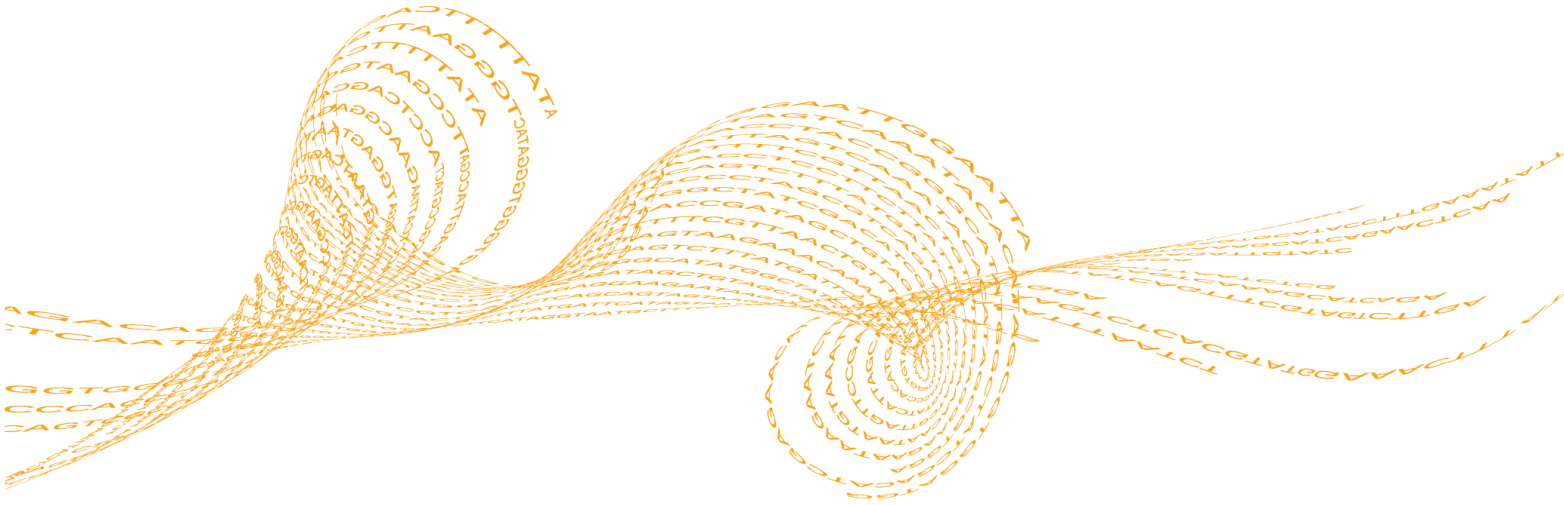
TruSight RNA Pan-Cancer Panel

Protocol Guide

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



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Fragment RNA

Preparation

1 Save the following Elution 2-Frag-Prime program on the thermal cycler.
   - Choose the preheat lid option and set to 100°C
   - 94°C for 8 minutes
   - Hold at 4°C
   - Each well contains 17 µl

2 Set the centrifuge to 15°C to 25°C.

Procedure

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 Apply the seal and centrifuge at 280 × g for 1 minute.

WARNING

If starting with FFPE RNA, do not perform the following incubation procedure. Proceed immediately to *Synthesize First Strand cDNA* on page 4.

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

WARNING

FSA contains Actinomycin D, a toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. See the safety data sheet (SDS) for environmental, health, and safety information. For more information, see *Technical Assistance* on page 23.

Preparation

1 Save the following Synthesize 1st Strand program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 25°C for 10 minutes
   - 42°C for 15 minutes
   - 70°C for 15 minutes
   - Hold at 4°C
   - Each well contains 25 µl

Procedure

1 Add 50 µl Protoscript II to FSA. Pipette or invert to mix. Then apply the seal and 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

Preparation

1 Save the following Synthesize 2nd Strand program on the thermal cycler:
- Choose the preheat lid option and set to 30°C
- 16°C for 30 minutes
- Hold at 4°C
- Each well contains 50 µl

Procedure

1 Add 5 µl RSB.

2 Add 20 µl SMM.

3 Pipette to mix.

4 Apply the seal and 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 Apply the seal and 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 and wait until the liquid is clear (~5 minutes).

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 on the magnetic stand for 5 minutes.

17 Remove from the magnetic stand.

18 Add 20 µl RSB.

19 Apply the seal and 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 and wait until the liquid is clear (~5 minutes).

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

Preparation

1 Save the following ATAIL70 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 37°C for 30 minutes
   - 70°C for 5 minutes
   - Hold at 4°C
   - Each well contains 30 µl

Procedure

1 Add 12.5 µl ATL.

2 Pipette to mix.

3 Apply the seal and 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

Preparation

1 Save the following LIG30 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 30°C for 10 minutes
- Hold at 4°C
- Each well contains 37.5 µl

Procedure

1 Add the following reagents in the order listed.
- RSB (2.5 µl)
- LIG (2.5 µl)
- RNA adapters (2.5 µl)

2 Pipette to mix.

3 Apply the seal and 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 Apply the seal and centrifuge at 280 × g for 1 minute.

8 Add 42 µl AMPure XP Beads to each well of CAP.

9 Transfer entire volume (42 µl) from ALP plate to CAP.

10 Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).

