

Ion AmpliSeq™ Library Preparation on the Ion Chef™ System

USER GUIDE

for use with:

Ion AmpliSeq™ Chef-Ready Kits

Oncomine™ Chef-Ready Kits

Ion AmpliSeq™ On-Demand Panels

Ion AmpliSeq™ Ready-to-Use Panels

Ion AmpliSeq™ Made-to-Order and Community Panels

Ion AmpliSeq™ Sample ID Panel

Ion Chef™ System

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Revision	Date	Description
L	13 May 2024	<ul style="list-style-type: none">• Updated protocol. See “Library denaturation” on page 46.• Added troubleshooting high polyclonality. See “Elevated polyclonality” on page 48.
K.0	22 September 2023	<ul style="list-style-type: none">• Added instructions for denaturing libraries for templating workflows. See “Library denaturation” on page 46.• Added a troubleshooting tip regarding high polyclonality. See “Tips” on page 48.
J.0	1 August 2023	<ul style="list-style-type: none">• Updated Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. A29024) component Ion AmpliSeq™ Chef Supplies DL8 (Part No. A29027) to include the PCR Plate Frame.• Updated instructions to include loading the PCR Plate Frame. See “Load the Ion Chef™ InstrumentDO NOT USE-useGUID-E57267F9-A1E4-4F78-B802-29D0DDB3D10C” on page 28.• Updated instructions to include unloading the PCR Plate Frame. See “Unload the Ion Chef™ Instrument” on page 38.

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

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Product information



CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the “Safety” appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

The Ion AmpliSeq™ Kit for Chef DL8 (DNA to Library, 8 samples/run) is used for automated library preparation of Ion AmpliSeq™ libraries using the Ion Chef™ System. The kit provides sufficient material for the preparation of 32 libraries (4 Ion Chef™ runs).

Key features of the kit include:

- Automated workflow for library preparation of up to 8 barcoded Ion AmpliSeq™ libraries per Ion Chef™ run using a single 1- or 2-pool Ion AmpliSeq™ panel.
- Easy-to-use cartridge format with reagent barcoding for full reagent tracking.
- Proven performance of Ion AmpliSeq™ technology, compatible with DNA extracted from formalin-fixed, paraffin-embedded tissue and other challenging sources.

Software compatibility

The Ion AmpliSeq™ Kit for Chef DL8 is compatible with Torrent Suite™ Software 5.0 and later. We recommend updating your Torrent Server, Ion Chef™, and Ion sequencer instrument software to the latest available versions before using this kit.

If you are using the OncoPrint™ Comprehensive Assay Plus or the OncoPrint™ Myeloid Research Assay, see the user guide for your assay for software compatibility requirements.

Ion AmpliSeq™ Kit for Chef DL8 (library preparation)

IMPORTANT! Store all consumables and cartridges under the recommended conditions and in an upright position. Ion AmpliSeq™ Chef Solutions DL8 cartridges are shipped at ambient temperature, but need to be stored at 2°C to 8°C upon arrival.

The Ion AmpliSeq™ Kit for Chef DL8 (Cat. No. [A29024](#)) contains materials required for performing 4 Ion Chef™ runs, with up to 8 Ion AmpliSeq™ libraries prepared per run. Upon arrival, inspect all consumables and contact Technical Support if any of the components have been damaged during shipping.

Component	Part No.	Quantity per kit	Storage
Ion AmpliSeq™ Chef Reagents DL8	A29025	4 cartridges	-30°C to -10°C
Ion AmpliSeq™ Chef Solutions DL8	A29026	4 cartridges	2°C to 8°C ^[1]
Ion AmpliSeq™ Chef Supplies DL8 (per insert) <ul style="list-style-type: none"> • Ion AmpliSeq™ Tip Cartridge L8 • PCR Frame Seal • PCR Plate Frame • Enrichment Cartridge 	A29027	1 box with 4 inserts	15°C to 30°C
IonCode™ 0101–0132 in 96 Well PCR Plates (dried) Set includes 4 PCR plates: <ul style="list-style-type: none"> • IonCode™ 0101–0108 in 96 Well PCR Plate (red) • IonCode™ 0109–0116 in 96 Well PCR Plate (yellow) • IonCode™ 0117–0124 in 96 Well PCR Plate (green) • IonCode™ 0125–0132 in 96 Well PCR Plate (blue) 	A29028	1 set of 4 plates	15°C to 30°C

^[1] Ion AmpliSeq™ Chef Solutions DL8 cartridges are shipped at ambient temperature, but need to be stored at 2°C to 8°C upon arrival.

IMPORTANT! Use scissors to open the plastic bag containing the PCR Frame Seal. Tearing the bag open may damage the seal.

Ion AmpliSeq™ and Oncomine™ Chef-Ready Kits

- Ion AmpliSeq™ and Oncomine™ Chef-Ready Kits contain assays packaged with the reagents and supplies from the stand-alone Ion AmpliSeq™ Kit for Chef DL8 (see “Ion AmpliSeq™ Kit for Chef DL8 (library preparation)” on page 8 for details).
- Oncomine™ tumor specific panels (AmpliSeq.com) can be used with the Ion AmpliSeq™ Kit for Chef DL8.
- Each Chef-Ready Kit contains sufficient reagents to prepare 32 libraries (4 runs) on the Ion Chef™ System.
- For assays that include both DNA and RNA panels, DNA and RNA libraries must be prepared on separate runs of the Ion Chef™ System, each with a different set of IonCode™ Barcode Adapters.
- Store Ion AmpliSeq™ and Oncomine™ panels at –30°C to –10°C . Store the Ion AmpliSeq™ Kit for Chef DL8 as directed (see “Ion AmpliSeq™ Kit for Chef DL8 (library preparation)” on page 8).

Assays	Primer pools ^[1]	Panel type	Quantity
Ion AmpliSeq™ Chef-Ready Kits			
Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GS Chef Ready (Cat. No. A51306)	2	RNA	8 tubes
Ion AmpliSeq™ Cancer Hotspot Research Panel v2, Chef-Ready Kit (Cat. No. A32914)	1	DNA ^[2]	
Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-Ready Kit (Cat. No. A31446)	1	RNA ^[3]	
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit (Cat. No. A36412)	1	RNA ^[4]	
Oncomine™ Chef-Ready Kits			
Oncomine™ BRCA Research Assay Chef-Ready Library Preparation (Cat. No. A32841)	2	DNA	8 tubes
Oncomine™ Tumor Mutation Load Assay – Chef-Ready Library Preparation (Cat. No. A37910)	2	DNA	
Oncomine™ Immune Response Research Assay (Cat. No. A32928)	1	RNA	
Oncomine™ Myeloid Research Assay – Chef-Ready Library Preparation (Cat. No. A36941)	2 (DNA) 1 (RNA)	DNA and RNA	
Oncomine™ Comprehensive Assay v3C (Cat. No. A35806)	2 (DNA) 2 (RNA)	DNA and RNA	
Oncomine™ Comprehensive Assay Plus – Automated Library Preparation (Cat. No. A49667)	2 (DNA) 2 (RNA)	DNA and RNA	

(continued)

Assays	Primer pools ^[1]	Panel type	Quantity
OncoPrint™ Childhood Cancer Research Assay–Chef-Ready (Cat. No. A36486)	2 (DNA) 2 (RNA)	DNA and RNA	8 tubes
OncoPrint™ Focus Assay, Chef-Ready (Cat. No. A42008)	1 (DNA) 1 (RNA)	DNA and RNA	

^[1] Each primer pool is supplied at the appropriate volume and concentration for directly loading into an Ion AmpliSeq™ Kit for Chef DL8 cartridge.

^[2] 207 primer pairs per pool

^[3] 20,800 primer pairs per pool

^[4] 23,930 primer pairs per pool

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Item	Source
Ion Chef™ System	4484177
P20 and P200 µL pipettors, and filtered tips	fisherscientific.com
(Optional) Non-interruptible Power Supply (UPS) ^[1]	MLS
Nuclease-free Water	AM9932
Low TE (10 mM Tris, 0.1 mM EDTA, pH 8.0)	50-843-203 (fisherscientific.com)
Required for libraries from RNA	
One of the following: <ul style="list-style-type: none"> SuperScript™ IV VILO™ Master Mix SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme Ion Torrent™ NGS Reverse Transcription Kit^[2] SuperScript™ VILO™ cDNA Synthesis Kit^[3] 	<ul style="list-style-type: none"> 11756050 11766050 A45003 11754050
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Veriti™ Thermal Cycler <i>or</i> equivalent	4375786

^[1] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use a non-interruptible power supply that provides 2500 W output or higher.

^[2] Recommended for the OncoPrint™ Comprehensive Assay Plus – Automated Library Preparation and the OncoPrint™ tumor specific RNA panel.

^[3] Recommended for the Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-Ready Kit, Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit, and the OncoPrint™ Focus Assay, Chef-Ready Kit.

Ion AmpliSeq™ panels

Ion AmpliSeq™ panels provide pools of primers for the amplification of target regions. The primers contain proprietary modifications that enable removal of primer sequences during library preparation for efficient target assessment during sequencing. Multiple primer pools can be used to create overlapping amplicons that enable complete coverage of large targets. Panels are designed to be used with nucleic acid from various sources, including nucleic acid from formalin-fixed paraffin-embedded (FFPE) tissue and cell-free DNA (cfDNA).

Order Ion AmpliSeq™ panels using Ion AmpliSeq™ Designer. Users who are interested in inherited disease or germline research applications can design and order panels using a content selection engine or by uploading their own gene list. The gene designs in the On-Demand catalog have been optimized for high performance. For additional information, see Appendix B, “Ion AmpliSeq™ panels” or visit [AmpliSeq.com](https://www.illumina.com/ion-ampliseq).

The following types of panels are compatible with the Ion Chef™ System.

Product and description	In stock panels		Made-to-order panels	Fixed panels	
	Oncomine™ tumor specific panels	Ion AmpliSeq™ On-Demand Panels	Ion AmpliSeq™ Custom Panels	Ion AmpliSeq™ Community Panels	Ion AmpliSeq™ Ready-to-Use Panels
Genome	Human	Human	Any	Human	Human
Nucleic Acid	DNA/RNA	DNA only	DNA/RNA	DNA/RNA	DNA/RNA
Sample type	FFPE	Genomic DNA	Any	Any	Any
Target type	Genes	Genes	Genes, regions, hotspots, amplicons, expression, fusions	Genes, regions, hotspots, amplicons, expression, fusions	Genes, regions, hotspots, amplicons, expression, fusions

Recommended materials and equipment

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Item	Source
Additional equipment	
Fisher Scientific™ Mini Plate Spinner Centrifuge, or equivalent 96-well plate centrifuge	14-100-143 (fisherscientific.com)
Recommended for nucleic acid isolation	
Ion AmpliSeq™ Direct FFPE DNA Kit	A31133
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
RecoverAll™ Multi-Sample RNA/DNA Workflow	A26069 and A26135
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
PureLink™ Genomic DNA Mini Kit	K182001
PureLink™ RNA Mini Kit	12183018A
Recommended for nucleic acid quantification	
TaqMan™ RNase P Detection Reagents Kit (<i>Recommended for gDNA</i>)	4316831
Qubit™ 4 Fluorometer ^[1] and one or more of the following kits: <ul style="list-style-type: none"> Qubit™ dsDNA HS Assay Kit (DNA) Qubit™ RNA HS Assay Kit (RNA) 	Q33238 <ul style="list-style-type: none"> Q32851, Q32854 Q32852, Q32855
(<i>Optional</i>) Ion Library TaqMan™ Quantitation Kit ^[2]	4468802
Additional material for the removal of deaminated bases from purified FFPE DNA	
Uracil DNA Glycosylase (UDG), heat labile	78310100UN

^[1] Qubit™ 2.0 Fluorometer and later are supported.

^[2] Recommended for checking concentration of combined library.

Ion AmpliSeq™ Kit for Chef DL8 workflow

The following workflow summarizes the preparation of DNA and RNA Ion AmpliSeq™ libraries using the Ion AmpliSeq™ Kit for Chef DL8 and the Ion Chef™ System.

①

Prepare DNA Samples

Remove deaminated bases from purified FFPE DNA (page 16)

FFPE preservation methods can lead to significant cytosine deamination within the isolated DNA, and result in decreased sequencing quality. As a result, we recommend removing deaminated cytosines before sequencing.

Dilute the DNA samples (page 17)

DNA samples are diluted to an optimal concentration.

Add DNA to the IonCode™ PCR plate (page 18)

DNA is added to the barcode plate for library preparation. When complete, proceed to “Run samples” ③.



②

Prepare RNA Samples

Isolate and dilute RNA samples (page 19)

Guidelines for isolated RNA are described. RNA is diluted to an optimal concentration.

Reverse transcribe RNA (page 20)

RNA is added to the barcode plate and reverse transcribed to generate cDNA for library preparation. When complete, proceed to “Run samples” ③.



3

Run samples

Create a sample set (page 24)

You can use the Sample Set to create a Planned Run. The information from the Sample Set and individual samples within the Sample Set are prepopulated in the Planned Run workflow bar steps and Planned Run template.

**Add Ion AmpliSeq™ 2X Primer Pools to Positions A and B of the Reagents cartridge (page 26)**

Add Ion AmpliSeq™ Panel to the gDNA or cDNA samples for library preparation.

**Load the Ion Chef™
InstrumentDO NOT USE-useGUID-E57267F9-
A1E4-4F78-B802-29D0DDB3D10C (page 28)**

Install reagents and consumables on the Ion Chef™ Instrument.

Start the Ion Chef™ run (page 31)

Enter panel specific amplification parameters, perform automated library preparation and pooling of barcoded sample libraries on the Ion Chef™ Instrument.

**Unload the Ion Chef™ Instrument (page 38)**

Unload completed library pools for sequencing and clean the Ion Chef™ Instrument.

2

Prepare DNA samples

■ Isolate DNA samples	15
■ Remove deaminated bases from purified FFPE DNA	16
■ Dilute the DNA samples	17
■ Add DNA to the IonCode™ PCR plate	18

We recommend that you create a sample set, thaw reagents, prepare the instrument, and add primer pools to the Reagents cartridge ahead of time so that samples are ready to be loaded into the instrument immediately after transfer to the IonCode™ 96-well PCR plate (see Chapter 4, “Run the Ion Chef™ System”).

For information on preparing RNA samples, go to Chapter 3, “Prepare RNA samples”.

IMPORTANT! If you are using an OncoPrint™ Chef-Ready Kit, follow the DNA sample preparation procedure in the user guide for your assay.

