

# Performance Characteristics of the D5000 and High Sensitivity D5000 ScreenTape Assays for the 4150 TapeStation System

## Introduction

The Agilent 4150 TapeStation system provides fully automated, fast, and reliable nucleic acid electrophoresis for individual sample throughput from 1 to 16 samples per run using ready-to-use consumables and minimal handling effort. The entire DNA and RNA ScreenTape portfolio as well as the workflow and software of the Agilent 4200 TapeStation system are fully compatible with the 4150 TapeStation system, which enables a seamless transition between the two systems. The Agilent D5000 ScreenTape assays are established for quantitative DNA analysis in a size range from 100 to 5000 bp to complete the DNA ScreenTape portfolio together with the Agilent D1000 assays and the Agilent Genomic DNA ScreenTape assay. Therefore, the Agilent 4150 TapeStation system together with the D5000 ScreenTape assays are ideally suited for quality control of next-generation sequencing (NGS) libraries or DNA fragments larger than 1000 bp at low sample throughput demand.

This Technical Overview focuses on the specification characteristics of the D5000 and High Sensitivity D5000 (HS D5000) ScreenTape assays on the Agilent 4150 TapeStation system by the evaluation of sensitivity, sizing, quantification, and molarity analysis. The performance of both assays was compared between the Agilent 4150 and the Agilent 4200 TapeStation systems. In addition, the Agilent 2100 Bioanalyzer system with the corresponding Agilent DNA 7500 and High Sensitivity DNA (HS DNA) assays was included for a comparison of all three systems.

## Analytical Specifications

Table 1 summarizes the analytical specifications of the D5000 and HS D5000 ScreenTape assays for both the 4150 and the 4200 TapeStation systems and the specifications of the DNA 7500 and HS DNA assays for the 2100 Bioanalyzer system<sup>1</sup>.

## Experimental

### Material

The 4150 TapeStation system (part number G2992AA) and the 4200 TapeStation system (part number G2991AA) with the D5000 ScreenTape (part number 5067-5588), D5000 reagents (part number 5067-5589), HS D5000 ScreenTape (part number 5067-5592), and HS D5000 reagents

(part number 5067-5593) as well as the 2100 Bioanalyzer system (part number G2939BA) using the DNA 7500 kit (part number 5067-1506) and HS DNA kit (part number 5067-4626) were obtained from Agilent Technologies (Waldbronn, Germany). NoLimits DNA fragments were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Mouse genomic DNA was obtained from Promega, the M220 Focused-ultrasonicator from Covaris Inc. (Woburn, MA, USA) and the Agencourt AMPure XP kit from Beckman Coulter Inc. (Fullerton, CA, 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). Mouse genomic DNA was sheared with a Covaris ultrasonicator followed by

AMPure XP size selection. The obtained DNA smears were similarly diluted with TE buffer to achieve different concentrations across the quantitative range.

### DNA analysis

DNA samples were analyzed using the D5000 and HS D5000 ScreenTape assays on the 4150 and 4200 TapeStation systems or the DNA 7500 and HS DNA assays together with the 2100 Bioanalyzer system, according to manufacturer instructions<sup>2,3,4,5</sup>. For data analysis, Agilent TapeStation software revisions 3.1 (4150 TapeStation system), A.02.02 (SR1) (4200 TapeStation system), and Agilent 2100 Expert software revision B.02.10 (2100 Bioanalyzer system) were applied.

**Table 1.** Analytical specifications of the D5000 and HS D5000 ScreenTape assays (4150 and 4200 TapeStation systems) and the DNA 7500 and HS DNA assays (2100 Bioanalyzer system).

