

Analysis of high molecular weight genomic DNA using the Agilent 2200 TapeStation and Genomic DNA ScreenTape

Application Note

Nucleic Acid Analysis

Authors

Arunkumar Padmanaban
Agilent Technologies, Inc.
Bangalore, India

Donna McDade Walker
Agilent Technologies, Inc.
Edinburgh, UK



Introduction

This Application Note demonstrates the use and application of the Agilent Genomic DNA ScreenTape assay in conjunction with the Agilent 2200 TapeStation system for easy sizing, quantification and quality assessment of genomic DNA samples. Intact, high quality genomic DNA in combination with accurate sample quantification is required for numerous applications, particularly array comparative genome hybridization and next generation sequencing.



Agilent Technologies

Experimental

Cell Culture

The HEK293 cell line was purchased (CRL1573, ATCC) and cultured using MEM medium containing 10 % FBS and 1 % Pen/Strep (Sigma Aldrich, India). After incubating at 37 °C in a 5 % CO₂ atmosphere for four days, the cells were harvested at 80 % confluence. Each cell pellet containing approximately 1 million cells was frozen at -80 °C until further use.

Genomic DNA Extraction

Genomic DNA (gDNA) was extracted from the HEK293 cell pellets using four extraction kits: The Agilent DNA Extraction Kit (200600) was obtained from Agilent Technologies. Additional extraction kits were DNeasy Blood and Tissue kit (69504; Qiagen), PureLink Genomic DNA mini kit (K1820-00; Invitrogen) and the Wizard SV Genomic DNA kit (A2360; Promega). All were purchased from respective vendors and used following manufacturer guidelines. Extractions using Qiagen and Invitrogen kits were carried out in triplicate and extraction using Promega and Agilent kits were carried out in duplicate.

Genomic DNA Analysis: Quantification

The extracted genomic DNA was quantified using the Qubit dsDNA BR assay kit (Q32850) using the Qubit 1.0 Fluorometer following the manufacturer guidelines. The genomic DNA was also quantified using the NanoDrop 1000 spectrophotometer.

Quantification, Sizing and Integrity Analysis using the Agilent Genomic DNA ScreenTape assay

The 2200 TapeStation system (G2964AA), Genomic DNA ScreenTape (5067-5365) and Genomic DNA Reagents (5067-5366) were obtained from Agilent Technologies. The extracted gDNA was analyzed using the Genomic DNA ScreenTape assay. The samples were prepared by mixing 1 µL of gDNA sample with 10 µL of Genomic DNA Sample buffer. A 3 µL amount of Genomic DNA Ladder was placed in the first tube of an 8-way strip followed by the samples. The prepared strip was vortexed on high speed for 5 seconds, centrifuged and placed in the 2200 TapeStation instrument. The samples were analyzed as triplicates for each individual extracted sample.

Results and Discussion

The efficiency of four different genomic DNA extraction kits from different vendors was assessed using the Agilent Genomic DNA ScreenTape assay in conjunction with the 2200 TapeStation system. We have named these

protocols A, B, C, and Agilent. In each case, the genomic DNA concentration, molecular weight, sample integrity, and thus overall yield and quality was determined.

Genomic DNA Quantification

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 and NanoDrop spectrophotometer to compare results. The mean values obtained from the Qubit, NanoDrop and the Genomic DNA ScreenTape assay was plotted, compared and presented in Figure 1.

