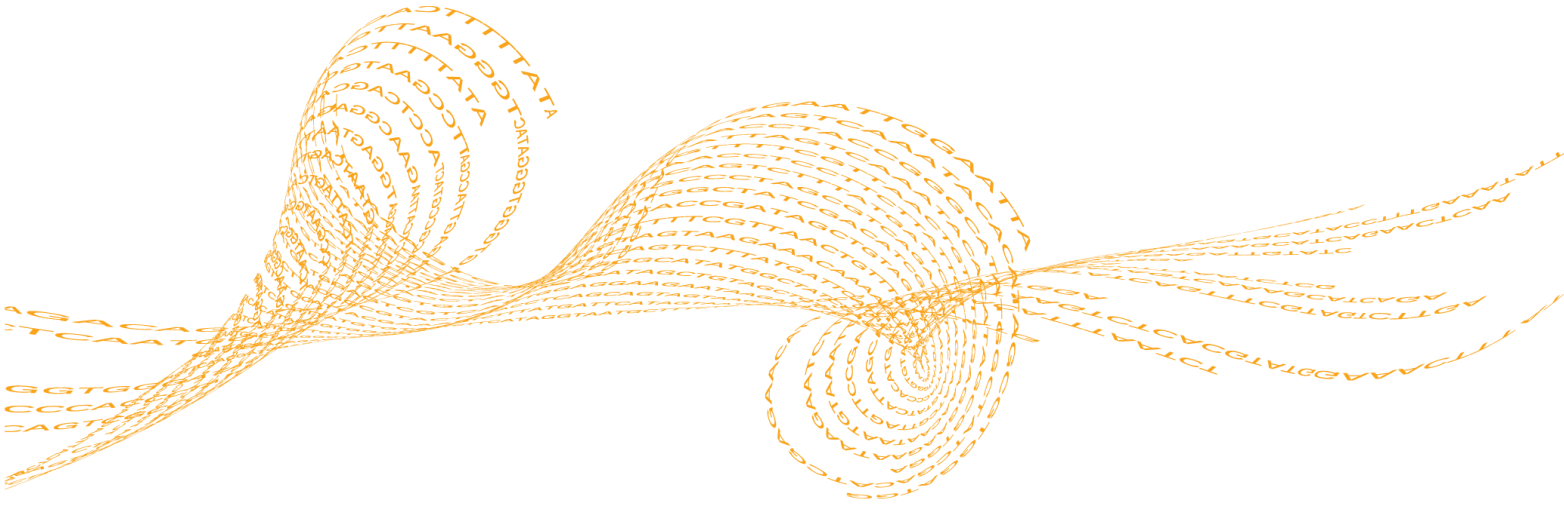
TruSeq Targeted RNA Expression

Protocol Guide

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



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Synthesize cDNA

Preparation

1. Save 1 of the following programs on a thermal cycler:
   - For intact total RNA, save the CDNASYN1 program:
     - Choose the preheat lid option and set to 100°C
     - 25°C for 5 minutes
     - 42°C for 15 minutes
     - 95°C for 10 minutes
     - Hold at 4°C
   - For degraded RNA or both RNA input types, save the CDNASYN2 program:
     - Choose the preheat lid option and set to 100°C
     - 25°C for 10 minutes
     - 42°C for 30 minutes
     - 95°C for 10 minutes
     - Hold at 4°C
2. Label a new 96-well HSP plate according to the input RNA:
   - CDP1 for intact total RNA.
   - CPD for degraded RNA.

Procedure

1. Vortex RCS1 for 5 seconds.
2. Centrifuge at 600 × g for 5 seconds.
3. Dilute according to your input RNA:
   - Dilute 50 ng intact total RNA with nuclease-free water to 5 µl.
   - Dilute ≥ 200 ng degraded RNA with nuclease-free water to 3 µl.
4. Add diluted RNA to the appropriate plate:
   - Add 5 µl diluted intact total RNA to the CDP1 plate.
   - Add 3 µl diluted degraded RNA to the CDP plate.
5. Combine the following volumes in a new 1.7 ml microcentrifuge tube. Multiply each volume by the number of samples being prepared.

Reagent	Intact Total RNA Volume (µl)	Degraded RNA Volume (µl)
RCS1	4.4	4.4
ProtoScript II Reverse Transcriptase	1.1	2.2
10X DTT (0.1M)*	0	1.1
Total volume per pool	5.5	7.7

* Included with ProtoScript II Reverse Transcriptase reagent.