Analytical Specifications	4150 and 4200 TapeStation Systems		2100 Bioanalyzer System	
	D5000 ScreenTape Assay	High Sensitivity D5000 ScreenTape Assay	DNA 7500 Assay	High Sensitivity DNA Assay
Sizing range	100 to 5000 bp	100 to 5000 bp	100 to 7500 bp	50 to 7000 bp
Sensitivity <sup>1</sup>	0.1 ng/μL	5 pg/μL	0.5 ng/μL	5 pg/μL
Sizing accuracy <sup>2</sup>	± 10 %	± 15 %	± 10 %	± 10 %
Sizing precision <sup>2</sup>	5 % CV	10 % CV	5 % CV	5 % CV
Quantitative range	0.1 to 50 ng/μL	10 to 1000 pg/μL	0.5 to 50 ng/μL <sup>2</sup>	5 to 500 pg/μL <sup>2</sup>
Quantitative accuracy	± 20 %	± 25 %	± 20 % <sup>2</sup>	± 20 % <sup>2</sup>
Quantitative precision	0.1 to 1 ng/μL: 15 % CV 1 to 50 ng/μL: 10 % CV	15 % CV	100 to 1000 bp: 10 % CV <sup>2</sup> 1000 to 7500 bp: 5 % CV <sup>2</sup>	50 to 2000 bp: 15 % CV <sup>2</sup> 2000 to 7000 bp: 10 % CV <sup>2</sup>

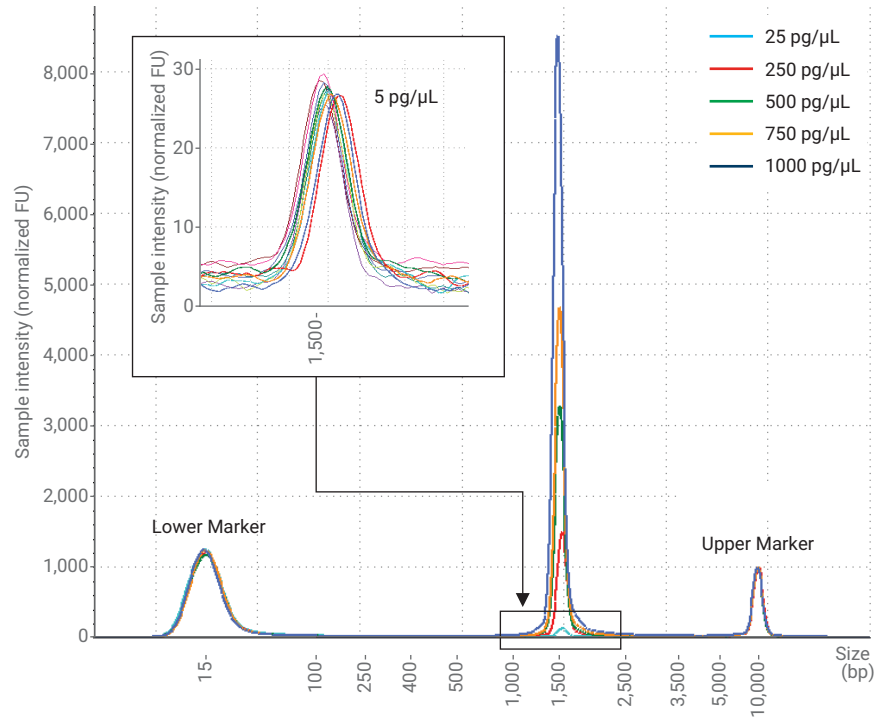
<sup>1</sup> S/N ratio >3 for a single peak.

<sup>2</sup> Determined by analyzing the respective ladder as sample.

## Results and Discussion

### Sensitivity

An electropherogram overlay of a 1500 bp DNA fragment dilution series up to 1000 pg/ $\mu$ L analyzed with the HS D5000 ScreenTape assay on the 4150 TapeStation system is displayed in Figure 1. The enlarged section shows electropherogram overlays of the lowest concentration (5 pg/ $\mu$ L), which is the specified limit of detection. The DNA fragment is clearly detected down to 5 pg/ $\mu$ L, exhibiting a signal-to-noise ratio (S/N) greater than three. Thereby, the specified sensitivity of the HS D5000 ScreenTape assay is verified.



