Application Note

Genomics

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Quality Assessment of Genomic DNA for Biobanking Samples with the Agilent Femto Pulse System

Authors

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Abstract

Quality assurance and quality control of nucleic acid samples are essential for biobanks and genomic handling facilities, as well as for many molecular biology applications. The revolutionary Agilent Femto Pulse system is the only instrument capable of replacing pulsed-field gel electrophoresis for the analysis of high molecular weight (HMW) gDNA. The Femto Pulse system provides researchers with extreme sensitivity and fast separation times, with the capability of quantifying and qualifying a single cell amount of gDNA in under 70 minutes. The Agilent Genomic DNA 165 kb kit was designed for the separation of HMW gDNA, providing reproducible sizing, quantification, and quality control assessment with the genomic quality number (GQN).

Introduction

Characterization of DNA integrity is critical to biobanking quality control assessment. Understanding genomic DNA (gDNA) quality is a unique niche of DNA integrity and is essential for the success of downstream processes. With the rising interest in long-read and whole-genome sequencing technologies, comparative genomic hybridization, and mapping technologies using linked reads, the knowledge of gDNA quality, fragmentation, sizing, and concentration is a necessity for successful outcomes.

gDNA quality is assessed in part by determining the degree of fragmentation and degradation of the sample. Several factors affect sample quality such as: sample storage, extraction methods, repeated freeze-thawing, 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. Physical shearing occurs during extensive, harsh mixing conditions, repeated freeze-thawing, and ice crystal formation. Disruption of the cellular environment around DNA will trigger enzymatic and chemical shearing through free radical oxidation, depurination, and nuclease degradation of gDNA resulting in a smear of short fragments. Chemical shearing can also occur during extraction of DNA with harsh chemicals. Gentle mixing with wide-bore tips can help thwart physical fragmentation of gDNA, while chemical and enzymatic processes can be stopped by freezing, dehydration, or addition of chelating agents such as EDTA. Considering the many ways DNA integrity can be altered, records of nucleic acid quality are recommended before long-term storage of gDNA samples.

Typically, overnight pulsed-field gel electrophoresis (PFGE) has been utilized to analyze gDNA over 50 kb in size. The Agilent Femto Pulse system with the Agilent genomic DNA 165 kb kit is the only solution on the market capable of replacing PFGE for assessing high molecular weight (HMW) gDNA. Separations are completed in as little as 70 minutes, thus saving time and money in determining sample quality[2]. In addition, Agilent designed ProSize data analysis software to allow for easy analysis of gDNA quality with the genomic quality number (GQN). The user defines a size threshold deemed appropriate for their specific application. ProSize then calculates the GQN based on the fraction of the total measured concentration of the sample that lies above the 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.

Experimental

Coriell samples no. 40 (gDNA sample 1) and no. 92 (gDNA sample 2) were separated on two different Femto Pulse systems with the Genomic DNA 165 kb kit (FP-1002-0275) to demonstrate reliability between instruments and degradation over time. Sheared gDNA from PacBio was separated on the Femto Pulse system with the Agilent Genomic DNA 165 kb kit to demonstrate GQN flexibility. Promega human gDNA (#G1521) was separated on the Femto Pulse system with the Genomic DNA 165 kb kit to show peak size and characteristic fragment length.

Results and discussion

Sizing

The Femto Pulse system utilizes a pulsed-field method for separating high molecular weight gDNA. This allows an average size to be determined for gDNA greater than 50 kb. In addition, the electropherogram provides a visual, displaying the size distribution of the sample. Without the pulsed-field method, samples greater than around 50 kb are displayed as sharp peaks and sized as greater than 60 kb. gDNA sample 1 was analyzed on the Femto Pulse system with the Genomic DNA 165 kb kit and had an average smear size of 44,800 bp, with a size distribution ranging from 1,200 to 300,000 bp (Figure 1).

Reliability between instruments

Two different gDNA samples were analyzed on the Femto Pulse system with the Agilent Genomic DNA 165 kb kit. Both samples were analyzed at several concentrations on two different Femto Pulse instruments to demonstrate reliability and consistency of sizing, concentration, and gDNA quality analysis between instruments and over a wide concentration range (Figure 2). The precision, on each instrument and across the dilution series, was below 8 % for sizing and 16 % for quantification, well within the specifications of the kit. In addition, the GQNs demonstrate extremely tight precision with 3 % CV.

