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Ion AmpliSeq™ Exome RDY Library Preparation

USER GUIDE

for use with:
Ion AmpliSeq™ Exome RDY Kit 1×8
Ion AmpliSeq™ Exome RDY Kit 4×2

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Revision	Date	Description
F.0	30 October 2023	• Updated storage temparature for the Ion AmpliSeq™ Exome RDY Panel. • Replaced Agencourt™ AMPure™ XP Kit with Beckman Coulter™ AMPure XP 5 mL and Beckman Coulter™ AMPure XP 60 mL.
E.0	17 January 2018	• New catalog numbers added for the Ion AmpliSeq™ Exome RDY Kits, and sequencing chips removed from the kits • Updated for the Qubit™ 4 Fluorometer
D.0	19 September 2017	• Updated FuPa digestion and adapter ligation conditions • Added support for IonCode™ Barcode Adapters • Reagent volumes in the Ion AmpliSeq™ Library Kit Plus updated • Guidance for qPCR program for library quantification aligned with *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub No. MAN0006735) • Updated weblinks • Reorganized chapter structure for ease of use

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

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Contents

- **CHAPTER 1 Product information** 5
  - Product description 5
  - Ion AmpliSeq™ Exome RDY Kits 6
    - Kit contents and storage 6
  - Ion Xpress™ Barcode Adapters Kits 7
  - IonCode™ Barcode Adapters 1–384 Kit 7
  - Required materials not supplied 8
  - Recommended materials and equipment (optional) 9
  - Ion AmpliSeq™ Exome RDY workflow starting from genomic DNA 10
  - Procedure overview 11
- **CHAPTER 2 Prepare Ion AmpliSeq™ Exome RDY libraries** 12
  - Procedure guidelines 12
  - Guidelines for DNA quantification, and amount and quality of DNA needed 13
  - Amplify the targets 13
  - Combine target amplification reactions 14
  - Partially digest amplicons 15
  - Ligate adapters to the amplicons and purify 15
    - Ion Xpress™ Barcode Adapters adapters only: Combine and dilute adapters 16
    - Set up and run the ligation reaction 16
    - Purify the unamplified library 17
    - Proceed to library quantification 17
- **CHAPTER 3 Quantify the unamplified library by qPCR** 18
  - Elute and dilute the library 18
  - Quantify the library and dilute 19
  - (Optional) Combine exome libraries 20
  - Store libraries 20

CHAPTER 4 Quantify the amplified library with the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument 21

Amplify the library 21
Purify the amplified library 22
First-round purification 22
Second-round purification 23
Qubit™ Fluorometer: Quantify the library and calculate the dilution factor 24
Agilent™ 2100 Bioanalyzer™: Quantify the library and calculate the dilution factor 25
(Optional) Combine exome libraries 25
Store libraries 25

APPENDIX A Tips and troubleshooting 26

Tips 26
Modifications to the standard workflow 26
Shortcuts 26
Limited samples 26
Troubleshooting 27
Library yield and quantification 27
Low amplicon uniformity 28
Other 30

APPENDIX B Strategies for combining Ion AmpliSeq™ Exome RDY libraries 32

Ion Chip capacities for Ion AmpliSeq™ Exome RDY libraries 32

APPENDIX C Data analysis 33

Torrent Coverage Analysis plugin 33
Configure and run the Torrent Coverage Analysis plugin 33
Coverage Analysis Report 35
Torrent Variant Caller plugin 39
Configure and run the Torrent Variant Caller plugin 39

APPENDIX D Safety 41

Chemical safety 42
Biological hazard safety 43

Documentation and support 44

Customer and technical support 44
Limited product warranty 44

Product information

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

Product description

This guide covers the following products:

- Ion AmpliSeq™ Exome RDY Kit 1×8 (Cat. No. A38262)
- Ion AmpliSeq™ Exome RDY Kit 4×2 (Cat. No. A38264)
- Ion Xpress™ Barcode Adapters Kits (various Cat. Nos.)
- IonCode™ Barcode Adapters 1–384 Kit (Cat. No. A29751)

The **Ion AmpliSeq™ Exome RDY Kits** contain reagents for the rapid preparation of eight exome libraries for sequencing using the one of the Ion GeneStudio™ S5 Series Sequencers, the Ion S5™/Ion S5™ XL Sequencer, or the Ion Proton™ Sequencer. These kits include Exome RDY plates that have 12 primer pools that are dried into 2 rows of 4 plates (Cat. No. A38264) or 8 rows of one plate (Cat. No. A38262) to minimize pipetting steps and facilitate automation. The kits include the Exome RDY Panel plates, and the Ion AmpliSeq™ Library Kit Plus.

The **Ion Xpress™ Barcode Adapters Kits** and the **IonCode™ Barcode Adapters 1–384 Kit**, ordered separately, enable the preparation of barcoded libraries using the library kits that are listed above. Multiple barcoded libraries can be combined and loaded onto a single Ion chip to minimize sequencing run time and cost. Ion Xpress™ Barcode Adapters Kits require addition of the Ion P1 Adapter to barcodes before ligation. The IonCode™ Barcode Adapters 1–384 Kit pre-mixes each barcode adapter and the Ion P1 Adapter to simplify ligation reaction setup.

Ion AmpliSeq™ Exome RDY Kits

The Ion AmpliSeq™ Exome RDY Kits (Cat. Nos. A38262 and A38264) include the Ion AmpliSeq™ Library Kit Plus and the Ion AmpliSeq™ Exome RDY Panel, which provide reagents for preparing eight exome libraries.

Kit contents and storage

Ion AmpliSeq™ Library Kit Plus (Cat. No. 4488990; sufficient for preparing eight exome libraries)

Component	Amount	Storage
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 (pink cap)	48 µL	
1X Library Amp Mix (black cap)	1.2 mL	
Low TE	6 mL	15°C to 30°C[1]

[1] Can be stored at –30°C to –10°C.

Ion AmpliSeq™ Exome RDY Panel (one format provided per kit)

Component, format	Amount	Storage
Ion AmpliSeq™ Exome RDY Panel, 1×8 (Part No. 4489838)	1 plate for 8 libraries	2°C to 8°C
Ion AmpliSeq™ Exome RDY Panel, 4×2 (Part No. 4489840)	4 plates for 2 libraries each	

Ion Xpress™ Barcode Adapters Kits

Each Ion Xpress™ Barcode Adapters Kit kit provides 16 different barcode adapters, sufficient for ~200 Ion AmpliSeq™ Exome libraries. These barcode adapters, or IonCode™ Barcode Adapters, are required to run multiple libraries per sequencing chip, and are ordered separately.

<table>
<tr><th>Component</th><th>Cap color</th><th>Quantity</th><th>Volume per tube</th><th>Storage</th></tr>
<tr><td>Ion Xpress™ P1 Adapter</td><td>Violet</td><td>1 tube</td><td>320 µL</td><td rowspan="2">–30°C to –10°C</td></tr>
<tr><td>Ion Xpress™ Barcode X</td><td>White</td><td>16 tubes (1 per barcode)</td><td>20 µL each</td></tr>
</table>

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)

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	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)	–30°C to –10°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
One of the following thermal cyclers: • SimpliAmp™ Thermal Cycler • Applied Biosystems™ 2720 Thermal Cycler • ProFlex™ 96-well PCR System • GeneAmp™ PCR System 9700[1] or Dual 96-well Thermal Cycler	See web product pages
One of the following: • Ion Library TaqMan® Quantitation Kit and real-time PCR instrument • Qubit™ 4 Fluorometer[2] and Qubit™ dsDNA HS Assay Kit (DNA) • Agilent™ 2100 Bioanalyzer™ and Agilent™ High Sensitivity DNA Kit	4468802 Q33238, Q32851/Q32854 Agilent G2939AA, 5067-4626
One of the following: • IonCode™ Barcode Adapters 1–384 Kit • Ion Xpress™ Barcode Adapters Kit	A29751 Variable
(Optional) MicroAmp™ Optical 96-Well Reaction Plate with Barcode	N8010560, 4306737
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Film Compression Pad	4312639
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5-mL	13-698-791 (**fisherscientific.com**)
One of the following: • Beckman Coulter™ AMPure XP 5 mL • Beckman Coulter™ AMPure XP 60 mL	A63880 A63881 (**fisherscientific.com**)
DynaMag™–96 Side Magnet, or other plate magnet	12331D
Nuclease-free Water	AM9932
Ethanol, Absolute, Molecular Biology Grade	BP2818500 (**fisherscientific.com**)
Pipettors, 2–200 µL, and low-retention filtered pipette tips	**fisherscientific.com**

