iontorrent

Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit

USER GUIDE

for use with:
Ion OneTouch™ 2 System
Ion Chef™ System
Ion GeneStudio™ S5 System
Ion GeneStudio™ S5 Plus System
Ion GeneStudio™ S5 Prime System
Ion S5™ System
Ion S5™ XL System
Ion Proton™ System

Catalog Numbers A36553, A36554, and A36555
Publication Number MAN0017343
Revision B.0

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ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0017343

Revision	Date	Description
B.0	5 December 2018	• Protocol updates • Support added for the Ion AmpliSeq™ RNA ERCC Companion Panel • Support added for the Ion GeneStudio™ S5 Systems
A.0	30 November 2017	New document

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1 Product information

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

Product description

The Ion AmpliSeq™ Gene Expression Core Panel provides gene-level expression information from a single multiplexed panel targeting over 20,000 genes (> 90% of the RefSeq gene database). Library preparation with the kit requires 10 ng of total RNA input, without the need for poly (A) selection or ribosomal RNA depletion.

This guide describes the manual procedure for using the Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel from the Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit to prepare libraries from RNA.

In addition, instructions for using the Ion AmpliSeq™ RNA ERCC Companion Panel are provided. The Ion AmpliSeq™ RNA ERCC Companion Panel in combination with the ERCC RNA Spike-In Mix enables performance assessment of libraries in gene expression experiments on Ion Torrent™ sequencing platforms. (For details, see Appendix B, "Ion AmpliSeq™ RNA ERCC Companion Panel").

Software compatibility

The Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel is compatible with Torrent Suite™ Software version 5.8 and later. Be sure to update your Torrent Server to the latest available version of Torrent Suite™ Software before using this kit.

Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit

The Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit (Cat. No. A36412, ordered separately) provides reagents for using the Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel on the Ion Chef™ System. The Ion AmpliSeq™ Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit consists of Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel tubes, which are ready to load into an Ion AmpliSeq™ Chef Reagents DL8 cartridge, and the Ion AmpliSeq™ Kit for Chef DL8, which contains all the reagents and supplies sufficient for preparing 32 libraries. See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for more information.

Compatible kits

The panel is optimized to work with the following kits:

- Ion AmpliSeq™ Library Kit Plus
- Ion Xpress™ Barcode Adapters
- IonCode™ Barcode Adaptors
- Ion AmpliSeq™ RNA ERCC Companion Panel and ERCC RNA Spike-In Mix
- Templating kits for:
  - Ion Chef™ System
  - Ion OneTouch™ 2 System
- Sequencing kits for:
  - Ion GeneStudio™ S5 System
  - Ion GeneStudio™ S5 Plus System
  - Ion GeneStudio™ S5 Plus System
  - Ion S5™ System
  - Ion S5™ XL Systems
  - Ion Proton™ System

Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit

The Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit (Cat. Nos. A36553, A36554, and A36555) provides reagents for 24, 96, and 384 libraries, respectively.

Component	Number of kits		
	Cat. No. A36553 (24 reactions)	Cat. No. A36554 (96 reactions)	Cat. No. A36555 (384 reactions)
Ion AmpliSeq™ Library Kit Plus	1	4	16
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel	1	4	16

The kit components for each kit are shown in the following table. The amount of each component is indicated for a single kit.

Contents	Amount	Storage
Ion AmpliSeq™ Library Kit Plus [1]		
5X Ion AmpliSeq™ HiFi Mix (red cap)	120 µL	–30°C to –10°C
FuPa Reagent (brown cap)	48 µL	
Switch Solution (yellow cap)	96 µL	
DNA Ligase (blue cap)	48 µL	
25X Library Amp Primers (white cap)	48 µL	

Contents	Amount	Storage
1X Library Amp Mix (black cap)	1.2 mL	–30°C to –10°C
Low TE (clear cap)	6 mL	Room temperature (15—30°C)
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel[2]		
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel	192 µL	–30°C to –10°C

[1] Kit is shipped on frozen gel packs. Store as indicated.
[2] Shipped on dry ice. Store as indicated.

IonCode™ Barcode Adapters 1–384 Kit

The IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751) provides 384 different pre-mixed adapters in a convenient 96-well plate format. These barcode adapters, or Ion Xpress™ Barcode Adapters, are required to run multiple libraries per sequencing chip, and are ordered separately.

Component	Quantity	No. of reactions	Storage
IonCode™ Barcode Adapters 1–384 Kit: • IonCode™ 0101–0196 in 96-well PCR Plate (red) • IonCode™ 0201–0296 in 96-well PCR Plate (yellow) • IonCode™ 0301–0396 in 96-well PCR Plate (green) • IonCode™ 0401–0496 in 96-well PCR Plate (blue)	4 × 96-well plates (20 µL/well)	3,840 (10 reactions per barcode)	–30°C to –10°C

Ion Xpress™ Barcode Adapters Kits

Each kit provides 16 different barcode adapters, sufficient for ~640 Ion AmpliSeq™ libraries. These barcode adapters, or IonCode™ Barcode Adapters, are required to run multiple libraries per sequencing chip, and are ordered separately.

Component	Cap color	Quantity	Volume per tube	Storage
Ion Xpress™ P1 Adapter	Violet	1 tube	320 µL	–30ºC to –10ºC
Ion Xpress™ Barcode X	White	16 tubes (1 per barcode)	20 µL each	

The following Ion Xpress™ Barcode Adapters Kits are available:

- Ion Xpress™ Barcode Adapters 1–16 (Cat. No. 4471250)
- Ion Xpress™ Barcode Adapters 17–32 (Cat. No. 4474009)
- Ion Xpress™ Barcode Adapters 33–48 (Cat. No. 4474518)
- Ion Xpress™ Barcode Adapters 49–64 (Cat. No. 4474519)
- Ion Xpress™ Barcode Adapters 65–80 (Cat. No. 4474520)
- Ion Xpress™ Barcode Adapters 81–96 (Cat. No. 4474521)
- Ion Xpress™ Barcode Adapters 1–96 (Cat. No. 4474517; Complete set of adapters)

Required materials and equipment not supplied

Unless otherwise specified, all materials are available from Thermo Fisher Scientific (**www.thermofisher.com**). MLS: Fisher Scientific (**www.fisherscientific.com**) or major laboratory suppliers.

