AmpliSeq for Illumina Exome Checklist

Quantify and Dilute DNA

- ☐ 1 Quantify DNA using a fluorometric method, such as Qubit or PicoGreen.
- ☐ 2 Dilute DNA to desired final concentration using Low TE.

Amplify Targets

- ☐ 1 Combine the following volumes per sample in a 1.5 ml tube.
  - 5X AmpliSeq HiFi Mix (14 µl)
  - 50–100 ng DNA (≤ 56 µl)
  - Nuclease-free water (to 70 µl)
- ☐ 2 Pipette or vortex briefly to mix, and then centrifuge briefly.
- ☐ 3 For each sample, transfer 70 µl master mix into a new well of an 8-tube strip.
- ☐ 4 Unseal the AmpliSeq Exome Panel plate.
- ☐ 5 For each sample, add 5 µl master mix to each well in one row of the AmpliSeq Exome Panel plate (12 wells) without changing tips.
- ☐ 6 Seal the plate, vortex briefly, and then centrifuge briefly.
- ☐ 7 Place on the thermal cycler and run the AMP_10 program.

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 10°C for up to 24 hours. For longer durations, store at -25°C to -15°C.

Partially Digest Amplicons

- ☐ 1 Briefly centrifuge to collect contents.
- ☐ 2 For each row, use a multichannel pipette to combine the 12 wells into the column 6 well, without changing tips.
- ☐ 3 Add 6 µl FuPa Reagent to each well in column 6.
- ☐ 4 Vortex briefly, and then centrifuge briefly.
- ☐ 5 Place on the thermal cycler, cover with a compression pad, and run the FUPA program.

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 10°C for up to 1 hour. For longer periods, store at -25°C to -15°C.

Ligate Indexes

☐ 1 Add the following volumes ***in the order listed*** to each well

Reagent	Volume (µl)
Switch Solution	12
AmpliSeq CD Indexes Large Volume or UD Indexes for Illumina	6
DNA Ligase	6
Total Volume per sample	**80**

☐ 2 Seal the plate.
☐ 3 Vortex briefly, and then centrifuge briefly.
☐ 4 Place on the thermal cycler, cover with a compression pad, and run the LIGATE program.

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 10°C for up to 1 hour. For longer periods, store at -25°C to -15°C.

Clean Up Library

☐ 1 Briefly centrifuge the plate to collect contents.
☐ 2 Add 80 µl AMPure XP beads to each library.
☐ 3 Vortex briefly.
☐ 4 Inspect each well to make sure that the mixture is homogeneous.
☐ 5 Centrifuge briefly.
☐ 6 Incubate at room temperature for 5 minutes.
☐ 7 Place on a magnetic stand until the mixture is clear.
☐ 8 Remove and discard supernatant.
☐ 9 Wash with 150 µl 70% EtOH.
☐ 10 Wash again with 150 µl 70% EtOH.
☐ 11 Centrifuge briefly.
☐ 12 Place on the magnetic stand.
☐ 13 Immediately remove all residual EtOH as follows.
  ☐ a Use a 20 µl pipette to remove residual EtOH.
  ☐ b Air-dry on the magnetic stand.
  ☐ c Inspect each well to make sure that the EtOH has evaporated.
  ☐ d If EtOH remains, continue to air-dry until EtOH is no longer visible.
☐ 14 If you are using the AmpliSeq Library Equalizer for Illumina, proceed to *Equalize Libraries* on page 4. Otherwise, continue to *Amplify Library* on page 1.

Amplify Library

☐ 1 For each reaction, combine the following volumes.

Reagent	Volume (µl)
1X Lib Amp Mix (black cap)	45
10X Library Amp Primers (pink cap)	5
Total Volume per reaction	**50**

☐ 2 Vortex briefly, and then centrifuge briefly.
☐ 3 Remove the plate from the magnetic stand.
☐ 4 Add 50 µl amplification master mix to each library well, and then seal the plate.
☐ 5 Vortex briefly, and then centrifuge briefly.
☐ 6 Place on the thermal cycler, cover with a compression pad, and run the AMP_7 program.

SAFE STOPPING POINT

If you are stopping, leave the plate on the thermal cycler at 10°C for up to 24 hours. For longer durations, store at -25°C to -15°C.

