

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

Lidia Prieto-Lafuente¹, Claire MacDonald¹, Emma Brown¹, Rainer Nitsche² and Alex Siebold³

1) Agilent Technologies UK Ltd., Edinburgh, UK. 2) Agilent Technologies, Waldbronn, Germany 3) Agilent Technologies, Inc., La Jolla, CA, USA



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 analysis of DNA fragments (100 bp to 5,000 bp) within the Next Generation Sequencing (NGS) workflow. Here, we focus on quantification, sizing, and sensitivity of both D5000 ScreenTape assays. In addition, the performance was compared to the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation system.

Material and Methods

Material

DNA ladder (New England Biolabs), NoLimits DNA Fragments (500 & 3,000 bp), DNA ladder (Thermo Fisher Scientific Inc.), Mouse gDNA (Promega), Swiss Webster Male Mouse gDNA (Zyagen), M220 ultrasonicator (Covaris), Agencourt AMPure XP kit (Beckman Coulter), 4200 TapeStation system, D5000 ScreenTape, D5000 Reagents, HS D5000 ScreenTape, HS D5000 Reagents, 2100 Bioanalyzer system, DNA 7500 and HS DNA kit (Agilent Technologies).

Sample workflow

A dilution series of 2 DNA fragments was prepared. The two DNA ladders were directly used as samples. Genomic DNA was sheared with an ultrasonicator followed by size selection. A dilution series of the obtained DNA was prepared.

Results

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

Analytical specifications	D5000 ScreenTape Assay	High Sensitivity D5000 ScreenTape Assay
Sizing range	100 bp – 5000 bp	100 bp – 5000 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 – 1000 pg/μL

- Signal/noise ratio >3 for a single peak
- Determined using D5000/High Sensitivity D5000 Ladder as sample
- Measured against 2200 TapeStation system

Sensitivity

A dilution series of 2 DNA fragments was analyzed with the 4200 TapeStation system using the High Sensitivity D5000 ScreenTape assay. The fragments are clearly detected down to 5 pg/μL, with a signal-to-noise ratio (S/N) greater than 3 (Fig 1).