Item	Source
SuperScript™ VILO™ cDNA Synthesis Kit	11754050
Other materials	
One of the following: • Ion Xpress™ Barcode Adapters 1-16 Kit • IonCode™ Barcode Adapters 1–384 Kit	• 4471250 • A29751 or A31174
MicroAmp™ Optical 96-well Reaction Plates	• N8010560 • 4306737 (with barcode)
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Ion Library Quantitation Kit	4468802
Agencourt™ AMPure™ XP Reagent	A63882

Item	Source
100% Ethanol	MLS
Nuclease-Free Water	AM9932
1.5-mL Eppendorf LoBind™ Tubes	022431021
Qubit™ 4 Fluorometer or equivalent.[1]	Q33216
Magnetic Stand-96 or DynaMag™-96 Side Magnet	AM10027, 12331D
One of the following: • SimpliAmp™ Thermal Cycler • AB™ 2720 Thermal Cycler • Veriti™ 96-well Thermal Cycler • ProFlex™ 96-well PCR System • GeneAmp™ PCR System 9700 Single or Dual 96-well Thermal Cycler[1]	Various
Microcentrifuge (for quick 2000 × *g* centrifugations)	MLS

[1] The Qubit™ 3.0 Fluorometer and Qubit™ 2.0 Fluorometer are supported but no longer available for purchase.

Recommended materials and equipment

Unless otherwise specified, all materials are available from Thermo Fisher Scientific (**www.thermofisher.com**). MLS: Fisher Scientific (**www.fisherscientific.com**) or major laboratory suppliers.

Item	Source
Recommended **RNA isolation kits**	
MagMAX™-96 Total RNA Isolation Kit	4463365
PureLink™ RNA Mini Kit	12183020
Recommended **for quantification**	
Qubit™ RNA HS Assay Kit	Q32852
Agilent™ 2100 Bioanalyzer™ Instrument	G2939AA
Agilent™ High Sensitivity DNA Kit	5067-4626
Agencourt™ AMPure™ XP Reagent	A63882
Other materials	
(Optional) Universal RNA — Mouse Normal Tissues	Biochain Institute R4334566-1
(Optional) Ion AmpliSeq™ RNA ERCC Companion Panel and ERCC RNA Spike-In Mix	A36552 and 4453740

2 Methods

Note:
See the *Ion AmpliSeq™ Library Preparation on the Ion Chef™ System User Guide* (Pub. No. MAN0013432) for instructions to run the Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel on the Ion Chef™ Instrument.

Before first use

Data analysis

Ion AmpliSeq™ Transcriptome library analysis has two components:

- Mapping of sequence reads to the `AmpliSeq_Mouse_Transcriptome_V1_Reference.fasta` file.
- Quantifying matches per amplicon using defined amplicon regions from the `AmpliSeq_Mouse_Transcriptome_V1_Designed.bed` file.

Torrent Suite™ Software aligns reads to the reference and the ampliSeqRNA plugin determines valid matches to amplicon target regions in the panel using the AmpliSeq_Mouse_Transcriptome_V1_Designed.bed file.

Download panel files from Ampliseq.com

You can either download the panel files from Ampliseq.com and manually import them, or you can link your Torrent Server to your Ampliseq.com account and upload them from the **Plan** ▸ **Templates** page. See the *Torrent Suite™ Software Help*.

1. Sign in to your Ampliseq.com account (**AmpliSeq.com**).
2. Select **Ready-to-Use Panels**, then **RNA**.

3. Find the **Ion AmpliSeq Transcriptome Mouse Gene Expression Research Panel** and click **Download panel files**.
   A compressed folder that is named **Ion_AmpliSeq_Mouse_Transcriptome_V1.zip** downloads.

4. Extract the files from the compressed folder.

5. Find the `AmpliSeq_Mouse_Transcriptome_V1_Reference.fasta` and `AmpliSeq_Mouse_Transcriptome_V1_Designed.bed` files, then save them to a storage location.
   You can also contact Technical support for help. See "Customer and technical support" on page 41.

6. See "Import the Ion AmpliSeq™ Transcriptome mapping reference" on page 12 to install the `AmpliSeq_Mouse_Transcriptome_V1_Reference.fasta` and "Upload a target regions file" on page 13 to install the `AmpliSeq_Mouse_Transcriptome_V1_Designed.bed` file.

Import the Ion AmpliSeq™ Transcriptome mapping reference

The mouse transcriptome reference is not a preloaded reference in Torrent Suite™ Software; however, you can import it as a custom reference.

1. Download the `AmpliSeq_Mouse_Transcriptome_V1_Reference.fasta` file from ampliseq.com or your Field Applications Specialist (FAS). See "Download panel files from Ampliseq.com" on page 11 for more information.

2. In Torrent Suite™ Software, click **(Settings) ▸ References**.

3. Click **Import Custom Reference**.

4. In the **Add Reference Sequence** dialog box, upload the `AmpliSeq_Mouse_Transcriptome_V1_Reference.fasta` file from your local storage. The transcript index creation takes a few minutes.
   a. Select the **Upload File** tab.
   b. Click **Select File**, then navigate to and select the file from your local storage.
   c. Click **Open**.