Perform Second Cleanup

- ☐ 1 Briefly centrifuge the plate to collect contents.
- ☐ 2 Add 25 µl AMPure XP beads to each well.
- ☐ 3 Vortex briefly, and then centrifuge briefly.
- ☐ 4 Incubate at room temperature for 5 minutes.
- ☐ 5 Place the plate on a magnetic stand until the liquid is clear.
- ☐ 6 Transfer the ***entire*** supernatant (~75 µl), ***which contains the desired amplicon library***, to a new plate.
- ☐ 7 Add 60 µl AMPure XP beads.
- ☐ 8 Vortex briefly, and then centrifuge briefly.
- ☐ 9 Incubate at room temperature for 5 minutes.
- ☐ 10 Place on the magnetic stand until the liquid is clear.
- ☐ 11 Without disturbing the beads, remove and discard supernatant.
- ☐ 12 Wash with 150 µl 70% EtOH.
- ☐ 13 Wash again with 150 µl 70% EtOH.
- ☐ 14 Use a 20 µl pipette to remove and discard residual EtOH.
- ☐ 15 Air-dry on the magnetic stand for 5 minutes.
- ☐ 16 Remove from the magnetic stand.
- ☐ 17 Add 30 µl Low TE to each well.
- ☐ 18 Vortex briefly, and then centrifuge briefly.
- ☐ 19 Place on the magnetic stand and wait until the liquid is clear (~5 minutes).
- ☐ 20 Transfer 27 µl supernatant to a new plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C.

Check Libraries

- ☐ 1 Place the plate on the magnetic stand. Keep the plate on the stand while performing normalization and pooling.
- ☐ 2 Assess library quality.
- ☐ 3 Quantify the library.

Dilute Libraries to the Starting Concentration

- ☐ 1 Calculate the molarity value of the library or pooled libraries.
- ☐ 2 Using the molarity value, calculate the volumes of Low TE and library needed to dilute libraries to the starting concentration.

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
NextSeq 550 and NextSeq 500	2	1.1–1.9

- ☐ 3 Dilute libraries using Low TE:
  - **Libraries quantified as a pool**—Dilute the pool to the starting concentration.
  - **Libraries quantified individually**—Dilute each library to the starting concentration. Add 10 µl each diluted library to a tube.
- ☐ 4 Dilute to the final loading concentration.

Equalize Libraries

- ☐ 1 Use the following steps to normalize library concentration without quantification using the AmpliSeq Library Equalizer for Illumina.

Amplify Library

- ☐ 1 Remove the plate with purified libraries from the magnetic stand.
- ☐ 2 For each reaction, combine the following volumes.

Reagent	Volume (µl)
1X Lib Amp Mix (black cap)	45
10X Library Amp Primers (pink cap)	5

- ☐ 3 Vortex briefly, and then centrifuge briefly.
- ☐ 4 Add 50 µl amplification master mix to each library well.
- ☐ 5 Place on the thermal cycler, cover with a compression pad (if applicable), and run the EQUAL program.

Wash Equalizer Beads

- ☐ 1 For each reaction, combine the following volumes:
  - Equalizer Beads (7 µl)
  - Equalizer Wash Buffer (14 µl)
- ☐ 2 Pipette to mix.
- ☐ 3 Place on the magnetic stand until liquid is clear.
- ☐ 4 Remove and discard all supernatant.
- ☐ 5 Remove from the magnetic stand.
- ☐ 6 For each reaction, add 7 µl Equalizer Wash Buffer. Pipette to resuspend.

Add Equalizer Capture

- ☐ 1 Briefly centrifuge the library plate to collect contents, and then unseal.
- ☐ 2 Place on the magnetic stand until liquid is clear.
- ☐ 3 Transfer 45 µl of supernatant to a new plate.
- ☐ 4 Add 10 µl Equalizer Capture.
- ☐ 5 Seal the plate, vortex to mix, and then briefly centrifuge to collect contents.
- ☐ 6 Incubate at room temperature for 5 minutes.

Perform Second Cleanup

- ☐ 1 Unseal the plate.
- ☐ 2 Vortex or pipette washed Equalizer Beads to mix.
- ☐ 3 Add 6 µl Equalizer Beads .
- ☐ 4 Seal the plate, vortex thoroughly, and then centrifuge briefly.
- ☐ 5 Incubate at room temperature for 5 minutes.
- ☐ 6 Place on the magnetic stand until liquid is clear.
- ☐ 7 Unseal the plate.
- ☐ 8 Remove and discard all supernatant.

Elute Library

- ☐ 1 Remove the plate from the magnetic stand.
- ☐ 2 Add 30 µl Equalizer Elution Buffer .
- ☐ 3 Seal the plate, vortex thoroughly, and then centrifuge briefly.
- ☐ 4 Elute the library by incubating on a thermal cycler at 45°C for 5 minutes.
- ☐ 5 Place on the magnetic stand until liquid is clear.
- ☐ 6 Unseal the plate.
- ☐ 7 Transfer 27 µl supernatant to a new plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C.

Denature and Dilute Libraries

☐ 1 Denature and dilute libraries for loading on the sequencing instrument you are using.