Performance of the Agilent D1000 and the Agilent High Sensitivity D1000 ScreenTape Assay for the Agilent 4200 TapeStation System

Technical Overview

Introduction

The Agilent 4200 TapeStation system is an automated system for fast and reliable DNA and RNA electrophoresis. In comparison to the Agilent 2200 TapeStation system, it offers fully scalable throughput from 1 to 96 samples, and increased walk away time, which are essential features for high throughput customers. The Agilent D1000 ScreenTape assays were developed to separate and analyze DNA molecules ranging from 35 to 1,000 bp and provide size and concentration values. The 4200 TapeStation system, together with the D1000 ScreenTape assays, is ideally suited for the analysis of PCR products, fragmented DNA, and DNA libraries.

This Technical Overview focuses on the performance of both D1000 ScreenTape assays in evaluating the accuracy and precision of quantification and sizing, as well as the sensitivity of these assays. In addition, the data were compared to the 2200 TapeStation system to demonstrate the full compatibility of both systems.

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Table 1 summarizes the analytical specifications of the D1000 and the Agilent High Sensitivity D1000 ScreenTape assays.

Experimental

Material

Two commercially available DNA ladders (source undisclosed) and NoLimits DNA fragments, the Qubit 1.0 Fluorometer, and NanoDrop 1000 were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Mouse genomic DNA was purchased from Promega, and the M220 Focused-ultrasonicator from Covaris, Inc. (Woburn, MA, USA).

The Agilent 4200 TapeStation (p/n G2991AA) and Agilent 2200 TapeStation (p/n G2964AA or G2965AA) systems with Agilent D1000 ScreenTape (p/n 5067-5582), Agilent D1000 ScreenTape Reagents (p/n 5067-5583), Agilent High Sensitivity D1000 ScreenTape (p/n 5067-5584), and Agilent High Sensitivity D1000 ScreenTape Reagents (p/n 5067-5585) were obtained from Agilent Technologies (Waldbronn, Germany).

Sample preparation

The DNA fragments and ladders were diluted with water so that the concentration fit into the specified quantitative range of both DNA assays (Table 1). Mouse gDNA was sheared with an ultrasonicator to prepare the DNA smear sample.

DNA analysis

DNA analysis was performed using the D1000 or the High Sensitivity D1000 ScreenTape assay in combination with the 2200 and 4200 TapeStation systems, according to the manufacturer's instructions[1-4].

Table 1. Analytical specifications of the Agilent D1000 and the Agilent High Sensitivity D1000 ScreenTape assays for the Agilent 4200 TapeStation system.

Analytical specifications	Agilent D1000 ScreenTape Assay	Agilent High Sensitivity D1000 ScreenTape Assay
Sizing range	35–1,000 bp	35–1,000 bp
Typical resolution	35–300 bp: 15 % 300–1,000 bp: 10 %	35–300 bp: 15 % 300–1,000 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	0.1–1 ng/µL: 15 % CV 1–50 ng/µL: 10 % CV	15 % CV
Quantitative accuracy[4]	± 20 %	± 20 %
Quantitative range	0.1–50 ng/µL	10–1,000 pg/µL
Maximum sample buffer strength	20 mM KCl, 60 mM Phosphate buffer, 60 mM Guanidine-HCl, 240 mM NaCl, 60 mM Acetate	7 mM KCl, 20 mM Phosphate buffer, 20 mM Guanidine-HCl, 80 mM NaCl, 20 mM Acetate

[1] Signal-to-noise (S/N) > 3 (single peak)
[2] Measured using one ladder per ScreenTape device
[3] Sizing accuracy for analysis with electronic ladder: ± 20 %
[4] Measured against the Agilent 2200 TapeStation system

Results and Discussion

Sensitivity

A dilution series of a single 600 bp DNA fragment from 1 ng/µL to 5 pg/µL was analyzed with the 4200 TapeStation system using the High Sensitivity D1000 ScreenTape assay. Figure 1 shows the electropherogram overlay for this dilution series, where the signal peaks are clearly visible for all tested concentrations. The enlarged image shows the electropherogram overlay of the repeated analysis of 5 pg/µL DNA (n = 8). The peaks are clearly visible above the background signal, demonstrating the specified sensitivity of 5 pg/µL for the Agilent High Sensitivity D1000 ScreenTape assay (Table 1). The lower limit of detection for the D1000 ScreenTape assay is specified to be 0.1 ng/µL for a single fragment at a signal-to-noise (S/N) ratio of larger than 3 (data not shown).

