Ion AmpliSeq™ Library Preparation on the Ion Chef™ System

Catalog Numbers A29024, A35121, 4484177, A32914, A29998, A31446, A32841

Pub. No. MAN0013433 **Rev.** D.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

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.

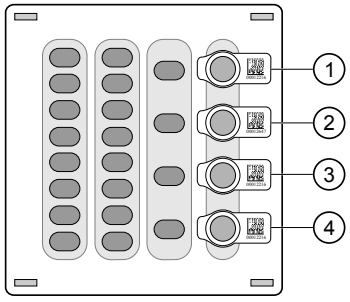
Thaw the reagents and prepare the instrument

- 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.

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.



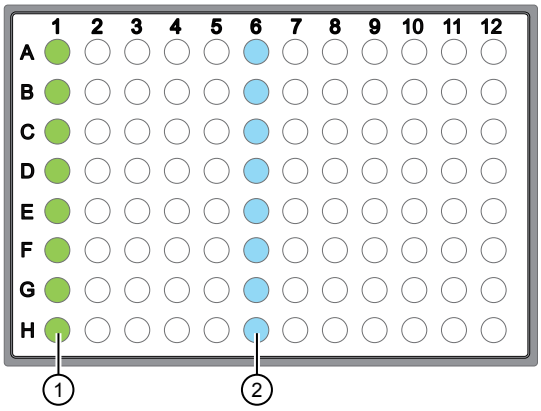
① Position A (150 µL Primer Pool 1 at 2X concentration)
② Position B (150 µL Primer Pool 1 or 2 at 2X concentration)
③ Position C (Empty tube)
④ Position D (Output tube)

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

If you are using	Action
Chef-ready panels	1. Vortex the Primer Pool Tubes to mix, then centrifuge. 2. Remove the caps, then replace the tubes in positions A and B of the Reagents cartridge with the Chef-ready panel tubes.
2X panels, aliquot only	• For a 1-pool panel, pipet 150 µL of the 2X Primer Pool into each of the Position A and B tubes. • For a 2-pool panel, pipet 150 µL of the 2X Primer Pool 1 into the Position A tube, and 150 µL of 2X Primer Pool 2 into the Position B tube.
5X panels, dilute and aliquot	• For a 1-pool panel, dilute to 2X by adding 120 µL of 5X Primer Pool to 180 µL of Nuclease-free Water. Then pipet 150 µL of the 2X Primer Pool into each of the Position A and B tubes. • For a 2-pool panel, pipet 60 µL of 5X Primer Pool 1 into the Position A tube, and 60 µL of 5X Primer Pool 2 into the Position B tube. Then 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.

3. Carefully inspect each well for air bubbles. Remove any air bubbles by gentle pipetting. Alternatively, centrifuge the plate briefly in a plate centrifuge.
4. 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.

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, verify the Server and Sample set information, then touch **Next**.
7. Enter the appropriate number of primer pools, target amplification cycles, and an anneal/extension time for your run.

Primer pairs per pool	Recommended number of amplification cycles (10 ng DNA, 3,000 copies)		Anneal/extension time
	High quality DNA	Low quality DNA (FFPE DNA or cfDNA)	
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

Exceptions to the amplification parameters recommended in the parameter table

Ion AmpliSeq™ panel	Primer pairs/pool	Description of change
Ion AmpliSeq™ Pharmacogenomics Research Panel, Chef-ready (Cat. No. A29998)	119	Add 2 amplification cycles (21 cycles for high-quality DNA, 24 cycles for FFPE DNA)
Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-ready (Cat. No. A31446)	20,800	• Use 12 cycles for high-quality RNA • Use 17 amplification cycles for FFPE RNA instead of 16 cycles
Ion AmpliSeq™ panels using a 375-bp amplicon design	—	Add 4 minutes to the anneal/extend time recommended in the table

8. Touch **Start Run**.
9. After approximately 7 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. 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.

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.
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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Manufacturer: Multiple Life Technologies Corporation manufacturing sites are responsible for manufacturing the products associated with the workflow covered in this guide.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288

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

Revision history: Pub. No. MAN0013433

Revision	Date	Description
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 (see "Start the Ion Chef™ run" on page 2)
C.0	9 March 2017	• Anneal and extension time recommendation updated for 375 bp amplicon designs • Graphics updated
B.0	20 October 2015	• Rebranding • Updated guidance for removing library tube from instrument after a run
A.0	30 August 2015	New quick reference