Performance of the Agilent D5000 and High Sensitivity D5000 ScreenTape Assays for the Agilent 4200 TapeStation System

Technical Overview

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Introduction

The Agilent 4200 TapeStation system provides automated, fast, and reliable DNA and RNA electrophoresis for up to 96 samples using prepackaged reagents and minimal manual handling. The Agilent D5000 ScreenTape and High Sensitivity D5000 ScreenTape assays have been developed for the separation and analysis of DNA fragments from 100 bp to 5,000 bp, a size range that complements and resides between the Agilent D1000 ScreenTape and the Genomic DNA ScreenTape assays. The 4200 TapeStation system and the DNA ScreenTape assays can be used at several steps of the Next Generation Sequencing (NGS) workflow. With the emergence of methodologies, such as the use of Transposomes, NGS library sizes are tending to increase beyond 1,000 bp, even for the short-read NGS technologies.

This Technical Overview focuses on the performance of both D5000 ScreenTape assays with respect to the accuracy and precision of quantification and sizing, as well as the sensitivity of these assays. Data analysis for quantification and molarity determination was compared against the corresponding assay for the Agilent 2100 Bioanalyzer system. Additionally, performance of both the D5000 and High Sensitivity D5000 assays on the 4200 TapeStation was compared to the 2200 TapeStation system.

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Table 1 summarizes the analytical specifications of both the D5000 and High Sensitivity D5000 ScreenTape assays.

Experimental

Materials

Two commercially available DNA ladders from New England Biolabs (Hitchin, Uk), Thermo Fisher Scientific Inc., and two NoLimits DNA Fragments (500 and 3,000 bp) from Thermo Fisher Scientific Inc. (Waltham, MA, USA) were used for this study. Mouse genomic DNA was obtained from Promega (Madison, WI, USA) and Swiss Webster Male Mouse genomic DNA was obtained from Zyagen (San Diego, CA, USA). The M220 Focused-ultrasonicator from Covaris Inc. (Woburn, MA, USA), and the Agencourt AMPure XP kit from Beckman Coulter Inc. (Fullerton, CA, USA) were used. The Agilent 4200 TapeStation system (p/n G2991AA), D5000 ScreenTape (p/n 5067-5588), D5000 Reagents (p/n 5067-5589), High Sensitivity D5000 ScreenTape (p/n 5067-5592), High Sensitivity D5000 Reagents (p/n 5067-5593), the Agilent 2100 Bioanalyzer system (p/n G2943CA), DNA 7500 Kit (p/n 5607-1506) and High Sensitivity DNA kit (p/n 5067-4626) were obtained from Agilent Technologies (Waldbronn, Germany).

Sample preparation

A dilution series of the 500 bp and a 3,000 bp DNA fragment was prepared. The two commercially available ladders were directly used as samples. Genomic DNA was sheared with a Covaris ultrasonicator followed by AMPure XP size selection. A dilution series of the obtained DNA sample or smear was prepared.

DNA analysis

The 4200 and 2200 TapeStation systems were used with the D5000 and High Sensitivity D5000 ScreenTape assays. Where indicated, performance was compared against the 2100 Bioanalyzer system using the DNA 7500 and High Sensitivity DNA assays. The DNA analysis was performed according to the manufacturer's protocols.

Table 1. Analytical specifications of the Agilent D5000 ScreenTape and the Agilent High Sensitivity D5000 ScreenTape assay for the Agilent 4200 TapeStation system.

Analytical specifications	Agilent D5000 ScreenTape Assay	Agilent High Sensitivity D5000 ScreenTape Assay
Sizing range	100 bp–5,000 bp	100 bp–5,000 bp
Typical resolution	400–5,000 bp: 15 %	400–5,000 bp: 15 %
Sensitivity[1]	0.1 ng/µL	5 pg/µL
Sizing precision[2]	5 %CV	10 %CV
Sizing accuracy[2]	±10 %	±15 %
Quantitative precision[2]	0.1–1 ng/µL 15 %CV 1–50 ng/µL 10 %CV	15 %CV
Quantitative accuracy[3]	±20 %	±25 %
Quantitative range	0.1–50 ng/µL	10–1,000 pg/µL
Maximum sample buffer strength	250 mM KCl 250 mM *Tris*-HCl 125 mM NaCl 50 mM Acetate 25 mM $MgCl_2$ 25 mM BSA 25 mM Guanidine-HCl	25 mM KCl 25 mM *Tris*-HCl 12.5 mM NaCl 5 mM Acetate 2.5 mM $MgCl_2$ 2.5 mM BSA 2.5 mM Guanidine-HCl