DNA Sizing

To determine the sizing accuracy of both D1000 ScreenTape assays, two commercially available DNA ladders were analyzed with the 4200 TapeStation system and the 2200 TapeStation system. The obtained sizes were plotted against the nominal sizes as supplied by the manufacturers. Ten different DNA fragments ranging from 80 to 1,000 bp, depending on the DNA ladder, were analyzed. For all tested DNA fragments, the determined sizing accuracy with the 4200 TapeStation system and the 2200 TapeStation system was below 10 % for both the standard and the high sensitivity assays (Figure 2).

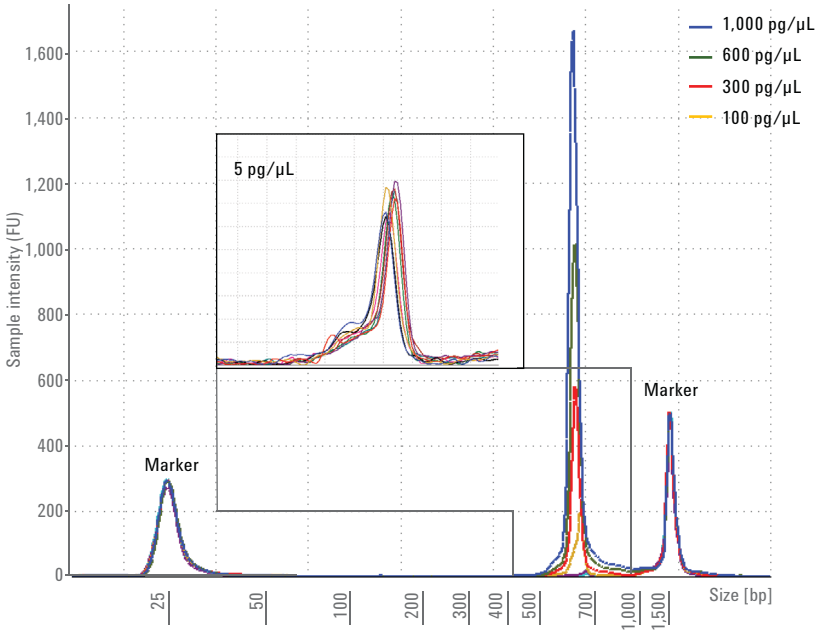

Figure 1. A 600 bp DNA fragment dilution series from 1 ng/µL to 5 pg/µL was analyzed with the Agilent High Sensitivity D1000 ScreenTape assay and the Agilent 4200 TapeStation system. The electropherogram overlay is shown. The enlarged image shows an overlay of eight individual electropherograms at a DNA concentration of 5 pg/µL.

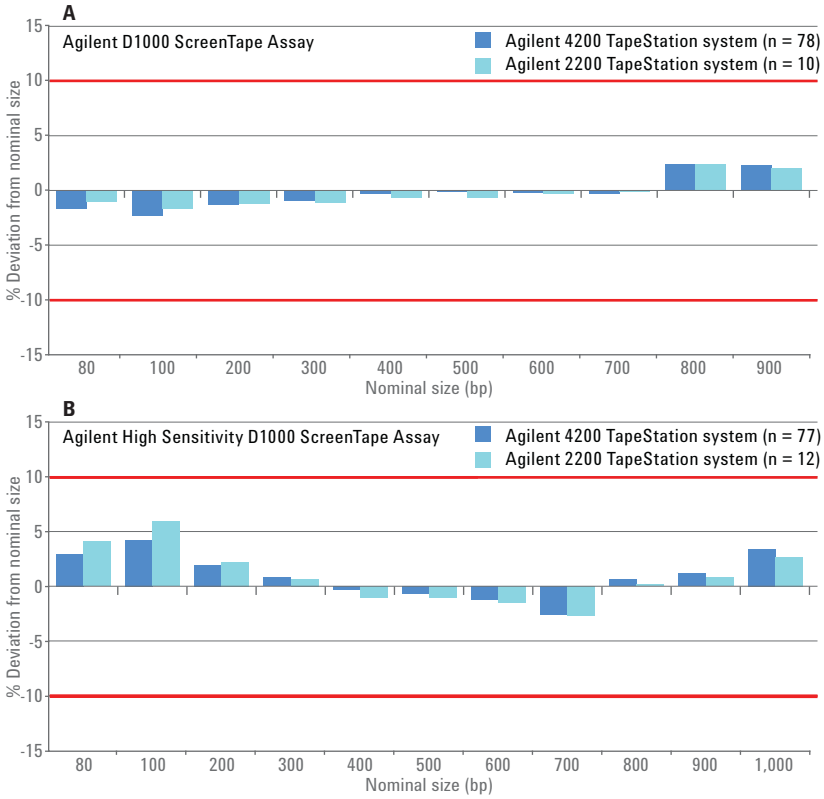

Figure 2. The sizing accuracy of the DNA analysis with the Agilent 4200 TapeStation and Agilent 2200 TapeStation systems was determined using two commercial DNA ladders. The % deviation from the nominal sizes as supplied by the manufacturer is shown.

