Application Note

Genomics

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Assessment of Genomic DNA Quality with the Agilent 5200 Fragment Analyzer System

Authors

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Abstract

The successful outcome of techniques such as next-generation sequencing (NGS) rely on the quality of genomic DNA (gDNA) starting material. The Agilent 5200 Fragment Analyzer system offers effortless analysis of genomic DNA (gDNA) with the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit. gDNA up to 60,000 bp in size and over a wide concentration range can be analyzed with both kits. Agilent ProSize data analysis software provides an electropherogram and a digital gel image for easy visual inspection and assigns a genomic quality number (GQN) for quick evaluation of gDNA quality. The 5200 Fragment Analyzer system, equipped with the Agilent FA 12-Capillary Array Short, 33 cm or Agilent FA 12-Capillary Array Ultrashort, 22 cm offers consistent sizing, quantification, and GQN assessment of gDNA with both the Agilent Genomic DNA 50 kb kit and Agilent HS Genomic DNA 50 kb kit.

Introduction

The quality and concentration of genomic DNA (gDNA) starting material is crucial for successful downstream long-read and whole genome next-generation sequencing (NGS). Quality analysis for gDNA with varying ranges of concentrations can be performed on the Agilent 5200 Fragment Analyzer system with the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit. The Genomic DNA 50kb kit offers a concentration range of 25 to 250 ng/µL input gDNA, while the HS Genomic DNA 50 kb kit has a lower concentration range of 0.3 to 12 ng/µL input gDNA for low concentrated samples. We compared the sizing, quantification, and genomic quality number (GQN) consistency of different gDNA samples on the 5200 Fragment Analyzer system equipped with a short or ultrashort array using the described kits.

Experimental

The experiments in this study were done using a 5200 Fragment Analyzer system and can be replicated with comparable results on Agilent 5300 and 5400 Fragment Analyzer systems.

The 5200 Fragment Analyzer system equipped with the Agilent FA 12-Capillary Array Short, 33 cm (short array) (p/n A2300-1250-3355) or Agilent FA 12-Capillary Array Ultrashort, 22 cm (ultrashort array) (p/n A2300-1250-2247) was used to analyze cotton gDNA (Zyagen, #PLG-1022), *E. coli* gDNA (New England Biolabs, #14380), and Coriell #78 gDNA (Coriell Institute for Medical Research) smears with the Agilent Genomic DNA 50 kb kit (p/n DNF-467) or the Agilent HS Genomic DNA 50 kb kit (p/n DNF-468). The Smear Analysis function in ProSize data analysis software was used to determine the average smear size. The GQN size threshold was set at 1,000 and 10,000 bp for cotton, *E. coli*, and Coriell #78 gDNA samples.

Results and discussion

Genomic DNA size

gDNA samples were separated on the 5200 Fragment Analyzer system with the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit on both the short and ultrashort capillary arrays (Figure 1). Both kits utilized the same ladder fragments containing a 48,500 bp fragment, as seen on the X-axis of the electropherograms, allowing for accurate sizing of large gDNA samples up to 60,000 bp. Each kit utilizes a different ladder concentration appropriate for its concentration range.

The average smear size was similar between the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit on both the short and ultrashort arrays for the cotton, *E. coli*, and Coriell gDNA, with a 9, 8, and 6 % CV respectively (Figure 1). These results indicated that gDNA ranging in size from 75 to 60,000 bp can be analyzed with either kit on either capillary array with the ability to obtain similar and reproducible sizing results.

