Fragmenting High Molecular Weight DNA for PacBio® Long-Read Sequencing Using Pipette Shearing

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ABSTRACT

DNA fragmentation is an important step in preparing DNA libraries for PacBio long-read sequencing. While there are many methods to fragment DNA, pipette shearing is one of the simplest methods that does not require specialized equipment or reagent kits. In this app note, we describe how to automate pipette shearing using the Opentrons Flex™ liquid handler.

Key Features

- Automated pipette shearing generates DNA fragments that meet specific requirements for PacBio sequencing.
- Automated pipette shearing is effective for both intact and degraded DNA samples.

INTRODUCTION

Long-read DNA sequencing with PacBio instruments has the benefits of providing less ambiguous assembly, easier library preparation, and faster sequencing runs compared to short-read sequencing. While both sequencing methods require fragmenting DNA before preparing libraries, PacBio long-read sequencing requires shearing DNA into larger fragments generally from 10 kb to 25 kb.

Fragment sizes appropriate for PacBio sequencing can be generated using mechanical methods, including sonication, nebulization, and hydrodynamic shearing. However, many mechanical methods use specialized equipment solely for the purpose of shearing DNA.

Pipette shearing can replace the need to use specialized equipment. Because pipette shearing can involve hundreds of mixes, it lends itself well to automation.

Here, we demonstrate how pipette shearing breaks high molecular weight (HMW) DNA into fragments suitable for PacBio long-read library preparation using intact and degraded DNA as starting material.

METHODS

Sample preparation:
HMW DNA was isolated from blood using the Nanobind HMW DNA extraction kit (PacBio, Cat. No. 103-260-000). Degraded DNA was extracted from the NA12878 cell line.

DNA fragmentation:
The DNA was fragmented using pipette shearing on the Opentrons Flex liquid handling robot. The DNA was diluted in Buffer LTE (PacBio, Cat. No. 103-228-900) and placed in a Nest 2 mL Deepwell plate (Opentrons, Cat. No. 999-00103). Shearing was performed as indicated in Table 1.

Table 1. Pipette shearing conditions.

DNA concentration	10 ng/µL
Total Volume	300 µL, 500 µl
Pipette speed	1000 µL/s
Volume pipetted	200 µL
Number of mixes	800 cycles

Data analysis:
DNA before and after shearing was analyzed using automated pulsed-field capillary electrophoresis system on a Femto Pulse (Agilent, PN: M5330AA) instrument using the gDNA 165kb Analysis kit, 275 samples (Agilent, Cat No. FP-1002-0275).

RESULTS AND DISCUSSION

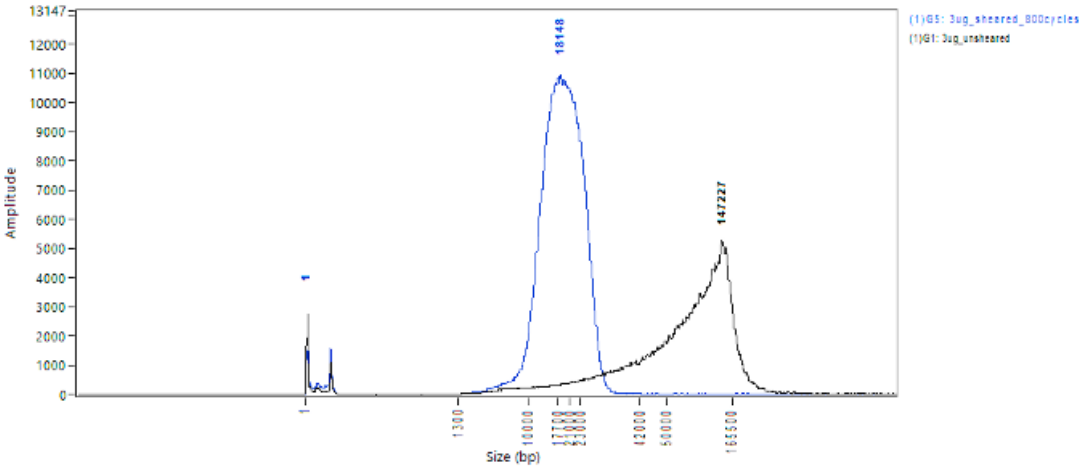
We fragmented 3 µg and 5 µg samples of HMW DNA with pipette shearing and analyzed their fragment lengths before and after shearing. The 3 µg and 5 µg samples were diluted in 300 µL and 500 µL of Buffer LTE, respectively.

