Application Note

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Genomic DNA Extractions Compared with the Agilent Femto Pulse System

Authors

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Abstract

The Agilent Femto Pulse system's revolutionary design presents the only instrument capable of automating pulsed-field gel electrophoresis (PFGE) analysis of high molecular weight (HMW) gDNA. The Femto Pulse system enables qualitative analysis of gDNA size and integrity in a fraction of the time compared to PFGE analysis. Advances in the third-generation sequencing or long-read sequencing platforms for applications ranging from small genome analysis to *de novo* assembly of complex genomes impose high-quality standards for HMW gDNA samples. Various methods are available for isolation of gDNA from eukaryotic and prokaryotic sources that are tailored towards the specific downstream application. Therefore, the size and quality of the isolated gDNA is affected by the different extraction methods, driving a need for reliable quality assessment of gDNA samples. The Agilent Genomic DNA 165 kb kit was designed for separation of HMW gDNA on the Femto Pulse system, providing accurate and reproducible sizing and quality assessment. Sizing and quality of gDNA is compared between five different gDNA extraction methods on the Femto Pulse system with the Genomic DNA 165 kb assay.

Introduction

Large-insert libraries such as those used for 10X Genomics, Oxford Nanopore, and PacBio Technologies require specific quality conditions of the starting genomic DNA (gDNA) material for successful outcomes. Several factors affect sample quality, including: extraction method, sample storage, repeated freeze-thawing cycles, and denaturation[1]. Physical, enzymatic, and chemical shearing of gDNA into smaller fragments can occur at many different points during extraction and handling of gDNA. Therefore, accurate and reliable assessment of gDNA quality is essential for the success of these downstream sequencing processes. The gDNA quality is assessed in part by determining the degree of fragmentation and degradation of the sample. Typically, overnight pulsed-field gel electrophoresis (PFGE) has been utilized to assess the integrity of high molecular weight (HMW) gDNA. The Femto Pulse system with the Genomic DNA 165 kb kit is the only solution on the market capable of replacing PFGE with an automated separation system for assessing HMW gDNA in as little as 70 minutes.

Experimental

Yeast *Saccharomyces cerevisiae* strain BY4741 (Dharmacon, p/n YSC1048) was grown in YPD broth (ThermoFisher Scientific, p/n A1374501) for 24 hours. The cells were harvested by centrifugation and gDNA was extracted following several protocols. Method A followed the classical phenol-chloroform-isoamyl alcohol (25:24:1) (Thermo Fisher Scientific, p/n AC327111000) extraction protocol[2] and methods B, C, D, and E were performed according to the manufacturer's instruction from four different commercial kits: Wizard genomic DNA purification kit (Promega Life Sciences, p/n A1120), Quick-DNA universal kit (Zymo Research, p/n D4068T), MegaLong gDNA kit (G-Biosciences, p/n 786-146), genomic-tip 20/G, and genomic DNA buffer set (Qiagen, p/n 10223 and 19060). Method B was a liquid extraction, C and E were a spin column extraction, and method D was a membrane extraction. Extracted gDNA was separated by PFGE and on the Agilent Femto Pulse system (p/n FPv1-CE2) with the Agilent Genomic DNA 165 kb kit (p/n FP-1002-0275) utilizing either the FP-1002-22 gDNA 165 kb method (fast method) or FP-1002E22 extended gDNA 165 kb method (extended method). The Genomic DNA 165 kb fast and extended methods are selected in the Femto Pulse system operational software. Approximately 50 ng/µL of gDNA was loaded onto a 0.8 % agarose gel (Lonza SeaKem Agarose, p/n 50152) and separated by PFGE with the Pippin-Pulse preset protocol, 5 to 150 kb, for 18 hours (Sage Sciences). The Agilent FP 165 kb Ladder (165, 50, 42, 23, 21, 17.7, and 10 kb) (p/n FP-7002-U035) and low range PFG marker (New England Biolabs, p/n N0350S) was used. After separation, the agarose gel was poststained with Agilent FP Intercalating Dye (p/n FP-6001-U030) and visualized with a UV transilluminator.

