High Throughput Genomic DNA Assessment by the Agilent 4200 TapeStation System

Technical Overview

Introduction

The Agilent 4200 TapeStation system is a high throughput automated system for fast and reliable DNA and RNA electrophoresis. Uniquely, the system offers scalable sample processing from 1 to 96 samples at a constant cost per sample. In conjunction with the Agilent Genomic DNA ScreenTape assay, the system can separate and analyze genomic DNA (gDNA) from 200 up to greater than 60,000 base pairs. It provides accurate quantification and sizing data as well as an automated numerical assessment of gDNA quality based on the DNA integrity number (DIN)\(^1\) and is therefore the ideal QC tool for next generation sequencing (NGS) and array comparative genomic hybridization (aCGH) workflows.

This Technical Overview focuses on the performance of the Genomic DNA ScreenTape assay with respect to gDNA integrity analysis and sample sensitivity, as well as sizing and quantification accuracy and precision. Correlating this data with the Agilent 2200 TapeStation system demonstrates the full compatibility of both systems.

Table 1 summarizes the analytical specifications of the Genomic DNA ScreenTape assay.

<table>
<thead>
<tr>
<th>Analytical specifications</th>
<th>Agilent Genomic DNA ScreenTape Assay</th>
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<tbody>
<tr>
<td>Sizing range</td>
<td>200 to &gt;60,000 bp</td>
</tr>
<tr>
<td>Sensitivity(^1)</td>
<td>0.5 ng/µL</td>
</tr>
<tr>
<td>Sizing precision(^2)</td>
<td>200 bp–15,000 bp: 15 %CV</td>
</tr>
<tr>
<td>Sizing accuracy(^2)</td>
<td>200 bp–15,000 bp: ± 15 %</td>
</tr>
<tr>
<td>Quantitative precision(^3)</td>
<td>15 %CV</td>
</tr>
<tr>
<td>Quantitative accuracy(^3)</td>
<td>± 20 %</td>
</tr>
<tr>
<td>Quantitative range</td>
<td>10–100 ng/µL</td>
</tr>
<tr>
<td>DIN functional range(^4)</td>
<td>5–300 ng/µL</td>
</tr>
<tr>
<td>Carryover</td>
<td>N/A</td>
</tr>
</tbody>
</table>

1 Signal-to-noise > 3 (single peak)
2 Determined using the Genomic DNA ladder as sample
3 Average result from various genomic DNA sample types
4 DIN = DNA Integrity Number
Experimental

Material
Mouse Genomic DNA was obtained from Clontech Laboratories, Inc. (Mountain View, CA, USA). Human Genomic DNA from Promega Corp. (Madison, WI, USA). The ND2000 NanoDrop UV/Vis spectrophotometer was purchased from Thermo Fischer Scientific Inc. (Wilmington, DE, USA). The Agilent 4200 TapeStation system (p/n G2991AA), Agilent 2200 TapeStation system (p/n G2964AA or G2965AA) with Genomic DNA ScreenTape (p/n 5067-5365) and Genomic DNA Reagents (p/n 5067-5368) were obtained from Agilent Technologies (Waldbronn, Germany).

Sample preparation
Commercially available mouse gDNA was sheared mechanically by aspiration through a needle to generate samples with different gDNA integrity. The gDNA concentration was determined using UV-Vis spectrophotometry. Based on the quantification, a dilution series of the gDNA samples was prepared.

Genomic DNA analysis
The gDNA analysis was performed using the Genomic DNA ScreenTape assay and both the 2200 and 4200 TapeStation systems according to the manufacturer[2,3]. On both platforms, TapeStation software revisions A.02.01 were used for data analysis. In brief, 1 µL gDNA was mixed with 10 µL Genomic DNA sample buffer. The consumables and the prepared samples were placed in the TapeStation instrument. Samples were tested on the 2200 TapeStation system analyzing them on a single Genomic DNA ScreenTape device. Samples on the 4200 TapeStation system were loaded from 96-well plates.

Results and Discussion

gDNA Integrity Analysis
Three different mouse gDNA samples with different DNA integrity were analyzed using the 4200 TapeStation system. The 4200 TapeStation Software displays the results as an electropherogram, a gel image, and data table. The DIN value is automatically determined, and directly displayed under the individual lane of the gel image (Figure 1). The DIN is calculated using a scale from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN a strongly degraded gDNA sample.