**Figure 1.** Electropherogram overlay of a 1500 bp DNA fragment dilution series (5 pg/ $\mu$ L - 1000 pg/ $\mu$ L) analyzed with the HS D5000 ScreenTape assay on the 4150 TapeStation system. The close-up shows the overlay of individual electropherograms at the specified limit of detection of 5 pg/ $\mu$ L (n=10).

## Sizing

The D5000 ScreenTape assays provide sizing information of DNA in a range from 100 to 5000 bp. The specified sizing accuracy and precision presented in Table 1 were previously confirmed with two commercially available DNA ladders on the 4200 TapeStation system<sup>6</sup>.

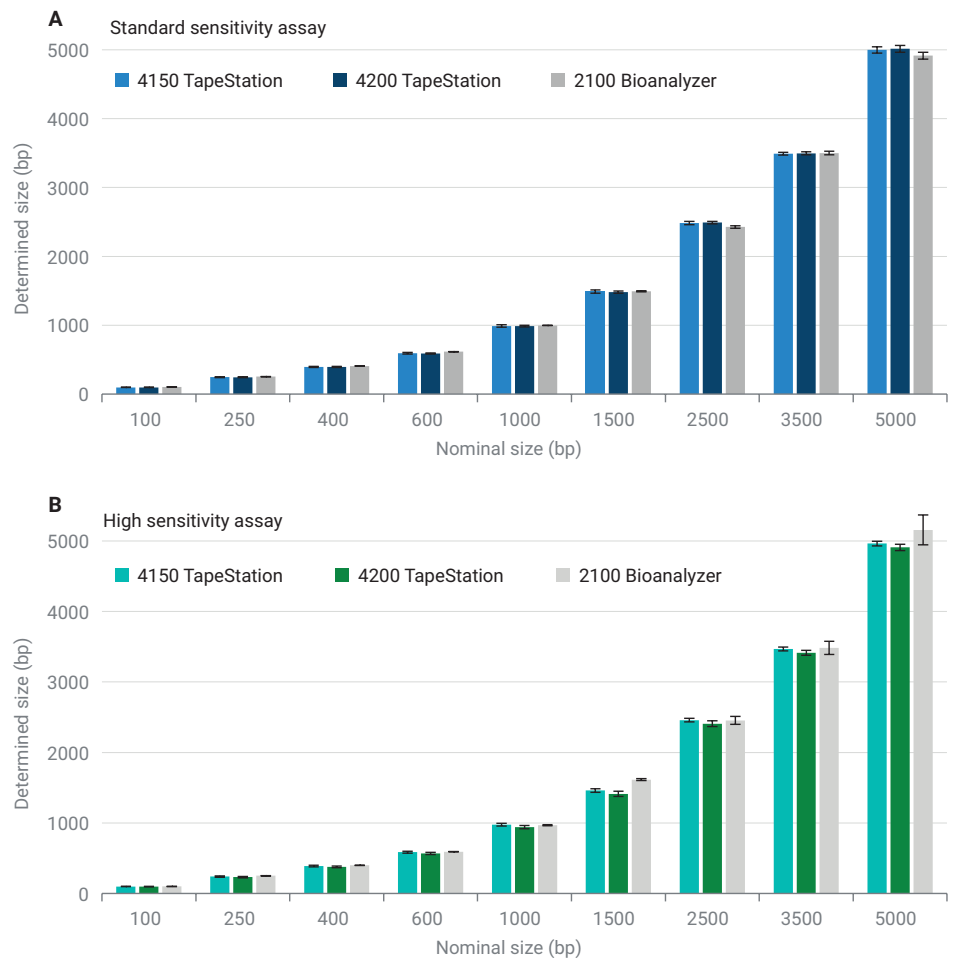
To evaluate the sizing performance of the ScreenTape assays on the 4150 TapeStation system the corresponding assay ladders were measured as samples. For the direct comparison of the three systems the same ladders were analyzed on the 4200 TapeStation system and on the 2100 Bioanalyzer system using the corresponding DNA 7500 and HS DNA assays. The sizing results of the individual ladder fragments were plotted against the nominal sizes as supplied by the manufacturer (Figure 2).

