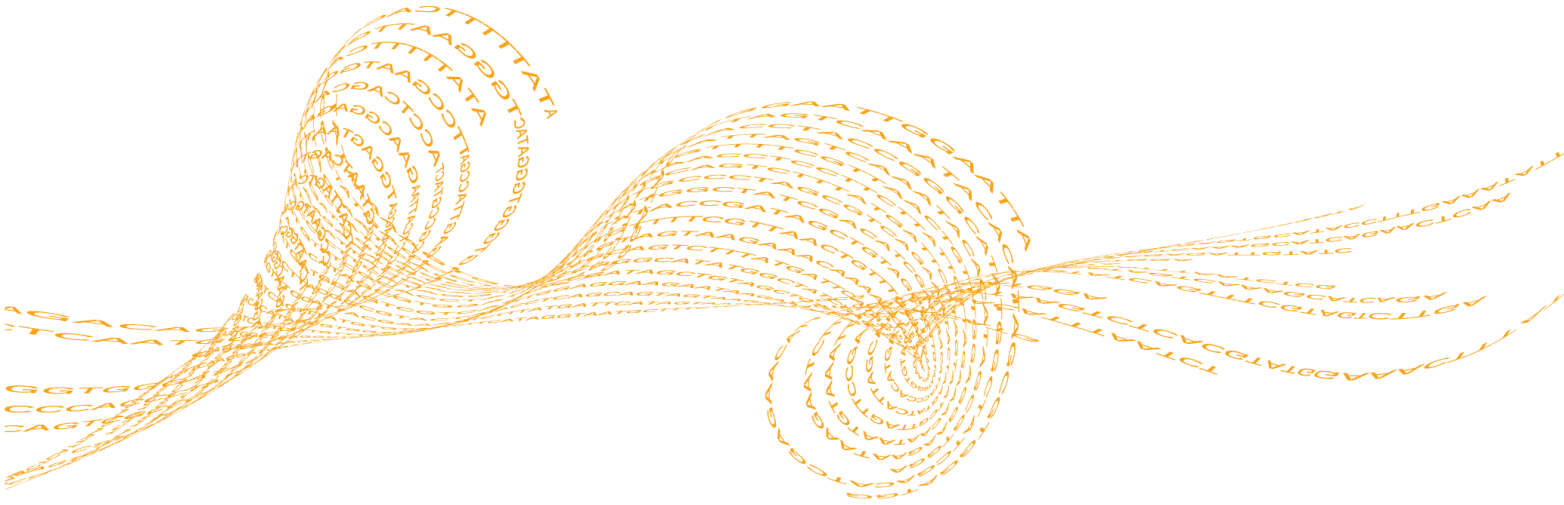
TruSeq Small RNA Library Prep

Protocol Guide

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



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Ligate Adapters

Preparation

1. Preheat a thermal cycler to 70°C.
2. Choose the thermal cycler preheat lid option and set to 100°C.

Procedure

1. Combine the following volumes in a new 200 µl PCR tube on ice:
   - RA3 (1 µl)
   - 1 µg total RNA in nuclease-free water (5 µl)
2. Pipette to mix, and then centrifuge briefly.
3. Place on the preheated thermal cycler.
4. Incubate at 70°C for 2 minutes.
5. Remove from the thermal cycler and place on ice.
6. Preheat the thermal cycler to 28°C.
7. Combine the following volumes in a new 200 µl PCR tube on ice. Multiply each volume by the number of samples being prepared. Make 10% extra reagent if you are preparing multiple samples.
   - HML (2 µl)
   - RNase Inhibitor (1 µl)
   - T4 RNA Ligase 2, Deletion Mutant (1 µl)
8. Pipette to mix, and then centrifuge briefly.
9. Add 4 µl to the tube of RA3/total RNA mixture.
10. Pipette to mix.
11. Place on the preheated thermal cycler.
12. Incubate at 28°C for 1 hour.
13. Add 1 µl STP and pipette to mix.
14. Continue incubating at 28°C for 15 minutes.
15. Remove from the thermal cycler and place on ice.
16. Preheat the thermal cycler to 70°C.
17. Add 1.1 × N µl RA5 to a new 200 µl PCR tube.
18. Place on the preheated thermal cycler.
19. Incubate at 70°C for 2 minutes.
20. Remove from the thermal cycler and place on ice.
21. Preheat the thermal cycler to 28°C.
22. Add 1.1 × N µl 10mM ATP to the tube of RA5.
23. Pipette to mix.
24. Add 1.1 × N µl T4 RNA Ligase to the RA5/ATP mixture.

