Technical Overview

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Performance Equivalence of the D1000 ScreenTape Assays on the Agilent TapeStation Systems

Introduction

Agilent TapeStation systems are well-established automated electrophoresis systems for fast and reliable analysis of nucleic acids for multiple applications. The platform integrates a benchtop electrophoresis-based instrument, data-processing TapeStation software, and reagents, used in concert with application-specific ScreenTape sample-processing consumable devices. DNA and RNA samples can be analyzed with minimal handling effort and fully scalable throughput from 1 to 96 samples. The entire Agilent ScreenTape portfolio for TapeStation systems is a vital tool for sample quality control (QC) at different checkpoints in next-generation sequencing (NGS) workflows.

To provide users with continued improvements and benefits to the TapeStation platform, Agilent has recently introduced a technology update to the Agilent 4200 TapeStation instrument. For example, all instruments manufactured from spring 2021 are equipped with a modified ScreenTape nest, enabling users to easily exchange the electrode cartridge during the yearly, mandatory, preventive maintenance service. Thus, the modified ScreenTape nest secures an optimized cost of ownership. In addition, a modified instrument optical system allows for a better match with the application-specific fluorescent dyes. All existing ScreenTape applications as well as plastic consumables are fully compatible with the new 4200 TapeStation system.

This technical overview highlights the performance of the Agilent D1000 ScreenTape assay and the Agilent High Sensitivity D1000 ScreenTape assay on the new 4200 TapeStation system. Analytical assay specifications like sensitivity, sizing, quantification, and molarity delivered by the new instrument were evaluated and compared with the results obtained on the legacy 4200 and 4150 TapeStation systems to demonstrate data equivalency between all three models.

Analytical specifications

Sizing, quantification, and molarity were compared between three TapeStation models using the provided corresponding ScreenTape ladders as samples, a 300 bp DNA fragment, and sheared human genomic DNA (gDNA). Table 1 summarizes the analytical specifications of the D1000 and High Sensitivity D1000 ScreenTape assays.

Table 1. Comparison of analytical specifications of the Agilent D1000 ScreenTape assay and the Agilent High Sensitivity D1000 ScreenTape assay.

Analytical Specifications	Agilent D1000 ScreenTape Assay	Agilent High Sensitivity D1000 ScreenTape Assay
Sizing Range	35 to 1,000 bp	35 to 1,000 bp
Typical Resolution	35 to 300 bp: 15% 300 to 1,000 bp: 10%	35 to 300 bp: 15% 300 to 1,000 bp: 10%
Sensitivity[1]	0.1 ng/µL	5 pg/µL
Sizing Precision[2]	5% CV	5% CV
Sizing Accuracy[2,3]	±10%	±10%
Quantitative Precision	0.1 to 1 ng/µL: 15% CV 1 to 50 ng/µL: 10% CV	15% CV
Quantitative Accuracy[2]	±20%	±20%
Quantitative Range	0.1 to 50 ng/µL	10 to 1,000 pg/µL

[1]Signal-to-noise >3 (single peak)
[2]Measured using one ladder per ScreenTape device
[3]Sizing accuracy for analysis with electronic ladder: ±20%

Experimental

Materials

The 4150 TapeStation (p/n G2992AA), legacy 4200 TapeStation (p/n G2991AA), and new 4200 TapeStation (p/n G2991BA) systems with the Agilent D1000 ScreenTape (p/n 5067-5582), D1000 Reagents (p/n 5067-5583), Agilent High Sensitivity D1000 ScreenTape (p/n 5067-5584), and High Sensitivity D1000 Reagents (p/n 5067-5585) were obtained from Agilent Technologies Inc. NoLimits 300 bp DNA fragments (p/n SM1621), the Qubit 2.0 Fluorometer, and Qubit 1X dsDNA HS (High Sensitivity) Assay Kit (p/n Q33231) were purchased from Thermo Fisher Scientific Inc. Human genomic DNA (p/n G304A) was acquired from Promega. The M220 Focused-ultrasonicator and the microTUBEs were obtained from Covaris Inc.

Sample preparation

DNA fragments were prepared by diluting the commercially available DNA from Promega in 10 mM Tris-HCl (pH = 8.0) to achieve a desired number of concentrations within the quantitative ranges of both D1000 ScreenTape assays (Table 1). Human gDNA provided by the vendor was utilized to generate DNA smears. The gDNA was sheared in the microTUBEs on the Covaris instrument according to the manufacturer's recommendations. A shearing time of 110 seconds was selected to achieve a target peak size of approximately 300 bp. The final smear sample was diluted in 10 mM Tris-HCl (pH = 8.0) to prepare different concentrations satisfying the respective specifications[1]. Nominal concentrations of all analyzed samples were determined on the Qubit Fluorometer with Qubit 1X dsDNA HS Assay Kit. The respective DNA ScreenTape ladders were used as samples without any additional treatment.

DNA analysis

The D1000 and High Sensitivity D1000 ScreenTape assays were utilized for sample analysis on three new 4200 TapeStation instruments, and on single legacy 4200 and 4150 TapeStation systems, respectively. Sample preparation for both assays were performed according to the Agilent quick guide instructions[2,3]. The DNA samples were analyzed in replicates of nine on all TapeStation systems using Agilent TapeStation software 4.1.

Results and discussion

Sensitivity

A dilution series of a 300 bp DNA fragment with six concentrations from 5 to 1,000 pg/µL was analyzed on a new 4200 TapeStation system using the High Sensitivity D1000 ScreenTape assay. The electropherogram overlay of all used concentrations demonstrated single and distinct peaks (Figure 1).

