TruSight RNA Fusion Panel Checklist

Fragment RNA

- ☐1 Dilute the total RNA in nuclease-free ultrapure water to a final volume of 8.5 µl in the DFP plate.
- ☐2 Add 8.5 µl EPH.
- ☐3 Pipette to mix.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on the thermal cycler and run the Elution 2-Frag-Prime program.
- ☐6 Centrifuge at 280 × g for 1 minute.

Synthesize First Strand cDNA

- ☐1 Add 50 µl Protoscript II to FSA. Pipette or invert to mix, and then centrifuge briefly.
- ☐2 Add 8 µl Protoscript II and FSA mixture.
- ☐3 Pipette to mix.
- ☐4 Place on the thermal cycler and run the Synthesize 1st Strand program.

Synthesize Second Strand cDNA

- ☐1 Add 5 µl RSB.
- ☐2 Add 20 µl SMM.
- ☐3 Pipette to mix.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place on the preprogrammed thermal cycler and run the Synthesize 2nd Strand program.
- ☐6 Place on the bench and let stand to bring to room temperature (~5 minutes).
- ☐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 Wash 2 times with 200 µl 80% EtOH.
- ☐15 Use a 20 µl pipette to remove residual EtOH.
- ☐16 Air-dry for 5 minutes.
- ☐17 Remove from the magnetic stand.
- ☐18 Add 20 µl RSB.
- ☐19 Shake at 1800 rpm for 2 minutes.
- ☐20 Incubate at room temperature for 2 minutes.
- ☐21 Centrifuge at 280 × g for 1 minute.
- ☐22 Place on a magnetic stand until liquid is clear.
- ☐23 Transfer 17.5 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 7 days.

Adenylate 3′ Ends

- ☐1 Add 12.5 µl ATL.
- ☐2 Pipette to mix.
- ☐3 Centrifuge at 280 × g for 1 minute.
- ☐4 Place on the thermal cycler and start the program ATAIL70.
- ☐5 Place on ice for 1 minute or until cooled to 2°C to 8°C.

Ligate Adapters

- ☐1 Add the following.
  - RSB (2.5 µl)
  - LIG (2.5 µl)
  - RNA adapters (2.5 µl)
- ☐2 Pipette to mix.
- ☐3 Centrifuge at 280 × g for 1 minute.
- ☐4 Place on the thermal cycler and start the program LIG30.
- ☐5 Add 5 µl STL.
- ☐6 Pipette to mix.
- ☐7 Centrifuge at 280 × g for 1 minute.
- ☐8 Add 42 µl AMPure XP Beads to each well of CAP.
- ☐9 Transfer entire volume from ALP plate to CAP.
- ☐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 22.5 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 20 µl supernatant to the PCR plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Perform First PCR Amplification

- ☐1 Place the PCR plate on ice and add 5 µl PPC.
- ☐2 Add 25 µl PMM.
- ☐3 Pipette to mix.
- ☐4 Place on the thermal cycler and run the PCR program.
- ☐5 Add 50 µl AMPure XP Beads to the PPP plate for each well corresponding to a sample in the PCR plate.
- ☐6 Centrifuge PCR plate at 280 × g for 1 minute.
- ☐7 Transfer the entire volume (50 µl) to the PPP 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 12.5 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 12 µl supernatant to the TSP1 plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Check Libraries

- ☐1 If using a Standard Sensitivity NGS Fragment Analysis Kit, run 2 µl undiluted 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 ~250–300 bp.
- ☐4 Calculate the concentration of the library using a region selection of 160–700 bp.

Hybridize Probes

- ☐1 Dilute 200 ng of each library in 10 µl RSB.
- ☐2 Add the following to the RAH1 plate for a final volume of 25 µl.
  - 200 ng library (in 10 µl RSB)
  - CT3 (12.5 µl)
  - RFO (2.5 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.

Capture Hybridized Probes

- ☐1 Centrifuge RAH1 at 280 × g for 1 minute.
- ☐2 Add 62.5 µl SMB.
- ☐3 Shake at 1200 rpm for 5 minutes.
- ☐4 Incubate at room temperature for 25 minutes.
- ☐5 Centrifuge at 280 × g for 1 minute.
- ☐6 Place on a magnetic stand until liquid is clear.
- ☐7 Remove and discard all supernatant.
- ☐8 Remove from the magnetic stand.
- ☐9 Add 50 µl EEW.
- ☐10 Centrifuge at 280 × g for 10 seconds.
- ☐11 Pipette to mix.
- ☐12 Shake at 1800 rpm for 4 minutes.
- ☐13 Place on the thermal cycler and start the program RNA BIND.
- ☐14 Place on a magnetic stand until liquid is clear.
- ☐15 Remove and discard all supernatant.
- ☐16 Remove from the magnetic stand.
- ☐17 Repeat steps 9–16 for a total of 2 washes.
- ☐18 Mix 9.5 µl EE1 and 0.5 µl HP3, and then vortex.
- ☐19 Add 10 µl elution premix.
- ☐20 Centrifuge at 280 × g for 10 seconds.
- ☐21 Shake at 1800 rpm for 2 minutes.
- ☐22 Incubate at room temperature for 2 minutes.
- ☐23 Centrifuge at 280 × g for 1 minute.
- ☐24 Place on a magnetic stand until liquid is clear.
- ☐25 Transfer 9 µl supernatant to the RAH2 plate.
- ☐26 Add 1.7 µl ET2.
- ☐27 Shake at 1200 rpm for 1 minute.
- ☐28 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Perform Second Hybridization

- ☐1 Add the following.
  - CT3 (12.5 µl)
  - RFO (2.5 µl)
- ☐2 Shake at 1200 rpm for 1 minute.
- ☐3 Centrifuge at 280 × g for 1 minute.
- ☐4 Place on the thermal cycler and run the RNA HYB program.