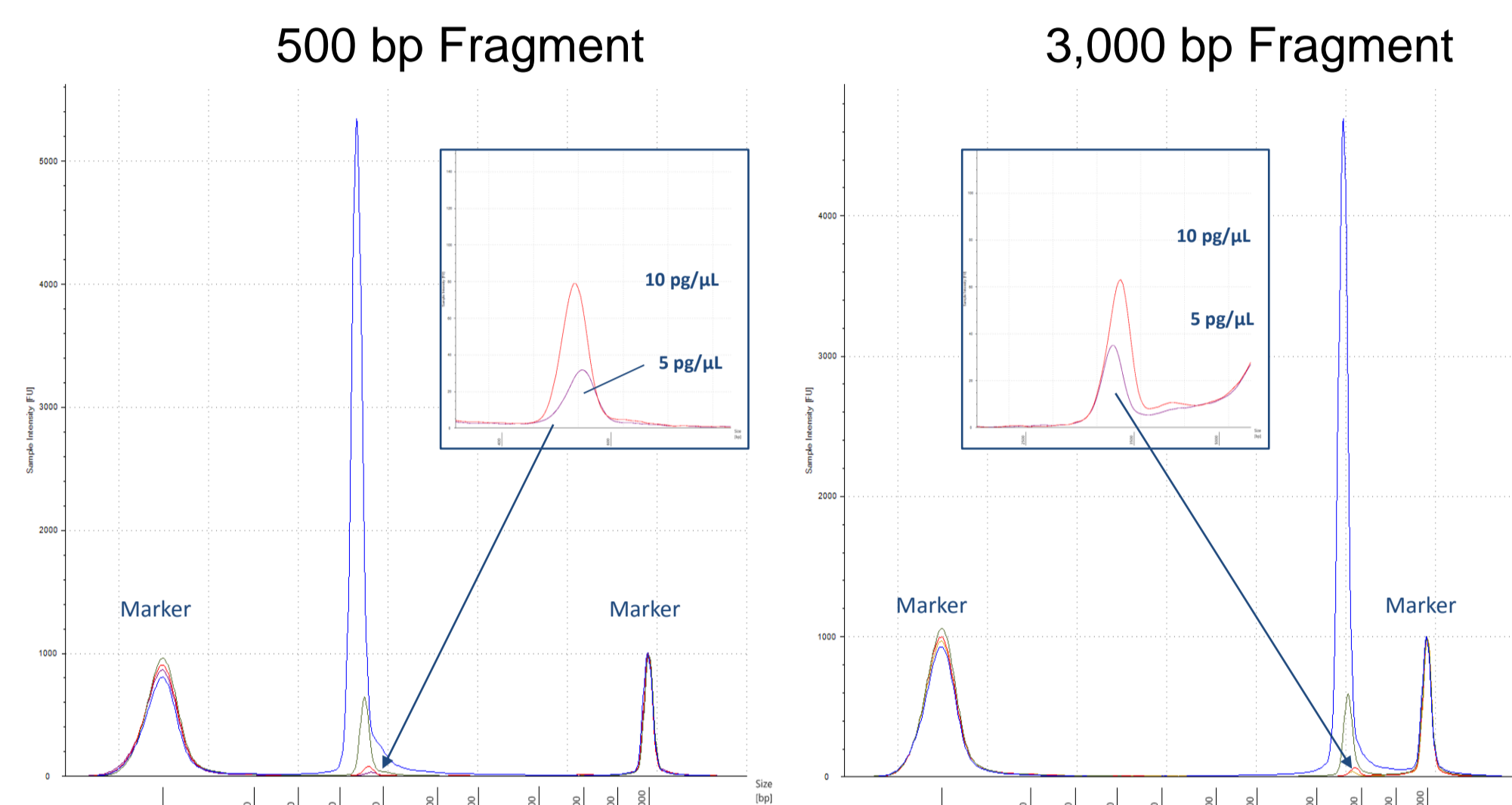


Figure 1. Electropherogram overlay of both DNA fragments (1,000, 100, 10 and 5 pg/μL) analyzed with the High Sensitivity D5000 assay and the 4200 TapeStation system.

DNA sizing

To determine the sizing accuracy and precision of the D5000 ScreenTape assay, two DNA ladders were analyzed with the 4200 TapeStation system. The determined sizing accuracy was clearly below the specified 10% for the D5000 ScreenTape assay (Fig 2).

