TruSeq RNA Exome Checklist

For Research Use Only. Not for use in diagnostic procedures.

Fragment RNA

- ☐ 1 Dilute the total RNA in nuclease-free ultrapure water to 8.5 µl in the DFP plate.
- ☐ 2 Add 8.5 µl EPH.
- ☐ 3 Shake at 1600 rpm for 20 seconds.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and run the Elution 2-Frag-Prime program.
- ☐ 6 Remove from thermal cycler when it reaches 4° C and centrifuge briefly.

Synthesize First Strand cDNA

- ☐ 1 Add 50 µl SuperScript II to FSA. Pipette to mix, and then centrifuge briefly.
- ☐ 2 Add 8 µl SuperScript II and FSA mixture.
- ☐ 3 Shake at 1600 rpm for 20 seconds.
- ☐ 4 Place on the thermal cycler and run the Synthesize 1st Strand program.
- ☐ 5 When the thermal cycler reaches 4°C, remove the DFP plate from the thermal cycler.

Synthesize Second Strand cDNA

- ☐ 1 Add 5 µl RSB.
- ☐ 2 Add 20 µl SMM.
- ☐ 3 Shake at 1600 rpm for 20 seconds.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and incubate at 16°C for 1 hour.
- ☐ 6 Place on the bench and let stand to bring to room temperature.
- ☐ 7 Add 90 µl AMPure XP beads to the CCP plate.
- ☐ 8 Transfer all to the CCP plate.
- ☐ 9 Shake at 1800 rpm for 2 minutes.
- ☐ 10 Incubate at room temperature for 5 minutes.
- ☐ 11 Centrifuge at 280 × g for 1 minute.
- ☐ 12 Place on a magnetic stand until liquid is clear.
- ☐ 13 Remove and discard 135 µl supernatant.
- ☐ 14 Leave the CCP plate on the magnetic stand when performing the following wash step.
- ☐ 15 Wash 2 times with 200 µl 80% EtOH.
- ☐ 16 Use a 20 µl pipette to remove residual EtOH.
- ☐ 17 Air-dry for 5 minutes.
- ☐ 18 Remove from the magnetic stand.
- ☐ 19 Add 17.5 µl RSB.
- ☐ 20 Shake at 1800 rpm for 2 minutes.
- ☐ 21 Incubate at room temperature for 2 minutes.
- ☐ 22 Centrifuge at 280 × g for 1 minute.
- ☐ 23 Place on a magnetic stand until liquid is clear.
- ☐ 24 Transfer 15 µl supernatant to the ALP plate.

SAFE STOPPING POINT

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

Adenylate 3′ Ends

- ☐ 1 Add 2.5 µl RSB.
- ☐ 2 Add 12.5 µl ATL.
- ☐ 3 Shake at 1800 rpm for 2 minutes.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the 37°C microheating system for 30 minutes.
- ☐ 6 Move to the 70°C microheating system for 5 minutes.
- ☐ 7 Place on ice for 1 minute.

Ligate Adapters

- ☐ 1 Add the following.
  - RSB (2.5 µl)
  - LIG (2.5 µl)
  - RNA adapters (2.5 µl)
- ☐ 2 Shake at 1800 rpm for 2 minutes.
- ☐ 3 Centrifuge at 280 × g for 1 minute.
- ☐ 4 Place on the 30°C microheating system for 10 minutes, and then place on ice.
- ☐ 5 Add 5 µl STL.
- ☐ 6 Shake at 1800 rpm for 2 minutes.
- ☐ 7 Centrifuge at 280 × g for 1 minute.
- ☐ 8 Perform steps 9 through 24 using the **Round 1** volumes.
- ☐ 9 Add AMPure XP beads.

	Round 1	Round 2
AMPure XP beads	42 µl	50 µl

- ☐ 10 Shake at 1800 rpm for 2 minutes.
- ☐ 11 Incubate at room temperature for 5 minutes.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Place on a magnetic stand until liquid is clear.
- ☐ 14 Remove and discard all supernatant.
- ☐ 15 Wash 2 times with 200 µl 80% EtOH.
- ☐ 16 Use a 20 µl pipette to remove residual EtOH.
- ☐ 17 Air-dry for 5 minutes.
- ☐ 18 Remove from the magnetic stand.
- ☐ 19 Add RSB.

	Round 1	Round 2
RSB	52.5 µl	22.5 µl

- ☐ 20 Shake at 1800 rpm for 2 minutes.
- ☐ 21 Incubate at room temperature for 2 minutes.
- ☐ 22 Centrifuge at 280 × g for 1 minute.
- ☐ 23 Place on a magnetic stand until liquid is clear.
- ☐ 24 Transfer 50 µl supernatant to the CAP plate.
- ☐ 25 Repeat steps 9 through 24 with the new plate using the **Round 2** volumes.
- ☐ 26 Transfer 20 µl supernatant to the PCR plate.