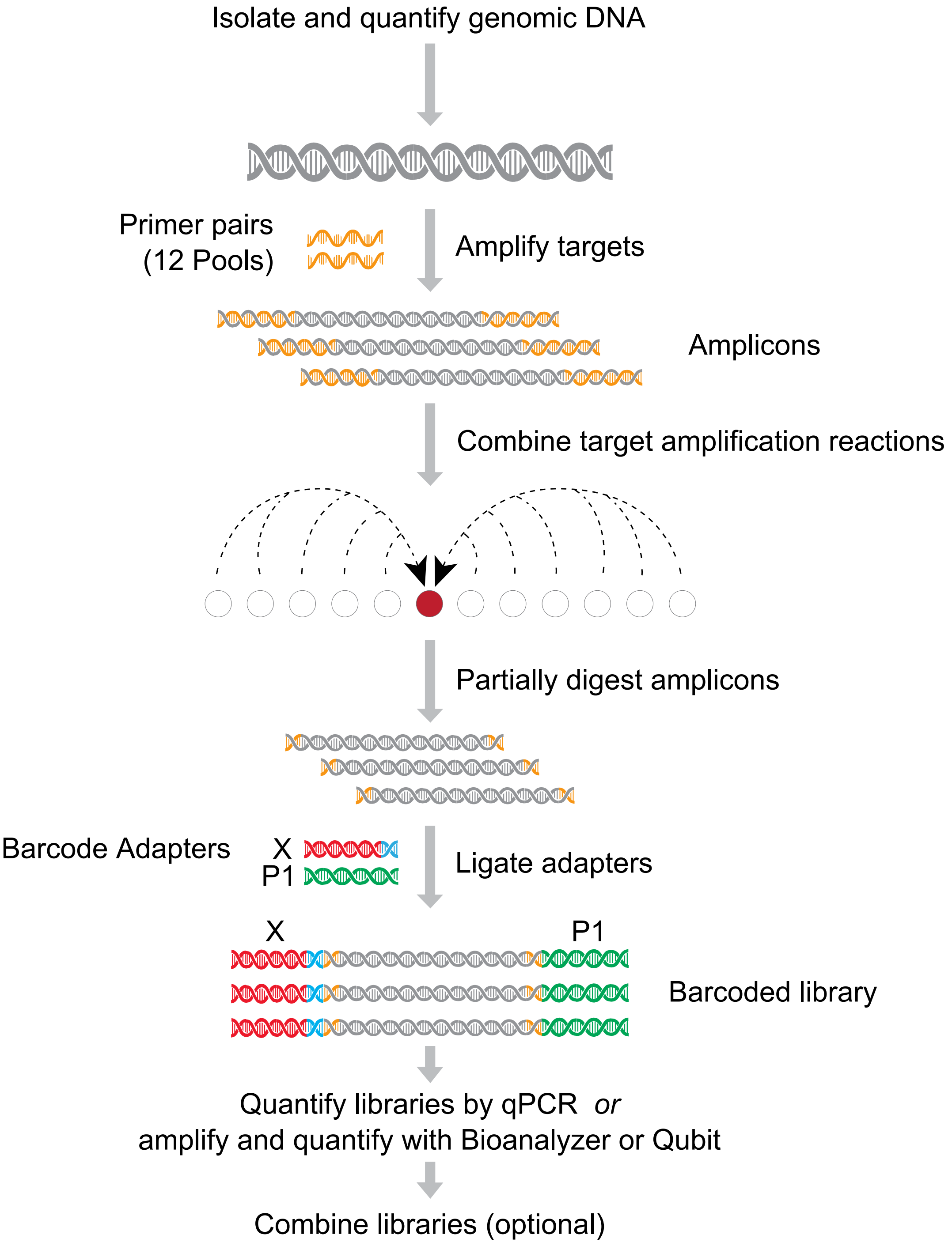
[1] Supported but no longer available for purchase.
[2] Qubit™ 3.0 Fluorometer and Qubit™ 2.0 Fluorometer are supported but no longer available for purchase.

Recommended materials and equipment (optional)

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Additional equipment	
Real-time PCR instrument (e.g., Applied Biosystems™ 7900HT, 7500, StepOne™, StepOnePlus™, ViiA™ 7 Systems, QuantStudio™ 3 or 5 System, or QuantStudio™ 12K Flex Real–Time PCR System	See web product pages
Fisher Scientific™ Mini Plate Spinner Centrifuge, or equivalent 96-well plate centrifuge	14-100-143 (**fisherscientific.com**)
Nucleic acid isolation	
MagMAX™ DNA Multi-Sample Kit	4413020
PureLink™ Genomic DNA Mini Kit	K182000
Nucleic acid quantification	
TaqMan® RNase P Detection Reagents Kit	4316831

Ion AmpliSeq™ Exome RDY workflow starting from genomic DNA



Procedure overview

1. Amplify target regions from 50–100 ng of genomic DNA (gDNA) in the Ion AmpliSeq™ Exome RDY plates using the 5X Ion AmpliSeq™ HiFi Mix.
2. Combine target amplification reactions, then partially digest amplicons with FuPa Reagent.
3. Ligate barcode adapters with Switch Solution and DNA Ligase, then purify.
4. Normalize or quantify the libraries using one of two options:
    - Without further amplification, quantify libraries by qPCR and dilute to 100 pM.
    - Quantify libraries using the Qubit™ Fluorometer or the Agilent™ 2100 Bioanalyzer™ instrument and dilute to 100 pM. If you use this method with one of these instruments, which does not specifically detect amplifiable molecules, library amplification and purification are required before quantification.

    Note: Quantitative PCR is the most simple and robust workflow, and is recommended for samples of unknown quality or quantity. Qubit™ fluorometry is the most economical, but lacks specificity. Agilent™ 2100 Bioanalyzer™ assessment generates the most information for troubleshooting.

5. Up to three barcoded libraries can be combined before template preparation and sequencing. Combining libraries maximizes chip use while minimizing cost and labor.

Prepare Ion AmpliSeq™ Exome RDY libraries

- Procedure guidelines 12
- Guidelines for DNA quantification, and amount and quality of DNA needed 13
- Amplify the targets 13
- Combine target amplification reactions 14
- Partially digest amplicons 15
- Ligate adapters to the amplicons and purify 15

Procedure guidelines

- Thaw components that contain enzymes—such as 5X Ion AmpliSeq™ HiFi Mix, FuPa Reagent, DNA Ligase, and 1X Library Amp Mix—on ice, and keep on ice during the procedure. All other components may be thawed at room temperature. Gently vortex and centrifuge all reagents 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.
- Use good laboratory practices to minimize cross-contamination of products. If possible, perform PCR setup in an area or room that is free of amplicon contamination. Always change pipette tips between samples.
- Use a calibrated thermal cycler specified in “Required materials not supplied” on page 8.
- Pipet viscous solutions slowly and ensure complete mixing by vigorous vortexing or pipetting up and down several times.

Guidelines for DNA quantification, and amount and quality of DNA needed

- 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 with highly degraded DNA samples.
- We recommend that you not use formalin-fixed, paraffin-embedded (FFPE) tissue as a source of genomic DNA.
- Densitometric quantification methods (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA. Use of these methods can lead to gross overestimation of the concentration of sample DNA, under-seeding of the target amplification reaction, low library yields, and poor chip loading.
- For each exome library, use 50–100 ng of genomic DNA (gDNA). The maximum volume of DNA is 56 µL.
- If DNA is not limiting, we recommend using 100 ng per sample. If libraries are to be amplified before quantification with the Qubit™ Fluorometer, or the Agilent™ 2100 Bioanalyzer™ instrument, we recommend 50 ng DNA per sample.

Amplify the targets

1. For each sample, prepare a master mix:

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	14 µL
50–100 ng gDNA (non-FFPE)	≤ 56 µL
Nuclease-free Water	**to 70 µL**

2. Mix the master mix thoroughly by vortexing, or pipetting up and down 5 times, then centrifuge briefly to collect droplets.

3. Remove the seal from the Ion AmpliSeq™ Exome RDY plate.

4. For each sample, use a low volume pipettor to dispense carefully 5-µL aliquots of master mix into a single horizontal row (12 wells) of the plate without changing the tip.

   Note: A blue dye is added to the primers to help identify wells that contain dry primers. Each row in the 1×8 format (Part. No. 4489838) contains primers. Only rows "C" and "F" in the 4×2 format (Part. No. 4489840) contain primers.

5. Apply a MicroAmp™ Clear Adhesive Film, ensure a tight seal, then briefly centrifuge the plate to collect droplets.

6. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program for 5-µL volume.

IMPORTANT! Use of the recommended plates, seals, compression pads, and a Thermo Fisher Scientific thermal cycler are critical for best performance.

Stage	Step	Temp	Time
Hold	Activate enzyme	99°C	2 minutes
Cycle (10 cycles)	Denature	99°C	15 seconds
	Anneal/extend	60°C	16 minutes
Hold	—	10°C	Hold

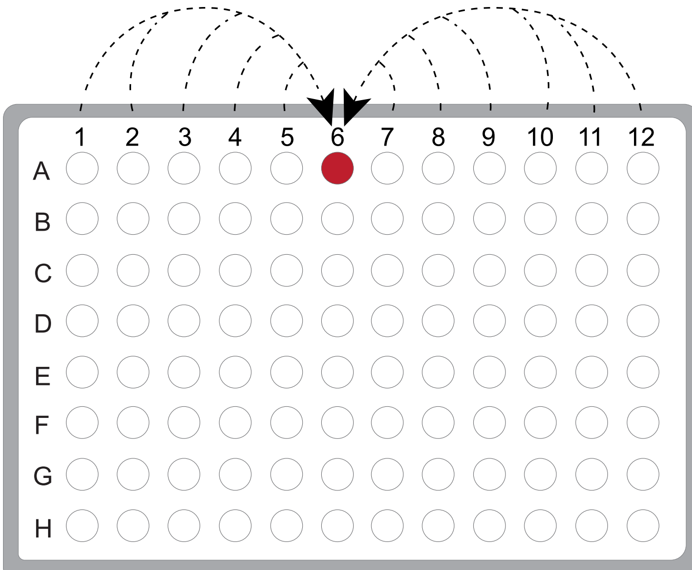
STOPPING POINT You can store PCR products at 10°C overnight. For longer lengths of time, store at –30°C to –10°C.

Combine target amplification reactions

1. Centrifuge the plate briefly to collect droplets, then carefully remove the adhesive film.

2. Combine the 12 target amplification reactions for each sample (row) by transferring the contents from wells 1–5 and 7–12 into the column 6 well, without changing the tip.

Note: Due to evaporation, final volume of the combined reactions can range from 50 µL to 60 µL.



Note: Graphic shows row A wells combined. Combine target amplification reactions for each row on an 8-library plate, or rows C and F on a 2-library plate.