An enlarged section shows the overlay of the technical replicates (n = 9) at a concentration of 5 pg/µL, corresponding to the specified limit of detection (Table 1). The respective fragment peak of 300 bp was clearly detected for all 9 replicates with signal-to-noise ratio greater than 3. Thereby, the sensitivity of the High Sensitivity D1000 ScreenTape assay using a commercially available fragment was confirmed on the new 4200 TapeStation system. Likewise, the sensitivity of 0.1 ng/µL was verified for the D1000 ScreenTape assay (data not shown).

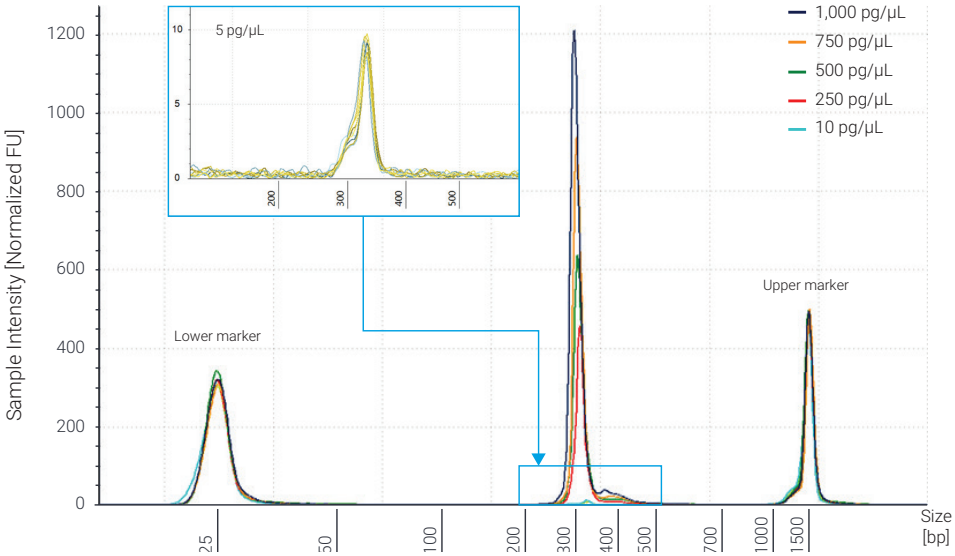

Figure 1. A 300 bp DNA fragment dilution series (5 to 1,000 pg/µL) was analyzed using the Agilent High Sensitivity D1000 ScreenTape assay on the new Agilent 4200 TapeStation system. The enlarged image shows the overlay of individual electropherograms at the specified limit of detection of 5 pg/µL (n = 9).

Sizing

The analytical specifications of the D1000 and High Sensitivity D1000 ScreenTape assays are summarized in Table 1. Differing in quantitative range, both D1000 assays allow for accurate separation of DNA fragments and smears ranging from 35 to 1,000 bp in length. Previously, sizing specifications of the D1000 DNA assays were evaluated on the legacy 4200 TapeStation system using two commercially available ladders[4]. In this study, sizing performance on the new 4200 TapeStation instrument was evaluated using the D1000 and High Sensitivity D1000 ScreenTape assay ladders as samples, as described for the 4150 TapeStation system[5], and the results of all TapeStation models were compared.

Absolute sizes were determined on three new 4200 TapeStation instruments and compared with the results obtained on both the legacy 4200 and 4150 TapeStation systems for all fragments constituting the corresponding ladders. The sizing performance of all instruments is presented as an individual bar chart for each assay with nominal ladder fragment sizes supplied by Agilent on the X-axes (Figure 2).