Results and Discussion

Sizing with the fast and extended Genomic DNA 165 kb methods on the Femto Pulse system

The Femto Pulse system software offers two separation methods for the Genomic DNA 165 kb assay. The fast method is a 70-minute pulsed-field capillary electrophoresis (CE) separation that is best suited for separation and sizing of gDNA smears up to 80 kb. The extended method features a different pulsed-field CE separation method that lasts for 3.5 hours and is specialized for enhanced separation and sizing of gDNA smears greater than 80 kb. Genomic DNA greater than 80 kb, separated with the fast method, will be displayed as a sharp, compact peak similar to a DNA fragment trace around 165 kb[3]. This is due, in part, to the amount of time available to resolve the sample during pulsed-field separation. The extended method utilizes an alternative pulsed-field CE method over an extended separation time and will display the same gDNA sample as a wider smear that represents the entire size range of the sample. The extended method is recommended for gDNA samples greater than 80 kb that separate on the fast method as a compact peak around 165 kb.

The Femto Pulse system Genomic DNA 165 kb extended method was utilized to accommodate separation of extracted gDNA greater than 80 kb (Figure 1). Sizing of the gDNA was analyzed with the Smear Analysis tab on Agilent ProSize data analysis software. The smear size accounts for the distribution of the concentration over the entire smear range. The yeast gDNA smear size and integrity varied depending on the extraction method used. Genomic DNA samples decreased in smear size with the extraction methods A, B, C, D, and E (Table 1). The liquid extraction methods A and B resulted in the largest intact gDNA smear. Genomic DNA extracted with the traditional liquid phenol-chloroform extraction method (A) produced larger intact gDNA, averaging 163,670 bp, compared to the other liquid extraction method B at 103,111 bp. Method C, a spin column gDNA extraction method, and method D, a membrane gDNA extraction method, displayed similar smear sizing results in the middle of the five different extraction methods.

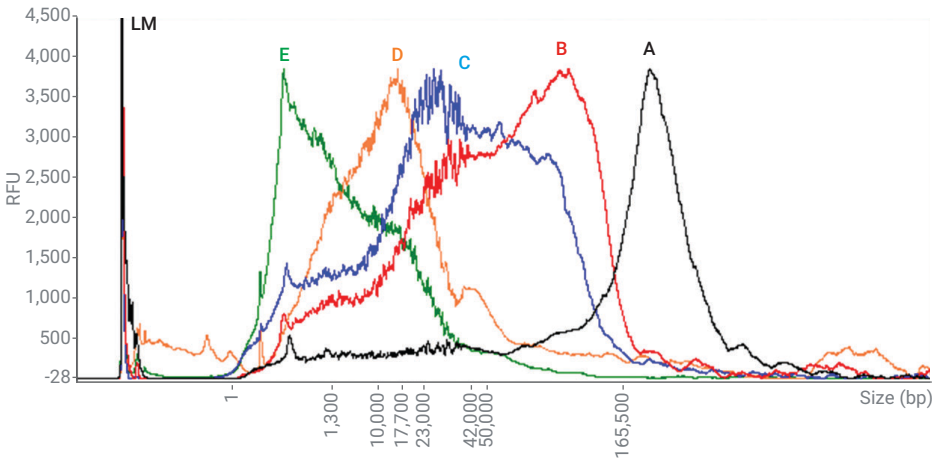

Figure 1. Electropherogram traces from the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit extended method. Yeast *Saccharomyces cerevisiae* gDNA was extracted by five different methods: A) phenol chloroform; B) liquid extraction; C) and E) spin column extraction; and D) membrane extraction. LM = lower marker.

Table 1. Average smear size, percent CV, and $GQN_{50\,kb}$ of yeast *Saccharomyces cerevisiae* gDNA from five different extraction methods analyzed on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. *n = 3.

Extraction Method	Average Size* (bp)	% CV of Size	Average $GQN_{50\,kb}$*
A	163,670	1.9%	8.2
B	103,111	1.2%	7.4
C	76,778	2.8%	6.1
D	62,165	5.1%	3.0
E	27,388	4.7%	1.1

Method E, a spin column gDNA extraction method, when analyzed using the Genomic DNA 165 kb extended method resulted in an electropherogram trace with the entire gDNA sample less than 80 kb. Therefore, the method E sample was analyzed using both the fast and extended separation methods for comparison (Figure 2). Both methods assigned similar smear sizes to sample E of 30,579 and 27,388 bp (fast method and extended method, respectively). In this example, the 1-hour fast method would be favored over the extended method due to a shortened run time.

