High-Pass DNA Size Selection: Method Comparison with ONT MinION

sage science
Application Note: BluePippin

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BluePippin High-Pass Plus DNA size selection is used prior to library construction for Oxford Nanopore MinION sequencing producing a sequencing result with 2.7X higher N50 than untreated DNA.

Introduction

Third-generation sequencing technologies have enabled the reading of single molecules up to hundreds of kilobases in length. One of the challenges to exploiting this technology is that DNA extraction methods will often over-fragment DNA, and the smaller molecules will preferentially occupy the pores. This effectively reduces average read lengths and N50 scores for a run. For this reason, it can be very advantageous to remove smaller DNA fragments from input samples. One such method is Sage Science's BluePippin High-Pass Plus, which uses agarose gel electrophoresis to filter out molecules below a defined threshold and collect the larger molecules from a sheared DNA sample.

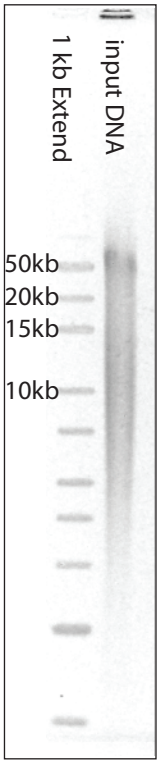

Figure 1. DNA Shear

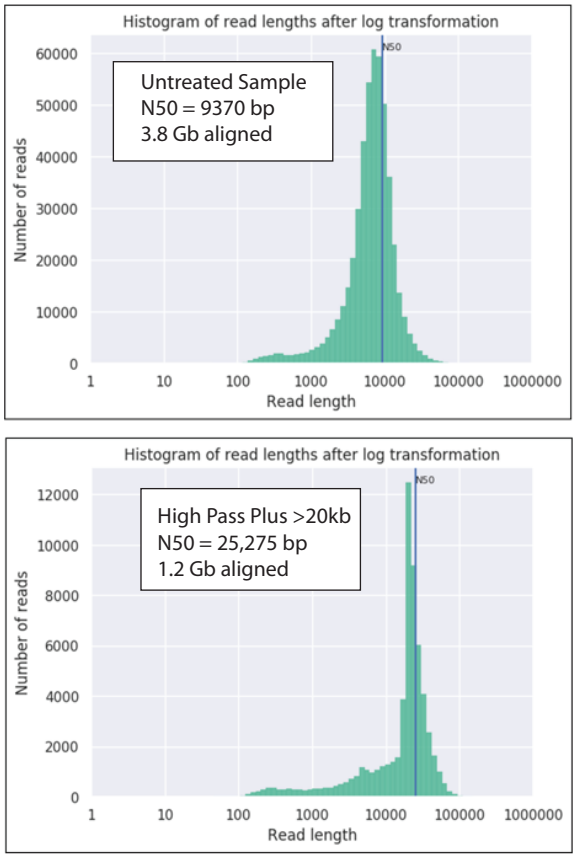

Figure 2. MinION Read Length Histograms

Methods

Human DNA (Human White Blood Cells extracted with a Qiagen Genomic-tip™) was sheared using a shearing protocol that combines various Covaris gTube™ shearing protocols to produce a sheared fragment distribution between ~5kb to ~100 kb (Figure 1). Split aliquots of the sheared DNA were used to prepare MinION libraries. One DNA aliquot was treated with the >20kb High-Pass size selection using the High Pass Plus™ gel cassette with the BluePippin system. The size selected and untreated DNA were constructed into sequencing libraries were prepared using the Oxford Nanopore Native Barcoding Ligation Protocol (SQK-LSK109, EXP-NBD104, EXP-NBD114). Sequencing was undertaken on a MinION for each condition. A comparison of the read length histograms are shown in Figure 2.

Discussion

The High Pass Plus™ gel cassette was specifically designed for the High Pass method and can be run with either a pre-set >15 or >20 kb electrophoresis protocol on the BluePippin. The >20kb High Pass run time is ~3:15 and the cassette includes a larger elution module than standard BluePippin cassettes, to maximize DNA recovery for single molecule sequencing applications.

The MinION results indicate a significant improvement of the N50 in the size selected sample compared to the untreated sample (from ~9kb to ~25kb). There was a 70% reduction in sequence yield with the size selected library since for the purpose of this study, sheared input DNA was intentionally prepared to contain large amount of DNA below 20kb. Yield and N50s can be improved with higher molecular weight shearing profiles. This study does show the High-Pass Plus™ size selection provides a sharp cut off at 20kb as indicated by a >20kb N50.