The sizing results were highly accurate, within  $\pm 2\%$  deviation from the nominal size for the D5000 and  $\pm 4\%$  for the HS D5000 ScreenTape assay on the 4150 TapeStation system. Comparably, sizing accuracy was below  $\pm 8\%$  deviation on all applied assays of the 4200 TapeStation and the 2100 Bioanalyzer systems. Thereby, the specified sizing accuracy was met for all assays and systems.

For demonstration of precise sizing, the ladders were analyzed as replicates of 18 on the two TapeStation systems or as replicates of 6 on the 2100 Bioanalyzer system. Sizing precision, which is displayed as error bars in Figure 2, was below 3% CV for the standard and below 5% CV for the high sensitivity assays for all three systems, which matches with the specifications of each assay.

In addition to the assay ladders, sheared DNA samples were analyzed using the region functionality to confirm the performance of the assays for DNA smears, which are typical samples within NGS workflows. The average region size

for the smears was highly comparable across all three systems, with average size deviations below 10% using the respective standard sensitivity assays. Also, the average sizing precision met the specified range of 5% CV for the mentioned assays (data not shown).



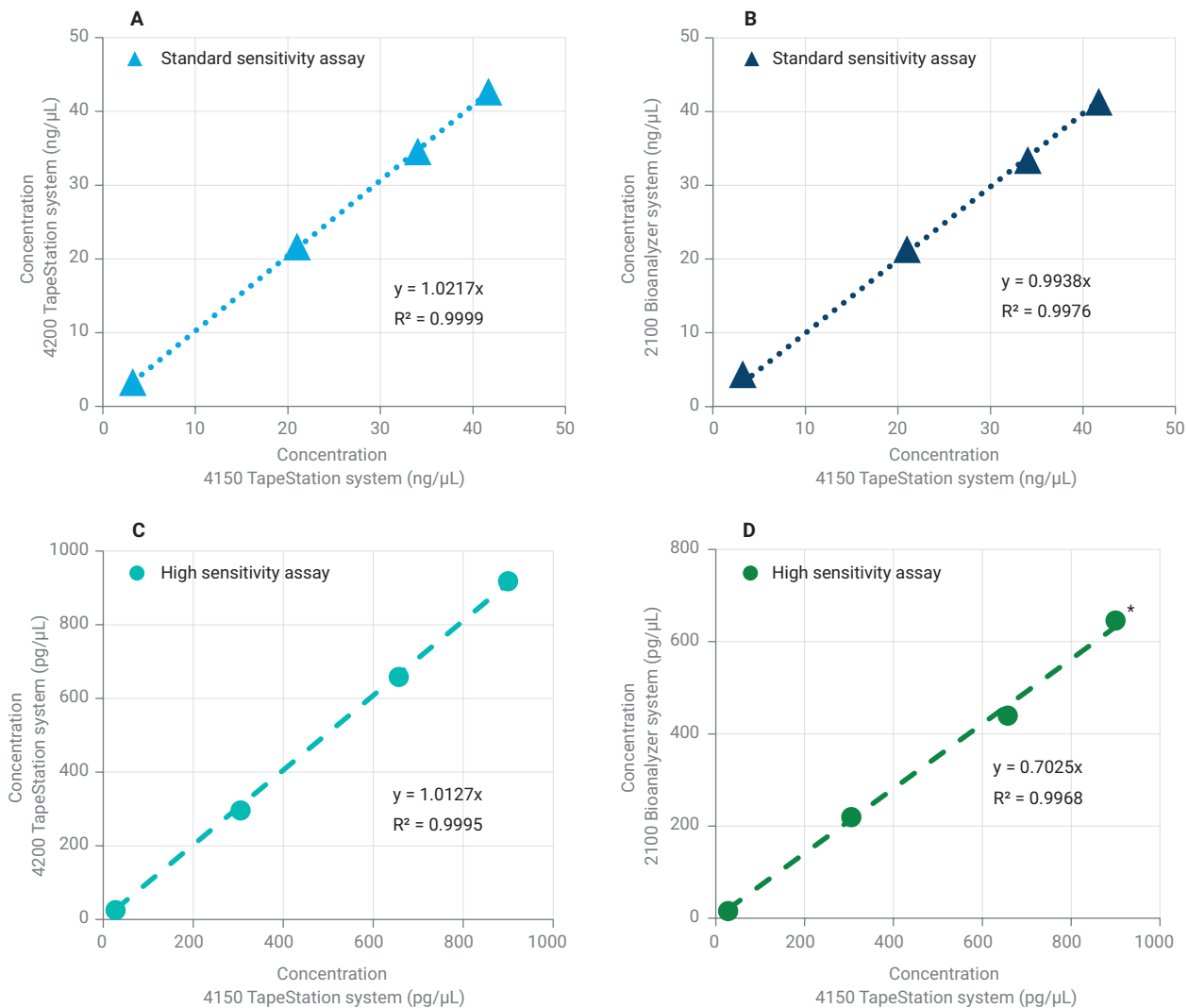
**Figure 2.** Sizing results for nine DNA fragments of the corresponding ScreenTape assay ladders analyzed with the 4150 and 4200 TapeStation systems (n=18) and the 2100 Bioanalyzer system (n=6). A) Sizing results of the D5000 ScreenTape assay (4150 and 4200 TapeStation systems) and the DNA 7500 assay (2100 Bioanalyzer system) compared to nominal sizes. B) Sizing results comparing the High Sensitivity D5000 ScreenTape assay (4150 and 4200 TapeStation systems) and the HS DNA assay (2100 Bioanalyzer system) to nominal sizes.