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Enrich DNA Fragments

- ☐ 1 Place on ice and add 5 µl PPC.
- ☐ 2 Add 25 µl PMM.
- ☐ 3 Shake at 1600 rpm for 20 seconds.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and run the PCR program.
- ☐ 6 Add 50 µl AMPure XP beads.
- ☐ 7 Transfer contents from PCR plate to the CPP plate.
- ☐ 8 Shake at 1800 rpm for 2 minutes.
- ☐ 9 Incubate at room temperature for 5 minutes.
- ☐ 10 Centrifuge at 280 × g for 1 minute.
- ☐ 11 Place on a magnetic stand until liquid is clear.
- ☐ 12 Remove and discard all supernatant.
- ☐ 13 Wash 2 times with 200 µl 80% EtOH.
- ☐ 14 Use a 20 µl pipette to remove residual EtOH.
- ☐ 15 Air-dry for 5 minutes.
- ☐ 16 Remove from the magnetic stand.
- ☐ 17 Add 17.5 µl RSB.
- ☐ 18 Shake at 1800 rpm for 2 minutes.
- ☐ 19 Incubate at room temperature for 2 minutes.
- ☐ 20 Centrifuge at 280 × g for 1 minute.
- ☐ 21 Place on a magnetic stand until liquid is clear.
- ☐ 22 Transfer 15 µl supernatant to the TSP1 plate.

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Check Libraries

- ☐ 1 If using a Standard Sensitivity NGS Fragment Analysis Kit:
  - ☐ a Dilute the DNA library 1:1 with RSB.
  - ☐ b Run 1 µl diluted DNA library.
- ☐ 2 If using a DNA 1000 chip, run 1 µl undiluted DNA library.
- ☐ 3 Check the size and purity of the sample. Expect the final product to be a band at ~260 bp.

First Hybridization of Probes

- ☐ 1 Add the following to the REH1 plate.
  - DNA library sample or pool (45 µl)
  - CT3 (50 µl)
  - CEX (5 µl)
- ☐ 2 For single-plex, add the following to the REH1 plate:
  - DNA library sample 200ng (11.25 µl)
  - CT3 (12.5 µl)
  - CEX (1.25 µl)
- ☐ 3 Shake at 1200 rpm for 1 minute.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and run the RNA HYB program.
  - For 2-4 plex pools, each well contains 100 µl.
  - For single-plex pools, each well contains 25 µl.
- ☐ 6 Remove from the thermal cycler immediately after the 90-minute incubation.

First Capture of Hybridized Probes

- ☐ 1 Centrifuge at 280 × g for 1 minute.
- ☐ 2 Transfer all to the REW1 plate.
- ☐ 3 Add 250 µl (62.5 µl for 1-plex) SMB.
- ☐ 4 Shake at 1200 rpm for 5 minutes.
- ☐ 5 Incubate at room temperature for 25 minutes.
- ☐ 6 Centrifuge at 280 × g for 1 minute.
- ☐ 7 Place on a magnetic stand until liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Remove from the magnetic stand.
- ☐ 10 Wash two times with 200 µl EWS.
- ☐ 11 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex to mix.
- ☐ 12 For 1-plex, create elution premix in a 1.7 microcentrifuge tube, and then vortex to mix.
  - EE1 (9.5 µl)
  - HP3 (0.5 µl)
- ☐ 13 Add 23 µl (10 µl for 1-plex) elution premix.
- ☐ 14 Shake at 1800 rpm for 2 minutes.
- ☐ 15 Incubate at room temperature for 2 minutes.
- ☐ 16 Centrifuge at 280 × g for 1 minute.
- ☐ 17 Place on a magnetic stand until liquid is clear.
- ☐ 18 Transfer 21 µl (9 µl for 1-plex) supernatant to the REH2 plate.
- ☐ 19 Add 4 µl (1.7 µl for 1-plex) ET2.
- ☐ 20 Shake at 1200 rpm for 1 minute.
- ☐ 21 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

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Second Hybridization of Probes

- ☐ 1 Add the following.
  - RSB (20 µl)
  - CT3 (50 µl)
  - CEX (5 µl)
- ☐ 2 For 1-plex, add the following.
  - RSB (0.55 µl)
  - CTE (12.5 µl)
  - CEX (1.25 µl)
- ☐ 3 Shake at 1200 rpm for 1 minute.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and run the RNA HYB program.
- ☐ 6 Remove from the thermal cycler immediately after the 90-minute incubation.

Second Capture of Hybridized Probes

- ☐ 1 Centrifuge at 280 × g for 1 minute.
- ☐ 2 Transfer all supernatant to the REW2 plate.
- ☐ 3 Add 250 µl (62.5 µl for 1-plex) SMB.
- ☐ 4 Shake at 1200 rpm for 5 minutes.
- ☐ 5 Incubate at room temperature for 25 minutes.
- ☐ 6 Centrifuge at 280 × g for 1 minute.
- ☐ 7 Place on a magnetic stand until liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Remove from the magnetic stand.
- ☐ 10 Wash two times with 200 µl EWS.
- ☐ 11 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex to mix.
- ☐ 12 For 1-plex, create elution premix in a 1.7 ml microcentrifuge tube, and then vortex to mix.
  - EE1 (9.5 µl)
  - HP3 (0.5 µl)
- ☐ 13 Add 23 µl (10 µl for 1-plex) elution premix.
- ☐ 14 Shake at 1800 rpm for 2 minutes.
- ☐ 15 Incubate at room temperature for 2 minutes.
- ☐ 16 Centrifuge at 280 × g for 1 minute.
- ☐ 17 Place on a magnetic stand until liquid is clear.
- ☐ 18 Transfer 21 µl (9 µl for 1-plex) supernatant to the REC1 plate.
- ☐ 19 Add 4 µl (1 µl for 1-plex) ET2.
- ☐ 20 Shake at 1800 rpm for 1 minute.
- ☐ 21 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