25 Pipette to mix.

26 Add 3 µl to the tube of RA3 mixture.

27 Pipette to mix.

28 Place on the preheated thermal cycler.

29 Incubate at 28°C for 1 hour.

30 Remove from the thermal cycler and place on ice.

Reverse Transcribe and Amplify Libraries

Preparation

1. Preheat the thermal cycler to 70°C.
2. Choose the thermal cycler preheat lid option and set to 100°C.
3. Label a new 200 µl PCR tube 12.5 mM dNTP Mix.

Procedure

1. Combine the following volumes in the 12.5 mM dNTP Mix tube to dilute to 12.5 mM. Multiply each volume by the number of samples being prepared. Prepare 10% extra reagent if you are preparing multiple libraries.
   - 25 mM dNTP Mix (0.5 µl)
   - Ultrapure water (0.5 µl)
2. Pipette to mix, and then centrifuge briefly.
3. Set aside on ice.
4. Add 6 µl each adapter-ligated RNA library to a new 200 µl PCR tube.
5. Add 1 µl RNA RT Primer to the tube of adapter-ligated RNA.
6. Pipette to mix, and then centrifuge briefly.
7. Place on the preheated thermal cycler.
8. Incubate at 70°C for 2 minutes.
9. Remove from the thermal cycler and place on ice.
10. Preheat the thermal cycler to 50°C.
11. Combine the following volumes in a new 200 µl PCR tube on ice. Multiply each volume by the number of libraries being prepared. Make 10% extra reagent if you are preparing multiple libraries.
    - 5X First Strand Buffer (2 µl)
    - 12.5 mM dNTP Mix (0.5 µl)
    - 100 mM DTT (1 µl)
    - RNase Inhibitor (1 µl)
    - SuperScript II Reverse Transcriptase (1 µl)
12. Pipette to mix, and then centrifuge briefly.
13. Add 5.5 µl to the tube of adapter-ligated RNA/primer mix.
14. Pipette to mix, and then centrifuge briefly.
15. Place on the preheated thermal cycler.
16. Incubate at 50°C for 1 hour.
17. Remove from the thermal cycler and place on ice.
18. Combine the following reagents in a new 200 µl PCR tube on ice to prepare the PCR master mix. Multiply each volume by the number of libraries being prepared. Make 10% extra reagent if you are preparing multiple libraries with the same index.
    - Ultrapure water (8.5 µl)
    - PML (25 µl)

- RP1 (2 µl)
- RPIX (2 µl)

19 Pipette to mix, and then centrifuge briefly.

20 Place on ice.

21 Add 37.5 µl PCR master mix to the adapter-ligated RNA mixture.

22 Pipette to mix, and then centrifuge briefly.

23 Place on ice.

24 Place on the preheated thermal cycler.

25 Incubate using the following program on the thermal cycler:
- Choose the preheat lid option and set to 100°C.
- 98°C for 30 seconds
- 11 cycles of:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 15 seconds
- 72°C for 10 minutes
- 4°C hold

26 Run each library on a High Sensitivity DNA chip.

SAFE STOPPING POINT

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

Purify cDNA Construct

Preparation

1. [Optional] Label a new 200 µl PCR tube 0.1X Pellet Paint.
2. Determine the volume of 1X TBE Buffer needed for gel electrophoresis. Dilute the 5X Novex TBE Buffer to 1X.
3. Place 6% Novex TBE gel into the gel electrophoresis unit per manufacturer instructions.

