

Performance Characteristics of the D1000 and High Sensitivity D1000 ScreenTape Assays for the 4150 TapeStation System

Introduction

The Agilent 4150 TapeStation system enables fully automated electrophoresis for fast and reliable DNA and RNA analysis with ready-to-use consumables and minimal handling effort. The system offers full scalability, with throughput of any number of samples between 1 and 16 per run. The entire Agilent DNA and RNA ScreenTape assay portfolio for the Agilent 4200 TapeStation system is fully compatible with the 4150 TapeStation system. Further workflow and software compatibility enables seamless transition between the two systems. The Agilent D1000 ScreenTape assays are developed for the analysis of DNA sizing from 35 to 1,000 bp, and quantity down to 5 pg/ μ L. Consequently, the 4150 TapeStation system together with the D1000 ScreenTape assays is ideally suited for fast DNA sample quality control at low sample throughput in next-generation sequencing (NGS) workflows, delivering highly precise analytical evaluations.

This Technical Overview focuses on the performance of the D1000 and High Sensitivity D1000 (HS D1000) ScreenTape assays on the 4150 TapeStation system with respect to sensitivity, sizing, and quantification. Performance of both assays was compared to results obtained with the 4200 TapeStation system. In addition, the performance was compared to the corresponding Agilent DNA 1000 and High Sensitivity DNA (HS DNA) assays using the Agilent 2100 Bioanalyzer system, which is a well established platform for DNA quality control.

Analytical Specifications

Table 1 summarizes the analytical specifications of the D1000 and HS D1000 ScreenTape assays for the 4150 and the 4200 TapeStation systems, and the specifications of the DNA 1000 and HS DNA assays for the 2100 Bioanalyzer system¹.

Experimental

Materials

The 4150 TapeStation system (p/n G2992AA) and 4200 TapeStation system (p/n G2991AA) with the D1000 ScreenTape (p/n 5067-5582), D1000 Reagents (p/n 5067-5583), High Sensitivity D1000 ScreenTape (p/n 5067-5584), and High Sensitivity D1000 Reagents (p/n 5067-5585) as well as the 2100 Bioanalyzer system (p/n G2939BA) using the DNA 1000 Kit (p/n 5067-1504) and High Sensitivity

DNA Kit (p/n 5067-4626) were obtained from Agilent Technologies (Waldbronn, Germany). NoLimits DNA fragments and the Qubit 2.0 Fluorometer were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Mouse genomic DNA was obtained from Promega, and the M220 Focused-ultrasonicator was from Covaris Inc. (Woburn, MA, USA).

Sample preparation

The DNA fragments were diluted with TE buffer so that the concentrations covered the entire specified quantitative range of the DNA assays (Table 1). Nominal concentrations were determined spectrofluorometrically. Mouse genomic DNA was sheared with a Covaris ultrasonicator. The obtained DNA smear was similarly diluted with TE buffer to achieve different concentrations across the quantitative range.

DNA analysis

DNA samples were analyzed using the D1000 and HS D1000 ScreenTape assays on the 4150 and 4200 TapeStation systems or the DNA 1000 and HS DNA assays together with the 2100 Bioanalyzer system, according to the manufacturer's instructions. The samples were analyzed in replicates of 18 on three different 4150 and 4200 TapeStation instruments, and in replicates of six on two different 2100 Bioanalyzer instruments each. For data analysis, Agilent TapeStation software revisions 3.1 (4150 TapeStation system), A.02.02 (SR1) (4200 TapeStation system), and 2100 Expert software revision B.02.10 (2100 Bioanalyzer system) were applied.

Table 1. Analytical specifications of the D1000 and HS D1000 ScreenTape assays (4150 and 4200 TapeStation systems) and the DNA 1000 and HS DNA assays (2100 Bioanalyzer system).