Isolate DNA samples

Guidelines for isolating and quantifying gDNA

- High-quality gDNA can be prepared and quantified using commercially available kits. See “Required materials not supplied” on page 10 for kits that are recommended for isolating gDNA from fresh and FFPE (Formalin-fixed, paraffin-embedded) samples.
- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA. See *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNase P Detection Reagents Kit* (Pub. No. MAN0007732), available at thermofisher.com.
- The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used for quantification, particularly for FFPE DNA, and highly degraded DNA samples.
- Methods such as densitometry (for example, NanoDrop™ spectrophotometers) are not recommended, because they are not specific for gDNA, and are therefore sensitive to small fragments of hydrolyzed RNA. Using densitometry can lead to overestimation of the sample gDNA concentration, under-seeding of the target amplification reaction, low library yields, and poor chip loading.
- The Direct FFPE DNA preparation can be stored for up to 6 months at –20°C before library preparation.

Guidelines for the amount of DNA needed per target amplification reaction

- Select one of the following guidelines for input DNA.
 - Oncomine™ kits only: for each target amplification reaction, use 300–30,000 copies of DNA (10 ng of mammalian gDNA) from normal or FFPE tissue.
 - All other kits: for each target amplification reaction, use 300–30,000 copies of DNA (1–100 ng of mammalian gDNA) from normal tissue, FFPE tissue, or cfDNA.
- Increasing the amount of DNA results in higher-quality libraries, especially when DNA quality or quantity is unknown. We recommend using 1 ng gDNA (300 copies) only with high-quality, well-quantified samples.

Remove deaminated bases from purified FFPE DNA

IMPORTANT! This procedure is required for the Oncomine™ Comprehensive Assay Plus and Oncomine™ Tumor Mutation Load Assay. Follow the instructions in the user guide for your assay.

If using DNA isolated from sources other than FFPE tissue, proceed directly to “Dilute the DNA samples” on page 17.

FFPE preservation methods can lead to significant cytosine deamination of the isolated DNA, resulting in decreased sequencing quality. Deaminated cytosine (uracil) bases can be enzymatically removed by treatment with Uracil DNA Glycosylase (UDG) before the target amplification reaction.

Note: We recommend treating all samples including FFPE and high quality DNA (for example, commercial controls or DNA isolated from cell lines) with UDG to remove deaminated bases before target amplification.

1. For each FFPE DNA sample, add the following components to a single well of a 96-well PCR plate.

Component	Volume
20 ng FFPE DNA	≤9 µL
UDG, heat-labile	1 µL
Low TE	to 10 µL

2. Mix the reaction by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Alternatively, seal the plate, vortex for 5 seconds to mix the reactions, then centrifuge briefly to collect the contents.

Note: To prevent evaporation during UDG treatment, use an applicator tool to press the film securely around each reaction well and around the perimeter of the plate.

- Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

Temperature	Time
37°C	2 minutes
50°C	10 minutes
4°C	Hold (≤1 hour)

- Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.

STOPPING POINT Reactions can be stored at –20°C long term.

- Carefully remove the plate seal, add 20 µL of nuclease-free water to each well, mix the reaction by pipetting at least half the total volume up and down at least 5 times, then proceed immediately to “Add DNA to the IonCode™ PCR plate” on page 18. Add 15 µL of the UDG treated DNA to the target amplification reaction (for 10 ng input).

Dilute the DNA samples

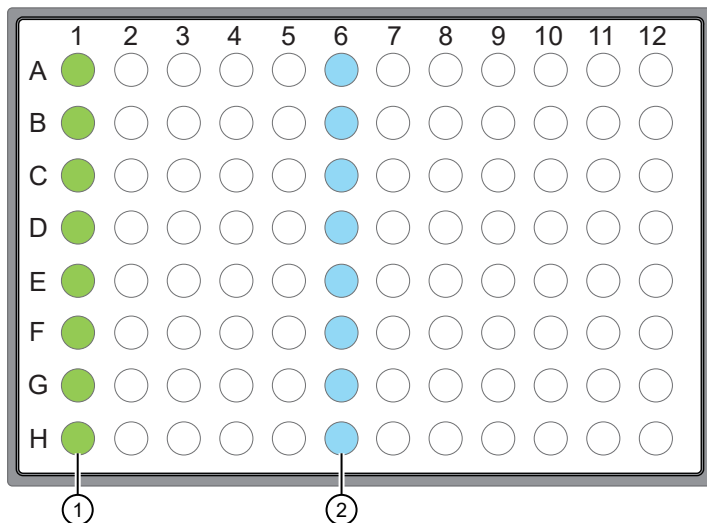
Dilute samples to 0.67 ng/µL with Nuclease-free Water. Prepare 15 µL of each diluted sample (10 ng) to make an Ion AmpliSeq™ Chef library.

Note:

- If you are using an Oncomine™ Chef-Ready Kit, see the user guide for your assay for instructions for diluting the DNA samples.
 - If you choose to use more gDNA, increase the concentration, not the volume. For example, to apply 100 ng of gDNA per library, use 15 µL of gDNA at 6.7 ng/µL.
 - If you choose to use less gDNA, decrease the concentration, not the volume. For example, to apply 1 ng of gDNA per library, use 15 µL of gDNA at 0.067 ng/µL.
 - If you are using Direct FFPE DNA, do not dilute the lysate.
-

Add DNA to the IonCode™ PCR plate

1. Remove, then discard 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.

Proceed to Chapter 4, “Run the Ion Chef™ System”.

3

Prepare RNA samples

■ Isolate and dilute RNA samples	19
■ Reverse transcribe RNA	20

For information on preparing DNA samples, go to Chapter 2, “Prepare DNA samples”.

IMPORTANT! If you are using an Oncomine™ Chef-Ready Kit or the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GS Chef Ready, follow the RNA sample preparation procedure in the user guide for your assay.

Isolate and dilute RNA samples

Guidelines for isolating and quantifying RNA

- High-quality RNA can be prepared and quantified using commercially available kits. See “Required materials not supplied” on page 10 for kits recommended for isolating RNA from fresh and FFPE samples.
- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32855) for quantifying RNA.
- Methods such as densitometry (for example, NanoDrop™ spectrophotometers) are not recommended, because they are sensitive to small fragments of hydrolyzed RNA. Using densitometry can lead to overestimation of the sample RNA concentration, under-seeding of the target amplification reaction, low library yields, and poor chip loading.

Dilute the RNA samples

Dilute samples with Nuclease-free Water according to the following table, depending on the reverse transcription enzyme you are using. You must prepare either 12 µL, 7 µL, or 10.5 µL of each diluted sample (10 ng) for reverse transcription reactions before an Ion AmpliSeq™ Chef library preparation run.

Reverse transcriptase	RNA concentration	Volume of RNA required per reaction
SuperScript™ IV VILO™ Master Mix	0.83 ng/µL	12 µL
Ion Torrent™ NGS Reverse Transcription Kit	1.43 ng/µL	7 µL
SuperScript™ VILO™ cDNA Synthesis Kit ^[1]	0.95 ng/µL	10.5 µL

^[1] Recommended for the Human and Mouse Ion AmpliSeq™ Transcriptome panels (Cat. Nos. [A31446](#) and [A36412](#)).

Note: If you choose to use more RNA, increase the concentration, not the volume. For example, for the SuperScript™ VILO™ Master Mix reaction, to apply 100 ng of RNA per library, use 12 µL of RNA at 8.33 ng/µL, or 10.5 µL of RNA at 9.5 ng/µL.

Proceed to reverse transcription (“Reverse transcribe RNA” or “Reverse transcribe RNA using the SuperScript™ VILO™ cDNA Synthesis Kit” on page 57).

Reverse transcribe RNA

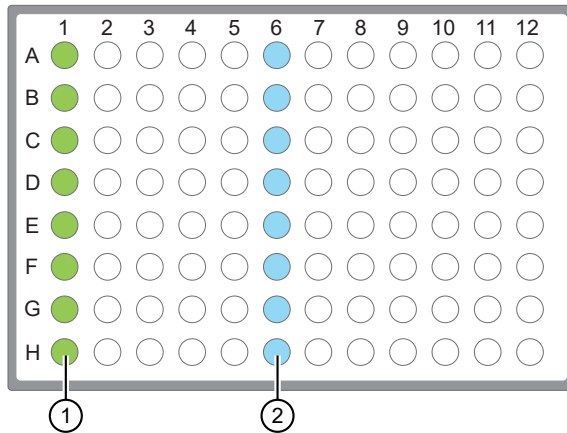
RNA samples must be manually converted to cDNA before library preparation on the Ion Chef™ Instrument. We recommend using the Ion Torrent™ NGS Reverse Transcription Kit or SuperScript™ IV VILO™ Master Mix (ordered separately), which simplifies reaction setup and provides improved performance. If you are using an Oncomine™ Chef-Ready Kit, see the user guide for your assay to determine the correct reverse transcriptase.

Note:

- For setting up reverse transcription reactions with the SuperScript™ III Reverse Transcriptase, see “Reverse transcribe RNA using the SuperScript™ VILO™ cDNA Synthesis Kit” on page 57.
 - Set up the reverse transcription reactions on ice or a cold block.
-
1. If you are using the ERCC RNA Spike-In Mix and the Ion AmpliSeq™ RNA ERCC Companion Panel proceed to “Add ERCC RNA Spike-In Mix 1 to RNA samples” on page 77.
 2. If RNA was prepared from FFPE tissue and not previously heat-treated, preheat at 80°C for 10 minutes, then cool to room temperature.
 3. Remove, then discard the plate seal from an IonCode™ 96 Well PCR Plate.
 4. Add reverse transcription mix.
 - Pipet 3 µL of the SuperScript™ IV VILO™ Master Mix into wells A1 to H1 of the plate.
 - Pipet 2 µL of the NGS 5X Reaction Buffer into wells A1 to H1 of the plate, then pipet 1 µL of the NGS 10X RT Enzyme Mix into wells A1 to H1 of the plate.

5. Add RNA.

- If you are using the Ion Torrent™ NGS Reverse Transcription Kit, pipet 7 μL of each RNA sample (1.43 ng/ μL , 10 ng total) into column 1 wells containing the reverse transcription mix to give a 10- μL reaction volume.
- If you are using the SuperScript™ VILO™ Master Mix, pipet 12 μL of each RNA sample (0.83 ng/ μL , 10 ng total) into column 1 wells containing the RT Master Mix to give a 15- μL reaction volume.



- ① Each column 1 well contains 3 μL SuperScript™ IV VILO™ Master Mix or , and 12 μL of diluted RNA sample (0.833 ng/ μL , 10 ng total), or 12 μL 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 is in H6. All appear light blue in the actual plates.
6. If you are processing fewer than 8 samples, it is preferable to add replicates or positive control samples to the run. Otherwise, pipet 12 μL of Nuclease-free Water as non-template control into column 1 wells that do not contain an RNA sample.

Note: If you are processing 5 or fewer samples, we recommend that you quantify your output combined library by qPCR to ensure that an optimal concentration is used in templating reactions.

7. Seal the plate with a MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge the plate to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

IMPORTANT! 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 Deck Scan.

- Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to synthesize cDNA.

Temperature	Time
25°C	10 minutes
50°C	10 minutes
85°C	5 minutes
10°C	Hold

STOPPING POINT Samples can be stored at 10°C overnight (12–16 hours) in the thermal cycler. For longer term, store at –30°C to –10°C.

- Remove the plate from the thermal cycler, then gently tap the plate to collect any condensate. Alternatively, centrifuge briefly to collect droplets.

Proceed to “Thaw the reagents and prepare the instrument” on page 23.

4

Run the Ion Chef™ System

■ Thaw the reagents and prepare the instrument	23
■ Create a Sample Set—Imported file	24
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■ Set up the Ion Chef™ System	26
■ Start the Ion Chef™ run	31
■ Unload the Ion Chef™ Instrument	38
■ Clean the Ion Chef™ Instrument	41

IMPORTANT! If you are using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GS Chef Ready, follow the recommendation in the *Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay User Guide* (Pub. No. [MAN0024915](#)).

Thaw the reagents and prepare the instrument

- Before the run, thaw one Ion AmpliSeq™ Chef Reagents DL8 cartridge at room temperature for 20 minutes.
- If needed, thaw the Ion AmpliSeq™ primer pools.
- If not performed after a previous run, unload and clean the Ion Chef™ Instrument (see “Clean the Ion Chef™ Instrument” on page 41).
- 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.

Create a Sample Set—Imported file

IMPORTANT!

- If you are using the OncoPrint™ Comprehensive Assay Plus or the OncoPrint™ Myeloid Research Assay – Chef-Ready, follow the recommendations for creating sample sets in the user guide for your assay.
- If you are using Torrent Suite™ Software version 5.16 or later, you must create a sample set.

You can use the Sample Set to create a Planned Run. The information from the Sample Set and individual samples within the Sample Set are prepopulated in the Planned Run workflow bar steps and Planned Run template. For more information, see the help system for your version of Torrent Suite™ Software.

To create an Ion AmpliSeq™ on Ion Chef™ Sample Set, you can either import samples from a CSV file, or enter them manually.

To download a template CSV file, see “Create a samples file from a template” on page 59.

To create a sample set manually, see “Create a Sample Set manually” on page 25.

Note: For more information, see the help system for your version of Torrent Suite™ Software.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.



2. In the **Import Samples** screen, click **Sample File Format**.
A CSV template file downloads to your computer in the Downloads folder.
3. Open the CSV template file, then enter the sample names, PCR plate positions, and barcodes used. Save the file to your computer with a new name.
You can also enter sample names in the CSV file, then supply the plate position, barcode, and other information later by editing the Sample Set in the **Sample Sets** screen.
4. Click **Select File**, select your new CSV file, then click **Open**.
5. Click **Add Sample Set**.
6. Complete the **Add Sample Set** dialog box.
 - a. Enter a Sample Set name.

- b. Select the appropriate **Group Type**.
- c. Set **Library Prep Type** to **AmpliSeq on Chef**.
- d. Set **Library Prep Kit** to **Ion AmpliSeq Kit on Chef DL8**.
- e. Type or scan the PCR plate serial number.
- f. In **Description**, enter additional information.
- g. Click **Save & Finish**

A new Sample Set is created.

Proceed to “Set up the Ion Chef™ SystemDO NOT USE-useGUID-E57267F9-A1E4-4F78-B802-29D0DDB3D10C” on page 26.

Create a Sample Set manually

IMPORTANT!

- If you are using the OncoPrint™ Comprehensive Assay Plus or the OncoPrint™ Myeloid Research Assay – Chef-Ready, follow the recommendations for creating sample sets in the user guide for your assay.
- If you are using Torrent Suite™ Software version 5.16 or later, you must create a sample set.

You can use the Sample Set to create a Planned Run. The information from the Sample Set and individual samples within the Sample Set are prepopulated in the Planned Run workflow bar steps and Planned Run template. For more information, see the help system for your version of Torrent Suite™ Software.

Create a Sample Set manually by entering sample information into the Torrent Suite™ Software without the use of an external CSV file. This method of entering sample information is useful for creating small Samples Sets.

To create a Sample Set manually, enter individual samples into the software, then create a new Sample Set and add your samples to it. Alternatively, you can add your new samples to an existing Sample Set.

For Sample Sets that contain numerous samples, you may want to import samples using a CSV file. For more information, see “Create a Sample Set—Imported file” on page 24.