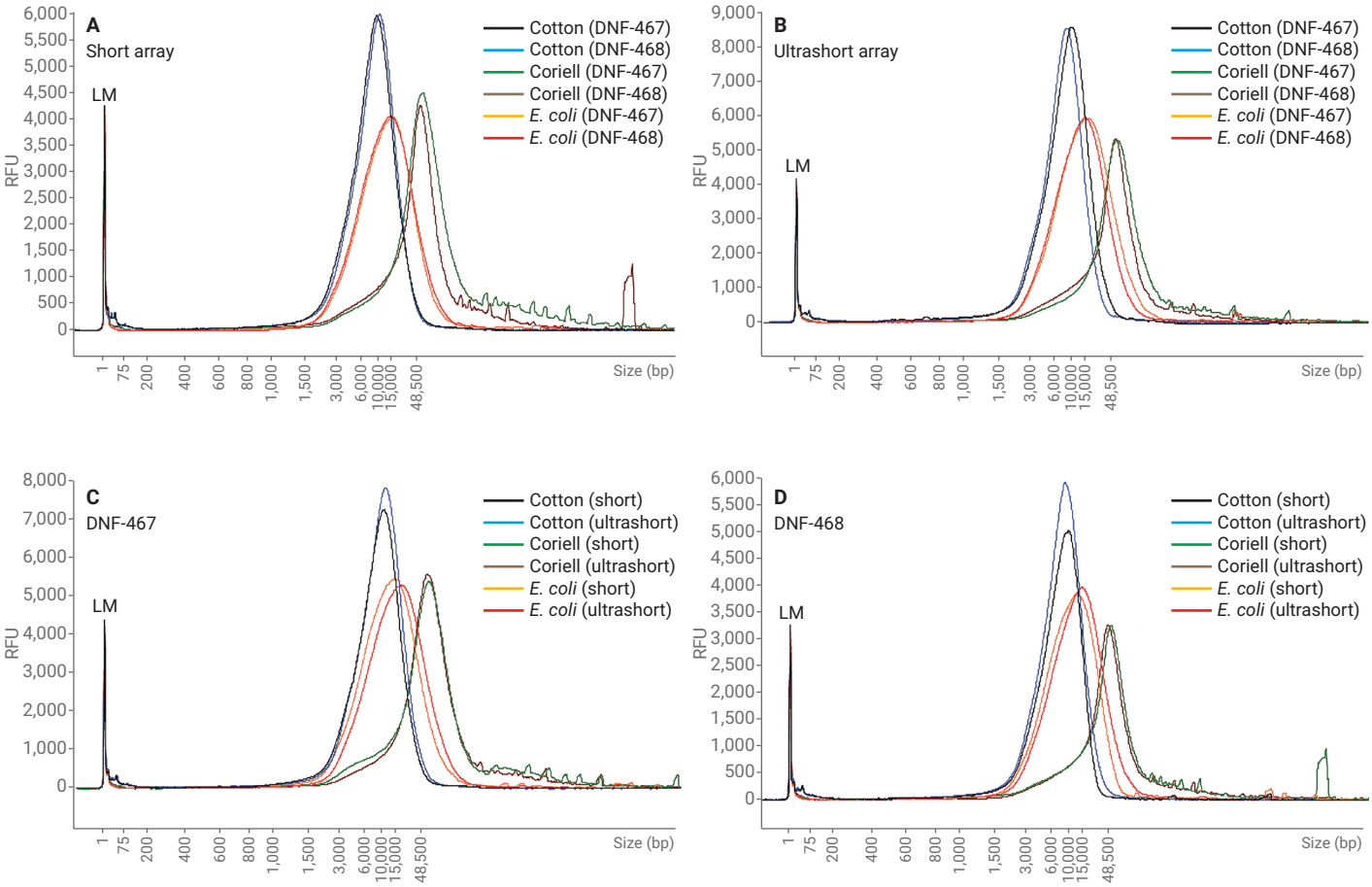

Figure 1. Cotton, *E. coli*, and Coriell gDNA separations on the 5200 Fragment Analyzer system. Comparison of the Genomic DNA 50 kb kit (DNF-467) and HS Genomic DNA 50 kb kit (DNF-468) on the (A) short and (B) ultrashort arrays. Comparison of the short and ultrashort arrays with the (C) Genomic DNA 50 kb kit (DNF-467): average size of cotton gDNA 10,721 and 11,423 bp; *E. coli* gDNA 19,928 and 20,232 bp; Coriell 53,331 and 53,087 bp on the short and ultrashort arrays respectively. (D) HS Genomic DNA 50 kb kit (DNF-468): average size of cotton gDNA 9,686 and 9,932 bp; *E. coli* gDNA 16,645 and 19,525 bp; Coriell 51,662 and 48,334 bp on the short and ultrashort arrays, respectively. LM = lower marker

