QUICK REFERENCE

Ion AmpliSeq™ Exome Library Preparation

(Recommended protocol: qPCR Quantitation)

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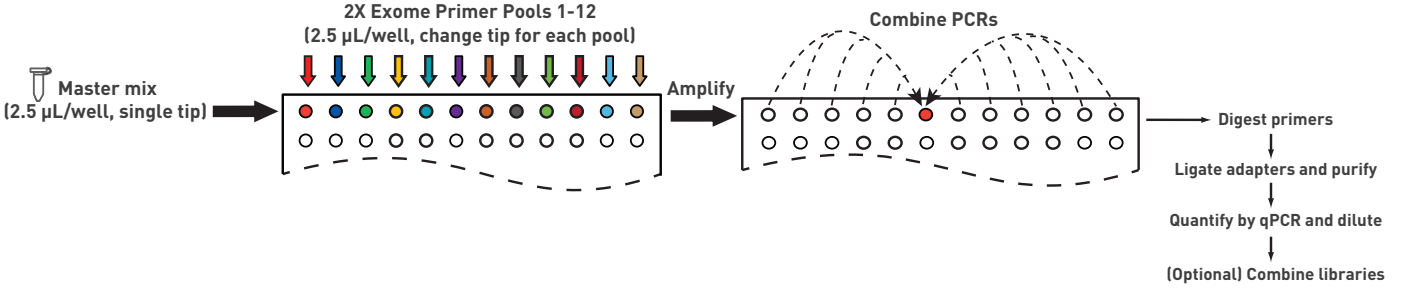
This guide provides a protocol for preparing libraries using the Ion AmpliSeq™ Exome Kit (Cat. nos. 4487084, 4488991, 4489839) for sequencing on the Ion Proton™ System. For additional tips and troubleshooting, see the Ion AmpliSeq™ Library Preparation User Guide (Pub. no. MAN0006735), available on the **Ion Community**.

IMPORTANT! You must upgrade to Torrent Suite™ software version 4.0.2 or later before template preparation and sequencing. For updates and supportive information on Ion AmpliSeq™ Exome, please see **http://bit.ly/ampliseqexome**.



Quantify DNA

IMPORTANT! We recommend the TaqMan® RNase P Detection Reagents Kit (Cat. no. 4316831) for quantifying amplifiable DNA. See the *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan® RNase P Detection Reagents Kit* (Pub. no. MAN0007732), available on the Ion Community. The Qubit® dsDNA HS Assay Kit (Cat. no. Q32851 or Q32854) may also be used. Methods such as densitometry are not recommended.

Amplify 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)*	Y µL
Nuclease-free Water	(21 – Y) µL
Total	35 µL

* Where DNA is not limiting, we recommend 100 ng.

2. Mix thoroughly. For each sample, use a low volume pipettor and a **single** tip to carefully dispense 2.5-µL aliquots into 12 wells of a 96-well plate (Cat. no. N8010560 or 4306737).

3. Using a low volume pipettor and a **new** tip for each well, carefully add 2.5 µL of the appropriate 2X Exome Primer Pool (tubes 1–12) to each well with master mix as shown above for a total reaction volume of 5 µL.

4. Apply a MicroAmp® clear adhesive film (Cat. no. 4306311), ensure a tight seal, and briefly centrifuge the plate to collect droplets.

5. Place a MicroAmp® Compression Pad on the plate, load the plate in the thermal cycler, and run the following program for 5 µL volume.

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

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

STOPPING POINT PCR products may be stored at 10°C overnight. For longer periods, store at –20°C.

Partially digest primer sequences

1. Briefly centrifuge the plate to collect droplets. Slowly remove the plate seal and combine the 12 PCRs for each sample (row) by carefully transferring the material from wells 1–5 and 7–12 into the column 6 well, using a single tip, as shown above.
2. Add 6 µL of FuPa Reagent (brown cap) to each combined sample, to bring the total sample volume to approximately 60 µL.
3. Seal the plate with a MicroAmp® adhesive film, vortex thoroughly, and briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times prior to sealing the plate.
4. Place a MicroAmp® Compression Pad on the plate, load the plate in the thermal cycler, and run the following program.