1. In the **Plan** tab, click **Samples**, then click **Add or Update Sample Set/Samples**.
2. Click **Enter New Sample**.
 - a. Complete the **Add Sample** dialog box. For information about defining the samples, see the help system for your version of Torrent Suite™ Software.
 - b. Click **Done**.
Your new samples and sample attributes appear in the **Enter Samples** list.

- c. Enter additional samples if needed.
3. Add the samples to a sample set. Do one of the following:
 - Click **Add to existing Sample Set**, then select an existing Sample Set to contain the samples.
 - Click **Create Sample Set**. For information about defining the Sample Set, see the help system for your version of Torrent Suite™ Software.
 4. Click **Save Sample Set**.
The set name appears in the Sample Sets list.

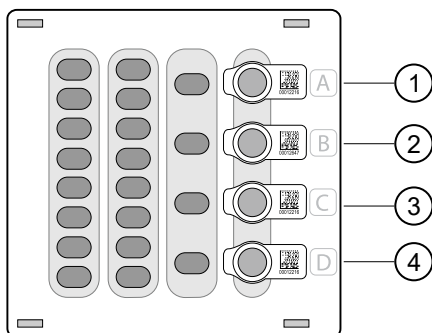
Proceed to “Set up the Ion Chef™ System DO NOT USE-useGUID-E57267F9-A1E4-4F78-B802-29D0DDB3D10C” on page 26.

Set up the Ion Chef™ System

Note: For assays, with DNA and RNA panels, DNA and RNA libraries must be prepared on separate runs of the Ion Chef™ System, each with a different set of IonCode™ Barcode Adapters.

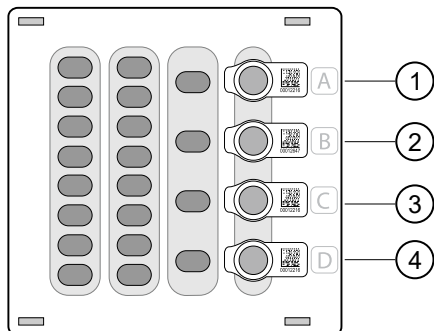
Add Ion AmpliSeq™ 2X Primer Pools to Positions A and B of the Reagents cartridge

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)

- OncoPrint™ 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 Ion AmpliSeq™ Chef Reagents DL8 cartridge using the following guidelines:

Note: Use the same volumes if you are processing fewer than 8 samples.

If you are using	Action
Chef-Ready panels^[1]	
1 or 2 primer pools	<ol style="list-style-type: none"> 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 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"> 1. 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"> 1. Pipet 150 µL of the 2X Primer Pool 1 into the Position A tube. 2. 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"> 1. Dilute to 2X by adding 120 µL of the 5X Primer Pool to 180 µL of Nuclease-free Water. 2. Pipet 150 µL of the 2X Primer Pool into each of the Position A and B tubes.

(continued)

If you are using	Action
2 primer pools	<ol style="list-style-type: none"> 1. Pipet 60 µL of the 5X Primer Pool 1 into the Position A tube. 2. Pipet 60 µL of the 5X Primer Pool 2 into the Position B tube. 3. Pipet 90 µL of Nuclease-free Water into each of the Position A and Position B tubes. 4. Using a new tip for each tube, pipet up and down 5 times to mix.

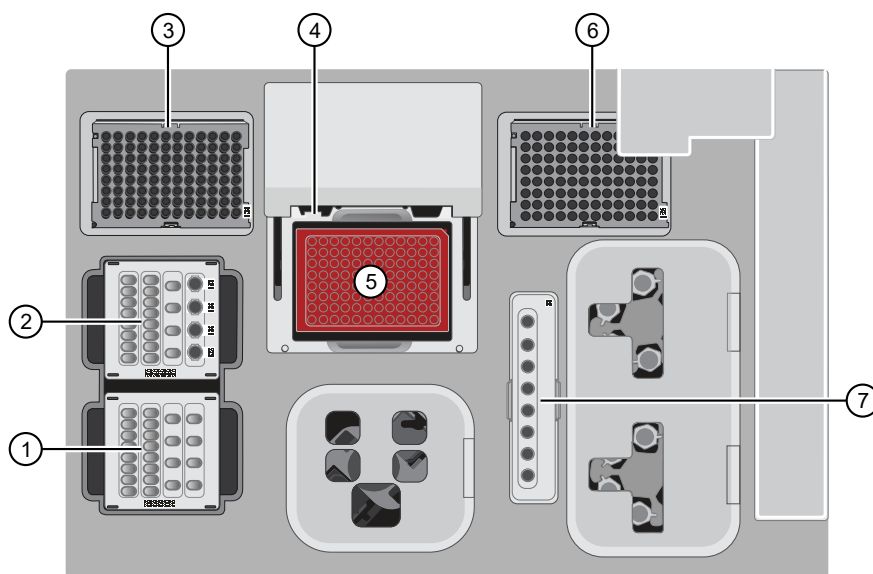
[1] Not all Chef-Ready panels are provided at 2X.

Note:

- For standard panels, the tube in Position D contains 700 µL of combined barcoded libraries at completion of the run.
- For the OncoPrint™ Comprehensive Assay Plus, the tubes in Position C (samples A1–A4) and Position D (samples A5–A8) contain 350 µL of combined barcoded libraries at the completion of the run.


Load the Ion Chef™ Instrument

Use the following procedure to load the Ion Chef™ Instrument. A fully loaded deck is shown in the following figure:

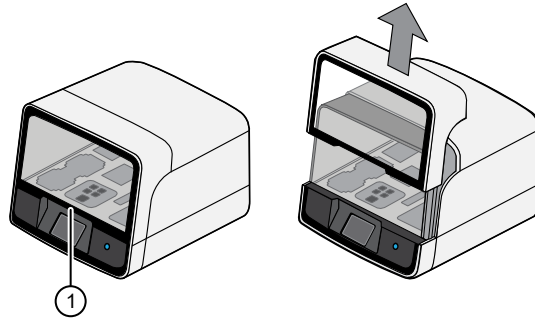


- | | |
|--|--|
| ① Ion AmpliSeq™ Chef Solutions DL8 cartridge | ⑤ IonCode™ 96 Well PCR Plate and PCR Plate Frame |
| ② Ion AmpliSeq™ Chef Reagents DL8 cartridge | ⑥ Empty Tip Cartridge L8 |
| ③ Ion AmpliSeq™ Tip Cartridge L8 | ⑦ Enrichment Cartridge |
| ④ PCR Frame Seal | |

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. Open the Ion Chef™ Instrument door.
 - a. On the instrument touchscreen, touch  (Open Door) in the upper right corner, then wait for the door latch to open.
 - b. Lift the instrument door to the top of the travel until the latch engages.

IMPORTANT! Lift the door from the center.



① Center of door

2. Load the Ion AmpliSeq™ Chef Solutions DL8 cartridge into the Solutions station.
 - a. Gently tap the cartridge on the bench to force the reagents to the bottoms of the tubes.
 - b. Load the cartridge into the Solutions station so that it snaps into place and is level on the deck.
3. 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.
4. Load the Ion AmpliSeq™ Chef Reagents DL8 cartridge into the Reagents station.
 - a. Gently tap the cartridge on the bench to force the reagents to the bottoms of the tubes, then verify that all the liquid is at the bottom, and not splashed on the side of the tubes.
 - b. Load the cartridge into the Reagents station so that it snaps into place and is level on the deck.

IMPORTANT! Ensure that 4 flagged tubes are uncapped and loaded in Positions A–D of the Reagents cartridge, and 2X Primer Pools are loaded in Positions A and B.

5. Load an empty Tip Cartridge L8 from a previous run into the Used Pipette Tip station.
6. 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.

IMPORTANT! The run fails if you do not close the locking bracket.

7. If you are preparing libraries from RNA, carefully remove the plate seal from the IonCode™ 96 Well PCR Plate containing cDNA.

IMPORTANT!

- Start from the left side of the plate to minimize the chance of damaging the barcode on the right side of the plate.
 - To prevent spattering of reverse transcription reactions during removal of the plate seal, we recommend that you position the plate in a 96-well rack, then carefully peel the seal with one hand while firmly holding the plate and rack down on the bench with your other hand. Inspect the column 1 wells for droplets on the side of wells. Apply a new plate seal and recentrifuge the plate to collect droplets to the bottom of the wells, if needed.
-

8. (*RNA samples only*) If the samples were reverse transcribed using the Ion Torrent™ NGS Reverse Transcription Kit, raise the volume to 15 µL.

- a. Add 5 µL of nuclease-free water to each sample.
 - b. Seal the plate with a MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge the plate to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
-

IMPORTANT! 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 Deck Scan.

- c. Carefully remove the plate seal.
9. Load the IonCode™ 96 Well PCR Plate containing gDNA or cDNA onto the thermal cycler sample block, with position A1 in the upper left corner, then press down to seat it.
 10. With the white dot on the PCR Plate Frame facing upward, load the PCR Plate Frame into the thermal cycler sample block pressing down firmly on each corner, then insert a new PCR Frame Seal underneath the automated heated cover. Ensure that the PCR Plate Frame is pressed completely down onto the thermal cycler block and that the PCR Plate Frame sits lower than the PCR Plate.
-

IMPORTANT! When the PCR Frame Seal is positioned correctly, its tabs project upward and contact the heated cover.

11. Load the Enrichment Cartridge into the Enrichment station, then press down on the cartridge to ensure that it is level with the instrument deck.
 12. 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.
-

IMPORTANT! After closing the door, ensure that both sides of the door are locked down.

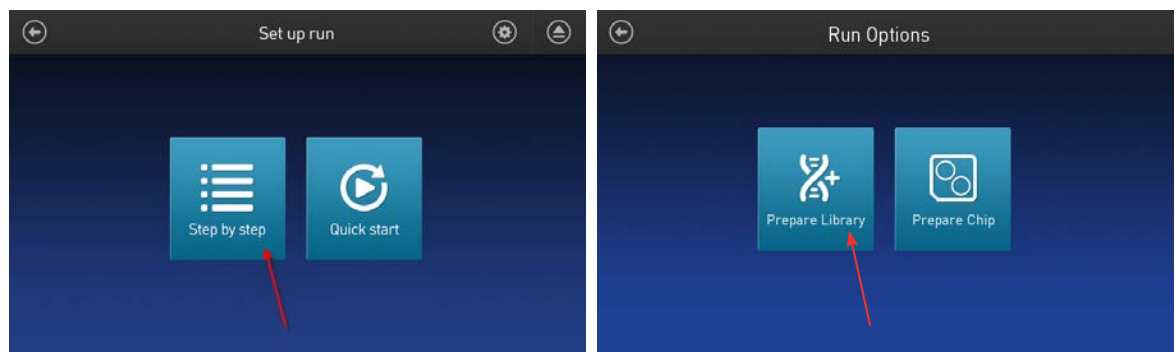
Start the Ion Chef™ run

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**.



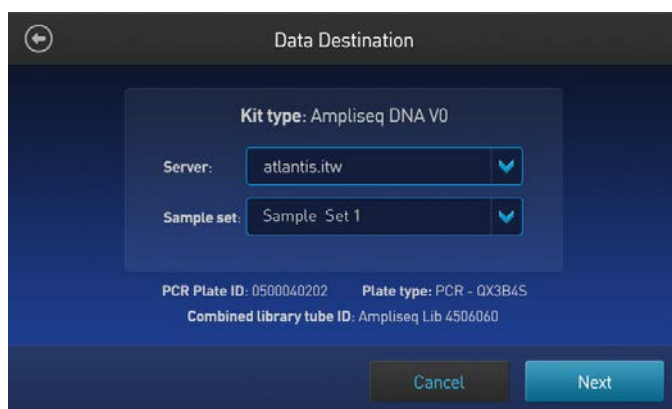
3. Ensure that you have completely loaded the Ion Chef™ deck with Ion AmpliSeq™ Kit for Chef DL8 consumables by advancing through the Step by Step deck loading steps on the instrument touch screen.
4. Touch **Start check** on the **Close Door** screen. The Ion Chef™ Instrument performs a Deck Scan.



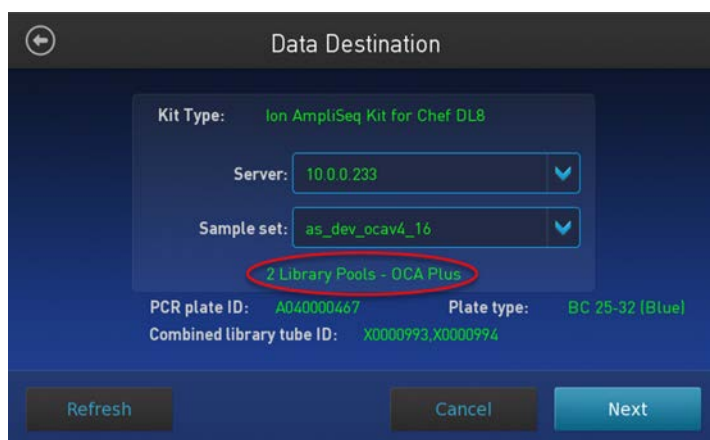
5. After Deck Scan completes (~3 minutes), touch **Next**.
6. On the Data Destination screen, ensure that the Server and Sample set information is correct, then touch **Next**.

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 "Create a Sample Set—Imported file" on page 24 or "Create a Sample Set manually" on page 25.)

- Example of a generic assay setup.



- Example of a correct OncoPrint™ Comprehensive Assay Plus setup—When starting the library preparation run on the Ion Chef™ Instrument ensure that the correct Kit Type and Sample set are selected, and that **2 Library Pools - OCA Plus** is displayed in order to properly prepare OncoPrint™ Comprehensive Assay Plus—Chef Ready libraries. If 2 Library Pools - OCA Plus is not displayed the default library preparation script is run which results in all 8 libraries combined into a single pool. The 2 Library Pools - OCA Plus library preparation script is only available in Torrent Suite™ Software 5.16 or later.



Ensure that *2 Library Pools - OCA Plus* appears below the Sample set dropdown list.

- Example of a correct Oncomine™ Myeloid Assay setup—When starting the library preparation run on the Ion Chef™ Instrument ensure that the correct Kit Type and Sample set are selected, and that **Myeloid** is displayed in order to properly prepare Oncomine™ Myeloid Assay–Chef Ready libraries. If Myeloid is not displayed the default library preparation script is run which may result in low quality libraries. The Myeloid specific library preparation script is only available in Torrent Suite™ Software 5.14 or later.



Ensure that Myeloid appears below the Sample set dropdown list.

- Enter the appropriate number of primer pools, target amplification cycles, and an anneal/extension time for your run.