The NanoDrop gives higher concentrations than the 2200 TapeStation and Qubit as this system cannot distinguish double stranded DNA from single stranded DNA or RNA. UV based measurement is also affected by other factors, such as salt, that can influence absorbance reading. In contrast, the Qubit and the Genomic DNA ScreenTape assay use a fluorescence

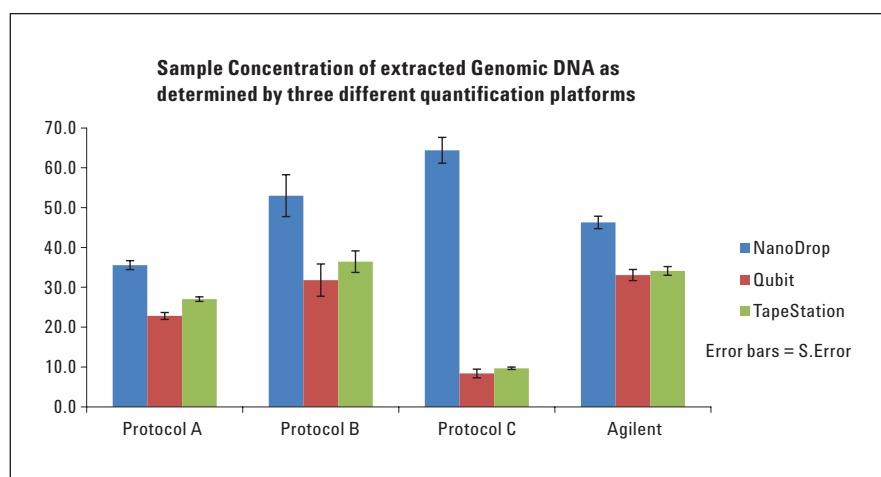


Figure 1
Mean quantification data (in ng/µL) for each extraction kit as reported by the Agilent 2200 TapeStation, Nanodrop, and Qubit instruments

dye that binds specifically to double stranded DNA. This means that the 2200 TapeStation only reports the concentration of the genomic DNA. The data shows that the ScreenTape and Qubit provide very similar results with a minimum average difference of 1 % and a maximum average difference of 5 %. The CV percentages are similar across all three platforms for each kit illustrating differences between each triplicate extracted sample rather than any performance variances in the platforms.

Genomic DNA Size Distribution Analysis

Using the Genomic DNA ScreenTape assay to quantify samples provides important additional information on the sample size distribution and quality of the genomic DNA extracted. This is information that is not available when using photometric or fluorescent measurement techniques. The gel image in Figure 2 was easily exported from the 2200 TapeStation software and shows the differences in quality of genomic DNA obtained from the four different extraction methods.

Figure 2 illustrates triplicates of the samples from each extraction kit and provides information about the size distribution in each case. The differences in genomic DNA sample integrity is easily visualized; highly intact genomic DNA appears as a sharp, compact band whereas degraded genomic DNA can be seen as a diffuse smear extending into the lower molecular weight range of the ScreenTape lane. These results were presented in less than 2 minutes per sample, enabling a rapid assessment of the integrity of the genomic DNA sample.

The data in the box and whisker plot in Figure 3 was produced using the size information obtained from the 2200 TapeStation software and exported to CSV. Plotting this data provides an easy check of the DNA shearing due