6. Invert to mix.
7. Centrifuge at 600 × g for 5 seconds.
8. Distribute evenly into an 8-tube strip.

9 Add the volume appropriate for your plate:
   - Add 5 µl to the CDP1 plate.
   - Add 7 µl to the CDP plate.

10 Shake at 1600 rpm for 20 seconds.

11 Centrifuge at 280 × g for 1 minute.

12 Place on the thermal cycler and run the CDNASYN1 or CDNASYN2 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

Hybridize Oligo Pool

Preparation

1. Save the following program as ANNEAL on a thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 70°C for 5 minutes
   - 68°C for 1 minute
   - 65°C for 2.5 minutes
   - 60°C for 2.5 minutes
   - 55°C for 4 minutes
   - 50°C for 4 minutes
   - 45°C for 4 minutes
   - 40°C for 4 minutes
   - 35°C for 4 minutes
   - 30°C for 4 minutes
   - Hold at 30°C
2. Unseal the CDP or CDP1 plate.

Procedure

1. Combine the following volumes in a new 1.7 ml microcentrifuge tube. Multiply each volume by the number of reactions being prepared.

Reagent	Volume (µl)
TOP	5.5
Additional TOP or TE buffer	5.5
Total volume per reaction	11

2. Vortex for 5 seconds.
3. Centrifuge at 600 × g for 5 seconds.
4. Distribute into an 8-tube strip.
5. Add 10 µl to the CDP or CDP1 plate.
6. Shake at 1600 rpm for 20 seconds.
7. Incubate at room temperature for 1 minute.
8. Vortex OB1 for 5 seconds.
9. Add 30 µl OB1 to the CDP or CDP1 plate.
10. Shake at 1600 rpm for 1 minute.
11. Place on the thermal cycler and run the ANNEAL program.
12. Centrifuge briefly.

Wash, Extend, and Ligate Bound Oligos

Preparation

1. Place the midi-plate insert inside the microheating system and preheat to 37°C.
2. Label a new 96-well midi plate HYP.
3. Unseal the CDP or CDP1 plate.

Procedure

1. Transfer all supernatant to the HYP plate.
2. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
3. Remove and discard all of the supernatant.
4. Move from the magnetic stand to a bench.
5. Add 100 µl AM1 to each well to resuspend the pellet.
6. Shake at 1800 rpm for 2 minutes.
7. Centrifuge at 280 × g for 5 seconds.
8. Unseal and place on a magnetic stand until the liquid is clear (~2 minutes).
9. Remove and discard all supernatant.
10. Move from the magnetic stand to a bench.
11. Add 175 µl UB1.
12. Shake at 1800 rpm for 2 minutes.
13. Centrifuge at 280 × g for 5 seconds.
14. Unseal and place on a magnetic stand until the liquid is clear (~2 minutes).
15. Invert ELM4 to mix.
16. Remove and discard all supernatant.
17. Move from the magnetic stand to a bench.
18. Add 40 of ELM4.
19. Shake at 1800 rpm for 2 minutes.
20. Centrifuge at 280 × g for 5 seconds.
21. Place on the 37°C preheated microheating system and incubate for 45 minutes.
22. Remove the adhesive seal from the plate.
23. Unseal and place on a magnetic stand until the liquid is clear (~2 minutes).
24. Remove and discard all supernatant.
25. Add 50 µl of UB1.

Amplify Libraries

Preparation

1 Label a new 96-well HSP plate IAP.

2 Determine the required number (X) of PCR cycles using the following table:

RNA Input	Number of PCR Cycles (X)	
	High Quality (RIN ≥ 8.0)	Degraded (RIN < 8.0)
12–48 amplicons	32–36	34–38
49–96 amplicons	30–32	32–34
97–200 amplicons	27–30	30–32
201–384 amplicons	25–27	28–30
385–1000 amplicons	23–25	26–28

3 Save the following program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 95°C for 2 minutes
- X cycles of:
  - 98°C for 30 seconds
  - 62°C for 30 seconds
  - 72°C for 60 seconds
- 72°C for 5 minutes
- Hold at 10°C

Procedure

1 Arrange Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.

2 Arrange Index 2 (i5) adapters in rows A–H of the TruSeq Index Plate Fixture.

3 Place the plate on the TruSeq Index Plate Fixture.

4 Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.

5 Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.

