AUTOMATED GENOMIC DNA QC ENSURES HIGH QUALITY DATA FROM DOWNSTREAM WORKFLOWS

Arunkumar Padmanaban, Ruediger Salowsky, Delphine Rabiller and Donna McDade Walker

Agilent Technologies

Introduction

The success of several genomics study depends primarily on the quality of starting material, which in most cases is the genomic DNA. The quality and quantity of the extracted genomic DNA affects the downstream applications like microarray studies, library constructions and gene expression studies. Since, these are expensive and time consuming applications the QC of the genomic DNA has become a mandatory at several stages of the experiment. The integrity of genomic DNA was traditionally studied using Agarose gel, which is more manual, cumbersome and involves exposure to hazardous chemicals like ethidium bromide.

Figure 1: The Agilent 2200 TapeStation System

The new Agilent Genomic DNA ScreenTape assay along with the Agilent 2200 TapeStation system enables analysis of high molecular weight genomic DNA (gDNA). The Genomic DNA ScreenTape device has 16 individual, pre-packaged separation channels and can size DNA fragments between 200 and 60000bp. The 2200 TapeStation instrument offers flexibility to utilize 8-way strip tubes as well as 96-well plates for low and high-throughput requirements. With minimal sample preparation, automated loading and a variable throughput system, digital results can be presented in a gel image, data table and electropherogram within approximately 1 minute per sample. Here in this study, we demonstrate the capability of the Agilent 2200 TapeStation system in accurate, reproducible sizing and quantification of gDNA samples.

Materials & Methods

Instrument and reagent kits: Agilent 2200 TapeStation system (G2964AA), Agilent Genomic DNA ScreenTape kit was obtained from Agilent Technologies (Waldbornn, Germany). Agilent DNA extraction kit (200600-1) was obtained from Agilent technologies and used in accordance to manufacture guidelines. HEK293 cell line was purchased from HEK 293 from ATCC (ATCC, USA) and cultured in cultured using MEM medium containing 10% FBS and 1% Pen/Strep and incubated at 37°C in a 5% CO2 atmosphere.

The cells were harvested at 80% confluent and frozen at -80 °C until further use. Qiagen - DNeasy Blood and Tissue kit (69504), Invitrogen – PureLink Genomic DNA mini kit (K1820-00), Promega – Wizard SV Genomic DNA kit (A2360) was purchased from respective vendors and used following manufacturer guidelines. Qubit dsDNA BR Assay Kit (Q32850) was purchased and used with Qubit® 1.0 Fluorometer following the manufacturer guidelines. Benchtop sonicator was purchased from Crest Ultrasonics Corp (Trenton, NJ)

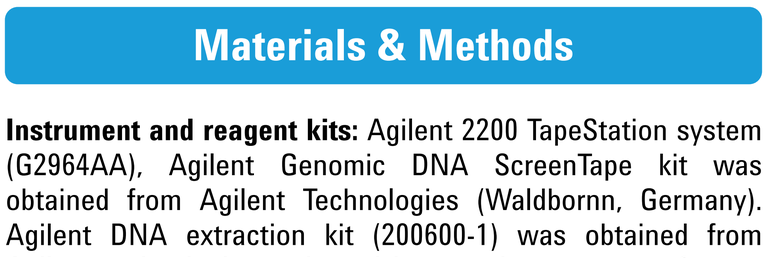


Figure 2: Schematic representation of the workflow

Genomic DNA was extracted using four extraction kits using a cell pellets containing one million cells of HEK293. Extraction for each kit was carried out in triplicates. The extracted gDNA was then analyzed using the Genomic DNA ScreenTape assay.

The genomic DNA extracted using Qiagen extraction kit was sonicated to artificially fragment the gDNA to smaller sizes. A volume of 20µl of extracted gDNA was aliquoted into a vial and placed in the sonicator. The gDNA was then sampled at time points 0, 2, 5 8 and 20 minutes. The size distribution of the sonicated samples was analyzed using the Genomic DNA ScreenTape assay.

The genomic DNA samples are prepared by mixing 1µl of sample with 10µl of gDNA sample buffer. The mixture is then mixed briefly and vortexed and placed in the Agilent 2200 TapeStation.

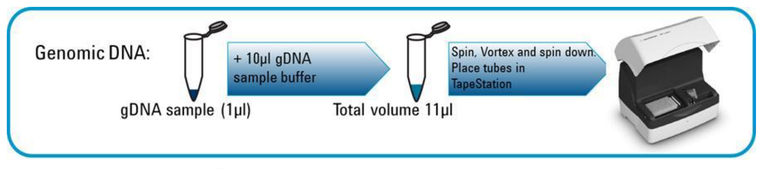


Figure 3: gDNA sample preparation protocol

Results & Discussion

The performance of the Genomic DNA ScreenTape assay was assessed by analyzing gDNA extracted using extraction kits from different vendors. The precision in sizing the gDNA and quantification is demonstrated by running triplicates. The ability of the Genomic DNA ScreenTape assay in discriminating the integrity of gDNA was assessed by running artificially degraded gDNA samples.