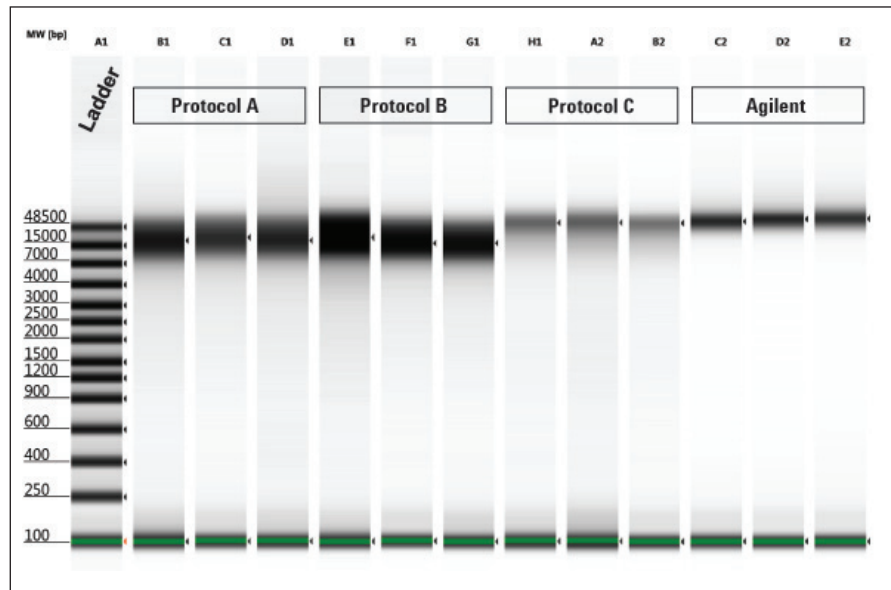


Figure 2
Gel image representing triplicate analyses of gDNA samples obtained from four extraction kits.

to the extraction method. The plot illustrates the molecular weight size distribution of extracted genomic DNA from each of the kits as reported by the Genomic DNA ScreenTape. The whiskers represents the molecular weight spread of the gDNA obtained using the four extraction methods. One of the replicate extractions from Protocol B produced sheared genomic

DNA, which is represented as outlying data in Figure 3. This could be detected using the Genomic DNA ScreenTape but would not have been identified using only the Qubit or Nanodrop. The graph indicates that a higher average molecular weight and therefore a more intact sample of genomic DNA was obtained when using the Agilent DNA Extraction Kit.

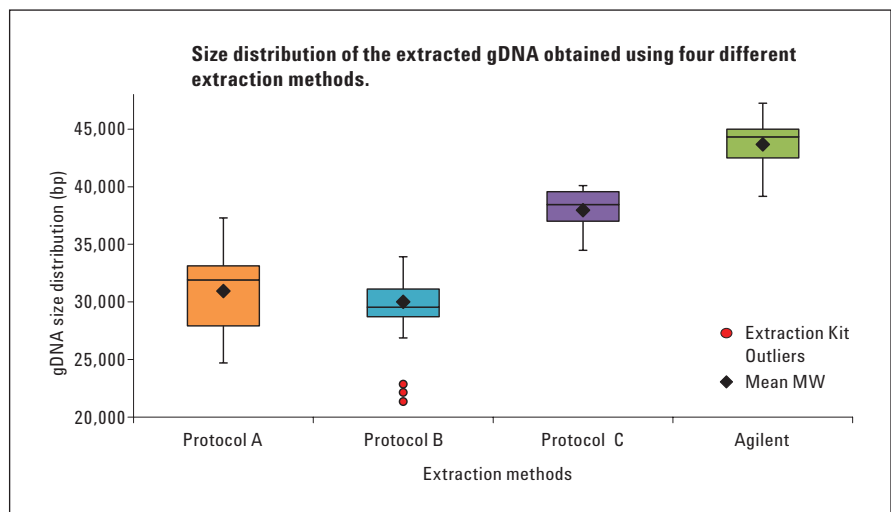


Figure 3
Size distribution of extracted genomic DNA from four kits.

The bubble chart in Figure 4 shows the relationship between the average molecular weight size of the overall sample trace versus the molecular weight size of the peak maxima, with the bubble size indicating the concentration of the sample for each of the four extraction kits. This information illustrates the overall extraction efficiency for each kit, with a large bubble located in the top right hand quadrant of the chart the most desirable outcome. The data was collated from the CSV export functionality of the 2200 TapeStation software.

The chart shows that protocol A and B extract smaller molecular weight sized double stranded genomic DNA fragments with average molecular weights of less than 25,000 bp. Genomic DNA obtained using Protocol C is of high molecular weight intact DNA but of low sample concentration and thus low overall yield. The Agilent DNA Extraction Kit gives the highest sample yield consisting of approximately 30 ng/ μ L of double stranded, intact high molecular weight genomic DNA with sizes around 45,000 bp.

Genomic DNA Integrity Analysis

The Genomic DNA ScreenTape assay allows the comparison of multiple samples to examine the differences in sample size, distribution and peak shape. This feature is of particular importance when comparing past and present samples to a chosen standard. The profile overlay in Figure 5 illustrates these parameters for each of the different extraction methods.