Ampliseq Workflow Options

Estimated completion time: 05:30 PM

of primer pools: 1

Target amplification cycles: 17

Anneal & extension time: 4

Cancel Start Run

Recommended number of amplification cycles for Ion AmpliSeq™ panels

Primer pairs per pool (excluding gene fusion primer pairs) ^[1]	Recommended number of amplification cycles (10 ng DNA/RNA, 3,000 copies) ^[2]		Anneal/Extension time ^[3]
	High quality DNA/RNA	Low quality DNA/RNA (FFPE DNA/RNA or cfDNA/RNA)	
Panels for gene fusion detection only	28	31	4 minutes
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

Recommended number of amplification cycles for Ion AmpliSeq panels (continued)

Primer pairs per pool (excluding gene fusion primer pairs) ^[1]	Recommended number of amplification cycles (10 ng DNA/RNA, 3,000 copies) ^[2]		Anneal/Extension time ^[3]
	High quality DNA/RNA	Low quality DNA/RNA (FFPE DNA/RNA or cfDNA/RNA)	
3,073–6,144	14	17	16 minutes
6,145–24,576	13	16	16 minutes

^[1] When using RNA panels with a mix of primer pairs for gene expression and gene fusion detection, use the number of gene expression primer pairs only to determine the appropriate number of amplification cycles.

^[2] Source: *Ion AmpliSeq™ Library Preparation User Guide* (Pub. No. MAN0006735). Because nucleic acid is divided between two amplification reactions on the Ion Chef™ Instrument, one additional cycle is recommended above the standard manual Ion AmpliSeq™ protocol. Additional cycles can be added if sample quantity or quality is questionable.

^[3] 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 (Cat. No. A31446)	13	17	16
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit (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) ^[2]	13 (DNA) 23 (RNA)	16 (DNA) 29 (RNA)	16 (DNA) 4 (RNA)

Recommended number of amplification cycles for Ion AmpliSeq and Oncomine Research Assay Chef-Ready panels (continued)

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]	
Oncomine™ Childhood Cancer Research Assay–Chef-Ready (Cat. No. A36486)	16 (DNA) 28 (RNA)	19 (DNA) 31 (RNA)	8 (DNA) 4 (RNA)
Oncomine™ 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] Due to the disparity in the required number of target amplification cycles for high quality and FFPE RNA we do NOT recommend running both high quality and FFPE samples on the same plate using the same input amount. If preparing a positive control (high quality) along with FFPE RNA samples, reduce the positive control sample input to 2 ng and use the FFPE cycling parameters.

The number of amplification cycles that are recommended in the preceding tables are based on 10 ng DNA/RNA input. Adjust the number of amplification cycles for lower or higher DNA/RNA input:

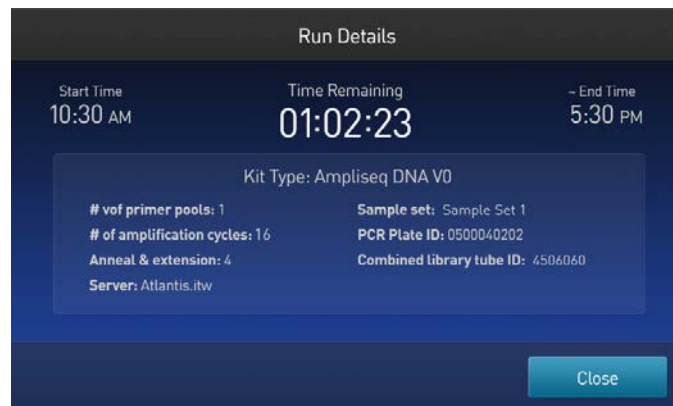
Amount of DNA/RNA starting material	Adjustment to number of amplification cycles
1 ng (300 copies)	+3
10 ng (3,000 copies)	0
100 ng (30,000 copies)	-3

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! After a run, the Ion Chef™ Instrument holds the barcoded libraries in the tube that is loaded 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. To avoid fluid loss due to evaporation, remove and cap the tube of combined barcoded libraries 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.

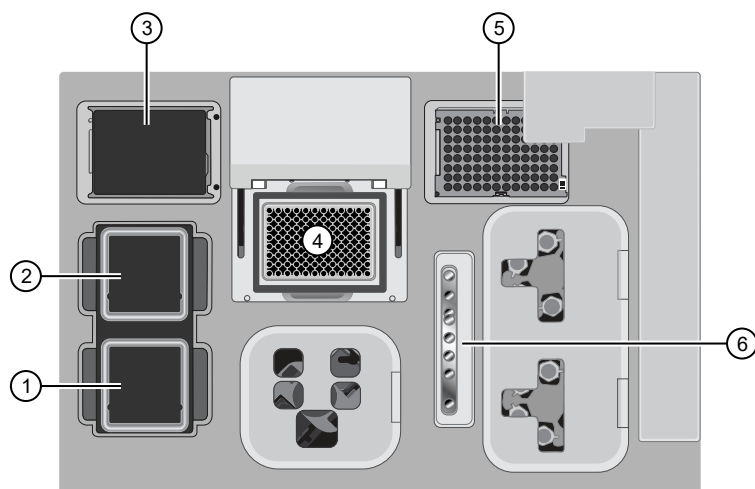
Note: Touch **Run Details** to view run information.



You can also monitor the run on the Torrent Browser by selecting **Monitor ▶ Ion Chef**, and viewing the **Library Prep Progress** bar and **Library Prep Status** associated with your Sample Set.


Unload the Ion Chef™ Instrument

Remove the tube containing the combined library at approximately 100-pM concentration from Position D of the Reagents cartridge, and remove used consumables from the instrument from the indicated stations. If you are using the Oncomine™ Comprehensive Assay Plus, also remove the tube from Position C of the Reagents cartridge.

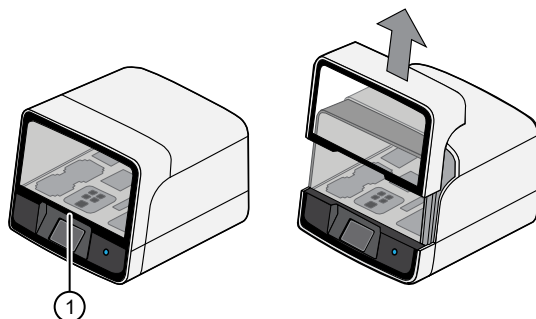


- ① Solutions station
- ② Reagents station
- ③ New Pipette Tip station: move the empty Tip Cartridge to the Used Pipette Tip station
- ④ Thermal cycler sample block
- ⑤ Used Pipette Tip station
- ⑥ Enrichment station

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.

IMPORTANT! Lift the door from the center.



- ① Center of door

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.

Note: The libraries are at approximately 100 pM (total combined library concentration) and can be stored 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 template kit user guide for detailed instructions for template preparation.

For Ion Chef™ templating workflows, we recommend that libraries be denatured. For more information, see “Library denaturation” on page 46.

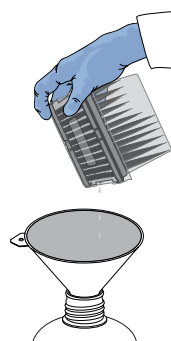
3. Remove, then discard the Ion AmpliSeq™ Chef Solutions DL8 cartridge.
4. Remove, then discard the IonCode™ 96 Well PCR Plate, PCR Plate Frame, and seal from the thermal cycler sample block in unison.

IMPORTANT! Do not attempt to separate the PCR Plate Frame from the plate and seal, as this may cause PCR product to splash and contaminate the instrument deck.

5. Remove, then discard the box of used pipette tips from the Used Pipette Tip station.

Note:

- Handle the disposable reservoir in the Used Tip station with care. During the run, liquid waste collects in the reservoir.
 - Appropriately discard the liquid waste by tipping the reservoir on the corner with the slot and pouring the waste into a waste container through that slot.
 - Do not reuse the Used pipette tip rack.
-



6. Move the empty Tip Cartridge L8 from the New Pipette Tip station to the Used Pipette Tip station.

IMPORTANT! Do not discard the empty Ion AmpliSeq™ Tip Cartridge L8.

7. Remove and discard the Enrichment Cartridge.

(Optional) Combine libraries from multiple Ion Chef™ runs

To combine Ion AmpliSeq™ libraries from multiple Ion Chef™ runs, see Appendix D, “Strategies for combining Ion AmpliSeq™ libraries”.

Clean the Ion Chef™ Instrument

About the cleaning protocol

The Ion Chef™ System includes an automated cleaning function that must be performed following every run. The cleaning routine is initiated from the Ion Chef™ Instrument touchscreen and is designed to minimize potential contamination. During the routine, the instrument irradiates the deck with ultraviolet light for 1 minute after all consumables have been removed from the instrument.

IMPORTANT! Although the Ion Chef™ Instrument cleaning routine provides some protection against contamination, it is not a substitute for good laboratory technique or precautions. When preparing DNA libraries for use or when preparing the Ion Chef™ Instrument, make certain to observe sterile laboratory procedures at all times to help ensure minimal contamination.

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**.

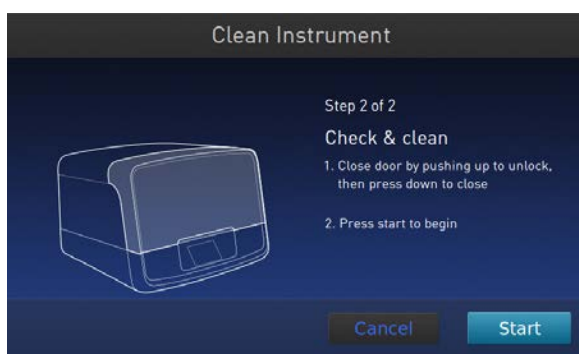


Note: You can also clean the instrument at any time starting from the home touchscreen. Tap **Settings**, then tap **Clean Ion Chef**.

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.

5

Templating, sequencing and data analysis

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Create a Planned Run with Sample Sets

You must create a Sample Set before you plan an instrument run. See “Create a Sample Set—Imported file” on page 24.

IMPORTANT! If you are using the OncoPrint™ Comprehensive Assay Plus, follow the instructions for creating a planned run in the *OncoPrint™ Comprehensive Assay Plus User Guide* (Pub. No. [MAN0018490](#)).

Note that a Sample Set that uses an Ion AmpliSeq™ library preparation kit must also use the corresponding barcode kit that comes with the library preparation kit.

1. In the **Plan** tab, click **Samples**.
2. In the **Sample Sets** screen, select one or more Sample Sets to add to the Planned Run.
 - To plan a run using a single Sample Set, click **⚙ (Actions) ▶ Plan Run** in the row of the Sample Set.

Select	Set Name	Date	# Samples	Description	Grouping	Lib Prep Type	Lib Prep Kit	PCR Plate Serial #	Combined Tube Label	Status	
▶	Sample Set A	2017/12/04 01:19 PM	3		Self					created	⚙
▶	2015-11-24 MSW1	2017/10/18 04:45 PM	48		DNA and Fusions		Ion AmpliSeq Kit for Chef CL8			lib	Edit Set Plan Run Library Prep Summary Delete Set
▶	SteveSample	2017/09/08 03:09 PM	1		Self					cre	
▶	CX165_MB	2017/01/26 12:15 PM	3							cre	

- To plan a run using multiple Sample Sets, select the checkboxes next to the Sample Sets that you want to add to the Planned Run, then click **Plan Run**.



IMPORTANT! Ensure that all selected Sample Sets use the same barcode kit. To verify the barcode kit that is used, expand the Sample Set entry to view its details.



The **Select a Run Template to apply to this experiment** dialog box lists Planned Run templates that support your Sample Set.

- In the **Plan Run from Sample Set** dialog box, select a run template to use for the experiment, then click **Plan Run**.

If you do not see the template that you are looking for, select **Show All Templates**, then look again for the template.

The **Create Plan** workflow opens to the **Barcoding** step with the Sample Sets that you selected:



- In the **Barcoding** step in the workflow bar, enter or select the required information. Depending on your sequencing application, options can vary.

Option	Description
Analysis Parameters	Select Default to accept default analysis parameter settings (<i>recommended</i>). Advanced users can customize analysis parameters by selecting Custom , then editing appropriate analysis options. For more information, see the help system for your version of Torrent Suite™ Software.
Reference Library	Select the reference library file that is appropriate for your sample. Depending on your application, you may have to select separate DNA, RNA, and fusions reference library files.
Target Regions	Select the target regions BED file appropriate for your sample. Depending on your application, you may have to select separate DNA and fusions target regions files. Ensure that you are using the current BED or VCF files.
Hotspots	Select the hotspots (BED or VCF) file appropriate for your sample. Ensure that you are using the current BED or VCF files.

5. Select the **Use same reference & BED files for all barcodes** checkbox if you are using the same reference, target regions, and hotspots files across all barcoded samples in the Planned Run.
If you are using different reference and/or BED files for one or more of your barcoded samples, deselect the **Use same reference & BED files for all barcodes** checkbox.
6. In **Sample Tube Label**, scan or enter the barcode of the Ion Chef™ sample tubes to be used in the run.
7. In **Chip Barcode**, scan or enter the barcode that is printed on the chip that is used for this run.
8. Complete the samples table.
 - You can save the samples table to a CSV file, fill out all required sample information, and then upload the samples table to populate the **Samples Table** automatically.
 - a. Click **Save Samples Table** to save the CSV file to your computer.
 - b. Edit the CSV file by entering all required sample information into the appropriate sample information columns, then save the CSV file to your computer.
 - c. Click **Load Samples Table**, then select an appropriate CSV file containing sample information specific for this Planned Run.
 - d. Click **Load** to populate the samples table with sample information supplied by the CSV file.
 - Alternatively, you can manually enter sample information into the samples table. Depending on your sequencing application, options can vary.

Options	Description
Barcode	For barcoded samples, select a barcode.
Sample Name (Required)	Select a sample that is a part of the selected sample set or sets.
Control Type	Click the Control Type column heading to expand the column, then select the control type from the dropdown list.
Sample ID	<i>(Optional)</i> Review sample ID information for each sample. To edit Sample ID , you must edit the Sample Set as described in “Create a Sample Set—Imported file” on page 24 or “Create a Sample Set manually” on page 25.
Sample Description	<i>(Optional)</i> Review sample description for each sample. To modify Sample Description , you must edit the Sample Set as described in “Create a Sample Set—Imported file” on page 24 or “Create a Sample Set manually” on page 25.
DNA/Fusions	For DNA and fusions application, select DNA or Fusions for each sample.
Reference	If using different reference and BED files for one or more samples, click the Reference column heading to expand the sections, then select reference, target regions, and hotspots files for each sample.
Annotations	Click the Annotations column heading to expand the annotations specific for your application (for example, cancer type or embryo ID), then complete the required information.

(continued)

Options	Description
Ion Reporter workflow	Select the Ion Reporter™ Software analysis workflow specific for your run. If you do not see your analysis workflow, select the Show All Workflows checkbox in the column heading.
Relation	Select sample relationship group.
Gender	Select Male , Female , or Unknown .
IR Set ID	Set the IR Set ID to the same value for related samples. After file transfer, in Ion Reporter™ Software, samples with the same IR Set ID are considered related samples and are launched in the same analysis (for example, normal sample and its corresponding tumor sample). Do not give unrelated samples the same IR Set ID value even if the value is zero or blank.

