A Novel Assay for Automated Electrophoretic Analysis of Genomic DNA

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Introduction

The success of any genomic study depends primarily on the quality of the starting material, like genomic DNA (gDNA). The integrity of extracted gDNA affects downstream applications like microarray hybridization and next generation sequencing library construction. Since these are expensive and time-consuming applications, a quality control (QC) of gDNA has become highly recommended.

The Agilent Genomic DNA ScreenTape provides a reproducible QC method for assessing integrity and quantity of gDNA using electrophoretic separation with the convenience of an automated system.

Figure 1: The Agilent 2200 TapeStation System

With minimal sample preparation, automated loading and a variable throughput system, digital results can be presented as a gel image, data table and in an electropherogram view. The ability to overlap and compare electropherograms within the software enables discrimination of sample quality across different degradation states, sample types and concentrations.

Here, we present data showing that the Genomic DNA ScreenTape can easily verify the integrity of gDNA starting material with high sensitivity, precision and accuracy.

Materials & Methods

Different gDNA samples were analyzed using the Genomic DNA ScreenTape assay. Quantification was compared to the Nanodrop™ UV spectrophotometer (Thermo Scientific) and the Qubit BR dsDNA fluorescence assay (Life Technologies). gDNA was artifically fragmented using ultra sonication to compare different degradation levels.

Screentape Analysis Protocol:

The gDNA samples were prepared by mixing 1 µL of sample with 10 µL of Genomic DNA Sample Buffer, vortexed for 5 seconds and placed in the Agilent 2200 TapeStation (see Figure 2).

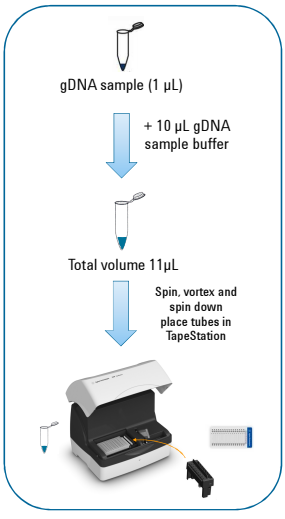

Figure 2: Genomic DNA sample preparation protocol

Results & Discussion

Genomic DNA quantification and sizing

Three different gDNA samples were analyzed in concentrations from 5 to 100 ng/µL using the Genomic DNA ScreenTape assay. The average quantification and sizing result was calculated from 18 replicate samples (distributed over 18 individual ScreenTapes). The obtained quantification result was compared to the Nanodrop and the Qubit assay. The three gDNA samples were (1) enzymatically degraded, (2) sheared by the use of a column-based cleanup procedure (Qiagen Dneasy) and (3) intact.

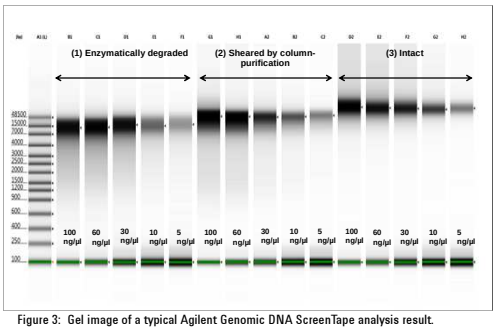

Figure 3: Gel image of a typical Agilent Genomic DNA ScreenTape analysis result.

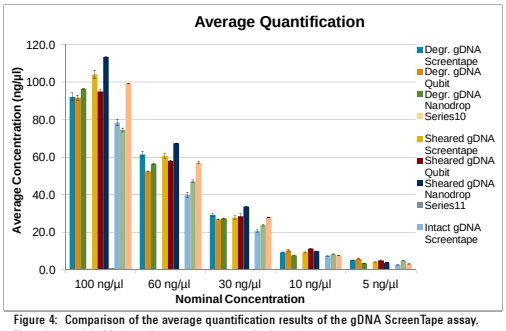

Figure 4: Comparison of the average quantification results of the gDNA ScreenTape assay, Nanodrop and Qubit (error bars represent standard error)