Partially digest amplicons

IMPORTANT! FuPa Reagent is viscous. Pipet slowly and mix thoroughly. Perform this step on ice or a cold block, then quickly proceed to incubation.

1. **Add 6 µL of FuPa Reagent** (brown cap) to each combined target amplification reaction, to bring the total volume to approximately 60 µL.
2. Seal the plate with 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.
3. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

Termperature	Time
50°C	20 minutes
55°C	20 minutes
60°C	20 minutes
10°C	Hold

STOPPING POINT Hold the reactions for up to 1 hour at 10°C. For longer lengths of time, store the plate at –30°C to –10°C.

Ligate adapters to the amplicons and purify

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

Ion Xpress™ Barcode 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™ Barcode Adapters 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. Store diluted adapters at –20°C.

Combine the volumes indicated in the following table. Add 6 µL of this barcode adapter mix to the ligation reaction in step 3 on the following page.

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 selected

Set up and run 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 listed to each well containing digested amplicons.

   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)	12 µL
2	IonCode™ Barcode Adapters *or* diluted Ion Xpress™ Barcode Adapters mix (for barcoded libraries)	6 µL
3	DNA Ligase (blue cap)	6 µL
—	**Total volume** (including ~60 µL of digested amplicon)	**~84 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™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program:

Temperature	Time
22°C	30 minutes
68°C	5 minutes
72°C	5 minutes
10°C	Hold

STOPPING POINT You can store PCR products at 10°C overnight. For longer lengths of time, store at –30°C to –10°C.

Purify the unamplified library

IMPORTANT!
- Bring the Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.
- Use freshly prepared 70% ethanol for the next steps. Combine 230 µL of ethanol with 100 µL of Nuclease-free Water per sample.
- Do NOT substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

1. Carefully remove the plate seal, then add **80 µL (about 1X sample volume) of Agencourt™ AMPure™ XP Reagent** to each library. Pipet up and down 5 times to mix the bead suspension thoroughly with the DNA.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the plate in a DynaMag™–96 Side Magnet (Cat. No. 12331D), then incubate for 5 minutes or until solution is clear. Carefully remove, then discard the supernatant without disturbing the pellet.
4. Add **150 µL of freshly prepared 70% ethanol**, move the plate side to side in the magnet to wash the beads, remove, then discard the supernatant. Do not disturb the pellet.
5. Repeat step 4 for a second wash.
6. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes. **Do not overdry**.

Proceed to library quantification

Proceed to one of the following:

- Option 1: Quantify the library by qPCR, Chapter 3 (page 18).
- Option 2: Quantify the amplified library with the Qubit™ 2.0, Qubit™ 3.0, or Qubit™ 4 Fluorometer, or with the Agilent™ 2100 Bioanalyzer™ instrument, Chapter 4 (page 21).

Quantify the unamplified library by qPCR

- Elute and dilute the library 18
- Quantify the library and dilute 19
- (Optional) Combine exome libraries 20
- Store libraries 20

Elute the library, then determine the concentration by qPCR with the Ion Library TaqMan® Quantitation Kit (Cat. No. 4468802). Libraries that have not undergone a second round of amplification typically have yields of 100–500 pM. However, yield is not indicative of library quality. After quantification, determine the dilution factor that results in a concentration of ~100 pM, which is suitable for template preparation using an Ion template kit.

Note: Ion Library TaqMan® Quantitation Kit can also be used to quantify libraries that have been amplified using the procedure described in "Amplify the library" on page 21.

Elute and dilute the library

1. Remove the plate with purified libraries from the plate magnet, then add 50 µL of Low TE to the pellet to disperse the beads.
2. Seal the plate with 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.
3. Incubate at room temperature for at least 2 minutes.
4. Place the plate on the magnet for at least 2 minutes.
5. Prepare a 100-fold dilution for quantification. Remove 2 µL of supernatant, containing the library, then combine with 198 µL of Nuclease-free Water.

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

Quantify the library and dilute

Determine the concentration of each Ion AmpliSeq™ library by qPCR with the Ion Library TaqMan® Quantitation Kit (Cat. No. 4468802) using the steps that follow. Each sample, standard, and negative control is analyzed in duplicate 20-µL reactions.

Note: Exome libraries typically have yields of 100–500 pM.

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

2. Prepare reaction mixtures. For each sample, control, and standard, combine 20 µL of Ion Library qPCR Master Mix and 2 µL of Ion Library TaqMan® Quantitation Assay, 20X , then mix thoroughly. Dispense 11-µL aliquots into the wells of a 96-well standard PCR plate.

3. Add 9 µL of the diluted (1:100) Ion AmpliSeq™ library or 9 µL of each control dilution to each well (two wells per sample as noted before). Total reaction volume is 20 µL.

4. Program your real-time instrument:
   - Enter the concentrations of the control library standards.
   - Use ROX™ reference dye as the passive reference dye.
   - Select a reaction volume of 20 µL.
   - You can use the Ion Library qPCR Master Mix on various Thermo Fisher Scientific instruments, as listed in the following table.

<table>
<tr><th>Real-time PCR System</th><th>Stage</th><th>Temperature</th><th>Time</th></tr>
<tr><td rowspan="4">7300 Real-Time PCR System
7500 Real-Time PCR System
7900HT Real-Time PCR System
7900HT Fast Real-Time PCR System
ViiA™ 7 Real-Time PCR System
QuantStudio™ 3 or 5 Real-Time PCR System</td><td>Hold (optional)</td><td>50°C</td><td>2 minutes</td></tr>
<tr><td>Hold (polymerase activation)</td><td>95°C</td><td>2 minutes</td></tr>
<tr><td rowspan="2">Cycle (40 cycles)</td><td>95°C</td><td>15 seconds</td></tr>
<tr><td>60°C</td><td>1 minute</td></tr>
</table>

5. Following qPCR, calculate the average concentration of the undiluted Ion AmpliSeq™ library by multiplying the concentration that is determined with qPCR by 100.

6. Based on the calculated library concentration, determine the dilution that results in a concentration of ~100 pM.

   For example:
   - The undiluted library concentration is 300 pM.
   - The library dilution factor is 300 pM/100 pM = 3.
   - Therefore, 1 µL of library that is mixed with 2 µL of Low TE (1:3 dilution) yields ~100 pM.

7. Dilute library to ~100 pM as described, then proceed to combining libraries or template preparation.

Note: A library that yields less than 100 pM can be rescued with library amplification. Combine 25 µL of unamplified library with 71 µL of 1X Library Amp Mix and 4 µL of 25X Library Amp Primers. Perform 5–10 library amplification cycles (see step 3 of "Amplify the library" on page 21 for cycling conditions).

(Optional) Combine exome libraries

You can combine two barcoded exome libraries on a single Ion PI™ or Ion 540™ Chip, or four barcoded exome libraries on a single Ion 550™ Chip, for a targeted average coverage depth of approximately 100X.

Note: We recommend a minimum average coverage depth of 100X. Loading fewer libraries per chip yields higher coverage depth. See Appendix B, "Strategies for combining Ion AmpliSeq™ Exome RDY libraries" for more information.

Store libraries

Store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

Quantify the amplified library with the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument

- Amplify the library 21
- Purify the amplified library 22
- Qubit™ Fluorometer: Quantify the library and calculate the dilution factor 24
- Agilent™ 2100 Bioanalyzer™: Quantify the library and calculate the dilution factor 25
- (Optional) Combine exome libraries 25
- Store libraries 25

Ion AmpliSeq™ Exome RDY libraries must be amplified before quantification to enrich amplifiable material and obtain sufficient material for accurate quantification. Amplify the library using 1X Library Amp Mix, then purify. Quantify the library using the Qubit™ 2.0, Qubit™ 3.0, or Qubit™ 4 Fluorometer, or the Agilent™ 2100 Bioanalyzer™ instrument. Amplified libraries typically have yields of 300–1,500 ng/mL. Yield is not indicative of library quality. After quantification, determine the dilution factor that results in a concentration of ~100 pM, which is appropriate for template preparation using an Ion template kit.

Alternatively, the Ion Library TaqMan® Quantitation Kit can be used to quantify amplified libraries.

Amplify the library

1. Remove the plate containing the Ion AmpliSeq™ 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.

   Note: The 1X Library Amp Mix and 25X Library Amp Primers can be combined before addition.

2. Seal the plate with MicroAmp™ Clear Adhesive Film, 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.

   Note: Library amplification takes place in the presence of the AMPure™ XP beads.

3. Place a MicroAmp™ Optical Film Compression Pad on the plate, load in the thermal cycler, and 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

STOPPING POINT You can store samples at 10°C overnight. For longer lengths of time, store at –30°C to –10°C.

Proceed to "Purify the amplified library".

Purify the amplified library

Perform a two-round purification process with the Agencourt™ AMPure™ XP Reagent:

- **First round at 0.5X bead-to-sample-volume ratio:** High molecular-weight DNA is bound to beads, while amplicons and primers remain in solution. **Save the supernatant.**
- **Second round at 1.2X bead-to-original-sample-volume ratio:** Amplicons are bound to beads, and primers remain in solution. **Save the bead pellet, and elute the amplicons from the beads.**

IMPORTANT!

- Bring Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the solution slowly.
- Use freshly prepared 70% ethanol for the next steps. Combine 230 µL of ethanol with 100 µL of Nuclease-free Water per sample.
- Do **NOT** substitute a Dynabeads™-based purification reagent for the Agencourt™ Agencourt™ AMPure™ XP Reagent.

First-round purification

1. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.

2. **Add 25 µL (0.5X sample volume) 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 mix the bead suspension thoroughly with the DNA.