5. Complete the information required in the following fields.

Field	Description
Short name *(required)*	Enter a recognizable short form of the genome name.
Description *(required)*	Enter a longer, more descriptive reference genome name.
Version *(optional)*	Enter a version number and the accession number (if there is one).
Notes *(optional)*	Use this field to record any notes about the reference genome.

6. Click **Import Reference**.

The reference genome and associated information is added to the **Reference Sequences** table.

Upload a target regions file

You must upload the `AmpliSeq_Mouse_Transcriptome_V1_Designed.bed` to Torrent Suite™ Software before you can create a Planned Run.

1. Click **(Settings)** ▸ **References** , then click **Target Regions** in the left navigation menu.
2. In the **Target Regions** screen, click **Add Target Regions**.
3. In the **New Target Region**s screen, click **Select File**, then navigate to the `AmpliSeq_Mouse_Transcriptome_V1_Designed.bed` file to be uploaded.
4. Select the `AmpliSeq_Mouse_Transcriptome_V1` reference sequence from the Reference dropdown list.
5. *(Optional)* Add a description and notes.
6. Click **Upload Target Regions File**.
   Wait while the file is validated. The status is updated to **Successfully Completed** after the upload finishes.

   Note: For large files, validation can take several minutes. Refresh your browser to check that validation is complete.

Before you begin

- Thaw components that contain enzymes—such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, and DNA Ligase—on ice, and keep on ice during procedure. All other components, including primer pools, can be thawed at room temperature. Gently vortex and centrifuge before use.
- If there is visible precipitate in the Switch Solution or the tube cap after thawing, vortex or pipet up and down at room temperature to resuspend.
- Minimize freeze-thawing of Ion AmpliSeq™ Gene Expression Core Panel and 5X VILO™ Reaction Mix by aliquoting if needed for your experiments.
- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is separate from template preparation. Always change pipette tips between samples.
- Do not reuse MicroAmp™ Clear Adhesive Films.
- Pipet viscous solutions slowly and ensure complete mixing by vigorous vortexing or pipetting up and down several times.

Guidelines for isolating and quantifying RNA

- A list of recommended RNA isolation kits is provided in "Required materials and equipment not supplied" on page 8.

IMPORTANT! We strongly recommend DNase treatment of RNA samples. Follow the DNase treatment instructions in RNA isolation kit manual, or use TURBO DNA-*free*™ Kit (Cat. No. AM1907) for DNase treatment. Although DNase-treated total RNA is the recommended input, you can also start from poly(A)+ RNA or rRNA-depleted RNA. When poly (A)+ RNA is used, amplicons corresponding to genes without poly(A) tail will not be amplified.

- A list of recommended kits and instruments for quantifying RNA is provided in "Required materials and equipment not supplied" on page 8. We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32852) for quantifying unfixed RNA.

Reverse transcribe RNA

1. If you are using the ERCC RNA Spike-In Mix and the Ion AmpliSeq™ RNA ERCC Companion Panel proceed to Appendix B, "Ion AmpliSeq™ RNA ERCC Companion Panel".

2. For each sample, add the following components into a single well of a 96-well PCR plate on ice. Prepare a master mix for multiple reactions, adding the enzyme last.

Component	Volume
5X VILO™ RT Reaction Mix	1.0 µL
10X SuperScript™ III Enzyme Mix	0.5 µL
DNase-treated total RNA (10 ng)[1]	≤ 3.5 µL
Nuclease-Free water	to 5 µL
Total	**5 µL**

[1] Input amount can range from 0.1–100 ng for high quality RNA. PCR cycles must be adjusted accordingly.

3. Seal the plate with MicroAmp™ adhesive film, vortex thoroughly, then centrifuge to collect droplets.

4. 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
4°C	Hold[1]

[1] Samples can be held at 4°C overnight.

STOPPING POINT Samples can be stored at 4°C overnight. For longer periods, store at –20°C.

Amplify targets

1. For each reaction, combine the following components on ice. Prepare a master mix for multiple reactions, adding the enzyme last.

Component	Volume per reaction
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel	8 µL
Nuclease-Free Water	3 µL
Total	**15 µL**

2. If a master mix was prepared, gently vortex PCR master mix, then centrifuge briefly to collect droplets.

3. Remove the plate seal from the reverse transcription reaction, then add 15 µL of PCR master mix to each reaction well of the plate.

4. Seal the plate, vortex thoroughly, then centrifuge to collect droplets.

   Note: Use a new adhesive film to avoid cross-contamination. Due to the long PCR incubation time and small reaction volumes, be sure to seal the plate well and/or use a compression pad to minimize evaporation.

5. Load the plate in the thermal cycler, then run the following program.

Stage	Temperature	Time
Hold	99°C	2 minutes
Cycle; (set number according to the following table)	99°C	15 seconds
	60°C	16 minutes
Hold	10°C	Hold[1]

[1] You can hold samples at 4°C overnight.

Amount	Number of cycles
0.1 – 1 ng	16
10 ng	12
100 ng	10

IMPORTANT! Use recommended input amount and number of PCR cycles to avoid bias in gene expression levels due to PCR saturation.

STOPPING POINT You can store PCR products at 4°C overnight. For longer periods, store at –20°C.

Partially digest primer sequences

1. Carefully remove the plate seal, then add 2 µL of FuPa Reagent (brown cap) to each amplified sample.
2. Seal the plate, vortex thoroughly, then centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
3. Load the plate in the thermal cycler, then run the following program.

Temperature	Time
50°C	10 minutes
55°C	10 minutes
60°C	20 minutes
10°C	Hold (up to 1 hour)

IMPORTANT! Do not freeze samples at this point. Proceed to next step within 1 hour.