The sizing precision of both DNA assays with the 4200 TapeStation system, in comparison to the 2200 TapeStation system, were evaluated and shown in Figure 3.

The determined sizing precision for the DNA analysis with standard sensitivity as well as high sensitivity assays was below 5 % CV for both TapeStation systems (Figure 3, Table 1). The 5 % CV sizing precision specified for the D1000 and the High Sensitivity D1000 ScreenTape assay was determined using one DNA ladder per ScreenTape device. The slightly higher %CV values for the 4200 TapeStation system might be caused by using multiple tapes for this test compared to only one tape with the 2200 TapeStation system.

This excellent sizing precision is not only obtained for a commercial DNA ladder, but can also be achieved when analyzing a more complex DNA sample. To prove this, a DNA sample with a distribution of multiple fragment sizes (smear), typical for samples within the Next Generation Sequencing (NGS) workflows, was analyzed with the D1000 and the High Sensitivity D1000 ScreenTape assay and the 4200 TapeStation system at different concentrations across the specified concentration range of the two assays. The obtained sizing precision for the DNA smear sample was below 3 % CV for all tested concentrations (data not shown).

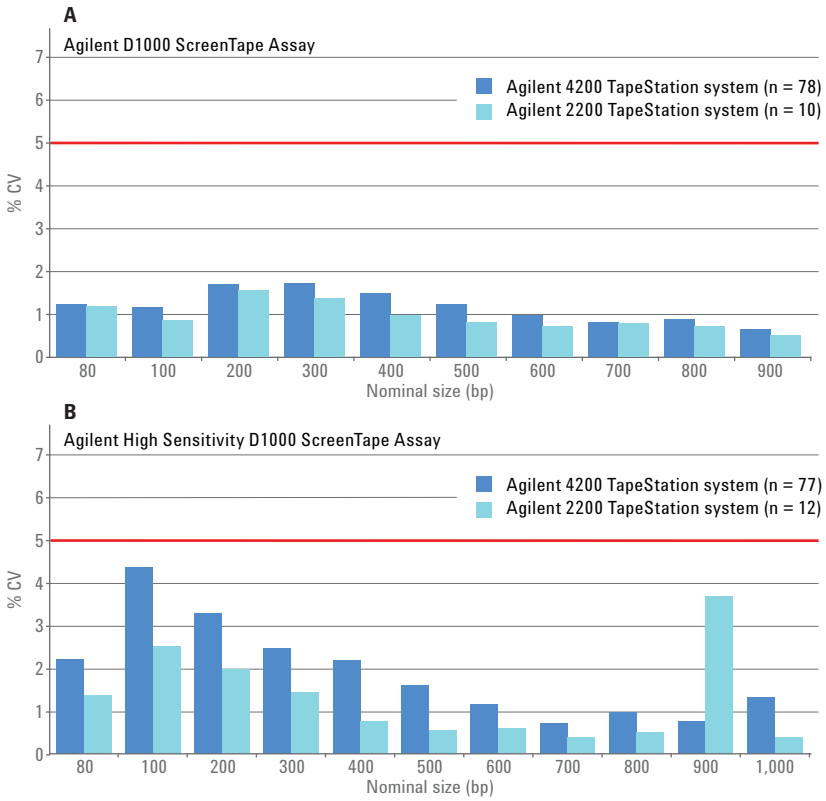

Figure 3. The sizing precision of the standard and high sensitivity DNA analysis with the Agilent 4200 TapeStation system and the Agilent 2200 TapeStation system was determined using commercial DNA ladders.

DNA Quantification

In addition to sizing, the D1000 and High Sensitivity D1000 ScreenTape assays also provide quantification data for individual DNA fragments and smears within a sample. To demonstrate the quantification accuracy and precision of the 4200 TapeStation system and its correlation with the 2200 TapeStation system, a dilution series of a 600 bp DNA fragment was quantified with both systems. For the D1000 ScreenTape assay, the concentration ranged from 0.1 to 50 ng/µL, and for the High Sensitivity ScreenTape assay from 10 to 1,000 pg/µL. The DNA concentrations determined with both TapeStation systems were plotted against each other using a logarithmic scale (Figure 4).