In assessing the quality of DNA shearing, we focused on the genomic quality number (GQN), which gives an indication of how intact the DNA is, and specific size and distribution requirements for PacBio long-read DNA fragments. These requirements include:

- Mean fragment size between 15-20 kb
- Narrow distribution between 10-30 kb
- Approximate range of sheared fragments 5-40 kb

Intact HMW DNA samples used in this study had GQN of 8 (for the 3 µg sample) and 8.8 (for the 5 µg sample) at a 30 kb threshold. DNA after shearing met all requirements specified above and indicates that pipette shearing provides DNA fragments of a suitable size for PacBio sequencing (Figure 1).

(A) 3 µg HMW DNA, before and after shearing



(B) 5 µg HMW DNA, before and after shearing

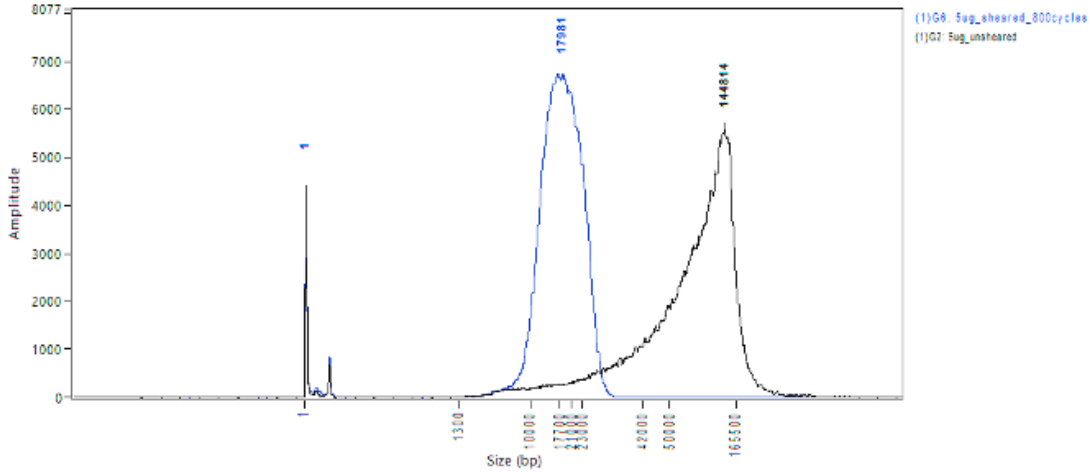
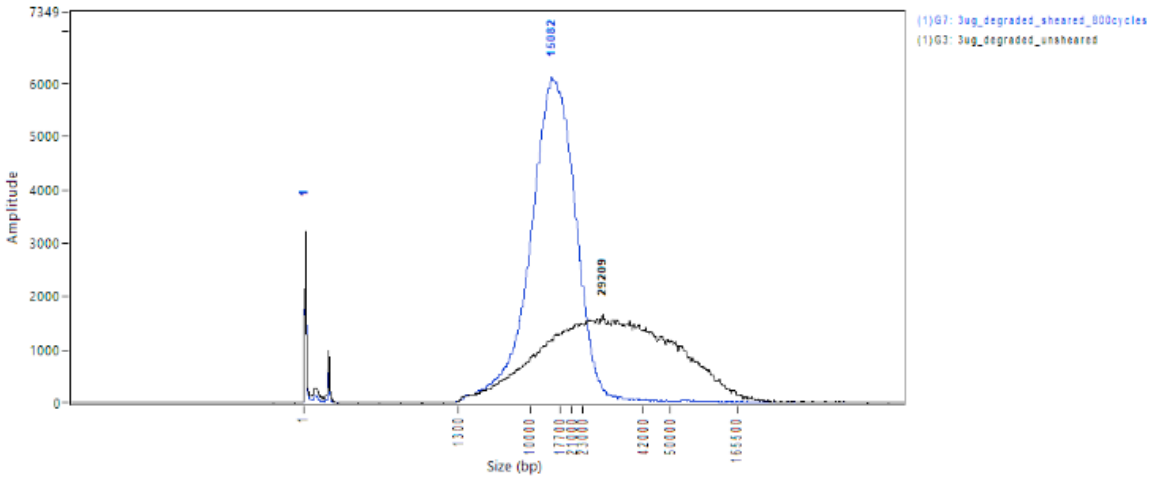