Ligate adapters to the amplicons and purify

When sequencing multiple libraries on a single chip, you *must* ligate a different barcode adapter to each library. DNA and RNA libraries from the same sample also require different barcodes.

IonCode™ Adapters are provided at the appropriate concentration and include forward and reverse adapters in a single well. No further handling is necessary.

Ion Xpress™ adapters require handling and dilution as described below.

IMPORTANT! When handling barcoded adapters, be careful to avoid cross contamination by changing gloves frequently and opening one tube at a time.

Ion Xpress™ adapters only: Combine and dilute adapters

For each barcode X selected, prepare a mix of Ion P1 Adapter and Ion Xpress™ Barcode X at a final dilution of 1:4 for each adapter. For example, combine the volumes indicated in the following table. Scale volumes as necessary. Use 2 µL of this barcode adapter mix in step 3 below.

Component	Volume
Ion P1 Adapter	2 µL
Ion Xpress™ Barcode X[1]	2 µL
Nuclease-free Water	4 µL
Total	**8 µL**

[1] X = barcode chosen

Note: Store diluted adapters at –20°C.

Perform the ligation reaction

1. If there is visible precipitate in the Switch Solution or the tube cap after thawing, vortex or pipet up and down at room temperature to resuspend before pipetting.

2. Briefly centrifuge the plate to collect the contents.

3. Carefully remove the plate seal, then add the following components in the order that is listed to each well containing digested amplicons. If preparing multiple non-barcoded libraries, a master mix of Switch Solution and adapters can be combined before addition.

   IMPORTANT! Add the DNA Ligase last. Do not combine DNA Ligase and adapters before adding to digested amplicons.

Order of addition	Component	Volume
1	Switch Solution (yellow cap)	4 µL
2	IonCode™ Adapters *or* diluted Ion Xpress™ barcode adapter mix (for barcoded libraries)	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume** (including ~22 µL of digested amplicon)	**~30 µL**

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

5. Place a MicroAmp™ Compression Pad on the plate, load in the thermal cycler, then run the following program:

Temperature	Time
22°C	30 minutes
72°C	5 minutes
10°C	Hold (up to 1 hour)

STOPPING POINT Samples can be stored for up to 24 hours at 10°C on the thermal cycler. For longer periods, store at –20°C.

Purify the unamplified library

IMPORTANT! Bring the AMPure™ XP Reagent to room temperature, then vortex thoroughly to disperse the beads before use. Pipet the suspension slowly.

IMPORTANT! Use freshly prepared 70% ethanol for the next steps. Combine 230 µL of 100% ethanol with 100 µL of Nuclease-Free Water per sample.

1. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times to thoroughly mix the bead suspension with the DNA.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the plate in a magnetic rack such as the DynaMag™-96 Side Magnet (Cat. No. 12331D), then incubate for 2 minutes or until solution clears. Carefully remove, then discard the supernatant without disturbing the pellet.
4. Add 150 µL of freshly prepared 70% ethanol and move the plate side-to-side in the two positions of the magnet to wash the beads, then remove and discard the supernatant without disturbing the pellet.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down 5 times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.
5. Repeat step 4 for a second wash.
6. Use a 10- or 20-µL pipettor to remove all ethanol droplets from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 2 minutes.

Proceed immediately to “Quantify and dilute the library“ on page 19.

Quantify and dilute the library

Determine library quantification method

The following kits are recommended for library quantification.

- Ion Library TaqMan® Quantitation Kit
- Agilent™ 2100 Bioanalyzer™ Instrument

Option 1: Quantify library by qPCR

Elute the unamplified library

1. Remove the plate containing the Ion AmpliSeq™ Transcriptome library from the magnet, then add 50 µL of Low TE to the pellet to disperse the beads. Seal the plate, vortex thoroughly, then centrifuge down to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

2. Place the plate in the magnet for at least 2 minutes. Transfer 45 µL of the supernatant to new wells on the same plate.

   Note: Use a smaller volume (45 µL instead of 50 µL) to avoid bead carryover during transferring. You may also leave the plate on the magnet before transferring final libraries for quantification or template preparation.

Quantify library by qPCR and calculate dilution factor

1. Prepare five 10-fold serial dilutions of the *E. coli* DH10B Ion Control Library (~68 pM; from the Ion Library TaqMan® Quantitation Kit) at 6.8 pM, 0.68 pM, 0.068 pM, 0.0068 pM, and 0.00068 pM (standards 1–5). Mark these as standards, then use these concentrations in the qPCR instrument software.

2. Dilute each Ion AmpliSeq™ Transcriptome library using the following recommendations.

Amount	Recommended dilutions
10 ng	1:2,500 or 1:10,000

3. Prepare reaction mixtures for 3 wells for each library and standard sample. Use the following tables to calculate the required volume for the master mix.

Component	Volume per reaction	
	96-well plate	384-well plate
2X TaqMan® Master Mix	10 µL	5 µL
20X Ion TaqMan® Assay	1 µL	0.5 µL
Total	**11 µL**	**5.5 µL**

4. Perform one of the following actions based on your choice of plates:

Option	Action
96-well reaction plates	Dispense 11 µL of the master mix into each well, then add 9 µL of your diluted library and standards.
384-well reaction plates	Dispense 5.5 µL of the master mix into each well, then add 4.5 µL of your diluted library and standards.

5. Load the plate in the real-time instrument, then run the following program.

Stage	Temperature	Time
Hold	50°C	2 minutes
Hold	95°C	20 seconds
Cycle (40 cycles)	95°C	1 second
	60°C	20 seconds

6. Following qPCR, calculate the average concentration of the undiluted Ion AmpliSeq™ Transcriptome library by multiplying the concentration that is determined with qPCR by the library dilution that is used in the assay.

