Technical Overview

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# Comparison of DNA Assays Using the 4200 TapeStation System and 2100 Bioanalyzer System

## Introduction

The Agilent 2100 Bioanalyzer system is a well established system for DNA quality control in multiple workflows. Specifically in NGS workflows, the sample throughput has dramatically increased, creating the need for high-throughput systems for DNA sample quality control. The Agilent 4200 TapeStation system has scalable throughput from 1 to 96 samples and walk-away operation, which are essential features for high-throughput analysis. The 4200 TapeStation system fully automates sample processing for DNA electrophoresis, including sample loading, separation, and imaging. Both platforms offer several DNA assays appropriate for a wide size and concentration range for the analysis of PCR products, fragmented DNA, and DNA libraries[1].

This Technical Overview compares the performance of the Agilent D1000 ScreenTape assay and Agilent High Sensitivity D1000 ScreenTape assay (HS D1000 ScreenTape assay) analyzed on the 4200 TapeStation system directly with the Agilent DNA 1000 assay and Agilent High Sensitivity DNA assay (HS DNA assay) of the 2100 Bioanalyzer system. Analytical specifications were compared by evaluating accuracy and precision of quantification and sizing with a suitable sample set of DNA fragments and sheared DNA.

## Analytical Specifications

Table 1 summarizes the analytical specifications of the D1000 and HS D1000 ScreenTape assays for the 4200 TapeStation system and the specifications of the DNA 1000 and HS DNA assays for the 2100 Bioanalyzer system. The analytical specifications of the ScreenTape assays were previously systematically validated for sensitivity, sizing, quantification, and molarity[2].

## Experimental

### Technical Details

The 4200 TapeStation system (p/n G2991AA) with D1000 ScreenTape (p/n 5067-5582) and reagents (p/n 5067-5583), High Sensitivity D1000 ScreenTape (p/n 5067-5584) and reagents (p/n 5067-5585), as well as the 2100 Bioanalyzer system (p/n G2943C) using the DNA 1000 Kit (5607-1504) and High Sensitivity DNA kit (p/n 5067-4626) were obtained from Agilent Technologies (Waldbronn, Germany).

NoLimits DNA fragments and lambda DNA were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Lambda DNA was sheared with the M220 Focused-ultrasonicator from Covaris, Inc. (Woburn, MA, USA) to produce DNA smear samples with a size distribution between 50 and 1,000 bp. Unless stated, the manufacturer's protocols and guidelines were followed. DNA samples were diluted with water to match the specified quantitative range of the DNA assays (Table 1).

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

| | Agilent 4200 TapeStation System | | Agilent 2100 Bioanalyzer System | |
|---|---|---|---|---|
| **Analytical specifications** | **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 |
| Sizing accuracy* | ±10 %** | ±10 %** | ±10 % | ±10 % |
| Sizing precision* | 5 % CV | 5 % CV | 5 % CV | 5 % CV |
| Quantitative range (DNA fragments) | 0.1–50 ng/µL | 10–1,000 pg/µL | 0.5–50 ng/µL* | 5–500 pg/µL* |
| Quantitative range (DNA smears) | 5–100 ng/µL | 0.5–15 ng/µL | - | 0.1–10 ng/µL |
| Quantitative accuracy | ±20 %*** | ±20 %*** | ±20 %* | ±20 %* |
| Quantitative precision | 0.1–1 ng/µL: 15 % CV<br>1–50 ng/µL: 10 % CV | 15 % CV | 25–500 bp: 15 % CV*<br>500–1,000 bp: 5 % CV* | 50–2,000 bp: ± 15 %*<br>2,000–7,000 bp: ± 10 %* |

* Determined by analyzing the respective ladder as sample.
** Accuracy of software ladder ±20 %.
*** Measured against the Agilent 2200 TapeStation system.

## Results and Discussion

### Sizing

The D1000 and HS D1000 ScreenTape assays as well as the DNA 1000 assay allow the analysis of DNA samples with a maximal size of 1,000 bp. Conversely, the HS DNA assay of the 2100 Bioanalyzer system provides a wider sizing range, from 50–7,000 bp.