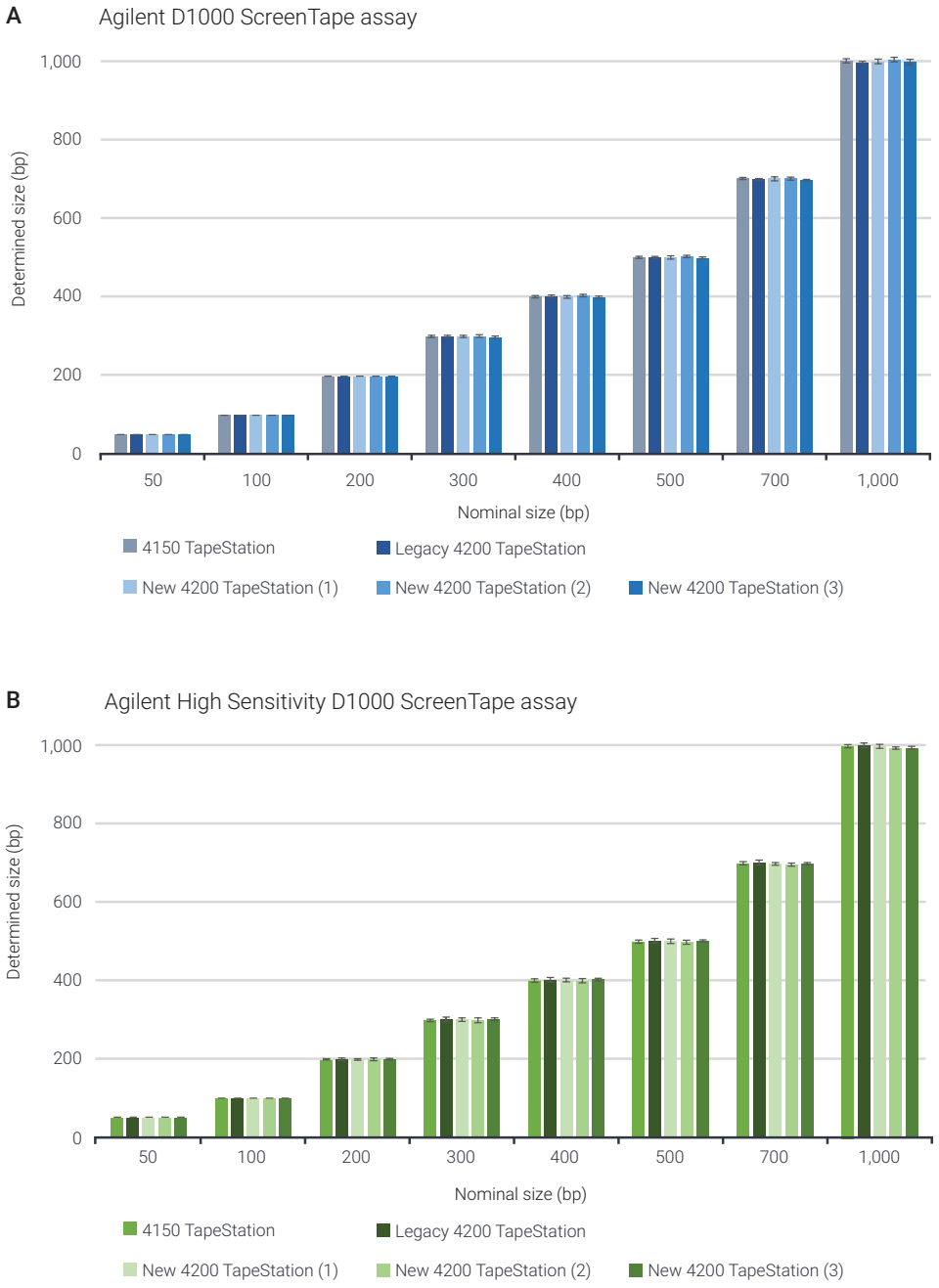

Figure 2. Sizing results for eight DNA fragments of the corresponding Agilent ScreenTape assay ladder analyzed on three new Agilent 4200 TapeStation instruments and on both the legacy Agilent 4200 TapeStation instrument and the Agilent 4150 TapeStation instrument (n = 9) compared to nominal sizes. (A) Agilent D1000 ScreenTape assay and (B) Agilent High Sensitivity D1000 ScreenTape assay.

Sizing accuracy and precision for the D1000 and the High Sensitivity D1000 ScreenTape assays were evaluated on the new 4200 TapeStation instruments in direct comparison with values obtained on the legacy 4200 and the 4150 TapeStation instruments. Sizing accuracy was ±2.6% or less for both ScreenTape assays on all TapeStation instruments, well within assay specifications (±10%). Sizing precision did not exceed a coefficient of variation (%CV) of 1.1% for the D1000, and 2.1% for the High Sensitivity D1000 ScreenTape assays on all three different TapeStation models, which is within the specified sizing precision of 5% for both assays.

The average size of a sample is a critical parameter of an NGS library to be prepared for multiplex sequencing, as it is required for molarity calculation. The average size of a library can be easily determined with the region analysis function of the TapeStation analysis software. In comparison to peak size determination, which is well suited for a symmetrical size distribution, the region analysis is more accurate for sheared DNA with a tailing on either side. A set region, flanking the entire smear, provides an average smear size that excludes a potential bias associated with uneven size distribution. Figure 3 illustrates how the region functionality of the TapeStation system can be applied to a sheared DNA sample. The average size of a smear is automatically reported by the TapeStation analysis software in the region table.

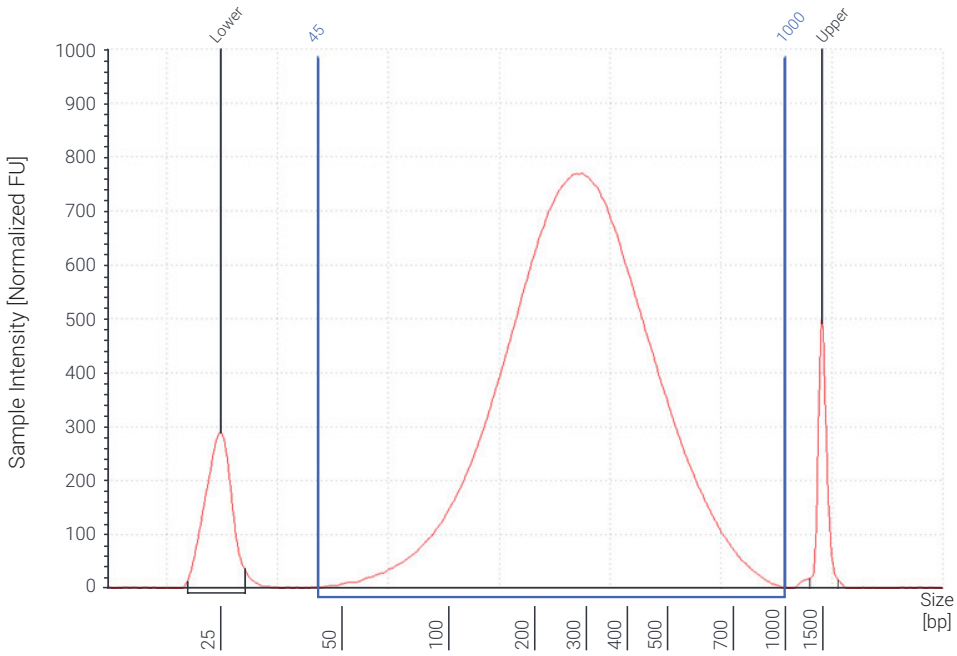

Figure 3. An example electropherogram of a sheared DNA sample analyzed on the new Agilent 4200 TapeStation system with the Agilent High Sensitivity D1000 ScreenTape assay using Agilent TapeStation analysis software region function. The region from 45 to 1,000 bp was established to flank the entire smear.