Genomic DNA size distribution analysis

The genomic DNA extracted using the different kits was prepared by mixing with the loading buffer and analyzed with the Genomic DNA ScreenTape device. A typical gel image of the analyzed samples is presented in Figure 4 showing sample replicates from different extraction kits. The figure shows samples ran on individual lanes of the Genomic DNA ScreenTape device.

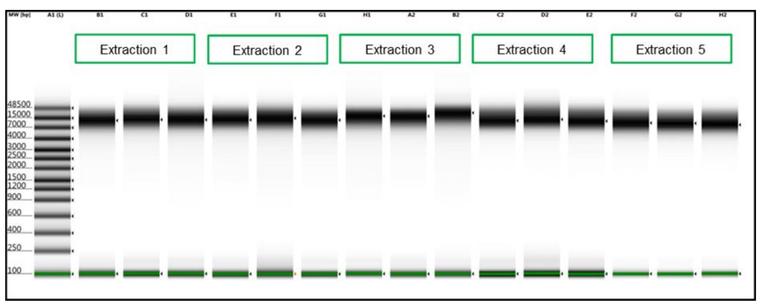


Figure 4: A typical gDNA analysis using the Genomic DNA ScreenTape

The inter-assay and intra-assay precision of the Genomic DNA ScreenTape assay was analyzed using the data from the replicates. Table 1 presents the inter-assay and intra-assay values demonstrating the Genomic DNA ScreenTape assays are within the stated specification of ±20%

Reproducibility CV%	Intra - Assay	Inter - assay
Quantitation	7.8	10.5
Sizing	9.4	17.7

Table 1: Sizing and quantification precision of the Genomic DNA ScreenTape assay

The Genomic DNA ScreenTape assay uses the lower maker to quantify the samples. The samples were run as triplicates and the quantification data was collated for each extraction kit. The samples were also quantified using the Qubit dsDNA broad range assay. The mean values obtained from Qubit and the Genomic DNA ScreenTape assay was plotted and compared and presented in the Figure 5.

The quantification accuracy of the Genomic DNA ScreenTape assay was compared to Qubit Fluorometer. A accuracy of >80% was obtained for gDNA samples extracted using different kits.

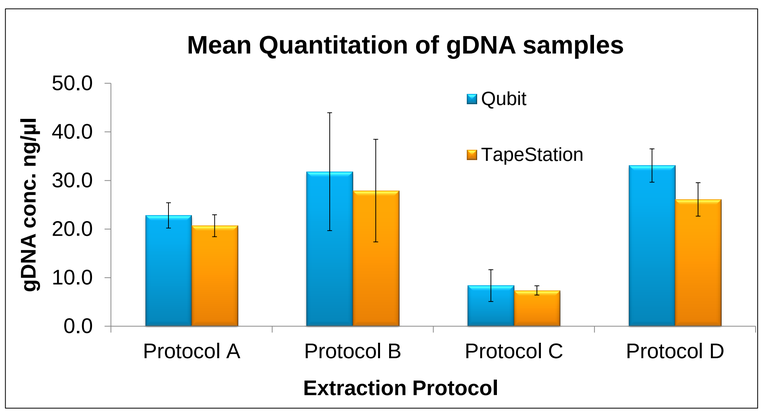


Figure 5: Quantification comparison of Qubit and 2200 TapeStation.

Genomic DNA quantitation

The quantification reproducibility across three days of replicates was analysed and presented in the Figure 6. The small error bars demonstrates the reproducibility of the Genomic DNA ScreenTape assay in quantifying gDNA samples.

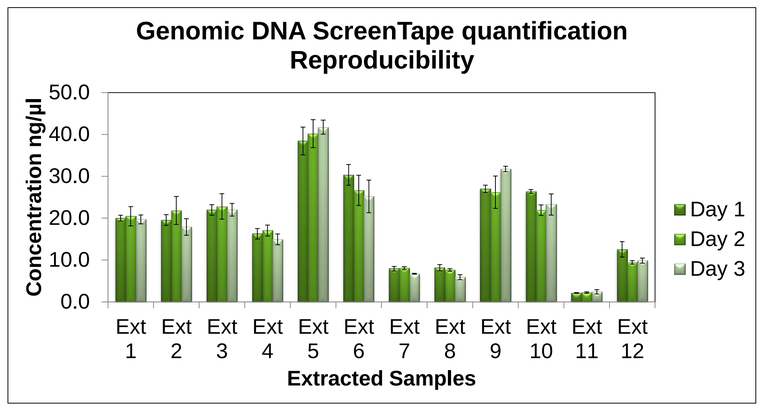


Figure 6: Gel image of fragmented gDNA analysis.