[1] S/N > 3 for a single peak

[2] Determined using the Agilent D5000/High Sensitivity D5000 ladder as sample

[3] Measured against the Agilent 2200 TapeStation system

Results and Discussion

Sensitivity

A dilution series of a discrete 500 bp and a 3,000 bp DNA fragment was analyzed with the 4200 TapeStation system using the High Sensitivity D5000 ScreenTape assay. The 2100 Bioanalyzer system and the High Sensitivity DNA assay were used to verify the DNA concentrations (data not shown).

Figure 1 shows the electropherogram overlay of both fragment dilution series analyzed with the High Sensitivity D5000 ScreenTape assay. The signal peaks are clearly visible for all the tested DNA concentrations. The inset electropherogram demonstrates the sensitivity of the High Sensitivity D5000 ScreenTape assay, the DNA fragment is clearly detected down to a concentration of 5 pg/µL, with a signal-to-noise ratio (S/N) greater than 3. This confirms the specified sensitivity for the High Sensitivity D5000 ScreenTape assay (Table 1).

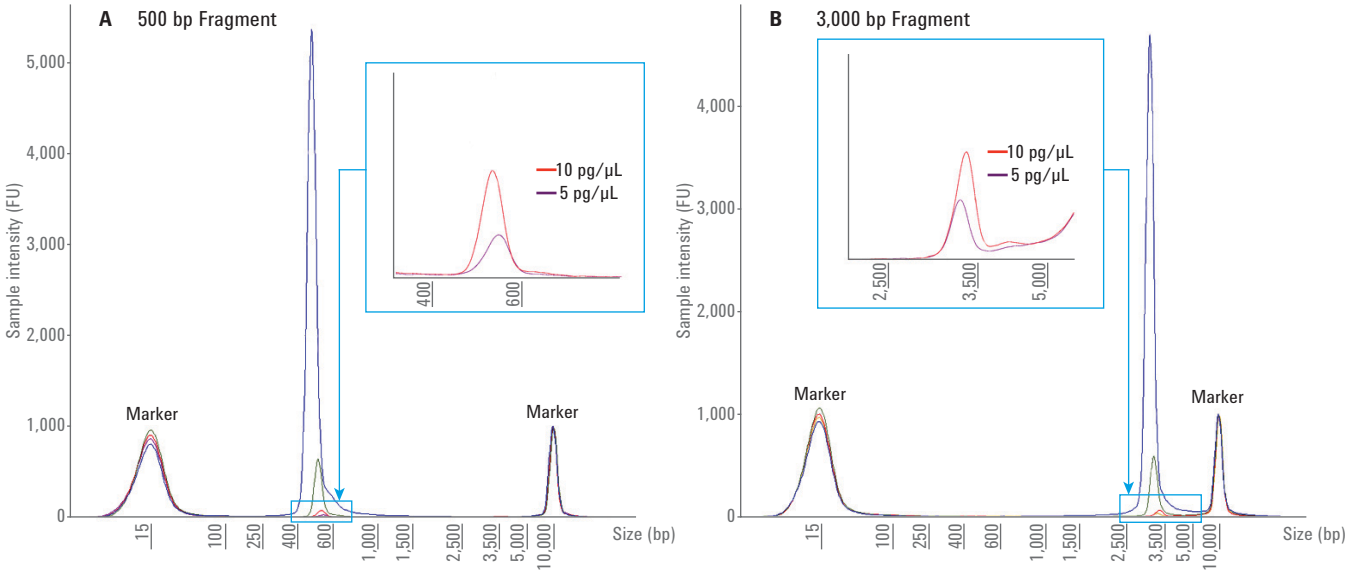

Figure 1. The electropherogram overlay of a dilution series (1,000, 100, 10 and 5 pg/µL) of the (A) 500 bp DNA fragment and (B) 3,000 bp fragment analyzed with the High Sensitivity D5000 assay and the 4200 TapeStation system is shown. The inserts show the two lowest concentrations for both fragments.