7. If the library concentration is greater than 70 pM, normalize the final library concentration to 70 pM, then pool barcoded libraries for templating and sequencing by combining an equal volume of each barcoded library. Alternatively, if one or more libraries is <70 pM, dilute each library to the same concentration, and pool by combining an equal volume of each.

   Expected yield: 0.5–5.0 nM

Option 2: Quantify the library using Agilent™ 2100 Bioanalyzer™ Instrument

Note: We do not recommend this option for libraries prepared from RNA.

Amplify the library

1. Remove the plate containing the Ion AmpliSeq™ Transcriptome library from the magnet, then add 50 µL of 1X Library Amp Mix and 2 µL of 25X Library Amp Primers to each bead pellet. Pipet the mixture up and down 5 times to mix thoroughly.

2. Place the plate back on the magnet for at least 2 minutes or until solution clears, then carefully transfer ~50 µL of supernatant from each well to clean plate without disturbing the pellet.

   Note: *(Optional)* Alternatively, amplify the library in the presence of the AMPure™ XP Reagent.

3. Seal the plate with MicroAmp™ Adhesive Film, place a MicroAmp™ Compression Pad on the plate, load in the thermocycler, then run the following program:

Stage	Temperature	Time
Hold	98°C	2 minutes
5 cycles	98°C	15 seconds
	64°C	1 minute
Hold	10°C	Hold (up to 1 hour)

STOPPING POINT *(Optional)* You can store samples at –20°C.

Purify the amplified library

1. Add 25 µL of Agencourt™ AMPure™ XP Reagent (at room temperature) to each plate well containing ~50 µL of sample, then pipet up and down 5 times to thoroughly mix the bead suspension with the DNA.

2. Incubate the mixture for 5 minutes at room temperature.

3. Place the plate in a DynaMag™-96 Side Magnet for at least 3 minutes or until solution is completely clear.

4. Carefully transfer the supernatant to a new well on the same plate without disturbing the pellet. Discard the pellet.

5. Remove the plate from the magnet. To the supernatant from previous step, add 60 µL of Agencourt™ AMPure™ XP Reagent, then pipet up and down 5 times to thoroughly mix the bead suspension with the DNA.

6. Incubate the mixture for 5 minutes at room temperature.

7. Place the plate in the magnet for 5 minutes or until the solution is clear. Carefully remove, then discard the supernatant without disturbing the pellet.

8. Add 150 µL of freshly prepared 70% ethanol to each well, then move the plate side to side in the magnet to wash the beads. Remove, then discard the supernatant without disturbing the pellet.

9. Repeat step 8 for a second wash.

10. Use a 10- or 20-µL pipette to remove all ethanol droplets from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 2 minutes.

11. Remove the plate containing the Ion AmpliSeq™ Transcriptome library from the magnet, then add 50 µL of Low TE to the pellet to disperse the beads. Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge down to collect droplets.

12. Place the plate on the magnet for at least 2 minutes. Transfer 45 µL of the supernatant to new a well on the same plate.

    Note: Use slightly smaller volume (45 µL instead of 50 µL) to avoid bead carryover during transfer. You can also leave the plate on the magnet before transferring final libraries for quantification or template preparation.

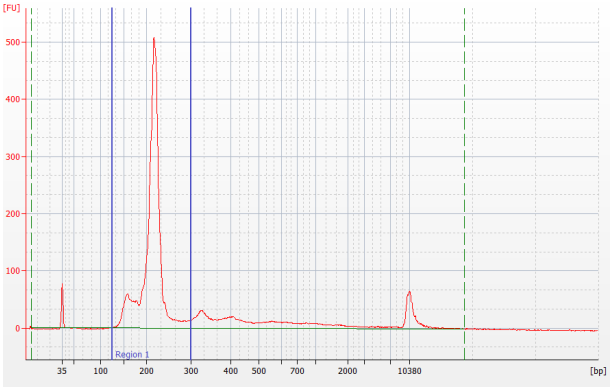
Quantify the library using the Agilent™ 2100 Bioanalyzer™ instrument and calculate dilution factor

1. Analyze 1 µL of amplified library on the Agilent™ 2100 Bioanalyzer™ instrument with the Agilent™ High Sensitivity DNA Kit (Cat. No. 5067-4626).

2. Determine the molar concentration of the amplified library using the Bioanalyzer™ software.

    Note: Ensure that the upper and lower marker peaks are identified and assigned correctly. Follow the manufacturer's instructions to perform a region analysis (smear analysis) in the 125–300 bp size range.

3. If the library concentration is > 20,000 pM, dilute the library 1:10, then repeat the quantification to obtain a more accurate measurement. Ion AmpliSeq™ Transcriptome libraries typically have yields of 1,000–50,000 pM.



Example trace of amplified Ion AmpliSeq™ Transcriptome library.

4. Based on the calculated library concentration, determine the dilution that results in a concentration of ~70 pM.

5. Dilute library to ~70 pM as described, pool barcoded libraries by combining an equal volume of each.

Templating and sequencing

Proceed to template preparation and sequencing, using the following guidelines. Detailed information is in the user guide for your template preparation kit.

Planned Run guidelines

- Use the following guidelines to create a Planned Run.

Template	Description
Ion AmpliSeq Transcriptome Mouse Gene Expression Panel OT2-Proton	For use withIon OneTouch™ 2 System and Ion Proton™ System.
Ion AmpliSeq Transcriptome Mouse Gene Expression Panel Manual Chef-S5	For use with manual Ion Chef™ System and Ion S5™ System or Ion GeneStudio™ S5 Systems.
Ion AmpliSeq Transcriptome Mouse Gene Expression Panel Chef-S5	For use with automaticIon Chef™ System and Ion S5™ System or Ion GeneStudio™ S5 Systems.