Analytical specification	4150 and 4200 TapeStation Systems		2100 Bioanalyzer System	
	D1000 ScreenTape Assay	High Sensitivity D1000 ScreenTape Assay	DNA 1000 Assay	High Sensitivity DNA Assay
Sizing range	35–1,000 bp	35–1,000 bp	25–1,000 bp	50–7,000 bp
Sensitivity ¹	0.1 ng/μL	5 pg/μL	0.5 ng/μL	5 pg/μL
Sizing accuracy ²	±10 % ³	±10 % ³	±10 %	±10 %
Sizing precision ²	5 % CV	5 % CV	5 % CV	5 % CV
Quantitative range	0.1–50 ng/μL	10–1,000 pg/μL	0.5–50 ng/μL ²	5–500 pg/μL ²
Quantitative accuracy	±20 %	±20 %	±20 % ²	±20 % ²
Quantitative precision	0.1–1 ng/μL: 15 % CV 1–50 ng/μL: 10 % CV	15% CV	25–500 bp: 15 % CV ² 500–1,000 bp: 5 % CV ²	50–2,000 bp: 15 % CV ² 2,000–7,000 bp: 10 % CV ²

¹ S/N >3 for a single peak

² Determined by analyzing the respective ladder as sample

³ Accuracy of software ladder ±20 %

Results and Discussion

Sensitivity

A dilution series of a 300 bp DNA fragment was analyzed with the 4150 TapeStation system using the High Sensitivity D1000 ScreenTape assay.

Figure 1 shows the electropherogram overlay of the dilution series with distinct signal peaks over the entire concentration range up to 1,000 pg/μL. Electropherogram overlays of the lowest concentration (5 pg/μL) are shown in the enlarged section. The DNA fragment is clearly detected down to 5 pg/μL, which is the specified limit of detection, at a signal-to-noise ratio (S/N) greater than three. Therefore, the sensitivity of 5 pg/μL for the HS D1000 ScreenTape assay is confirmed on the 4150 TapeStation system.

Sizing

The D1000 and HS D1000 ScreenTape assays enable sizing analysis of DNA from 35 to 1,000 bp. The sizing specifications presented in Table 1 were previously evaluated with two commercially available DNA ladders on the 4200 TapeStation system².

To determine the sizing performance of the D1000 and HS D1000 ScreenTape assays on the 4150 TapeStation system, the corresponding ScreenTape assay ladders were used as samples. The same ladders were analyzed on the 4200 TapeStation and 2100 Bioanalyzer systems for a direct comparison. Figure 2 shows the sizing results plotted against the nominal sizes supplied by the manufacturer.

Sizing accuracy was within $\pm 2\%$ for the ScreenTape assays on the 4150 and 4200 TapeStation systems, and below 6% for the DNA 1000 assay or 2% for the HS DNA assay on the 2100 Bioanalyzer system, which is within the specified sizing accuracy of $\pm 10\%$ for all assays and systems.

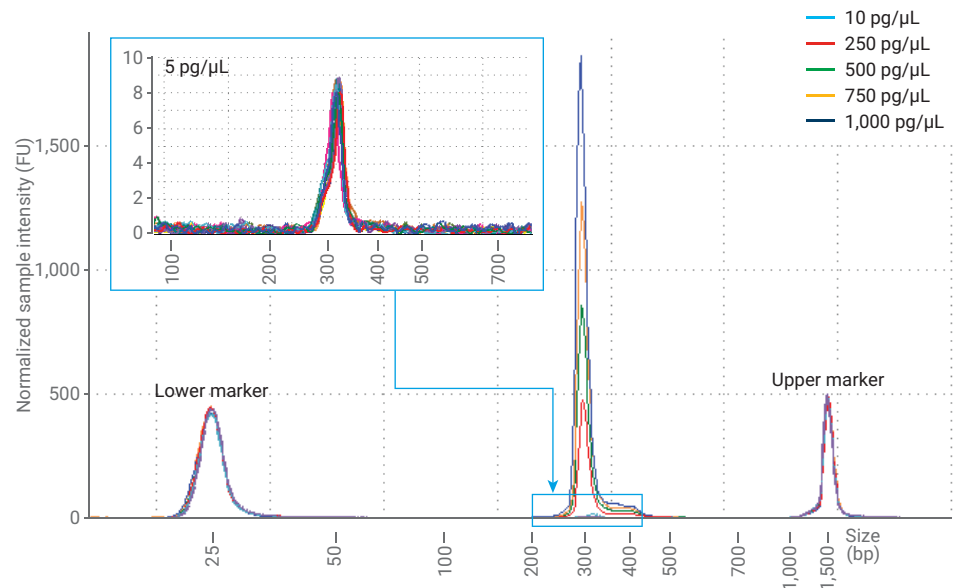


Figure 1. A 300 bp DNA fragment dilution series (5–1,000 pg/μL) was analyzed using the High Sensitivity D1000 ScreenTape assay on the 4150 TapeStation system and the electropherogram overlay is displayed. The enlarged image shows the overlay of individual electropherograms at the specified limit of detection of 5 pg/μL (n = 14).