Femto Pulse system comparison to pulsed-field gel electrophoresis (PFGE)

In the past, a 16 to 18-hour PFGE analysis was the only option for determining quality and size of gDNA over 50 kb. With advances in technology, the Femto Pulse system is the only instrument capable of assessing the size and quality of gDNA up to 165 kb, in as little as 70 minutes. Replacing overnight PFGE with the Femto Pulse system eliminates several days dedicated to quality control checks, thus saving time and money. ProSize data analysis software automatically provides peak size, an electropherogram, and a digital gel image for quick determination of sample quality and size. In contrast, PFGE runs require user assessment with additional software to acquire a calculated size. In addition, the Femto Pulse system reduces sample input to picogram levels, saving precious sample material.

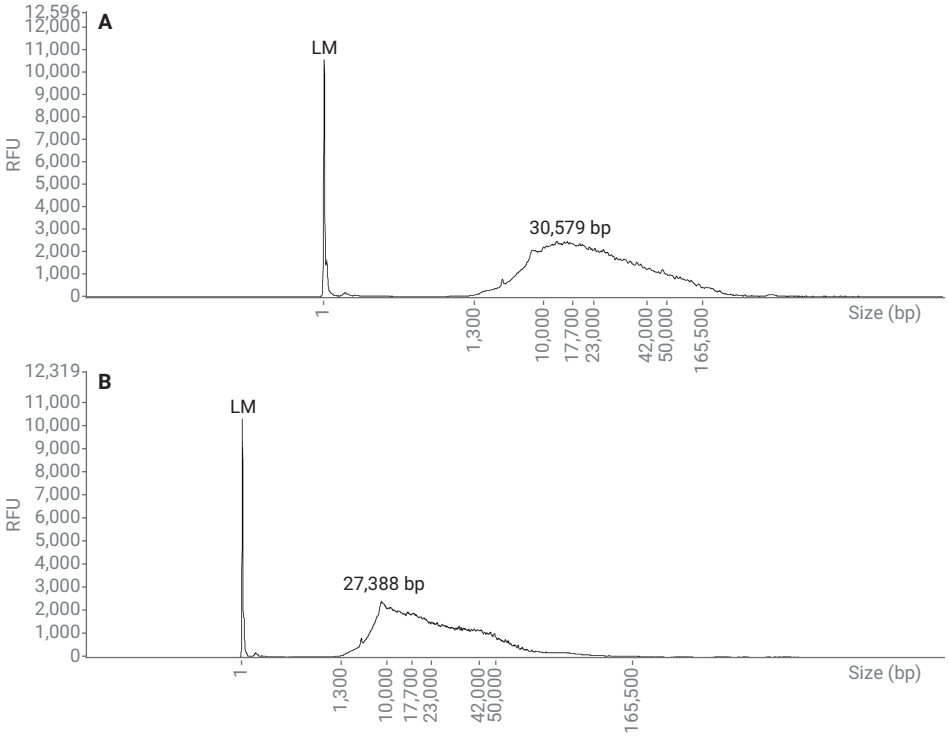

Figure 2. Yeast *Saccharomyces cerevisiae* gDNA extracted by method E (spin column) and separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit by the (A) fast method (B) or the extended method. Both separation methods resulted in similar smear sizing for gDNA less than 80 kb. LM = lower marker.

Comparison between the ProSize digital gel image from the Femto Pulse system extended method and the PFGE gel resulted in a similar size of the concentrated band area of the gDNA for all extraction methods tested (Figure 3). The high sensitivity of the Femto Pulse system enabled detection of the entire range of the gDNA smear that may not be detected on the PFGE due to low concentration. In addition, the superior resolution of the Femto Pulse system aided in distinguishing between the similarly sized gDNA samples from methods B, C, and D.

Genomic quality number (GQN)

The quality of gDNA after extraction can vary greatly depending on the extraction method, sample handling, and tissue type. Thus, quality control analysis plays a vital role in identifying highly intact gDNA prior to sample use. The genomic quality number (GQN) was designed for ProSize to allow for easy and customizable gDNA quality assessment. The user defines a size threshold deemed appropriate for their specific application. ProSize then calculates a GQN value based on the fraction of the total measured concentration that lies above the specified size threshold. The GQN scores the sample on a scale of 0 to 10, with 0 indicating none of the sample exceeds the threshold and 10 indicating 100 % of the sample lies above the threshold value.

