Total RNA extraction from human blood

June 2019

This info sheet describes a method to extract total RNA from human blood. The blood was collected and stabilised in PAXgene® Blood RNA Tubes and extracted with PAXgene® Blood RNA Kit. Prior to sequencing, the extracted RNA was globin depleted using GLOBINclear™ Kit. The sequencing performance was assessed using the PCR cDNA Sequencing Kit.

Materials

- 2.5 ml of human blood collected in PAXgene® Blood RNA Tubes
- PAXgene® Blood RNA Kit
- 15 ml Falcon tubes
- Centrifuge with capacity for 15 ml Falcon tubes
- ThermoMixer
- Vortex
- Mini-centrifuge (capacity for 15000 x g)
- Ethanol
- Ice and ice bucket

Method

Step 1

After blood collection, invert the PAXgene® Blood RNA Tubes with the blood and incubate the tubes at room temperature for at least 2 hours. If the tubes were stored in the fridge or freezer after blood collection, ensure the tubes equilibrate at room temperature at least 2 hours before starting the RNA extraction.

Step 2

Transfer the content of the tube to a 15 ml Falcon tube and centrifuge for 10 minutes at 3500 x g.

Step 3

Decant the supernatant and add 4 ml of RNase-free water (RNFW); vortex until the pellet is dissolved.

Step 4

Centrifuge for 10 minutes at 3500 x g.

Step 5

Decant the supernatant and use a sterile wipe to absorb the remaining liquid from the tube walls, being careful not to disturb the pellet.

Step 6

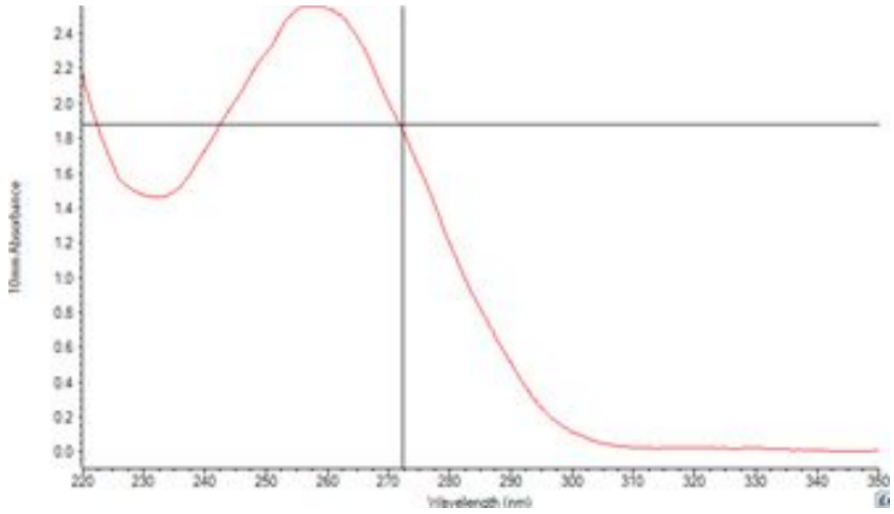
Follow the recommended protocol (steps 4 to 21, pages 47-50). On step 5, the ThermoMixer was set to 900 rpm. On steps 6, 9-11, and 14-19, the centrifuge was set to 15000 x g.