- Select the ampliSeqRNA plugin.
- (Optional) If you are using the Ion AmpliSeq™ RNA ERCC Companion Panel, select the ERCC_Analysis.

ampliSeqRNA plugin configuration

The configuration options for the ampliSeqRNA plugin are described in the following table. This plugin cannot be configured globally.

Note: You can change the **Reference Genome** used in the plugin run, if you edit the run report, then reanalyze the data (or reads) from the completed run.

Setting	Description
The following settings can be configured when you or select the ampliSeqRNA plugin as part of a Planned Run or Planned Run template.	
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses if they have a relatively low number of reads, such as those that can result from barcode contamination. A warning appears in the barcode summary report if any barcodes were discounted from the analysis. This setting is ignored for runs not using barcodes. Typically, the Filter Barcodes option is not needed if your Planned Run specifies which samples to associate with specific barcodes.
ERCC Tracking	Leave deselected.

Setting	Description
The following settings can be configured when you run the ampliSeqRNA plugin manually.	
Library Type	ampliSeqRNA is selected automatically and is currently the only **Library Type** that the ampliSeqRNA plugin is designed to work with. Note: If the Planned Run specified a different application, a dialog box will warn you that the plugin may not be appropriate for the run.
Targeted Regions	This is set to the target regions file used in the Planned Run. **Note:** You can override the default Target Regions setting that each barcode uses. This might be useful to specify a subset of genes of interest, or to correct the original Planned Run.
Filter Barcodes	Select this checkbox to remove whole barcodes from subsequent analyses. Typically, the **Filter Barcodes** option is not needed if your Planned Run specifies which samples to associate with specific barcodes.
ERCC Tracking	Leave deselected.

ERCC_Analysis plugin configuration

Configure the ERCC_Analysis plugin as described in the following table.

Parameter	Value
Passing R-squared value	0.90
Minimum transcript counts	10
ERCC pool used	Pool1[1]
Barcodes of interest	Select the barcodes that you used to generate the Ion AmpliSeq™ libraries.

[1] Pool 1 must be used with the Ion AmpliSeq™ RNA ERCC Companion Panel.

A

Tips and troubleshooting

Troubleshooting with control RNA

You can use Universal RNA — Mouse Normal Tissues (Biochain Institute Cat. No. R4334566-1) with the Ion AmpliSeq™ Transcriptome Mouse Gene Expression Core Panel as a general troubleshooting strategy. Use 10 ng of total RNA to follow the procedures that are outlined in this user guide. When 8 libraries are sequenced on an Ion PI™ Chip and analyzed using the ampliSeqRNA analysis plugin, you can expect about 90% of the reads on target for this control RNA library. Additionally you can expect 65–70% of the targets in this panel to be detected at ≥ 10 reads, representing gene expression levels covering 5 log units of dynamic range.

Observation	Possible cause	Recommended action
Low library yield	Input RNA was incorrectly quantified.	Requantify input RNA using a Qubit™ Fluorometer or Agilent™ RNA LabChip™ Kit. If neither is available, quantify with a NanoDrop™ Spectrophotometer, a less accurate alternative.
	Input RNA was less than 10 ng.	Add more RNA or increase target amplification cycles (See the table of recommendations in step 5 of "Amplify targets" on page 15 for cycle numbers based on input amounts).
	RT reaction was inefficient.	Make master mix if possible. For individual reaction setup, make sure correct volume of 5X VILO™ buffer and 10X SuperScript™ III enzyme mix is added into each reaction.
	PCR, digestion, or ligation reactions were inefficient.	Ensure proper dispensing and mixing of viscous components at each step.
	AMPure™ XP Reagent was overdried.	Do not dry the AMPure™ XP Reagent for more than 5 minutes.
Library yield is high	Input RNA was incorrectly quantified.	Requantify input RNA using a Qubit™ Fluorometer or Agilent™ RNA LabChip™ Kit. If neither is available, quantify with NanoDrop™ as a less accurate alternative.
	Input RNA was more than 10 ng.	Add less RNA or decrease target amplification cycles. **Note:** Do not use more than 100 ng of input RNA in the reverse transcription reaction; this can cause non-linear target amplification.
Number of on-target reads is lower than expected	Either the input RNA was less than 10 ng, or the PCR amplification cycles were less than optimal.	Add more RNA or increase target amplification cycles.

Observation	Possible cause	Recommended action
Number of on-target reads is lower than expected	RNA was degraded.	Use highest quality RNA possible. For degraded RNA, use up to 16 PCR cycles.
Barcode representation is uneven	Library was inaccurately quantitated.	Use correct dilution factor when calculating concentration.
	Library was inaccurately combined.	Dilute libraries to 100 pM, then combine equal volumes. If library concentration is less than 100 pM, dilute to a fixed concentration, for example, 50 pM, then combine equal volumes. Re-quantify the library pool to confirm the expected concentration.
The fraction of polyclonal ISPs (>40%) is high	Library was over-seeded.	Decrease amount of library added to the template preparation reaction by 50%.
	Library was incorrectly quantified.	Ensure that library was accurately quantified.
Library yields from replicate RNA samples are inconsistent	Sample evaporated in thermal cycler.	Seal the 96-well MicroAmp™ plates well with MicroAmp™ Adhesive Film Applicator (Cat. No. 4333183) and use a MicroAmp™ Compression Pad (Cat. No. 4312639).

B

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**.

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[1] and Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit[2] only.

Software compatibility

The Ion AmpliSeq™ RNA ERCC Companion Panelis 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 Torrent Server to the latest available version of Torrent Suite™ Software before using this kit.