## Quantification

The D5000 ScreenTape assays are suitable for quantification of DNA fragments in a range from 0.1 to 50 ng/μL for the D5000 ScreenTape assay and 10 to 1000 pg/μL for the HS D5000 ScreenTape assay. The quantitative range of the corresponding assays on the 2100 Bioanalyzer system is slightly different; limited to 0.5 ng/μL concerning the DNA 7500 assay and the HS DNA assay quantifies in a range from 5 to 500 pg/μL (Table 1).

To evaluate the quantitative specifications of the ScreenTape assays on the 4150 TapeStation system, a dilution series of a 1500 bp fragment covering the entire concentration range was analyzed. The same quantity analyses were performed on the 4200 TapeStation and the 2100 Bioanalyzer systems for comparison.

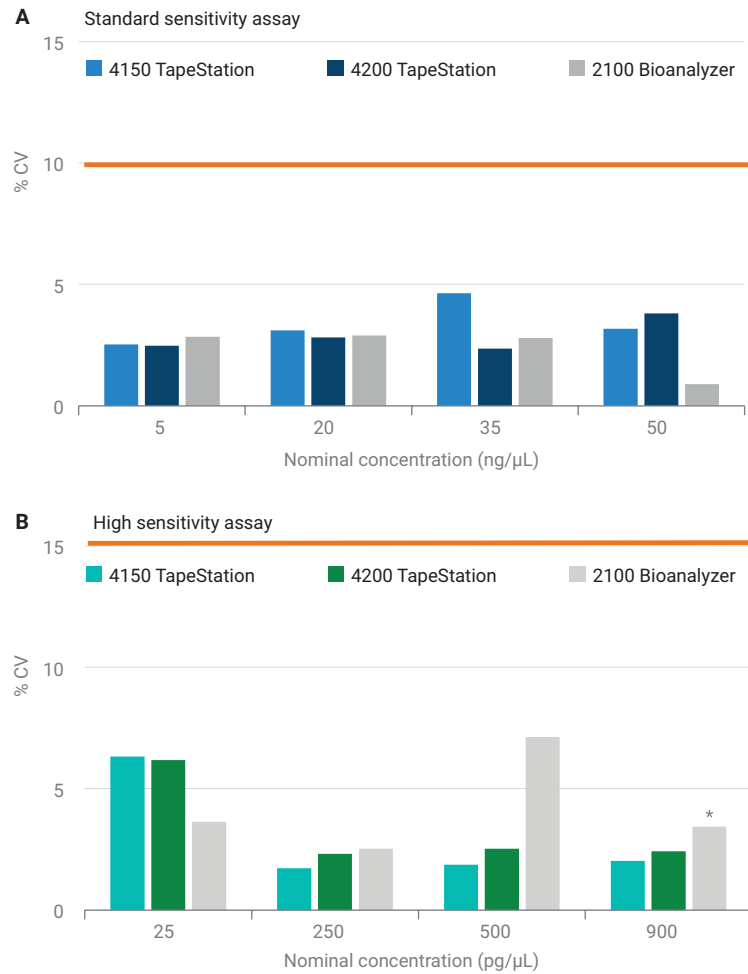
The quantification data obtained with the 4150 TapeStation system were plotted against the results from the two reference systems as displayed in Figure 3. The assessed concentration series correlated excellently between all three systems for both the standard and the high sensitivity assays, exhibiting R<sup>2</sup> values from 99.7 to 99.9 %.



**Figure 3.** Quantification of a 1500 bp fragment in dilution series from 25 to 50,000 pg/μL. Quantification results from the 4150 TapeStation system (X-axis) compared to the 4200 TapeStation system (Y-axis) using the D5000 ScreenTape (A) and the HS D5000 ScreenTape assays (C). Comparison of the quantification on the 4150 TapeStation system (X-axis) and the 2100 Bioanalyzer system (Y-axis) using the respective standard sensitivity D5000 ScreenTape and DNA 7500 assays (B) and high sensitivity HS D5000 and HS DNA assays (D). \* Sample concentration outside the specified quantification range of the HS DNA assay of the 2100 Bioanalyzer system.

The quantitative precision and the corresponding specifications for the ScreenTape assays are displayed in Figure 4. The specified precision for the DNA 7500 assay of the 2100 Bioanalyzer assays is different in the size range covering the 1500 bp fragment (5 % CV). The relative standard deviation (% CV) was below 5 % within the concentration range of 5 to 50 ng/μL for the standard sensitivity assays and below 8 % for the high sensitivity assays for all three systems. Therefore, all assays and systems met the specifications.

To demonstrate accurate and precise quantification for more complex samples, a dilution series of a DNA smear within the concentration range of the D5000 ScreenTape assay was analyzed. The quantification results of the smear samples were comparable between the three systems and precision was below 10 % CV (data not shown).

