Performance of the D1000 and High Sensitivity D1000 ScreenTape Assays for the Agilent 2200 TapeStation System

Technical Overview

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Introduction

The Agilent 2200 TapeStation system provides automated, fast and reliable DNA, RNA and protein electrophoresis for up to 96 samples using pre-packaged reagents and minimal manual handling. The Agilent D1000 and High Sensitivity D1000 ScreenTape assays have been developed for the separation and analysis of DNA fragments from 35 bp to 1000 bp for the 2200 TapeStation system.

This Technical Overview focuses on the performance of both D1000 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 further directly compared to the respective assay for the Agilent 2100 Bioanalyzer system. Table 1 summarizes the analytical specifications of both the D1000 and High Sensitivity D1000 ScreenTape assays.

Table 1. Analytical specifications of the D1000 and the High Sensitivity D1000 ScreenTape assay for the Agilent 2200 TapeStation system.

	D1000 ScreenTape	High Sensitivity D1000 ScreenTape
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	5 % CV	5 % CV
Sizing accuracy[2]	± 10 %	± 10 %
Quantitative precision	0.1–1 ng/µL 15 % CV 1–50 ng/µL 10 % CV	15 % CV
Quantitative accuracy[3]	± 20 %	± 20 %
Quantitative range	0.1–50 ng/µL	10–1,000 pg/µL

[1] Signal:noise ratio > 3 for a single peak
[2] Accuracy for software ladder: ±20 %
[3] Measured against the 2100 Bioanalyzer

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Experimental

Material

Two commercially available DNA ladders (source undisclosed) and NoLimits DNA fragments from Fermentas Molecular Biology Tools (part of Thermo Fisher Scientific) were used throughout the study. Mouse and human genomic DNA (Promega) was sheared with the M220 Focused-ultrasonicator (Covaris) and used together with the Agencourt AMPure XP kit (Beckman Coulter Genomics) and Dynabeads MyOne Streptavidin T1 (Life Technologies) in the generation of NGS library fragments for the SureSelect target enrichment workflow. SureSelect Reagent kit (G9611A) and SureSelectXT Human All Exon V4+UTRs capture library (5190-4636) were obtained from Agilent Technologies for the target enrichment of generated NGS libraries.

Sample preparation

NGS libraries for Illumina HiSeq and MiSeq multiplexed platforms were prepared following the guidelines outlined in the Agilent SureSelectXT Target Enrichment System for the Illumina Paired-End Sequencing Library (version 1.4.1) protocol. These libraries were enriched with the SureSelectXT Human All Exon V4+UTRs kit in accordance with the protocol.

DNA analysis

The 2200 TapeStation (G2964AA) and the 2200 TapeStation Nucleic Acid (G2965AA) systems were used together with D1000 ScreenTape (5067-5582) and D1000 Reagents (5067-5583), High Sensitivity D1000 ScreenTape (5067-5584) and High Sensitivity D1000 Reagents (5067-5585). Performance was compared against the 2100 Bioanalyzer system (G2943CA) using DNA 1000 Kit (5607-1504) and High Sensitivity DNA Kit (5067-4626). The DNA analysis was performed according to the protocols.

Results and Discussion

Sensitivity

The specified sensitivity for the D1000 ScreenTape assay is 0.1 ng/µL and for the High Sensitivity D1000 ScreenTape assay, 5 pg/µL (Table 1). A dilution series of a single 150 bp DNA fragment from 500 to 5 pg/µL was analyzed with the 2200 TapeStation system using the High Sensitivity D1000 ScreenTape assay to verify the sensitivity. Figure 1 shows the electropherogram overlay for this dilution series, where the signal peaks are clearly visible for the dilution steps and the sensitivity of the assay is demonstrated by the enlarged electropherogram image of the 5 pg/µL image.