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

Ligate adapters to the amplicons and purify

Set up and run the ligation reaction

1. Carefully remove the plate seal and add 6 µL of diluted barcode adapters to each well of digested amplicons:

Diluted barcode adapter component	Volume
Ion P1 Adapter	1.5 µL
Ion Xpress™ Barcode Adapter	1.5 µL
Nuclease-free Water	3.0 µL

2. Add 12 µL of Switch Solution to each well. If there is visible precipitate in the Switch Solution, vortex or pipet up and down at room temperature to resuspend.
3. Add 6 µL of DNA Ligase to each well to bring the total reaction volume to approximately 80 µL.

   Note: Do not pre-combine DNA Ligase and barcode adapters prior to adding to reaction mixture.
4. Seal the plate with MicroAmp® adhesive film, vortex thoroughly and briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times prior to sealing the plate.
5. Place a MicroAmp® Compression Pad on the plate, load the plate in the thermal cycler, and run the following program:

Temperature	Time
22°C	30 min
72°C	10 min
10°C	Hold (for up to 1 hour)

STOPPING POINT Samples may be stored at –20°C.

Purify the unamplified library

IMPORTANT! Bring AMPure® XP reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet solution slowly.

Use freshly prepared 70% ethanol for the next steps. Combine 460 µL of ethanol with 200 µL of Nuclease-free Water per sample.

1. Carefully remove the plate seal and add 80 µL of Agencourt® AMPure® XP Reagent to each library. 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 (Cat. no. 12331D), and incubate for 5 minutes or until solution clears. Carefully remove and 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. 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.**
7. Remove the plate containing the Ion AmpliSeq™ Exome library from the magnet, and add 50 µL of Low TE.
8. Seal the plate with a MicroAmp® adhesive file, vortex thoroughly, and spin down to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times prior to sealing the plate.
9. Place the plate in the magnet for at least 2 minutes. The supernatant contains the library. Remove 5 µL of the supernatant, and combine with 495 µL of Nuclease-free Water for quantification.

Quantify the library and dilute

Determine the concentration of each Ion AmpliSeq™ library by qPCR with the Ion Library Quantitation Kit (Cat. no. 4468802) using the steps below. Each sample, standard, and negative control should be analyzed in duplicate 20-µL reactions.

Exome libraries typically have yields of 100–500 pM.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Ion Control Library (~68 pM; from the Ion Library Quantitation Kit) at 6.8 pM, 0.68 pM, and 0.068 pM. Mark these as standards and use these concentrations in the qPCR instrument software.
2. Prepare reaction mixtures. For each sample, control, and standard, combine 20 µL of 2X TaqMan® MasterMix and 2 µL of 20X Ion TaqMan® Assay and mix thoroughly. Dispense 11-µL aliquots into the wells of a 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 as follows:
   - Enter the concentrations of the control library standards.
   - Use ROX™ Reference Dye as the passive reference dye.
   - Select a reaction volume of 20 µL.
   - Select FAM™ dye/MGB as the TaqMan® probe reporter/quencher.
   - The Ion Library TaqMan® qPCR Mix can be used on a variety of Life Technologies instruments, as listed below. The fast cycling program was developed using the StepOnePlus™ System in Fast mode.

Real-Time PCR System	Stage	Temp	Time
7900 HT System 7900 HT Fast System (Fast 96-Well, Standard 96-Well, or 384-Well Block Modules) ViiA™ 7 System StepOne™ System StepOnePlus™ System	Hold (optional)	50°C	2 min
	Hold	95°C	20 sec
	Cycle (40 cycles)	95°C	1 sec
		60°C	20 sec
7500 Fast System 7500 System	Hold (optional)	50°C	2 min
	Hold	95°C	20 sec
	Cycle (40 cycles)	95°C	3 sec
		60°C	30 sec

5. Following qPCR, calculate the average concentration of the undiluted Ion AmpliSeq™ library by multiplying the concentration 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 mixed with 2 µL of Low TE (1:3 dilution) yields ~100 pM.
7. Dilute library to ~100 pM as described and proceed to combining libraries or template preparation.

(Optional) Combine amplicon libraries

Up to 3 exome libraries can be combined and run on a single Ion PI™ Chip, depending on the coverage depth desired.

