Increased control over longer DNA fragmentation patterns using lower temperature with sparQ DNA Frag & Library Prep Kit

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ABSTRACT

Sequencing on Illumina and other short-read sequencing platforms often require DNA fragments between 250 to 550 bp. Longer DNA fragments benefit applications such as de novo genome assembly as well as the sequencing of repetitive and homologous regions of the genome. Enzymatic fragmentation of high input (500 ng or higher) DNA samples takes less time to achieve longer fragments, making it difficult to control the fragmentation pattern. In this application note we describe how fragmentation of high input DNA samples at a lower temperature provides better control over fragmentation patterns without introducing bias.

INTRODUCTION

Short-read sequencing technology requires fragmenting DNA into small and uniform sizes (250 to 550 bp) in order to sequence the entire genome of interest. Historically, DNA was sheared using mechanical fragmentation methods like ultrasonication which is expensive and unamenable for high-throughput library preparation. In contrast, enzymatic DNA fragmentation methods enable fully automated, high-throughput library preparation in a cost saving manner.

The sparQ DNA Frag & Library Prep Kit from Quantabio was developed to overcome limitations of other enzymatic fragmentation technologies like sequencing bias, lack of reproducibility and loss of sample complexity. The enzymatic fragmentation method incorporated in the sparQ DNA Frag & Library Prep Kit is tunable and highly reproducible. The fragmentation reaction within the kit is optimized at 32°C. The length of the fragmentation reaction and the amount of input DNA predominantly determine the DNA fragment size. At a certain fragmentation time, the DNA fragment size is inversely proportional to the amount of input DNA. Therefore, it is challenging to control the fragmentation profile of high input DNA (500 to 1000 ng) samples when trying to achieve longer fragment size.

Overcoming the above mentioned challenge is especially important in several applications, such as de novo genome assembly where longer insert sizes are required, and also in PCR-free library preparation where higher amounts of input DNA are required. In this application note, we demonstrate that by reducing the temperature of the fragmentation reaction of high input DNA samples to 25°C, we slowed down the fragmentation kinetics without introducing GC bias and providing better control over the fragmentation time to generate reproducible fragmentation patterns.

METHODS

Input DNA

Mixed bacterial genomic DNA (1:1:1 ratio: *Fusobacterium nucleatum*, *Escherichia coli* and *Bordetella pertussis*, from ATCC) and high molecular weight human genomic DNA (hgDNA,11691112001, Roche) were used for this study.

Library preparation

Either 500 ng mixed bacterial genomic DNA or 1,000 ng hgDNA was used as input for the library preparation using sparQ DNA Frag & Library Prep Kit. DNA samples were fragmented into different sizes by incubating the mixture of DNA samples and DNA Frag & Polishing master mix either at 32°C as described in the product manual (IFU-122.1_REV_05_95194_sparQ_DNA_Frag_Library_Prep_Kit_1120), 27°C (data not shown) or at 25°C in a thermocycler. PCR-free libraries were prepared by adding 0.5 µM sparQ UDI adapters in the presence of DNA Ligation master mix and incubating the ligation reaction at 20°C for 15 min. Libraries were then purified using sparQ PureMag Beads and eluted in 10 µl elution buffer (10 mM Tris-HCl, pH 8.0).

Library quantification and validation

To verify the size of the fragmented DNA, 1 µl of the fragmented DNA was run on a D5000 ScreenTape on the 4200 TapeStation® System (Agilent).

PCR-free libraries with Y-shaped adapters do not migrate in TapeStation or Bioanalyzer® electrophoretic traces according to its size (please see the application note entitled "Quality Control for PCR-free WGS libraries" for further details). To better assess the size range of the libraries,1 µl of the PCR-free libraries was amplified for 2 to 6 PCR cycles and 1 µl of the amplified and purified libraries was run on D5000 ScreenTape on the 4200 TapeStation System (Agilent).

Libraries were quantified using Qubit® dsDNA BR Assay Kit (Thermo Fisher Scientific) using manufacturer's recommendations: 2 µl purified library was added to 198 µl of Qubit working solution and mixed by vortexing. The tubes were incubated at room temperature for 2 min before loading the tubes into a Qubit 3.0 Fluorometer.

Sequencing

Libraries were normalized to 4 nM using EB buffer (QIAGEN®) and then pooled together. Library pools were sequenced using MiSeq® Reagent Kit v2 (300 cycles, # MS-102-2002, Illumina) according to the manufacturer's recommendations.

Data analysis

Sequencing data was analyzed with CLC Genomics Workbench 20.0.4 software (QIAGEN®).

RESULTS

Effect of lower temperature on fragmentation kinetics

In order to understand the effect of enzymatic fragmentation at lower temperature on the DNA fragmentation pattern, 1 µg hgDNA samples were fragmented at different time lengths either at 32°C as mentioned in the product manual or at 25°C.