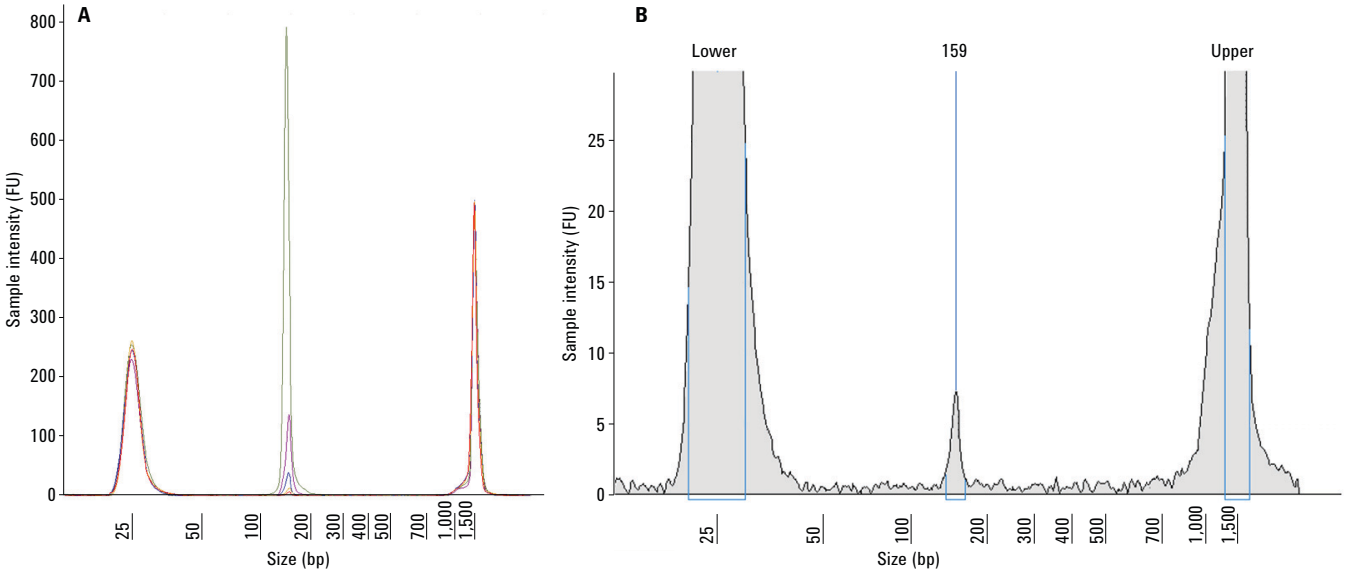

Figure 1. Panel A shows an electropherogram overlay of the analysis of a dilution series using a 150 bp DNA fragment. 500 (green), 100 (purple), 25 (blue), 10 (yellow) and 5 pg/µL (red) are shown. Panel B shows the electropherogram of only the 5 pg/µL 150 bp fragment. This peak is clearly visible above the background signal, thus showing the specified sensitivity for the Agilent High Sensitivity D1000 ScreenTape assay.

DNA sizing

In order to determine the sizing accuracy of the D1000 ScreenTape assay, two commercially available DNA ladders were analyzed with the 2200 TapeStation system and the obtained sizes were plotted against the nominal sizes as supplied by the manufacturer. Fifteen different DNA fragment sizes from 35 to 1,000 bp were covered. For all tested fragments, the sizing accuracy was below 10 % (Figure 2), which is in agreement with the specified sizing accuracy of ± 10 % (Table 1).

The same two commercial DNA ladders and the D1000 ladder were used to verify the sizing precision of the D1000 ScreenTape assay, as shown in Figure 3.

The calculated sizing precision for the tested DNA ladders was well within the specified value of 5% CV (Table 1).

DNA Quantification

In addition to sizing, D1000 and High Sensitivity D1000 ScreenTape assays also provide quantification data for individual DNA fragments within a sample.

To demonstrate the quantification accuracy, a dilution series of a 750 bp DNA fragment with concentrations ranging from 10 pg to 50 ng/µL was quantified with the 2200 TapeStation system using the D1000 and High Sensitivity D1000 ScreenTape assays. The same samples were also analyzed with the 2100 Bioanalyzer system using the DNA 1000 and High Sensitivity DNA assays. The DNA concentrations determined with both systems were plotted against each other using a logarithmic scale where the average of n = 60 for each data point is shown (Figure 4).

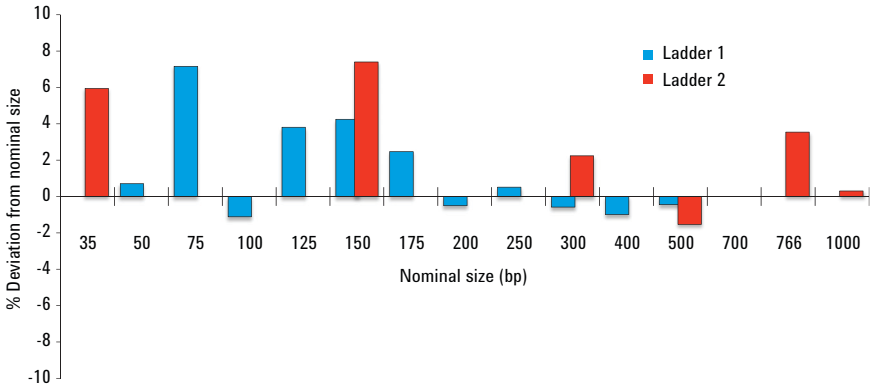

Figure 2. The sizing accuracy of the Agilent D1000 ScreenTape assay was determined using two commercial DNA ladders, Ladder 1 (blue, n = 56) and Ladder 2 (red, n = 46).

