Technical note

HIGH-THROUGHPUT DNA SHEARING FOR HIFI WHOLE GENOME SEQUENCING FROM WHOLE BLOOD SAMPLES

Introduction

Whole blood-derived DNA provides high-quality input for whole genome sequencing (WGS) with PacBio® HiFi technology but requires shearing prior to library preparation. This technical note describes one set of experimental conditions for shearing high molecular weight genomic DNA (gDNA) derived from human whole blood using MP Biomedicals' *FastPrep-96* instrument. These results demonstrate the *FastPrep-96* as a high-throughput DNA shearing option for WGS.

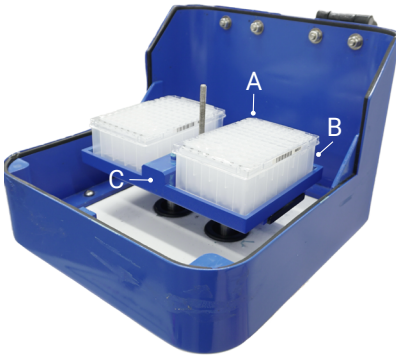

Figure 1. *FastPrep-96* plate setup. A) PCR plate B) 96 deep-well plate C) *FastPrep-96* adapter.

The *FastPrep-96* is a high-throughput, high-speed homogenizer capable of performing up to 1,800 oscillations per minute with a 1.5-inch stroke. The high forces created by the instrument can be used to shear DNA in solution without the need for adding beads. DNA is simply dispensed into a 0.2 mL hard-shell, semi-skirted PCR plate that is fitted on top of a 96 deep-well plate to secure it in the adapter (figure 1). Each adapter can hold two 96-well plates for a total processing throughput of 192 samples per run. Each run takes only a few minutes, and the resulting fragmented DNA can be used directly for library prep using the PacBio SMRTbell® prep kit 3.0 and protocol. Settings such as speed and time can be increased to produce smaller average fragment lengths, while increasing or decreasing the DNA concentration will increase or decrease the average fragment lengths respectively.

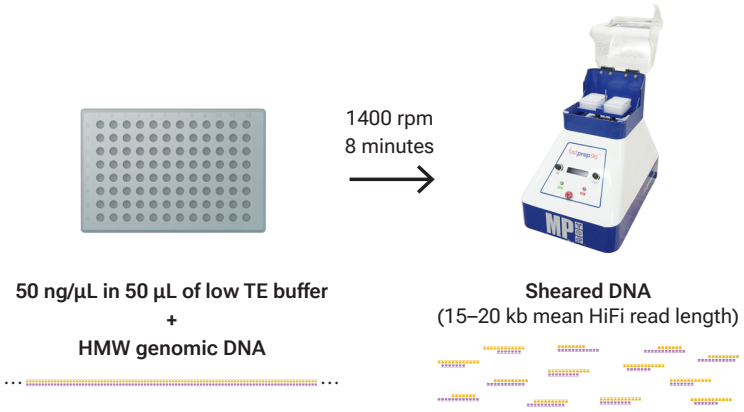

Figure 2. *FastPrep-96* workflow for sheared DNA.

Required materials and equipment

Material	Part number
FastPrep-96	MP Bio 116010500
MicroAmp 96-well clear reaction plate	Thermo Fisher 4483354
MicroAmp optical 8-cap strips	Thermo Fisher 4323032
96-deep-well plate	MP Bio 116010540 (*FastPrep 96* PCR plate holder for DNA shearing)
Centrifuge	Thermo Fisher 75004381 or similar

Protocol

1. Dispense ~2.5 µg of DNA suspended in low TE buffer (50 ng/µL x 50 µL) to each respective well on the 96-well PCR plate. Please note that using different DNA concentrations may result in varying average fragment sizes. It is recommended to normalize concentrations to produce consistent shearing profiles between samples.
2. Carefully seal the respective PCR plate with optical 8-cap strips. A tight seal is needed to prevent sample loss during shaking.
3. Fit the 96-well PCR plate into the top of 96-deep-well plate so that it is secure. The skirt of the PCR plate should be fastened around the edge of the deep-well plate.
4. For mean DNA fragment lengths of approximately 17.5kb, set the instrument to 1,400 rpm for 240 seconds.
5. Set the instrument to 1,400 rpm for 240 seconds and start the run.
6. Once the operation is complete, briefly spin down the PCR plate at 4,000 rpm for 1 minute to collect liquid at the bottom of the wells.
7. Proceed to QC on the *Agilent Femto Pulse* system or begin library prep using the PacBio SMRTbell prep kit 3.0. If not proceeding with library prep immediately, store samples at 4°C for up to two weeks.

Results

The following speed and time combinations (table 1) were tested with a human whole blood sample extracted according to the PacBio Nanobind® protocol. For each condition, aliquots of the sample were dispensed in wells across the PCR plate (A1, B10, C3, D6, E7, F10, G4, H12).