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

4. Place the plate in a DynaMag™–96 Side Magnet for at least 5 minutes or until the solution is clear.

5. Carefully transfer the supernatant from each well to a single well of a new 96-well PCR plate, without disturbing the pellet. Discard the pellet.

   IMPORTANT! The **supernatant** contains the desired amplicons. Do not discard!

Second-round purification

1. To the supernatant from step 5 in the previous procedure, **add 60 µL (1.2X original sample volume) of Agencourt™ AMPure™ XP Reagent**. Pipet up and down 5 times to mix the bead suspension thoroughly with the DNA.
2. Incubate the mixture for 5 minutes at room temperature.
3. Place the plate in the magnet for 3 minutes or until the solution is clear. Carefully remove, then discard the supernatant without disturbing the pellet.

   IMPORTANT! The amplicons are bound to the **beads**. **Save the bead pellet.**

4. **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.

   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. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes. Do not overdry.
7. Remove the plate from the magnet, then **add 50 µL of Low TE** to the pellet to disperse the beads. Pipet the mixture up and down 5 times to mix thoroughly.
8. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, and centrifuge to collect droplets. Alternatively, mix by setting a pipettor to 40 µL and pipet the mixture up and down at least 5 times prior to sealing the plate.
9. Incubate at room temperature for at least 2 minutes.
10. Place the plate in the magnet for at least 2 minutes, then analyze an aliquot of the supernatant as described in:
    - "Qubit™ Fluorometer: Quantify the library and calculate the dilution factor", or
    - Agilent™ 2100 Bioanalyzer™: Quantify the library and calculate the dilution factor"

    IMPORTANT! The **supernatant** contains the desired amplicons. Do not discard!

Qubit™ Fluorometer: Quantify the library and calculate the dilution factor

Analyze 10 µL of each amplified library using the Qubit™ dsDNA HS Assay Kit (Cat. Nos. Q32851 or Q32854) with the Qubit™ 2.0, Qubit™ 3.0, or Qubit™ 4 Fluorometer (Cat. No. Q33238). For more information, see the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326), available at **thermofisher.com**.

Note: Exome libraries typically have yields of 300–1,500 ng/mL.

---

1. Determine the amplified library concentration:
    a. Make a 1:200 working dilution of Qubit™ dsDNA HS Reagent using the Qubit™ dsDNA HS Buffer. Prepare 200 µL of diluted reagent for each library and standard.
    b. Combine 10 µL of the amplified Ion AmpliSeq™ exome library with 190 µL of diluted Qubit™ dsDNA HS Reagent, mix well, then incubate for at least 2 minutes.
    c. Prepare each Qubit™ dsDNA HS Standard by diluting 10 µL with 190 µL of diluted Qubit™ dsDNA HS Reagent.
    d. Measure the concentration on the Qubit™ 2.0, Qubit™ 3.0, or Qubit™ 4 Fluorometer.
    e. *(Qubit™ 2.0 Fluorometer only)* Calculate the concentration of the undiluted library by multiplying by 20. Alternatively, use the "Calculate Stock Conc." feature on your instrument.
2. Based on the calculated library concentration, determine the dilution that results in a concentration of ~22 ng/mL (which is equivalent to ~100 pM).

    For example:

    - The library concentration is 660 ng/mL.
    - The dilution factor is 660 ng/mL divided by 22 ng/mL = 30.
    - Therefore, 1 µL of library that is mixed with 29 µL of Low TE (1:30 dilution) yields approximately 22 ng/mL.
3. Dilute library to ~22 ng/mL (~100 pM), then proceed to combining libraries or template preparation.

Agilent™ 2100 Bioanalyzer™: Quantify the library and calculate the dilution factor

Analyze 1 µL of amplified library on the Agilent™ 2100 Bioanalyzer™ instrument with the Agilent™ High Sensitivity DNA Kit (Cat. No. 5067-4626). Exome libraries typically have yields of 1,000–10,000 pM.

1. Determine the molar concentration of the amplified library using the Bioanalyzer™ software. Ensure that the upper and lower marker peaks are identified and assigned correctly. Follow the instructions of the manufacturer to perform a region analysis (smear analysis) in the 200–350 bp size range. Higher molecular weight product can occur that does not affect performance.

2. Based on the calculated library concentration, determine the dilution that results in a concentration of ~100 pM.

   For example:

   - The library concentration is 3000 pM.
   - The dilution factor is 3000 pM/100 pM = 30.
   - Therefore, 1 µL of library that is mixed with 29 µL of Low TE (1:30 dilution) yields approximately 100 pM.

3. Dilute library to ~100 pM as described and proceed to combining libraries or template preparation.

(Optional) Combine exome libraries

You can combine two barcoded exome libraries on a single Ion PI™ or Ion 540™ Chip, or four barcoded exome libraries on a single Ion 550™ Chip, for a targeted average coverage depth of approximately 100X.

Note: We recommend a minimum average coverage depth of 100X. Loading fewer libraries per chip yields higher coverage depth. See Appendix B, "Strategies for combining Ion AmpliSeq™ Exome RDY libraries" for more information.

Store libraries

Store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

Tips and troubleshooting

Tips

- Plate seals can be firmly applied using the applicator in the MicroAmp™ Optical Adhesive Film Kit. Plate seals can be removed with much less effort when hot. Try removing seals right after taking the plate out of thermal cycler.
- Combine and dilute barcode adapters in large batches and aliquot into 96-well plates.
- If library yield is below 100 pM, libraries can still be sequenced by adding a proportionally larger volume to a combined library or template preparation.
- Ion AmpliSeq™ Exome RDY Kits are compatible with the Ion Library Equalizer™ Kit. See the *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003) for more information. To assure reliability, library yields should be consistently above the minimum concentrations stated in this user guide.

Modifications to the standard workflow

The following modifications to the standard protocol are designed to allow advanced users to modify and customize the standard Ion AmpliSeq™ Exome RDY protocol successfully.

IMPORTANT! These modifications are unsupported and sometimes can decrease performance.

Shortcuts

- When using qPCR quantification, careful removal of ethanol after the final wash eliminates the need for drying AMPure™ XP beads. However, excess residual ethanol can inhibit the library amplification reaction.

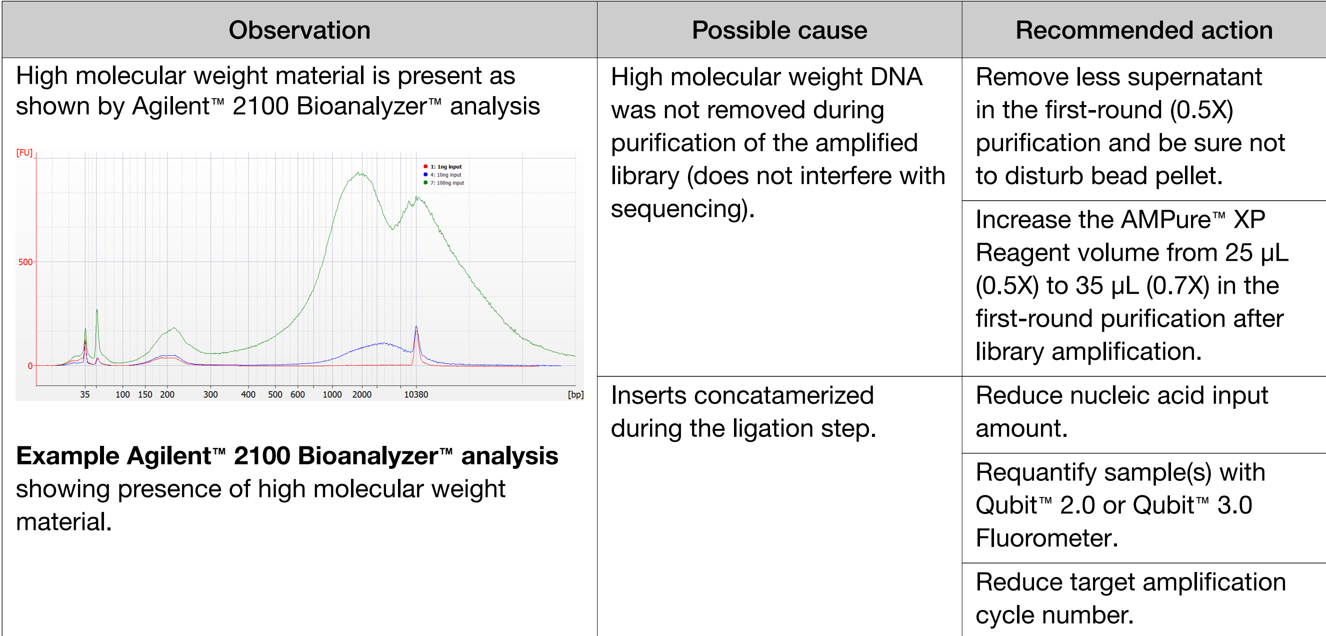
Limited samples

- DNA from high-quality FFPE tissue can be used with the Ion AmpliSeq™ Exome RDY Kits. Uniformity and representation of longer amplicons can decrease.
- When using the Qubit™ Fluorometer or Agilent™ 2100 Bioanalyzer™ instrument, amplified libraries with undetectable product can still be quantified by qPCR (see Chapter 3, "Quantify the unamplified library by qPCR").

