

Performance Characteristics of the Genomic DNA ScreenTape Assay for the 4150 TapeStation System

Introduction

The Agilent 4150 TapeStation system provides a fast and reliable automated electrophoresis solution for DNA and RNA quality control. The system allows the analysis of between 1 and 16 individual samples with automated sample processing and ready-to-use consumables to minimize handling effort. Plus, since the entire ScreenTape portfolio as well as the TapeStation software is compatible with the 4150 TapeStation system users can seamlessly transition between the 4150 and the 4200 TapeStation system. The Agilent Genomic DNA ScreenTape assay was developed for the size-based separation and quantitative analysis of genomic DNA (gDNA). It also provides an objective assessment of sample quality, based on the DNA integrity number (DIN). Together the Genomic DNA ScreenTape assay and the Agilent 4150 TapeStation system provide the perfect tool for gDNA quality control for low throughput demand.

This Technical Overview evaluates the performance characteristics of the Genomic DNA ScreenTape assay on the Agilent 4150 TapeStation system. These performance characteristics included gDNA integrity analysis, sensitivity, as well as accurate and precise sizing and quantification. For a compatibility demonstration, the performance on the 4150 TapeStation system was measured against the 4200 TapeStation system.

Analytical Specifications

Table 1 summarizes the analytical specifications of the Genomic DNA ScreenTape assay for both the 4150 and the 4200 TapeStation systems¹.

Table 1. Analytical specifications of the Genomic DNA ScreenTape assay.

Analytical Specifications	Genomic DNA ScreenTape Assay
Sizing range	200 to >60,000 bp
Sensitivity ¹	0.5 ng/μL
Sizing accuracy ²	200– 5,000 bp: ±15 %
Sizing precision ²	200–15,000 bp: 15 % CV
Quantitative accuracy	±20 %
Quantitative precision	15 % CV
Quantitative range	10–100 ng/μL
DIN functional range ³	5–300 ng/μL

¹ S/N ratio >3 for a single peak

² Determined by analyzing the genomic DNA ladder as sample

³ DIN—DNA Integrity Number

Experimental

Material

The Agilent 4150 TapeStation system (p/n G2992AA) and the Agilent 4200 TapeStation system (p/n G2991AA) with the Agilent Genomic DNA ScreenTape (p/n 5067-5365) and the Agilent Genomic DNA Reagents (p/n 5067-5366) were obtained from Agilent Technologies (Waldbronn, Germany). Fermentas Lambda DNA was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Human genomic DNA was obtained from Promega (Waltham, MA, USA), a Digital Sonifier from Branson Ultrasonics (Danbury, CT, USA), and insulin syringes Omnican 50 from B. Braun Melsungen AG (Melsungen, Germany).

Sample preparation

Fermentas lambda DNA was diluted with TE buffer and the resulting concentrations covered the entire specified quantitative range of the assay (Table 1). Commercially available gDNA was degraded using a combination of ultrasonication and shearing to produce four gDNA samples at different stages of degradation, covering the whole integrity range.

DNA analysis

DNA samples were analyzed using the Genomic DNA ScreenTape assay on the 4150 and 4200 TapeStation systems, according to the manufacturer's instructions¹. The samples were analyzed in replicates of 18 on three different 4150 and 4200 TapeStation instruments each. For data analysis, Agilent TapeStation software revisions 3.1 (4150 TapeStation system), and A.02.02 (SR1) (4200 TapeStation system) were applied.

Results and Discussion

Integrity analysis

Genomic DNA (gDNA) serves as starting material for numerous molecular analysis methods and especially in next-generation sequencing (NGS) workflows. Since the integrity of gDNA critically affects the success of any downstream application, quality control of the input material is an essential step to ensure high-quality results.

A software algorithm was established for the Genomic DNA ScreenTape assay and the TapeStation systems, which delivers the DNA integrity number (DIN) as objective quality metric for standardized DNA integrity assessment. Signal distribution across the entire size range of the assay is considered resulting in a numerical DIN ranging from 10 (highly intact DNA) to 1 (strongly degraded DNA)². DIN can be assessed within a wide concentration range from 5 to 300 ng/ μ L. The DIN as quality metric using the assay together with the 4200 TapeStation system has previously been validated³.

Figure 1 shows the analysis of four differentially degraded gDNA samples in triplicate performed with the 4150 TapeStation system and the Genomic ScreenTape assay. The results are displayed in the gel image view along with the automatically assessed DIN and the electropherogram view. Highly intact DNA shows up as a narrow peak above the highest marker peak of 48,500 bp on the gel image as well as in the electropherogram. In contrast, increasing degradation shifts the DNA to smaller sizes along with a broader distribution, which appears as a smear.