Procedure

1. Combine the following volumes in the 0.1X Pellet Paint tube. Multiply each volume by the number of libraries being prepared. Make 10% extra reagent if you are preparing multiple libraries.
   - 1X Pellet Paint NF Co-Precipitant (0.2 µl)
   - Ultrapure water (1.8 µl)
2. Pipette to mix, and then centrifuge briefly.
3. Combine 2 µl CRL and 2 µl DNA loading dye in a new 1.5 ml microcentrifuge tube.
4. Pipette to mix.
5. Combine 1 µl HRL and 1 µl DNA loading dye in a new 1.5 ml microcentrifuge tube.
6. Pipette to mix.
7. Combine all amplified cDNA construct (typically 48–50 µl) and 10 µl DNA Loading Dye in a new 1.5 ml microcentrifuge tube.
8. Pipette to mix.
9. Load 2 gel lanes with 2 µl CRL/loading dye mixture.
10. Load 1 gel lane with 2 µl HRL/loading dye mixture.
11. Load 2 gel lanes with 25 µl each of amplified cDNA construct/loading dye mixture.
12. Run the gel for 60 minutes at 145 V or until the blue front dye leaves the gel.
13. Remove the gel from the unit.
14. Open the cassette according to manufacturer instructions and stain the gel with ethidium bromide in a clean container for 2–3 minutes.
15. Place the gel breaker tube into a 2 ml microcentrifuge tube.
16. View the gel on a Dark Reader transilluminator or a UV transilluminator.
17. Using a razor blade, cut out the bands from the 2 lanes that correspond to the adapter-ligated constructs derived from the 22 nt and 30 nt small RNA fragments.
18. Place the band into the 0.5 ml gel breaker tube.
19. Centrifuge the nested tubes at 20,000 × g for 2 minutes to move the gel through the holes into the 2 ml tube.
20. If you are concentrating the final library, skip the remaining steps and proceed to *Add 300 µl ultrapure water to the gel debris in the 2 ml tube.* on page 8.
21. Add 200 µl ultrapure water to the gel debris.

22 Rotate for at least 2 hours to elute the DNA.

23 Transfer the eluate and gel debris to the top of a 5 µm filter.

24 Centrifuge at 10 seconds at 600 × g.

25 Add 300 µl ultrapure water to the gel debris in the 2 ml tube.

26 Rotate for at least 2 hours to elute the DNA.

27 Transfer the eluate and gel debris to the top of a 5 µm filter.

28 Centrifuge at 600 × g for 10 seconds, and then discard the filter.

29 Add the following volumes to the eluate:
- Glycogen (2 µl)
- 3M NaOAc (30 µl)
- [Optional] 0.1X Pellet Paint (2 µl)
- 100% ethanol (975 µl)

30 Centrifuge at 20,000 × g at 20 minutes at 4°C.

31 Remove and discard the supernatant. Leave the pellet intact.

32 If the pellet becomes loose, centrifuge at 20,000 × g for 2 minutes.

33 Wash the pellet with 500 µl 70% ethanol.

34 Centrifuge at 20,000 × g for 2 minutes.

35 Remove and discard the supernatant. Leave the pellet intact.

36 With the lid open, place the tube in a 37°C heat block until the pellet is dry (~7 minutes).

37 Resuspend the pellet in 10 µl 10 mM Tris-HC1, pH 8.5.

Check Libraries

1. Load 1 µl resuspended construct on an Agilent Technologies 2100 Bioanalyzer using a DNA-specific chip, such as the DNA 1000 or High Sensitivity DNA.
2. Check the size, purity, and concentration of the library.

Normalize Libraries

Procedure

1. Normalize library concentration to 2 nM using Tris-HCl 10 mM, pH 8.5.
2. For storage, add Tween 20 to the library for a final concentration of 0.1% Tween 20.

SAFE STOPPING POINT

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

Acronyms

Acronym	Definition
cDNA	Complementary DNA
CRL	Custom RNA Ladder
HML	Ligation Buffer
HRL	High Resolution Ladder
PCR	Polymerase Chain Reaction
PML	PCR Mix
RA3	RNA 3' Adapter
RA5	RNA 5' Adapter
RIN	RNA Integrity Number
RP1	RNA PCR Primer
RPI	RNA PCR Primer Index
RTP	RNA RT Primer
STP	Stop Solution
UHR	Universal Human Reference

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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