



Alpha and beta globin mRNA depletion from total RNA extracted from human blood

June, 2019

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

Materials

- Total RNA extracted from human blood
- [GLOBINclear™-Human Kit](#)
- Ethanol
- Isopropanol
- Vortex
- 1.5 ml Eppendorf tubes
- Incubator with capacity for 50°C and 58°C
- Magnetic rack

Optional (if RNA concentration ≤ 70 ng/ μ l)

- [Glycogen](#)
- 5 M ammonium acetate
- -80°C freezer
- Refrigerated mini-centrifuge with capacity for 12000 x g
- TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

Method

● Step 1

If the total RNA concentration is below 70 ng/μl continue to step 2, otherwise continue to step 10.

● Step 2

Transfer an adequate volume of the total RNA sample (ideally close to 10 μg) to a 1.5 ml Eppendorf tube and add 0.1 volume of 5 M ammonium acetate, 5 μg glycogen, and 3 volumes of ethanol.

● Step 3

Invert 10 times and place the mixture at -80°C for 30 minutes.

● Step 4

Centrifuge at 12000 x g for 30 minutes at 4°C.

● Step 5

Discard the supernatant and add 1 ml of ice cold 70% ethanol; invert the tube to mix.

● Step 6

Centrifuge at 12000 x g for 10 minutes at 4°C.

● Step 7

Discard supernatant.

● Step 8

Repeat steps 5 to 7.

- Step 9

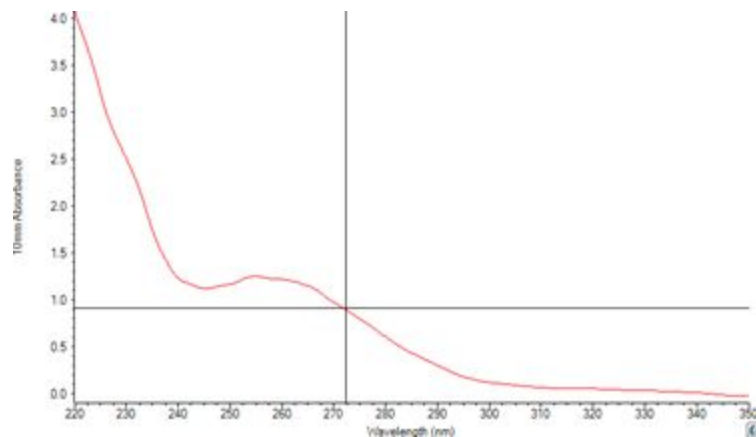
Dissolve the RNA pellet in 15 µl of TE and use 1 µl to assess concentration.

- Step 10

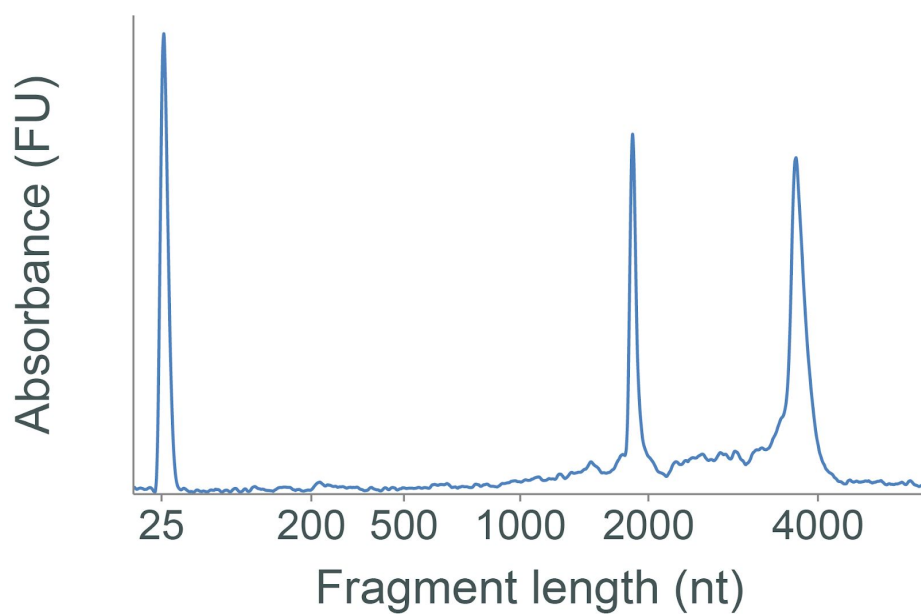
Follow the recommended [protocol](#) (steps B1 to F7, pages 7-12 of the handbook).

Results

- **Yield:** ~50-90% of input
- **OD 260/280:** 2.03
- **OD 260/230:** 0.48 (this low value could be due to carry-over of reagents or magnetic beads during the globin depletion process).



Agilent Bioanalyzer RNA 6000 Nano Kit, RIN: 9.2



Sequencing performance

Libraries for nanopore sequencing were prepared from 50 ng of both total RNA and globin depleted RNA, 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:

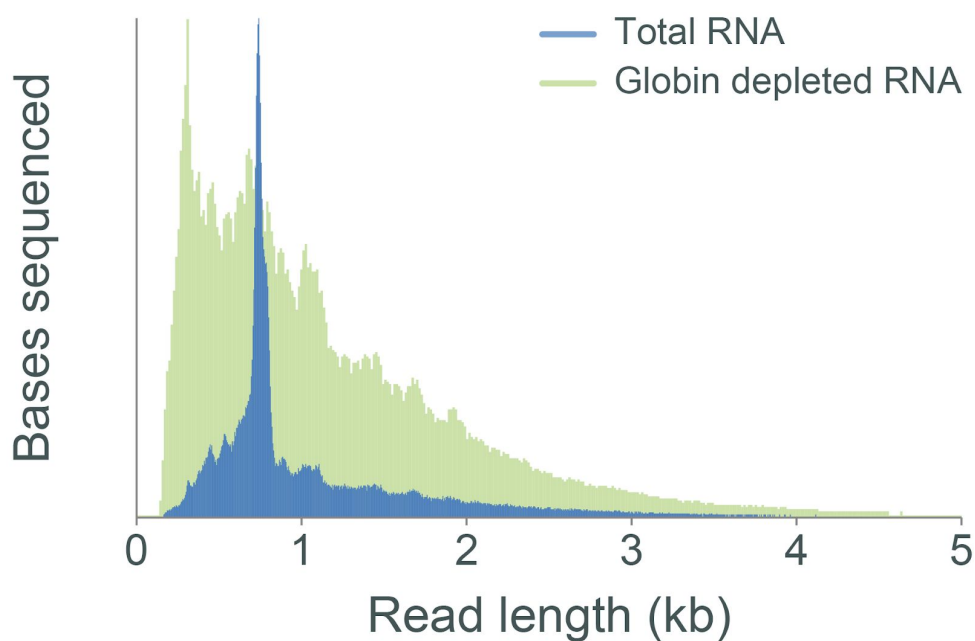


Figure 1. The length distribution of reads from total RNA and globin depleted RNA from human blood. The read length distribution from total RNA is dominated by a group of alpha and beta globin transcripts around 800 bp in length. The globin depletion effectively removes the bulk of these transcripts.