

# Automated Unbiased Metagenomic DNA Extraction for Long-Read Nanopore Sequencing

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## Overview

**Purpose:** Unbiased isolation of high molecular weight (HMW) DNA from metagenomic samples for long-read nanopore sequencing.

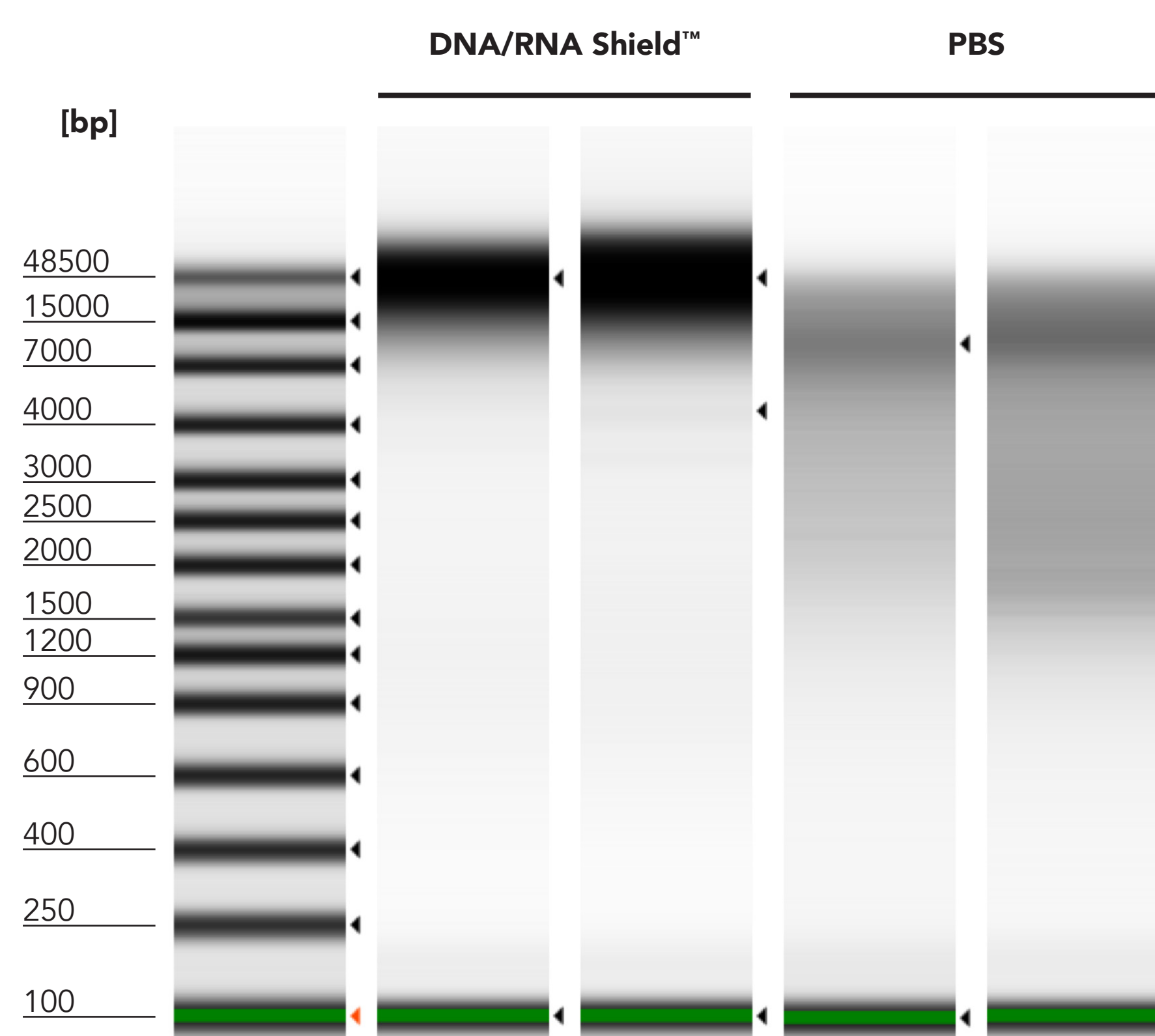
**Methods:** DNA protection and DNase inactivation using DNA/RNA Shield™ (Zymo Research, Irvine). Cell lysis by lysozyme (Sigma-Aldrich, St. Louis). DNA isolation from ZymoBIOMICS® Microbial Community Standard (Zymo Research, Irvine) using ZymoBIOMICS® 96 MagBead DNA Kit (Zymo Research, Irvine) and Microlab® STAR™ (Hamilton, Reno). Sequencing on MinION™ (Oxford Nanopore Technologies, UK).

**Results:** HMW metagenomic DNA suitable for nanopore sequencing with average read-lengths of 8 kb and unbiased microbial composition profile.

## Introduction

The advantages of nanopore sequencing include the capacity to generate very long-reads with remarkable speed and portability, spanning tandem-repeat regions resolving ambiguity during genome assembly<sup>1</sup>. However, extracting inhibitor-free HMW DNA suitable for long-read sequencing has always been a challenge due to DNA fragmentation and contaminant introduction. Furthermore, applying this to metagenomic samples increases difficulty as composition bias can be introduced during DNA extraction<sup>2</sup> and shearing due to the presence of DNases. Here we present an automated HMW DNA extraction pipeline that combines magnetic bead-based DNA extraction with the Microlab STAR™. Magnetic bead chemistry allows for the retention of HMW DNA, cleaner elutions, and is amenable to high throughput liquid handling applications. Eluted DNA from the pipeline was sequenced using the MinION™ platform; achieving 1M reads, 8 Gb throughput, with average read-lengths of 8 kb, with over 100 kb recorded. Our results demonstrate that the extracted microbial profile closely matches the theoretical composition. Overall, we have developed an automated method for HMW DNA extraction that shows unbiased microbial lysis that is compatible with long-read nanopore sequencing.

