Promega

Product Application

Oxford Nanopore DNA Sequencing of High Molecular Weight DNA

Use DNA purified with the Wizard® HMW DNA Extraction Kit for long read sequencing on the Oxford Nanopore MinION.

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 TM604, available at: **www.promega.com/protocols**

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

Kit: Wizard® HMW DNA Extraction Kit (Cat.# A2920)

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

Sample Type(s): Human blood, *E. coli*, spinach leaf

Input: 1µg

Materials Required:

- Wizard® HMW DNA Extraction Kit (Cat.# A2920)
- ProNex® Size-Selective Purification System (Cat.# NG2001)
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5332)
- Quantus™ Fluorometer (Cat.# E6150)
- QuantiFluor® ONE dsDNA System (Cat.# E4871)
- Heat block(s)
- Thermal cycler
- Low bind 1.5ml tubes
- 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 human blood according to TM604, Section 3.A. Isolating HMW DNA from Whole Blood.
2. Process *E. coli* according to TM604, Section 3.D. Isolating HMW DNA from Gram-Positive and Gram-Negative Bacteria.
3. Process spinach leaf according to TM604, Section 3.C. Isolating HMW Genomic DNA from Plant Tissue.
   - All DNA samples were rehydrated overnight at room temperature.

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

A. 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 flicking the tube.
   4. Incubate the sample at room temperature for 10 minutes.

5. Spin down the sample and pellet on a magnet. Carefully remove and discard the supernatant.
6. Leaving the sample on the magnetic stand, add 200µl of Wash Buffer to the sample and allow it to incubate for 30-60 seconds. Remove and discard the Wash Buffer.
7. Repeat previous step.
8. Spin down and place the tube back on the magnet. Pipette off any residual Wash Buffer.
9. Allow the sample to air dry for 5 minutes.
10. Remove the sample from the magnetic stand.
11. Add 61µl of Nuclease Free Water and resuspend the pellet. Incubate the sample at room temperature for 10 minutes to elute the DNA.
12. Pellet the beads on a magnet until the eluate is clear and colorless. Transfer eluted DNA to a clean low bind tube.

B. In the adapter ligation and clean-up steps:
   1. Use 100µl of ProNex® Chemistry instead of 40µl of AMPure® XP bead addition.
   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].
   4. Quantify eluted samples after clean-up steps using QuantiFluor® ONE dsDNA System with a Quantus™ Fluorometer.

Results:

Genomic DNA was purified from human blood, *E. coli* and spinach leaf using the Wizard® HMW DNA Extraction Kit. 0.5µg of DNA was electrophoresed using a Bio-Rad CHEF Pulsed-Field Gel Electrophoresis (PFGE) system to visualize the size of the DNA. *E. coli* bacteria samples were ~50kb – 300kb, human blood samples were ~50kb – 250kb and spinach leaf samples were ~ 50kb – 150kb (data not shown).

1µg of DNA from each sample was sequenced according to the Genomic DNA by Ligation (SQK-LSK109) Protocol[1] using the adaptations noted above. Samples were run using the MinKNOW software (release 19.12.5) with fast basecalling for 72 hours, or the run was stopped when less than 100 pores were available for sequencing. For each library an EPI2ME (v3.2.2) experiment was created and analyzed. The human blood samples were analyzed using the Fastq Human Alignment GRCH38 r3.2.2 workflow and the *E. coli* and spinach leaf using the Fastq Custom Alignment r.3.2.2 with appropriate reference genomes for alignment. N50 scores were generated by NanoPlot 1.28.4 running under Python 3.6.10.

DNA extracted with the Wizard® HMW DNA Extraction Kit and sequenced using Oxford Nanopore sequencing technology results in high-quality long read data with N50 values > 50kb and maximum read lengths > 500,000bp (Table 1).

The size of the DNA based on a Pulsed Field Gel Electrophoresis (PFGE) image has a notable impact on sequencing metrics such as N50 values and sequence length values (mean, median, maximum). Samples with higher molecular weight DNA have higher N50 and sequence length values (Figures 1, 2).

Table 1. Oxford Nanopore sequencing QC metrics from EPI2ME workflows.