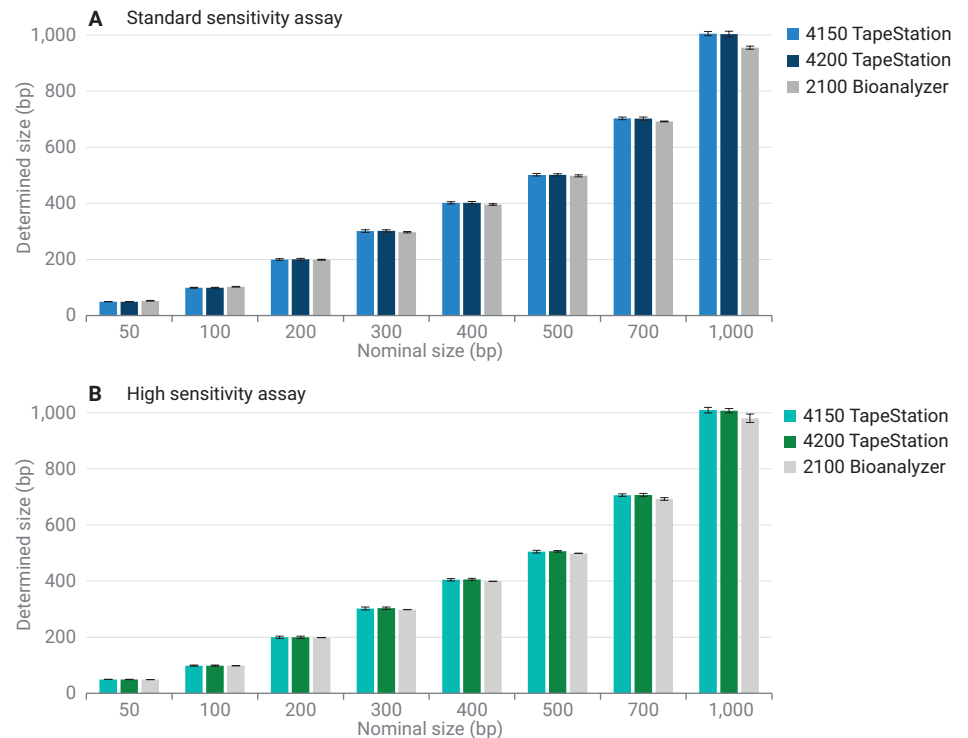


Figure 2. Sizing results for eight DNA fragments of the corresponding ScreenTape assay ladder analyzed with the 4150 and 4200 TapeStation systems (n = 18) and the 2100 Bioanalyzer system (n = 6). A) Sizing results from the D1000 ScreenTape assay (4150 and 4200 TapeStation systems) and the DNA 1000 assay (2100 Bioanalyzer system) compared to nominal sizes. B) Sizing results comparing the High Sensitivity D1000 ScreenTape assay (4150 and 4200 TapeStation systems) and the High Sensitivity DNA assay (2100 Bioanalyzer system) to nominal sizes.

Sizing precision was evaluated with 18 replicates per fragment for the 4150 and 4200 TapeStation systems or six replicates per fragment for the 2100 Bioanalyzer system, and is displayed by the error bars in Figure 2. The sizing precision was below 2 % CV for the standard, and below 3 % for the high sensitivity assays on the three systems, whereby the specifications of each assay (5 % CV) were met.

To demonstrate highly accurate and precise sizing for more complex samples, a DNA smear, which is a typical sample within NGS workflows, was analyzed. The average region size for the smear obtained from the region functionality was 295 or 301 bp using the D1000 or the HS D1000 assay, respectively, on the 4150 TapeStation system. Sizing results from the 4200 TapeStation and 2100 Bioanalyzer systems were highly comparable, with

a deviation below 6 % compared to the sizing results of the 4150 TapeStation system. Sizing precision was within the specified range of 5 % CV for all assays (data not shown).

The sizing analysis performed with the ScreenTape assays and the 4150 TapeStation system showed a high accuracy and precision for both the ladder as well as the sheared DNA sample, and was equivalent to the results obtained with the 4200 TapeStation and 2100 Bioanalyzer systems.