Input genomic DNA concentration

Both the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit are utilized for gDNA analysis, each covering a different sample input concentration. The Genomic DNA 50 kb kit covers gDNA samples at high concentrations of 25 to 250 ng/µL, with a single 200-fold dilution. In contrast, the HS Genomic DNA 50 kb kit targets gDNA samples at lower concentrations of 0.3 to 12 ng/µL, with a 12-fold dilution. There are several advantages of having a choice between two gDNA kits with different input concentration ranges. The Genomic DNA 50 kb kit saves time by eliminating an additional dilution step for highly concentrated samples and the HS Genomic DNA 50 kb kit enables the direct analysis of low concentrated samples. The different dilution factors of each kit allow for an overlap of in-well sample concentrations. Therefore, in this study, the input gDNA sample concentrations were normalized for each kit so that the in-well concentrations were the same, allowing for a quantitative comparison between kits.

Cotton, *E. coli*, and Coriell samples were each prepared at different concentrations. The in-well gDNA concentrations determined from the same kit using the short or ultrashort array were found to be very consistent between arrays for each sample (Figure 2A). Comparison of the gDNA concentrations between the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit were similar on both the short and ultrashort array for each sample (Figure 2B). The percent CVs throughout all the analysis methods for each cotton, *E. coli*, and Coriell sample were 13, 10, and 14 % respectively. These results indicate that both the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit can be utilized to determine gDNA concentrations with either the short or ultrashort array.

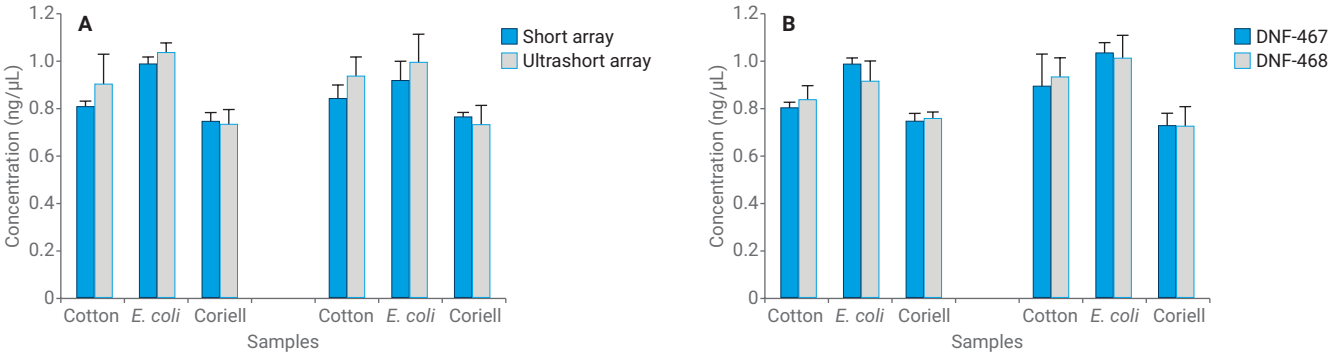

Figure 2. Average genomic DNA in-well concentration analyzed on the 5200 Fragment Analyzer system. (A) Comparison of short and ultrashort arrays. (B) Comparison of the Genomic DNA 50 kb kit (DNF-467) and HS Genomic DNA 50 kb kit. (DNF-468). n = 5.

Genomic DNA quality number (GQN)

It is important to do a preliminary assessment of extracted DNA to ensure it is of suitable quality for successful library preparation and downstream sequencing. The genomic quality number was designed 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 threshold and 10 indicating 100 % of the sample lies above the threshold value. Since gDNA size is affected by extraction methods, fixation (formalin-fixed paraffin-embedded samples), and degradation with time, the ability to define the size threshold gives the user the advantage of determining what is quality gDNA for their particular application. The GQN value allows for a fast and easy assessment of the quality of any type of gDNA sample.

Highly intact gDNA can range in size depending on the species, as demonstrated between cotton, *E. coli*, and Coriell gDNA (Figure 1). The GQN size threshold was set at 1,000 bp (Figure 3A) and 10,000 bp (Figure 3B) for cotton, *E. coli*, and Coriell gDNA samples demonstrating the GQN flexibility when evaluating the quality of gDNA. The higher 10,000 bp size threshold gave a lower GQN for the smaller sized cotton (3.6) and *E. coli* (5.9) samples compared to Coriell (8.6), as expected due to their varying size. The lower 1,000 bp size threshold reported a similar GQN for all three samples because the majority of the sample lies above the size threshold parameter. The GQN values were consistent between both the Genomic DNA 50 kb kit and the HS Genomic DNA 50 kb kit on both the short and ultrashort arrays for all samples.