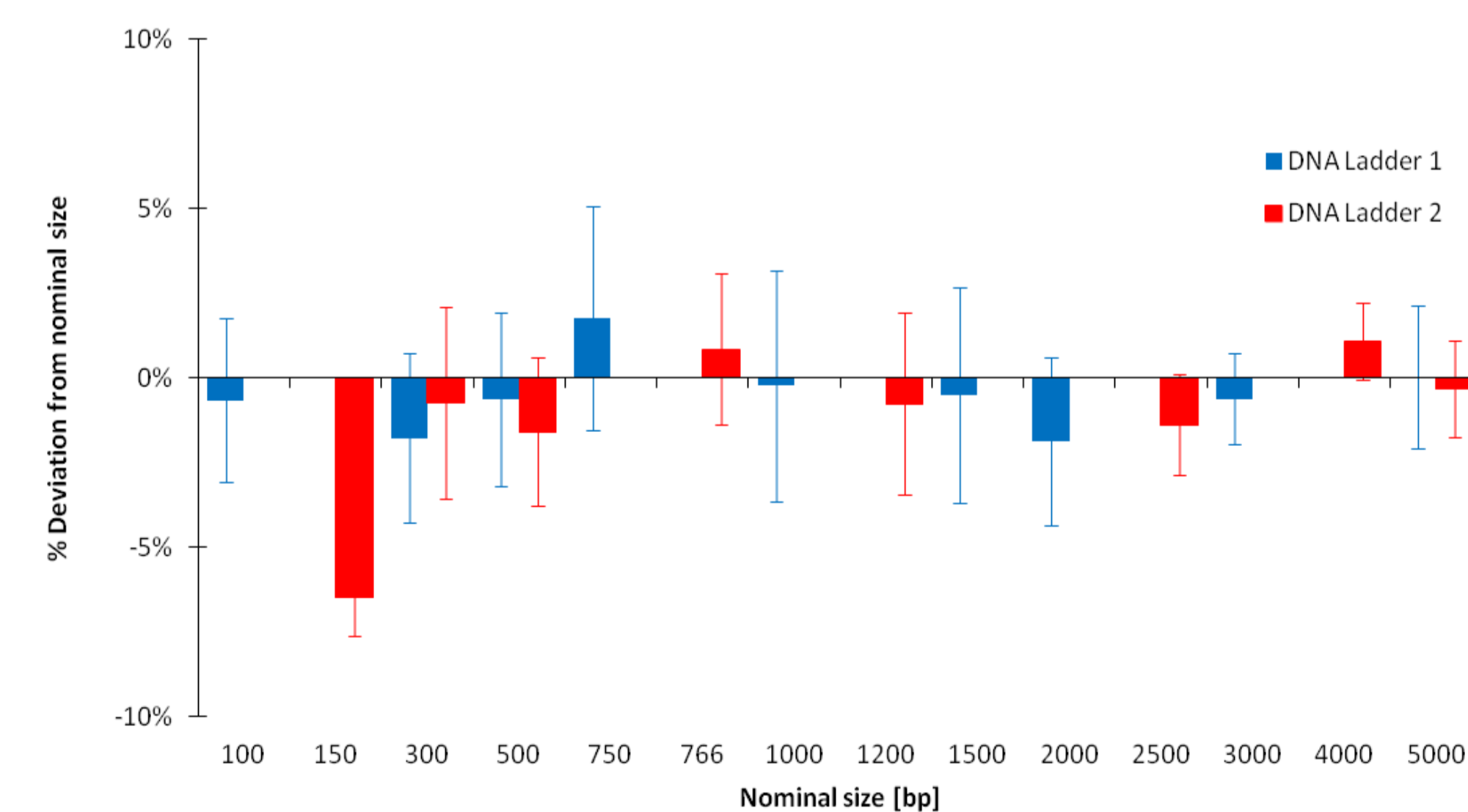


Figure 2. The sizing accuracy of the D5000 ScreenTape assay was determined using two DNA ladders (n = 6), in triplicate on two instruments. The obtained sizes were used to calculate the % deviation from the nominal sizes supplied by the manufacturer.

The calculated sizing precision for the two DNA ladders was within the specified value of 5 % CV for the D5000 ScreenTape assay (Fig 3).