Fragmentation at lower temperature (25°C) clearly slowed down fragmentation kinetics as shown in Figure 1. For example, fragmentation of hgDNA to an average fragment size of 550 bp took only 5 minutes when fragmentation was done at 32°C. In contrast, fragmentation at 25°C took approximately 12 min to achieve the same average fragment size. By slowing down the fragmentation reaction, it is possible to achieve better control over the fragmentation, especially when larger fragments are needed from higher input DNA.

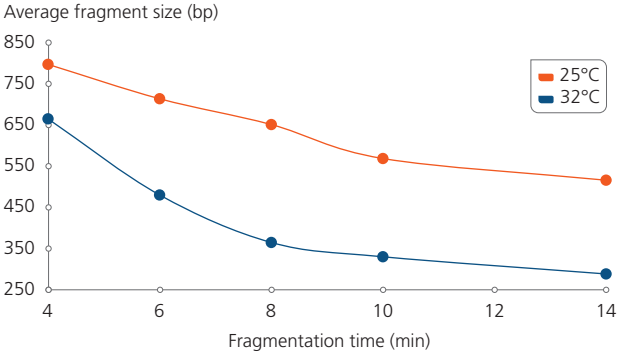

Figure 1 **Fragmentation at lower temperature slowed down fragmentation kinetics.**

Effect of low temperature on final library size

In order to check if enzymatic fragmentation at lower temperature has any effect on overall library size distribution, PCR-free libraries were prepared by ligating sparQ UDI Adapters following the protocol as mentioned in the product manual. Before analyzing on the TapeStation, 1 µl of the PCR-free libraries was amplified with 2 PCR cycles.

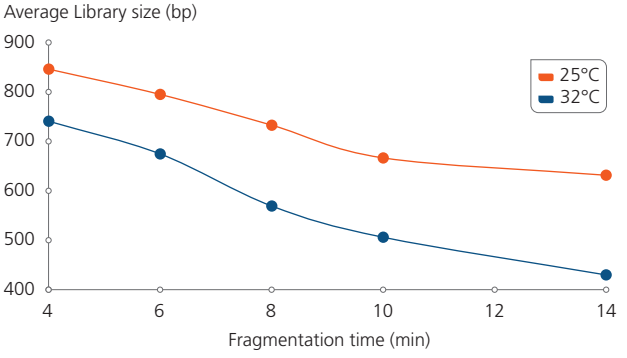

Figure 2 **Fragmentation at a lower temperature produces libraries with a larger average size than samples processed with the same fragmentation time at 32°C.**

Ligation of sparQ UDI Adapters increases the size of the DNA fragments by approximately 120 bp. As shown in figure 2, the size of the libraries was on average 120 bp larger than the fragment size shown in figure 1. Overall, adapter ligation was not inhibited by low temperature DNA fragmentation.

GC coverage with low temperature fragmentation

Many enzymatic fragmentation technologies suffer from sequence bias introduced during fragmentation. Quantabio developed a robust and tunable enzymatic fragmentation technology that causes minimal to no sequencing bias at the enzymatic fragmentation step. In order to evaluate if fragmentation at a lower temperature (25°C) generated any sequencing bias, 500 ng mixed bacterial genomic DNA (1:1:1 ratio: *Fusobacterium nucleatum*, *Escherichia coli* and *Bordetella pertussis*) was used as input. This mixed bacterial genomic DNA approach is critical to understand GC bias over a wide range of GC-content (10 to 85%).