- Click **Next** to continue the steps to create the Planned Run.

The software takes you to the next step in the workflow bar. For more information, see the help system for your version of Torrent Suite™ Software.

- Click **Save & Finish**.

The Planned Run is added to the Planned Runs table and can be used in an instrument run.

Library denaturation

For Ion Chef™ templating workflows library denaturation decreases (if present) the formation of heteroduplexes that can contribute to high polyclonality. For information about troubleshooting high polyclonality, see “Elevated polyclonality” on page 48.

Note: Library denaturation is not recommended for the OncoPrint™ Comprehensive Assay Plus, DNA, Chef Ready (Part No. A45617). This assay contains unique complex endpoints that require additional bioinformatic optimization before implementation of library denaturation. For this assay, proceed to templating, sequencing, and data analysis without denaturing the libraries. For more information about this assay, see the *OncoPrint™ Comprehensive Assay Plus User Guide* (Pub. No. [MAN0018490](#)).

- Following the recommendations in the library preparation or assay specific user guide, dilute and pool libraries to a final volume of at least 50 µL.
- When you are ready to template, transfer 50 µL of the pooled library into a 200 µL PCR tube or 96-well PCR plate.

Note: When using a PCR tube, ensure the proper tube rack is used to prevent tube crushing. For more information, see the thermal cycler user guide.

3. Load the 200 µL PCR tube or plate in a thermal cycler and run the following program.

Temperature	Time
99°C	5 minutes
4°C	2 minutes
4°C	Hold (up to 10 minutes)

4. Immediately start templating by adding 50 µL of Low TE to the denatured pooled library, mix, then transfer 25 µL to the templating tube.

Note: Denatured samples are stable at 4°C for up to 24 hours. However, we recommend that you begin templating within 30 minutes. Repeated denaturing is not recommended.

5. Proceed to templating, sequencing, and data analysis. Follow the instructions for your assay. If your assay does not have a user guide, contact support for assistance.

Assays	User Guide
Ion AmpliSeq™ automated library preparation on the Ion Chef™ System	
Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-Ready Kit (Cat. No. A31446)	<i>Ion AmpliSeq™ Transcriptome Human Gene Expression Kit User Guide</i> (Pub. No. MAN0010742)
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit (Cat. No. A36412)	<i>Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit User Guide</i> (Pub. No. MAN0017343)
Oncomine™ Research Assay automated library preparation on the Ion Chef™ System	
Oncomine™ BRCA Research Assay Chef-Ready Library Preparation (Cat. No. A32841)	<i>Oncomine™ BRCA Research Assay User Guide</i> (Pub. No. MAN0014634)
Oncomine™ Tumor Mutation Load Assay – Chef-Ready Library Preparation (Cat. No. A37910)	<i>Oncomine™ Tumor Mutation Load Assay User Guide</i> (Pub. No. MAN0017042)
Oncomine™ Immune Response Research Assay (Cat. No. A32928)	<i>Oncomine™ Immune Response Research Assay User Guide</i> (Pub. No. MAN0015867)
Oncomine™ Myeloid Research Assay – Chef-Ready Library Preparation (Cat. No. A36941)	<i>Oncomine™ Myeloid Research Assay User Guide</i> (Pub. No. MAN0016113)
Oncomine™ Comprehensive Assay v3C (Cat. No. A35806)	<i>Oncomine™ Comprehensive Assay v3 User Guide</i> (Pub. No. MAN0015885)
Oncomine™ Comprehensive Assay Plus – Automated Library Preparation (Cat. No. A49667)	<i>Oncomine™ Comprehensive Assay Plus User Guide</i> (Pub. No. MAN0018490)
Oncomine™ Childhood Cancer Research Assay–Chef-Ready (Cat. No. A36486)	<i>Oncomine™ Childhood Cancer Research Assay User Guide</i> (Pub. No. MAN0017117)
Oncomine™ Focus Assay, Chef-Ready (Cat. No. A42008)	<i>Oncomine™ Focus Assay, Part I: Library Preparation User Guide</i> (Pub. No. MAN0013530)



Tips and troubleshooting

View troubleshooting and FAQs online

Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently updated to ensure accurate and thorough content.

- For the Next-Generation Sequencing Support Center: <http://thermofisher.com/ngssupport>
- For general Ion AmpliSeq™ library FAQs: <http://thermofisher.com/4475345faqs>
- To browse the FAQ database and search using keywords: thermofisher.com/faqs

Tips

- Up to 100 ng of DNA or RNA can be used in the standard protocol if the sample quality or quantity is variable. Subtract up to 3 cycles from the target amplification if uniformity suffers.
- When using the Ion AmpliSeq™ Direct FFPE DNA Kit, samples may be processed in Eppendorf™ DNA LoBind™ Microcentrifuge Tubes before target amplification.
- When using the Ion AmpliSeq™ Comprehensive Cancer Panel, the 4 primer pools provided can be collapsed into 2 primer pools for library preparation on the Ion Chef™ System. Use the following procedure:
 - a. Mix equal volumes of Pool 1 and Pool 2 (new Pool 1).
 - b. Mix equal volumes of Pool 3 and Pool 4 (new Pool 2).
 - c. Adjust target amplification cycle number and anneal/extend time accordingly for the 2 new pools (~8,000 primer pairs/pool).

Note: 2-3% of amplicons may be compromised due to overlap.

Elevated polyclonality

Observation	Possible cause	Recommended action
Runs have elevated polyclonality	Too much library was added to the templating reaction.	High polyclonality originating from adding too much library cannot be eliminated by library denaturation. <ol style="list-style-type: none">1. Verify that the library has been diluted according to the assay user guide.2. Decrease the amount of library used for templating.

Observation	Possible cause	Recommended action
<p>Runs have elevated polyclonality (continued)</p>	<p>Over-cycling during target amplification and/or library amplification is creating heteroduplex molecules.</p>	<p>High polyclonality originating from over-cycling cannot be eliminated by decreasing library amount, but can be decreased through library denaturation.</p> <ul style="list-style-type: none"> • Verify that the correct amount of sample has been added. If high polyclonality persists despite library denaturation, the likely cause is too much library was added to the templating reaction. • Confirm that the libraries have been denatured. Denatured libraries remain stable for at least 24 hours at 4C°, do not repeat library denaturation protocol
		<p>Ensure that the thermal cycler has been calibrated and accurately reaches 99°C. Ramp rate for cooling to 4C° has been shown to have no effect on denaturing efficiency however, if desired, remove the tube from thermal cycler at 99°C after 5 minutes, then immediately place in wet ice to snap cool.</p>



Setup and operation of the Ion Chef™ System

Observation	Possible cause	Recommended action
Instrument does not display the home screen when powered on	Multiple causes are possible.	<ol style="list-style-type: none"> 1. Power off the instrument, wait 30 seconds, then power on the instrument. 2. If the instrument fails again, contact Technical Support.
The instrument door cannot be opened	An obstruction was present on or around the door mechanism.	Remove the obstruction blocking the door, then operate the instrument normally.
	A hardware or software error occurred.	Contact Technical Support to report the problem and for further assistance.
The instrument does not start a run	The instrument encountered a Deck Scan error (one or more consumables are absent or loaded improperly).	<ol style="list-style-type: none"> 1. Confirm that the touchscreen does not display any Deck Scan warnings. If alarms are present, note the one or more errors displayed, replace the missing consumable as directed, tap No when prompted, then tap Next to cancel the run. After returning to the home screen, restart the run. 2. If the error persists, ensure that: <ul style="list-style-type: none"> • All cartridges and PCR plate are loaded correctly and are level on the instrument deck. • The barcodes of the 4 flagged tubes in Positions A–D of the Reagents cartridge are visible and positioned correctly. • All tubes are both present <i>and</i> uncapped in the Reagents cartridge. 3. If the error persists after you check the consumables on the instrument deck, do one of the following: <ul style="list-style-type: none"> • If you are confident that the Ion Chef™ Instrument is set up correctly, and you are comfortable disregarding the warnings, tap YES following Deck Scan to proceed with the run. • If the instrument cannot start the run, contact Technical Support for further help.
	The instrument encountered an internal error.	<ol style="list-style-type: none"> 1. Record the error that is displayed on the instrument display, then tap OK. 2. Contact Technical Support to report the problem and for further help.
The instrument stops during a run	The instrument encountered an internal error.	<ol style="list-style-type: none"> 1. Record the error displayed on the instrument display, then tap OK. 2. Contact Technical Support to report the problem and for further assistance.
	A consumable was improperly loaded.	Ensure that all consumables are loaded according to the instructions provided.

Observation	Possible cause	Recommended action
Instrument displays one or more alerts during a run	The instrument detected one or more problems during the run.	After the instrument completes the run, contact Technical Support. If possible, capture an image of the alert or error message to help troubleshoot. IMPORTANT! The detected problem might impact the performance of the sequencing run.
	<ul style="list-style-type: none"> • Network connection to the server was interrupted. • User name or password was incorrect. 	<ol style="list-style-type: none"> 1. Tap the Instrument status button to view the alert(s). 2. In the Instrument status screen, confirm that the name of the Torrent Server connection is red. 3. Contact your network administrator to confirm that: <ul style="list-style-type: none"> • The Torrent Server can be accessed from the network port used by the Ion Chef™ Instrument. If not, troubleshoot the network connection. • The user name and password used by the Ion Chef™ Instrument are valid. If not, contact the server administrator to renew the credentials. 4. If the alert persists, capture an image of the alert or error message, if possible, to help troubleshoot, then contact Technical Support for further assistance.



Ion AmpliSeq™ panels

Order Ion AmpliSeq™ panels using Ion AmpliSeq™ Designer. For more information, visit [AmpliSeq.com](https://www.thermofisher.com).

OncoPrint™ tumor-specific panels

The Ion Torrent™ OncoPrint™ tumor-specific panel designs are small curated panels with verified performance that complement the on-market OncoPrint™ assays. They provide an end-to-end solution for research of specific tumors such as bladder, prostate, melanoma, and others from FFPE tissue samples.

The OncoPrint™ tumor-specific panels provide the following benefits.

- Small tumor-specific panels with relevant content for research
- End-to-end workflow support that includes bioinformatics and reporting
- Low sample input requirement and robust performance from FFPE tissue samples
- Specialized support for assay verification
- Panel customization

A variety of tumor-specific panels are available in Ion AmpliSeq™ Designer. The performance of each panel was verified on oncological research samples. A Copy Number Variant (CNV) baseline is provided with each panel for data analysis in Ion Reporter™ Software for samples that are sequenced on the Ion 530™ Chip or the Ion 540™ Chip.

For more information, visit <https://www.thermofisher.com/us/en/home/clinical/preclinical-companion-diagnostic-development/oncoPrint-oncology/oncoPrint-tumor-specific-panels.html>

Ion AmpliSeq™ On-Demand Panels

Ion AmpliSeq™ On-Demand Panels are designs that are suitable for common disease research areas and are customizable at the gene level. The designs include optimized amplicons with verified performance for germline analysis. You can create designs by selecting from a catalogue of > 5,000 pretested genes or browsing the Ion AmpliSeq™ Designer gene repository by research area of interest.

Each Ion AmpliSeq™ On-Demand Panel consists of 2 DNA primer pools, where the number of primer pairs per pool depends on the genes that are selected. The maximum number of genes that can be selected for design creation is 800. Each Ion AmpliSeq™ On-Demand Panel *order* is limited to 500 genes or 15,000 amplicons. The minimum number of amplicons per design is 24.

You can add genes that are not in the On-Demand catalog to your panel as a spike-in panel. Spike-in panels are high concentration made-to-order panels that are used to supplement on-demand designs with genes that are not available on-demand. Spike-in panels are created as 2-pool designs and are limited to 123 amplicons per pool.

Ion AmpliSeq™ On-Demand Panel designs are available in 8- or 32-reaction packs for library preparations on the Ion Chef™ System and in 24- or 96-reaction packs for manual library preparation. The panels support sequencing genomic targets from as little as 10 ng of DNA input.

Ion AmpliSeq™ fixed panels

Ion AmpliSeq™ fixed panels are fixed-design DNA and RNA panels that are created for germline or somatic analysis. These panels are verified for performance. They are suitable for a wide range of research areas, including inherited diseases, cancer research, human identification, infectious diseases, pharmacogenomics, developmental disorders, inflammation and immune response research, exome, and more.

Two types of fixed panels are available.

- Ion AmpliSeq™ Community Panels are fixed panels that are designed with input from leading disease researchers and are available to ship within a few weeks. These panels are verified for performance, and provide targeted sequencing for your research in cancer, inherited disease, and microbial and infectious disease.

When ordered in the tubes-only format, Ion AmpliSeq™ Community Panels contain 750 reactions (1 pool) or 1,500 reactions (2 pool) for panels with up to 96 amplicons; or 3,000 reactions (1 pool) or 6,000 reactions (2 pool) for panels with more than 96 amplicons.

When ordered in the tubes plus 384-well plate format, Ion AmpliSeq™ Community Panels contain 2,250 reactions (1 pool) or 4,500 reactions [2 pool] for panels with up to 96 amplicons; or 9,000 reactions (1 pool) or 18,000 reactions [2 pool] for panels with more than 96 amplicons. In this format reactions are split between tubes (1/3 rd) and plates (2/3 rd).

- Ion AmpliSeq™ Ready-to-Use Panels are fixed panels that are designed by Thermo Fisher Scientific researchers and are in stock and ready to ship immediately.

The number of reactions of Ion AmpliSeq™ Ready-to-Use Panels depends on the panel. You can view the number of available reactions on each product page on www.thermofisher.com.

Ion AmpliSeq™ Made-to-Order Panels

Ion AmpliSeq™ Made-to-Order Panels are designs that are customizable for any genome at the amplicon level. Panels can be designed against a number of preloaded reference genomes, or for any other organism by uploading a FASTA file reference sequence.

Each Ion AmpliSeq™ Made-to-Order Panel order includes primer pools at the standard 2X or 5X concentration, in some cases, 384-well plates containing all the individual primer pairs. Each plate well contains the forward and reverse primer in Low TE at a concentration of 307 µM per amplicon.

You can use the plates with panels in the following ways.

- Remake the entire panel.
- Remake a smaller panel containing a subset of primer pairs.
- Combine two panels.
- Make a 50X spike-in panel to add to other panels.

When ordered in the tubes-only format, Ion AmpliSeq™ Made-to-Order Panels contain 750 reactions (1 pool) or 1,500 reactions (2 pool) for panels with up to 96 amplicons; or 3,000 reactions (1 pool) or 6,000 reactions (2 pool) for panels with more than 96 amplicons.

When ordered in the tubes plus 384-well plate format, Ion AmpliSeq™ Made-to-Order Panels contain 2,250 reactions (1 pool) or 4,500 reactions [2 pool] for panels with up to 96 amplicons; or 9,000 reactions (1 pool) or 18,000 reactions [2 pool] for panels with more than 96 amplicons. In this format reactions are split between tubes (1/3 rd) and plates (2/3 rd).

