

### Direct RNA Sequencing of poly(A) RNA purified from Human Whole Blood

*Purify total RNA from human whole blood using the Maxwell® simplyRNA Blood Kit, then isolate mRNA using the PolyATtract® mRNA Isolation System for Oxford Nanopore Direct RNA Sequencing.*

**Kit:** Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)

**Analyses:**

- TapeStation
- Oxford Nanopore Direct RNA Sequencing

**Sample Type(s):** Whole blood from EDTA collection tube

**Materials Required:**

- Maxwell® RSC 48 Instrument (Cat.# AS8500)
- Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)
- PolyATtract® mRNA Isolation System (Cat.# Z5310)
- PolyATtract® System 1000 Magnetic Separation Stand (Cat.# Z5410)
- 65°C heat block

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM417 and TM021, available at:  
**[www.promega.com/protocols](http://www.promega.com/protocols)**

or contact Technical Services at:  
**[techserv@promega.com](mailto:techserv@promega.com)**

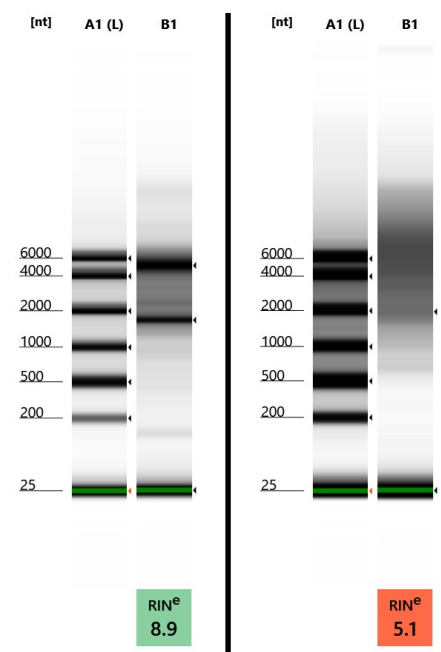
**Protocol:**

*Poly(A) RNA is only 2-5% of a total RNA sample. Oxford Nanopore Direct RNA Sequencing library preparation kit requires 500ng of poly(A) RNA. Please plan accordingly. We recommend starting with more than 1mg of total RNA before poly(A) isolation.*

1. Purify total RNA using the Maxwell® RSC simplyRNA Blood Kit according to Technical Manual TM417. Multiple purifications are required to isolate sufficient total RNA before polyA selection. Pool eluates from replicate purifications.
2. Isolate poly(A) RNA using the PolyATtract® mRNA Isolation System according to Technical Manual TM021. Follow the Small-Scale mRNA Isolation for total RNA samples up to 1mg, or the Large-Scale mRNA Isolation for 1-5mg of total RNA.
  - a. To increase the concentration of the poly(A) RNA, elute the poly(A) RNA in 25µl of Nuclease-Free Water, with a repeat elution of an additional 25µl of Nuclease-Free Water for a total volume of 50µl.

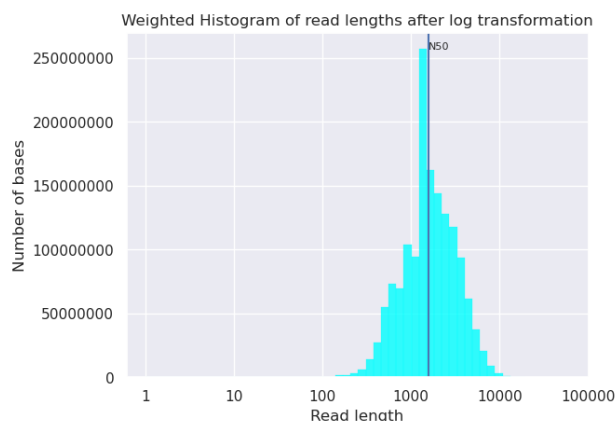
## Results:

Total RNA purified using the Maxwell® RSC simplyRNA Blood Kit, and poly(A) RNA selected using the PolyATtract® mRNA Isolation System is compatible with Nanopore Direct RNA Sequencing. Poly(A) RNA was sequenced using the Oxford Nanopore Direct RNA Sequencing Kit (SQK-RNA002)<sup>1</sup> on the MinION.



