Total RNA extraction from human cell lines

March 28 2019

This info sheet describes a method to extract total RNA from human cell lines (GM12878). The recommended method is based on work previously described by Workman *et al*, 2018.

Materials

- 5 x 10^7 cells
- Refrigerated centrifuge (with capacity for 50 ml Falcon tubes)
- Vortex
- 1.5 ml Eppendorf tubes
- 50 ml Falcon tubes
- 1x phosphate buffered saline (PBS)
- TRIzol
- Chloroform
- Isopropanol
- Ethanol
- TE buffer (1 mM EDTA, pH 8.0)

Method

Step 1

Harvest and pellet 5 x 10^7 cells in a 50 ml Falcon tube. If any liquid remains associated with the pellet, spin down the tube and aspirate the remaining supernatant.

Step 2

Add 500 µl of 1x PBS to pelleted cells.

Step 3

Centrifuge for 3 minutes at 300 x g and discard the supernatant.

Step 4

Add 4 ml of TRIzol to the cell pellet and vortex to homogenise.

Step 5

Incubate for 5 minutes at room temperature.

Step 6

Add 800 µl of chloroform and vortex.

Step 7

Incubate for 5 minutes at room temperature.

Step 8

Vortex and centrifuge for 15 minutes at 2000 x g at 4° C.

Step 9

Transfer the supernatant to a new 50 ml Falcon tube and add an equal volume of isopropanol.

Step 10

Invert the tube 15 times and incubate at room temperature for 15 minutes.

Step 11

Centrifuge for 20 minutes at 2000 x g at 4° C.

Step 12

Discard the supernatant.

Step 13

Add 4 ml of ice-cold 70% ethanol to wash the pellet.

Step 14

Centrifuge for 5 minutes at 2000 x g at 4° C.

Step 15

Discard the supernatant.

Step 16

Use a sterile paper wipe to absorb the ethanol from the tube walls.

Step 17

Allow the pellet to air-dry for 1 minute.

Step 18

Resuspend the pellet in 100 µl of TE by pipette mixing.

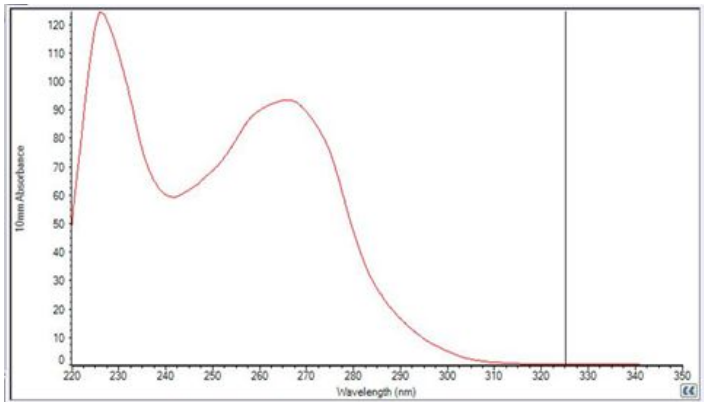
Step 19

Aliquot the sample as necessary and store aliquots at -80° C.