Prepare primer pools from plates

To create a primer pool from 384-well plates, combine and dilute the desired primer pairs to the appropriate concentration (per primer).

- For panels with up to 96 primer pairs per pool, combine and dilute to 400 nM per primer pair (2X:400 nM).
To prepare at 5X, combine and dilute to 1,000 nM per primer pair (5X:1,000 nM).
- For panels with 97–3,072 primer pairs per pool, combine and dilute to 100 nM per primer pair (2X:100 nM).
For panels up to 1,228 primer pair, to prepare at 5X, combine and dilute to 250 nM per primer pair (5X:250 nM).

For example, use these steps to create a primer pool with 2 µL of each primer pair.

1. Vortex the sealed primer plate, then briefly centrifuge to collect the contents.
2. Remove the plate seal, then transfer 2 µL of each desired primer pair into a tube.
3. Add Low TE (10 mM Tris, pH 8.0; 0.1 mM EDTA) to the tube to the appropriate final volume shown in the following table.

To make primer pools	Action
2X, 400 nM (12–96 primer pairs)	Add Low TE to a final volume of 1.536 mL. Can be prepared at 5X, 1,000 nM by increasing the final volume to 0.614 mL.
2X, 100 nM (97–3,072 primer pairs)	Add Low TE to a final volume of 6.140 mL. For panels up to 1,228 primer pairs, can be prepared at 5X, 250 nM by increasing the final volume to 2.456 mL.
50X spike-in panel, 2,500 nM (1–123 primer pairs)	Add Low TE to a final volume of 0.246 mL.

4. Mix thoroughly by vortexing, then briefly centrifuge to collect the contents.
The primer pools are ready to use.

Ion AmpliSeq™ Sample ID Panel

Use the Ion AmpliSeq™ Sample ID Panel to add (spike in) content to Ion AmpliSeq™ Ready-to-Use Panel, Ion AmpliSeq™ Made-to-Order Panel, or Ion AmpliSeq™ On-Demand Panel. For example, use the Ion AmpliSeq™ Sample ID Panel to add amplicons that create a unique identifier for human gDNA samples.

Panel	Conc.	Approx. library size (with adapters)	Quantity	No. of primer pairs	Storage ^[1]
Ion AmpliSeq™ Sample ID Panel (Cat. No. 4479790)	20X	150–220 bp	1 tube (96 rxns)	9	–30°C to –10°C

^[1] Shipped at ambient temperature. Store as indicated.

Use the Ion AmpliSeq™ Sample ID Panel

1. Add 15 µL of the Ion AmpliSeq™ Sample ID Panel and 135 µL of the primer panel to one or both primer pool tubes.
The Ion AmpliSeq™ Sample ID Panel can be used to match a tumor and normal sample. However, copy number variations in the tumor sample can distort the allele balance in the fingerprint.
2. When creating a new Planned Run in the Torrent Browser Planned Run Wizard, select the following settings.
 - a. In the **Kits** screen, select the **Control Sequence**, then select **Ion AmpliSeq Sample ID panel** from the list.
 - b. In the **Plugins** screen, select the **coverageAnalysis** plugin checkbox, then click **Configure**.
 - c. In the configuration dialog box, select the **Sample Tracking** checkbox.
When **Sample Tracking** is selected, the analysis produces a statistic for reads that are mapped to Sample ID targets so that the level of off-target reads is accurately represented in the **Coverage Analysis Report**.
When **Sample Tracking** is not selected, Sample ID reads are counted as off-target reads.
 - d. In the **Plugins** screen, select the **sampleID plugin**.
A **Sample ID Report** is generated after the run.

- After sequencing, select the **Data** tab in the Torrent Browser, then select **Completed Runs and Results**. Open the report for the run, then scroll down to the **Plugin Summary** section to find the sampleID plugin results.

Sample ID Report

IonXpress_001_R_2012_08_30_15_51_01_user_C02-620--R154979-E292_LT_LN_BT_BN_2-kr

M-TGACASRW

Number of mapped reads	664,406
Number of reads in sample ID regions	24,870
Percent reads in sample ID regions	3.74%
Total base reads in sample ID regions	74,069,093
Percent base reads in sample ID regions	3.55%
Male sample ID region reads	1,798
Female sample ID region reads	1,363

Sample ID Regions				Sample ID SNPs			
Bases in target regions	1,074	Bases in target regions	8				
Average base coverage depth	2,449.6	Average base coverage depth	2,620.2				
Uniformity of coverage	100.0%	Uniformity of coverage	100.0%				
Coverage at 1x	100.0%	Coverage at 1x	100.0%				
Coverage at 20x	100.0%	Coverage at 20x	100.0%				
Coverage at 100x	100.0%	Coverage at 100x	100.0%				

^ Allele Coverage for Sample Identification SNPs														
Chrom	Position	Target ID	TaqMan Assay ID	Call	Ref	AF	Cov	A Reads	C Reads	G Reads	T Reads	Deletions	+Cov	-Cov
chr3	193207380	SNP#1	C_25749280_10	T	T	99.9%	2170	0	3	0	2167	0	1119	1051
chr4	169663615	SNP#2	C_11245682_10	G	T	99.7%	2233	1	0	2219	7	6	961	1266
chr5	178690725	SNP#3	C_3153696_10	A	G	99.1%	2871	2829	25	17	0	0	1407	1464
chr7	137029838	SNP#4	C_3004178_10	C	T	99.8%	2674	0	2666	3	5	0	1244	1430
chr10	17193346	SNP#5	C_2822618	A	A	99.9%	1726	1722	0	2	0	2	895	829
chr12	6945914	SNP#6	C_2184724_1	S	C	66.3%	3369	1	2233	1133	2	0	1952	1417
chr18	9749879	SNP#7	C_1371205_10	R	G	56.6%	2535	1100	0	1433	1	1	1305	1229
chr22	33559508	SNP#8	C_11887110_1	W	T	51.8%	3395	1631	1	5	1756	2	1575	1818



Supplemental procedures

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Reverse transcribe RNA using the SuperScript™ VILO™ cDNA Synthesis Kit

Reverse transcription reactions can be performed with the SuperScript™ VILO™ cDNA Synthesis Kit, which contains the 10X SuperScript™ III Enzyme Mix. This enzyme mix requires that you prepare a reverse transcription Master Mix with 5X VILO™ Reaction Mix before use. Follow the procedure below.

Note: Set up the reverse transcription reaction on ice or a cold block.

1. If RNA was prepared from FFPE tissue and not previously heat-treated, pre-heat at 80°C for 10 minutes, then cool to room temperature.
2. Warm the 5X VILO™ Reaction Mix to room temperature, then vortex to mix.
3. If there is any visible precipitate in the 5X VILO™ Reaction Mix, vortex further until the mix is completely resuspended.
4. Prepare a reverse transcription Master Mix. For 8 samples, combine the following components in a 1.5-mL tube. Vortex for 1–3 seconds to mix, then centrifuge. Adjust volumes appropriately for fewer samples.

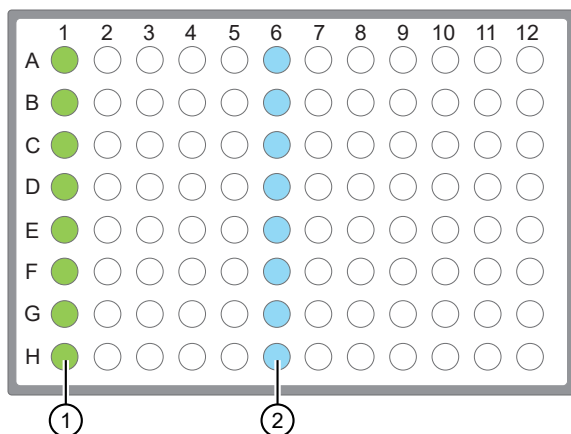
Component	Volume per reaction	Volume for 9 reactions ^[1]
5X VILO™ Reaction Mix (blue cap)	3 µL	27 µL
10X SuperScript™ III Enzyme Mix (black cap)	1.5 µL	13.5 µL

^[1] One reaction added as overage to compensate for pipetting error.

5. Remove, then discard the plate seal from an IonCode™ 96 Well PCR Plate.
6. Pipet 4.5 µL of the reverse transcription Master Mix into wells A1 to H1 of the plate.



- Pipet 10.5 µL of each RNA sample (0.95 ng/µL, 10 ng total) into column 1 wells containing the RT Master Mix to give a 15-µL reaction volume.



- Each column 1 well contains 4.5 µL reverse transcription Master Mix, and 10.5 µL of diluted RNA sample (0.95 ng/µL, 10 ng total).
- Each column 6 well contains a dried-down IonCode™ barcode. The lowest barcode number is in A6, and the highest is in H6. All appear light blue in the actual plates.

- If you are processing fewer than 8 samples, it is preferable to add replicates or positive control samples to the run. Otherwise, pipet 10.5 µL of Nuclease-free Water into column 1 wells that do not contain an RNA sample.

Note: If you are processing 5 or fewer samples, we recommend that you quantify your output combined library by qPCR to ensure that an optimal concentration is used in templating reactions.

- Seal the plate with a MicroAmp™ Clear Adhesive Film, vortex thoroughly, then centrifuge briefly to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

IMPORTANT! 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 Deck Scan.

- Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to synthesize cDNA.

Temperature	Time
42°C	30 minutes
85°C	5 minutes
10°C	Hold

STOPPING POINT Samples can be stored at 10°C overnight (12–16 hours) in the thermal cycler. For longer term, store at –30°C to –10°C.

- Remove the plate from the thermal cycler, then gently tap the plate to collect any condensate. Alternatively, centrifuge briefly to collect droplets.



Proceed to “Thaw the reagents and prepare the instrument” on page 23.

(Optional) Qubit™ Fluorometer—Quantify the FFPE DNA

When using the Ion AmpliSeq™ Direct FFPE DNA Kit, the DNA concentration can be estimated using a Qubit™ Fluorometer and the Qubit™ dsDNA HS Assay Kit (Cat. No. [Q32851](#)). See the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. [MAN0002326](#)) for more information.

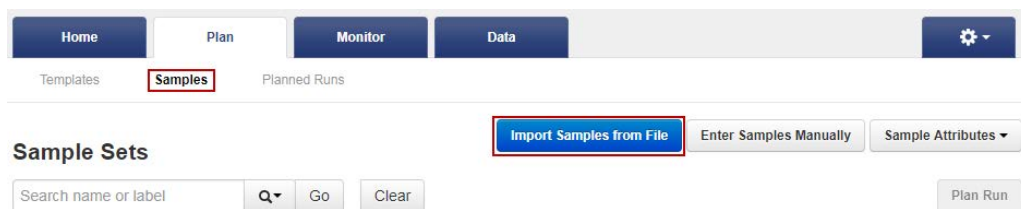
1. Set up the required number of 0.5-mL Qubit™ Assay tubes for standards and samples. The Qubit™ dsDNA HS Assay requires 2 standards.
2. Prepare sufficient Qubit™ working solution for all samples and standards by diluting Qubit™ dsDNA HS Reagent 1:200 in Qubit™ dsDNA HS Buffer.
3. Combine 2 µL of the FFPE DNA sample with 198 µL (200-µL final volume) of working solution, mix well, then incubate for at least 2 minutes.
4. Prepare each Qubit™ standard as directed in the user guide.
5. Measure the concentration of each sample and standard on the Qubit™ Fluorometer.
6. (*Qubit™ 2.0 Fluorometer only.*) Calculate the concentration of the undiluted sample by multiplying by the dilution factor. Alternatively, use the **Calculate Stock Conc.** feature on your instrument.

Proceed to “Dilute the DNA samples” on page 17.

Create a samples file from a template

If you do not already have a samples file on your computer to use to import samples from a CSV file, then you can download a samples template and use it to create a samples file.

1. In the **Plan** tab, click **Samples**, then click **Import Samples from File**.



2. In step 1 of the **Import Samples** section, click **Sample File Format** to download a sample CSV template.
 The sample file format CSV contains the version of the CSV file in the top row, and sample attributes in separate columns.
3. To create a new CSV file, copy and paste the contents of your existing sample CSV file into the new file format.



4. Open the CSV template and enter sample information into the cells.
For information on how to define the samples, see “Sample attributes” on page 60.
5. Save the file to your computer so that is available for use to create a Sample Set.

Sample attributes

When you create a Sample Set, you can enter sample attributes into Torrent Suite™ Software manually or import samples with a CSV file. Most of the sample information is optional except for **Sample Name**. However, some information is required if you transfer data to Ion Reporter™ Software, and those attributes required by Ion Reporter™ Software are indicated in the following table.

