Application Note

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Genomic DNA Sizing and Quality Control on the Agilent Femto Pulse System

Authors

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Abstract

The revolutionary design of the Agilent Femto Pulse system is the only instrument capable of replacing pulsed-field gel electrophoresis analysis of high molecular weight (HMW) gDNA. The Agilent Femto Pulse system has the added benefit of extreme sensitivity, with the ability to detect a single cell amount of gDNA. The Agilent Genomic DNA 165 kb kit was designed for separation of HMW gDNA, providing accurate and reproducible sizing and quality control.

Introduction

Large-insert libraries such as those used for 10X Genomics, Oxford Nanopore, and PacBio rely upon multiple quality control steps in their workflow platforms. Typically, overnight pulsed-field gel electrophoresis (PFGE) separations are utilized to assess gDNA over 50 kb in size. The Agilent Femto Pulse system is the only instrument on the market capable of replacing PFGE for assessing high molecular weight (HMW) gDNA, in as little as 70 minutes, thus saving time and money in the workflow process of large-insert libraries. The Agilent Genomic DNA 165 kb kit offers two methods for gDNA sample analysis: the FP-1002-22 - gDNA 165 kb (fast method) is a 70 minute pulsed-field CE separation method and the FP-1002E22 - Extended gDNA 165 kb (extended method) is a 3.5-hour pulsed-field CE separation method that provides enhanced separation and sizing for gDNA smears.

Experimental

Various intact and sheared gDNA from Promega and PacBio were separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit (p/n FP-1002-0275) utilizing either the FP-1002-22-gDNA 165 kb (fast method) or FP-1002E22-Extended gDNA 165 kb (extended method) and by pulsed-field gel electrophoresis. Approximately 50 ng/µL of gDNA was loaded onto a 0.8 % agarose gel (Lonza SeaKem Agarose #50152) and separated by pulsed-field gel electrophoresis with the Pippen-Pulsed method, 5 to 430 kb, for 18 hours with the Agilent FP 165 kb Ladder (p/n FP-7002-U035) (165, 50, 42, 23, 21, 17.7, 10 kb). After separation, the agarose gel was poststained with Intercalating Dye, 30 µL (p/n DNF-600-U030) and visualized with a UV transilluminator.

Results and discussion

Fast versus extended method

Two methods are available for gDNA sample analysis with the Agilent Genomic DNA 165 kb kit. The fast method is a 70 minute pulsed- field CE separation that is best suited for separation and sizing of gDNA smears ≤ 80 kb. The extended method features a different pulsed-field CE separation method for 3.5 hours that is unique for enhanced separation and sizing of gDNA smears ≥ 80 kb. Promega human gDNA was separated on the Femto Pulse system with both the fast method (Figure 1A) and the extended method (Figure 1B). gDNA separated with the fast method displayed a sharp, compact peak similar to a fragment trace around 165 kb. This is due, in part, to the amount of time available to resolve the sample during separation. The extended method utilizes an alternative pulsed-field CE method over an extended separation time and presented the gDNA sample