Troubleshooting

Library yield and quantification

Observation	Possible cause	Recommended action
Library concentration is low—general (Library concentration is NOT indicative of quality.)	Sample DNA was mis-quantified.	Requantify sample DNA using the TaqMan® RNase P Detection Reagents Kit.
	Residual ethanol in sample DNA inhibited target amplification.	Incubate uncapped tube in hood for 1 hour.
		Speed-vac tube at room temperature for 5 minutes.
	Residual ethanol from AMPure™ purification inhibited library amplification.	Carefully remove all drops of ethanol before library amplification, then centrifuge plate, if necessary.
	Sample DNA quality was low.	Add more DNA or increase target amplification cycles.
	PCR, digestion, or ligation was inefficient.	Ensure proper dispensing and mixing of viscous components at each step.
	Library was discarded during two-round bead purification of the amplified library.	Be sure to save the supernatant during first-round purification, and save the bead pellet during the second round.
	AMPure™ XP beads were over-dried.	Do not dry the AMPure™ XP beads more than 5 minutes.
	AMPure™ XP beads inhibited library amplification.	Transfer library off of beads prior to amplification.
	qPCR cycling time is too short.	Use standard qPCR cycling for library designs >175 bp instead of Fast cycling.
Library yield is high	Sample DNA was mis-quantified.	Requantify sample DNA using the TaqMan® RNase P Detection Reagents Kit.
	More than 100 ng of sample DNA was used.	Add less DNA, or decrease target amplification cycles.
Library concentration is high as measured on the Agilent™ 2100 Bioanalyzer™ Instrument	Markers are misassigned.	Ensure that markers are assigned correctly.

Observation	Possible cause	Recommended action
High molecular weight material is present as shown by Agilent™ 2100 Bioanalyzer™ analysis  **Example Agilent™ 2100 Bioanalyzer™ analysis** showing presence of high molecular weight material.	High molecular weight DNA was not removed during purification of the amplified library (does not interfere with sequencing).	Remove less supernatant in the first-round (0.5X) purification and be sure not to disturb bead pellet.
		Increase the AMPure™ XP Reagent volume from 25 µL (0.5X) to 35 µL (0.7X) in the first-round purification after library amplification.
	Inserts concatamerized during the ligation step.	Reduce nucleic acid input amount.
		Requantify sample(s) with Qubit™ 2.0 or Qubit™ 3.0 Fluorometer.
		Reduce target amplification cycle number.

Low amplicon uniformity

Observation	Possible cause	Recommended action
Short amplicons are under-represented	Purification was poor.	Vortex AMPure™ XP Reagent thoroughly before use, and be sure to dispense the full volume.
		100% ethanol is difficult to pipet accurately; it is essential to pre-wet pipette tips.
		In post-ligation library purification, increase AMPure™ XP Reagent volume from 80 µL (1X) to 96 µL (1.2X).
		In amplified library purification, increase AMPure™ XP Reagent volume in second round from 60 µL (1.2X) to 70 µL (1.4X).
	Digested amplicons were denatured.	Use the 60°C/20 minute temperature incubation during the amplicon digestion step.

Observation	Possible cause	Recommended action
Long amplicons are under-represented	Sample DNA was degraded.	FFPE DNA is not recommended for Ion AmpliSeq™ Exome RDY Panel.
	PCR was inefficient.	Double the anneal and extend time.
	Too few nucleotide flows were used.	Use at least 500 flows to sequence through exome amplicons.
	Sample was over-treated with FuPa Reagent.	Add no more than 6 µL FuPa Reagent per 60 µL combined target amplification reactions.
		Keep the plate on ice during FuPa Reagent addition, then transfer to a preheated thermal cycler immediately.
	Denaturation temperature was too high.	Use a 97°C enzyme activation/denaturation temperature instead of 99°C in target amplification reactions.
AT-rich amplicons are under-represented **Example of loss of AT-rich amplicons.** Within the Coverage Analysis Plugin, amplicon representation is plotted by GC content for an Ion AmpliSeq™ Panel. Amplicons with 23-50% GC show an excess failure rate (less than 20% of the mean read depth).	Target amplification was inefficient.	Double the anneal/extend time in the target amplification reaction.
		Decrease the anneal/extend temperature of the target amplification reaction from 60°C to 58°C.
	Digested amplicons were denatured.	Do not exceed 60°C during the amplicon digestion step.
GC-rich amplicons are under-represented **Example of loss of GC-rich amplicons.** Within the Coverage Analysis Plugin, amplicon representation is plotted by GC content for an	Denaturation was inadequate during target amplification.	Use a calibrated thermal cycler.
	Target amplification was inefficient.	Increase the anneal/extend temperature of the target amplification reaction from 60°C to 62°C for the first two cycles of the target amplification reaction.
	Library amplification was inefficient.	Do not amplify the library (not required for qPCR quantification).

Observation	Possible cause	Recommended action
Ion AmpliSeq™ Panel. Amplicons with 60-80% GC show an excess failure rate (less than 20% of the mean read depth).	Sample was over-treated with FuPa Reagent.	Add no more than 6 µL FuPa Reagent per 60 µL combined target amplification reaction.
		Keep the plate on ice during FuPa Reagent addition, then transfer to thermal cycler immediately.
Pool representation is not balanced **Example of pool imbalance.** Within the Coverage Analysis Plugin, mean read depth per primer pool is plotted for a 2-pool Ion AmpliSeq™ Panel. In this example, Primer Pool 1 has approximately half the reads of other pools.	Amount of DNA in target amplification reactions varied.	Be sure to make a master mix for each sample DNA.
	Pipetting was inaccurate when pools were combined after target amplification.	Centrifuge the plate after target amplification. Ensure that the entire volume of each target amplification reaction for a sample is removed and combined into a single pool.

Other

Observation	Possible cause	Recommended action
Adapter dimers are present on the Agilent™ 2100 Bioanalyzer™ instrument at 90–105 bp, or are present during sequencing **Adapter dimers.** Barcode adapters run at ≈53 bp, and barcode adapter dimers run at ≈105 bp.	Purification was inefficient.	In unamplified library purification, decrease AMPure™ XP Reagent volume from 80 µL (1X) to 60 µL (0.75X).
		In amplified library purification, decrease AMPure™ XP Reagent volume in the second round from 60 µL (1.2X) to 50 µL (1X).
	Adapter dimers formed during reaction setup, or during digestion.	Do not combine barcode adapters, DNA Ligase, and Switch Solution before addition.
		Use a 65°C temperature incubation instead of 60°C during the amplicon digestion step.
	Adapter concentration was too high.	Ensure that barcode adapters are diluted properly.

Observation	Possible cause	Recommended action
The number of on-target reads is lower than expected	Unknown.	Increase the number of target amplification cycles from 10 to 12, and/or increase the anneal/extend temperature of the target amplification reaction from 60°C to 62°C or 64°C for the first two cycles of the target amplification reaction.
		Lower the DNA input.
Barcode representation is uneven	Library quantification was inaccurate.	Use the Ion Library TaqMan® Quantitation Kit for the most specific and accurate library quantification.
	Library combination was inaccurate.	Dilute libraries to 100 pM, then combine equal volumes.
Percentage of polyclonal ISPs is high (>40%)	Library input was too high.	Decrease amount of library added to the template preparation reaction by 50%.
	Library was mis-quantified.	Ensure that library was quantified accurately.
	Other.	Check the appropriate template preparation user guide for more information.
Low quality ISPs are present at high percentage (>15%)	Library input was too low.	Double the volume of library used in template preparation.
		Use a fresh dilution of library prepared in a low-bind tube.
	Other.	Check the appropriate template preparation user guide for more information.

Strategies for combining Ion AmpliSeq™ Exome RDY libraries

Ion Chip capacities for Ion AmpliSeq™ Exome RDY libraries

Two barcoded exome libraries can be combined and run on a single Ion PI™ Chip v3, or Ion 540™ Chip, and up to four barcoded exome libraries can be combined and run on a single Ion 550™ Chip, depending on the coverage and depth desired. The number of exome libraries that can be accommodated in a single sequencing run depends on the size of the chip, the ability to quantify and combine barcoded libraries reliably, and the coverage required.

As the number of libraries on a single chip increases, the average coverage depth decreases. This relationship is shown in the following table. The numbers shown in the table presume high-accuracy quantification, and should serve as a guide for approximate capacities. We recommend determining real limits empirically.

An exome library can be run on multiple chips to increase coverage depth. Run data can be combined by using the CombineAlignments plugin.

Number of exome libraries per chip	Approximate average read depth[1]
Ion PI™ Chip v3 or Ion 540™ Chip	
1	250X
2	125X
Ion 550™ Chip	
1	400X
2	200X
3	133X
4	100X

[1] Actual read depth per sample can vary as a result of barcode balance and chip loading. Values based on 75 M reads for the Ion PI™ Chip v3 and Ion 540™ Chip, and 120 M reads for the Ion 550™ Chip.

Data analysis

For detailed information for setting up and performing data analysis with Torrent Suite™ Software, see *Torrent Suite™ Software Help* accessed from the Torrent Browser **Help** menu, or downloaded from **thermofisher.com** as a PDF. Here you will find information for run planning, data reporting, plugin configuration, variant calling, reference file management, and other topics.

See *Ion Reporter™ Software Help* for detailed information for using the suite of bioinformatics tools in Ion Reporter™ Software to streamline variant analysis and reporting.

The following is a brief description of configuring and running the Torrent Coverage Analysis and Torrent Variant Caller plugins.