DNA sizing

To determine the sizing accuracy of the D5000 ScreenTape assay, two commercially available DNA ladders were analyzed with the 4200 TapeStation system. The obtained sizes were compared to the nominal sizes as supplied by the manufacturer. Fourteen different DNA fragment sizes within the size range of the assay from 100 to 5,000 bp were covered. Some DNA fragment sizes were contained in both ladders. For all tested DNA fragment sizes, the sizing accuracy was clearly below 10 % (Figure 2), which is in agreement with the specified sizing accuracy of ± 10 % for the D5000 ScreenTape assay (Table 1). The sizing accuracy of the High Sensitivity D5000 ScreenTape assay was also within the specifications (data not shown).

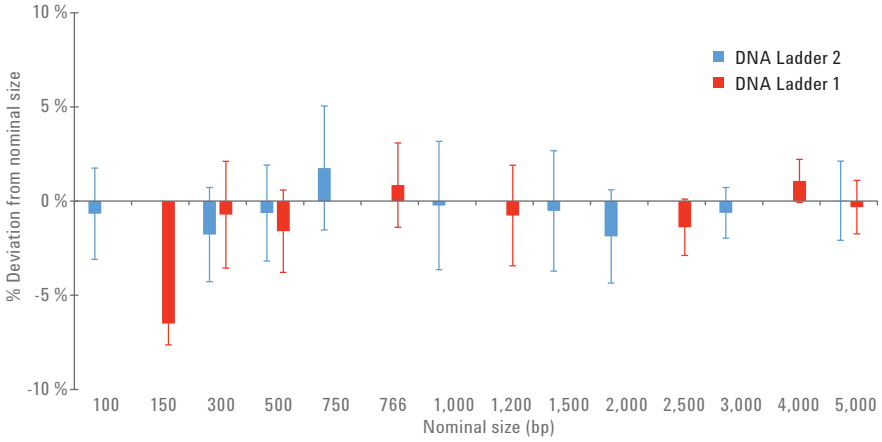

Figure 2. The sizing accuracy of the Agilent D5000 ScreenTape assay was determined using two commercial DNA ladders (n = 6). The analysis was performed in triplicate on two different instruments. The obtained sizes were used to calculate the % deviation from the nominal sizes as supplied by the manufacturer.

Figure 3 shows the same two commercial DNA ladders were used to verify the sizing precision of the D5000 ScreenTape assay.

The calculated sizing precision for the two DNA ladders was within the specified value of 5 % CV (Table 1) for the D5000 ScreenTape assay. The sizing precision of the High Sensitivity D5000 assay was also within the specifications (data not shown).

DNA Quantification

In addition to sizing, D5000 and High Sensitivity D5000 ScreenTape assays also provide quantification data for individual DNA fragments, as well as distributed DNA populations or smears.

To demonstrate quantification accuracy, the 500 and a 3,000 bp DNA fragment, with concentrations at 10, 100, and 500 pg/µL were quantified with the High Sensitivity D5000 assay, and concentrations ranging from 0.5 to 50 ng/µL with the D5000 ScreenTape assay. The same samples were also analyzed with the 2100 Bioanalyzer system using the High Sensitivity DNA and the DNA 7500 assays. The DNA concentrations determined with both systems were plotted against each other using a logarithmic scale where the average of n = 6 to 20 for each data point is shown (Figure 4).

Figure 4 shows an excellent correlation for DNA quantification of two single fragments at 500 and 3,000 bp between the 4200 TapeStation and the 2100 Bioanalyzer systems for both the standard sensitivity and the high sensitivity DNA assays.