- ☐ 1 Add 45 µl (18 µl for 1-plex) AMPure XP beads.
- ☐ 2 Shake at 1800 rpm for 1 minute.
- ☐ 3 Incubate at room temperature for 5 minutes.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on a magnetic stand until liquid is clear.
- ☐ 6 Remove and discard all supernatant.
- ☐ 7 Wash 2 times with 200 µl 80% EtOH.
- ☐ 8 Use a 20 µl pipette to remove residual EtOH.
- ☐ 9 Air-dry for 5 minutes.
- ☐ 10 Remove from the magnetic stand.
- ☐ 11 Add 27.5 µl RSB.
- ☐ 12 Shake at 1800 rpm for 1 minute.
- ☐ 13 Incubate at room temperature for 2 minutes.
- ☐ 14 Centrifuge at 280 × g for 1 minute.
- ☐ 15 Place on a magnetic stand until liquid is clear.
- ☐ 16 Transfer 25 µl supernatant to the REA plate.

SAFE STOPPING POINT

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Amplify Enriched Library

- ☐ 1 Remove seal and add 5 µl PPC.
- ☐ 2 Add 20 µl EPM.
- ☐ 3 Shake at 1200 rpm for 1 minute.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place the midi plate on the thermal cycler and run the EPM AMP program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days.

Clean Up Amplified Enriched Library

- ☐ 1 Centrifuge at 280 × g for 1 minute.
- ☐ 2 Transfer 50 µl to the REC2 plate.
- ☐ 3 Add 90 µl AMPure XP beads.
- ☐ 4 Shake RAC2 at 1800 rpm for 1 minute.
- ☐ 5 Incubate at room temperature for 5 minutes.
- ☐ 6 Centrifuge at 280 × g for 1 minute.
- ☐ 7 Place on a magnetic stand until liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Wash 2 times with 200 µl 80% EtOH.
- ☐ 10 Use a 20 µl pipette to remove residual EtOH.
- ☐ 11 Air-dry on the magnetic stand for 5 minutes.
- ☐ 12 Remove from the magnetic stand.
- ☐ 13 Add 32 µl (8 µl for 1-plex) RSB.
- ☐ 14 Shake at 1800 rpm for 1 minute.
- ☐ 15 Incubate at room temperature for 2 minutes.
- ☐ 16 Centrifuge at 280 × g for 1 minute.
- ☐ 17 Place on a magnetic stand until liquid is clear.
- ☐ 18 Transfer 30 µl (7.5 µl for 1-plex) supernatant to the REL plate.

SAFE STOPPING POINT

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

Check Enriched Libraries

- ☐ 1 Quantify the libraries according to the Illumina *Sequencing Library qPCR Quantification Guide (document # 11322363)* or fluorometric method.
- ☐ 2 Load 1 µl of the post-enriched library on one of the following:
  - Advanced Analytical Technologies Standard Sensitivity NGS Fragment Analysis Kit
  - Agilent High Sensitivity DNA Chip
- ☐ 3 Check the size of the library for a distribution of DNA fragments with a size range from ~200 bp–1 kb.
- ☐ 4 Proceed to cluster generation.

Acronyms

Acronym	Definition
ALP	Adapter Ligation Plate
ATL	A-Tailing Mix
CAP	Clean Up ALP Plate
CCP	cDNA Clean Up Plate
CEX	Coding Exome Oligos
CPP	Clean Up PCR Plate
CT3	Capture Target Buffer 3
DFP	Depleted RNA Fragmentation Plate
EE1	Enrichment Elution Buffer 1
EPH	Elute, Prime, Fragment High Mix
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
FSA	First Strand Synthesis Act D Mix
HP3	2N NaOH
IEM	Illumina Experiment Manager
LIG	Ligation Mix
LRM	Local Run Manager
PCR	Polymerase Chain Reaction Plate
PMM	PCR Master Mix
PPC	PCR Primer Cocktail
REA	RNA Exome Amplification Plate
REC1	RNA Exome Clean Up Plate 1
REC2	RNA Exome Clean Up Plate 2
REH1	RNA Exome Hyb Plate 1
REH2	RNA Exome Hyb Plate 2
REL	RNA Exome Library Plate
REW1	RNA Exome Wash Plate 1
REW2	RNA Exome Wash Plate 2
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SMM	Second Strand Marking Master Mix
STL	Stop Ligation Buffer
TSP	Target Sample Plate