Quality Control for PCR-Free WGS Libraries: A Guideline for TapeStation® Analysis of PCR-Free Library Preparation with Y-Shaped Adapters

Keywords: PCR-free library, quality control, Next Generation Sequencing, Y-shaped adapters, sparQ DNA Frag & Library Prep Kit, sparQ DNA Library Prep Kit, sparQ PureMag Beads

ABSTRACT

Quality control (QC) of PCR-free libraries using electrophoresis usually results in unreal library sizes due to large library sizes due to the structural features of Y-shaped adapters. In this application note, we describe a method to accurately determine the size of PCR-free libraries by adding a short PCR amplification step prior to automated DNA fragment analysis.

INTRODUCTION

Library quality control is an essential step in next-generation sequencing (NGS) workflows to ensure successful sequencing outcomes. QC analysis checks for DNA fragment length, yield, library size distribution and whether impurities like adapter dimers or other unwanted DNA fragments are present within the library. A gold standard method for library size distribution QC is the use of automated electrophoresis system like the TapeStation or Bioanalyzer® system (Agilent®), where DNA fragment migration serves as visual confirmation of sample quality.

One common library preparation method is to use a PCR-free library protocol, which can be useful when sequencing genomic regions with biased base composition (high GC or AT-content). The elimination of the PCR amplification step from the NGS workflow reduces nucleotide misincorporation and improves genome assembly, but the QC analysis of these libraries presents some challenges. PCR-free libraries with Y-shaped adapters migrate at a slower rate during electrophoresis compared with double-stranded size marker fragments due to their open and single stranded ends (Figure 1). Therefore, these libraries would require further analysis to generate accurate QC information.

In this application note, we present a method to conduct an accurate QC analysis of PCR-free libraries using the sparQ DNA Frag & Library Prep Kit or the sparQ DNA Library Prep Kit, without incurring additional cost for library preparation. HiFi PCR Master Mix and Primer Mix (included with the sparQ DNA library prep kits) were used to run a short PCR amplification on an aliquot of 1 µl of the PCR-free library before analyzing the PCR product on the TapeStation. The PCR cycling linearizes the library fragments by generating complementary double-stranded ends from the template libraries, resulting in optimal migration and showing the true fragment size of the PCR-free library on the TapeStation (Figure 1).

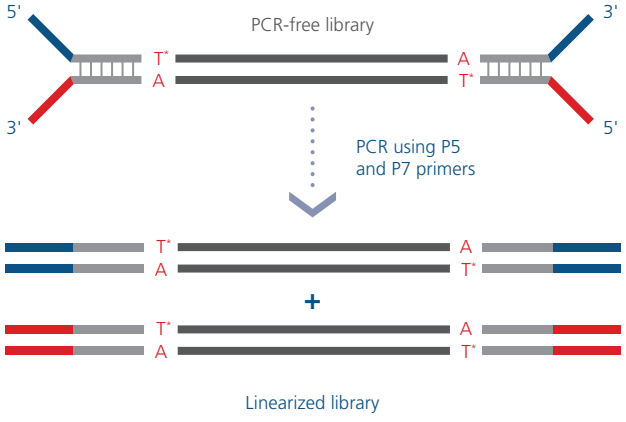

Figure 1 Exposed Y-shaped adapters become fully double stranded after PCR cycling.

METHODS

Sample input

Streptococcus agalactiae genomic DNA was isolated from single-colony bacterial cultures using QIAamp® DNA Mini Kit (QIAGEN®). For library preparation, a range between 400 and 1000 ng of isolated DNA was used as input.

Library Preparation

Libraries were prepared using the sparQ DNA Frag & Library Prep Kit following the product manual (IFU-122.1_REV_05_95194_sparQ_DNA_Frag_Library_Prep_Kit_1120). DNA samples

were fragmented for 3 min at 32°C to achieve a fragment size larger than 450 bp. PCR-free libraries were prepared by adding 0.5 µM sparQ UDI Adapters in the presence of DNA Ligation master mix. The ligation reaction was incubated at 20°C for 15 min. PCR-free libraries were purified using sparQ PureMag Beads and eluted in 10 µl elution buffer (10 mM Tris-HCl, pH 8.0).

Library QC

1. A short PCR amplification run was performed with 1 µl of the purified PCR-free library using HiFi PCR Master Mix (2X) and Primer Mix, components of the sparQ DNA Frag & Library Prep Kit. PCR master mix was prepared for each sample library according to Table 1.