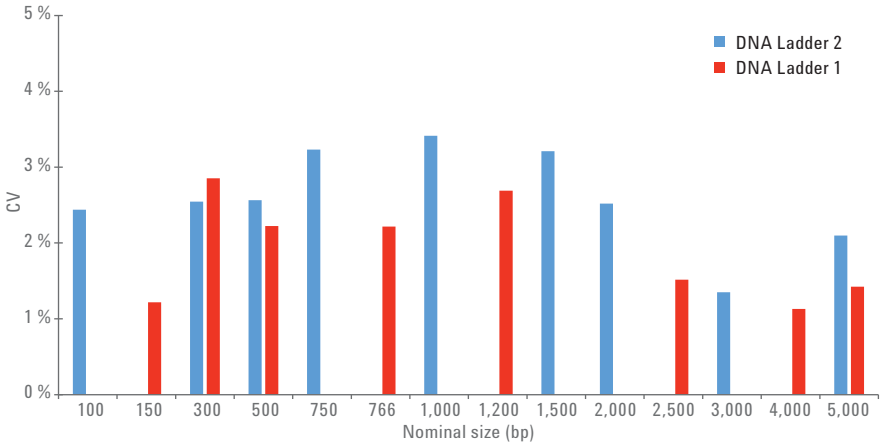

Figure 3. Sizing precision of the Agilent D5000 ScreenTape assay determined with two commercial ladders (n = 6). The analysis was performed in triplicates on two different instruments.

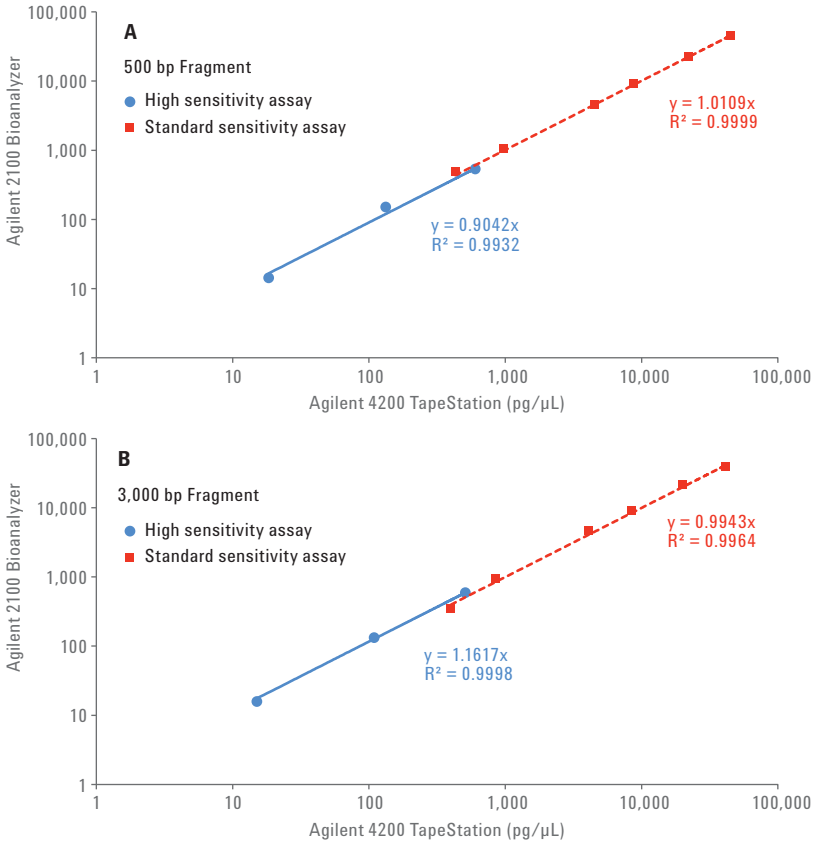

Figure 4. The quantification results of a dilution series of a 500 and 3,000 bp fragment are shown. The results from the Agilent 4200 TapeStation system were plotted against the results obtained with the Agilent 2100 Bioanalyzer system. A logarithmic scale is used for both graphs and the average of n = 6 to 20 per concentration is shown.

The quantitative precision of the D5000 assay was determined from the data in Figure 4. Figure 5 shows that the quantification precision is within specifications for both fragments analyzed (Table 1).

To demonstrate that quantification is also applicable across other applications, a typical sample from the NGS workflow was generated and analyzed using the region functionality for both the 4200 TapeStation and 2100 Bioanalyzer systems. Genomic DNA was sheared with an ultrasonicator followed by size selection with AMPure beads. A dilution series of the obtained size selected DNA smear was prepared with concentrations ranging from 325 to 3,000 pg/µL, and analyzed on the 2100 Bioanalyzer and the 4200 TapeStation systems using the high sensitivity assays.