Sheared gDNA samples of different concentrations were prepared to be within the previously specified quantitative range for smears[1]. The samples were separated with both the D1000 and the High Sensitivity D1000 ScreenTape assays on all three TapeStation models and demonstrated excellent sizing performance with respect to smear analysis. An identical smear analysis region ranging from 45 to 1,000 bp was set for both assays within the TapeStation analysis software.

Figure 4 shows the comparison of sizing results of the D1000 and High Sensitivity D1000 ScreenTape assays delivered by the 4150, legacy 4200, and three new 4200 TapeStation systems, respectively. The results of all TapeStation models were comparable throughout the nominal concentrations and highly consistent between the three new 4200 TapeStation instruments. The D1000 and High Sensitivity D1000 ScreenTape assays demonstrated reproducible sizing with a maximum %CV of 2.6% and 3.7%, respectively. Both DNA ScreenTape assays met the specified analytical specifications of ±5%.

Accurate and precise sizing performance of the D1000 and the High Sensitivity D1000 ScreenTape assays was fully validated with multiple sample types on the new 4200 TapeStation system. The sizing results obtained on the new instrument were equivalent to the results delivered by the other two TapeStation models.

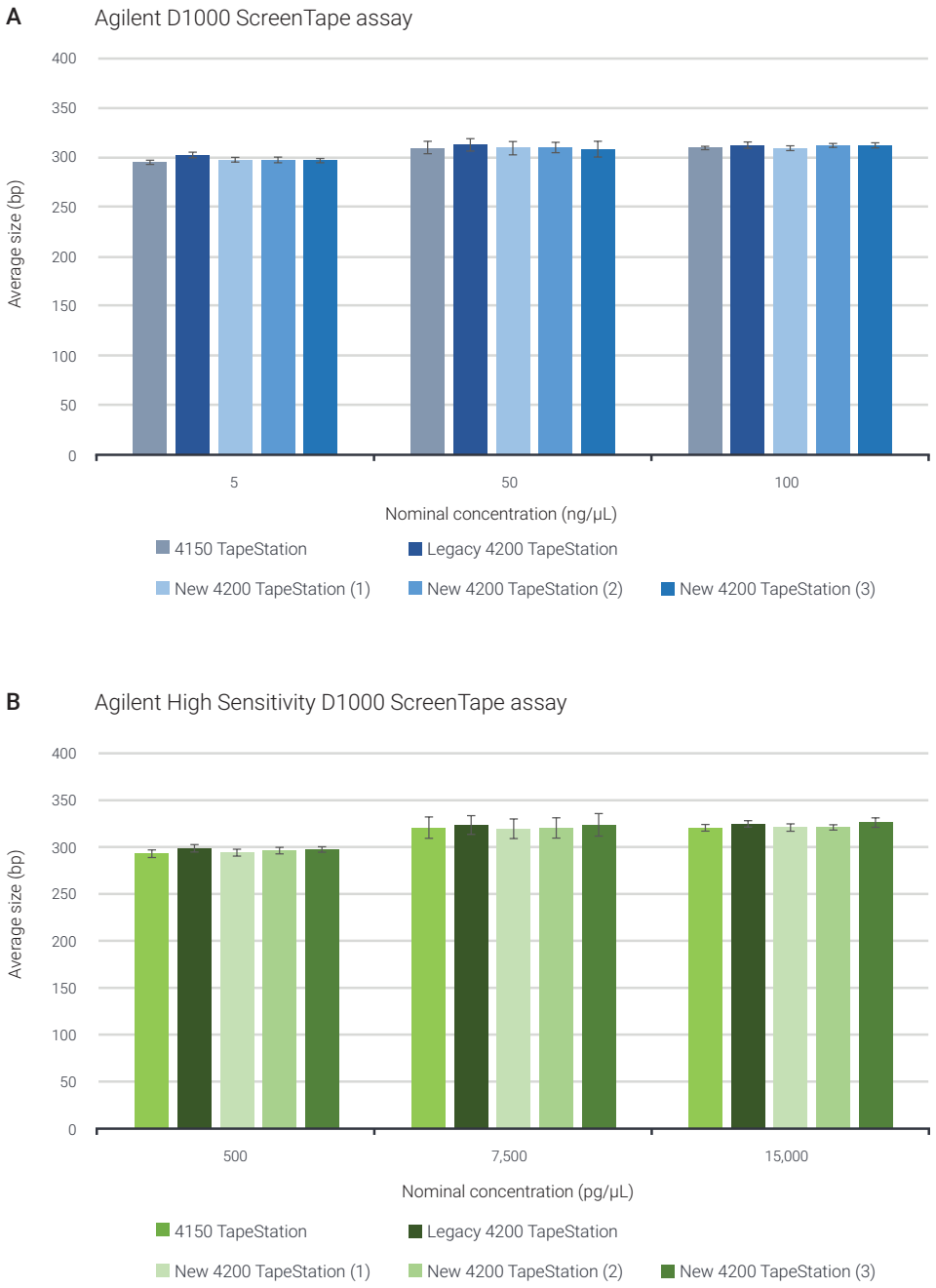

Figure 4. Comparative analysis of average size for sheared DNA throughout the respective dilution series. (A) Agilent D1000 ScreenTape assay. (B) Agilent High Sensitivity D1000 ScreenTape assay.

Quantification

The D1000 ScreenTape assays enable precise determination of sample concentration within quantitative ranges from 10 to 1,000 pg/µL for high sensitivity and from 0.1 to 50 ng/µL for standard sensitivity assays, respectively. Quantitative accuracy and precision for both ScreenTape assays are outlined in Table 1.

