RNA sequencing library construction for Illumina GA II

Jian Cao, Julie Ni, Wenxiu Ma, Vanessa Shiu, Luis A. Milla, Sangbin Park, Maria L. Spletter, Sheng Tang, Jun Zhang, Xing Wei, Seung K. Kim, and Matthew P. Scott

Abstract

This protocol is from:
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Please see the full manuscript for additional details.

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Materials

- T4 DNA Ligase Reaction Buffer - 6.0 ml B0202S by New England Biolabs
- Antarctic Phosphatase Reaction Buffer - 6.0 ml B0289S by New England Biolabs
- T4 RNA Ligase 1 (ssRNA Ligase) - 1,000 units M0204S by New England Biolabs
- Taq DNA Polymerase with Standard Taq Buffer - 400 units M0273S by New England Biolabs
- T4 Polynucleotide Kinase - 500 units M0201S by New England Biolabs
- Antarctic Phosphatase - 1,000 units M0289S by New England Biolabs

GlycoBlue™ Coprecipitant AM9516 by Thermo Scientific

SuperScript® III First-Strand Synthesis System 18080-051 by Thermo Scientific

QIAquick PCR Purification Kit 28104 by Qiagen

Protocol

Step 1.
Fragment 400ng of amplified mRNA to 10-200nt using 10x RNA fragmentation buffer (Ambion)

Step 2.
Purify using regular ethanol precipitation method with 0.35µl of GlycoBlue (Ambion).

Step 3.
Dephosphorylate the 3’ end the RNA samples using 10x Antarctic Phosphatase Buffer and 0.5 µl Antarctic Phosphatase (NEB) at 37 °C for 20 minutes.

DURATION
00:20:00

Step 4.
Heat inactivate at 75°C for 10 minutes.

DURATION
00:10:00

Step 5.
Phosphorylate the 5’ end of RNA samples was using 10x T4 DNA ligase buffer (it has 1mM ATP final) and T4 PNK (NEB) at 37 °C for 30 minutes.

DURATION
00:30:00

Step 6.
Purify the RNAs in the reactions using ammonium acetate and ethanol precipitation with 2µl of GlycoBlue (Ambion).

Step 7.
Ligate the RNA samples at 37 °C for one hour to 3’ linker (5’-/5rApp/CTG TAG GCA CCA TCA AT/3ddC/-3’) (synthesized by IDT) using:
T4 RNA ligase 1 (NEB),
5X ATP-free T4 RNA ligase buffer (16.5 mM DTT, 41.5% glycerol, 250 mM HEPES-KOH, pH8.3, 50 mM MgCl2, 50 µg/ml acetylated BSA),
and 10% DMSO .

DURATION
01:00:00

Step 8.
Purify the RNAs in the reactions using ammonium acetate and ethanol precipitation with 2µl of GlycoBlue (Ambion).

Step 9.
Run the RNA samples on 6% TBE-Urea PAGE Gel (Invitrogen).

Step 10.
Cut 100-200nt bands and elute **overnight** with 400µl stop solution (1M ammonium acetate and 10mM EDTA) at 4°C.

DURATION
18:00:00

Step 11.
Purify the RNAs in the supernatant using regular ethanol precipitation method with 2µl of GlycoBlue (Ambion).

Step 12.
Ligate the RNA samples at 37°C for 1 hour to 5’ linker (with bar code) using:
T4 RNA ligase 1 (NEB),
10x T4 RNA ligase 1 buffer (NEB),
and 10% DMSO .

DURATION
01:00:00

Step 13.
Purify the RNAs by ammonium acetate and ethanol precipitation and gel purification as described in steps 8-11.

NOTES
Tracey DePellegrin 30 Sep 2015

The 5’ barcoded linkers are synthesized by IDT.
IPC1: 5’-/5AmMC6/ ACG CTC TTC CGA TCT rCrUrGrG-3’
IPC2: 5’-/5AmMC6/ ACG CTC TTC CGA TCT rCrGrUrC-3’
Control 1: 5’-/5AmMC6/ ACG CTC TTC CGA TCT rArCrUrU-3’
Control 2: 5’-/5AmMC6/ ACG CTC TTC CGA TCT rCrCrCrU-3’

Step 14.
Reverse transcribe cDNA of the RNA samples using SuperScript III (Invitrogen) following manufacture’s protocol. The primer sequence used for reverse transcription is 5’-ATT GAT GGT GCC TAC AG-3’.

Step 15.
Amplify the cDNA samples using Taq (NEB) following manufacture’s protocol.

NOTES
Tracey DePellegrin 30 Sep 2015
Forward primer: 5’-GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T-3’.
Reverse primer: 5’-CAA GCA GAA GAC GGC ATA CGA GCT CTT CCG ATC TAT TGA TGG TGC CTA CAG-3’.

Step 16.
Purify the PCR products (200-300nt) using Qiagen PCR purification kit.

Step 17.
Dilute the purified PCR samples to 10nM and sequence using Illumina GA II sequencing system.