Component	Volume for 1 sample (µl)
HiFi PCR Master Mix (2X)	10
Primer Mix	0.6
Nuclease-free H_2O	8.4
PCR-free library	1
Total	20

Table 1 **PCR master mix.**

2. The PCR was run according to the cycling program detailed in Table 2.

Step	Temperature	Incubation Time	Cycles
Initial denaturation	98°C	2 min	1
Denaturation	98°C	20 sec	2–6
Anneal	60°C	30 sec	
Extend	72°C	30 sec	
Final extension	72°C	1 min	1
Hold	4°C	Hold	1

Table 2 **PCR cycling conditions.**

3. The size of the PCR-free library was verified by running 1 µl of the PCR product on a D1000 ScreenTape on the 4200 TapeStation System. The expected size of the library was ~600 bp.

RESULTS

An enzymatic-based fragmentation was performed, as the first step of the sparQ DNA Frag & Library Prep Kit, on eight samples of bacterial genomic DNA with different DNA input amounts (400 – 1000 ng). The fragmentation time and temperature, 3 minutes and 32°C respectively, were chosen to target 450-550 bp fragments. The fragmented DNA samples were then analyzed on the TapeStation system. All eight samples had an expected migration pattern, showing an average size of 500 bp (Figure 2A). Similarly, the electropherogram showed a fragment size distribution between 450 to 550 bp across all samples (Figure 2B).

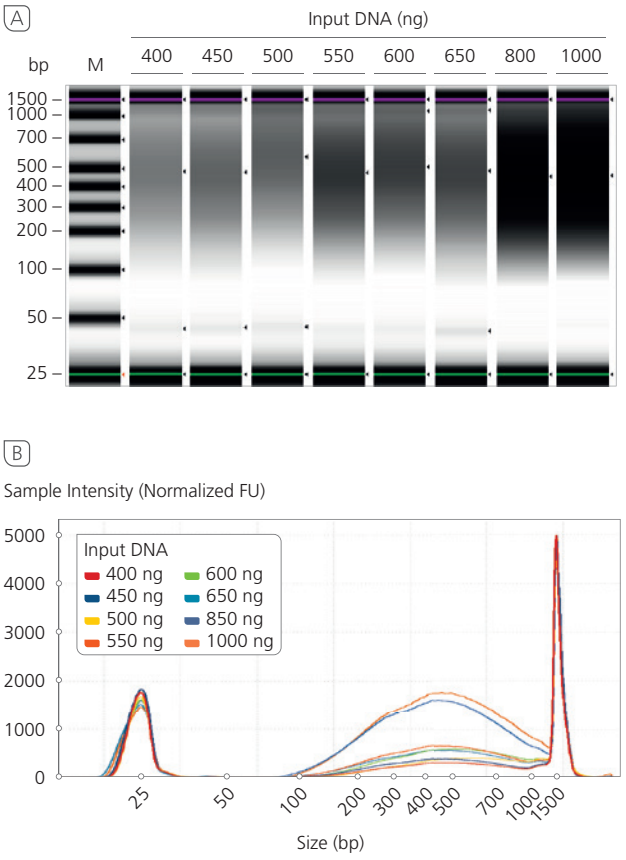

Figure 2 **Size distribution of fragmented DNA samples.**
A Gel electrophoresis of bacterial genomic DNA at different input amounts after enzymatic fragmentation. Samples were run on a D1000 ScreenTape on the 4200 TapeStation System. **B** Electropherogram of the fragmented bacterial genomic DNA at different input amounts.

After verifying the size distribution of these samples after fragmentation, the library preparation process continued with the ligation step using sparQ UDI Adapters (Y-shaped adapters for Illumina® sequencing platforms). Based on Figure 2, the expected full length of the libraries should be ~600 bp. However, TapeStation analysis showed two different bands, one at approximately 800 bp and the second one close to 1100 bp (Figure 3A). Therefore, these PCR-free libraries did not migrate properly in the gel electrophoresis system which resulted in larger library sizes and a multimodal size distribution (Figure 3B).

To accurately QC PCR-free libraries, a short PCR amplification run (2 – 6 cycles) was added using 1 µl of PCR-free library samples and the HiFi PCR Master Mix to ensure that the ends of the PCR-free libraries were fully complementary and double-stranded (Table 2). The PCR products were run on the TapeStation, which confirmed the expected average library length of ~600 bp and a monomodal library distribution with the majority of fragments between 550 – 600 bp as shown in Figure 4.