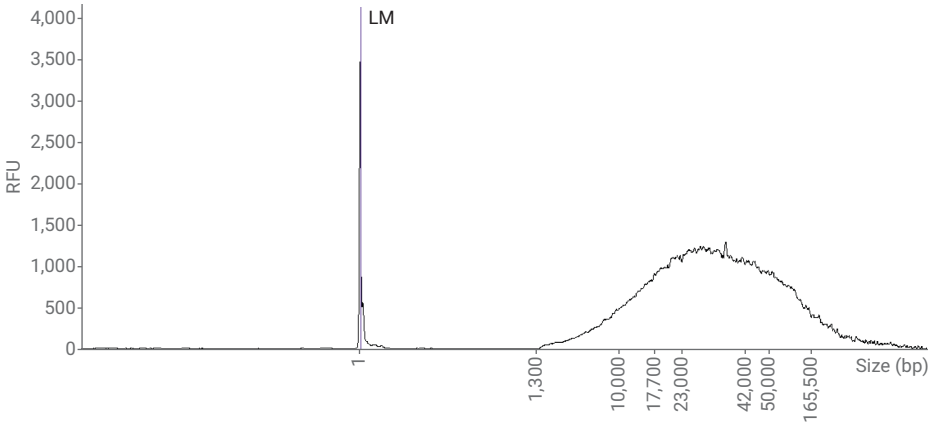


Figure 1. Analysis of gDNA sample 1 on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. LM = lower marker.