Sample Source	*E. coli* bacteria		Human blood		Spinach leaf	
Sample Name	J1	J6	B2	B5	S1	S2
QC Metrics						
Number of Active Pores	1548	1589	1339	1645	1431	1340
Total Yield (bp) Total Yield (GB)	1.797E+10 (17.97GB)	1.83E+10 (18.3GB)	4.69E+09 (4.69GB)	1.06E+10 (10.6GB)	4.01E+09 (4.01GB)	7.55E+09 (7.55GB)
Reads Analyzed	930,514	1,204,740	275,955	720,732	550,430	1,493,850
Avg Quality Score	10.2	10.9	11.1	11.2	9.5	9.7
Median Quality Score	10.4	11.2	11.3	11.5	9.3	9.5
Mean Seq Length	19,313	15,227	17,002	14,761	7,289	5,055
Median Read Length	5,285	3,548	4,223	3,531	1,550	1,209
Maximum read Length	524,901	429,464	256,162	467,728	211,034	222,897
N50 read length	**57,926**	**51,367**	**47,788**	**54,520**	**30,649**	**22,510**
Number of reads > 100kb	30,666	22,517	3612	14,556	808	509

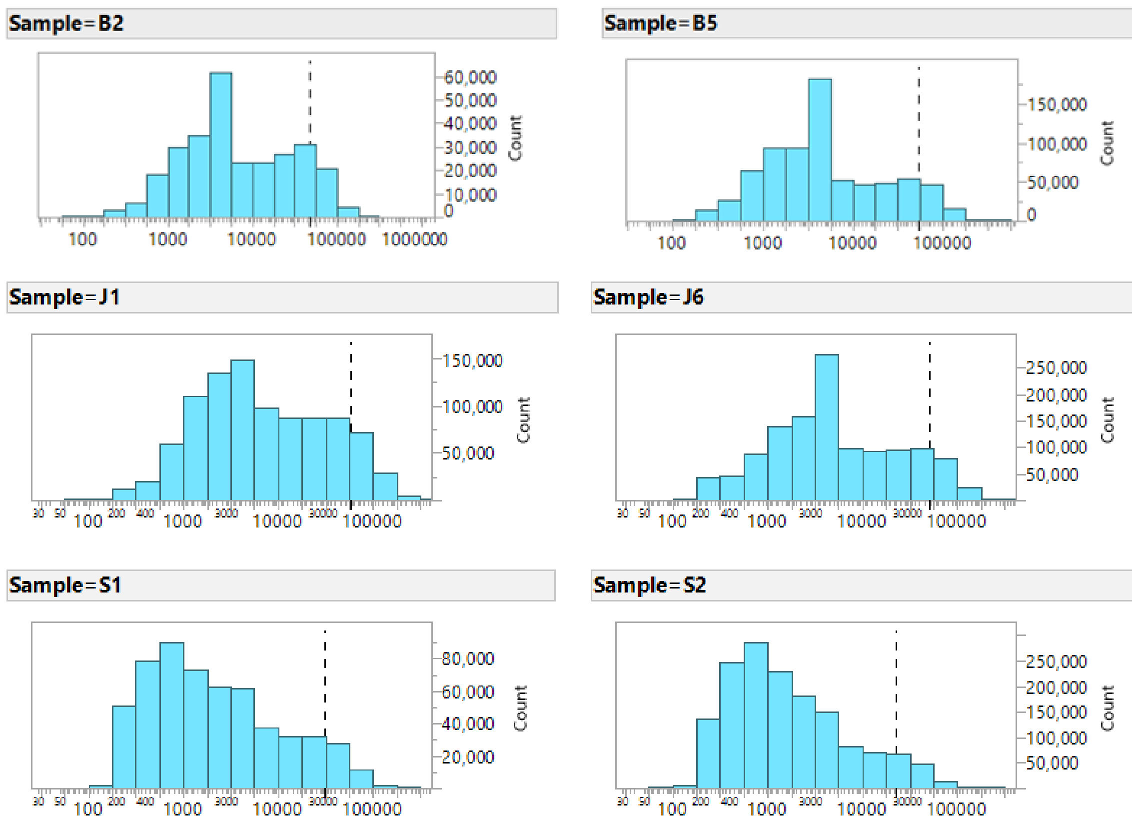

Figure 1. Histograms of sample read lengths using a log X-axis scale. Samples were purified according to TM604 for the Wizard® HMW DNA Extraction Kit and sequenced on an Oxford Nanopore MinION flow cell. Row 1: human blood samples (B2, B5); row 2: *E. coli* samples (J1, J6); row 3: spinach samples (S1, S2). The Y-axis indicates the number of reads (Count) and is scaled independently for each histogram. The N50 for each sample is indicated by the vertical dashed line.