Quantitative performance of the D1000 ScreenTape assays on the legacy 4200 TapeStation system was previously evaluated using the respective dilutions of a commercially available 600 bp DNA fragment[3]. Similarly, serial dilutions of a 300 bp DNA fragment covering the entire specified quantitative range were used to assess quantitative capabilities of the D1000 assays on the new 4200 TapeStation system. Direct comparison of the quantitative results between the new and the legacy 4200 TapeStation systems as well as between the new 4200 and the 4150 TapeStation systems is shown in Figure 5. As the quantitative ranges of the D1000 and High Sensitivity D1000 ScreenTape assays overlap, the data generated by both assays were presented together, on a logarithmic scale. The scatterplots demonstrated excellent linearity, confirming accurate measurements within the specified quantitative ranges (Table 1). Concentrations measured on the new 4200 TapeStation system were consistent with those reported by the other two TapeStation systems and the measurements showed strong correlation between all three models, with R^2 values of 99.9 to 100%.

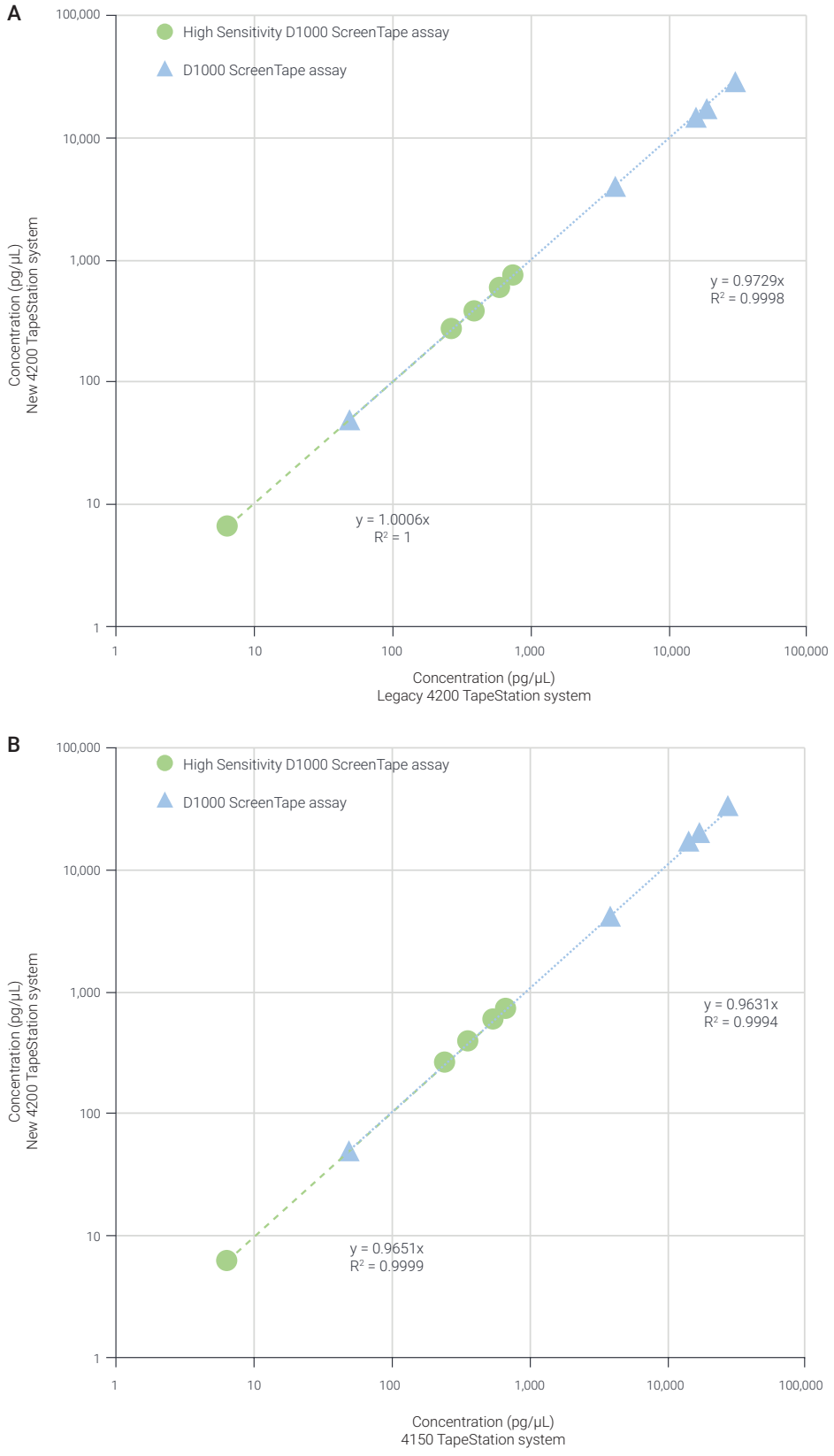

Figure 5. Quantification of a 300 bp fragment in a dilution series from 10 to 50,000 pg/µL. The fragments were analyzed with the Agilent High Sensitivity D1000 ScreenTape assay and the Agilent D1000 ScreenTape assay. (A) Comparison of the quantification on the legacy (X-axis) and new Agilent 4200 TapeStation systems (Y-axis). (B) Comparison of the quantification on the Agilent 4150 TapeStation system (X-axis) and the new 4200 TapeStation system (Y-axis).