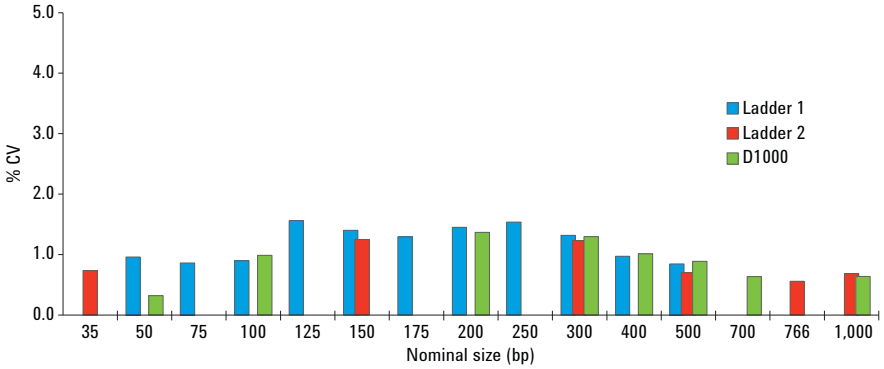

Figure 3. Sizing precision of the Agilent D1000 ScreenTape assay determined with the two commercial ladders (Ladder 1 and 2, n = 56 and 46, respectively) as well as the D1000 ScreenTape ladder (green, n = 48).

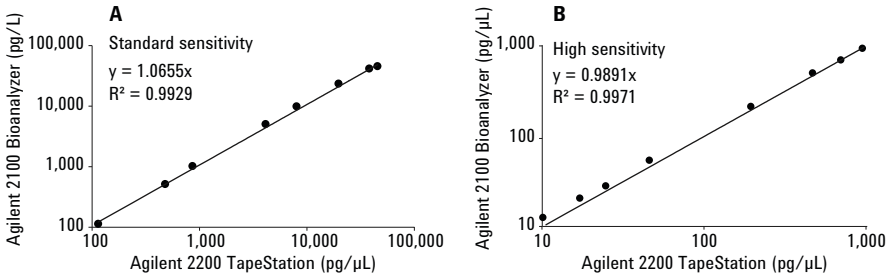

Figure 4. Panel A shows a plot of the concentrations of a 750 bp DNA fragment dilution series from the Agilent D1000 ScreenTape assay on the 2200 TapeStation system plotted against the concentrations of the same samples from the DNA 1000 assay on the 2100 Bioanalyzer system. Panel B shows a plot of concentrations for the 750 bp DNA fragment dilution series from the High Sensitivity D1000 ScreenTape assay on the 2200 TapeStation system plotted against the concentrations obtained from the High Sensitivity DNA assay on the 2100 Bioanalyzer system. A logarithmic scale is used for both graphs and the average of n = 60 per concentration point are shown.

Figure 4 shows an excellent correlation for DNA quantification of single fragments between the 2200 TapeStation and the 2100 Bioanalyzer systems for both the standard sensitivity and the high sensitivity DNA assays.

However, users often look at more complex DNA samples than just single fragments. For example, samples within the Next Generation Sequencing (NGS) workflows cover DNA fragment size distributions within libraries, commonly referred to as "smears". To address this, quantification of the D1000 ScreenTape assay was tested using DNA samples from different steps of the NGS library preparation workflow. Samples were obtained following the SureSelect target enrichment protocol for Illumina paired-end sequencing.

The gel image in Figure 5 shows the typical smear pattern obtained when analyzing samples within the NGS workflow. The graph demonstrates the quantification of the samples from the NGS workflow, again correlating the quantification between the 2200 TapeStation and the 2100 Bioanalyzer systems. Within the specified concentration range of 0.1 to 50 ng/µL good correlation was obtained for the majority of samples. Samples 7 and 11, which show the highest band intensity in the gel image, are marked in red in the graph. Despite having DNA concentrations above the quantitative range of both assays the quantification results are still comparable between the two systems.