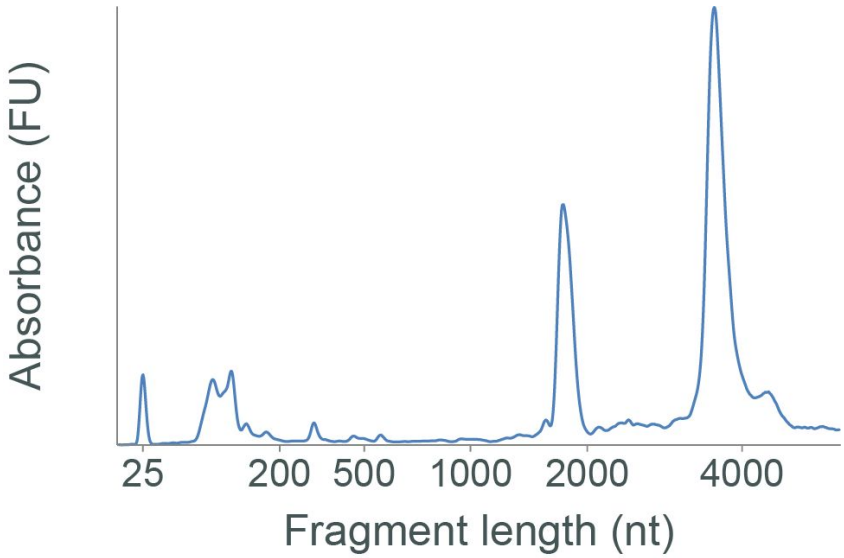
Results

- **Yield:** 230 µg
- **OD 260/280:** 1.89
- **OD 260/230:** 0.81

  Note: The lower than expected A260/230 ratio is indicative of TRIzol contamination. The performance of the PCR-cDNA Sequencing Kit is not adversely affected as long as the TRIzol concentration present in the reverse transcription and strand switching (first enzymatic step) is <0.5%.



- Agilent Bioanalyzer RNA 6000 Nano Kit, RIN: 9.7



Sequencing performance

Libraries for nanopore sequencing were prepared from 50 ng of total RNA ± 200 pg of ERCC RNA Spike-In Mix, using the PCR-cDNA Sequencing Kit (SQK-PCS109).

- Typical throughput: ⋆⋆⋆
  (8+ Gb or 7-12 M+ reads in 48 h on FLO-MIN106D).
- Read length profile:

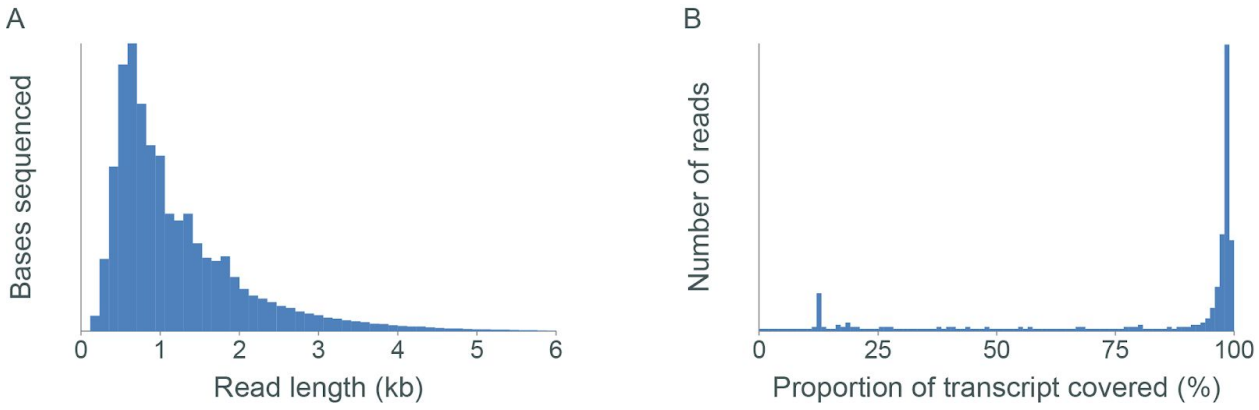


Figure 1. The length distribution of reads that align to NA12878 and the ERCC panel. Panel A: the length distribution of reads that align to the NA12878 reference genome. Panel B: the proportion of the ERCC transcript covered by a read aligning to that transcript. The observed lengths of the reads that aligned to the ERCC panel show the majority of reads cover almost the entire transcript (as is expected, as the SQK-PCS109 kit enriches for full-length RNA template molecules). This suggests that length distribution of reads in Panel A is representative of the length of the RNA molecules present in the sample and is not being biased during the library preparation process. The Spearman's rank correlation coefficient (rho) and the coefficient of determination (r^2) for the ERCC alignments were ~0.95, further suggesting that there is very little bias in the library preparation and sequencing.