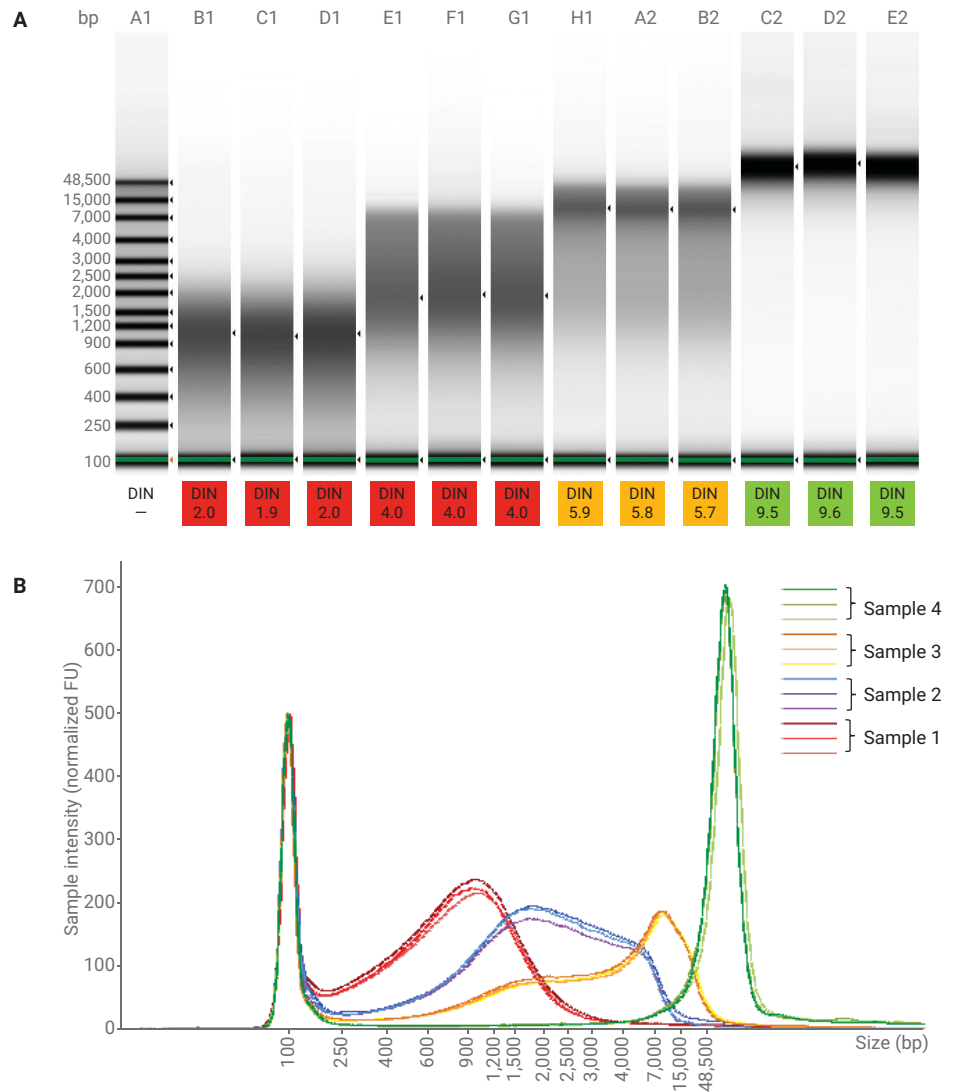


Figure 1. Four gDNA samples (30 ng/ μ L) at different degradation stages were analyzed on the 4150 TapeStation system together with the Genomic DNA ScreenTape assay to determine DNA integrity by the DNA integrity number (DIN). A) Gel image with corresponding DIN displayed below each lane including color coded result flagging. B) Electropherogram overlay of all 12 gDNA samples.

Genomic DNA integrity assessment on the 4150 TapeStation system was evaluated by analyzing the same DNA samples as a replicate of 18 on three different 4150 TapeStation instruments. The results were compared to data obtained from three different 4200 TapeStation instruments. The DIN values produced were highly reliable and reproducible across all the applied instruments. Furthermore, the DIN values were determined precisely with standard deviations below 0.2, as well as equivalent on the two different TapeStation systems (Figure 2).