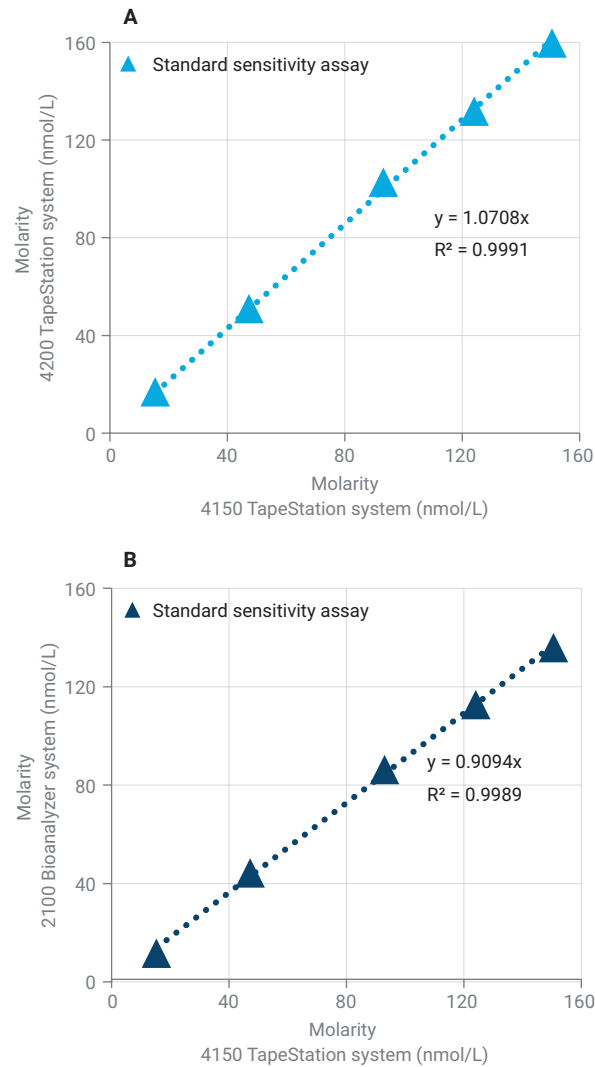


**Figure 4.** Quantification precision of the 1500 bp fragment in four concentrations analyzed with the 4150 TapeStation system (n = 18), the 4200 TapeStation system (n = 16), and the 2100 Bioanalyzer system (n = 6). The orange lines indicate the corresponding specified quantitative precision of the ScreenTape assays. A) Quantification precision of the D5000 ScreenTape assay (4150 and 4200 TapeStation systems) and the DNA 7500 assay (2100 Bioanalyzer system). B) Quantification precision of the HS D5000 ScreenTape assay (4150 and 4200 TapeStation systems) and the HS DNA assay (2100 Bioanalyzer system). \* Sample concentration outside the specified quantification range of the HS DNA assay of the 2100 Bioanalyzer system.

