0	2 August 2021	Corrected cycling conditions for the Ion AmpliSeq™ human and mouse transcriptome panels.
E.0	19 April 2021	<ul style="list-style-type: none">• Updated Sample set requirements for Torrent Suite™ Software 5.16.• Support added for Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit.• Information for Ion AmpliSeq™ Pharmacogenomics Research Panel, Chef-Ready Kit removed due to discontinuation.
D.0	15 May 2017	Last two rows of the amplification cycle table merged, anneal/extension time column added to table, and anneal/extension time recommendations modified for higher plexy panels.
C.0	9 March 2017	<ul style="list-style-type: none">• Anneal and extension time recommendation updated for 375 bp amplicon designs.• Graphics updated.
B.0	20 October 2015	<ul style="list-style-type: none">• Rebranding• Updated guidance for removing library tube from instrument after a run.
A.0	30 August 2015	New quick reference

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