Torrent Coverage Analysis plugin

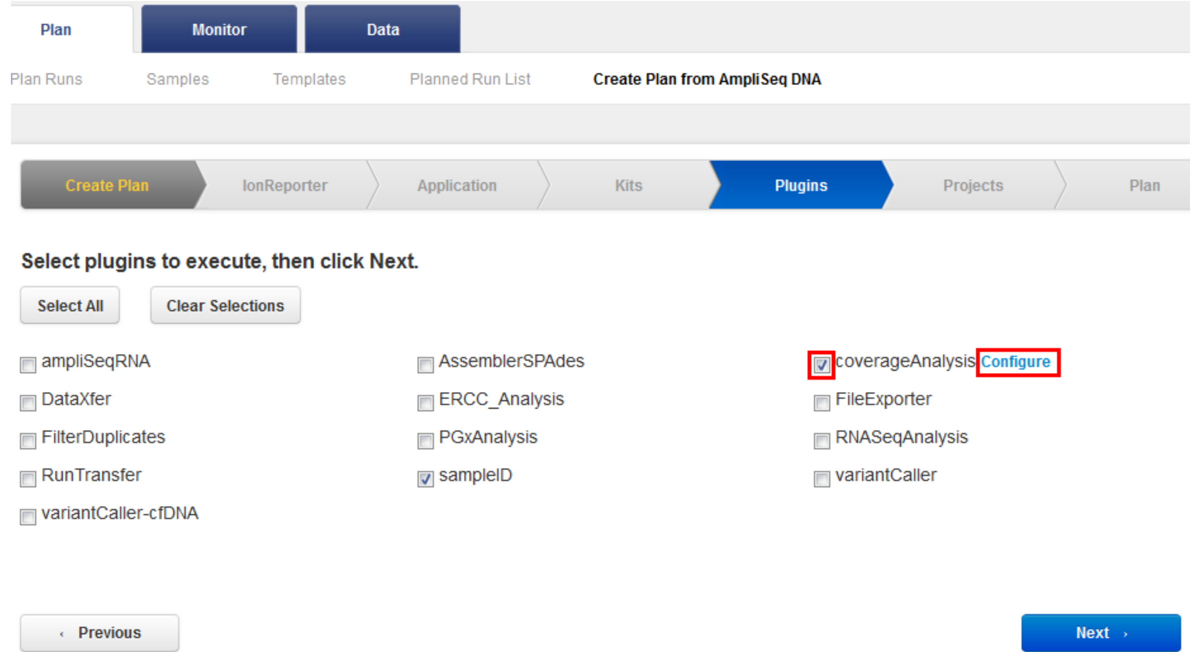
The Torrent Coverage Analysis plugin provides statistics and graphs describing the level of sequence coverage produced for targeted genomic regions.

Configure and run the Torrent Coverage Analysis plugin

There are two ways to run the Torrent Coverage Analysis plugin:

- Automatically, by selecting and configuring the plugin during run planning or
- Manually, allowing you to run the plugin at any time from a completed run report.

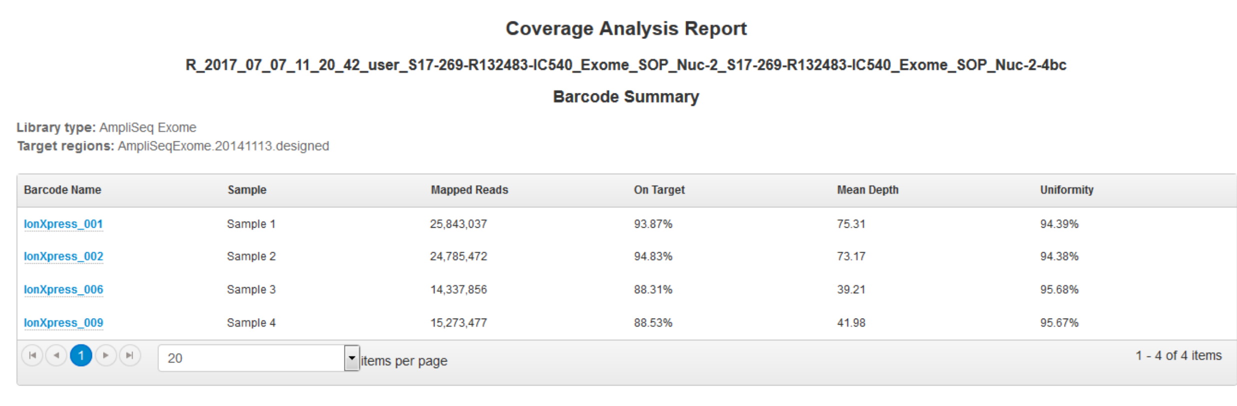
1. To run the Coverage Analysis plugin automatically during run analysis, select the **coverageAnalysis** checkbox in the **Plugins** step in the template or Planned Run Wizard. After selecting the checkbox, a **Configure** link appears:



2. Click **Configure**, then select settings appropriate to your run:
   a. Check **Sample Tracking** if you included the Ion AmpliSeq™ Sample ID Panel in your target amplification reaction.
   b. Check **Use Only Uniquely Mapped Reads** if you would like the plugin to examine only reads that map to a single location.
   c. Check **Minimum Aligned Length** or **Minimum Mapping Quality** to filter reads that do not meet specified alignment or quality values.
3. When you are satisfied with your selections, click **Save Changes**

Coverage Analysis Report

The run report includes basic run metrics, but more detail can be found by clicking the **coverageAnalysis.html** link, including options to download a barcode summary report, and an amplicon coverage matrix.



Coverage Analysis Report

R_2017_07_07_11_20_42_user_S17-269-R132483-IC540_Exome_SOP_Nuc-2_S17-269-R132483-IC540_Exome_SOP_Nuc-2-4bc

Barcode Summary

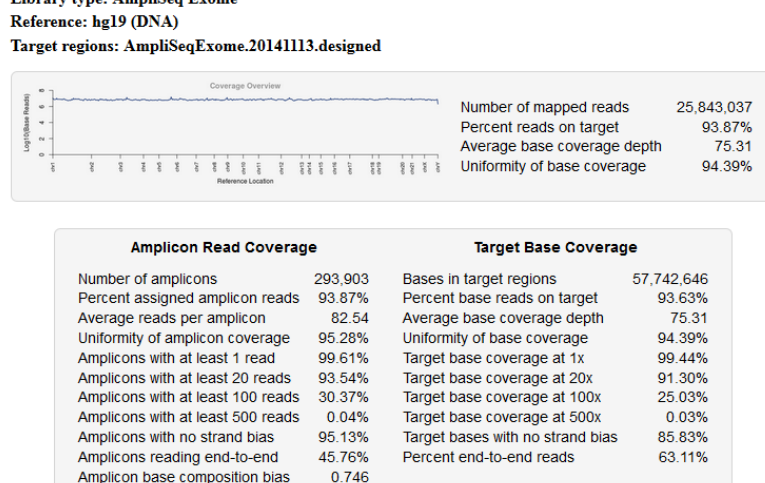
Library type: AmpliSeq Exome
Target regions: AmpliSeqExome.20141113.designed

Barcode Name	Sample	Mapped Reads	On Target	Mean Depth	Uniformity
IonXpress_001	Sample 1	25,843,037	93.87%	75.31	94.39%
IonXpress_002	Sample 2	24,785,472	94.83%	73.17	94.38%
IonXpress_006	Sample 3	14,337,856	88.31%	39.21	95.68%
IonXpress_009	Sample 4	15,273,477	88.53%	41.98	95.67%

20 items per page — 1 - 4 of 4 items

- Download Barcode Summary Report
- Download barcode/amplicon coverage matrix
- Download a ZIP report summary.

More detailed information can be obtained for each barcoded library by clicking the Barcode ID.



Coverage Analysis Report

Sample Name: Sample 1

IonXpress_001_R_2017_07_07_11_20_42_user_S17-269-R132483-IC540_Exome_SOP_Nuc-2_S17-269-R132483-IC540_Exome_SOP_Nuc-2-4bc

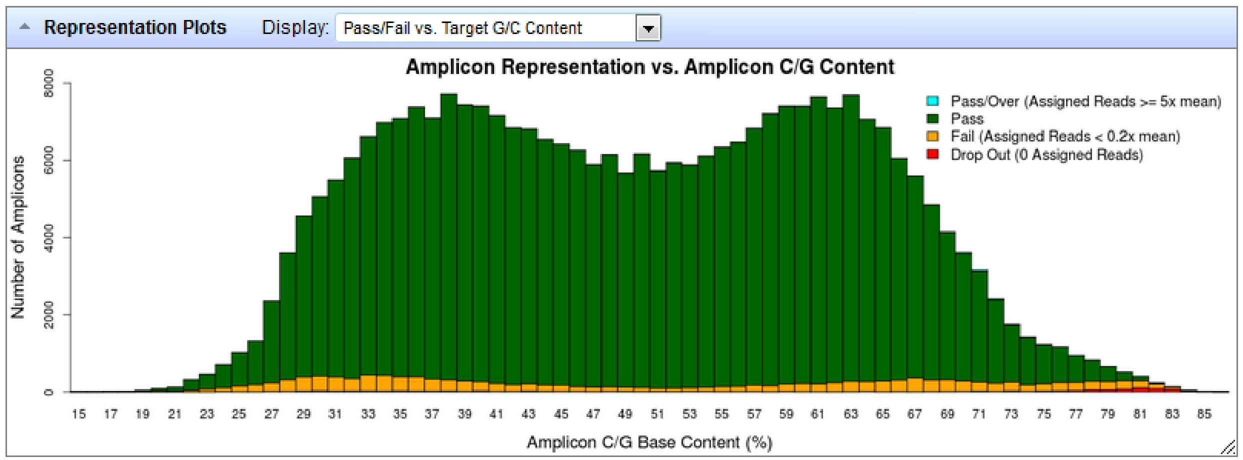
Library type: AmpliSeq Exome
Reference: hg19 (DNA)
Target regions: AmpliSeqExome.20141113.designed

Number of mapped reads	25,843,037
Percent reads on target	93.87%
Average base coverage depth	75.31
Uniformity of base coverage	94.39%