Sensitivity

A 48,500 bp lambda DNA sample at 0.5 ng/μL was analyzed with the 4150 TapeStation system using the Genomic DNA ScreenTape assay for sensitivity evaluation.

Figure 3 shows the electropherogram overlay of the sample focusing on the enlarged peaks in the insert section. The lambda DNA sample is clearly detected at the specified limit of detection of 0.5 ng/μL, exhibiting a signal-to-noise ratio (S/N) greater than three. This verifies the sensitivity of 0.5 ng/μL for the assay on the 4150 TapeStation system.

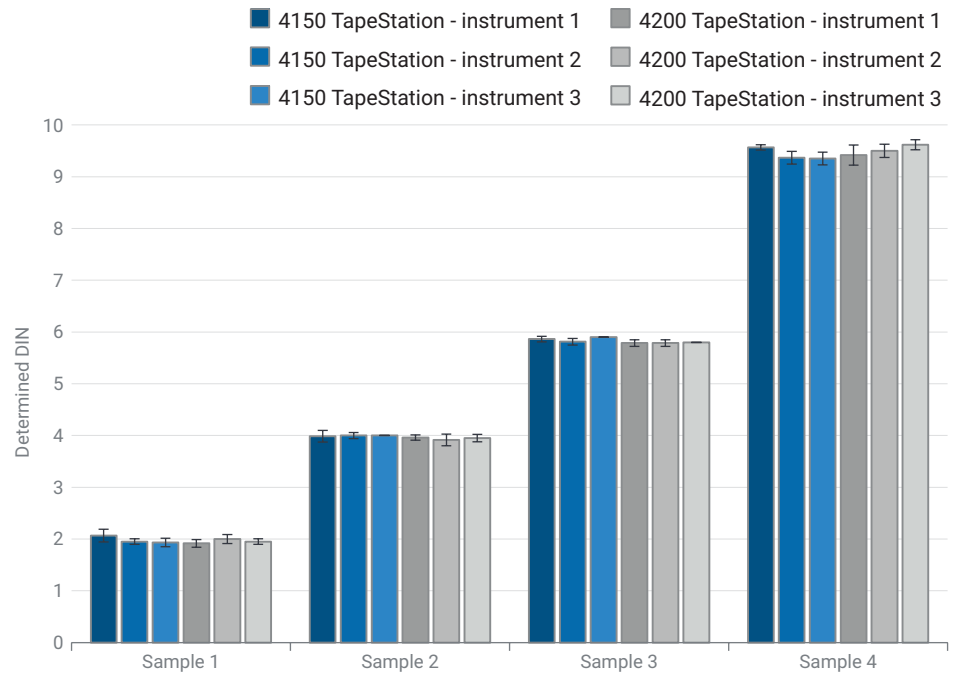


Figure 2. Averaged DIN values including standard deviations of four differentially degraded gDNA samples (n = 18) analyzed on each three different 4150 and 4200 TapeStation instruments using the Genomic DNA ScreenTape assay.

