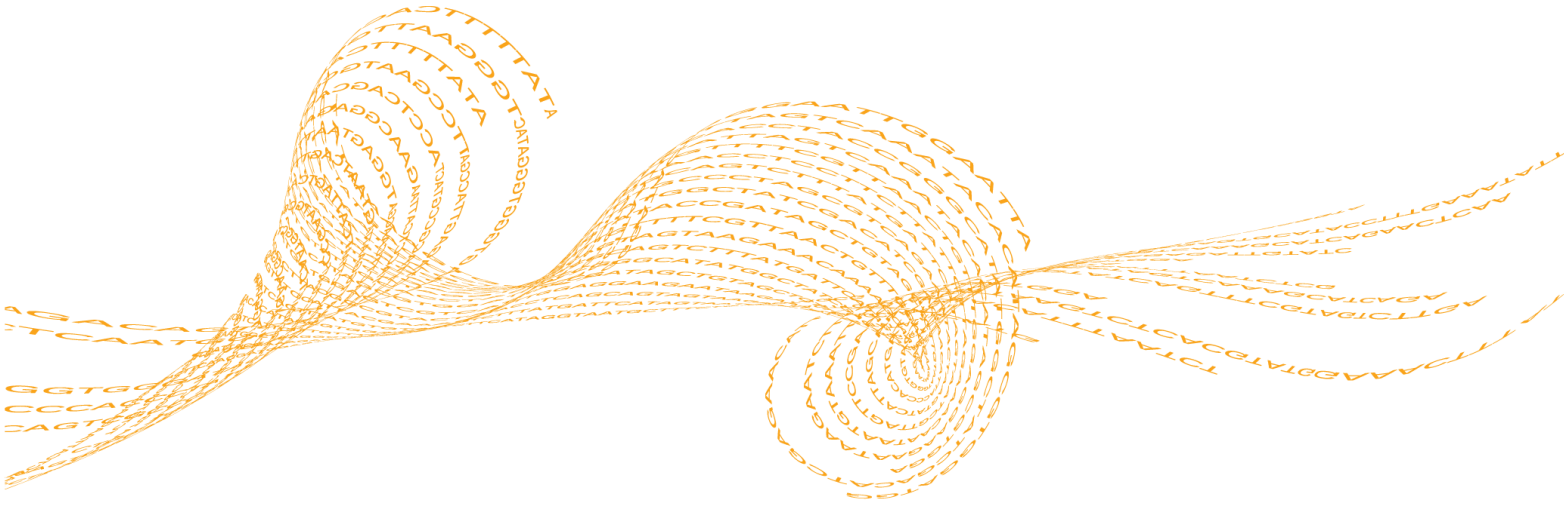
TruSeq Stranded mRNA Library Prep for NeoPrep

Protocol Guide

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



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Prepare Samples for Loading

Preparation

1 Save the following mRNA Denaturation program on a thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 65°C for 5 minutes
   - 25°C for 5 minutes
   - 25°C hold

Procedure

1 Dilute 25–100 ng total RNA with nuclease-free ultrapure water to a final volume of 12.5 µl in each well of a new PCR plate. Pipette to mix. Do not vortex.

2 Vortex RPB2 to resuspend.

3 Add 12.5 µl RPB2 to each well. Pipette to mix.

4 Place on the thermal cycler and run the mRNA Denaturation program.

Set Up Run and Load Library Card

Procedure

1. Vortex the reagent plate for 3 seconds.
2. Centrifuge at 600 × g for 5 seconds.
3. Select **Prepare Libraries** on the Library Prep Instrument Welcome screen.
4. Do the following and then select **Next**.
   - If running in BaseSpace mode, select a run.
   - If running in standalone mode, use the following options to select a protocol:
     - Click **Select by barcode**, and then scan the reagent plate barcode or enter the reagent plate serial number.
     - Click **Select by name**, and then select **TruSeq Stranded mRNA**.
5. Configure the run. Select **Next**.
6. Review the run and sample information. Select **Next**.
7. Enter the consumable tracking information. Select **Next**.
8. Place the library card on the library card stage.

   WARNING
   To avoid instrument damage, make sure that the library card guide is not on the library card.
9. Close the library card compartment door. Select **Verify Library Card**.
10. Place the library card guide on the library card.
11. Load the entire contents of the oil vial into the library card using the oil funnel.

    WARNING
    Use the required pipette tips. Other tips are not supported and can result in reagents not dispensing properly and run failure.