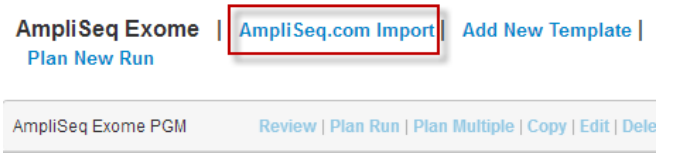
Store libraries

Libraries may be stored at 4–8°C for up to 1 month. For longer term, store at –20°C.

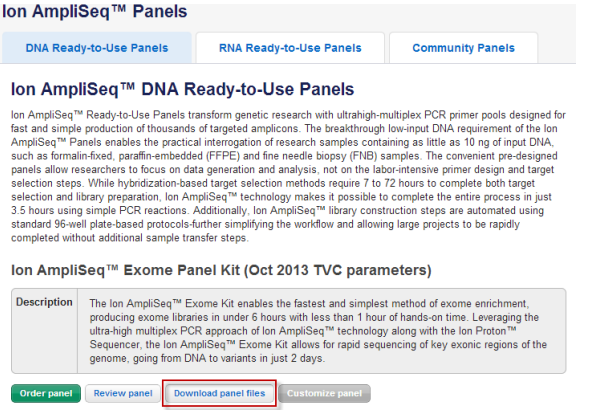
Download support files from AmpliSeq.com

When sequencing exome libraries for the first time:

1. In the Torrent Browser, under the **Plan** tab, click on **Plan Template Run,** navigate to AmpliSeq Exome section, and click on the **AmpliSeq.com Import** link.



2. Log into AmpliSeq.com. Under the **DNA Ready-to-Use Panels** tab, select **the Ion AmpliSeq™ Exome Panel Kit** and click **Download panel files** to download the necessary template and support files.



3. Verify that the Ion AmpliSeq™ Exome Panel template is now available in the Plan screen.

Template preparation

If using the Ion PI™ Template OT2 200 Kit v3 (Cat. no. 4488318), follow the instructions in that user guide.

If using the Ion PI™ Template OT2 200 Kit v2 (Cat. no. 4485146), follow the instructions in that user guide with the following exceptions to the "Prepare the amplification solution" section in Chapter 3:

- Substitute **Ion PI™ Ion Sphere™ Particles XT**, included in the Ion PI™ Reagents XT Kit (Cat. no. 4487053), instead of the standard Ion PI™ Ion Sphere™ Particles when preparing the amplification solution.
- Select the **Ion PI™ Template OT2 200 Kit v2 with XT** protocol on the OneTouch™ 2 instrument.

Set up a planned run

IMPORTANT! To set up a planned run for Ion AmpliSeq™ Exome libraries, you **must select** the Ion AmpliSeq™ Exome Panel template (downloaded as described above):

1. In the Torrent Browser, under the **Plan** tab, click on **Plan Template Run**, navigate to the **AmpliSeq Exome** section, and click on **Plan Run**.
2. In the Plan Run workflow, select the appropriate template and sequencing kits.

Sequencing

If using the Ion PI™ Sequencing 200 Kit v3 (Cat. no. 4488315), follow the instructions in that user guide.

If using the Ion PI™ Sequencing 200 Kit v2 (Cat. no. 4485149), follow the instructions in that user guide with the following exceptions to the "Load the Ion PI™ Chip v2" section in Chapter 4:

- For Ion PI™ Reagents XT Kit (Cat. no. 4487053) lots 1407166 and 1422806, use the Ion PI™ Loading Buffer included in the Ion PI™ Sequencing 200 Kit v2. For all other lots, substitute **Ion PI™Loading Buffer XT** included in the Ion PI™ Reagents XT Kit for the standard Ion PI™ Loading Buffer.
- For all Ion PI™ Reagents XT Kit lots, substitute **Ion PI™ Sequencing Polymerase v3**, included in the Ion PI™ Reagents XT Kit (Cat. no. 4487053), instead of the standard Ion PI™ Sequencing Polymerase when loading the sequencing polymerase (page 36 of the *Ion PI™ Sequencing 200 Kit v2 User Guide*).

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