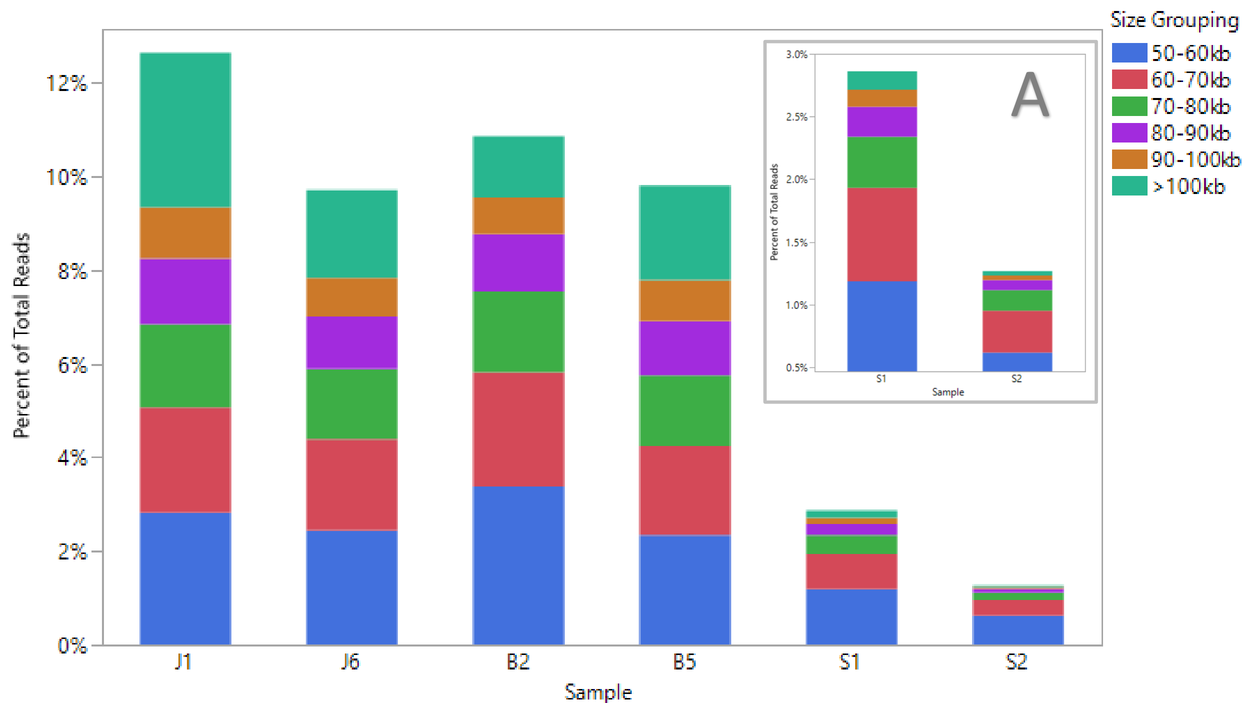

Figure 2. Percentage of total reads > 50kb categorized by 10kb size grouping for each sample. Human blood samples (B2, B5), *E. coli* samples (J1, J6) and spinach samples (S1, S2). Samples were purified according to TM604 for the Wizard® HMW DNA Extraction Kit and sequenced on an Oxford Nanopore MinION flow cell. 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 inset figure labeled "A" is a zoomed in image of both spinach leaf samples, with the percent of total reads on the Y-axis zoomed in between 0% and 3%.

Reference:

1. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK109) Protocol. Version GDE_9063_v109_revS_14Aug2019. Last update 28/01/2020. **Available at https://community.nanoporetech.com/protocols. Nanopore community account required.*