Attribute	Description
Sample Name (Required)	<p>The unique name of the sample.</p> <p>Use any combination of alphanumeric characters, plus spaces, periods (.), hyphens (-), and underscores (_).</p> <p>This attribute is used by Ion Reporter™ Software.</p> <p>A 255-character limit must be followed for Ion Reporter™ Software name validation. If you are using Ion Reporter™ Software, and the actual sample name already exists in that software, a string such as _v1 or _v2, and so on, is added to the sample name.</p>
Sample External ID	<p><i>(Optional)</i> If you manage samples in an external system, for example, a Laboratory Information Management System (LIMS), enter the identifier from that system.</p>
PCR Plate Position	<p>A unique identifier for the 96-well plate used for library preparation and templating.</p> <ul style="list-style-type: none"> • Select a plate position of A to H.
Barcode Kit	<p>The name of the barcode kit used to make a library from the sample. The same barcode kit must be used for all samples in a Sample Set.</p>
Barcode	<p>The name of the specific barcode in the selected barcode kit. Assign a unique barcode to each sample in a Sample Set.</p>
Sample ID	<p>A unique identification code (SampleID) for each barcode in a sample. This helps to track samples or possibly identify misassignment between samples and barcodes in a sequencing run. The SampleID is passed to Ion Reporter™ Software.</p> <p>If you manage samples in an external system (for example, a LIMS), you can use the identifier from that system.</p> <p>This attribute cannot be changed.</p> <p>Note: This attribute is optional in Torrent Suite™ Software. However, this value can be required for planned runs that include automatic upload of data to Ion Reporter™ Software.</p>
Control Type	<p>The control type used when preparing the sample. If a value is selected, the sample is identified as a control sample.</p> <ul style="list-style-type: none"> • MSI On-Chip Control • No Template Control



(continued)

Attribute	Description
Basic Annotations	
Description	Typically, one or two sentences that describe the sample.
Nucleotide Type	The nucleic acid type or RNA variant type that is associated with the sample: <ul style="list-style-type: none"> • Unspecified • DNA • RNA • TNA • Fusions
Sample Source	The source from which the sample is extracted. <ul style="list-style-type: none"> • Blood • FFPE • Other
Panel Pool Type	The type of pool that is used by the assay. <ul style="list-style-type: none"> • Dual Pool • Single Pool
Gender	The biological gender of the sample. This attribute is used by Ion Reporter™ Software. <ul style="list-style-type: none"> • Female • Male • Unknown <p>IMPORTANT! If you are using Ion Reporter™ Software, do not leave this blank. Select Unknown if the gender is not known. Several analysis workflows in Ion Reporter™ Software, for example, copy number variation detection and genetic disease research, are limited when the gender is not known. The analysis workflows can return unexpected results when the gender is incorrectly specified for a sample.</p>



(continued)

Attribute	Description
Type	<p>The relationship type for this sample. Type is used in conjunction with Relationship Group, described below. For example, a relationship group can contain two samples, one with a type Tumor and another with a type Normal.</p> <p>The following sample relationships are supported by Ion Reporter™ Software:</p> <ul style="list-style-type: none"> • Control • Father • Mother • Normal • Sample • Self—Use Self for both a single sample and for the proband sample in a trio. A single sample is not related to other samples and is analyzed by itself. • Tumor
Relationship Group	<p>Use Relationship Group to designate a group of multiple related samples within the same Sample Set. For example, DNA and RNA samples from the same sample would have the same Relationship Group number.</p> <p>Use a whole number to define a sample as part of a relationship group. It is used in conjunction with Type. For example, a Sample Set can contain 6 samples, consisting of 3 groups of 2 related samples each (of types Tumor and Normal). In this case, you would designate the two samples in each group as part of group 1, 2, or 3. This is identical to the Set ID in the IonReporterUploader plugin.</p>
Extra Annotations (used for specialized applications, such as preimplantation genetic screening (PGS) research or oncology research)	
Sample Collection Date	<p>The date that the blood sample was drawn.</p>
Sample Receipt Date	<p>The date that the laboratory received the blood sample.</p>
Cancer Type	<p>The type of cancer that is present in the sample.</p>
Population	<p>The super population code assignment for a sample, as defined by the human 1000 genomes project (www.internationalgenome.org/faq/which-populations-are-part-your-study/). The population is relevant to the analysis of samples by the TCRB-LR assay workflow in Ion Reporter™ Software. The TCRB-LR analysis workflow produces a haplotype group assignment for samples having a population attribute of "European".</p>
Mouse Strains	<p>The name of a mouse strain. Choose from a select number of the most common strains. In Ion Reporter™ Software 5.12, the selected mouse strain will not affect Ion Reporter™ Software analysis workflows.</p>
Cellularity %	<p>The percentage of tumor cells in the sample. This is a whole number between 1 and 100.</p>
Biopsy Days	<p>The post-fertilization time at which the biopsy was taken from an embryo. This is a whole number.</p>



(continued)

Attribute	Description
Cell Number	The cell count of the biopsied material.
Couple ID	An identifier for the couple. For use with the Reproductive research application.
Embryo ID	An identifier for the embryo. For use with the Reproductive research application.
User-defined Attributes	
<user defined>	If you create additional sample attributes, each attribute is listed here and in the CSV file. Attributes that are marked as mandatory must be entered for each sample. If you create an attribute of type integer, only numeric characters (whole numbers) can be entered for that attribute.



Strategies for combining Ion AmpliSeq™ libraries

- Combine barcoded libraries from multiple runs for sequencing on a single Ion chip 64
- Combine libraries prepared using different panels for equal coverage 65
- Combine unequal volumes of libraries for varying depth of coverage 66
- Ion Chip™ capacities for Ion AmpliSeq™ DNA libraries sequenced at equal depths 67
- Ion Chip™ capacities for Ion AmpliSeq™ RNA libraries 68

This section describes various strategies for super-pooling Ion AmpliSeq™ libraries prepared in multiple runs of the Ion Chef™ Instrument.

Combine barcoded libraries from multiple runs for sequencing on a single Ion chip

The Ion AmpliSeq™ Kit for Chef DL8 contains reagents and materials sufficient to prepare up to 32 uniquely barcoded libraries. If the same Ion AmpliSeq™ panel (or two panels with the same number of amplicons) was used over multiple runs with different barcodes, the combined libraries can be super-pooled by adding equal volumes of each. See “Ion Chip™ capacities for Ion AmpliSeq™ DNA libraries sequenced at equal depths” on page 67 and “Ion Chip™ capacities for Ion AmpliSeq™ RNA libraries” on page 68 to determine how many libraries you can sequence on various chips, given the number of primer pairs in the Ion AmpliSeq™ panel you used, and the coverage depth you require.

Note: Optional automated sample tracking is not supported if combined libraries prepared in multiple Ion AmpliSeq™ for Chef runs are super-pooled.

Combine libraries prepared using different panels for equal coverage

Use this method to combine libraries from different panels to be sequenced on a single chip at approximately the same depth. To prepare 100 µL of combined library from libraries prepared from two different panels, do the following.

1. Dilute each library to ~100-pM concentration.
 2. Combine the libraries using the following formula.
- Volume (µL) of library from Panel 1 = $100 \mu\text{L} \times \frac{\text{Number of primer pairs in Panel 1}}{\text{Total number of primer pairs in Panels 1 and 2}}$
 - Volume (µL) of library from Panel 2 = $100 \mu\text{L} \times \frac{\text{Number of primer pairs in Panel 2}}{\text{Total number of primer pairs in Panels 1 and 2}}$

Example:

Number of primer pairs in Panel 1 = 207

Number of primer pairs in Panel 2 = 20

Volume of library from Panel 1 = $100 \mu\text{L} \times (207/227) = 91 \mu\text{L}$

Volume of library from Panel 2 = $100 \mu\text{L} \times (20/227) = 9 \mu\text{L}$

Total volume of combined libraries = 100 µL

Combine unequal volumes of libraries for varying depth of coverage

Ion AmpliSeq™ libraries with unique barcodes may be super-pooled to produce disproportionate numbers of reads. Use this strategy for combining DNA and RNA libraries to adjust the number of reads as desired.

For example, when comparing tumor and normal sample pairs with the same panel, an average depth of coverage at ~250X may be preferred for the normal sample, while an average depth of coverage at ~2500X may be preferred for the tumor sample. In this case, 8 barcoded normal and 8 tumor libraries can be super-pooled at a 1:9 (normal:tumor) ratio by adding 10 µL of combined library from the normal sample run with 90 µL of the combined library from a tumor sample run.

Sample	Fractional volume/reads	
Combined libraries from Chef run #1 (normal)	0.1	<p>The bar chart displays the distribution of reads across 8 samples. For each sample, there are two bars: a blue bar for 'Normal' and a red bar for 'Tumor'. The y-axis represents the percentage of reads relative to the total volume of the combined library, ranging from 0 to 12. The x-axis lists the samples from 1 to 8. In every sample, the tumor library contributes approximately 10.9% of the reads, while the normal library contributes approximately 1.1%. The total percentage for each sample is 12%.</p>
Combined libraries from Chef run #2 (tumor)	0.9	
Sum	1.0	

Ion Chip™ capacities for Ion AmpliSeq™ DNA libraries sequenced at equal depths

The number of combined libraries that can be accommodated in a single sequencing run depends on the chip, the balance of barcoded library concentration, and the coverage that is required.

For a given chip, as the number of amplicons increases, the number of libraries that can be accommodated per sequencing run decreases. This relationship is shown in the following table. The numbers in the table serve as a guide for approximate capacities. As the number of libraries per chip increases, it becomes more difficult to balance the reads between libraries. In addition, libraries from FFPE tissue tend to produce more variable results. We suggest combining fewer libraries initially and determining real limits empirically.

	Ion Chip™									
	Ion 510™		Ion 520™		Ion 530™		Ion 540™		Ion 550™	
Average depth	150X	2500X	150X	2500X	150X	2500X	150X	2500X	150X	2500X
Expected coverage	95% >30X	95% >500X	95% >30X	95% >500X	95% >30X	95% >500X	95% >30X	95% >500X	95% >30X	95% >500X
Amplicons per library	Approximate number of libraries per chip									
12–24	272	26	>384	48	>384	152	>384	>384	>384	>384
25–48	152	14	272	26	>384	84	>384	272	>384	>384
49–96	84	8	152	14	>384	48	>384	152	>384	243
97–192	48	4	84	8	272	26	>384	84	>384	134
193–384	26	2	48	4	152	14	>384	48	>384	77
385–768	14	1	26	2	84	8	272	26	>384	41
769–1,536	8	—	14	1	48	4	152	14	243	22
1,537–3,072	4	—	8	—	26	2	84	8	134	13
3,073–6,144	2	—	4	—	14	1	48	4	77	7
6,145–12,288	1	—	2	—	8	—	26	2	41	3
12,289–24,576	—	—	1	—	4	—	14	1	22	2
24,577–49,152	—	—	—	—	2	—	8	—	13	1

Ion Chip™ capacities for Ion AmpliSeq™ RNA libraries

If there is a user guide for your RNA panel, follow the recommendations in the user guide for your panel.

The following guidelines can be used for panels without a user guide. These recommendations serve as suggestions. The actual capacity to multiplex libraries is determined by the expression levels of the genes included in your Ion AmpliSeq™ RNA panel. The expression levels of the individual genes can vary by input RNA type. We suggest using the formula for new panels and determining actual multiplexing limits empirically.

- We recommend that you plan for an average of 5,000 reads per amplicon for an Ion AmpliSeq™ RNA library targeting 1–200 genes. The actual sequencing depth that is required depends on the expression levels of the gene targets in your sample RNA, so scale the sequencing depth to accommodate your sample type and research needs.
- For panels containing fusion detection primer pairs, higher library multiplexing is possible because most targets are not present, and therefore do not create library molecules. For most fusion detection assays, only ~250,000 reads per library are required.
- Use the following formula and chip capacity table to provide initial guidance for multiplexing RNA-derived gene expression libraries on Ion sequencing chips.

$$\text{Number of libraries sequenced per chip} = \frac{\text{Chip capacity in reads}}{(\text{Sequencing depth}) \times (\text{Number of primer pairs})}$$

	Ion Chip™				
	Ion 510™	Ion 520™	Ion 530™	Ion 540™	Ion 550™
Chip capacity in reads (M)	2–3	3–5	15–20	60–80	100–130

Example:

Chip capacity of Ion 540™ Chip = ~60,000,000 reads

Sequencing depth desired = 5,000 reads per amplicon

Number of primer pairs = 100

$60,000,000 / (5,000 \times 100) = 120$ libraries per Ion 540™ Chip



Ion AmpliSeq™ Made-to-Order Panels

Ion AmpliSeq™ Made-to-Order Panels are designs that are customizable for any genome at the amplicon level. Panels can be designed against a number of preloaded reference genomes, or for any other organism by uploading a FASTA file reference sequence.

Each Ion AmpliSeq™ Made-to-Order Panel order includes primer pools at the standard 2X or 5X concentration, in some cases, 384-well plates containing all the individual primer pairs. Each plate well contains the forward and reverse primer in Low TE at a concentration of 307 μM per amplicon.

You can use the plates with panels in the following ways.

- Remake the entire panel.
- Remake a smaller panel containing a subset of primer pairs.
- Combine two panels.
- Make a 50X spike-in panel to add to other panels.

When ordered in the tubes-only format, Ion AmpliSeq™ Made-to-Order Panels contain 750 reactions (1 pool) or 1,500 reactions (2 pool) for panels with up to 96 amplicons; or 3,000 reactions (1 pool) or 6,000 reactions (2 pool) for panels with more than 96 amplicons.

When ordered in the tubes plus 384-well plate format, Ion AmpliSeq™ Made-to-Order Panels contain 2,250 reactions (1 pool) or 4,500 reactions [2 pool] for panels with up to 96 amplicons; or 9,000 reactions (1 pool) or 18,000 reactions [2 pool] for panels with more than 96 amplicons. In this format reactions are split between tubes (1/3 rd) and plates (2/3 rd).

Prepare primer pools from plates

To create a primer pool from 384-well plates, combine and dilute the desired primer pairs to the appropriate concentration (per primer).

- For panels with up to 96 primer pairs per pool, combine and dilute to 400 nM per primer pair (2X:400 nM).
To prepare at 5X, combine and dilute to 1,000 nM per primer pair (5X:1,000 nM).
- For panels with 97–3,072 primer pairs per pool, combine and dilute to 100 nM per primer pair (2X:100 nM).
For panels up to 1,228 primer pair, to prepare at 5X, combine and dilute to 250 nM per primer pair (5X:250 nM).

For example, use these steps to create a primer pool with 2 μL of each primer pair.



1. Vortex the sealed primer plate, then briefly centrifuge to collect the contents.
2. Remove the plate seal, then transfer 2 μ L of each desired primer pair into a tube.
3. Add Low TE (10 mM Tris, pH 8.0; 0.1 mM EDTA) to the tube to the appropriate final volume shown in the following table.

To make primer pools	Action
2X, 400 nM (12–96 primer pairs)	Add Low TE to a final volume of 1.536 mL. Can be prepared at 5X, 1,000 nM by increasing the final volume to 0.614 mL.
2X, 100 nM (97–3,072 primer pairs)	Add Low TE to a final volume of 6.140 mL. For panels up to 1,228 primer pairs, can be prepared at 5X, 250 nM by increasing the final volume to 2.456 mL.
50X spike-in panel, 2,500 nM (1–123 primer pairs)	Add Low TE to a final volume of 0.246 mL.

4. Mix thoroughly by vortexing, then briefly centrifuge to collect the contents.
The primer pools are ready to use.



Expand any Ion AmpliSeq™ panel with a spike-in panel

You can modify or expand an Ion AmpliSeq™ On-Demand Panel by adding a spike-in panel. Spike-in panels added to a Ion AmpliSeq™ panel add new content, control assays, and improve coverage uniformity (for example, by adding the same primer pair to 2 or more primer pools).

1. If needed, prepare a 50x (2500 nM) spike-in panel from individual primer pairs using the procedure in “Ion AmpliSeq™ Made-to-Order Panels” on page 53.
2. Add the spike-in panel (at 2,500 nM) to a primary Ion AmpliSeq™ panel using the following guidelines.

Number of primer pairs per pool			Target concentration (combined)	Instructions
Primary panel	Spike-in	Combined		
Chef-Ready primary panels				
Add 6.4 µL of the spike-in panel to the Chef-Ready panel tube.				
All other primary panels				
Less than 96	Up to 123	Less than 96	2X:400 nM	Add 16 µL of the spike-in panel per 100 µL of the primary panel
Less than 96	Up to 123	More than 96	2X:100 nM	Dilute parent panel from 400 nM to 100 nM, then add 4 µL spike-in panel per 100 µL of primary panel
Greater than 96	Up to 123	More than 96	2X:100 nM	Add 4 µL spike-in panel per 100 µL primary panel

3. Mix thoroughly by vortexing, then briefly centrifuge to collect the contents.
The modified primer pools are ready to use. No adjustment of volume is necessary.