[1] Cat. Nos. A26325, A26326, A26327, and A31446
[2] Cat. Nos. A36553, A36554, A36555, and A36412

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**.

Item	Source
ERCC RNA Spike-In Mix	4456740
One of the following: • 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	• 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 30.
- For instructions for installing the ERCC_Analysis plugin, see "Install the ERCC_Analysis plugin" on page 30.

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.

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 step 2 in "Reverse transcribe RNA" on page 14

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 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 Thermo Fisher Cloud.

1. Sign in to the **Thermo Fisher Cloud**.
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.

C

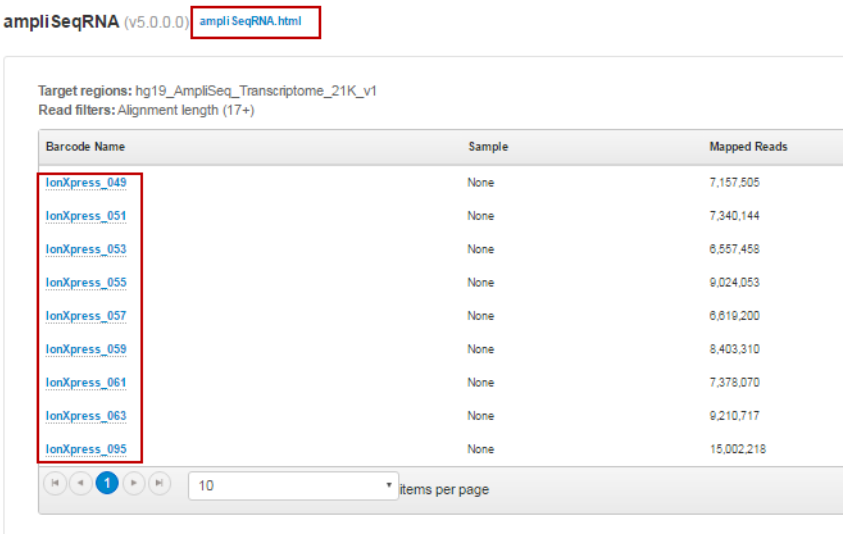
Data analysis

Review ampliSeqRNA plugin results

The ampliSeqRNA plugin generates an initial summary report that lists the samples, the number of mapped reads, the percent of valid reads, and the percent of targets detected. A series of log2 reads-per-million (RPM) pair correlation plots are included for rapid correlation analysis. Microsoft™ Excel™-compatible reports are also generated, including differential expression tables. Additional details about read coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

After the sequencing run completes, review the plugin results in the report summary.

1. In the **Data** tab, click **Completed Runs & Reports**.
2. In the list of runs, locate the run of interest, then click the link in the **Report Name** column.
3. In the left navigation menu, click **ampliSeqRNA** to view the plugin results.



- Click the **ampliSeqRNA.html** link to open the **ampliSeqRNA Report – Barcode Summary** for all barcodes.
- In the barcode table, click individual barcode names to see the results for an individual barcode.
- Click the **Distribution Plots**, **Correlation Heatmap**, **Correlation Plot**, and **Gene Heatmap** tabs to review the data graphically.

Graphical report	Description
Distribution Plots	
Reads Alignment Summary	A graphical summary of the number of mapped and unmapped reads across barcodes, as reported in the **Barcode Summary** table.
Distribution of Gene Reads	Distribution of genes across barcodes showing the frequency of numbers of genes having similar log10 read counts. All curves are plotted on the same axis scale. The counts data are fitted to a Gaussian kernel using the default R 'density' function.
Correlation Heatmap	A heatmap of Spearman correlation r-values for comparing log2 RPM reads pair correlation barcodes, with dendrogram reflecting ordering of barcodes as being most similar by these values.

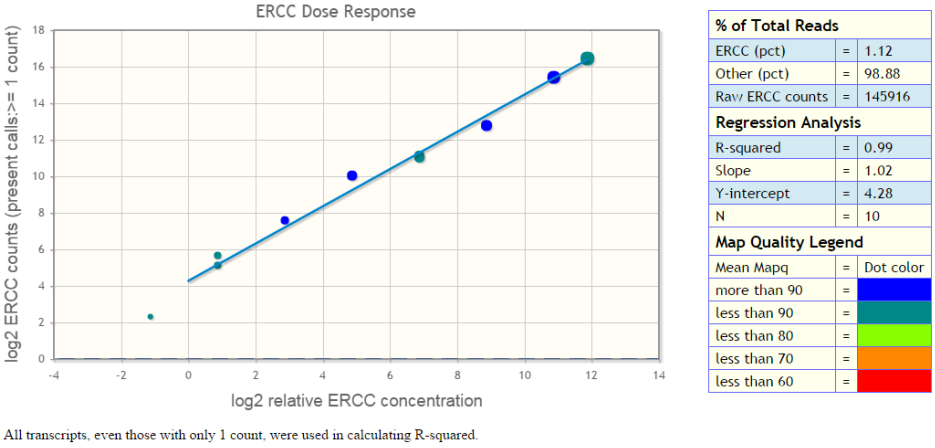
Graphical report	Description
Correlation Plot	
Barcode read pair correlation plot	Lower panels show log2(RPM+1) values plotted for each pair of barcodes, with linear least squares regression line overlaid and line slope reported. Upper panels show Pearson correlation r-values for the regression line. Diagonal panels show the frequency density plot for the individual log(RPM+1) values for each barcode. (If only one barcode has reads, a density plot is displayed.) Click the plot to open an expanded view.
Gene Heatmap	
Gene Representation Heatmap	Displays 250 genes showing the most variation in representation across barcodes as measured by the coefficient of variation (CV) of normalized read counts for genes that have at least one barcode with at least 100 RPM reads, plotted using log10 of those counts. For this plot, barcodes are omitted if they have $<10^5$ total reads.