Perform Second Capture

- ☐1 Centrifuge RAH2 at 280 × g for 1 minute.
- ☐2 Add 62.5 µl SMB.
- ☐3 Shake at 1200 rpm for 5 minutes.
- ☐4 Incubate at room temperature for 25 minutes.
- ☐5 Centrifuge at 280 × g for 1 minute.
- ☐6 Place on a magnetic stand until liquid is clear.
- ☐7 Remove and discard all supernatant.
- ☐8 Remove from the magnetic stand.
- ☐9 Add 50 µl EEW.
- ☐10 Centrifuge at 280 × g for 10 seconds.
- ☐11 Pipette to mix.
- ☐12 Shake at 1800 rpm for 4 minutes.
- ☐13 Place on the thermal cycler and start the program RNA BIND.
- ☐14 Place on a magnetic stand until liquid is clear.
- ☐15 Remove and discard all supernatant.
- ☐16 Remove from the magnetic stand.
- ☐17 Repeat steps 9–16 for a total of 2 washes.
- ☐18 Mix 9.5 µl EE1 and 0.5 µl HP3, and then vortex.
- ☐19 Add 10 µl elution premix.
- ☐20 Centrifuge at 280 × g for 10 seconds.
- ☐21 Shake at 1800 rpm for 2 minutes.
- ☐22 Incubate at room temperature for 2 minutes.
- ☐23 Centrifuge at 280 × g for 1 minute.
- ☐24 Place on a magnetic stand until liquid is clear.
- ☐25 Transfer 9 µl supernatant to the RAW1 plate.
- ☐26 Add 1.7 µl ET2.
- ☐27 Shake at 1200 rpm for 1 minute.
- ☐28 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

- ☐1 Add 20 µl AMPure XP Beads.
- ☐2 Shake at 1800 rpm for 2 minutes.
- ☐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 27.5 µl 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 2 minutes.
- ☐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 PCR2 plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Perform Second PCR Amplification

- ☐1 Add 5 µl PPC.
- ☐2 Add 20 µl EPM.
- ☐3 Pipette to mix.
- ☐4 Centrifuge at 280 × g for 1 minute.
- ☐5 Place 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 the PCR2 plate at 280 × g for 1 minute.
- ☐2 Add 90 µl AMPure XP Beads to the RAC2 plate.
- ☐3 Transfer 50 µl to the plate.
- ☐4 Shake RAC2 at 1800 rpm for 2 minutes.
- ☐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 140 µl 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 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 supernatant to the RAL plate.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Check Enriched Libraries

- ☐1 Quantify the libraries.
- ☐2 If using a Standard Sensitivity NGS Fragment Analysis Kit, run 2 µl of the postenriched library.
- ☐3 If using a DNA 1000 Chip, run 1 µl of the postenriched library.
- ☐4 Check the size and purity of the sample. Expect the final product to be a band at ~250–300 bp.
- ☐5 Check the size of the library for a distribution of DNA fragments with a size range from ~200 bp–1 kb.
- ☐6 Denature and dilute pooled libraries to the loading concentration for the instrument you are using. For loading recommendations, see the TruSight RNA Fusion Panel support page.

Acronyms

Acronym	Definition
ALP	Adapter Ligation Plate
ATL	A-Tailing Mix
CAP	Clean Up ALP Plate
CCP	cDNA Clean Up Plate
CPP	Clean Up PCR Plate
CT3	Capture Target Buffer 3
DFP	Depleted RNA Fragmentation Plate
EE1	Enrichment Elution Buffer 1
EEW	Enhanced Elution Wash Buffer
EPH	Elute, Prime, Fragment High Mix
EPM	Enhanced PCR Mix
ET2	Elute Target Buffer 2
FSA	First Strand Synthesis Act D Mix
HP3	2N NaOH
LIG	Ligation Mix
PCR	Polymerase Chain Reaction Plate
PMM	PCR Master Mix
PPC	PCR Primer Cocktail
RAA	RNA Access Amplification Plate
RAC1	RNA Access Clean Up Plate 1
RAC2	RNA Access Clean Up Plate 2
RAH1	RNA Access Hyb Plate 1
RAH2	RNA Access Hyb Plate 2
RAL	RNA Access Library Plate
RAW1	RNA Access Wash Plate 1
RFO	RNA Fusion Oligos
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SMM	Second Strand Marking Master Mix
STL	Stop Ligation Buffer
TSP	Target Sample Plate