Promega

Product Application

Oxford Nanopore Sequencing with Promega Purification Kits

Use Promega purifications kits to purify genomic DNA from fresh blood for high quality Oxford Nanopore long-read sequencing results.

Kits: Wizard® Genomic DNA Purification Kit (Cat.# A1120)
ReliaPrep™ Blood gDNA Miniprep System (Cat.# A5081)
Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520)
Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses: Pulsed-Field Gel Electrophoresis (PFGE)
Oxford Nanopore MinION Sequencing

Sample Type(s): Fresh human blood

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 Manuals TM050, TM330, TM455, TM419, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- QuantiFluor® ONE dsDNA System (Cat.# E4871)
- Quantus™ Fluorometer (Cat.# E5160)
- ProNex® Size-Selective Purification System (Cat.# NG2001)
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5332)
- Oxford Nanopore Ligation Sequencing Kit (Cat.# SQK-LSK109) and associated consumables
- Oxford Nanopore Flow Cell Priming Kit (Cat.# EXP-FLP002)
- Oxford Nanopore MinION Flow Cell R9.4.1 (Cat.# MIN-106D)
- Oxford Nanopore MinION Sequencing device (Cat.# MIN-101B)

Protocol:

1. Process fresh human blood according to the technical manual of the applicable purification kit.
2. Take the following precautions to maximize the size of high molecular weight (HMW) gDNA during the purification. Do not vortex samples (outside of steps listed in technical manuals) and use only pulse vortexing after sample lysis. Use wide-bore tips for sample pipetting steps after lysis. Take care to pipette slowly.

Note: The provided DNA Rehydration Solution included in the Wizard® Genomic DNA Purification Kit (Cat.# A1120) contains a low amount of EDTA (pH 8.0). EDTA can impact the library preparation efficiency for Oxford Nanopore Ligation Sequencing Kits and Rapid Sequencing Kits. Nuclease-Free Water can be used in place of DNA Rehydration Solution for these samples.

For Oxford Nanopore Sequencing, follow the *Genomic DNA by Ligation (SQK-LSK109) Protocol*[1] as written, with the following changes:

A. Omit the optional DNA fragmentation step.

B. Replace the AMPure® XP bead clean-up steps after the DNA repair and end-prep steps with the following ProNex® Chemistry protocol:

1. Allow the ProNex® Chemistry to equilibrate to room temperature for 30-60 minutes. Resuspend ProNex® Chemistry by vigorous vortexing for 10 seconds or longer.
2. Transfer the DNA sample (60µl) to a clean 1.5ml low-bind tube.
3. Add 96µl of ProNex® Chemistry to the sample and mix by pipetting 10 times.
4. Incubate the sample at room temperature for 10 minutes.
5. Place the sample on a magnetic stand for 2 minutes.
6. Carefully remove and discard the supernatant.
7. Leaving the sample on the magnetic stand, add 200µl of Wash Buffer to the sample and allow it to incubate 30-60 seconds. Remove and discard the Wash Buffer.
8. Repeat previous step.
9. Spin down and place the tube back on the magnet. Pipette off any residual Wash Buffer.
10. Allow the sample to air dry for 5 minutes.
11. Remove the sample from the magnetic stand.
12. Add 61µl of Nuclease-Free Water and resuspend pellet by pipetting. Incubate sample at room temperature for 5 minutes to elute the DNA.
13. Return the sample to the magnetic stand for 1 minute and transfer eluted DNA to a clean tube.

C. In the adapter ligation and clean-up steps:

1. Substitute the 40µl of AMPure® XP bead addition with 100µl of ProNex® Chemistry.
2. Use the Long Fragment Buffer (LFB) for wash steps.
3. Continue with wash steps as written in the Genomic DNA by Ligation (SQK-LSK109) Protocol[1].