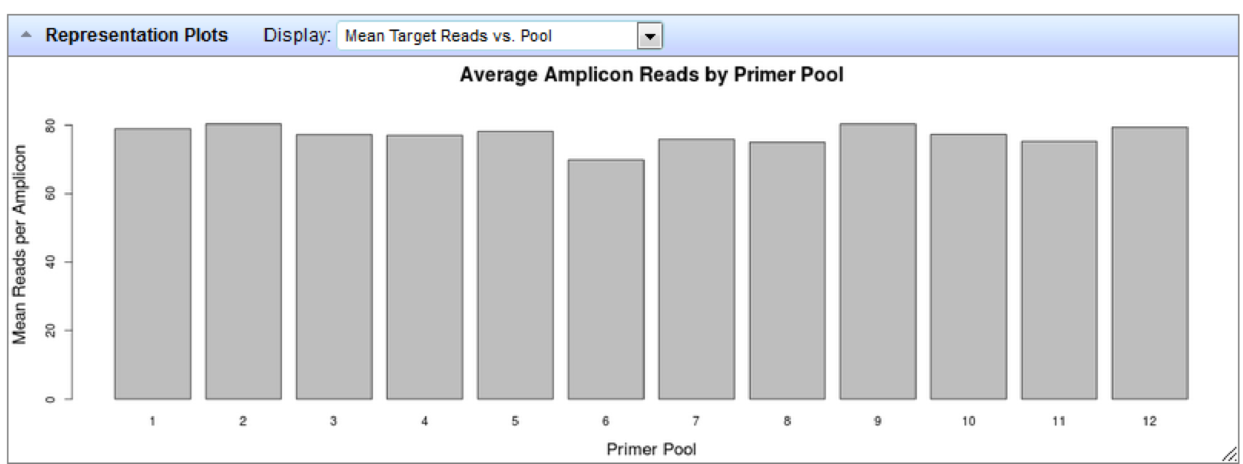
Amplicon Read Coverage		Target Base Coverage	
Number of amplicons	293,903	Bases in target regions	57,742,646
Percent assigned amplicon reads	93.87%	Percent base reads on target	93.63%
Average reads per amplicon	82.54	Average base coverage depth	75.31
Uniformity of amplicon coverage	95.28%	Uniformity of base coverage	94.39%
Amplicons with at least 1 read	99.61%	Target base coverage at 1x	99.44%
Amplicons with at least 20 reads	93.54%	Target base coverage at 20x	91.30%
Amplicons with at least 100 reads	30.37%	Target base coverage at 100x	25.03%
Amplicons with at least 500 reads	0.04%	Target base coverage at 500x	0.03%
Amplicons with no strand bias	95.13%	Target bases with no strand bias	85.83%
Amplicons reading end-to-end	45.76%	Percent end-to-end reads	63.11%
Amplicon base composition bias	0.746		

At the top of the Coverage Analysis Report is a table that shows amplicon statistics on the left side and base statistics on the right side. All metrics are defined with pop-up explanations. The plugin run options can be reviewed as pop-up help on the report title "Coverage Analysis Report".

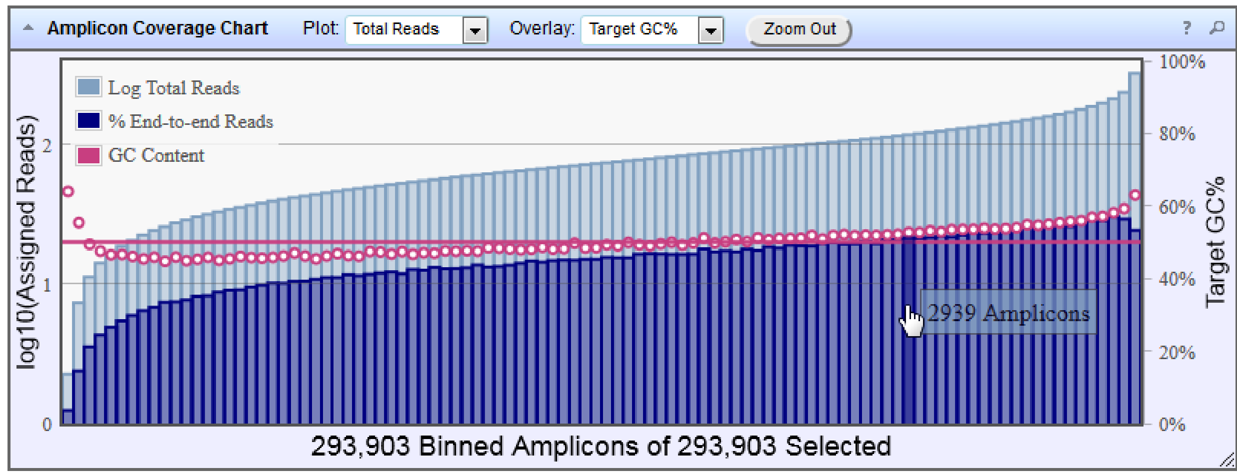
The Representation Plots (which are revealed by clicking the triangle in the blue bar) allow you to visualize amplicon representation by GC content and length. A mean target depth per primer pool representation plot is also available by selecting **Mean Target Reads vs. Pool** from the **Display** dropdown list.



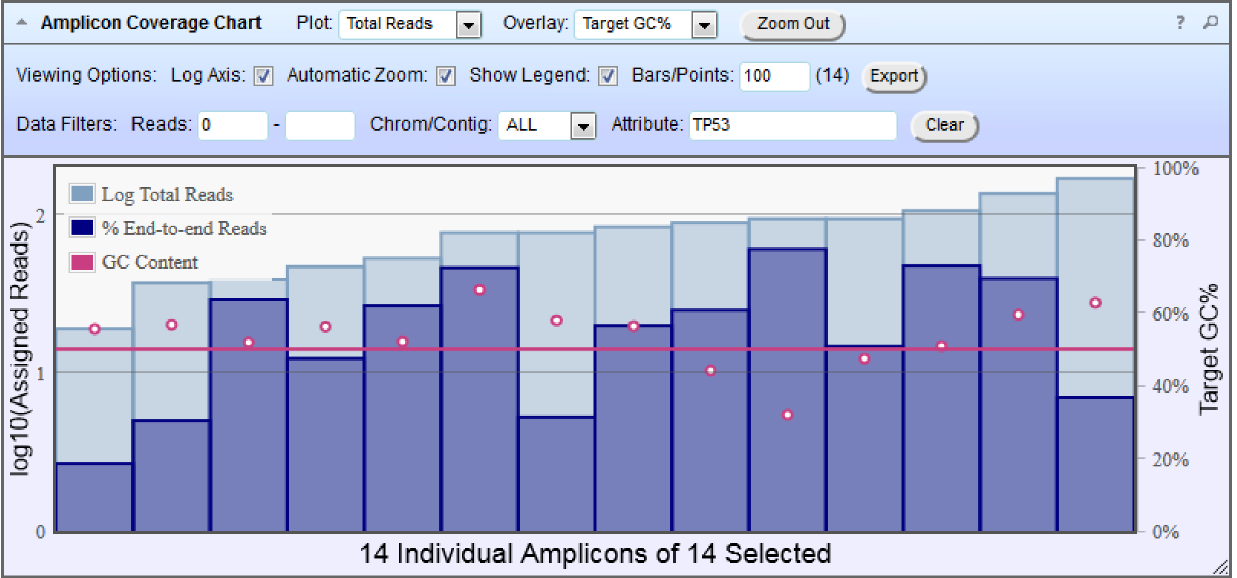
In the following example, the 12 exome primer pools are equally represented:



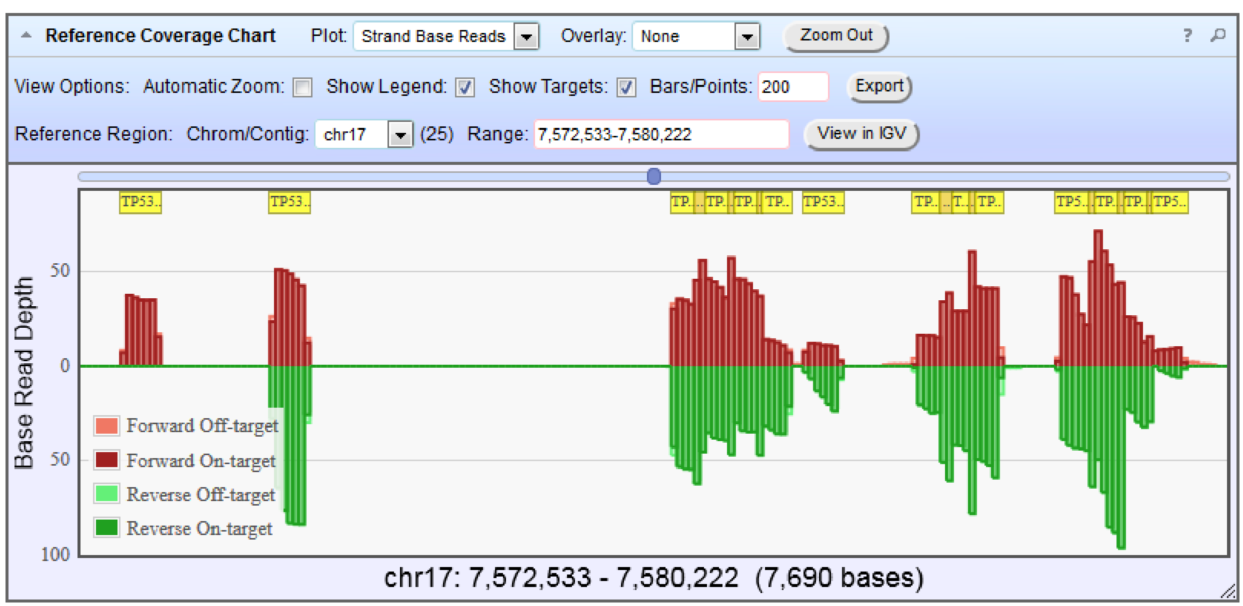
The Amplicon Coverage Chart shows amplicons that are binned by representation, low to high, with various overlays. Zoom into this graph to see how many amplicons are in each bin, and click a bar to get more information on that group of amplicons.