The sizing performance for the ScreenTape assays shown in Table 1 were previously evaluated with two commercially available DNA ladders[2]. To compare the sizing of the ScreenTape assays for the 4200 TapeStation system directly with the 2100 Bioanalyzer assays, three DNA fragments (300, 500, and 1,000 bp) were diluted to match the quantitative range of each assay, then analyzed. As recommended by the D1000 and HS D1000 ScreenTape assay protocol, a ladder was run on each ScreenTape. The sizing results were plotted against the nominal sizes supplied by the manufacturer (Figure 1).

The specified sizing accuracy (±10 %) was met for each DNA fragment with both electrophoresis platforms and all assays. The D1000 and HS D1000 ScreenTape assays showed sizing accuracy similar to the DNA 1000 and HS DNA assays with the 2100 Bioanalyzer system.

Figure 2 shows sizing precision evaluated with six replicates per fragment on each assay. The determined sizing precision for the D1000 ScreenTape assay (4200 TapeStation system) and the DNA 1000 assay (2100 Bioanalyzer system) for all fragments was below 1 % CV, which is within the specified range for both assays (5 % CV).

The sizing precision of the HS D1000 ScreenTape assay for all fragments was below 1 % CV and below 2 % for the HS DNA assay, which met the specifications of each assay (5 % CV).

The results for sizing obtained with the 4200 TapeStation system showed equivalent accuracy and precision compared to the 2100 Bioanalyzer system.

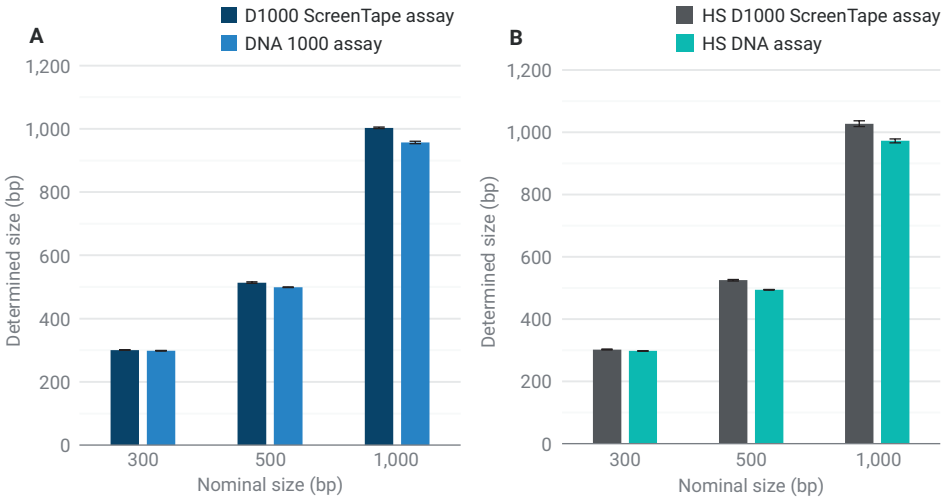


**Figure 1.** Sizing results for three DNA fragments (n = 6) analyzed with the 4200 TapeStation and 2100 Bioanalyzer systems. A) Sizing results of the D1000 ScreenTape assay compared with the DNA 1000 assay. B) Sizing results comparing the High Sensitivity D1000 ScreenTape assay with the High Sensitivity DNA assay.