    The loading angle of the pipette depends on the item being dispensed. The angle is specified in each step of the control software loading guide and is depicted in these procedures.
12. Insert pipette tips to the bottom of the wells of the prepared sample plate. Pipette up and down 1 time to mix.
13. Transfer 25 µl of prepared samples 1–8.
14. Transfer 25 µl of prepared samples 9–16.
15. If you are preparing < 16 samples, add 25 µl RSB to empty sample wells.
16. Transfer 125 µl of the large reagents i–iv.
17. Transfer 125 µl of the large reagents v–vii.
18. Vortex DMB until well-dispersed.
19. Add 80 µl DMB to the large reagent well viii.
20. Transfer 15 µl of small reagents 1–4, and then 5–8.
21. For small reagents 9–12:
    a. Use a clean 8-tube strip to pierce the foil on the reagent wells. Discard the 8-tube

strip.

b Transfer 15 µl of each reagent.

22 For small reagents 13–16:

a Use a clean 8-tube strip to pierce the foil on the reagent wells. Discard the 8-tube strip.

b Transfer 15 µl of each reagent.

23 Transfer 5 µl of small reagents a–d, and then e–h.

24 Transfer 3 µl of adapters A–H.

25 Transfer 3 µl of adapters I–P.

26 Remove the library card guide.

WARNING

To avoid instrument damage, make sure that the library card guide is removed from the library card.

27 Close the library card compartment door. Select **Start Run**.

28 When the run is complete, select **Next**.

Unload Libraries

WARNING
The used library card contains hazardous materials. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and a laboratory coat. Handle the used library card as chemical waste. Dispose of containers and any unused contents in accordance with the governmental safety standards for your region. For more information, see the SDS for this kit at support.illumina.com/sds.html.

Procedure

1 Add 10 µl RSB to each well of a new PCR plate labeled 1–16.

2 Open the library card compartment door and place the library card guide on the library card.

3 Use a 200 µl pipette to transfer 20 µl from library card collection wells 1L–8L, and then 9L–16L to corresponding wells 1–16 of the plate. Pipette to mix.

4 Centrifuge briefly.

5 Transfer the entire volume from plate wells 1–8, and then 9–16 to the center indent in the membrane of the corresponding library separation tubes 1–16.

6 Let stand for 10 seconds while the oil is absorbed in the tubes.

7 Transfer the entire volume from library separation tubes 1–8, and then 9–16 to the corresponding wells 1–16 of a new PCR plate.

8 Remove the library card and library card guide from the library card stage.

9 Discard the library card in accordance with applicable standards.

10 Close the library card compartment door, and then select **Home**.

11 Select from the following options:

Table 1 Post Run Options

Library Prep Instrument Quantification	Library Prep Instrument Normalization	Pooling Required	Then...
Yes	Yes	No	The protocol stops here. The final library is normalized to 10 nM. Proceed to cluster generation.
Yes	Yes	Yes	Proceed to *Pool Libraries*.
Yes	No	Yes or No	Proceed to *[Optional] Normalize Libraries Manually*.
No	No	Yes or No	Proceed to *[Optional] Check Libraries Manually*.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

[Optional] Check Libraries Manually

Procedure

1. Quantify the libraries using qPCR according to the Illumina *Sequencing Library qPCR Quantification Guide (document # 11322363)*.
2. If using a Standard Sensitivity NGS Fragment Analysis Kit on an Advanced Analytical Fragment Analyzer:
   a. Dilute the DNA library 1:1 with RSB.
   b. Run 1 µl diluted DNA library.
3. If using a DNA 1000 chip on an Agilent Technologies 2100 Bioanalyzer, run 1 µl undiluted DNA library.
4. Check the size and purity of the sample. Expect the final product to be a band at ~300 bp.

[Optional] Normalize Libraries Manually

Procedure

1. Transfer 5 µl from each well of the library plate to the corresponding wells of a midi plate.
2. Normalize each library to 10 nM with Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Pipette to mix.
3. Select from the following options:
   - For libraries that do not require pooling, the protocol stops here. Proceed to cluster generation.
   - For libraries that require pooling, proceed to *Pool Libraries*.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Pool Libraries

Procedure

1 Determine the number of samples to combine for each pool.

2 Transfer 5 µl of each library to be pooled from the library plate to a single well of a new PCR plate. Pipette to mix.

3 Proceed to cluster generation.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 2 months.

Acronyms

Acronym	Definition
DMB	Digital Microfluidics Beads
RPB2	RNA Purification Beads 2
RSB	Resuspension Buffer

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 2 Illumina General Contact Information

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

Table 3 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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