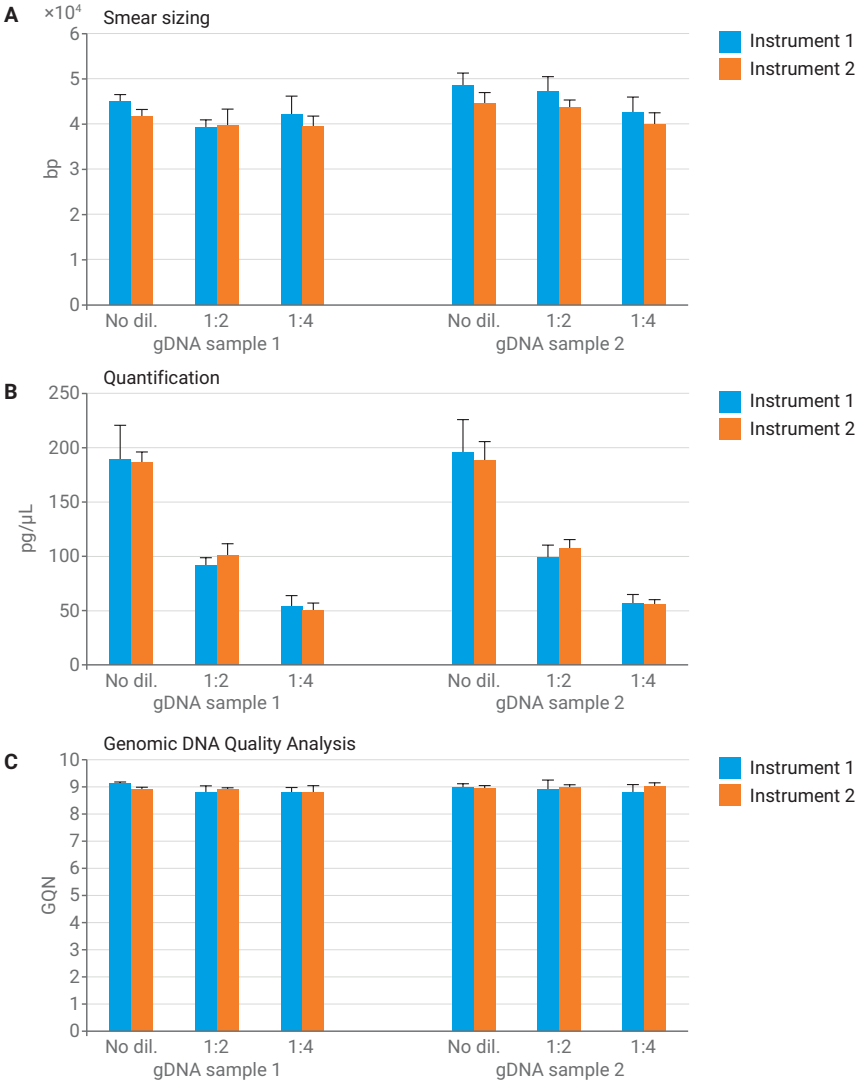


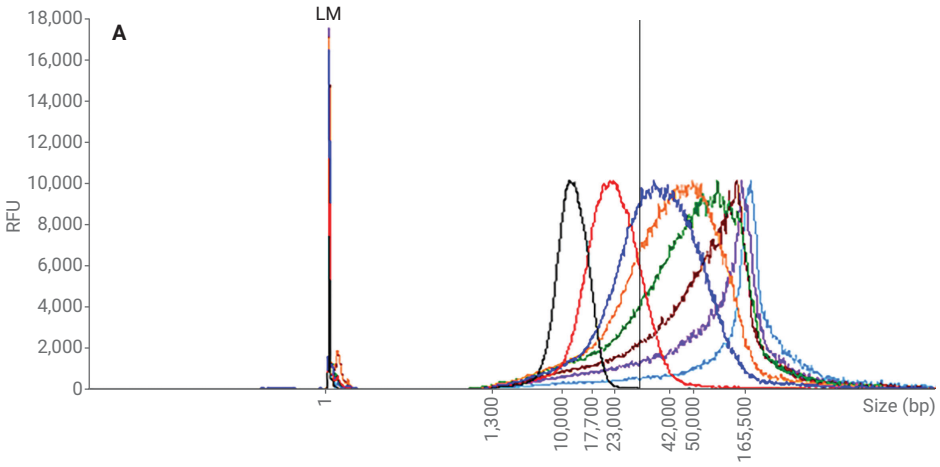
Figure 2. Analysis of two gDNA samples at different concentrations separated on two different Agilent Femto Pulse systems with the Agilent Genomic DNA 165 kb kit. Quantification, sizing, and gDNA quality analysis ($GQN_{10,000}$) were compared between two different instruments demonstrating reliability and reproducibility. n = 4.

Genomic quality number (GQN)

The GQN applies a numerical value to gDNA quality and represents the percent of sample above the size threshold. Highly intact gDNA can range in size depending on many factors including 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 downstream processes.

The GQN size threshold was set at 10,000 bp, based on the size distribution of gDNA samples A and B. The GQN remained consistent across the dilution series with a very tight range of 9.0 to 8.8 (Figure 2). A GQN of 9.0 refers to 90 % of the sample lying above the threshold level of 10,000 bp. Thus, 10 % of the sample lies below the threshold level. A decrease in GQN for a sample over time indicates degradation has taken place.

In Figure 3, the GQN size threshold value was set at 30,000 bp for the sheared gDNA PacBio 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.



B

Average smear size (bp)	$GQN_{30,000}$
12,147	0
23,339	1.5
45,304	6.4
57,789	7.1
73,267	7.8
94,045	7.8
109,968	8.2
164,292	8.8

Figure 3. Sheared gDNA from PacBio separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit. A) Separations on the electropherogram; B) Average smear size, and $GQN_{30,000}$, n = 2, LM = lower marker.

Detection of degradation

Slight degrees of degradation can be detected by the Femto Pulse system and are reflected in the GQN. A prepared plate with gDNA sample 1 was analyzed immediately after preparation and then again three days later after being left at room temperature. Degradation of sample 1 was evident on the Femto Pulse system separation by a small smear in front of the gDNA and a decreasing average smear size shift from 44.8 to 34.5 kb (Figure 4).

The degradation was also reflected in the decreasing $GQN_{10,000}$ from 9.1 to 8.4. ProSize also has the option of selecting a base pair range with the Smear Analysis tab enabling the user to detect the percent of degradation within the sample in the low molecular weight area. A degradation range of 200 to 1,200 bp was chosen for sample 1. This range included the area directly in front of the sample while excluding the lower marker. The percent of degradation increased from 0.1 to 1.4 % due to the room temperature environment (Figure 4). This has also been termed the smear ratio (SR) or "less than X kb"[1]. The smear ratio provides an estimate of the proportion of DNA that has been fragmented due to freeze-thaw shearing or chemical degradation.