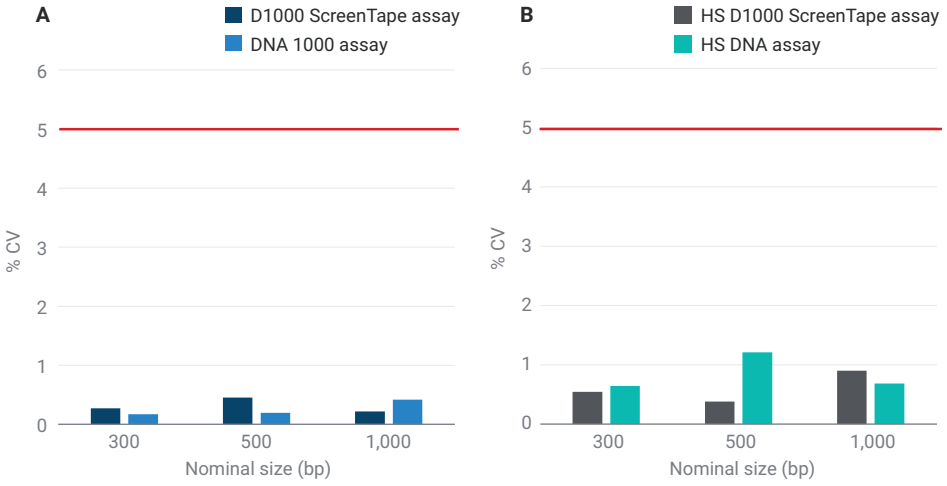


**Figure 2.** Sizing precision analyzed with three different size DNA fragments (n = 6) with the 4200 TapeStation and 2100 Bioanalyzer systems. A) Sizing precision of the D1000 ScreenTape assay compared with the DNA 1000 assay. B) Sizing precision of the High Sensitivity D1000 ScreenTape assay in comparison to the High Sensitivity DNA assay.

## Quantification

The quantification performance for sensitivity, precision, and accuracy was previously validated for the D1000 and HS D1000 ScreenTape assays, and showed excellent performance[2].

The quantitative range for the D1000 ScreenTape assay is specified from 0.1 to 50 ng/µL. This range provides a slightly higher sensitivity to the corresponding DNA 1000 assay of the 2100 Bioanalyzer system for sample concentrations between 0.5 and 50 ng/µL, as shown in Table 1. Quantification of DNA fragments with the HS D1000 ScreenTape assay ranges from 10 to 1,000 pg/µL, whereas the HS DNA assay of the 2100 Bioanalyzer system is suitable for concentrations between 5 and 500 pg/µL (Table 1).

Quantification performance was compared between the assays by analyzing three dilutions of a 500 bp DNA fragment to cover low, mid, and high concentrations within the range of the assays. The quantitative precision of all samples met the specification of the respective assay (data not shown). The 4200 TapeStation system results correlated well with the 2100 Bioanalyzer results, as shown in Figure 3.

## Molarity

Sample molarity is often used to determine the load volume of NGS libraries for pooling before sequencing. The calculation of molarity is based on average size and concentration of library samples. Thus, for accurate molarity results and successful library sequencing, sizing and quantification must be accurate. Excellent assay performance for NGS library samples was shown previously for the D1000 and HS D1000 ScreenTape assays[2]. It was also demonstrated that the assays are suitable for sample quality control in a high throughput sequencing environment[3,4].

The lower and upper size limits of the DNA assays (Table 1) apply for maximum peak sizes of DNA fragments. DNA libraries must match the assay range with the whole sample size to fit between the lower and upper marker of an assay. Table 1 shows that the quantitative range for DNA smears differs from the quantitative range for DNA fragments.

A dilution series of sheared DNA was analyzed with the D1000 and HS D1000 ScreenTape assays on the 4200 TapeStation system. A ladder was run on each ScreenTape to ensure the most accurate sizing.