Examples of spike-in volumes for different categories of parent and spike-in panel

Number of primer pairs per pool			Concentration (nM)			Volumes for spiking (µL)		Final concentration (nM)	
Parent	Spike-in	Combined	Parent	Spike-in	Target (combined)	Parent	Spike-in	Parent	Spike-in
50	20	70	400	2,500	400	160	25.6	345	345
80	60	140	100	2,500	100	160 (diluted)	6.4	96.2	96.2

Examples of spike-in volumes for different categories of parent and spike-in panel (continued)

Number of primer pairs per pool			Concentration (nM)			Volumes for spiking (μL)		Final concentration (nM)	
Parent	Spike-in	Combined	Parent	Spike-in	Target (combined)	Parent	Spike-in	Parent	Spike-in
3,000	120	3,120	100	2,500	98.5	160	6.4	96.2	96.2



Ion AmpliSeq™ Sample ID Panel

Using the Ion AmpliSeq™ Sample ID Panel

If your panel includes the Ion AmpliSeq™ Sample ID Panel, do not add additional panel. For additional details, see the user guide for your assay.

The Ion AmpliSeq™ Sample ID Panel is an Ion AmpliSeq™ Spike-in panel that is used to add amplicons to create a unique identifier for human gDNA samples. It is comprised of 9 primer pairs at a 20X concentration (1000 nM per primer). If you are using the Ion AmpliSeq™ Kit for Chef DL8, use the following procedure to create a sample signature in your panel.

1. For addition to a primary panel with greater than 96 primer pairs per pool, add 15 µL of the Ion AmpliSeq™ Sample ID Panel and 135 µL of the primer panel to one or both Primer Pool Tubes.

Note:

- For addition to a primary panel with less than 96 primer pairs per pool, optimization may be required to obtain the desired read coverage distribution of Sample ID amplicons relative to primary panel amplicons.
- If a primary panel has more than one primer pool, the Ion AmpliSeq™ Sample ID Panel can be added to one or all primer pools.
- The Ion AmpliSeq™ Sample ID Panel can be used to match a tumor and normal sample. However, copy number variations in the tumor sample can distort the allele balance in the fingerprint.

2. Select the following settings when creating a new Planned Run in the Torrent Browser Planned Run Wizard:

- a. In the **Kits** step, select the **Control Sequence**, then choose **Ion AmpliSeq Sample ID panel** from the dropdown list.
- b. In the **Plugins** step, select the **coverageAnalysis** plugin checkbox, then click **Configure** (see the help system for your version of Torrent Suite™ Software).
- c. In the configuration dialog, select the **Sample Tracking** checkbox.

Note: Selecting the **Sample Tracking** checkbox enables the analysis to produce a statistic for reads mapped to Sample ID targets so that the level of off-target reads is accurately represented in the Coverage Analysis Report. If **Sample Tracking** is not selected, Sample ID reads are counted as off-target reads.

- d. In the **Plugins** step, select the **sampleID plugin**. A Sample ID Report is then automatically generated after the run.



- Following sequencing, select the **Data** tab in the Torrent Browser, then select **Completed Runs and Results**. Open the report for the run, then scroll down to the Plugin Summary section to find the sample ID plugin results.

Sample ID Report

IonXpress_001_R_2012_08_30_15_51_01_user_C02-620--R154979-E292_LT_LN_BT_BN_2-kr

M-TGACASRW

Number of mapped reads	664,406
Number of reads in sample ID regions	24,870
Percent reads in sample ID regions	3.74%
Total base reads in sample ID regions	74,069,093
Percent base reads in sample ID regions	3.55%
Male sample ID region reads	1,798
Female sample ID region reads	1,363

Sample ID Regions				Sample ID SNPs			
Bases in target regions	1,074	Bases in target regions	8				
Average base coverage depth	2,449.6	Average base coverage depth	2,620.2				
Uniformity of coverage	100.0%	Uniformity of coverage	100.0%				
Coverage at 1x	100.0%	Coverage at 1x	100.0%				
Coverage at 20x	100.0%	Coverage at 20x	100.0%				
Coverage at 100x	100.0%	Coverage at 100x	100.0%				

^ Allele Coverage for Sample Identification SNPs														
Chrom	Position	Target ID	TaqMan Assay ID	Call	Ref	AF	Cov	A Reads	C Reads	G Reads	T Reads	Deletions	+Cov	-Cov
chr3	193207380	SNP#1	C_25749280_10	T	T	99.9%	2170	0	3	0	2167	0	1119	1051
chr4	169663615	SNP#2	C_11245682_10	G	T	99.7%	2233	1	0	2219	7	6	961	1266
chr5	178690725	SNP#3	C_3153696_10	A	G	99.1%	2871	2829	25	17	0	0	1407	1464
chr7	137029838	SNP#4	C_3004178_10	C	T	99.8%	2674	0	2666	3	5	0	1244	1430
chr10	17193346	SNP#5	C_2822618	A	A	99.9%	1726	1722	0	2	0	2	895	829
chr12	6945914	SNP#6	C_2184724_1	S	C	66.3%	3369	1	2233	1133	2	0	1952	1417
chr18	9749879	SNP#7	C_1371205_10	R	G	56.6%	2535	1100	0	1433	1	1	1305	1229
chr22	33559508	SNP#8	C_11887110_1	W	T	51.8%	3395	1631	1	5	1756	2	1575	1818



Ion AmpliSeq™ RNA ERCC Companion Panel



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](https://www.thermofisher.com/support).

Product description

The Ambion™ ERCC RNA Spike-In Mix provides a set of external RNA controls that enables performance assessment of RNA-Seq libraries in gene expression experiments on Ion Torrent™ sequencing platforms. With the addition of the Ion AmpliSeq™ RNA ERCC Companion Panel to your Ion AmpliSeq™ RNA panel, dynamic range and sensitivity can be similarly evaluated in Ion AmpliSeq™ RNA libraries that are prepared from RNA samples containing the ERCC RNA Spike-In Mix. Add ERCC RNA Spike-In Mix to each RNA sample, then amplify targets with an Ion AmpliSeq™ RNA panel plus Ion AmpliSeq™ RNA ERCC Companion Panel. Sequence the libraries on an Ion PGM™, Ion Proton™, Ion S5™, Ion S5™ XL, or Ion GeneStudio™ S5 System, then compare the Spike-In Mix data to known Spike-In Mix concentrations to evaluate the dynamic range and lower limit of detection of the platform. This user guide provides guidelines for preparing and analyzing Ion AmpliSeq™ RNA libraries with the ERCC RNA Spike-In Mix and the Ion AmpliSeq™ RNA ERCC Companion Panel.

Note: For use with the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit (Cat. Nos. [A26325](#), [A26326](#), [A26327](#), and [A31446](#)) and Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit (Cat. Nos. [A36553](#), [A36554](#), [A36555](#), and [A36412](#)) only.

Software compatibility

The Ion AmpliSeq™ RNA ERCC Companion Panel is compatible with Torrent Suite™ Software version 5.10 and later and the ERCC_Analysis plugin version 5.10.0.3 or later. Be sure to update your Ion Torrent™ Server to the latest available version of Torrent Suite™ Software before using this kit.



Contents and storage

The Ion AmpliSeq™ RNA ERCC Companion Panel provides enough reagent for 96 manual reactions or 48 automated reactions.

Contents	Amount	Storage
Ion AmpliSeq™ RNA ERCC Companion Panel	1 × 96 µL	–30°C to –10°C

Note: The Ion AmpliSeq™ RNA ERCC Companion Panel is shipped at ambient temperature. Store as indicated.

Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Item	Source
ERCC RNA Spike-In Mix	4456740
One of the following: <ul style="list-style-type: none">• Ion AmpliSeq™ Transcriptome Human Gene Expression Kit• Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit• Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-Ready Kit• Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit	<ul style="list-style-type: none">• A26325, A26326, or A26327• A36553, A36554, or A36555• A31446• A36412

Before you begin

Ensure that version 5.10.0.3 or later of the ERCC_Analysis plugin has been installed on the Torrent Suite™ Software. This version of the plugin requires Torrent Suite™ Software version 5.10 or later.

- To confirm the version that is installed on the Torrent Suite™ Software, see “Confirm ERCC_Analysis plugin version” on page 78.
- For instructions for installing the ERCC_Analysis plugin, see “Install the ERCC_Analysis plugin” on page 78.



Add ERCC RNA Spike-In Mix 1 to RNA samples

IMPORTANT! Ion AmpliSeq™ RNA panel size and content, as well as starting RNA quantity and quality, affect the percentage of ERCC mapped reads in the final libraries. We recommend using the following tables as starting points. Further adjustment of amount of ERCC RNA Spike-In Mix added to each sample may be needed.

1. Determine the amount of ERCC RNA Spike-In Mix 1 to add, using the following table as a guideline.

Amount of total RNA	Volume of diluted ERCC RNA Spike-In Mix 1
10 ng	1 µL (1:5,000 dilution)
20 ng	2 µL (1:5,000 dilution)
50 ng	1 µL (1:1,000 dilution)
100 ng	2 µL (1:1,000 dilution)

2. Prepare the appropriate dilution of ERCC RNA Spike-In Mix 1 needed using the following table. Scale the volumes accordingly if > 10 µL of the dilution is needed.

Dilution	ERCC RNA Spike-In Mix 1	Nuclease-free Water
1:10	1 µL undiluted	9 µL
1:100	1 µL of 1:10	9 µL
1:1,000	1 µL of 1:100	9 µL
1:5,000	2 µL of 1:1,000	8 µL
1:10,000	1 µL of 1:1,000	9 µL
1:50,000	1 µL of 1:5,000	9 µL

Note: Prepare a fresh dilution of the ERCC RNA Spike-In Mix for each procedure. Discard unused diluted Spike-In Mix.

3. Add the volume of the appropriate ERCC RNA Spike-In Mix 1 dilution, which is determined in step 1, to each RNA sample. Concentrate the RNA sample containing ERCC RNA Spike-In Mix 1 (centrifugal vacuum concentration recommended), if needed.
Proceed to “Add the Ion AmpliSeq™ RNA ERCC Companion Panel to your RNA panel” on page 78.



Add the Ion AmpliSeq™ RNA ERCC Companion Panel to your RNA panel

Note: Any Ion AmpliSeq™ Transcriptome Gene Expression panel can be modified through the addition of the Ion AmpliSeq™ RNA ERCC Companion Panel.

1. Add the Ion AmpliSeq™ RNA ERCC Companion Panel.


Reaction type	Amount
Manual reaction	Add 1 µL per reaction.
Automated (Ion Chef™ System)	Add 8 µL per pool (16 µL per Ion Chef™ Instrument run).

2. Mix thoroughly by vortexing, then centrifuge. The modified primer pools are ready to use.
3. Follow the standard library preparation protocol for Ion AmpliSeq™ RNA library preparation, using the change in panel volume specified in the table in step 1.
Return to in step 2

Supplemental procedures


Confirm ERCC_Analysis plugin version

If the ERCC_Analysis plugin has not been uploaded to the Torrent Suite™ Software, see “Install the ERCC_Analysis plugin”.



1. Sign in to the Ion Torrent™ Server connected to your sequencer via Torrent Suite™ Software.
2. Click  **(Settings)** ▶ **Plugins**
3. Scroll to the ERCC_Analysis plugin to see the installed version.

Install the ERCC_Analysis plugin

The ERCC Companion Panel requires you to upgrade the ERCC_Analysis plugin. You can find this plugin on the Connect.

1. Sign in to the apps.thermofisher.com.
2. Click the **Apps** icon (.
3. In **AppConnect**, under **Resource Libraries**, click **Plugins**.
4. Select **RNA-Seq** in the category bar.



5. Click  to download the ERCC_Analysis plugin. Select the checkbox to indicate that you agree to the terms and conditions, then click **Download Plugin**.
Either a compressed directory or a debian file that contains the plugin is downloaded to your local machine.
6. Click  (**Settings**) ▶ **Plugins** ▶ **Install or Upgrade Plugin** in Torrent Suite™ Software.
7. Click **Select File**, then browse to the location where you downloaded the plugin file, select the file, then click **Open**.
8. In the **Install or Upgrade Plugin** dialog box, click **Upload and Install**.

The plugin is now visible in Torrent Suite™ Software.






WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit thermofisher.com/support.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Caution, hot surface	Attention, surface chaude



(continued)

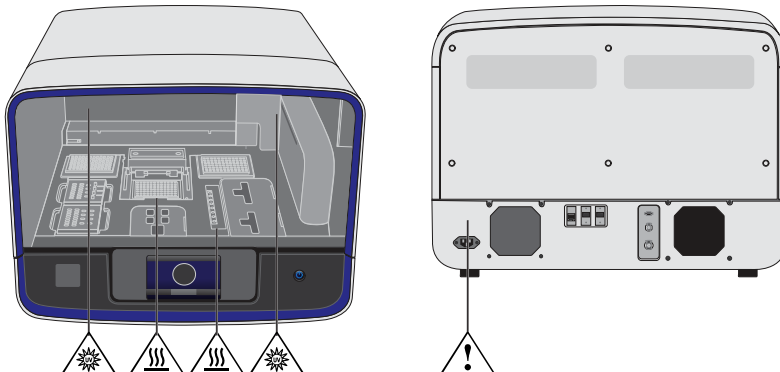
Symbol	English	Français
	Ultraviolet light	Rayonnement ultraviolet
	On/Off	On/Off (marche/arrêt)
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Do not dispose of this product in unsorted municipal waste	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.
	<p>CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.</p>	<p>MISE EN GARDE ! Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.</p>

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union Low Voltage Directive 2006/95/EC, EMC Directive 2014/30/EU, and R&TTE Directive 1999/5/EC.
	Indicates conformity with Australian standards for electromagnetic compatibility.



Location of safety labels on this instrument



Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General



CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.



Physical injury



CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.



CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.



WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.



Cleaning and decontamination



CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Ultraviolet (UV) Safety

The Ion Chef™ System uses a UV lamp which emits light at 254 nm. Under normal operating conditions, the UV lamp is powered on when performing the cleaning protocol. Safety interlocks are used to help ensure that the UV lamp is not powered when the door is open.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety compliance

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
IEC 61010-2-010 EN 61010-2-010	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</i>
IEC/EN 61010-2-020	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges</i>
IEC 61010-2-081 EN 61010-2-081	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes</i>



EMC

Reference	Description
Directive 2014/30/EU	<i>European Union "EMC Directive"</i>
FCC Part 15	<i>U.S. Standard "Industrial, Scientific, and Medical Equipment"</i>
AS/NZS 2064	<i>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</i>
ICES-001, Issue 3	<i>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</i>
US FCC CFR Title 47 Part 15.225, Subpart C	<i>Operation within the band 13.110–14.010 MHz.</i>
Industry Canada RSS 210, Issue 8, Annex 2 EN 302 291-1/2 V1.1.1	<i>Licence-Exempt Radio Apparatus: Category I Equipment</i>

Environmental design

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment



Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

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 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have questions, contact Life Technologies at www.thermofisher.com/support.

