APPLICATION NOTE

Using the DNAFluid+ Kit for viscous DNA to effectively pre-condition high molecular weight DNA for consistent shearing

Introduction

In the last few years, the Diagenode Megaruptor has become an essential tool for the rapidly emerging long read sequencing technologies, including PacBio Single Molecule, Real-Time (SMRT) sequencing. The Megaruptor utilizes a unique technology including shearing "hydropore" arrays which enable consistent and highly flexible mechanical shearing of high molecular weight DNA. High molecular weight genomic DNA (HMW gDNA) is often viscous, leading to challenges in handling and the precision of shearing for long read workflows. Here we present a new method for preparation of viscous DNA using the robust DNAFluid+ Kit, which effectively pre-conditions HMW gDNA for consistent shearing across samples of varying viscosity. This method is used in conjunction with PacBio Circulomics Nanobind technology for HMW gDNA extraction and PacBio Circulomics Short Read Eliminator (SRE) for size selection to generate SMRTbell libraries for HiFi sequencing.

Methods

HMW gDNA was extracted from GM12878 cells using the Circulomics Nanobind CBB Big DNA Kit (NB-900-001-01) and following the protocol for HMW DNA Extraction – Cultured Cells (Document ID: EXT-CLH-001). First, the sample was conditioned using the DNA Fluid+ Kit (speed 59). Then, it was diluted to 50 ng/µL and sheared using the Megaruptor 3 shearing Kit (cat. no. E07020001) (speed 31) on the Megaruptor 3 system. PacBio HiFi sequencing was performed on the sheared DNA using a 30 hr movie, SMRTbell Express Template Prep Kit 2.0, Binding Kit 2.0, Sequel II Sequencing Kit 2.0 and Circulomics SRE XS (SS-100-121-01) Kit.

To facilitate size selection using SRE XS, the post-nuclease AMPure PB cleanup in the HiFi SMRTbell library preparation protocol was eluted in 11 µL rather than the standard 31 µL. This volume was further adjusted to give a concentration of 100 ng/µL. This DNA sample was then used as the input to the Size Selection Protocol for SRE XS on page 16 of the Short Read Eliminator Kit Family Handbook v2.0. An equal volume of SRE XS (in this case 28 µL) was added instead of the standard 60 µL in step 2, before mixing and proceeding to the centrifugation in step 3 of the protocol. The SRE pellet was re-suspended in 11 µL of PacBio EB.

Results

The DNAFluid+ kit was used to pre-condition inhomogeneous HMW DNA to allow effective downstream shearing on the Megaruptor 3 system. DNA extracted from 5M GM12878 cells was inhomogeneous, with a concentration measurement CV (n = 3) of approximately 30%. Inhomogeneity due to viscosity was also reflected in discrepancies between Qubit and Nanodrop concentration measurements (Table 1). DNA with this inhomogeneity and concentration often clogs the shearing hydropores on the Megaruptor 3 system, leading to inconsistent shearing which can be resolved by using the DNAFluid+ Kit.

After the DNAFluid+ pre-conditioning, the mode DNA size decreased from 148,000bp to 47,500 bp (Figure 1a) and the concentration measurement CV decreased to 8% for Qubit and < 1 % for Nanodrop. These metrics demonstrate the DNAFluid+ workflow's ability to homogenize DNA and condition it for downstream shearing and sequencing.

The PacBio HiFi read length distribution shown in Figure 1b was generated using this workflow: 1) Nanobind extraction 2) DNA Fluid+ conditioning 3) Megaruptor 3 shearing and 4) HiFi sequencing on a PacBio Sequel II. A mean HiFi read length of 18.3 kb and fewer than 1 and 7% of reads shorter than 5 and 10kb in length, respectively, are commensurate with the production of high data yield HiFi sequencing.

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	Concentration (ng/µl)				UV/Vis abs		Size (bp)
Step	Qubit dsDNA	stdev	Nanodrop	stdev	260/230	260/280	Femtopulse (mode)*
Extraction	196	57	469	141	1.86	2.17	148000
DNAFluid+ shear	398	33	415	1	1.86	2.15	47500
Dilute then shear	47	2	53	1	1.83	2.55	18800

Table 1. Concentration and size metrics of Nanobind extracted DNA for PacBio HiFi sequencing.

*Mode refers to the most frequently occurring length, the peak value in the Femto trace.

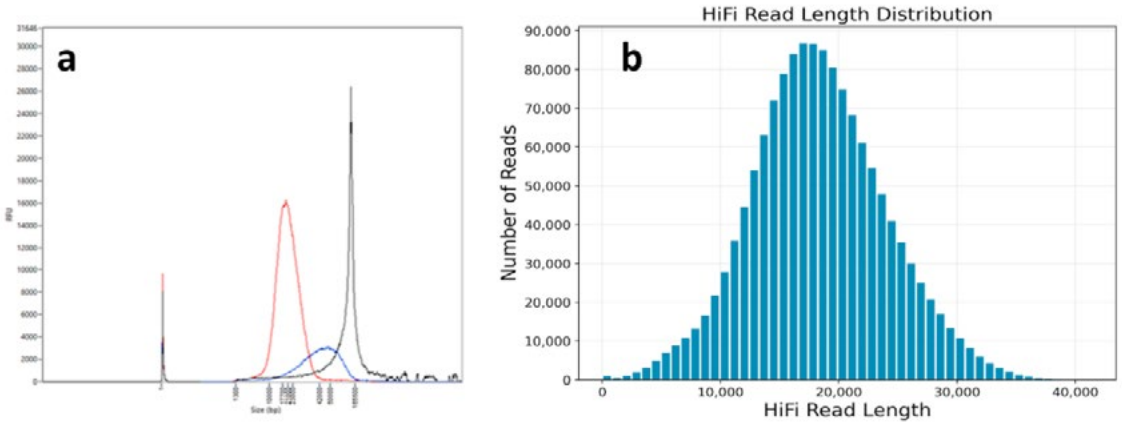

Figure 1. The Fluid+ and standard Megaruptor shearing modify the DNA size distribution in preparation for HiFi sequencing. a) Femto-Pulse traces showing the DNA size distributions as the DNA proceeds from extraction to library. FemtoPulse traces of: Nanobind extracted DNA (mode = 148000 bp), black trace;DNAFluid+ sheared DNA (mode = 47500 bp), blue trace; DNA diluted, then sheared at speed 29 (mode = 18800 bp), red trace. b) HiFi read length distribution. Total throughput = 26 Gb, Mean read length = 18300 bp.

Conclusion

These results show that PacBio Circulomics SRE XS is an excellent substitute for gel-based size selection and that the DNAFluid+ Kit works well to pre-condition inhomogeneous HMW DNA for Long Hydropore shearing without clogging. The DNAFluid+ Kit effectively pre-conditioned HMW gDNA to allow consistent shearing with the Megaruptor 3 Shearing kit (cat. no. E07020001) across samples of varying viscosity and showed excellent read length distribution of sequencing results.

References:

Short Read Eliminator Kit Family Handbook
https://www.circulomics.com/_files/ugd/5518db_411fba3c26c14b0189d33e9ade1da96f.pdf