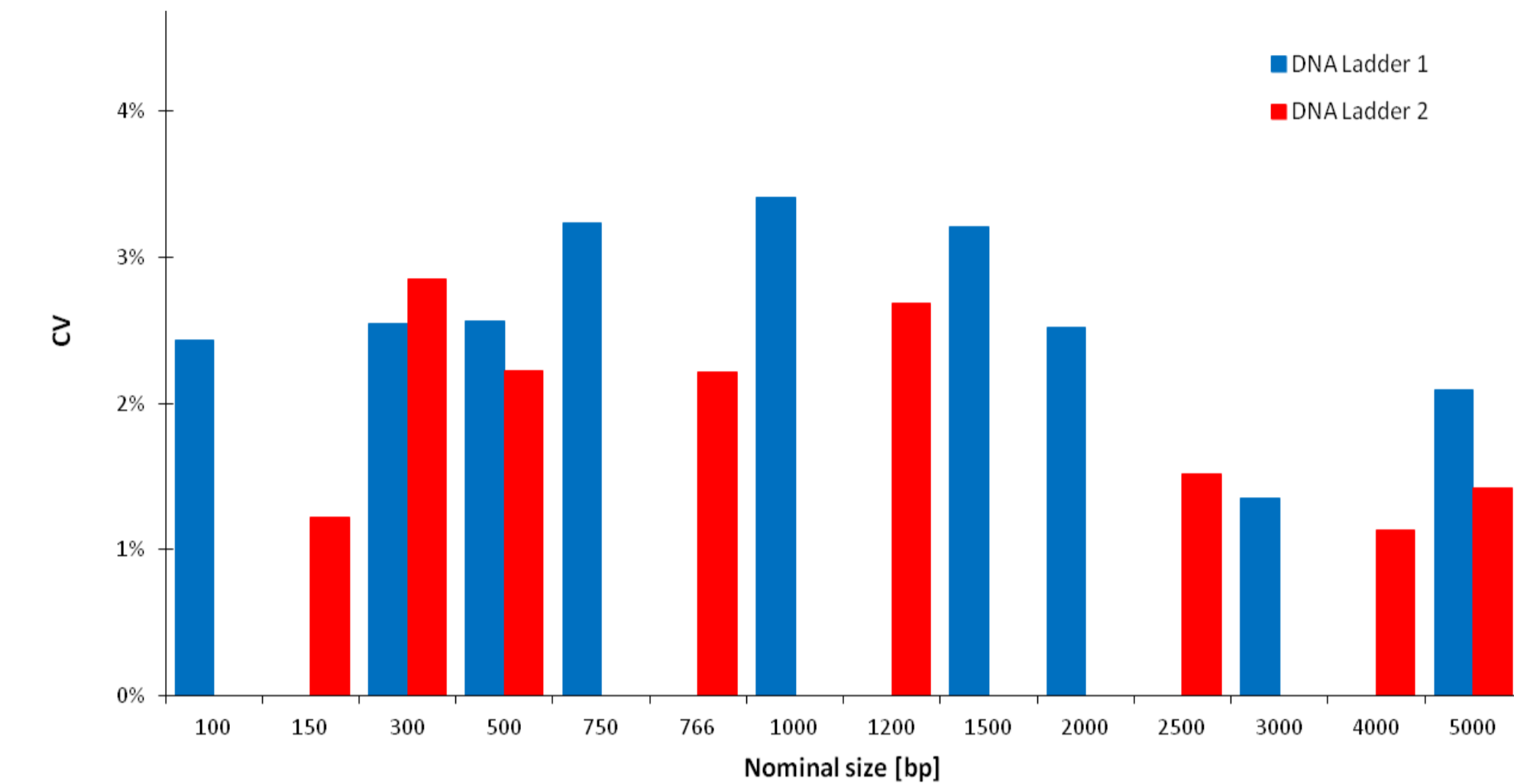


Figure 3. Sizing precision of the D5000 ScreenTape assay determined with two DNA ladders (n = 6), in triplicates on 2 instruments.

DNA Quantification

Both DNA fragments were quantified with the High Sensitivity D5000, and the D5000 ScreenTape assays and with the 2100 Bioanalyzer system using the High Sensitivity DNA and the DNA 7500 assays. An excellent correlation between the two systems for both the standard sensitivity and the high sensitivity DNA assays is observed (Fig 4).

