

Long-Read Sequencing of DNA Purified from Blood Using the Maxwell® RSC Instrument

Purify high molecular weight DNA from blood using the Maxwell® RSC Blood DNA Kit and the Maxwell® RSC Instrument for Oxford Nanopore long-read sequencing.

Kit:	Maxwell® RSC DNA Blood Kit (Cat.# AS1400)
Analyses:	Pulsed-field gel electrophoresis, Oxford Nanopore long-read sequencing
Sample Type(s):	Blood
Input:	300µl
Materials Required:	<ul style="list-style-type: none">▪ Maxwell® RSC DNA Blood Kit (Cat.# AS1400)▪ Maxwell® RSC Instrument (Cat.# AS4500)▪ Heat block▪ Wide-bore pipette tips

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 TM419, available at:

www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Protocol:

1. Follow the Maxwell® RSC Blood DNA Kit Technical Manual (TM419) with the following modifications to preserve high molecular weight DNA:
 - a. Do not vortex the sample after the addition of Lysis Buffer. Mix the sample by pipetting slowly with a wide-bore tip.
 - b. Pipette slowly and use only wide-bore tips when pipetting the sample after lysis.

Results:

Blood samples were processed according to the Maxwell® RSC Blood DNA Kit Technical Manual with and without the modifications listed above to preserve high molecular weight DNA. DNA from both samples was analyzed using pulsed-field gel electrophoresis (Figure 1). The sample purified with modifications to preserve high molecular weight DNA was then sequenced on the Oxford Nanopore MinION using the Rapid Sequencing (SQK-RAD004¹) library preparation kit (Table 1).

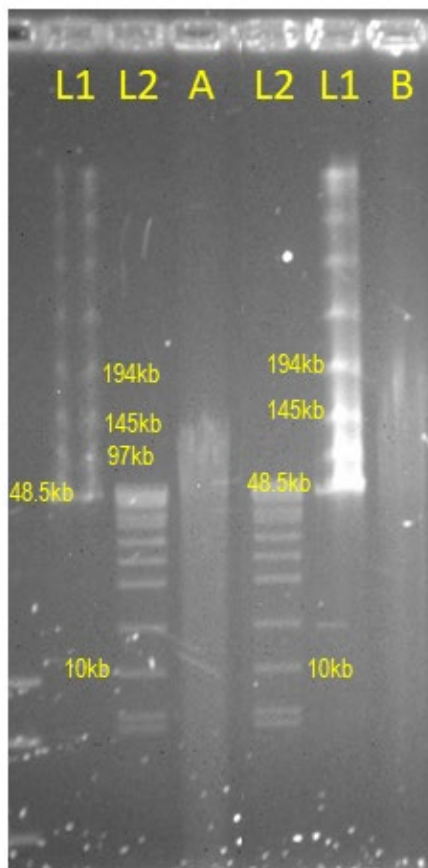


Table 1. Oxford Nanopore Sequencing Metrics.

Metric	Value
Time Sequenced	~8.5 hours
Number of Pores	1537
Total Yield (Gb)	2.47
Reads Analyzed	618,953
Avg Quality Score	10.6
Mean Seq Length	3994
Median Read Length	1631
Maximum Read Length	91,516
N50 Read Length	9380

Figure 1. Pulsed-Field Gel Electrophoresis (PFGE) image. Blood samples were processed according to the Maxwell® RSC Blood DNA Kit (Cat.# AS1400) Technical Manual with and without the modifications to preserve high molecular weight listed above. 500ng of each DNA sample was analyzed using PFGE. DNA size standards are loaded in lanes L1 and L2, and several size standards are labeled for reference. “A” refers to the standard processed sample; “B” refers to the sample processed with the modifications to preserve high molecular weight DNA.

References:

1. Oxford Nanopore Rapid Sequencing (SQK-RAD004) protocol checklist. Version RSE_9046_v1_revN_14Aug2019. Last update 02/10/2020.*

*Available at <https://community.nanoporetech.com/protocols>. Nanopore community account required.