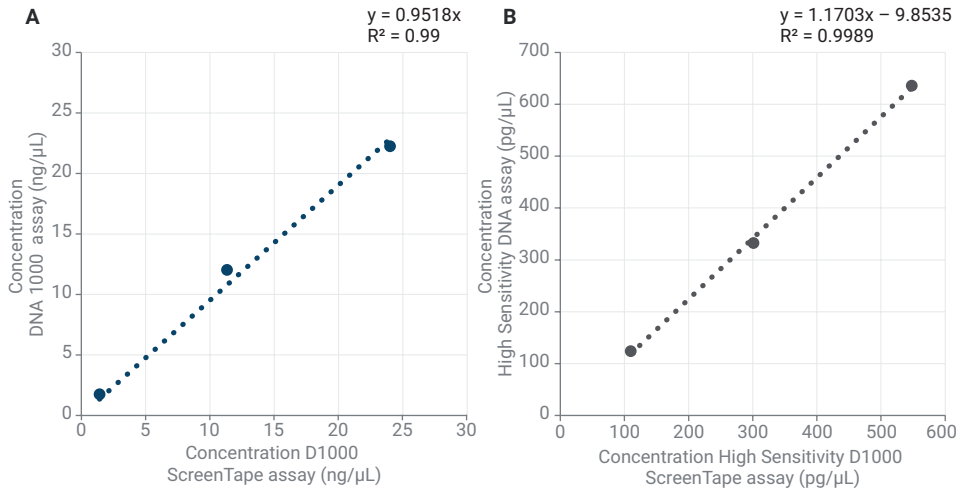


**Figure 3.** Quantification of a 500 bp fragment in three concentrations (n = 6) with the 4200 TapeStation system (on the X-axis) compared to the 2100 Bioanalyzer system (on the Y-axis). A) Concentrations measured with the D1000 ScreenTape assay compared to concentrations obtained from the DNA 1000 assay. B) Comparison of concentrations analyzed with the HS D1000 ScreenTape assay and with the HS DNA assay.

The molarity data was directly compared to the data of the same samples analyzed with the DNA 1000 and HS DNA assays of the 2100 Bioanalyzer system. The results showed excellent correlation for molarity analysis of sheared DNA with the 4200 TapeStation system compared to the 2100 Bioanalyzer system (Figure 4).

Figures 5 and 6 show that the electropherogram patterns of the ScreenTape assays for DNA smears are not identical to the pattern obtained with the 2100 Bioanalyzer system. This effect occurs due to different separation processes during gel electrophoresis on the Bioanalyzer chips and the ScreenTape devices. The position of the region on the X-axis depends on the size range of the assay.

Automatic integration and data processing of the TapeStation Analysis software and the 2100 Expert software generate highly comparable results for sizing, quantification, and molarity analysis of sheared DNA samples measured with the 4200 TapeStation and the 2100 Bioanalyzer systems.

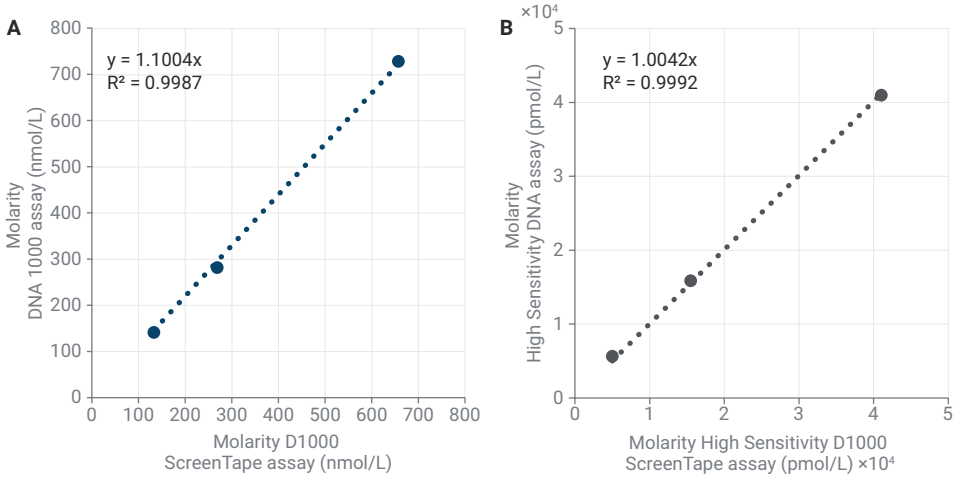


**Figure 4.** Correlation of molarity data of sheared DNA samples in three concentrations (n = 6). The results of the 4200 TapeStation system are plotted on the X-axis and the results obtained with the 2100 Bioanalyzer system on the Y-axis. The molarity was evaluated using region functionality. A) Sample molarities obtained with the D1000 ScreenTape assay compared with molarity data from the DNA 1000 assay. B) Correlated molarity data of sheared DNA samples analyzed with the HS D1000 ScreenTape assay and the HS DNA assay.