Genomic DNA integrity QC analysis

The performance of the Genomic DNA ScreenTape assay in discriminating different quality of gDNA was assessed by analyzing artificially fragmented sample. The gDNA extracted using the Qiagen extraction kit was subjected to sonication and samples with different levels of fragmentation was analyzed using the 2200 TapeStation instrument. The gel image in Figure 7 shows the fragmentation of gDNA over time. The single tight peak of gDNA started to break into smaller pieces that run as smears.

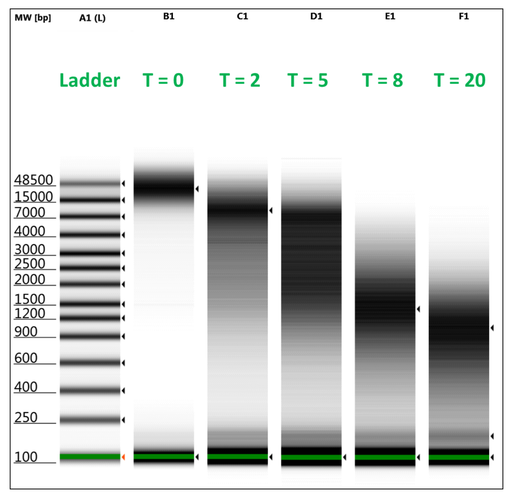


Figure 7: Gel image of fragmented gDNA analysis.

The electropherogram overlay shown in Figure 8 shows the fragmentation of the gDNA over the time period. The intact genomic DNA on sonication gets fragmented into smaller sizes. The randomly fragmented smaller sizes run as smear and the concentration of the smaller bands increases with the increase in the sonication time.

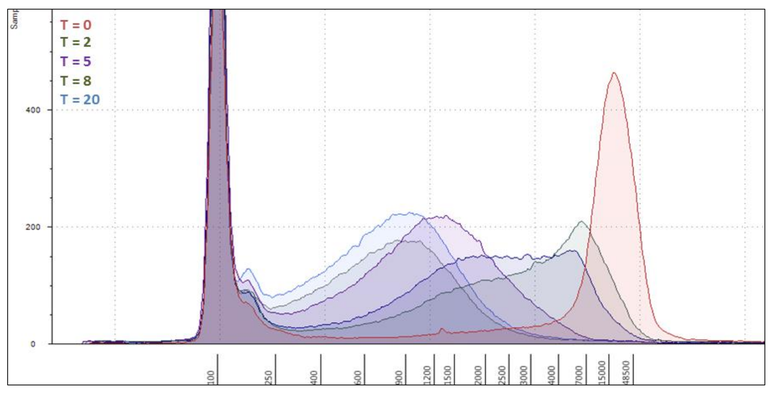
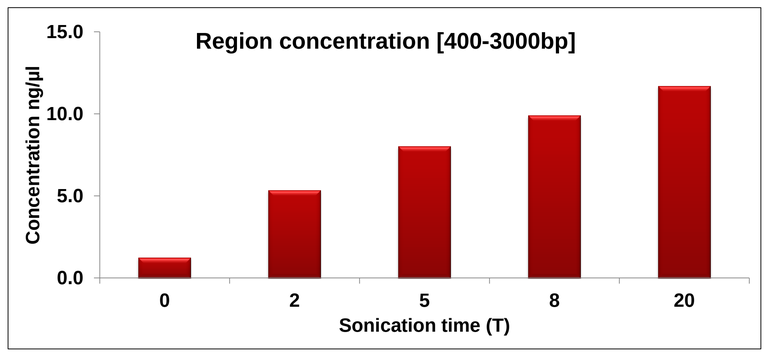


Figure 8: Electropherogram overlay of genomic DNA fragmentation.

The increase in concentration of the sample between the region 400 – 3000bp indicates the fragmentation of the genomic DNA and is presented in Figure 9.



Conclusions

- Agilent 2200 TapeStation and the Genomic DNA ScreenTape assay enables analysis of high molecular weight genomic DNA.
- The accuracy of sizing and quantification of the Genomic DNA ScreenTape assay is well within the specification of ±20%
- The sizing and quantification precision (CV) of the Genomic DNA ScreenTape assay is ±20%
- The Genomic DNA ScreenTape assay forms a suitable platform for gDNA integrity QC.