Conclusion

The Agilent 2200 TapeStation system in conjunction with the Agilent Genomic DNA ScreenTape assay provides an excellent solution for assessing the quantity, integrity and overall quality of genomic DNA starting material from

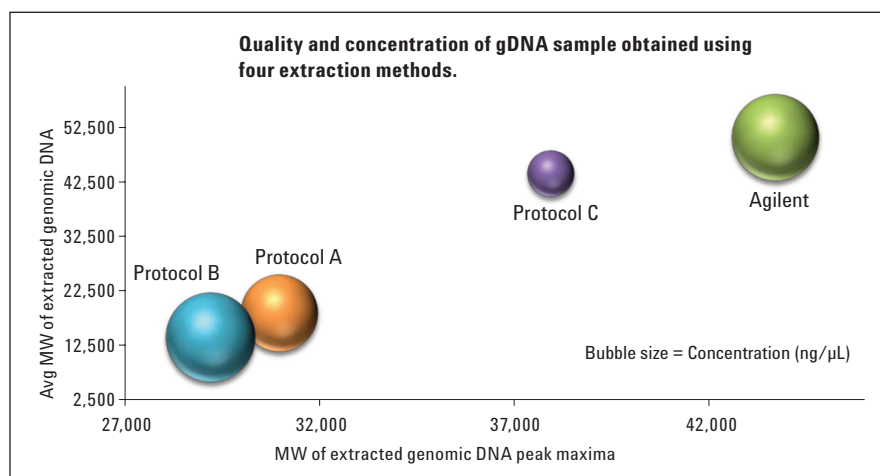


Figure 4
Size distribution and concentration of extracted genomic DNA from four extraction kits.

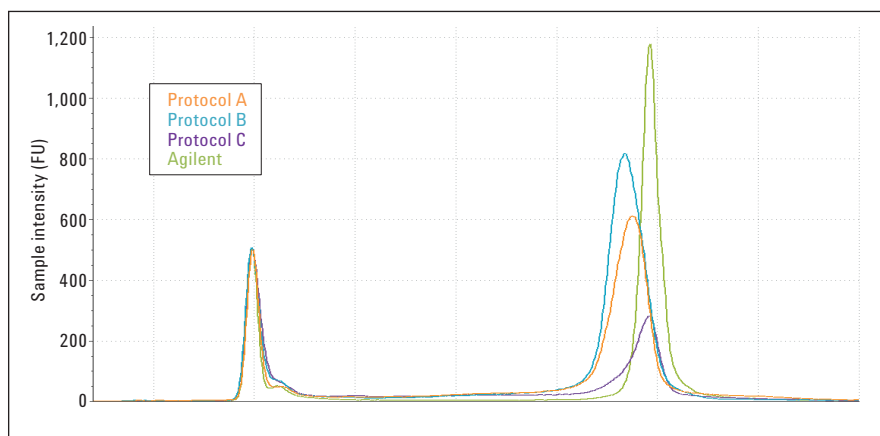


Figure 5
Electropherogram overlay of gDNA from the four extraction kits.

a single run, returning results in less than 2 minutes per sample and using only 1 μ L of genomic DNA. The 2200 TapeStation software easily enables the rapid assessment of experimental starting material by providing information about the efficiency of any given extraction method. The quantification of the Genomic DNA ScreenTape assay is highly comparable to the fluorescence-based Qubit instrument. Differences seen with UV based detection are attributed to the differences in the measurement method between the fluorescence based platforms and the spectrophotometric NanoDrop.

Contact Us:

US/Canada

Phone: Toll-free 1-800 227 9770

Email: agilent_inquiries@agilent.com

Europe

Email: info_agilent@agilent.com

Asia Pacific

Email: inquiry_lsca@agilent.com

www.agilent.com/genomics/tapestation

© Agilent Technologies, Inc., 2013
Published in USA, April 1, 2013
Publication Number 5990-1797EN



Agilent Technologies