Quantification

The D1000 and HS D1000 ScreenTape assays enable quantification of DNA fragments and NGS libraries. The linear quantification range, from 0.1 to 50 ng/μL for the D1000 ScreenTape assay, and 10 to 1,000 pg/μL for the HS D1000 ScreenTape assay, is summarized in Table 1. The quantitative

range of the corresponding assays on the 2100 Bioanalyzer system is slightly different. Quantification of the DNA 1000 assay is limited to 0.5 ng/μL, and the HS DNA assay is suitable for concentrations between 5 and 500 pg/μL (Table 1).

To demonstrate the specified accuracy and precision of the ScreenTape assays on the 4150 TapeStation system, serial dilutions of a 300 bp DNA fragment covering the entire concentration range were quantified. In addition, the same samples were analyzed on the 4200 TapeStation and the 2100 Bioanalyzer systems.

The quantitative results of the 4150 TapeStation system plotted against the data obtained from the 4200 TapeStation and 2100 Bioanalyzer systems showed excellent correlation for all assays (Figure 3).

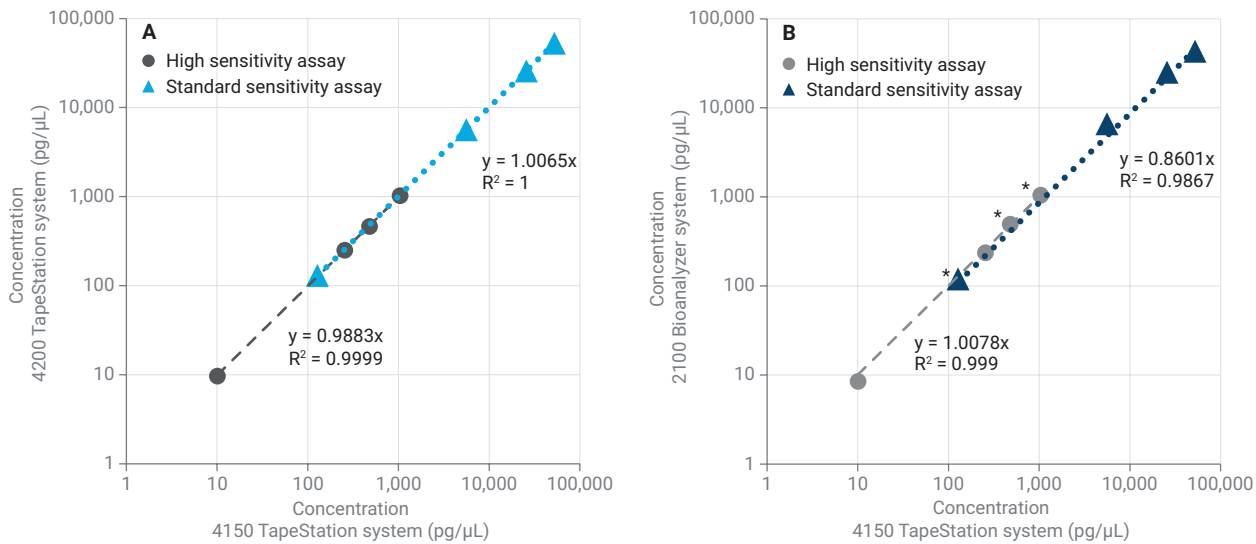


Figure 3. Quantification of a 300 bp fragment in a dilution series from 10–50,000 pg/μL. The fragments were analyzed with the high sensitivity assays HS D1000 ScreenTape assay (TapeStation systems) and HS DNA assay (2100 Bioanalyzer system), as well as the standard sensitivity assays D1000 ScreenTape assay (TapeStation systems) and DNA 1000 assay (2100 Bioanalyzer system). A) Quantification results from the 4150 TapeStation system (X-axis) compared to the 4200 TapeStation system (Y-axis). B) Comparison of the quantification on the 4150 TapeStation system (X-axis) and the 2100 Bioanalyzer system (Y-axis). * Sample concentration outside the specified quantification range of the DNA 1000 or HS DNA assay of the 2100 Bioanalyzer system.

Quantification results obtained with the 4150 TapeStation system were highly accurate, below 5 % deviation to the nominal value for the D1000 ScreenTape assay and below 7 % for the HS D1000 ScreenTape assay. Results from the 4200 TapeStation and 2100 Bioanalyzer systems were comparable, and the specification of 20 % quantitative accuracy was met for all assays.