6 Remove and discard all supernatant from the HYP plate.

7 Remove from the magnetic stand.

8 Add 22.5 µl diluted HP3.

9 Shake at 1800 rpm for 30 seconds.

10 Incubate at room temperature for at least 5 minutes.

11 Create the amplification mix:
- **96 libraries**—Add 56 µl TDP1 to 2.8 ml of PMM2.
- **48 libraries**—Combine 28 µl TDP1 and 1.4 ml PMM2 in a new 1.7 ml microcentrifuge tube.
- **16 libraries**—Combine 9.2 µl TDP1 and 460 µl PMM2 in a new 1.7 ml microcentrifuge tube.

12 Invert to mix.

13 Add 22 µl to the IAP plate.

14 Unseal the HYP plate.

15 Place on a magnetic stand until the liquid is clear (~2 minutes).

16 Transfer 20 µl supernatant from the HYP plate to the IAP plate.

17 Shake at 1600 rpm for 30 seconds.

18 Centrifuge at 280 × g for 1 minute.

19 Place on the thermal cycler and run the program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

Preparation

1. Prepare fresh 80% EtOH from absolute ethanol.
2. Vortex the AMPure XP Beads.
3. Label a new 96-well HSP plate LNP.
4. Label a new 96-well midi plate CLP.

Procedure

1. Add 85 µl AMPure XP Beads to the CLP plate.
2. Centrifuge the IAP plate at 280 × g for 1 minute.
3. Unseal the IAP plate.
4. Transfer all supernatant to the CLP plate.
5. Shake at 1800 rpm for 2 minutes.
6. Centrifuge the plate at 280 × g for 5 seconds.
7. Incubate room temperature for 15 minutes
8. Unseal and place on a magnetic stand until the liquid is clear (~5 minutes).
9. Remove and discard 135 µl supernatant.
10. Wash 2 times with 200 µl 80% EtOH.
11. Air dry on the magnetic stand for 15 minutes.
12. Remove from the magnetic stand.
13. Add 15 µl RSB.
14. Shake at 1800 rpm for 2 minutes.
15. Centrifuge at 280 × g for 5 seconds.
16. Return RSB to 2°C to 8°C storage.
17. Incubate the CLP plate at room temperature for 2 minutes.
18. Unseal and place on a magnetic stand until the liquid is clear (~5 minutes).
19. Transfer 12.5 µl supernatant to the LNP plate.

SAFE STOPPING POINT

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

Pool and Quantify Libraries

Procedure

1. Transfer 5 µl from the LNP plate to a new 2 ml microcentrifuge tube.
2. Vortex for 5 seconds.
3. Centrifuge at 600 × g for 5 seconds.
4. Load 1 µl pooled library onto the Standard Sensitivity NGS Fragment Analysis Kit or DNA 1000 Kit.
5. Determine the concentration of the pooled library.
6. Select the **Region Analysis** tab.
7. Drag the blue region lines to capture the 100–300 bp region.
8. Dilute each pooled library to 4 nM using RSB.
9. Denature and dilute the 4 nM library to the concentration for the sequencing instrument you are using. See the denature and dilute guide for your instrument.

SAFE STOPPING POINT

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

Acronyms

Acronym	Definition
A50X	Adapter Index
AM1	Wash 1 Buffer
CDP	cDNA Plate
CDP1	cDNA Plate 1
CLP	Clean Up Plate
cSNP	Coding SNP
DLSO	Downstream Locus-Specific Oligo
DV	Fragment distribution value
ELM4	Extension and Ligation Mix 4
EUC	Experienced User Card
FFPE	Formalin-fixed, paraffin-embedded
HP3	2N NaOH
HYP	Hybridization Plate
IAP	Index Adapter Plate
LNP	Library Normalization Plate
OB1	Paramagnetic Streptavidin Beads
P5	Flow cell binding site
P7	Flow cell binding site
PCR	Polymerase Chain Reaction
PMM2	PCR Master Mix 2
R7XX	Adapter Index
RCS1	Reverse Transcription cDNA Synthesis Master Mix 1
RIN	RNA Integrity Number
RQN	RNA Quality Number
RSB	Resuspension Buffer
SBS3	Read 2 sequencing primer binding site
smRNA	Small RNA sequencing primer binding site
TDP1	TruSeq DNA Polymerase 1

Acronym	Definition
TOP	Targeted Oligo Pool
UB1	Wash 2 Buffer
ULSO	Upstream Locus-Specific Oligo

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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