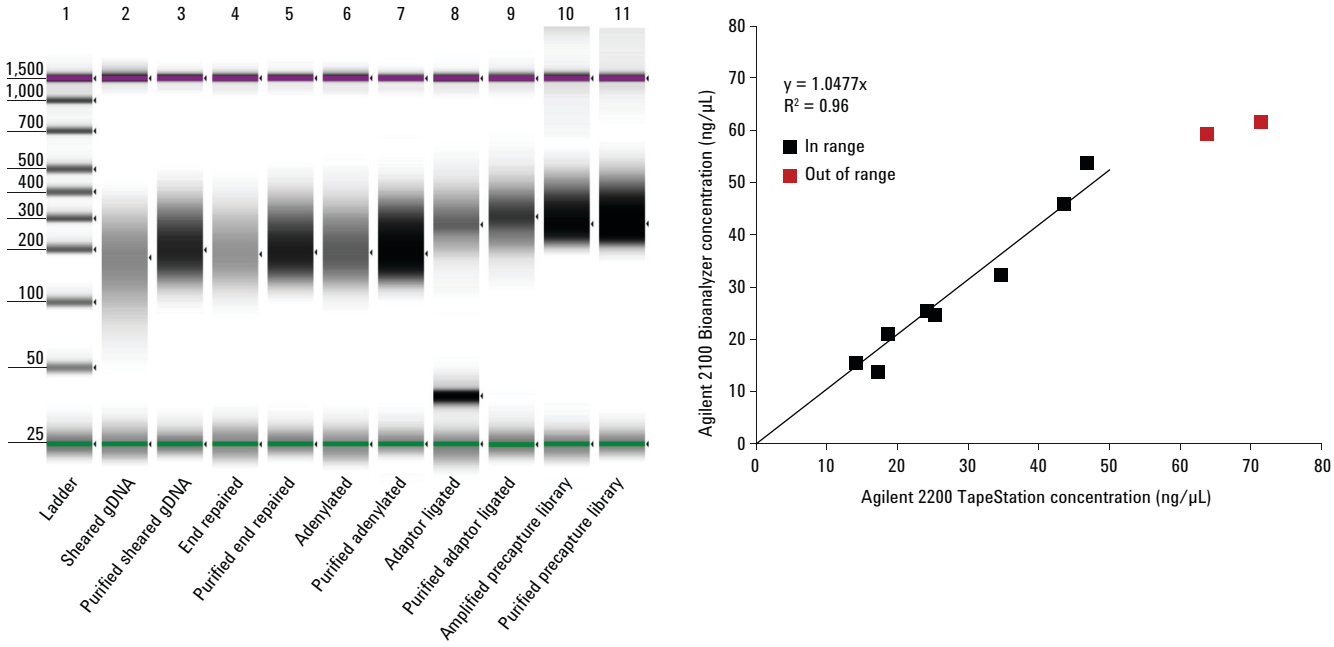

Figure 5. Gel image of the DNA analysis for samples from the NGS library workflow using the 2200 TapeStation system. The graph demonstrates the quantification correlation for the obtained NGS workflow samples correlating the 2200 TapeStation and the 2100 Bioanalyzer systems. The samples marked in red have a DNA concentration outside the specified quantitative range (> 50 ng/µL) of both instruments.

Molarity

The molarity of DNA samples is especially important for high sensitivity applications. For example, it is often applied to determine the DNA input for subsequent sequencing reactions within NGS protocols. Samples from the NGS library preparation, shown in Figure 5, were used to determine the correlation of the molarity values obtained with the 2200 TapeStation and the 2100 Bioanalyzer systems for the standard sensitivity assay (Figure 6A). Figure 6B shows the correlation obtained for the high sensitivity assays with the 2200 TapeStation and the 2100 Bioanalyzer systems using a dilution series of the final post-hybridized amplification product.

DNA molarities obtained with the 2200 TapeStation system are directly comparable with the results from the 2100 Bioanalyzer system.

Conclusion

This Technical Overview shows that the D1000 and High Sensitivity D1000 ScreenTape assays for the 2200 TapeStation system provide highly accurate and reproducible sizing and quantification of DNA fragments. Furthermore, it demonstrates that the assays can also be applied to determine the DNA molarity and quantification of complex DNA samples generated during library preparation within the NGS workflow. All concentration and molarity results have been shown to highly correlate with the equivalent assays on the 2100 Bioanalyzer system.

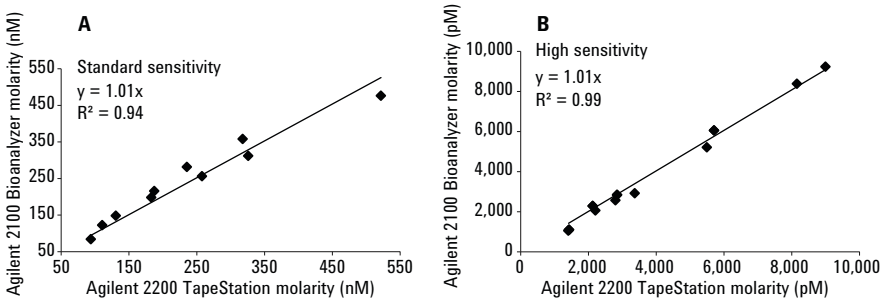

Figure 6. Panel A shows the correlation for molarity of samples from the SureSelect workflow obtained using the Agilent D1000 ScreenTape assay on the 2200 TapeStation system plotted against molarity data from the DNA 1000 assay on the 2100 Bioanalyzer system. Panel B shows DNA molarities of a dilution series of the final post-hybridized amplification product determined with the 2200 TapeStation and the High Sensitivity D1000 ScreenTape assay as well as with the 2100 Bioanalyzer and the High Sensitivity DNA assay.

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