Figure 4 displays the precision of the quantification and the corresponding specifications for the different concentration ranges. The relative standard deviation (% CV) was below 10 % for 0.1 ng/μL, below 6 % for the other concentrations of the standard sensitivity assays, and below 8 % for the high sensitivity assays. Therefore, all assays and systems met the specified precision. In general, % CV was slightly higher for the analyses performed with the 2100 Bioanalyzer system because of the lower replicate number of n = 6 compared to n = 18 for the TapeStation systems.

Additionally, the quantification results of the DNA smear samples were compared between the three systems. The smear was prepared at different concentrations covering the concentration ranges of the D1000 and the HS D1000 ScreenTape assays. Determined sample concentrations were highly correlative, and precision was below 6 % CV for the standard sensitivity assay and below 9 % CV for the high sensitivity assays (data not shown).

Accurate and precise quantification performance, which was successfully validated previously for the D1000 and HS D1000 ScreenTape assays on the 4200 TapeStation system², could be confirmed for the 4150 TapeStation system.

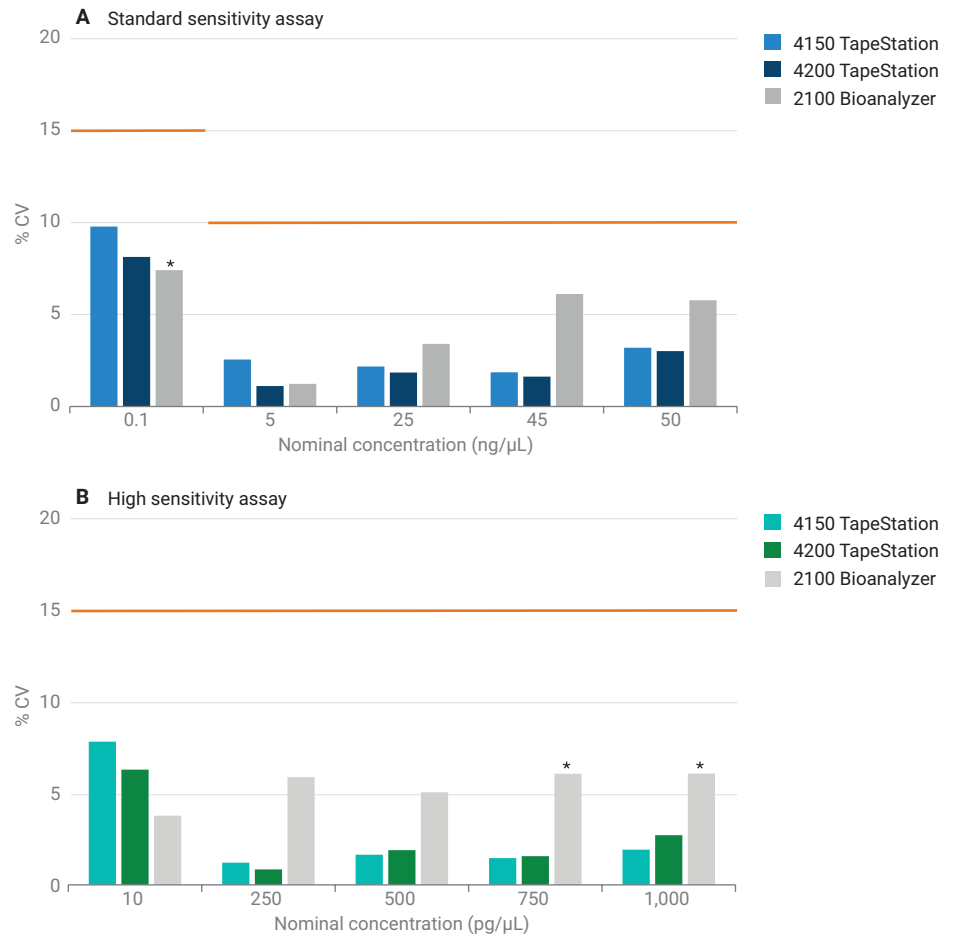


Figure 4. Quantification precision of the 300 bp fragment in five concentrations analyzed with the 4150 and the 4200 TapeStation systems (n = 18) and the 2100 Bioanalyzer system (n = 6). The orange lines indicate the corresponding specified quantitative precision. A) Quantification precision of the D1000 ScreenTape assay (TapeStation systems) and the DNA 1000 assay (2100 Bioanalyzer system). B) Quantification precision of the High Sensitivity D1000 ScreenTape assay (TapeStation systems) and the High Sensitivity DNA assay (2100 Bioanalyzer system). * Sample concentration outside the specified quantification range of the DNA 1000 or HS DNA assay of the 2100 Bioanalyzer system.