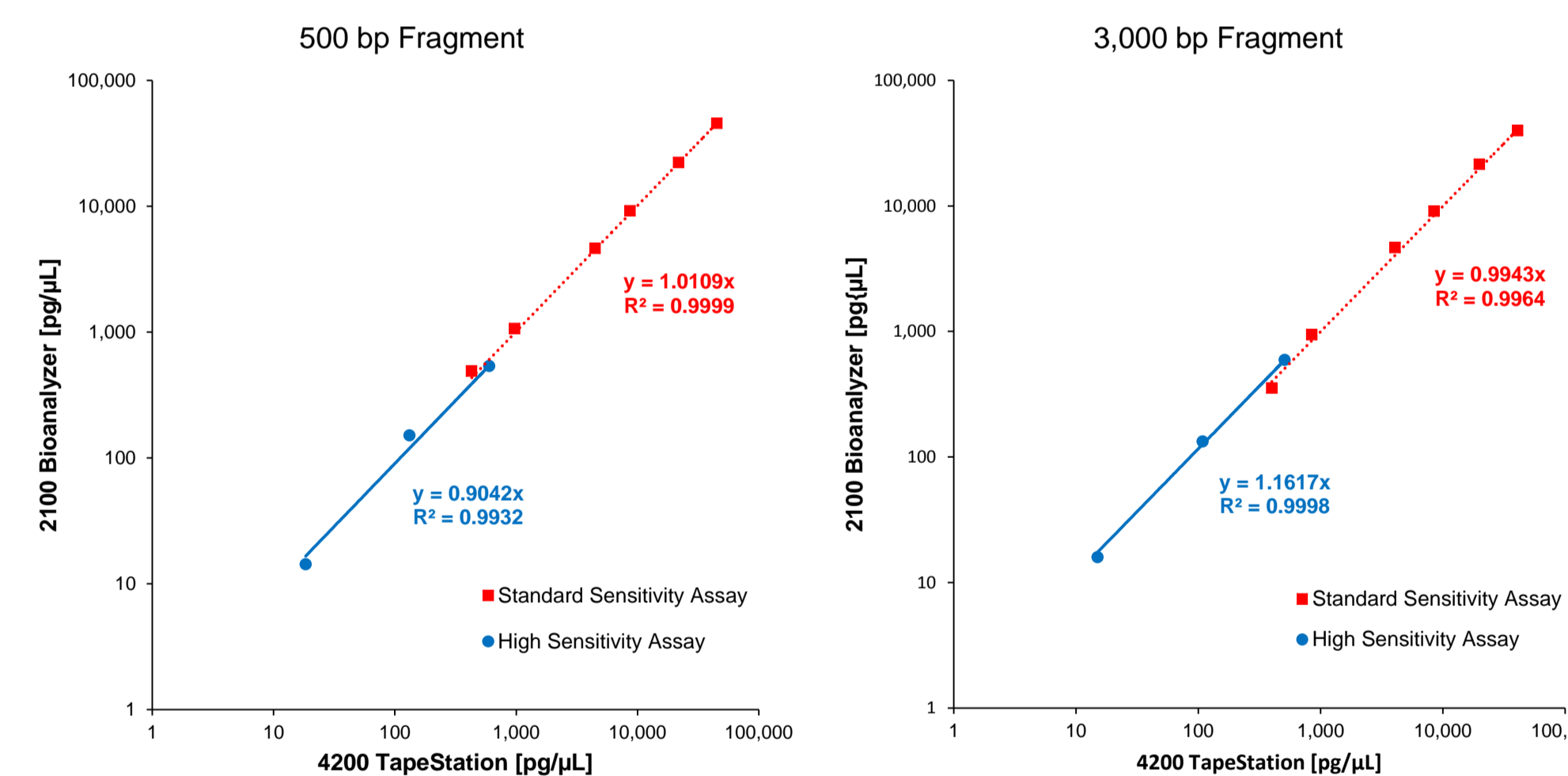


Figure 4. Quantification correlation of the 4200 TapeStation with the 2100 Bioanalyzer system. The measured concentration (n = 6 to 20), was plotted on a logarithmic scale.

The quantification precision is within the specified 10% CV for both fragments analyzed (Fig 5).

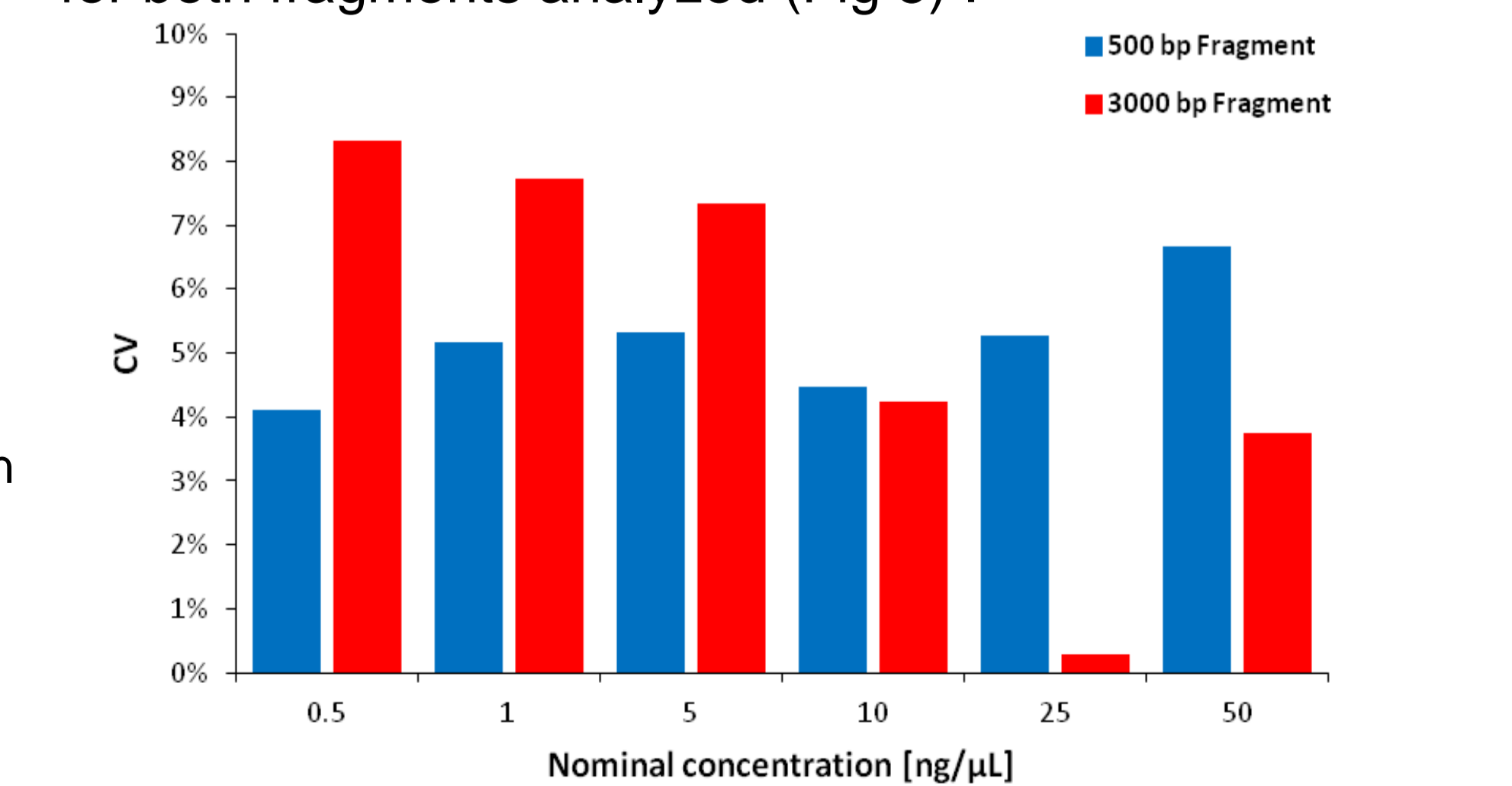


Figure 5. Quantitative precision for the analysis the two fragments with the Agilent D5000 ScreenTape assay (n=6 to 20).