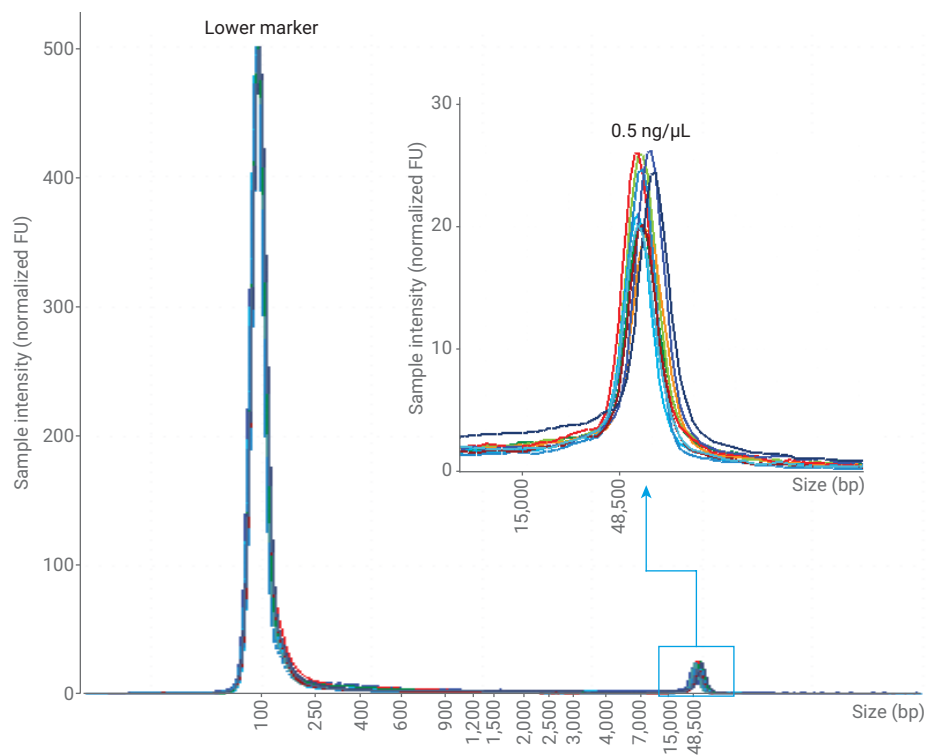


Figure 3. Electropherogram overlay of the lambda DNA sample analysis at the specified limit of detection (0.5 ng/μL) on the 4150 TapeStation system using the Genomic DNA ScreenTape assay. The enlarged image shows a close-up of the resulting peaks (n = 10).

Sizing

The Genomic DNA ScreenTape assay enables the analysis of DNA samples in a size range from 200 to over 60,000 bp. To demonstrate sizing accuracy and precision according to the specifications presented in Table 1 on the 4150 TapeStation system the Genomic DNA assay ladder was analyzed as a sample.

Sizing results of all the ladder fragments were highly accurate, with below 6 % deviation from the nominal size supplied by the manufacturer, which is within the specified range of $\pm 15\%$ (Figure 4). Additionally, the average sizing precision met the specification of 15 % CV (data not shown).

The sizing performance of the assay, which was verified previously on the 4200 TapeStation system³, was also confirmed for the 4150 TapeStation system.

Quantification

The Genomic DNA ScreenTape also provides quantitative analysis within the accounted linear range from 10 to 100 ng/ μL as shown in Table 1.

To validate the quantification performance of the assay on the 4150 TapeStation system, serial dilutions of the lambda DNA samples covering the entire concentration range were analyzed. The same dilution series was also quantified on the 4200 TapeStation system for a direct comparison.

The quantitative results of the two TapeStation systems are plotted against each other in Figure 5. The results showed excellent correlation with an R^2 value of 99.9 %. The concentrations obtained with the 4150 TapeStation system were highly accurate and deviated by below 8 % from the corresponding results achieved with the 4200 TapeStation system.

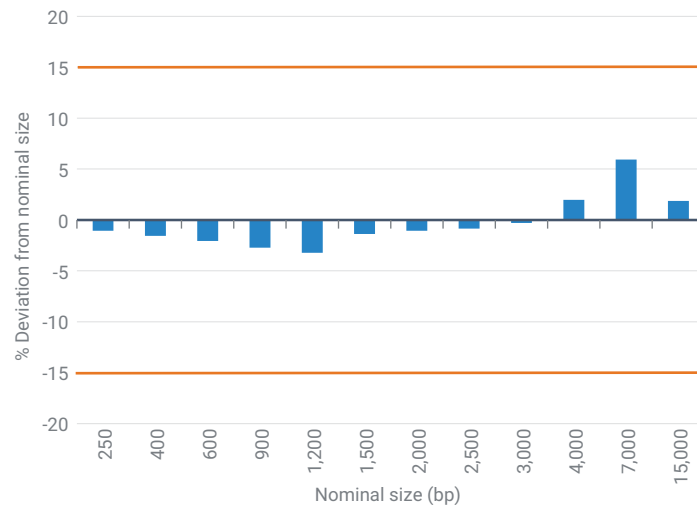


Figure 4. Sizing accuracy of the Genomic DNA assay ladder analyzed with the 4150 TapeStation system and the Genomic DNA ScreenTape assay. Percent deviation from the nominal size is displayed for 12 ladder fragments covering a size range from 250–15,000 bp. The orange line indicates the specified sizing accuracy of $\pm 15\%$.