Proper mixing and handling

Optimal handling and mixing of the gDNA sample is vital to attain accurate and precise quantitative measurements[1]. Before sampling, the stock gDNA must be acclimated to room temperature and then mixed by vortexing. Upon addition of the gDNA sample to the diluent marker in the 96-well plate, each well should be mixed by pipetting up and down 10 times with the appropriate volume indicated for the kit (20 µL volume for the HS Genomic DNA 50 kb kit or 100 µL for the Genomic DNA 50 kb kit). It is important to consider when mixing gDNA samples that repeat freeze-thaw events and repeated mixing will shear the gDNA. Mixing with wide-bore tips will decrease shearing due to mixing, while aliquoting out small volumes of gDNA samples will eliminate repeat freeze-thaw events. Regardless, it is important to stay consistent with the mixing technique of choice when working with gDNA in order to achieve reproducible results. Proper storage, handling, and mixing of the HS gDNA Ladder as recommended in the kit manual is also important to ensure the best possible results. When analyzing gDNA samples, always run the HS Genomic DNA 50 kB kit ladder in parallel with the samples to impart optimal sizing and quantification.

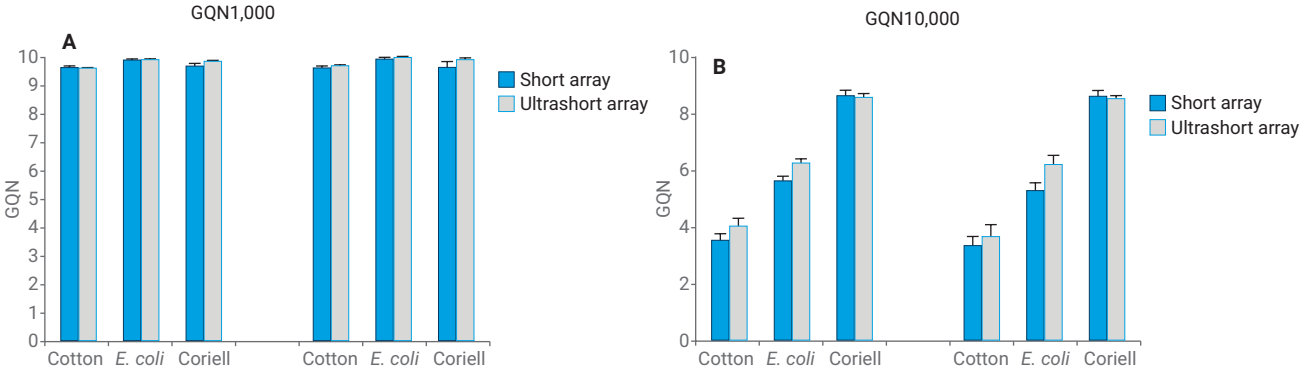

Figure 3. The average GQN for cotton, *E. coli*, and Coriell gDNA. Comparison of the Genomic DNA 50 kb kit (DNF-467) and the HS Genomic DNA 50 kb kit (DNF-468) on the short and ultrashort array. (A) $GQN_{1,000}$ (B) $GQN_{10,000}$. n = 5.

Conclusions

gDNA size, concentration, and GQN value remained consistent on the 5200 Fragment Analyzer system between the short array or ultrashort array and the Genomic DNA 50 kb kit and HS Genomic DNA 50 kb kit. The ultrashort array offered the convenience of a shortened run time, while reporting comparable gDNA size, concentration, and GQN values to the short array. The Genomic DNA 50 kb kit and HS Genomic DNA 50 kb kit both have the added benefit of an extended ladder up to 48,500 bp, with sizing through 60,000 bp. The Genomic DNA 50 kb kit is ideal for high concentration samples. Alternatively, the HS Genomic DNA 50 kb kit is optimal for lower concentrated, limited samples. The ability to set the size threshold for GQN allows for easy quality analysis of different-sized gDNA species.

Reference

1. Pocernich, C.; *et al.* Best Quantification Practices with the Agilent 5200 Fragment Analyzer System. *Agilent Technologies Application Note*, publication number 5994-0513EN, **2018**.

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