Figure 4 shows excellent correlation, with R^2 values of 99.9–100 % for DNA quantification of a single 600 bp DNA fragment between the 4200 TapeStation system and the 2200 TapeStation system for both the D1000 and the High Sensitivity D1000 ScreenTape assays. The quantification accuracy for the 4200 TapeStation system, as indicated by the % deviation from the concentrations obtained with the 2200 TapeStation system, was below 4 % for the D1000 ScreenTape assay and below 6 % for the High Sensitivity D1000 ScreenTape assay. The specified quantification accuracy for both D1000 assays is ± 20 % measured against the 2200 TapeStation system (Table 1).

Dilutions of a 300 bp DNA fragment were used to verify the DNA quantification precision across different concentrations on the 4200 TapeStation and 2200 TapeStation systems.

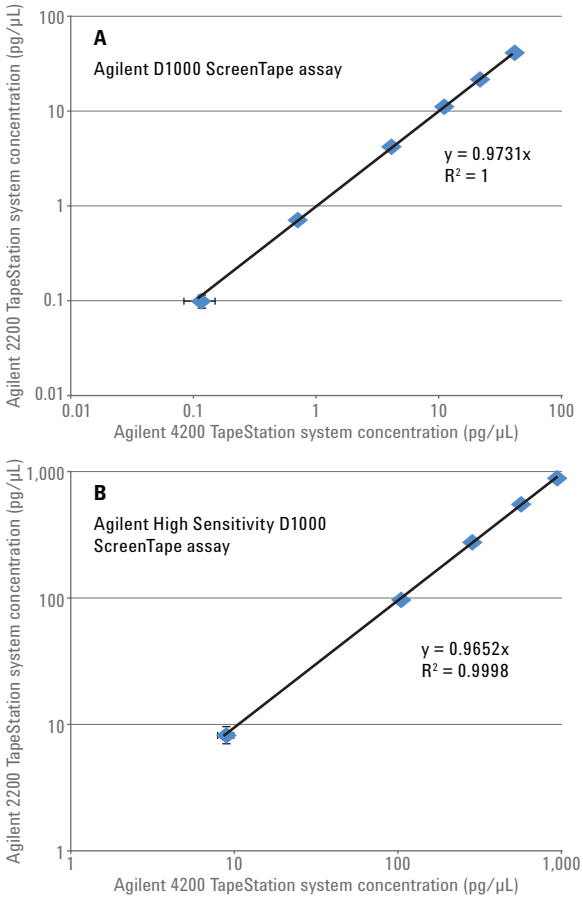

Figure 4. The concentrations of a 600 bp DNA fragment dilution series from the Agilent D1000 and Agilent High Sensitivity D1000 ScreenTape assays on the Agilent 4200 TapeStation system (n = 9 to 12) and the Agilent 2200 TapeStation system (n = 5 to 6) system were plotted against each other on a logarithmic scale.

The determined quantification precision for the DNA analysis with standard sensitivity was below 10 % CV for concentrations from 1 to 50 ng/µL, and below 15 % CV at 0.1 ng/µL (Figure 5). The quantification precision for the high sensitivity DNA analysis was below 15 % CV for all tested concentrations (Figure 6). Therefore, the calculated quantification precision for the D1000 ScreenTape and the High Sensitivity D1000 ScreenTape assays on the 4200 TapeStation system were well within specifications (Table 1).