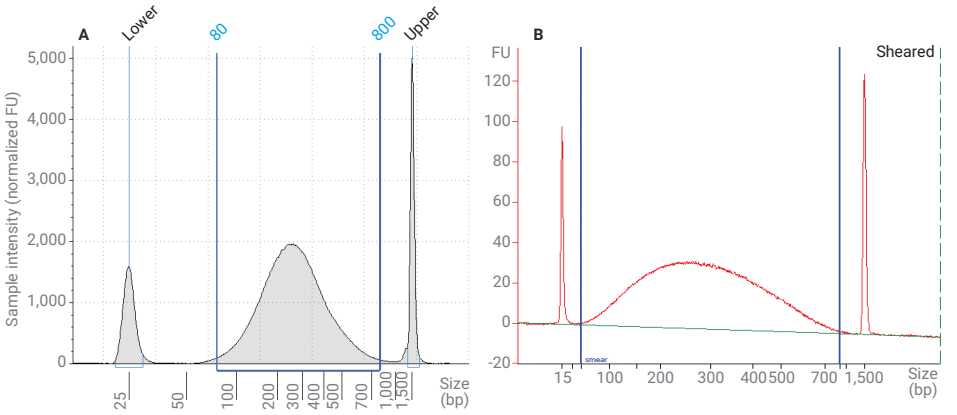


**Figure 5.** Electropherogram pattern of sheared DNA analyzed with the 4200 TapeStation and 2100 Bioanalyzer systems. A) Electropherogram of sheared DNA separated with the D1000 ScreenTape assay. B) Electropherogram of the same sample obtained with the DNA 1000 assay.

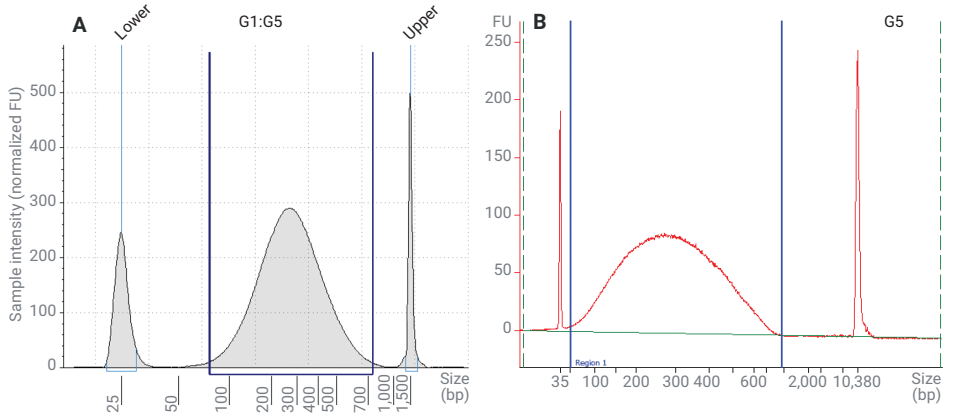


**Figure 6.** Electropherogram patterns of sheared DNA analyzed with the 4200 TapeStation and 2100 Bioanalyzer systems. A) Example electropherogram of a sheared DNA sample analyzed with the HS D1000 ScreenTape assay B) The same sample analyzed with the HS DNA assay.

## Conclusion

This Technical Overview shows that sizing, quantification, and molarity data of DNA ScreenTape assays for the Agilent 4200 TapeStation system highly correlate with data of equivalent assays on the Agilent 2100 Bioanalyzer system. Both systems show similar performance for sizing and quantification of DNA fragments and smears. Electropherogram patterns of sheared DNA analyzed with ScreenTape assays differ slightly from patterns obtained with corresponding assays of the 2100 Bioanalyzer system. The results for molarity, quantification, and sizing correlate highly between equivalent assays on both platforms for DNA smears. For the most accurate sizing and molarity results, it is recommended to run a ladder on each ScreenTape.

## References


1. Agilent Bioanalyzer and TapeStation Systems - Systems, Consumables and Supplies. *Agilent Technologies*, publication number 5991-6071EN, **2015**.
2. Performance of the Agilent D1000 and the Agilent High Sensitivity D1000 ScreenTape Assay for the Agilent 4200 TapeStation System. *Agilent Technologies Technical Overview*, publication number 5991-6903EN, **2016**.
3. Viering, E.; *et al.* Evaluating the Agilent 4200 TapeStation System for High Throughput Sequencing Quality Control. *Agilent Technologies Application Note*, publication number 5991-6892EN, **2016**.
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