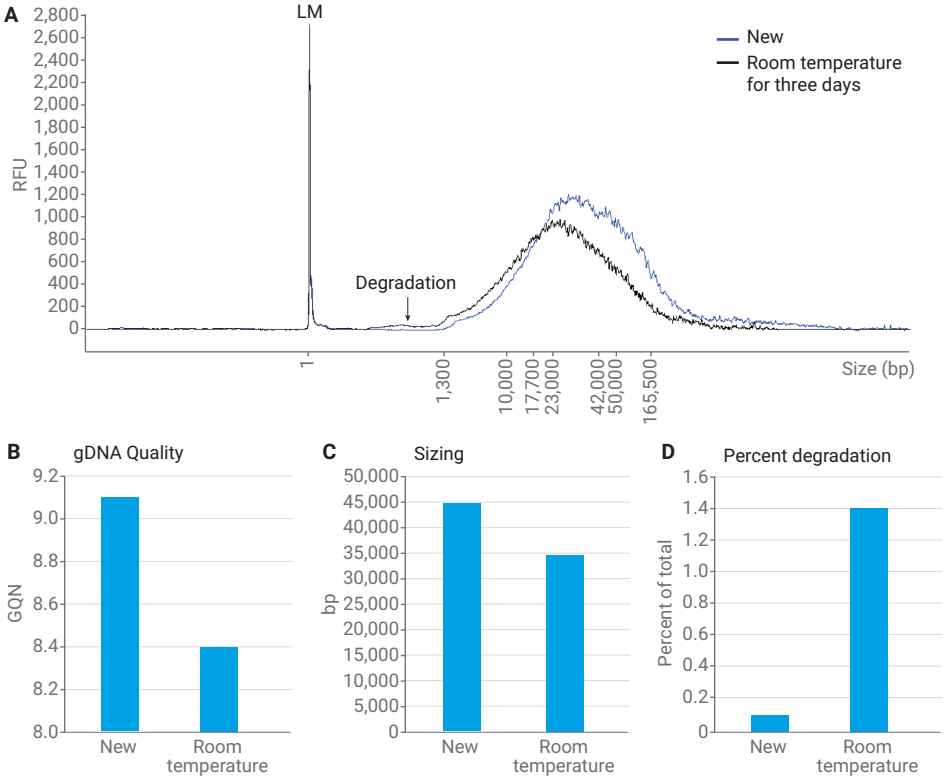


Figure 4. gDNA sample 1 was separated on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit (A) before (blue trace) and after (black trace) sitting at room temperature for three days. The electropherogram displays a degradation smear in front of the sample. gDNA quality analysis ($GQN_{10,000}$) (B) and size (C)decreased with an increase in percent degradation (D) in the low molecular weight region after sitting at room temperature for three days. n = 2; LM = lower marker.

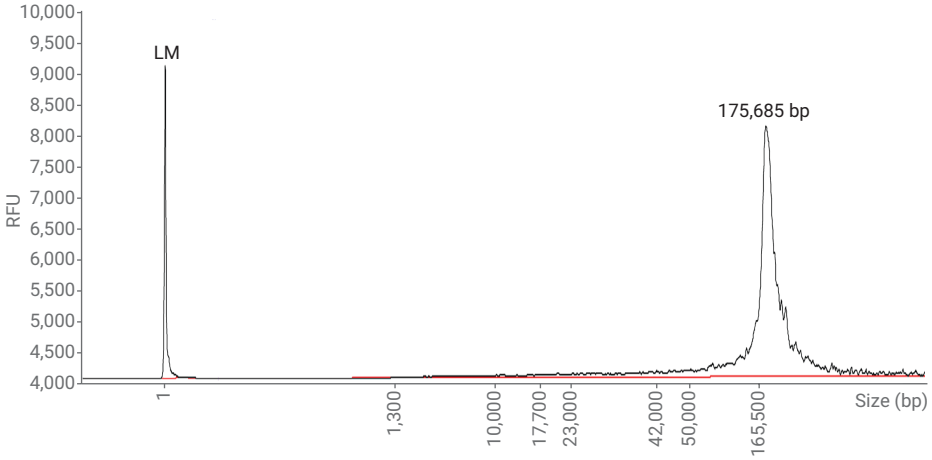


Figure 5. Separation of Promega human gDNA on the Agilent Femto Pulse system with the Agilent Genomic DNA 165 kb kit, peak size 175,685 bp. LM = lower marker.

Characteristic fragment length (CFL)

The characteristic fragment length (CFL) corresponds to the most abundant fragment of the sample[3]. In ProSize, it is reported as a peak size. The CFL is routinely reported when assessing DNA integrity with gel electrophoresis. A decreasing shift in the CFL is easily detected and can be used as an indicator of degradation due to physical shear stress. Promega human gDNA was separated on the Femto Pulse system with the Agilent Genomic DNA 165 kb kit. The electropherogram displayed a sharp peak at 175,685 bp, corresponding to the CFL for the sample (Figure 5). Not all samples display a definite CFL as seen in Figure 1. In these cases, changes in the average smear size can be utilized to track degradation instead of the most abundant peak size or CFL.

Conclusions

Records of the quality of HMW gDNA are becoming increasingly important with the rising interest in long-read and whole-genome sequencing technologies. The Femto Pulse system is the only instrument capable of replacing pulsed-field gel electrophoresis for the analysis of HMW gDNA over 60 kb in size in under 70 minutes with detection limits down to a single cell amount of DNA. Degradation of gDNA is easily assessed on the Femto Pulse system with the GQN, changes in average smear size or characteristic fragment length, and by an increase in the low molecular weight smear region.

References

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2. Pocernich, C.; Uthe, J.; Wong, K-S. Genomic DNA Sizing and Quality Control with the Agilent Femto Pulse System. *Agilent Technologies Application Note*, publication number 5994-0516EN, **2017**.
3. Cinselli, C. W.; *et al.* Combining Qualitative and Quantitative Imaging Evaluation for Assessment of Genomic DNA Integrity: The SPIDA Experience. *Anal. Biochem*. **2015**, *479*, 60–62.

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