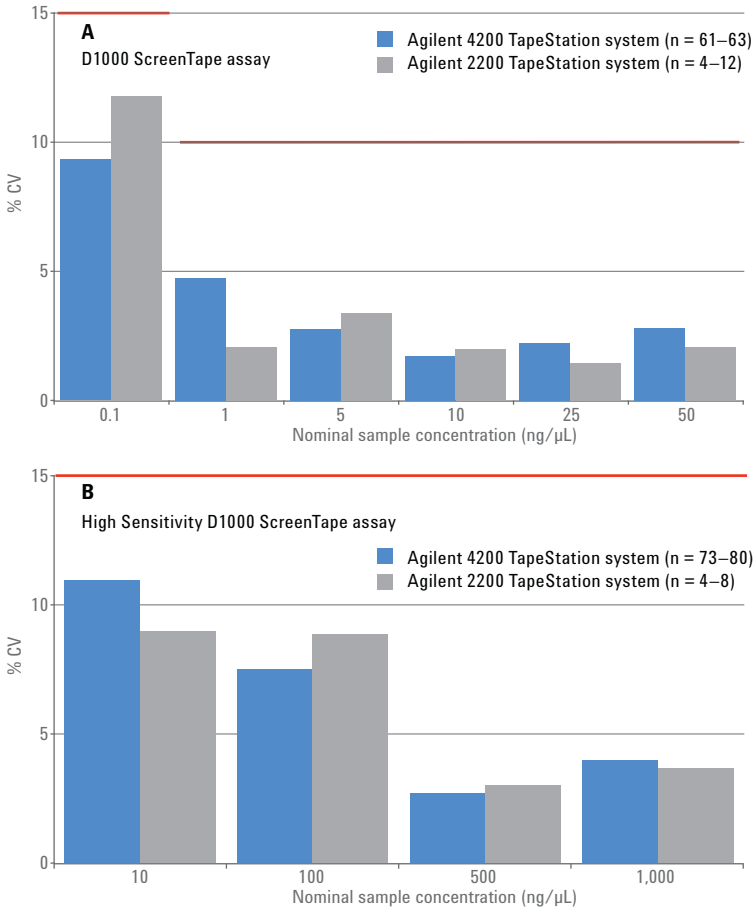

Figure 5. Quantification precision for a 300 bp DNA fragment dilution series from the Agilent D1000 ScreenTape and Agilent High Sensitivity D1000 ScreenTape assays on the Agilent 4200 and Agilent 2200 TapeStation systems.

Molarity

The molarity of DNA samples is important in determining the input amount of DNA for subsequent sequencing reactions for NGS protocols. A DNA smear sample was used to determine the correlation of the molarity values obtained with the 4200 TapeStation and 2200 TapeStation systems for the D1000 and the High Sensitivity D1000 ScreenTape assays (Figure 6).

Figure 6 shows excellent correlation, with R^2 values of 99.9–100% for the molarity of a DNA smear sample analyzed on the 4200 TapeStation and 2200 TapeStation systems with both the D1000 and the High Sensitivity D1000 ScreenTape assays.

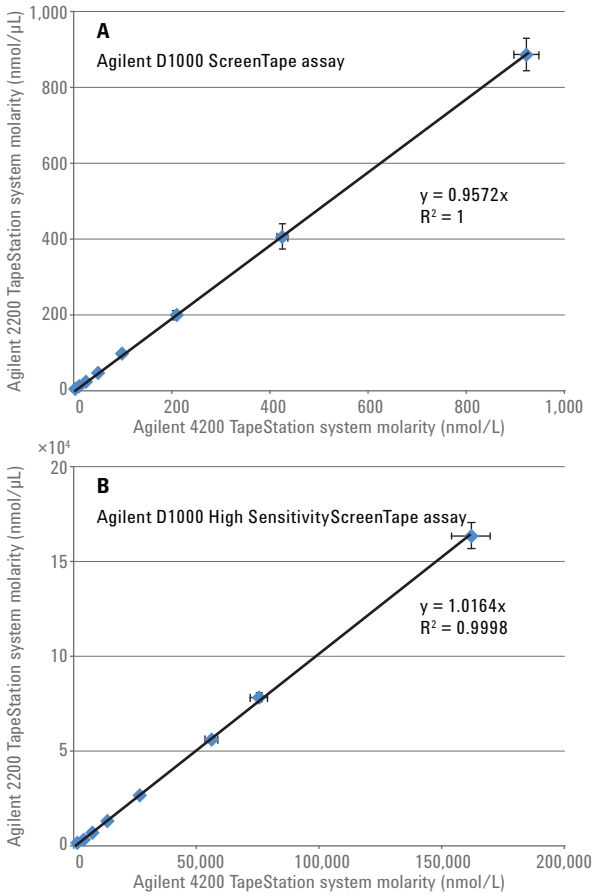

Figure 6. Molarity correlation of a DNA smear dilution series determined with the Agilent D1000 and Agilent High Sensitivity D1000 ScreenTape assays on the Agilent 4200 TapeStation and Agilent 2200 TapeStation systems (n = 11 to 12).

Conclusion

This Technical Overview shows that the Agilent D1000 and the Agilent High Sensitivity D1000 ScreenTape assays for the Agilent 4200 TapeStation system provide efficient and reliable DNA analysis of DNA fragments, including highly accurate and reproducible sizing and quantification. Furthermore, it demonstrates that the D1000 ScreenTape assays can also be applied to determine the DNA concentration and molarity of DNA smear samples.

The data obtained with the 4200 TapeStation system exhibits equivalency with the data generated from the Agilent 2200 TapeStation system. In contrast to the 2200 TapeStation system, the 4200 platform maximizes efficiency by offering electrophoresis of up to 96 samples in an unattended manner.

References

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3. D1000 ScreenTape Assay Quick Guide for 4200 TapeStation System, *Agilent Technologies*, publication number G2991-90030, **2015.**
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