Molarity

In NGS workflows, equimolar sample pooling is important to ensure high-quality sequencing results. Since molarity is calculated from the average size and concentration of the library samples, both parameters must be determined accurately and precisely.

For a comparative analysis, the molarity of a sheared DNA dilution series obtained with the 4150 TapeStation system was plotted against the results from the 4200 TapeStation and 2100 Bioanalyzer systems, as shown in Figure 5. Molarity analysis showed excellent correlation, with R^2 values of 99.8–100 % between all three systems.

Figure 6 shows example electropherograms of the sheared DNA sample obtained with the HS D1000 assay using the 4150 and 4200 TapeStation systems as well as with the HS DNA assay and the 2100 Bioanalyzer system. The electropherogram pattern of the 4150 and 4200 TapeStation systems is equivalent, as their overlay shows. However, the pattern differs from the electropherogram obtained with the

2100 Bioanalyzer system. As previously discussed and demonstrated, this effect occurs due to technical differences between the systems; however, resulting data is highly comparable³. Likewise, the high correlation for sizing, quantification, and molarity analysis of the DNA smears shown in this Technical Overview confirms the equal performance of both TapeStation systems and the 2100 Bioanalyzer system.

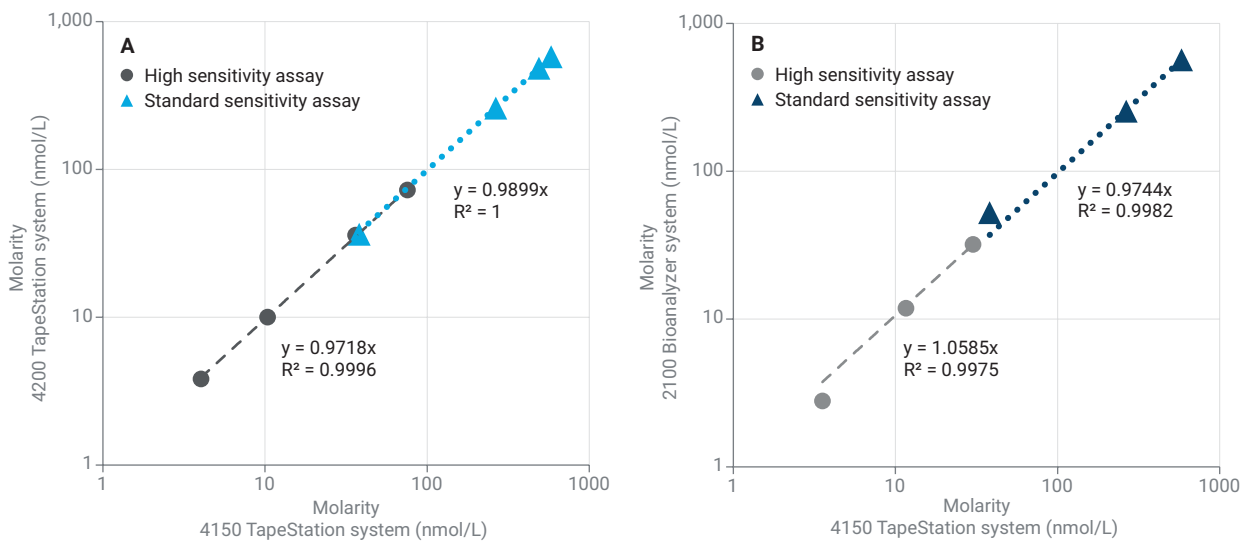


Figure 5. Molarity correlation of sheared DNA samples in serial dilutions obtained with the high sensitivity assays HS D1000 ScreenTape assay (TapeStation systems) and HS DNA assay (2100 Bioanalyzer system) as well as the standard sensitivity assays D1000 ScreenTape assay (TapeStation systems) and DNA 1000 assay (2100 Bioanalyzer system). Molarity was calculated using the region functionality. A) Comparison of the sample molarities obtained with the 4150 TapeStation system (X-axis) compared to the 4200 TapeStation system (Y-axis). B) Correlated molarity data from the 4150 TapeStation system (X-axis) and the 2100 Bioanalyzer system (Y-axis).