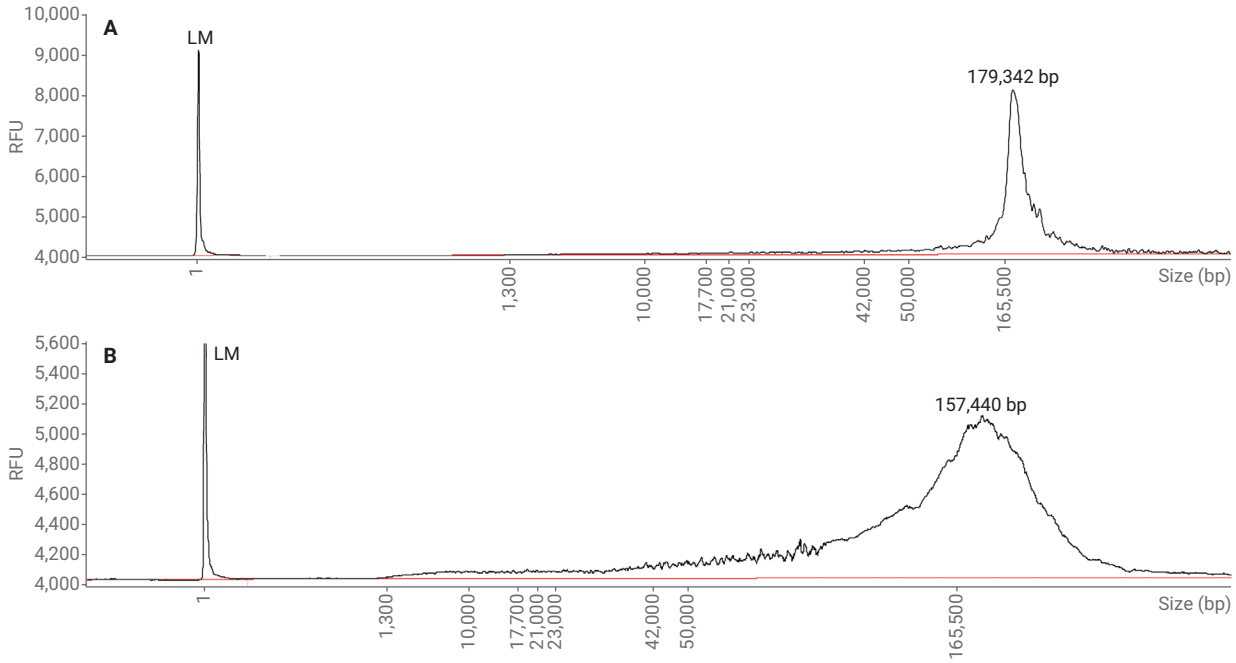


Figure 1. Separation of Promega human gDNA on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. (A) 70 minute fast method, smear size 179,342 ± 4402 bp, 2.5 % CV; (B) 3.5-hour extended method, smear size 157,440 ± 3017 bp, 1.9 % CV, n=4. LM = lower marker.

as a wide smear. The fast method reported a larger smear size (179,342 bp) compared to the extended method (157,440 bp). Due to better resolved separation, the extended method resulted in a broader smear representing the entire sizing range of the sample. Therefore, this method is recommended for larger gDNA samples separating as a compact peak around 165 kb.

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

In the past, 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 Agilent Femto Pulse system is the only instrument capable of separating and determining the quality of gDNA in as little as 70 minutes. The Agilent Femto Pulse system can replace all overnight PFGE steps in gDNA library preparations, saving money and time by eliminating several days dedicated to quality control checks. ProSize data analysis software automatically provides a peak size, an electropherogram, and 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 Agilent Femto Pulse system reduces sample input to picogram levels, saving precious sample material.

Various gDNA samples were separated by two different methods: the Agilent Femto Pulse system with the fast method and pulsed-field gel electrophoresis (Figure 2). The Agilent Femto Pulse system FP 165 kb Ladder was also separated on the PFGE for sizing comparison between the two methods. Comparison of the Agilent Femto Pulse system and PFGE gel images revealed that the smaller gDNA samples, A and D, separated similarly, with sizes of 165,547 and 102,017 bp, respectively. The larger gDNA samples B and C on the Agilent Femto Pulse system gel image were displayed as a tight band next to the 165 kb ladder peak with a smear extending above it, indicating a much larger sample. In addition, the Agilent Femto Pulse system electropherogram displayed a sharp peak at 165 kb for samples B and C. A long smear extending past the 165 kb ladder fragment was also displayed by the PFGE image. The tight band next to the 165 kb ladder fragment is a result of the shorter separation time with the fast method.

A large peak at 165 kb on the electropherogram or a dark band at 165 kb, with a smear above it on the gel image, indicates the presence of larger gDNA that would necessitate separation with the extended method on the Agilent Femto Pulse system. Certain gDNA samples slightly larger than 80 kb