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 on the magnetic stand for 5 minutes.

18 Remove from the magnetic stand.

19 Add 22.5 µl RSB.

20 Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).

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

Preparation

1 Save the following PMM AMP program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 98°C for 30 seconds
- 15 cycles of:
  - 98°C or 10 seconds
  - 60°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 4°C
- Each well contains 50 µl

Procedure

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 Apply the seal and centrifuge PCR plate at 280 × g for 1 minute.

7 Transfer the entire volume (50 µl) to the PPP plate.

8 Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).

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 on the magnetic stand for 5 minutes.

16 Remove from the magnetic stand.

17 Add 12.5 µl RSB.

18 Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).

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

Quantify Library

1. If using a Standard Sensitivity NGS Fragment Analysis Kit on an Advanced Analytical Fragment Analyzer, run 2 µl undiluted DNA library.
2. If using a DNA 1000 chip on an Agilent Technologies 2100 Bioanalyzer, 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

Preparation

1. Save the RNA HYB program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 95°C for 10 minutes
   - 18 cycles of 1 minute each, starting at 94°C, then decreasing 2°C per cycle
   - 58°C for 90 minutes
   - Hold at 58°C

Procedure

1. Dilute 200 ng of each library in 10 µl RSB.
2. Add the following items in the order listed to the RAH1 plate for a final volume of 25 µl.
   - 200 ng library (in 10 µl RSB)
   - CT3 (12.5 µl)
   - RPO (2.5 µl)
3. Apply the seal and 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. Each well contains 25 µl.

Capture Hybridized Probes

Preparation

1 Save the following RNA BIND program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 50°C for 20 minutes
- Hold at 50°C

Procedure

1 Centrifuge RAH1 at 280 × g for 1 minute.

2 Add 62.5 µl SMB.

3 Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).

7 Remove and discard all supernatant.

8 Remove from the magnetic stand.

9 Add 50 µl EEW.

10 Apply the seal and centrifuge at 280 × g for 10 seconds.

11 Pipette to mix.

12 Apply the seal and shake at 1800 rpm for 4 minutes.

13 Place on the thermal cycler and start the program RNA BIND. Each well contains 52.5 µl.

14 After 20 minutes, immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).

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 Apply the seal and 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 and wait until the liquid is clear (~2 minutes).

25 Transfer 9 µl supernatant to the RAH2 plate.

26 Add 1.7 µl ET2.

27 Apply the seal and 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

Procedure

1. Add the following reagents in the order listed.
   - CT3 (12.5 µl)
   - RPO (2.5 µl)
2. Apply the seal and 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. Each well contains 25.7 µl.

Perform Second Capture

Preparation

Procedure

1 Centrifuge RAH2 at 280 × g for 1 minute.

2 Add 62.5 µl SMB.

3 Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).

7 Remove and discard all supernatant.

8 Remove from the magnetic stand.

9 Add 50 µl EEW.

10 Apply the seal and centrifuge at 280 × g for 10 seconds.

11 Pipette to mix.

12 Apply the seal and shake at 1800 rpm for 4 minutes.

13 Place on the thermal cycler and start the program RNA BIND. Each well contains 53.2 µl.

14 After 20 minutes, immediately place on a magnetic stand and wait until the liquid is clear (~2 minutes).

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 Apply the seal and 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 and wait until the liquid is clear (~2 minutes).

25 Transfer 9 µl supernatant to the RAW1 plate.

26 Add 1.7 µl ET2.

27 Apply the seal and shake at 1200 rpm for 1 minute.

28 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

Preparation

Procedure

1. Add 20 µl AMPure XP Beads.
2. Apply the seal and 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 and wait until the liquid is clear (~2 minutes).
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 on the magnetic stand for 5 minutes.
10. Remove from the magnetic stand.
11. Add 27.5 µl RSB.
12. Apply the seal and 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 and wait until the liquid is clear (~2 minutes).
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

Preparation

1. Save the following EPM AMP program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 98°C for 30 seconds
   - 14 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 10°C
   - Each well contains 50 µl

Procedure

1. Add 5 µl PPC.
2. Add 20 µl EPM.
3. Pipette to mix.
4. Apply the seal and 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

Preparation

Procedure

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 from the PCR2 plate to the RAC2 plate.
4. Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).
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. Apply the seal and 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 and wait until the liquid is clear (2–5 minutes).
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

Quantify Libraries

1. Quantify the libraries using qPCR according to the Illumina *Sequencing Library qPCR Quantification Guide (document # 11322363).*

Check Library Quality

1. If using a Standard Sensitivity NGS Fragment Analysis Kit on an Advanced Analytical Fragment Analyzer, run 2 µl of the postenriched library.
2. If using a DNA 1000 Chip, run 1 µl of the postenriched library.
3. Check the size and purity of the sample. Expect the final product to be a band at ~250–300 bp.
4. Check the size of the library for a distribution of DNA fragments with a size range from ~200 bp–1 kb.
5. Denature and dilute pooled libraries to the loading concentration for the instrument you are using. For loading recommendations, see the TruSight RNA Pan-Cancer 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 Enrichment 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
RPO	RNA PanCancer Oligos
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SMM	Second Strand Marking Master Mix
STL	Stop Ligation Buffer
TSP	Target Sample Plate

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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