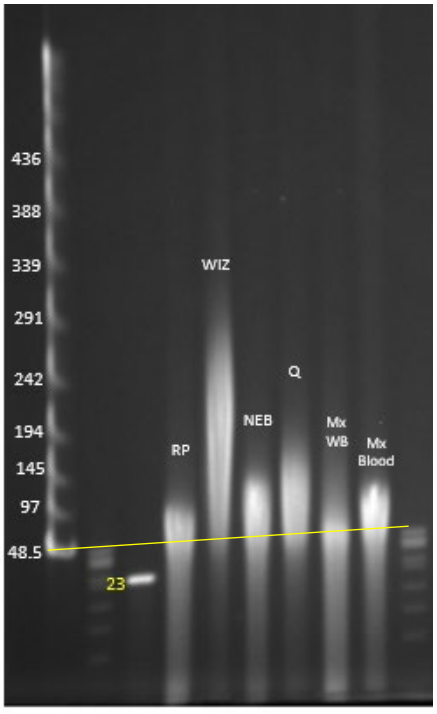
D. Quantify eluted samples after clean-up steps using QuantiFluor® ONE dsDNA System with a Quantus™ Fluorometer.

Results:

Human genomic DNA was purified using fresh blood samples from a single donor using various Promega purification kits (Wizard® Genomic DNA Purification Kit, ReliaPrep™ Blood gDNA MiniPrep System, Maxwell® RSC Whole Blood DNA Kit and Maxwell® RSC Blood DNA Kit) as well as Qiagen MagAttract DNA Kit and the NEB Monarch gDNA Kit. No samples were vortexed (outside of steps listed in technical manuals) and wide-bore tips were used for sample pipetting steps after lysis. DNA was quantified with QuantiFluor® ONE dsDNA System.

0.2µg of DNA from each purification kit was loaded on a 0.75% Pulsed Field Certified™ Agarose gel prepared with 0.5X KBB Buffer and separated by electrophoresis using the Pippin Pulse™ PFGE Electrophoresis Power Supply.
1µg of DNA from each purification kit was sequenced according to the Genomic DNA by Ligation (SQK-LSK109) Protocol[1] using ProNex® Chemistry in place of AMPure® XP bead clean-ups as noted above. Each library was run long enough to generate a minimum of 4.2GB total yield. The fast basecalling option was selected in MinKNOW. For each library, an EPI2ME experiment was created and analyzed using the Fastq Human Alignment GRCh38 Workflow Version 3.2.1.

Using methods that purify higher molecular weight genomic DNA based on a Pulsed-Field Gel Electrophoresis (PFGE) results in longer Oxford Nanopore sequencing read lengths (mean and maximum read length, N50 read length). Purification kits producing higher molecular weight DNA also result in higher percentages of long sequencing reads (reads greater than 50kb).



Lane	Sample
1	NEB PFGE Ladder
2	BioRad CHEF Size Std
3	Lambda/HindIII Marker
4	ReliaPrep™ Blood gDNA MiniPrep System
5	Wizard® Genomic DNA Purification Kit
6	NEB Monarch Genomic DNA Purification Kit
7	Qiagen MagAttract® HMW DNA Kit
8	Maxwell® RSC Whole Blood DNA Kit
9	Maxwell® RSC Blood DNA Kit
10	Bio-Rad CHEF Size Std

Figure 1. Pippin Pulsed-Field Gel Electrophoresis (PFGE) of purified genomic DNA. The table on the right lists the sample loaded in each lane. The yellow line indicates ~ 50kb across the gel image. Wizard® samples had the highest molecular weight smears, followed by Qiagen, then NEB and Maxwell® RSC Blood DNA (Cat.# AS1400), and then ReliaPrep™ (Cat.# A5081) and Maxwell® RSC Whole Blood DNA (Cat.# AS1520) with smears less than 100kb.