A dilution series of a size selected DNA smear was prepared with concentrations from 325 to 3,000 pg/μL, and analyzed on the 2100 Bioanalyzer and the 4200 TapeStation systems using the high sensitivity assays. A typical DNA smear pattern is observed. A good correlation between the 4200 TapeStation and the 2100 Bioanalyzer systems was obtained (Fig 6).

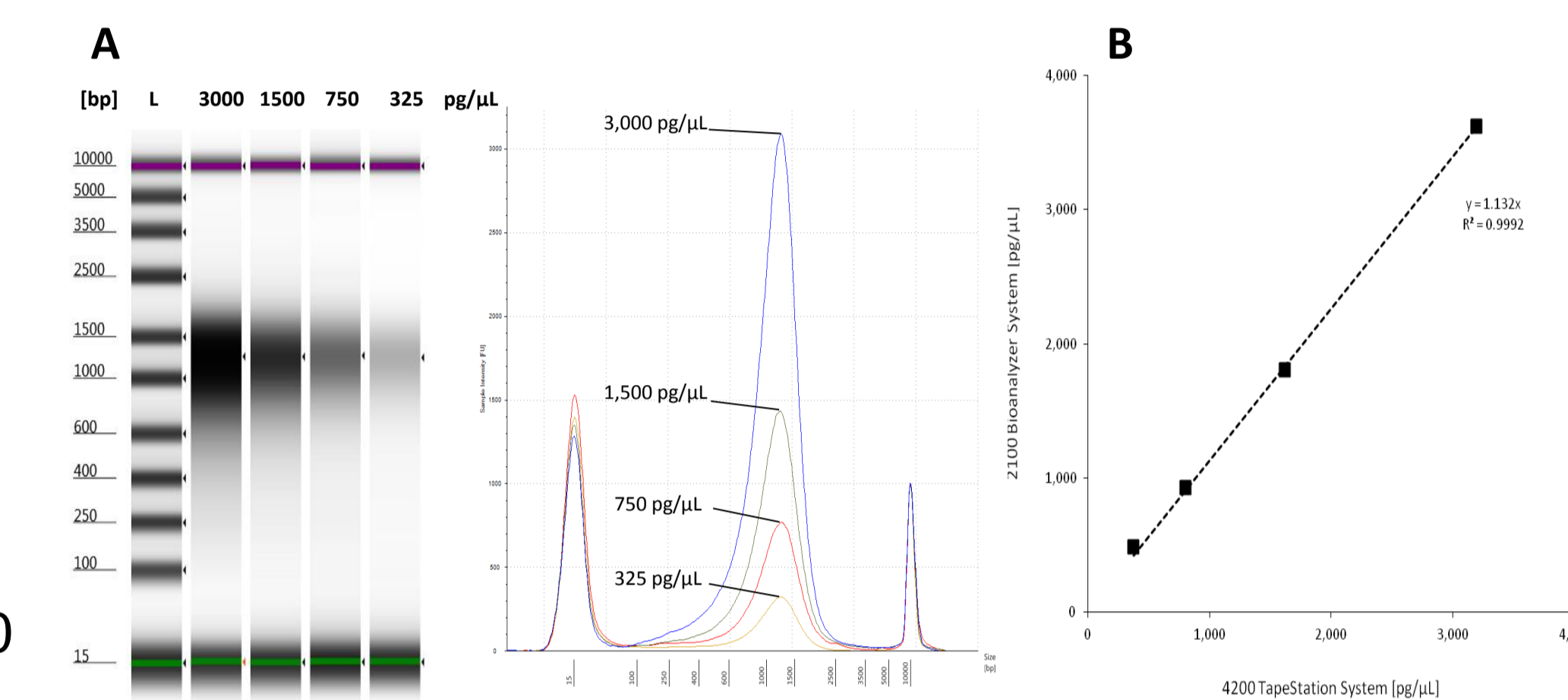


Figure 6. Smear quantification with the Agilent 4200 TapeStation system. A) Gel image and electropherogram overlay of a DNA smear (325 to 3,000 pg/μL) analyzed with the High Sensitivity D5000 ScreenTape assay. B) Correlation between the 2100 Bioanalyzer and the 4200 TapeStation systems.