The quantitative specifications presented in Table 1 were evaluated on the new 4200 TapeStation system in comparison with the legacy 4200 and 4150 TapeStation systems. Quantitative results on the new 4200 TapeStation instrument were achieved with accuracy relative to the legacy 4200 TapeStation instrument of ±3.2% or less for the D1000 and ±2.1% or less for the High Sensitivity D1000 ScreenTape assays, respectively. All TapeStation models demonstrated accurate quantitative results across the entire concentration range of both assays and met the corresponding analytical specifications (Table 1). Quantitative precision was below 5% for the D1000 and below 6% for the High Sensitivity D1000 ScreenTape assays for all concentrations. As shown in Figure 6, all systems met the specified quantitative precision for both assays. Highly comparable results were also delivered by analysis of sheared gDNA at different concentrations within the specified quantitative range of each assay (data not shown). Quantitative precision was 3% or less for the D1000 and 4% or less for the High Sensitivity D1000 ScreenTape assays. Overall, consistent and reliable quantitative results were delivered by all three TapeStation models.

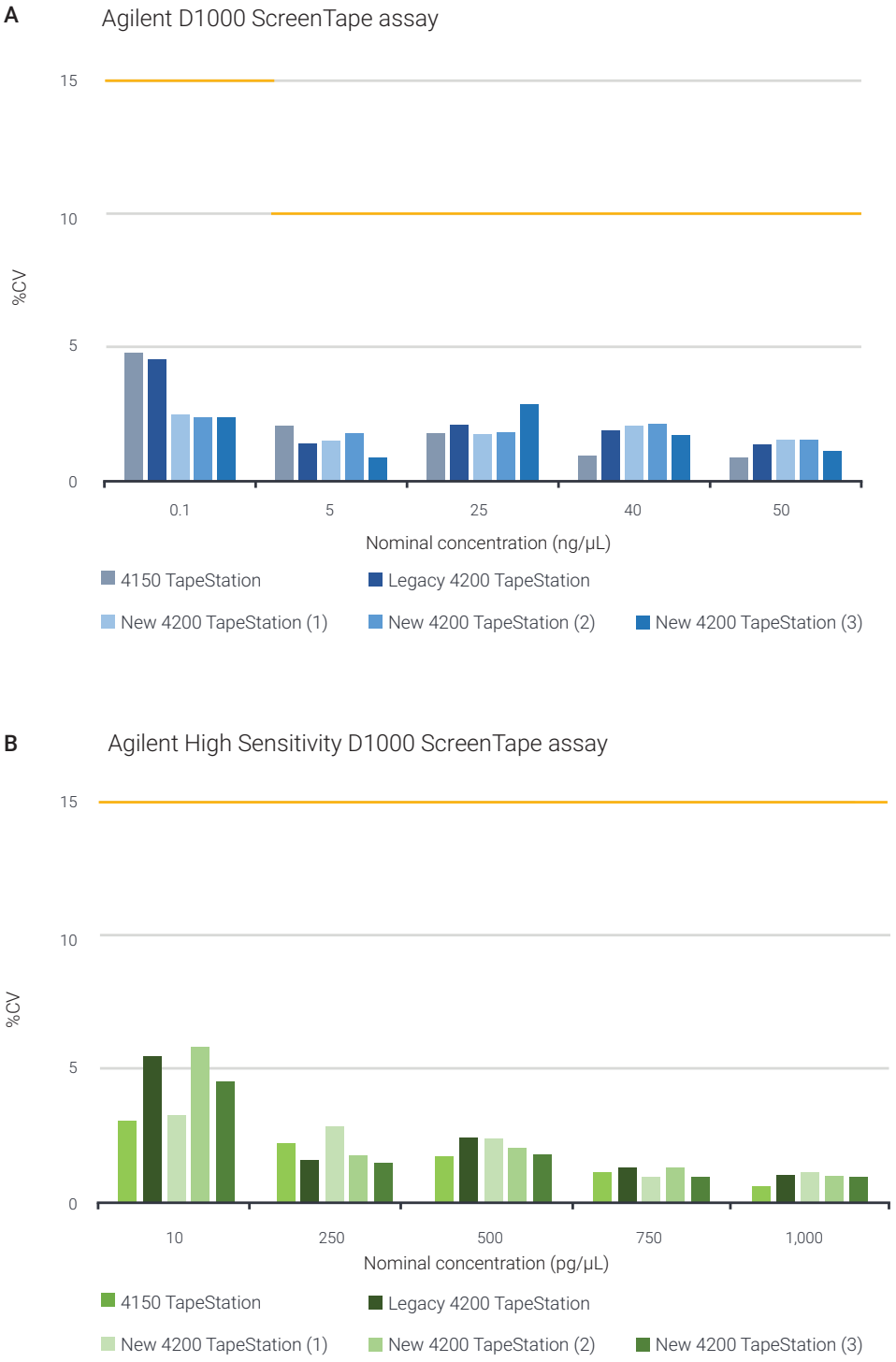

Figure 6. Quantification precision of the 300 bp fragment over five concentrations analyzed with .three new Agilent 4200 TapeStation instruments and on both the legacy Agilent 4200 TapeStation instrument and the Agilent 4150 TapeStation instrument. The orange lines indicate the corresponding specified quantitative precision. (A) Quantification precision of the Agilent D1000 ScreenTape assay. (B) Quantification precision of the Agilent High Sensitivity D1000 ScreenTape assay.

Molarity

Molarity is determined by both the average size and the concentration of a DNA smear. Even minor differences in both values greatly change the molar concentration of the sample. Accurate estimation of molarity is essential for successful multiplex sequencing. NGS sequencing protocols require normalization of the libraries prior to volumetric pooling. Only equimolar pooling can ensure an even read distribution of all samples. The TapeStation software automatically delivers molarity values, eliminating a need for additional calculations.

To assess the equivalence of the calculated molarity data on the new 4200 TapeStation system, a sheared gDNA sample with a nominal average size of 300 bp was analyzed with both the D1000 and High Sensitivity D1000 ScreenTape assays. The molarity values of the new 4200 TapeStation instrument were plotted pairwise against the values delivered by the legacy 4200 (Figure 7A) and 4150 TapeStation systems (Figure 7B). As shown in Figure 7, both plots demonstrated excellent molarity correlation by the regression analysis, with R^2 values of 99.9 to 100%.