## Molarity

In NGS workflows, sample molarity is the standard quantity metric for sample pooling of final NGS libraries prior to sequencing. For accurate molarity determination, both the average size and the concentration of the library samples must be assessed accurately and precisely.

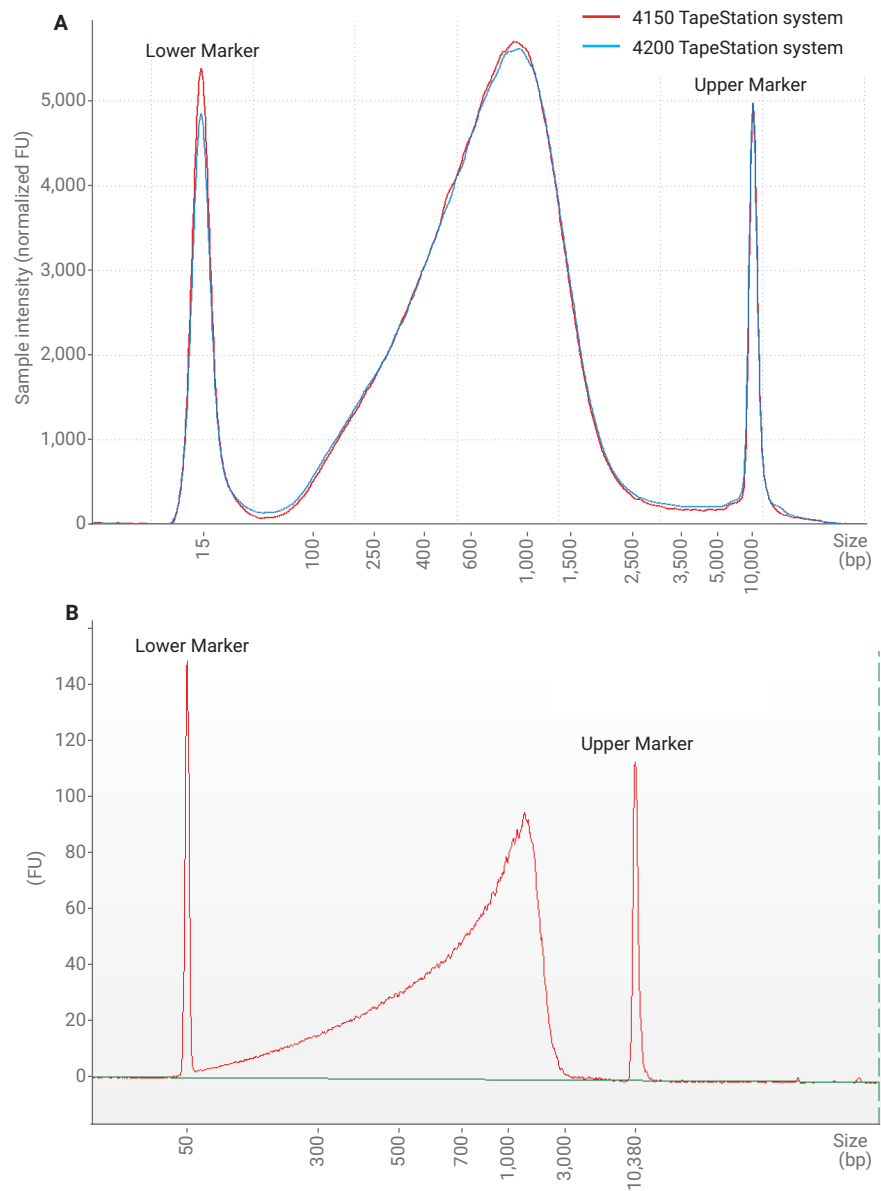
For the molarity correlation, the sheared DNA dilution series within the concentration range of the standard sensitivity assays was analyzed on the two TapeStation and the 2100 Bioanalyzer systems. The molarity results of the dilution series obtained with the 4150 TapeStation system were plotted against the results of the two reference systems and the direct comparison showed excellent  $R^2$  correlation values of 0.99 to 1 (Figure 5).