Molarity

Molarity is used to measure the DNA input for NGS sequencing. Therefore the correlation of the DNA smear molarity between the 4200 TapeStation and the 2100 Bioanalyzer systems for both the high sensitivity and the standard sensitivity assays was determined (Fig 7). Both molarities were directly comparable.

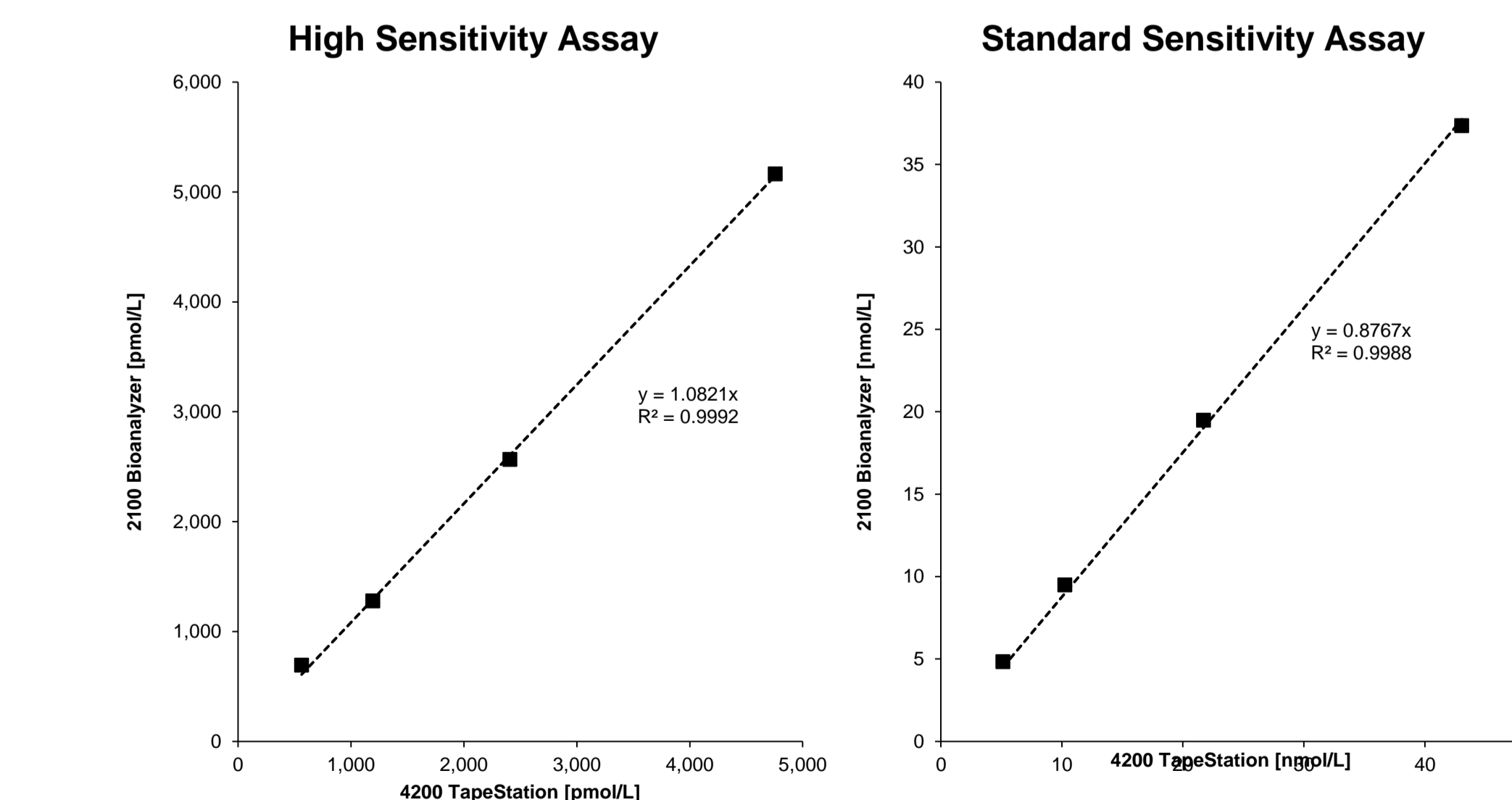


Figure 7. The correlation for molarity of the DNA smear obtained using the region functionality of both the 4200 TapeStation and the 2100 system is shown for the high sensitivity and the standard sensitivity assays.

Comparison of the 4200 and 2200 TapeStation systems Sizing

Sizing performance was compared between the two TapeStation platforms for both standard and high sensitivity assays analyzing D5000 and High Sensitivity D5000 ladders. A perfect sizing correlation between the systems for both sensitivity assays was obtained (Fig 8).