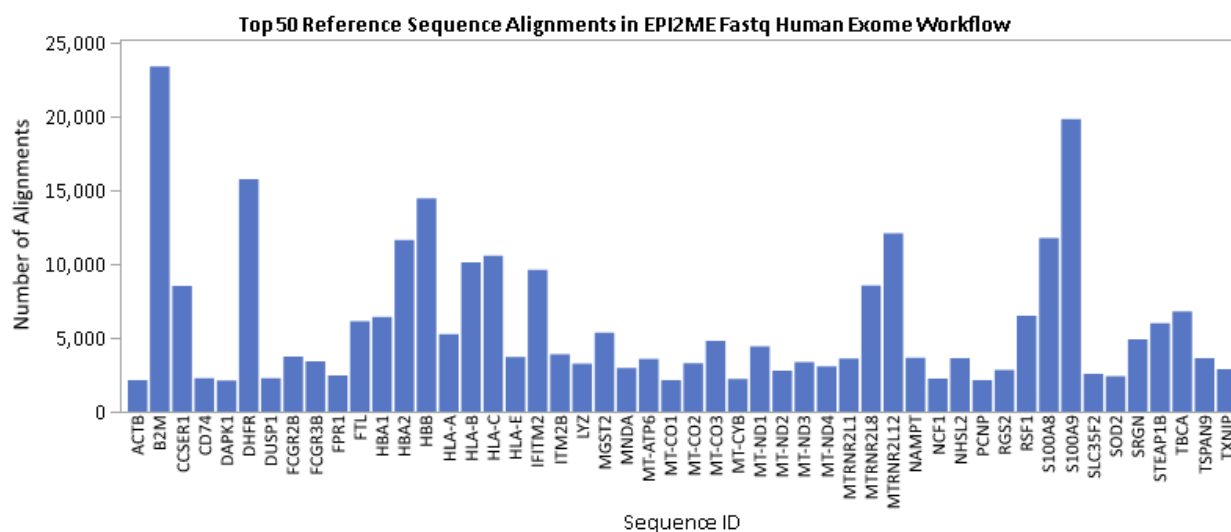
**Figure 1. TapeStation Electrophoresis of RNA samples.** **Left:** TapeStation electrophoresis of pooled total RNA purified from blood using Maxwell® RSC simplyBlood Kit (Cat.# AS1380). **Right:** TapeStation electrophoresis of isolated poly(A) RNA. Poly(A) RNA was isolated from the pooled RNA sample using the PolyATtract® mRNA Isolation System (Cat.# Z5310). The poly(A) RNA had a reduced RIN score (5.1), but this is expected for poly(A) selected RNA lacking the 28S and 18S rRNA bands

Pores Available	1048
Total Yield (Gb)	1.48
Reads Analyzed	1,183,592
Avg Quality Score	10.7
Median Quality Score	10.8
Mean Seq Length	1253
Median Read Length	996
Maximum Read Length	19,557
N50 read length	1565



**Figure 2. Oxford Nanopore Sequencing metrics and read length histogram.** **Left:** MinION Direct RNA Sequencing run metrics. **Right:** Histogram displaying the total number of RNA bases sequenced (y-axis) and read length on the x-axis. 432ng of poly(A) RNA was sequenced using the Direct RNA Sequencing Kit (SQK-RNA002) using the Oxford Nanopore MinION. The N50 read length of 1565 is similar to published<sup>2</sup> N50 read lengths.

The EPI2ME Fastq Human Exome workflow aligned the RNA sequences to the human reference genome assembly (GRCh38). See Figure 3. The top 50 expression mRNAs include targets expected to be common in human blood:  $\beta$ 2M (Beta-2 microglobulin – encodes a protein present on all nucleated cells), HBA2 (alpha-globin – encodes a protein of hemoglobin), HLA-B (major histocompatibility complex class 1B – encodes a protein in the immune system) and mitochondrial targets which are abundant in all human cell such as MT-CO1 (mitochondrially encoded cytochrome c oxidase I – encodes a protein in aerobic metabolism) and MT-RNR2L12 (mitochondrially encoded 16S RNA – encodes part of the mitochondrial large subunit ribosomal RNA).



**Figure 3. Top 50 Sequence IDs with the most aligned reads in the EPI2ME Fastq Human Exome Workflow.** 432ng of poly(A) RNA was sequenced using the Direct RNA Sequencing Kit (SQK-RNA002) on the Oxford Nanopore MinION, basecalled with GUPPY, and fastq files were uploaded to the Nanopore EPI2ME for alignment to the human reference assembly GRCh38.

### References:

1. Oxford Nanopore Technologies Direct RNA Sequencing (SQK-RNA002) Checklist. Version DRS\_9080\_v2\_revO\_14Aug2019. Last Update 10/06/2021.
2. Workman, R.E et al. Nanopore native RNA sequencing of a human poly(A) transcriptome. Nature Methods. December 2019. 16:1297-1305.