The gel image and the electropherograms in Figure 6 show a typical DNA smear pattern from NGS samples and libraries. The graph highlights the good correlation between the 4200 TapeStation and the 2100 Bioanalyzer systems.

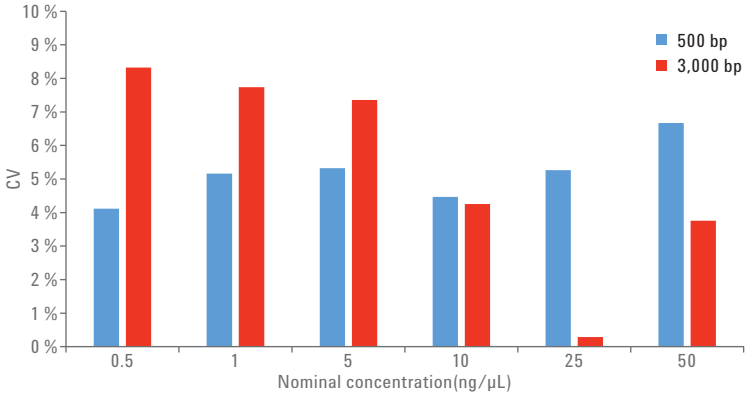

Figure 5. The quantitative precision for the analysis of a dilution series of the 500 and 3,000 bp fragments with the Agilent D5000 ScreenTape assay (n = 6 to 20).