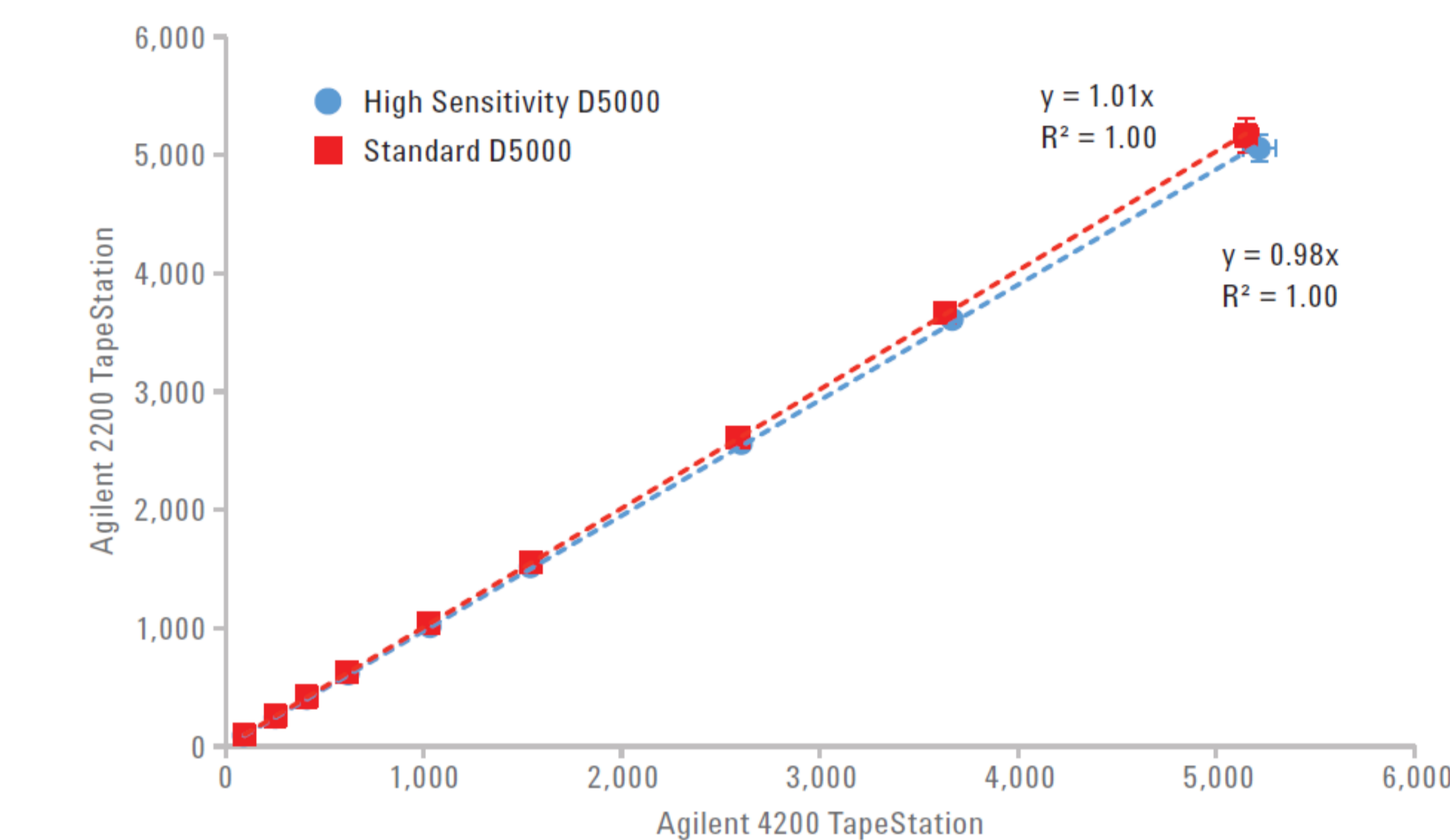


Figure 8. Correlation of D5000 ladder sizing on the 4200 and Agilent 2200 TapeStation systems for the high sensitivity and the standard sensitivity assays.

Conclusions

The Agilent D5000 ScreenTape and Agilent High Sensitivity D5000 ScreenTape assays for the Agilent 4200 TapeStation system offers:

- Highly accurate and reproducible sizing and quantification of DNA fragments ranging from 100 to 5,000 bp.
- Analysis of DNA average region size, molarity, and concentration of distributed DNA smears.
- Results equivalent to data from the Agilent 2100 Bioanalyzer system.
- Equivalent performance compared to the Agilent 2200 TapeStation system.



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