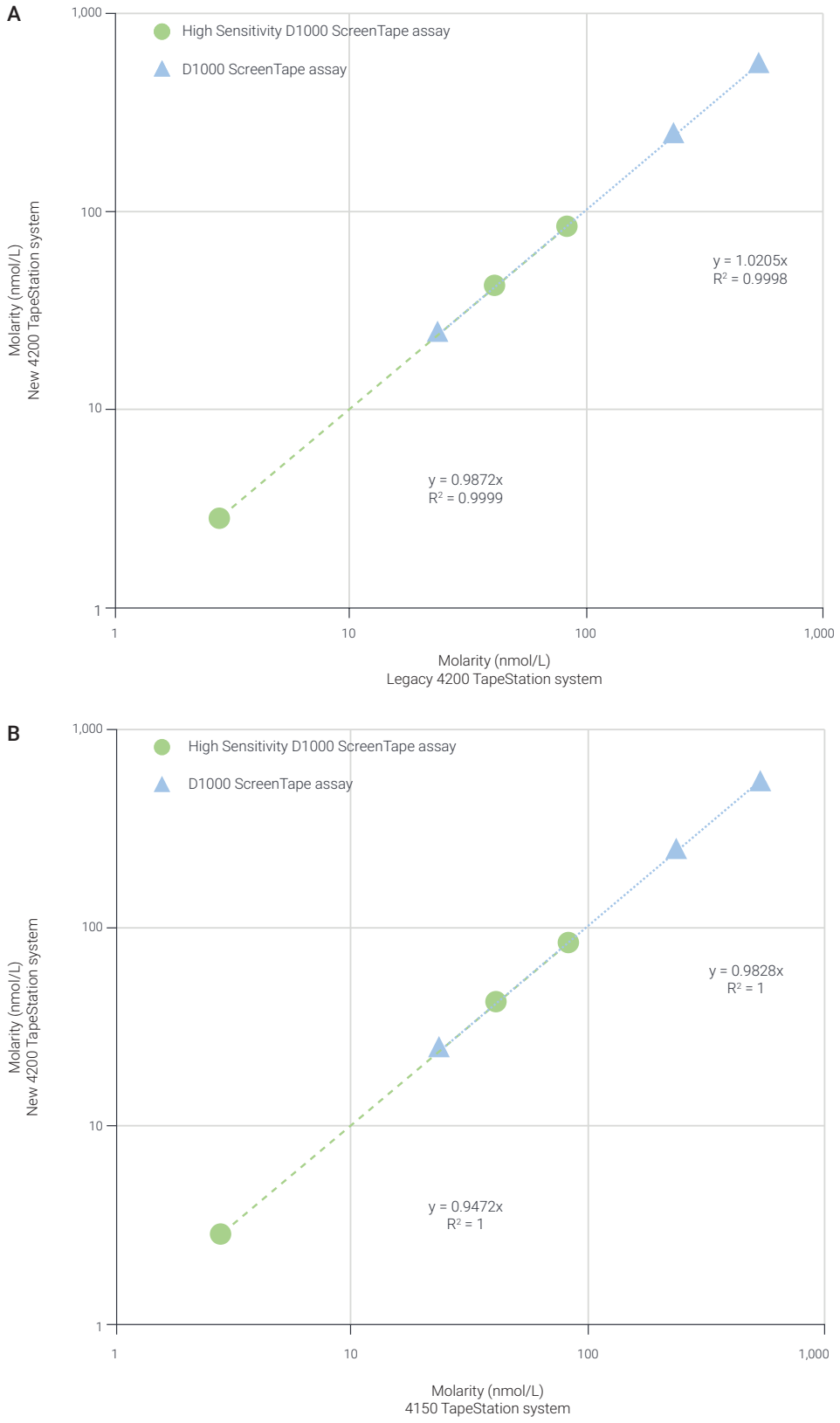

Figure 7. Molarity correlation of sheared gDNA samples in serial dilutions obtained with the Agilent High Sensitivity D1000 assay and Agilent D1000 ScreenTape assay. Molarity was calculated using the region functionality. (A) Comparison of the sample molarities obtained with the legacy (X-axis) compared to the new Agilent 4200 TapeStation systems (Y-axis). (B) Comparison of the sample molarities obtained with the Agilent 4150 TapeStation system (X-axis) and the new Agilent 4200 TapeStation system (Y-axis).

Figure 8 shows an example electropherogram overlay generated by a separation of sheared DNA using the High Sensitivity D1000 ScreenTape assay on all three TapeStation models. The new and legacy 4200 and the 4150 TapeStation systems provided an equivalent smear pattern. Likewise, the size distribution was observed to be the same between the three new 4200 TapeStation instruments (Figure 9). All TapeStation systems reported consistent molarity values and provided identical smear patterns, verifying high replicability across the instruments. By delivering identical results, the new and the legacy 4200 TapeStation systems demonstrated equal performance in relation to analysis of DNA smears.