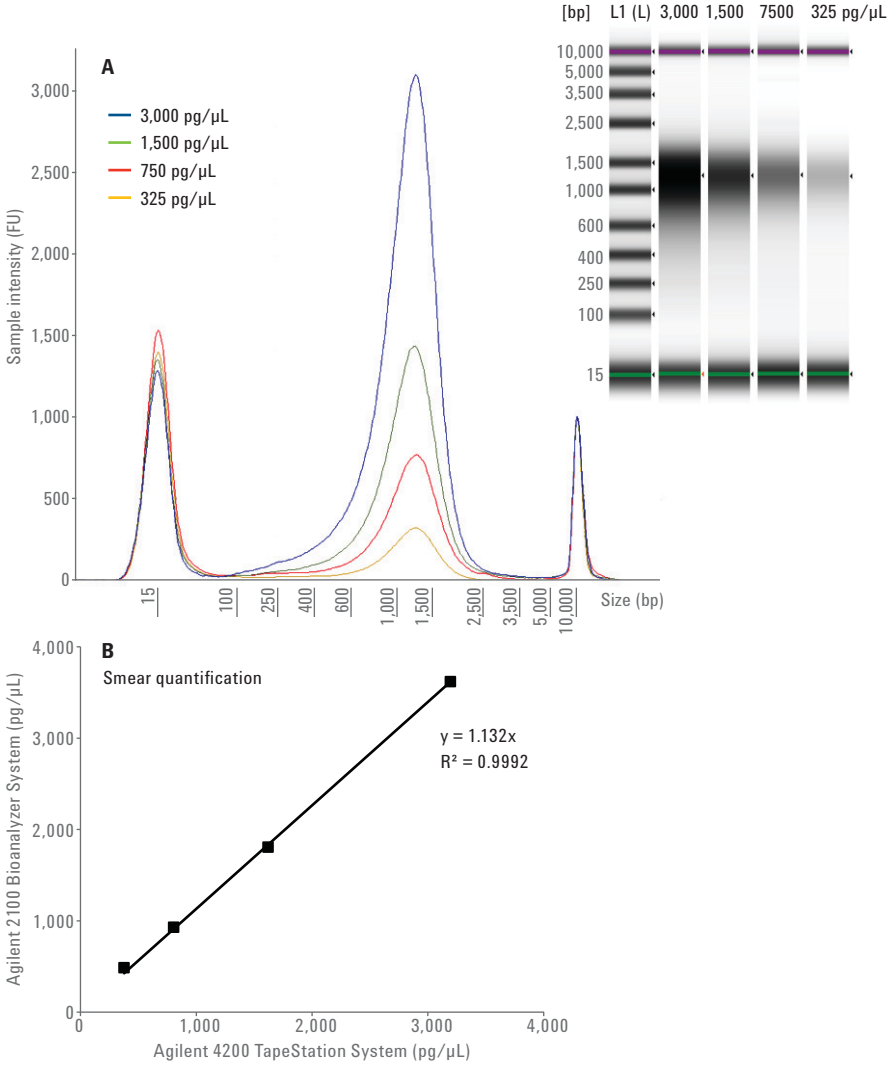

Figure 6. Smear quantification with the Agilent 4200 TapeStation system. A) Gel image and the electropherogram overlay of a dilution series of a DNA smear analyzed with the Agilent High Sensitivity D5000 ScreenTape assay. The concentrations ranged from 325 to 3,000 pg/µL. B) Correlation for the quantification results from the Agilent 2100 Bioanalyzer and the Agilent 4200 TapeStation systems.

Molarity

The molarity of DNA samples is especially important for downstream high sensitivity applications. For example, it is often used to measure the DNA input for sequencing reactions within NGS protocols. The samples from Figure 6 were used to determine the correlation of the molarity values obtained with the 4200 TapeStation and the 2100 Bioanalyzer systems for both the high sensitivity and the standard sensitivity assays (Figure 7).

Figure 7 clearly demonstrates that molarities determined for DNA smears with the 4200 TapeStation system are directly comparable with the results from the 2100 Bioanalyzer system.

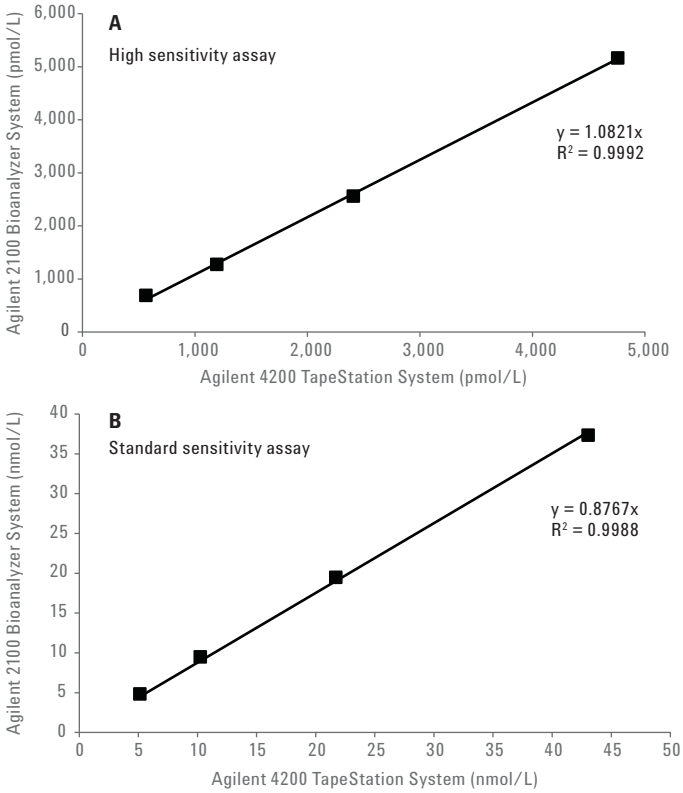

Figure 7. The correlation for molarity of the DNA smear obtained using the region functionality of both the Agilent 4200 TapeStation and the Agilent 2100 Bioanalyzer systems is shown for the high sensitivity and the standard sensitivity assays.

Comparison of the 4200 and 2200 TapeStation systems

In addition to comparing D5000 ScreenTape performance to the 2100 Bioanalyzer system, performance of the 4200 TapeStation was compared to the 2200 TapeStation system.

The same samples from Figures 6 and 7 were used to determine the correlation of the molarity values obtained with the 4200 TapeStation and the 2200 Tapestation systems for both the high sensitivity and standard sensitivity assays (Figure 8).

Finally, sizing performance was compared between the two platforms for both standard and high sensitivity assays. D5000 and High Sensitivity D5000 ladders were analyzed on the 4200 and 2200 TapeStation sytems. Figure 9 shows the perfect sizing correlation between platforms for both sensitivity assays.