- Click the links at the bottom of the report to download associated report files.

View the data analysis for the ERCC RNA Spike-In Mix

1. In the Torrent Suite™ Software, navigate to **Data ▸ Completed Runs & Results**, then select your run from the **Report Name** list.

   The ERCC_Analysis Plugin generates an ERCC Report as part of the run report. A sample report is shown below.



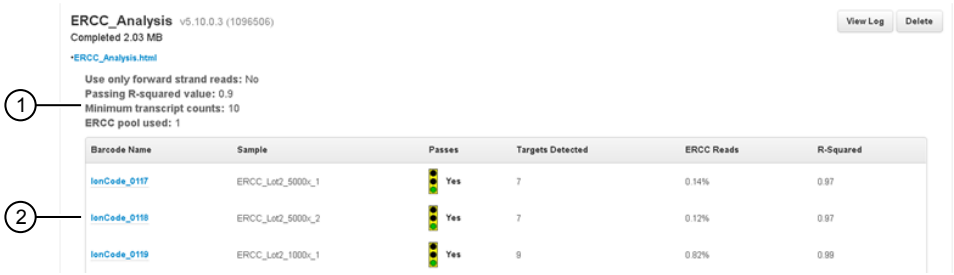
2. If you did not select the ERCC_Analysis plugin when setting up the Planned Run, run the plugin.
   a. Click **Select Plugins to Run ▸ ERCC_Analysis**

b. In the plugin configuration dialog, select appropriate parameters, then click **Submit**.

Parameter	Value
Passing R-squared value	0.90
Minimum transcript counts	10
ERCC pool used	Pool1[1]
Barcodes of interest	Select the barcodes that you used to generate the Ion AmpliSeq™ libraries.

[1] Pool 1 must be used with the Ion AmpliSeq™ RNA ERCC Companion Panel.

3. In the ERCC_Analysis section of the run report, Click a Barcode Name to view a regression plot of ERCC target read number versus relative concentration, with statistics, for the barcoded library you selected.



① ERCC_Analysis plugin parameters
② Barcode names for individual barcoded libraries

4. *(Optional)* Scroll on a data point to view details for that ERCC transcript.

Note: For a manual run of the ERCC_Analysis plugin, the counts per ERCC transcript are downloadable from the plugin output for each individual Ion AmpliSeq™ library. These counts can be easily compared to the concentrations provided in the following table.

ERCC RNAs targeted by the Ion AmpliSeq™ RNA ERCC Companion Panel and their concentrations in ERCC RNA Spike-In Mix 1.

ERCC ID	Concentration in Spike-In Mix 1 (attomole/µL)
ERCC-00031	1.83105469
ERCC-00042	468.75
ERCC-00069	1.83105469
ERCC-00084	29.296875
ERCC-00097	0.45776367
ERCC-00112	117.1875
ERCC-00131	117.1875
ERCC-00136	1,875

ERCC ID	Concentration in Spike-In Mix 1 (attomole/µL)
ERCC-00157	7.32421875
ERCC-00171	3,750

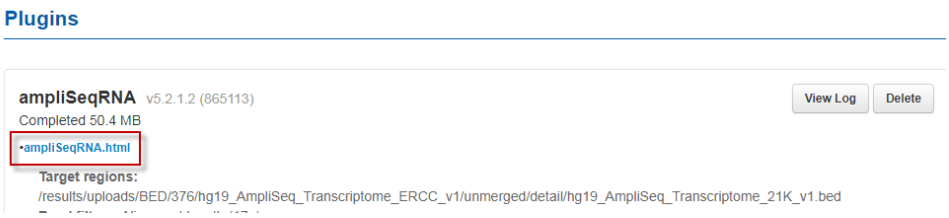
Note: For more information, see the *ERCC RNA Spike-In Control Mixes User Guide* (Pub. No. 4455352).

Analyze Ion AmpliSeq™ data using the Applied Biosystems™ Transcriptome Analysis Console

The Transcriptome Analysis Console (TAC) is used for the following types of analyses.

- Gene and Alternative Splicing analysis
- Comparison of genes from different samples

1. *(Optional)* To download the Transcriptome Analysis Console, search **www.thermofisher.com** for *Transcriptome Analysis Console*, then follow the instructions on the product page to download the software.
2. In the **Data** tab, click **Completed Runs & Reports**, then select your completed run.
3. In the **Plugin** section, click **ampliSeqRNA.html** to open the ampliSeq plugin report.



4. In the **ampliSeqRNA Report** dialog, scroll to the bottom, then click **Download CHP files normalized by RPM**.

   Note: Although the TAC can use CEL or CHP (CodeHealer Project) files, Torrent Suite™ Software generates only CHP files.

5. Open ZIP archive, then save.
6. Download AmpliSeq_Mouse_Transcriptome_V1.transcript.csv and AmpliSeq_Mouse_Transcriptome_V1.TAC_ARRAY_CONFIG files from **AmpliSeq.com**.
   a. Log into **AmpliSeq.com**.
   b. Enter *mouse transcriptome* in the search box.
   c. Select **Ampl_Mouse_Trans**.

d. In the next screen, scroll to the bottom, then click **Download panel files**.

e. In the Download Panel Files dialog, click **Download Now**.

7. Save all of the files in the TacLibraries directory that you selected in the **Preferences** tab of the TAC.

8. Follow the instructions in the *Transcriptome Analysis Console (TAC) User Guide* (Pub. No. 703150, **www.thermofisher.com**).

Safety

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, see the "Documentation and Support" section in this document.

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.

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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

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- 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/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.