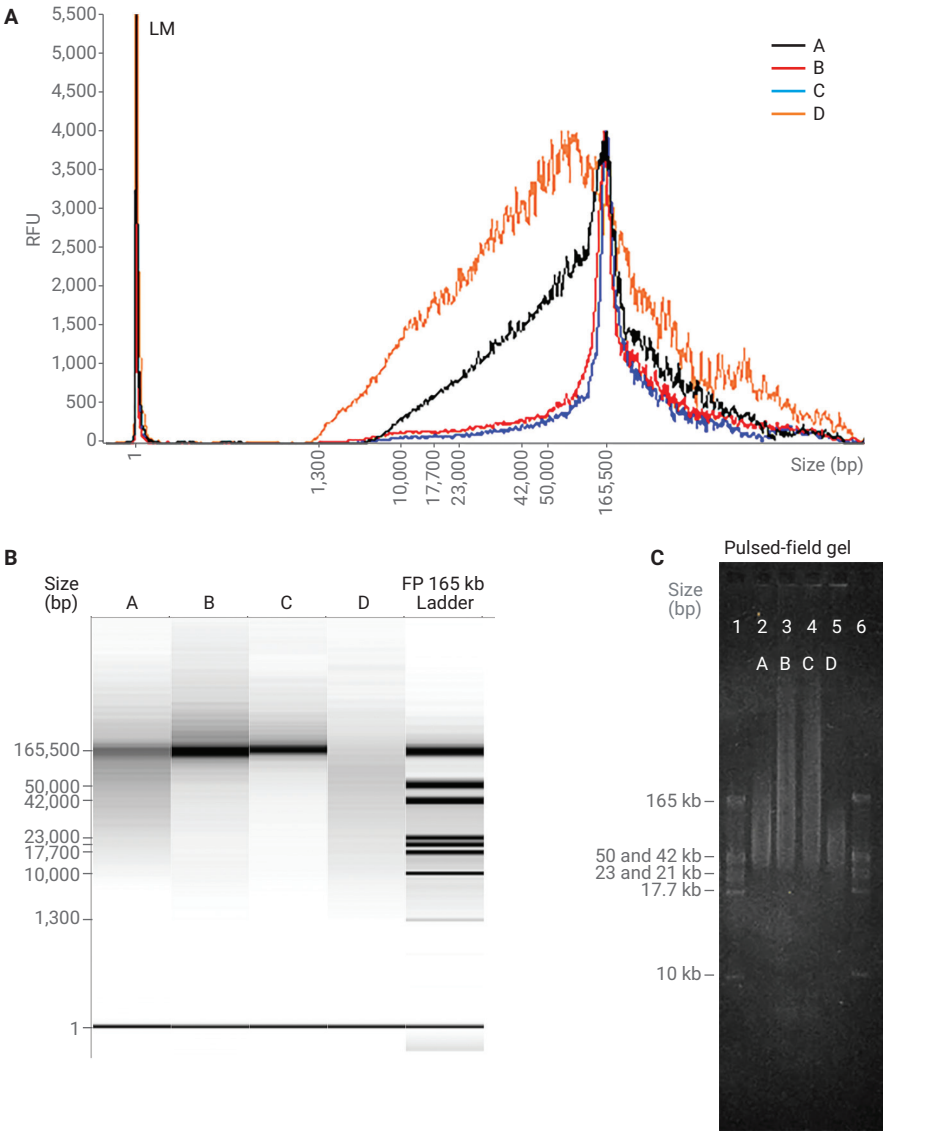
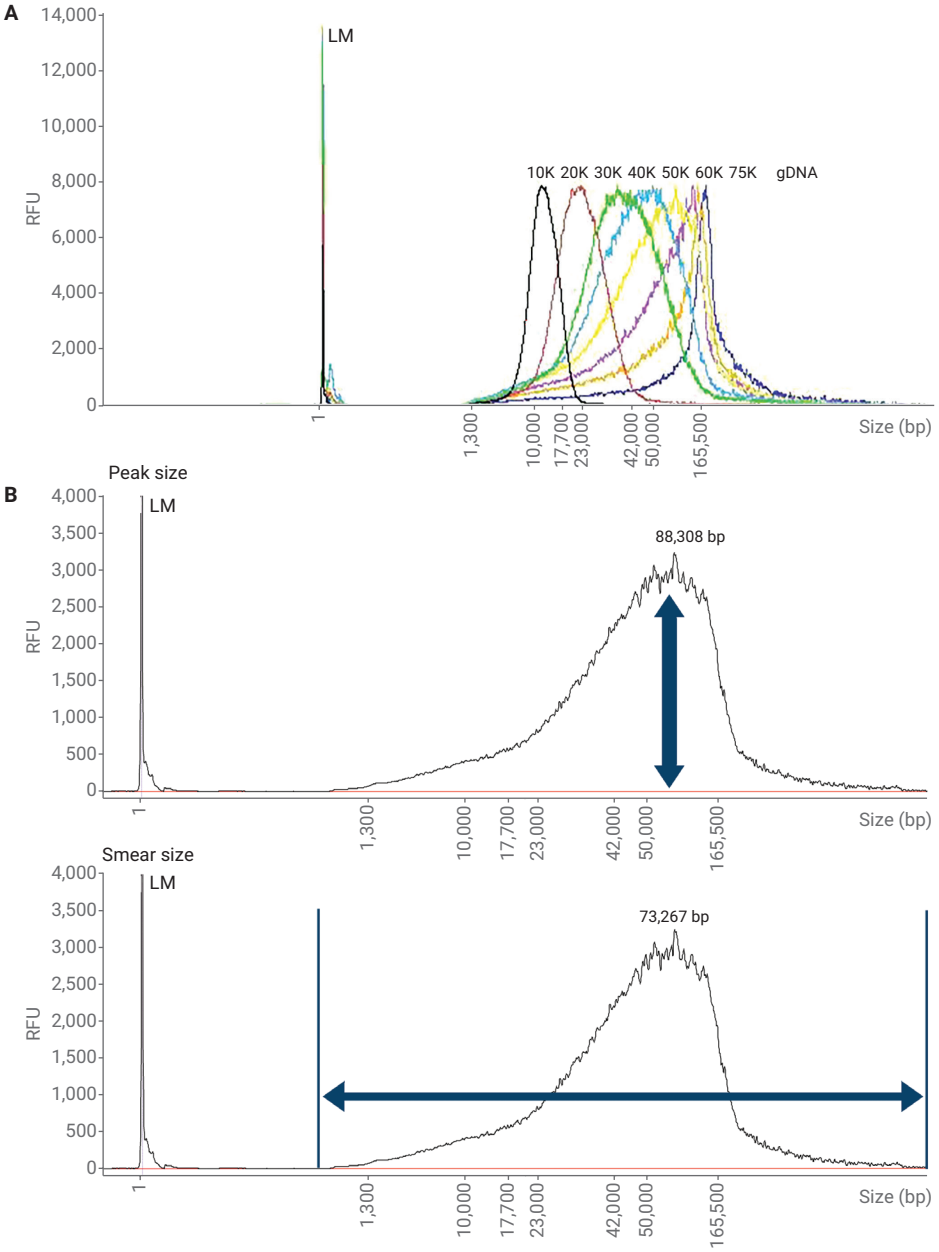