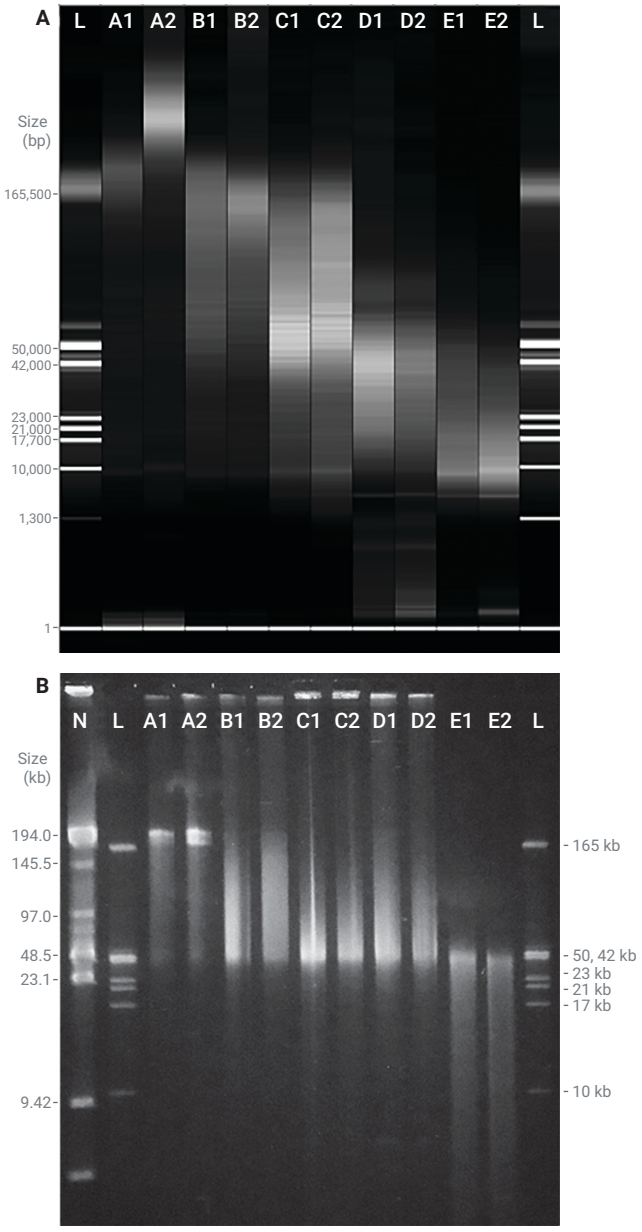

Figure 3. Yeast *Saccharomyces cerevisiae* gDNA extracted with five different methods: samples A1 and A2 are phenol chloroform extraction, B1 and B2 are liquid extraction, C1, C2, E1, and E2 are spin column extractions, and D1 and D2 are membrane extraction. (A) the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit extended method. (B) Gel image from pulsed-field gel electrophoresis. L = Agilent FP 165 kb Ladder; N = low-range PFG marker.

The ability to set a customizable GQN size threshold is a great advantage when working with differently sized gDNA. It enables the user to determine a suitable size threshold that can objectively direct decisions on which samples meet the user's requirements. The GQN size threshold was set at 50 kb for the yeast gDNA extracted from methods A, B, C, D, and E. The GQN decreased 8.2, 7.4, 6.1, 3.0, 1.1 with the decreasing size of the gDNA samples (methods A, B, C, D, and E, respectively, Table 1), as expected. The GQN also represents the percent of sample above the size threshold. For example, a GQN of 8.2 corresponds to 82 % of the sample lying above the threshold of 50 kb. Thus, 18 % of the sample lies below the threshold. Methods C and D resulted in similar sizes (77 and 62 kb, respectively), but had distinctly different GQN results of 6.1 and 3.0, respectively. In this case, because of the smear distribution, similarly sized gDNA did not have equal levels of quality as reflected by the GQN. Considering both size and quality is beneficial when determining the best gDNA extraction method and identifying suitable samples for downstream applications.

Conclusions

The Agilent Femto Pulse system is the only automated system capable of replacing overnight PFGE analysis of HMW gDNA in only 70 minutes, saving time and money. In addition, the Femto Pulse system reduces sample input to picogram levels, saving precious sample material. Agilent ProSize data analysis software reports a user-defined GQN quality score that objectively determines the percent of HMW gDNA in each sample. Considering both size and quality is beneficial when determining the best gDNA extraction method and identifying suitable samples for downstream applications. The smear size and quality of gDNA extracted with different methods was compared using the Femto Pulse system with the Agilent Genomic DNA 165 kb kit. The traditional phenol-chloroform extraction method yielded the largest and most intact gDNA compared to the other liquid, spin column, and membrane extraction methods investigated. The exceptionally high resolution of the Femto Pulse system allowed for the distinction of size distribution between similarly sized gDNA samples, which is unattainable with PFGE.

References

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