Figure 1. HMW DNA before and after shearing. (A) 3 µg HMW DNA before (black) and after (blue) shearing. After shearing, the DNA had an average fragment size of 18,337 bp. (B) 5 µg HMW DNA before (black) and after (blue) shearing. The average fragment size after shearing was 17,665 bp.

Because DNA samples can often come from less-than-optimal materials, it is likely that the DNA can degrade before shearing. One example of this is DNA from formalin-fixed paraffin-embedded tissues. To simulate if pipette shearing is suitable for these applications, we analyzed how degraded DNA performs using 3 µg and 5 µg samples, both with a GQN of 4.5 at a 30 kb threshold. Shearing from these samples also met the fragment size QC requirements for library construction (Figure 2).

(A) 3 µg degraded DNA, before and after shearing



(B) 5 µg degraded DNA, before and after shearing

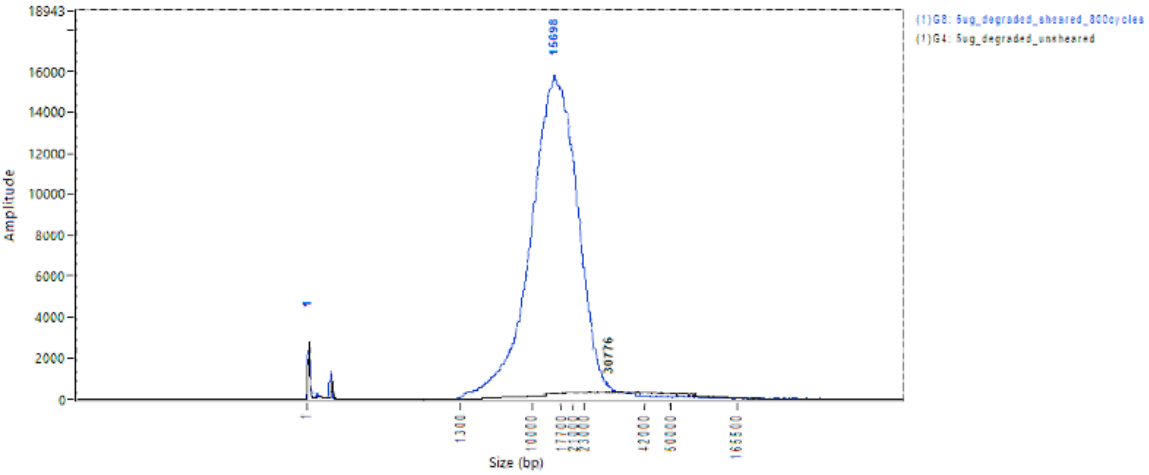


Figure 2. Degraded genomic DNA before and after shearing. (A) 3 µg degraded DNA before (black) and after (blue) shearing. The average fragment size after fragmenting was 15,751 bp. (B) 5 µg degraded DNA before (black) and after (blue) shearing. Fragmented DNA had an average size of 15,916 bp.

CONCLUSION

We found that automated pipette shearing of DNA using the Opentrons Flex is a viable alternative to other mechanical shearing methods. Fragmenting DNA from both HMW and degraded DNA samples met the specific requirements for DNA fragment sizes for PacBio sequencing.