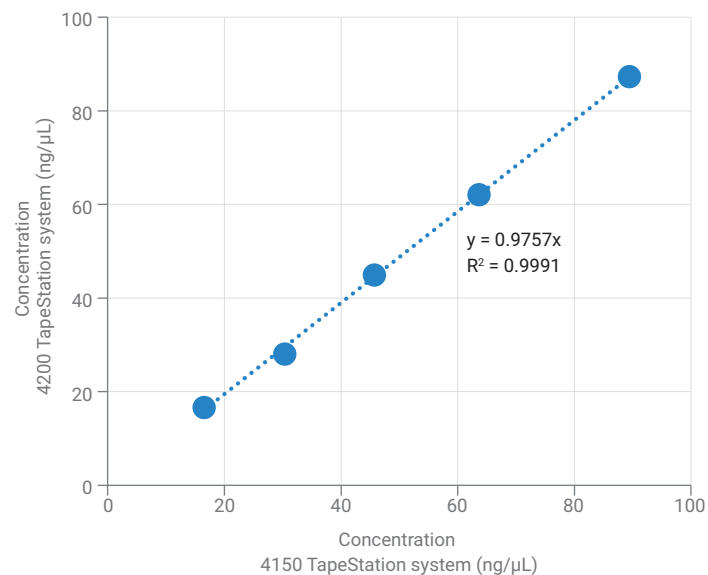


Figure 5. Quantification of lambda DNA in dilution series from 10–100 ng/ μL . The samples were quantified with the Genomic DNA ScreenTape assay on the 4150 TapeStation system (X-axis) and compared to data obtained with the 4200 TapeStation system (Y-axis).

Furthermore, quantification was precise with % CV values below 13 %, which was very comparable between the two TapeStation systems (Figure 6).

The quantitative accuracy and precision specifications of the assay, already demonstrated on the 4200 TapeStation system³, were confirmed on the 4150 TapeStation system.

Conclusion

This Technical Overview confirms the specifications of the Genomic DNA ScreenTape assay on the 4150 TapeStation system. The combination of the system and the assay ensures sensitivity down to 0.5 ng/μL as well as highly accurate and precise sizing and quantification of gDNA. Besides, the standardized DIN quality metric delivers reliable and consistent assessment of gDNA integrity in the large DIN functional range from 5 to 300 ng/μL. The objective quality measure of gDNA material is crucial for NGS workflows as well as biobank facilities. The performance of the Genomic DNA ScreenTape assay on the 4150 TapeStation was confirmed to be equivalent to the 4200 TapeStation system to assuring an easy switch and full compatibility between the two systems.

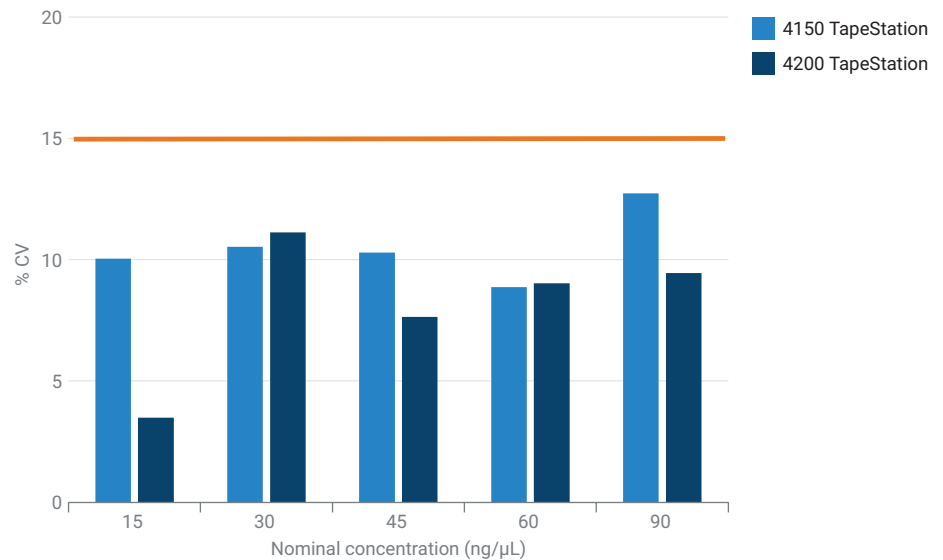


Figure 6. Quantification precision of the lambda DNA sample in five concentrations analyzed with the 4150 and the 4200 TapeStation systems (n = 18). The orange line indicates the corresponding specified quantitative precision of 15 %CV.

References

1. Agilent Genomic DNA ScreenTape Assay Quick Guide for 4200 TapeStation System. *Agilent Technologies*, publication number G2991-90040, **2015**.
2. DNA Integrity Number (DIN) with the Agilent 2200 TapeStation System and the Agilent Genomic ScreenTape Assay. *Agilent Technologies Technical Overview*, publication number 5991-5258EN, **2015**.
3. High Throughput Genomic DNA Assessment by the Agilent 4200 TapeStation System. *Agilent Technologies Technical Overview*, publication number 5991-6629EN, **2016**.

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