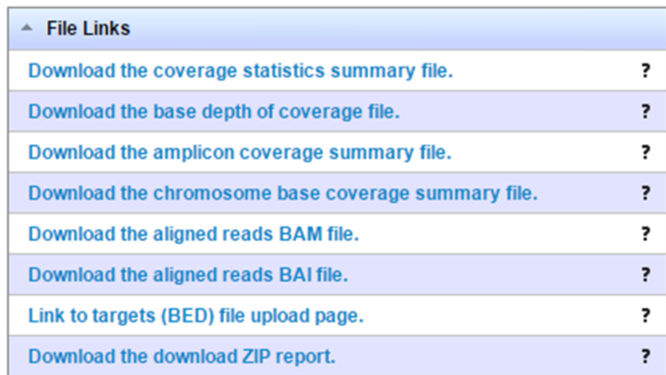
By clicking the magnifying glass in the upper right corner of the Amplicon Coverage Chart, you can change and filter what is shown on the chart by number of reads, chromosome, or gene. In this example, the TP53 gene was entered in the **Attribute** field.



The Reference Coverage Chart shows the strand-specific coverage in red and green for TP53 amplicons.



At the bottom of the Coverage Analysis Report, you can click links to download the data in this report and the BAM and BAI files that are required for IGV (Integrated Genomics Viewer).



Torrent Variant Caller plugin

For detailed instructions on running the Torrent Variant Caller, see "Torrent Variant Calling" in the *Torrent Suite™ Software Help*. The Torrent Variant Caller (TVC) plugin calls single-nucleotide polymorphisms (SNPs), multinucleotide polymorphisms (MNPs), insertions, and deletions in a sample across a reference or within a targeted subset of that reference.

This plugin provides optimized pre-set parameters for many experiment types, but is also customizable. After you find a parameter combination that works well on your data and has the balance of specificity and sensitivity that you want, you can save that parameter set and reuse it in your research. This is supported on both manual launches of the plugin and in automatic launches through the Planned Run template wizard.

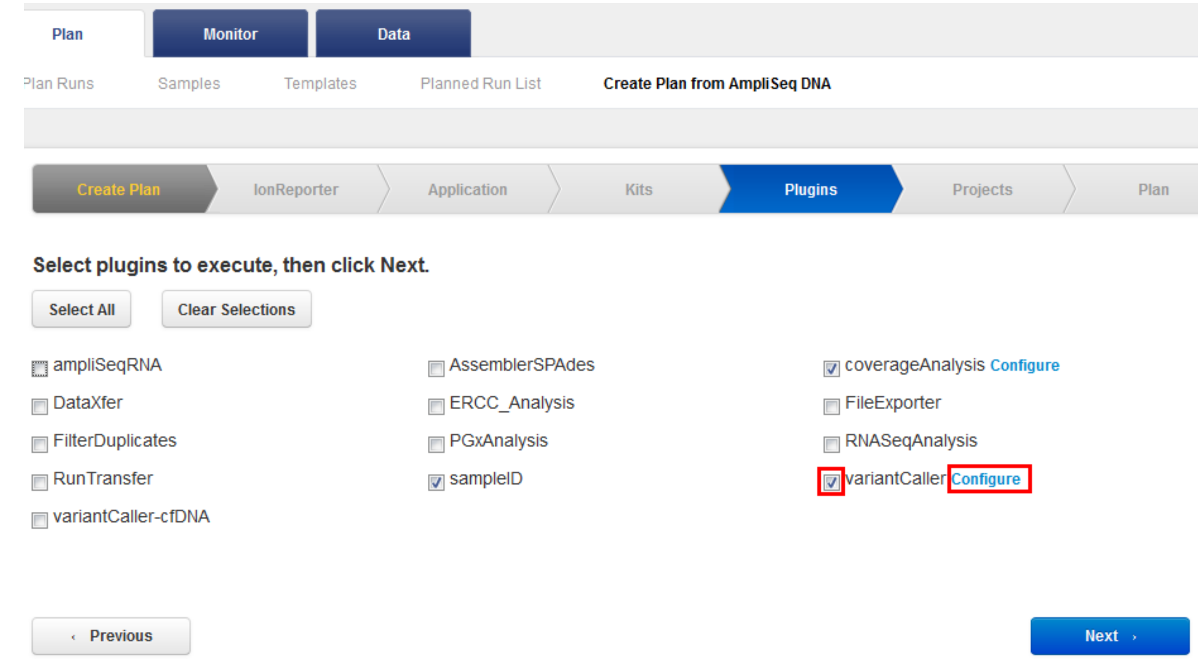
Note: The TVC plugin run uses the same target regions file and hotspots file as the main Torrent Suite™ Software analysis (if those files are present in the main analysis). You cannot change the target regions file or hotspots file through TVC configuration during Planned Run setup. You can use a different target regions file and hotspots file with a manual TVC launch from a completed run report.

Configure and run the Torrent Variant Caller plugin

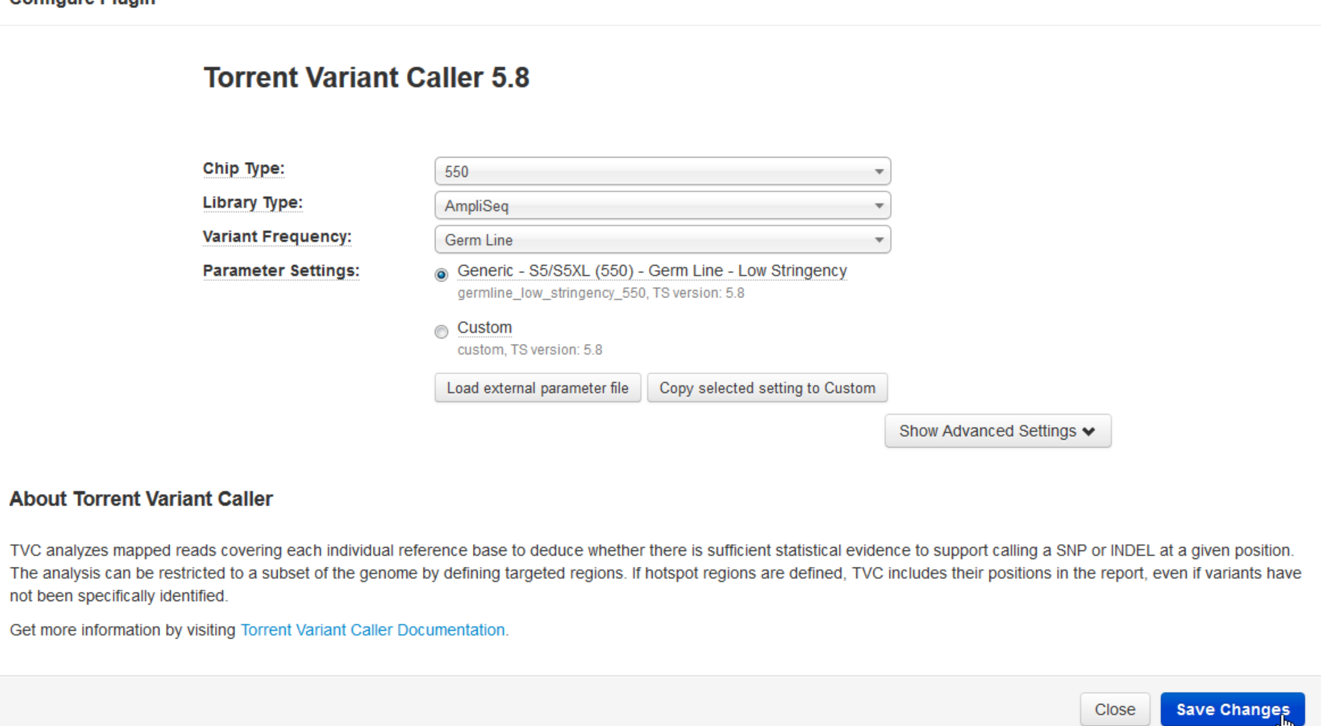
There are two ways to run the TVC plugin:

- Automatically, by selecting and configuring the plugin during run planning or
- Manually, allowing you to run the plugin at any time from a completed run report.

1. To run the TVC plugin automatically during run analysis, select the **variantCaller** checkbox in the **Plugins** step in the template or Create Plan Wizard. After selecting the checkbox, a **Configure** link appears:



2. Click **Configure** to open the Torrent Variant Caller configuration dialog:



3. Select **Chip Type**, **Library Type**, and **Variant Frequency**. Select the pre-defined **Parameter Settings** for your sequencing platform, create a custom parameter set, or load an external parameter file that is optimized for your Ion AmpliSeq™ panel, which can be downloaded from the panel page on **www.ampliseq.com**.

   The default parameter setting groups of Torrent Variant Caller are organized according to these attributes:

   - **Chip Type—**Parameter defaults are different for the different sequencing platforms and chips. Select Ion PGM™ and Ion Proton™ sequencers, or chips used on Ion S5™/Ion S5™ XL and Ion GeneStudio™ S5 Series sequencers.
   - **Library Type—**Parameter defaults are optimized for the different library types.
   - **Variant Frequency—**Somatic settings are optimized to detect low frequency variants. Germ-line settings are optimized for high frequency settings.

4. When you are finished with your selections, click **Save Changes**.

D

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, visit **thermofisher.com/support**.

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

WARNING! **4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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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  - 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 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**.