## DNA Extraction Results

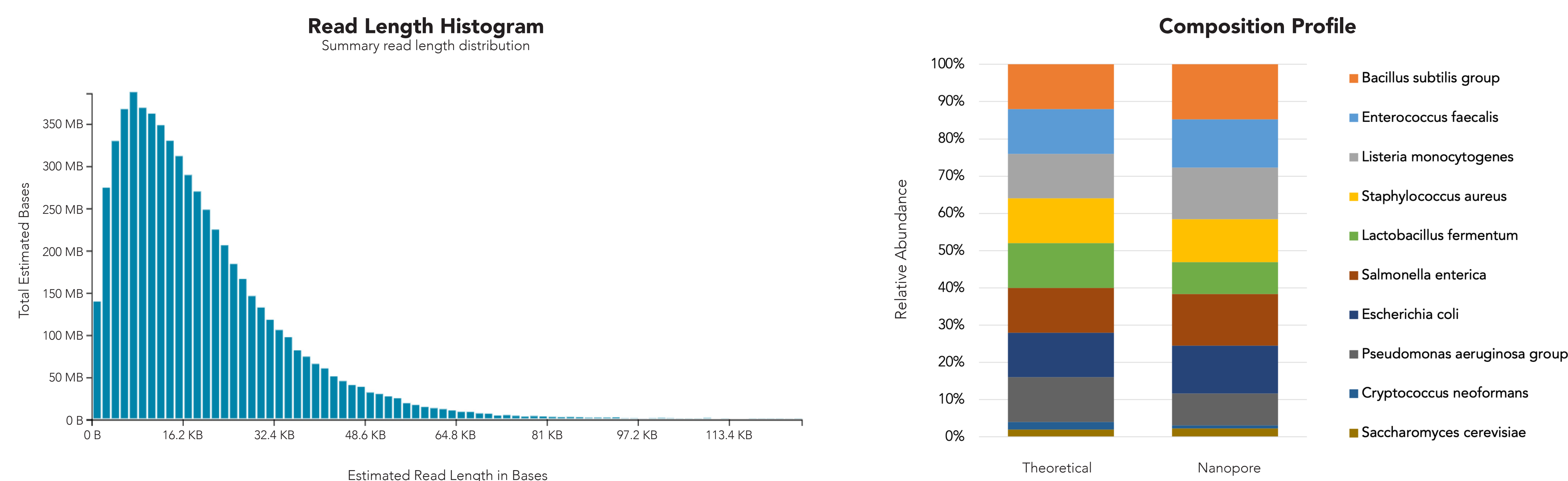


**Figure 1: DNA/RNA Shield™ preserves DNA integrity.** ZymoBIOMICS® Microbial Community Standard was pretreated with DNA/RNA Shield™ or PBS. Samples were then lysed by lysozyme. DNA was isolated and purified using ZymoBIOMICS® 96 MagBead DNA kit. DNA size was analyzed using Agilent 2200 TapeStation®.

## Workflow



## Nanopore Sequencing Results



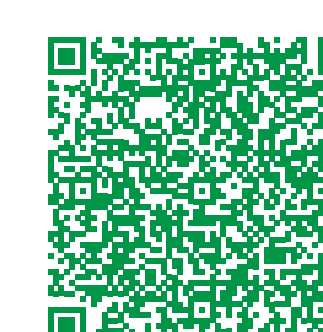
**Figure 2: Long-read sequencing of ZymoBIOMICS® Microbial Community Standard.** Sequencing was performed on Oxford Nanopore MinION™ device using an R9.4 flow cell. Library was prepared using the RBK-004 kit (Oxford Nanopore Technologies, UK) per manufacturer's protocol. Read length histograms were generated by MinKNOW 2.2 sequencing software (Oxford Nanopore Technologies, UK). Sequencing was performed for 48 hours and data was processed through bioinformatics whole genomics sequencing pipeline using the Centrifuge software for taxonomic assignment and classification. Composition of the ZymoBIOMICS® Microbial Standard was compared to the theoretical composition per manufacturer's specifications.

## Conclusions

- Pre-treating metagenomic samples with DNA/RNA Shield™ protects the DNA from fragmentation caused by nucleases during DNA extraction.
- The ZymoBIOMICS® MagBead DNA kit is capable of purifying HMW DNA which is compatible with long-read nanopore sequencing.
- This method produces an average read-length 8 kb, with greater than 100 kb fragments recorded.
- Combining both a pre-treatment step with DNA/RNA Shield™ and extraction with the ZymoBIOMICS® MagBead DNA Kit, this method captures the composition profile of metagenomic samples while minimizing the introduction of bias.



Learn more about the ZymoBIOMICS® MagBead DNA Kit



## References

1. Nicholls SM, Quick JC, Tang S, Loman NJ. Ultra-deep, long-read nanopore sequencing of mock microbial community standards. bioRxiv 487033; doi: <https://doi.org/10.1101/487033>
2. Brooks JP, Edwards DJ, Harwich MD, et al. The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies. BMC Microbiol. 2015;15:66. doi: 10.1186/s12866-015-0351-6