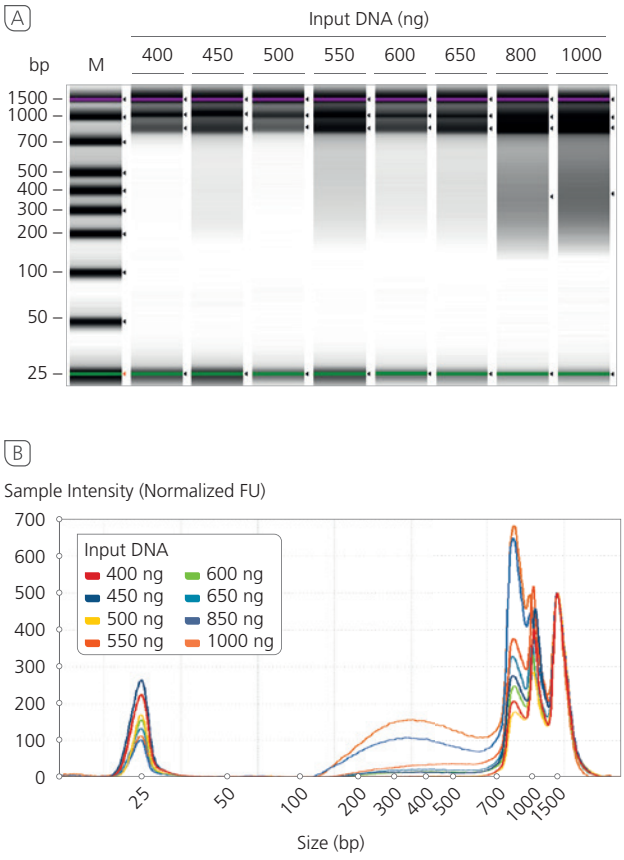

Figure 3 **Size distribution of PCR-free libraries.** **A** Gel electrophoresis of PCR-free libraries run on a D1000 ScreenTape on the 4200 TapeStation System. **B** Electropherogram of PCR-free libraries at different input amounts.

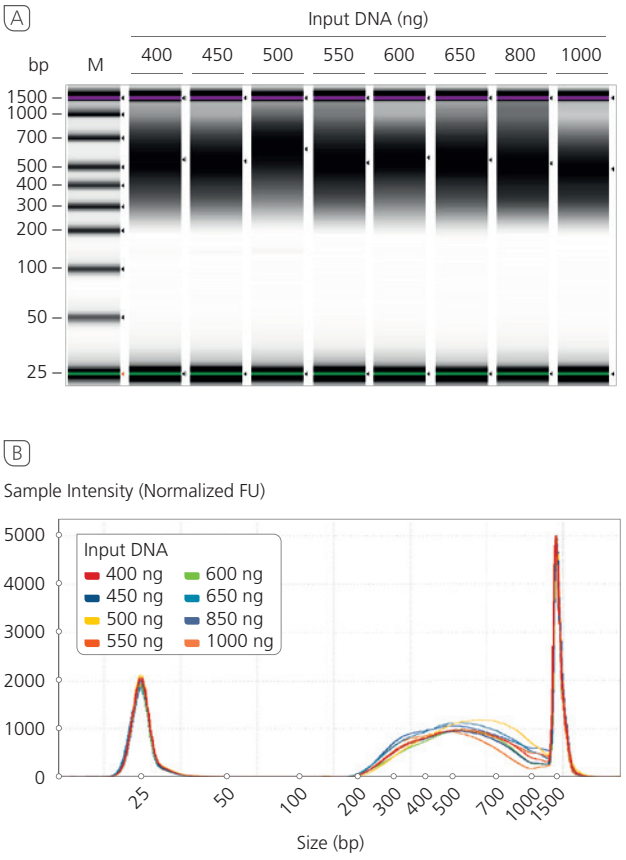

Figure 4 **Accurate size distribution of PCR-free libraries after a short PCR amplification.** **A** Gel electrophoresis of the PCR products from 1 µl of the amplified libraries run on a D1000 ScreenTape. **B** Electropherogram of the PCR products at different input amounts.

CONCLUSIONS

In this application note, we have detailed a protocol to accurately QC PCR-free libraries with Y-shaped adapters. This method offers the advantage of determining the library size distribution and quality of PCR-free libraries on the TapeStation system. Moreover, this method does not require additional cost since all the components needed for this QC analysis are included as part of the sparQ DNA Frag & Library Prep Kit and the sparQ DNA Library Prep Kit. Once the size distribution has been properly characterized, qPCR library quantification analysis can be performed to accurately determine the concentration and yield of the PCR-free libraries as the final QC step before sequencing.

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