Conclusion

This Technical Overview shows that the Agilent D5000 ScreenTape and Agilent High Sensitivity D5000 ScreenTape assays for the Agilent 4200 TapeStation system provide highly accurate and reproducible sizing and quantification of DNA fragments ranging from 100 to 5,000 bp. Furthermore, it demonstrates that the assays can also be applied to determine the DNA average region size, molarity, and concentration of distributed DNA smears. The sizing, concentration, and molarity results highly correlate with the data obtained from equivalent assays on the Agilent 2100 Bioanalyzer system. In addition, D5000 and High Sensitivity D5000 ScreenTape assays show equivalent performance in the 4200 and Agilent 2200 TapeStation systems.

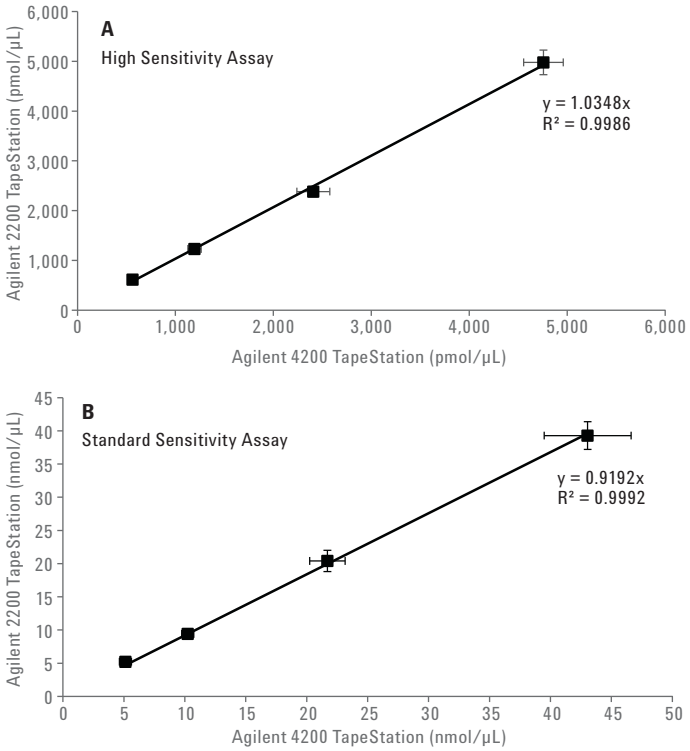

Figure 8. The correlation for molarity of the DNA smear obtained using the region functionality of both the Agilent 4200 TapeStation and the Agilent 2200 TapeStation system is shown for the high sensitivity and the standard sensitivity assays.

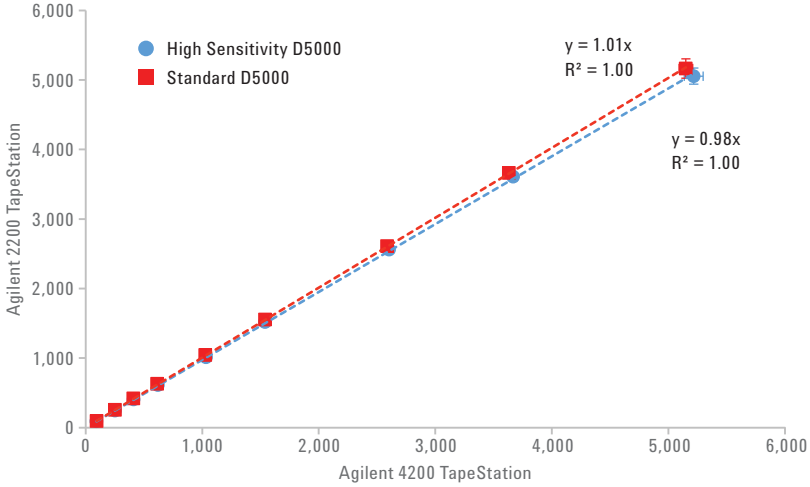

Figure 9. Correlation of Agilent D5000 ladder sizing on both the Agilent 4200 TapeStation and Agilent 2200 TapeStation systems is shown for the high sensitivity and the standard sensitivity assays.

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