**Figure 5.** Molarity correlation of sheared DNA samples in serial dilutions obtained with the standard sensitivity assays D5000 ScreenTape assay (TapeStation systems) and DNA 7500 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).

Figure 6 shows example electropherograms of the DNA smear analyses performed on the TapeStation and 2100 Bioanalyzer systems. The electropherogram pattern obtained from the two TapeStation systems is equivalent. However, it differs compared to the electropherogram pattern obtained from the 2100 Bioanalyzer analysis, which can be referred to different platform technologies<sup>7</sup>.

Nevertheless, data processing of the software platforms leads to highly comparable molarity results of the sheared DNA samples demonstrating a high comparability between the 4150 TapeStation, the 4200 TapeStation, and the 2100 Bioanalyzer systems. The excellent assay performance of the D5000 ScreenTape assays regarding NGS samples on the 4200 TapeStation as well as similar performance of the TapeStation systems compared to the 2100 Bioanalyzer system were previously demonstrated<sup>6</sup>.



**Figure 6.** Comparison of electropherogram patterns of a sheared DNA sample. A) Electropherogram overlay of sheared DNA analyzed with the D5000 ScreenTape assay on the 4150 and 4200 TapeStation systems. B) Electropherogram of the same sample obtained with the DNA 7500 assay and the 2100 Bioanalyzer system.



## Conclusion

This Technical Overview confirms the performance characteristics of the D5000 and the HS D5000 ScreenTape assays on the 4150 TapeStation system concerning assay sensitivity as well as highly accurate and precise sizing and quantification results for DNA fragments in a range from 100 to 5000 bp. Furthermore, average region size and quantity and molarity of sheared DNA samples, which are essential parameters for library quality control in NGS workflows, can be reliably assessed using the region functionality in the software. There was equivalent performance of the assays comparing the 4150 and the 4200 TapeStation systems, which ensures a seamless transition and full compatibility of the two systems. In addition, the results obtained with the 4150 TapeStation were highly correlated compared to the 2100 Bioanalyzer system, an established standard for DNA quality control.

## References

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