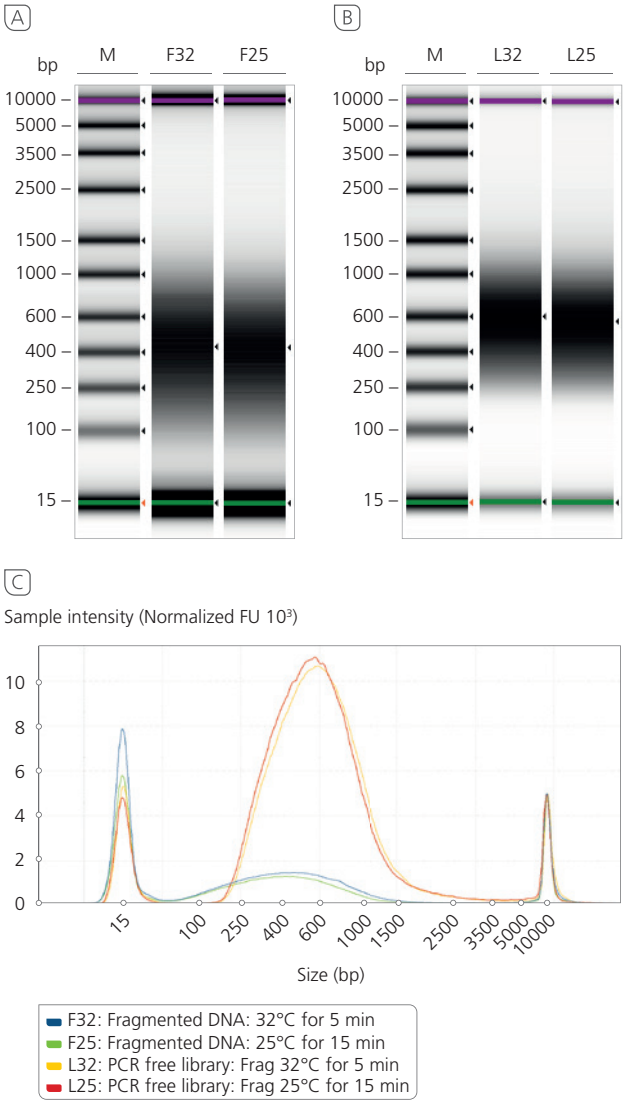

Figure 3 **Library preparation with mixed bacterial genomic DNA.** **A** 500 ng mixed bacterial genomic DNA was fragmented either at 32°C for 5 min (F32) or 25°C for 15 min (F25). **B** Libraries after 6 PCR cycles. **C** Size distribution of the fragmented DNA and amplified libraries.

Fragmentation of 500 ng mixed bacterial genomic DNA was performed at either 32°C for 5 min or 25°C for 15 min in order to achieve fragment sizes between 400-450 bp (Figure 3A). PCR-free libraries were prepared by ligating sparQ UDI adapters. To check the size of the libraries in TapeStation, 1 µl PCR-free libraries were amplified for 6 PCR cycles, followed by a PCR Cleanup step and then analyzed by TapeStation. The size of both libraries was between 550 to 600 bp (Figure 3B).

In order to check if a lower temperature fragmentation has any effect on the library yield, concentrations of each library were measured using Qubit dsDNA BR Assay Kit. The average yield of libraries obtained from the DNA samples fragmented at 32°C and 25°C was 710 (±65) and 790 (±55) ng, respectively.

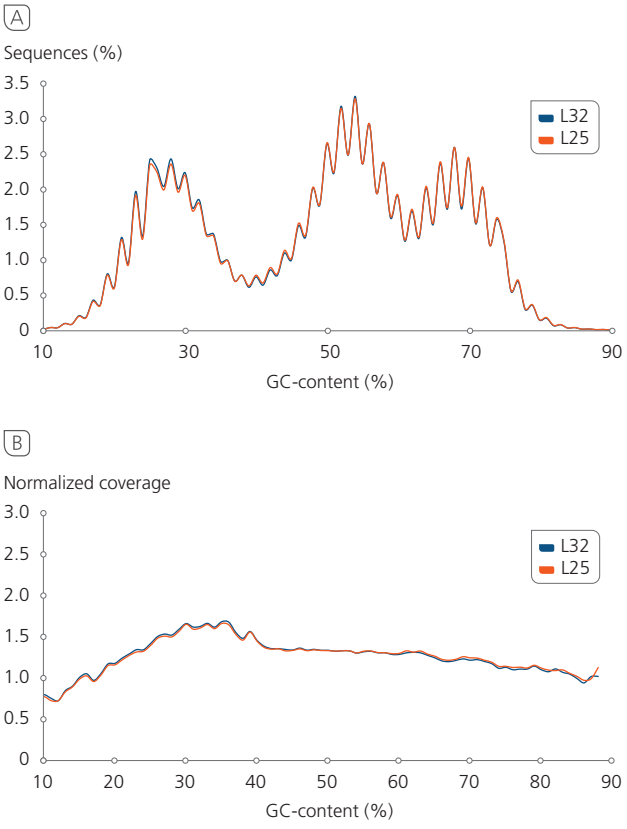

Figure 4 **Lower temperature fragmentation does not incorporate GC bias.** **A** Distribution of GC-content: the GC-content of a sequence is calculated as the number of GC-bases compared to all bases. **B** Mapped normalized coverage over GC-content.

CONCLUSIONS

DNA library preparation using sparQ DNA Frag & Library Prep Kit enables unbiased enzymatic fragmentation by incubating the DNA at 32°C for a desired period of time. This enzymatic fragmentation is both tunable and reproducible. In order to achieve longer fragments using higher input DNA samples, only a few minutes (1-5 min) incubation at 32°C is sufficient for fragmentation. However, it is often difficult to manage these shorter fragmentation times to reproducibly achieve the desired longer fragments. Fragmenting high input DNA samples at 25°C slows down the fragmentation process without introducing sequencing bias, allowing for greater reproducibility of larger inserts and more flexibility in the library preparation. This method can be adopted for high input DNA samples, especially when there is a need for longer DNA fragments.

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