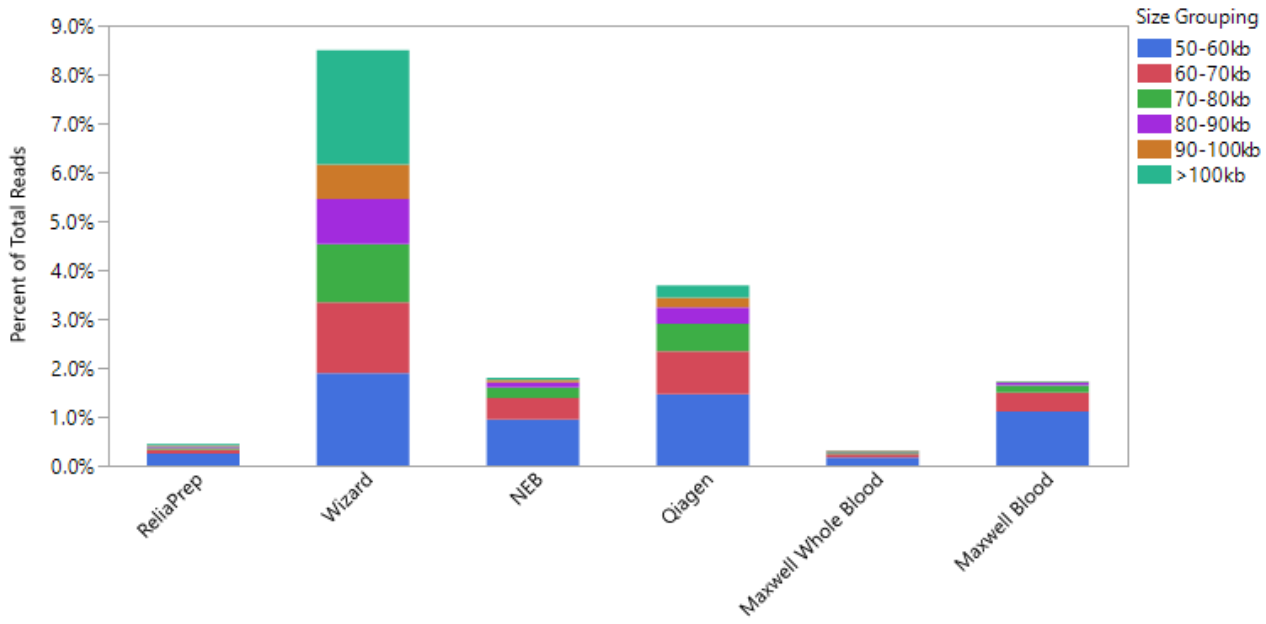


Figure 2. Percentage of total reads categorized by size grouping and purification method. Graph includes only reads 50kb or greater for each sample. The percentage of reads for each 10kb size grouping is displayed using different colors, see legend. The Wizard® sample has notably more long reads compared to the other purifications. The percentage of long reads matches the PFGE Gel (Figure 1) size distributions.

Table 1. Oxford Nanopore sequencing QC and Barcoding metrics [rev 3.2.1] from EPI2ME Fastq Human Alignment GRCh38 workflow.

QC Metrics	ReliaPrep™	Wizard®	NEB	Qiagen	Maxwell® RSC Whole Blood	Maxwell® RSC Blood
Total Yield	4.4 GB	5.0 GB	5.2 GB	5.4 GB	4.2 GB	4.2 GB
Reads Analyzed	436,971	343,644	481,807	465,142	604,616	355,300
Mean Quality Score	10.74	11.1	11.19	11.24	10.8	11.33
Median Quality Score	11.5	11.3	11.5	11.5	11.1	11.6
Mean Seq Length	10,003	14,512	10,850	11,636	6,973	11,727
Median Read Length	3555	3631	6590	5452	4372	6845
Maximum Read Length	236,038	422,802	172,538	310,305	216,178	143,346
N50 Read length	13,780	51,696	19,110	24,630	12,057	22,302
# Reads > 100kb	166	8,030	199	1,186	64	58
% Reads > 100kb	0.04%	2.3%	0.04%	0.25%	0.01%	0.02%

Reference:

1. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK109) Protocol. Version GDE_9063_v109_revN_14Aug2019. Last update 14/08/2019. **Available at https://community.nanoporetech.com/protocols. Nanopore community account required.*