Optional step

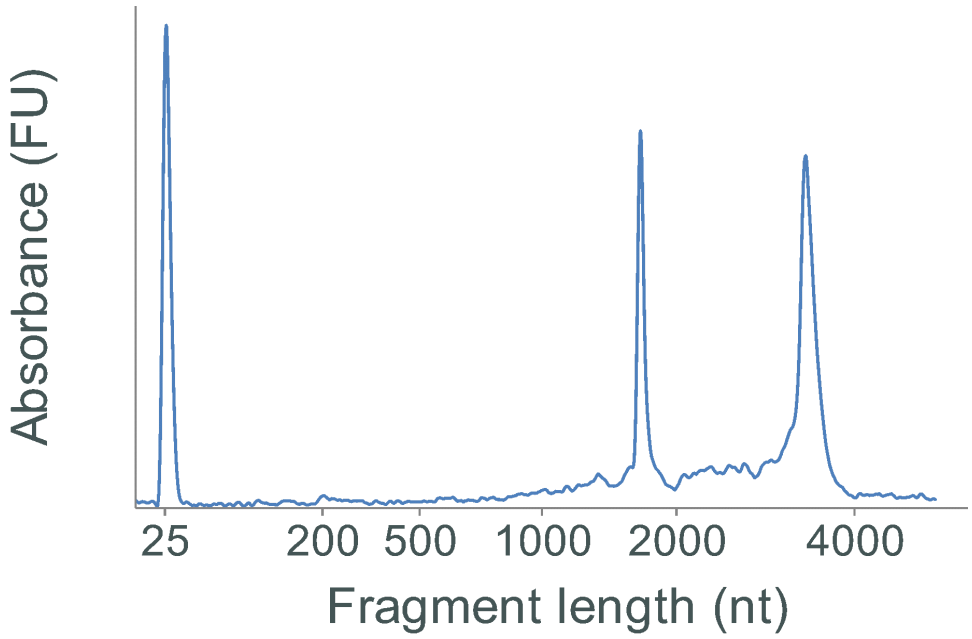
Deplete alpha and beta globin mRNA using the GLOBINclear TM -Human Kit following the recommended protocol. Note, >50% of input RNA was recovered after globin depletion.

Results

- **Yield:** 12-18 µg
- **OD 260/280:** 2.12
- **OD 260/230:** 1.73



- Agilent Bioanalyzer RNA 6000 Nano Kit, RIN: 8.9



Sequencing performance

Libraries for nanopore sequencing were prepared from 50 ng of globin depleted RNA ± 200 pg of ERCC RNA panel, using the PCR cDNA Sequencing Kit (SQK-PCS109):

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

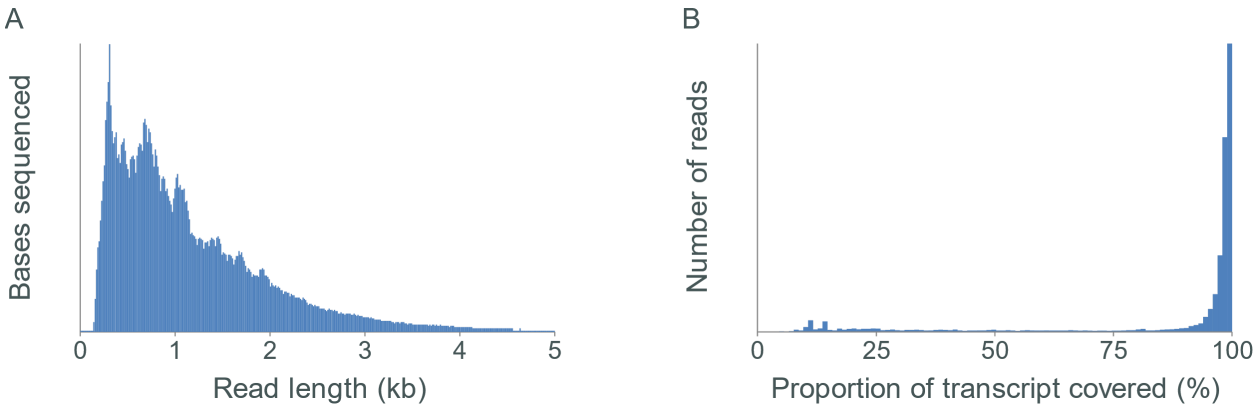


Figure 1. The length distribution of reads that align to NA12878 and the ERCC panel. Panel A: the length distribution of reads that align to the NA12878 reference genome. Panel B: the length distribution of reads that align to ERCC. The observed lengths of the reads that aligned to the ERCC panel show 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 >0.92, further suggesting that there is very little bias in the library preparation and sequencing.