Figure 2. Intact gDNA separated on: (A) electropherogram from the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit and the 70 minute fast method; (B) gel-image from the Agilent Femto Pulse system; (C) 18-hour pulsed-field slab gel electrophoresis. LM = lower marker.

can be separated with the fast method depending on the size distribution within the smear. For instance, bell-shaped smears with a smear size slightly larger than 80 kb, or larger smears with a majority of the distribution at a smaller size, will display highly resolved separations with the fast method.

Peak and smear sizes

Sheared gDNA samples from PacBio[1] ranging from 10,000 to 70,000 bp were separated on the Agilent Femto Pulse system with the fast method (Figure 3A). Peak and smear sizes were compared for each sample (Figure 3B and C). The peak size is the tallest/most concentrated portion of the sample, while the smear size accounts for the distribution of the concentration over the designated smear range. The gDNA samples with a typical bell curve or uniform distribution on either side of the highest peak reported similar peak and smear sizes. In contrast, gDNA samples with a larger area before the highest peak reported a smaller smear size compared to the peak size as expected. The most accurate sizing for a smear sample can be achieved with the Smear Analysis tab in ProSize. The Smear Analysis tab allows the user to set a base pair range for determination of the size and concentration of the smear.



C

Approximate size (bp)	Average peak size (bp)	Average smear size (bp)	$GQN_{30\ kb}$
10,000	11,301	12,147	0
20,000	21,601	23,339	1.5
30,000	36,727	45,304	6.4
40,000	53,934	57,789	7.1
50,000	88,308	73,267	7.8
60,000	144,124	94,045	7.8
70,000	152,633	109,968	8.2
gDNA1	173,183	164,292	8.8

Figure 3. Sheared gDNA separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit and the 70 minute fast method. (A) separations on the electropherogram; (B) peak versus smear size determination on an electropherogram; (C) Average peak size, smear size, and GQN set at threshold 30,000 bp, n = 2. LM = lower marker.

Genomic quality number (GQN)

The quality of gDNA starting material for 10X Genomics, Oxford Nanopore, and PacBio libraries is crucial for successful results. The quality of gDNA and degree of degradation 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 high-quality DNA prior to library preparation. Agilent designed the genomic quality number (GQN) for ProSize to allow for easy analysis of gDNA quality. 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 size threshold and 10 indicating 100 % of the sample lies above the size threshold value.