Speed (rpm)	Time (s)
1,400	240
1,400	480
1,800	240
1,800	480

Table 1. *FastPrep-96* speed and time combinations for shearing testing.

In addition to unsheared gDNA, all sheared samples at each of the *FastPrep-96* settings were sized on the *Agilent Femto Pulse* system. All positions showed nearly identical size profiles with the *Femto Pulse* system. Well B10 from each shearing condition was chosen for sequencing. SMRTbell prep kit 3.0 yields varied between 25 to 30%, which is well within the normal range as expected for manual library prep.

Libraries were pooled in an equal molar fashion and sequenced on the Sequel® IIe system using a single SMRT® Cell with an 85 pM loading concentration. Results from QC and sequencing (figures 3–4, table 2) show that the 1,400 rpm speed setting produced the optimal HiFi read length profiles. Shearing for 240 seconds produced a mean HiFi read length of 17,703 bp, while shearing for 480 seconds yielded a 16,855 bp mean read length. The faster 1,800 rpm setting at 240 and 480 seconds oversheared the DNA, resulting in lower mean HiFi read lengths of 13,184 bp and 11,658 bp respectively. Generally, when shearing DNA with the *FastPrep-96*, lower operating speeds for less time will result in larger mean fragment lengths.

Shearing conditions	Mean HiFi read count	Mean HiFi read length (bp)
1,400 rpm, 240 sec	474,152	17,703
1,400 rpm, 480 sec	595,335	16,855
1,800 rpm, 240 sec	552,835	13,184
1,800 rpm, 480 sec	582,245	11,658

Table 2. Mean HiFi read count and read length for each of the shearing conditions.

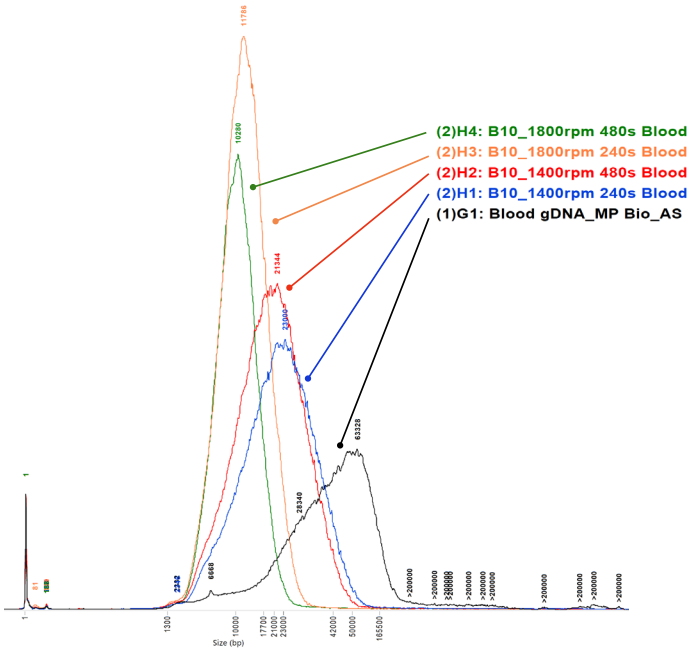

Figure 3. DNA fragment size distribution for each of the four shearing conditions and unsheared gDNA.

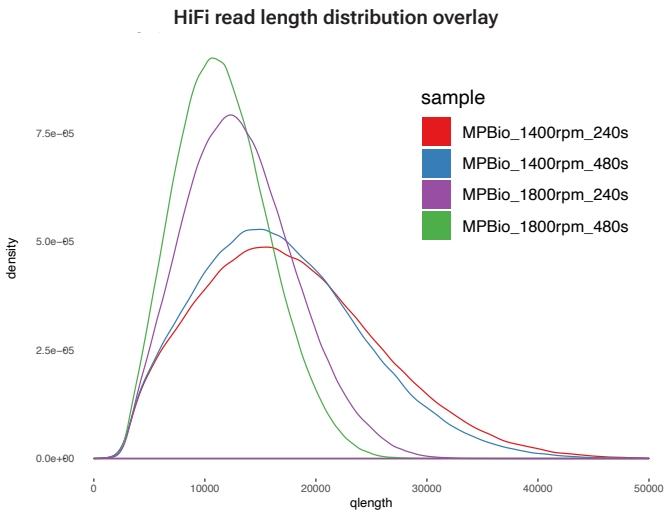

Figure 4. Base yield density by read length distribution for each of the four shearing conditions.

Conclusions

This technical note demonstrates the MP Bio *FastPrep-96* system's ability to shear HMW gDNA into the fragment sizes needed for HiFi whole genome sequencing. This simple workflow is time- and cost-efficient, and is an improved alternative to lower-throughput and more expensive consumable options. Instrument settings and DNA concentrations may need to be adjusted to fine-tune fragment size distributions for different sample types and/or applications.

For pricing and ordering information for the *FastPrep-96*, contact MP Biomedicals. For pricing and ordering information on for the SMRTbell prep kit 3.0, contact your local PacBio sales representative.

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