The three different mouse gDNA samples with different DNA integrity were analyzed in four to five different concentrations ranging from 5 to 100 ng/µL using the 4200 TapeStation system. The same samples were also analyzed using the 2200 TapeStation system for a direct comparison.

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Figure 1. Mouse gDNA samples with different DNA integrity at 50 ng/µL were analyzed using the Agilent 4200 TapeStation system and the Agilent Genomic DNA ScreenTape assay. The determined DIN values are shown under the gel image. The overlay shows the corresponding electropherograms.
Figure 2 summarizes the data obtained for the gDNA integrity analysis using the 4200 TapeStation and the 2200 TapeStation systems. The DIN obtained with both TapeStation systems is equivalent. In addition, the DIN is highly reproducible and independent of the loaded sample concentration. The standard deviation for the DIN was below 0.2 for the samples analyzed with the 4200 TapeStation system (n = 252) and in excellent agreement with data shown previously for the 2200 TapeStation platform.

**gDNA Quantification**

The Genomic DNA ScreenTape assay also provides quantification data for distributed DNA populations or smears. To demonstrate the quantification precision and accuracy, 15 male mouse gDNA samples with different gDNA integrity and with nominal concentrations ranging from 10 to 100 ng/µL were quantified as a replicate of six on 96-well plates using three different 4200 TapeStation instruments (n = 18). Figure 3 summarizes the quantification data obtained with the 4200 TapeStation system.

The quantification was reproducible across all samples and within specification for the quantitative precision (Table 1). Furthermore, Figure 3 demonstrates that quantitative precision is not dependent on the gDNA integrity of the measured sample.
The average quantitative accuracy of the 4200 TapeStation system for all measured gDNA samples compared to the concentration determined with the spectrophotometric measurement was within the specifications of the Genomic DNA ScreenTape assay (Table 1).

The same samples shown in Figure 3 were analyzed on a single Genomic DNA ScreenTape device using four different 2200 TapeStation instruments (n = 4) to compare the quantification performance of both TapeStation systems. The measured gDNA concentrations determined with both TapeStation systems were plotted against each other (Figure 4).

The obtained correlation for the quantification with both TapeStation systems was excellent, with an $R^2$ of 0.9971.

**Sensitivity**

Highly intact mouse gDNA at 0.5 ng/µL was analyzed repeatedly across a 96-well plate (n = 12) using the 4200 TapeStation system and the Genomic DNA ScreenTape assay. Figure 5 shows the electropherogram overlay of all 12 runs.

The signal peaks from 0.5 ng/µL gDNA are clearly visible above the baseline, with a signal-to-noise ratio greater than 3. This confirms the specified sensitivity for the Genomic DNA ScreenTape assay (Table 1).
gDNA Sizing
In addition to quantification, the Genomic DNA ScreenTape assay also provides sizing data for distributed DNA populations or smears.

To determine the sizing precision of the Genomic DNA ScreenTape assay, a human gDNA sample with medium DNA integrity was analyzed at five different concentrations ranging from 10 to 100 ng/µL with the 4200 TapeStation and the 2200 TapeStation systems, and the obtained average smear sizes were compared.

For the tested gDNA samples, the sizing was independent of the loaded gDNA concentration. The sizing precision (Figure 6) was in agreement with the specified sizing precision for the Genomic DNA ScreenTape assay (Table 1).

Conclusion
This Technical Overview describes the highly accurate and reproducible sizing and quantification of gDNA using the Genomic DNA ScreenTape assay and Agilent 4200 TapeStation. In addition, it details that the results obtained are equivalent to the medium throughput Agilent 2200 TapeStation system. The new high throughput 4200 TapeStation system along with the Genomic DNA ScreenTape assay therefore offers the same ease-of-use combined with high quality gDNA integrity, size, and quantification data, but also offers full 96-sample walkaway operation.

Figure 6. Human gDNA samples ranging from 10 to 100 ng/µL were analyzed with the Genomic DNA ScreenTape assay using the 4200 TapeStation (n = 10 to 72) and the 2200 TapeStation (n = 6 to 24) system.
References

1. DNA Integrity Number (DIN) with the Agilent 2200 TapeStation System and the Agilent Genomic DNA ScreenTape Assay, *Agilent Technologies*, publication number 5991-5258EN, 2014.