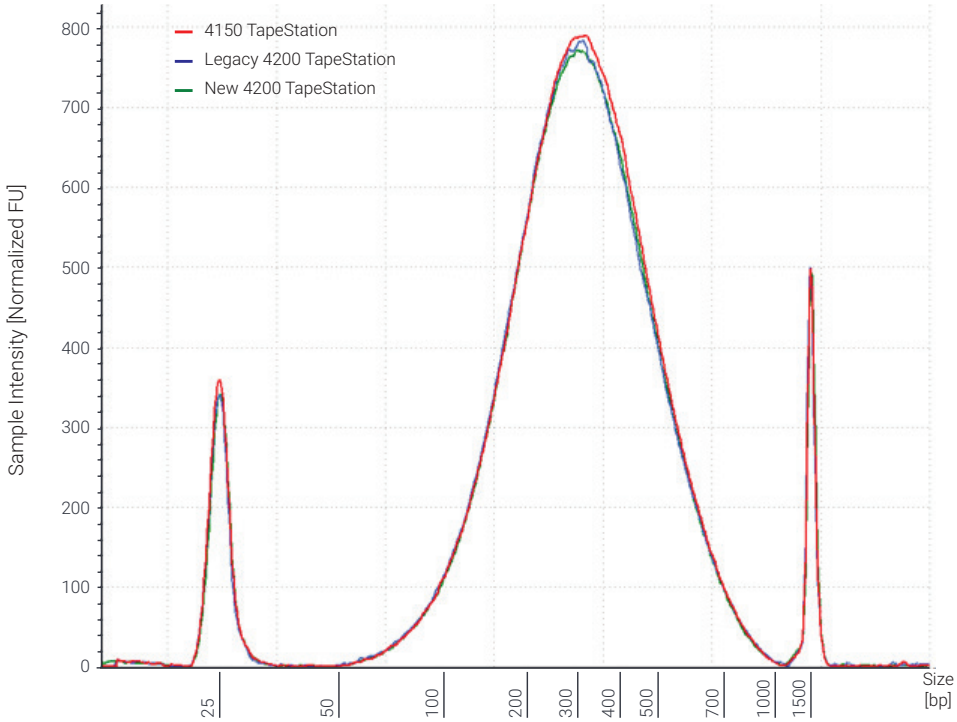

Figure 8. Electropherogram overlay of a smear sample analyzed using the Agilent High Sensitivity D1000 ScreenTape assay on the Agilent 4150 TapeStation system, legacy Agilent 4200 TapeStation system, and new Agilent 4200 TapeStation system.

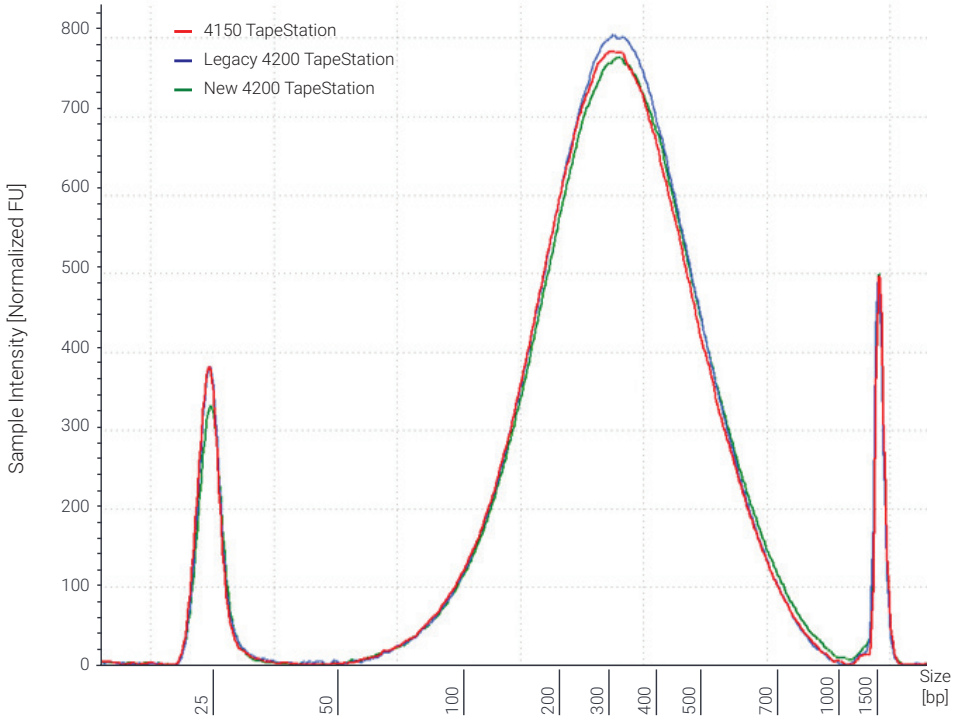

Figure 9. Electropherogram overlay of a smear sample analyzed using the Agilent High Sensitivity D1000 ScreenTape assay on three new Agilent 4200 TapeStation systems.

Conclusion

This technical overview demonstrates the equivalent performance of the Agilent D1000 ScreenTape assay and the Agilent High Sensitivity D1000 ScreenTape assay for the separation of DNA fragments and smears among Agilent TapeStation instruments. The electrophoretic analysis of the DNA fragments between 35 and 1,000 bp in length provided accurate sizing, precise quantification, and high sensitivity down to 5 pg/µL, fully meeting the assay analytical specifications. Furthermore, highly reliable and reproducible results were delivered for sheared gDNA samples with respect to average size, concentration, and molarity.

The performance of the D1000 ScreenTape assays was equivalent between the new and legacy Agilent 4200 TapeStation systems, ensuring full compatibility. In addition, the same high correlation for sizing, quantification, and molarity was noted between the new 4200 and the Agilent 4150 TapeStation systems. Overall, this technical overview confirms the analytical specifications of both the D1000 and High Sensitivity D1000 ScreenTape assays on the new 4200 TapeStation system and demonstrates data equivalency between the three different TapeStation models.

References

1. Comparison of DNA Assays Using the 4200 TapeStation Systems and 2100 Bioanalyzer System. *Agilent Technologies technical overview*, publication number 5991-9093EN, **2018**.
2. Agilent D1000 ScreenTape Quick Guide for TapeStation Systems. *Agilent Technologies*, publication number G2991-90031, **2018**.
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