Conclusion

This Technical Overview demonstrates the excellent performance of the 4150 TapeStation system with the D1000 and the HS D1000 ScreenTape assays with respect to assay sensitivity and accurate and precise sizing and quantification for DNA fragments from 35 to 1,000 bp. Moreover, complex samples such as sheared DNA can be analyzed reliably and reproducibly for average region size, quantity, and molarity, which are important parameters within NGS workflows. The performance of the ScreenTape assays was equivalent between the 4150 TapeStation system and the 4200 TapeStation system, ensuring full compatibility of the systems. Further results were closely correlated between the 4150 TapeStation system and the 2100 Bioanalyzer system, which represents an established standard for DNA quality control. Altogether, the high performance of the D1000 and the HS D1000 ScreenTape assays was proven on the 4150 TapeStation system, delivering highly consistent results compared to the 4200 TapeStation and the 2100 Bioanalyzer systems.

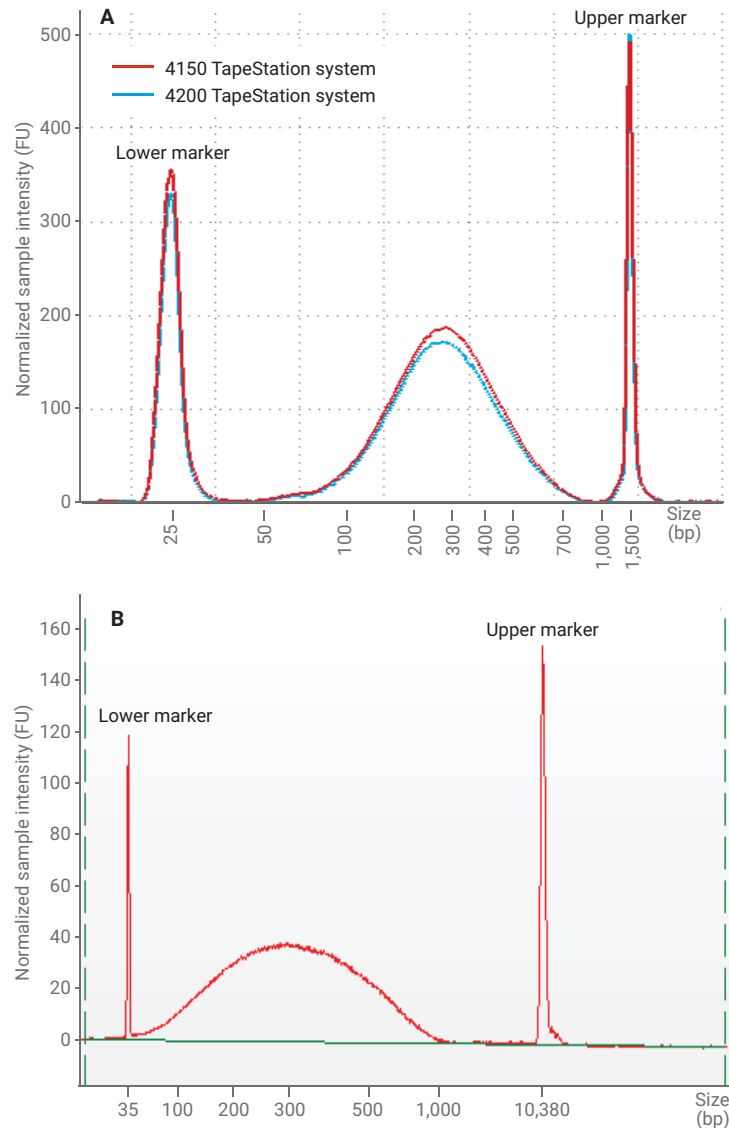


Figure 6. Comparison of electropherogram patterns of a sheared DNA sample. A) Electropherogram overlay of sheared DNA analyzed with the HS D1000 ScreenTape assay on the 4150 and 4200 TapeStation systems. B) Electropherogram of the same sample obtained with the HS DNA assay and the 2100 Bioanalyzer system.

References

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