Highly intact gDNA can range in size depending on the species. Thus, the ability to set the GQN size threshold is a great advantage when working with different-sized gDNA. It gives the user the ability to determine a size threshold that can objectively direct decisions on which samples to use for library preparations. The GQN can also quickly report the percent of sample above the size selection site. In Figure 3, the GQN size threshold value was set at 30,000 bp for all the gDNA samples demonstrating the GQN flexibility when evaluating the quality of gDNA. The 30,000 bp size threshold gave a lower GQN for the smaller sized samples compared to the larger sized gDNA samples, as expected due to their varying size.

Smear size throughout the concentration range

Reliable sizing is important in quality control of gDNA. The Agilent Femto Pulse system provides accurate gDNA sizing through 165 kb. To demonstrate that the smear size remains consistent over a wide concentration range, intact gDNA was separated on the Agilent Femto Pulse system with the fast method over the concentration range of 14 to 400 pg/µL (Figure 4A). Sizing remained similar over the entire concentration range (14 to 400 pg/µL), varying from 50,288–46,967 bp, respectively, with an average of 48,867 ± 1,166 bp (Figure 4B). The smear size precision remained very tight at 2.4 % CV over the entire concentration range.

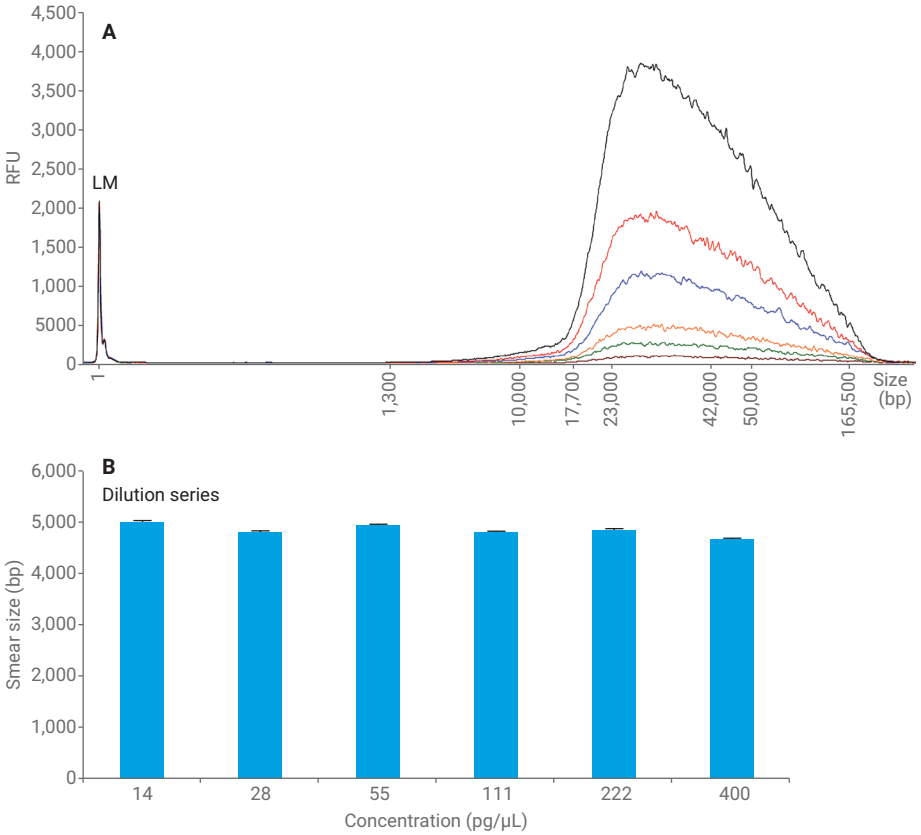


Figure 4. gDNA dilution series analyzed on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit and the 70 minute fast method. (A) separations on the electropherogram; (B) table displaying smear size per concentration. The average size over the range of concentrations was 48,727 ± 1,166 bp, 2.4 % CV, n = 24. LM = lower marker.

Conclusions

The Agilent Femto Pulse system is the only instrument on the market capable of replacing PFGE for analysis of high molecular weight gDNA. The Agilent Genomic DNA 165 kb kit offers a 70 minute fast method and a 3.5-hour extended method that allows for superior resolved separation. The Agilent Femto Pulse system provided consistent and reliable smear sizing over a wide concentration range. In addition, quick quality control analysis is available with the flexible GQN that utilizes a user-defined size threshold.

Reference

1. Sisneros, N.; *et al.*, Best Practices for Whole Genome Sequencing Using the Sequel System. *Pacific Biosciences poster*, **2017**.

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