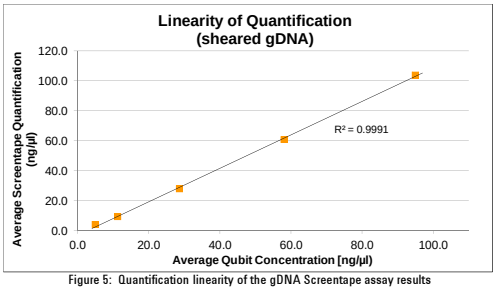

Figure 5: Quantification linearity of the gDNA Screentape assay results

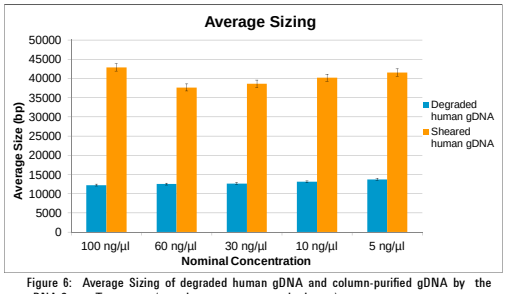

Figure 6: Average Sizing of degraded human gDNA and column-purified gDNA by the gDNA ScreenTape assay (error bars represent standard error)

Genomic DNA integrity QC analysis

The performance of the Genomic DNA ScreenTape assay in discriminating the different quality of gDNA was assessed by analyzing artificially fragmented samples. gDNA samples with different levels of fragmentation were analzyed on a gDNA ScreenTape. The gel image in Figure 7 shows the fragmentation gDNA over time.

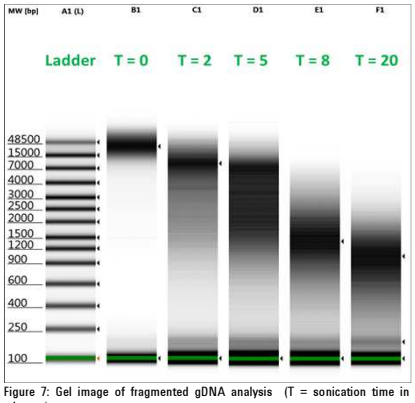

Figure 7: Gel image of fragmented gDNA analysis (T = sonication time in minutes)

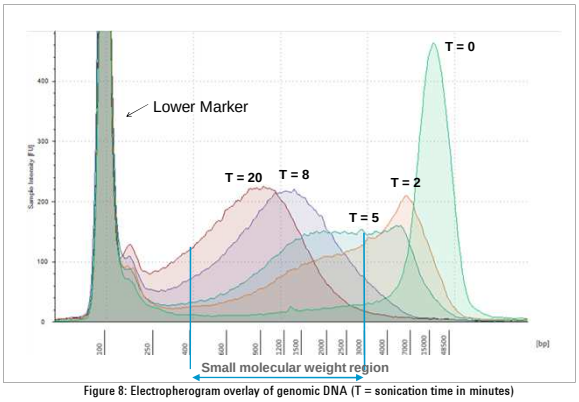

Figure 8: Electropherogram overlay of genomic DNA (T = sonication time in minutes)

Figure 8 shows the same gDNA samples in an electropherogram overlay view. Intact gDNA gets fragmented into smaller sizes in the course of the sonication, the randomly fragmented samples show as smears and can easily be compared in the software using the 'comparison' function of the Genomic DNA ScreenTape analysis software.

The increase in concentration of the sample within the region between 400bp and 3000bp due to sonication over time indicates the increase in fragmentation of the gDNA presented in Figure 9.

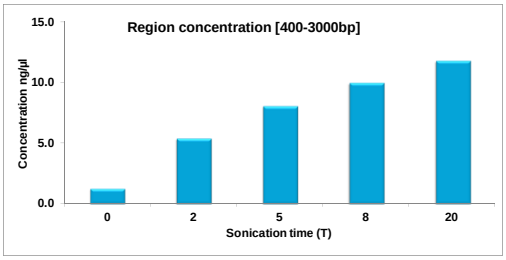

Figure 9: Small molecular weight DNA concentration dependence of sonication duration

Conclusions

- The Agilent 2200 TapeStation and Genomic DNA ScreenTape assay enables the analysis of gDNA and other high molecular weight DNA
- The quantification of the Genomic DNA ScreenTape assay is highly comparable to traditional quantification methods like UV and displays a precision (CV) of less than 20%
- The quantification of gDNA is linear over a broad sample concentration range
- The new Genomic DNA ScreenTape assay is an excellent platform for objectively ascertaining gDNA sample quantity, quality and integrity.