

Quality Assessment of NGS Libraries using Agilent Automated Electrophoresis Systems

Introduction

Next-generation sequencing (NGS) has become essential to many molecular biology laboratories for studying genetic variation associated with disease or other biological research. Generating robust NGS results relies largely on the quality of the library, making analysis of the libraries a crucial step in the sequencing workflow. Thus, many library preparation protocols recommend quality control (QC) of the input sample, at certain steps throughout the workflow, and of the final library to be sequenced. Knowledge of a library's size and concentration provides information that is crucial for preparing the sequencer to achieve optimal data output and enhance confidence in sequencing results.

Together, Agilent automated electrophoresis instruments, including the Agilent 2100 Bioanalyzer system, Agilent Fragment Analyzer systems, and Agilent TapeStation systems, are well-suited for QC of NGS libraries, providing high-quality quantification, qualification, and sizing of nucleic acids to allow for confident assessment of samples throughout the NGS workflow. Each system offers specific benefits suited to meet a variety of individual laboratory needs, such as throughput, sensitivity, speed, and resolution¹.

The use of each Agilent automated electrophoresis instrument in a variety of applications, including NGS sample QC, has been well documented². To demonstrate the equivalency of the Agilent automated electrophoresis systems to each other, NGS libraries were compared across each instrument. Each instrument offers a versatile assay portfolio that covers broad sizing and concentration ranges. The DNA analysis kits chosen for analysis of the NGS libraries in this paper have similar specifications (Table 1), with a sizing range well-suited for the NGS smear, allowing for comparison between the instruments. This technical overview highlights the capability of each system to accurately size and quantify NGS libraries.

Methods

Two NGS libraries were prepared for whole genome amplification (WGA) using the Agilent OnePGT Library Preparation kit for Illumina Sequencing and diluted to approximately 500 pg/μl in 1x TE. The concentration of the libraries was confirmed using the Qubit and the 1X dsDNA HS Assay Kit (Thermo Fisher Scientific p/n Q33230). Each library was analyzed in triplicate with the Agilent automated electrophoresis instruments, utilizing kits with similar sizing and quantification specifications (Table 1). Samples were analyzed on the Agilent 2100 Bioanalyzer system (p/n G2939BA) with the Agilent High Sensitivity DNA kit (p/n 5067-4626)³. The Agilent 5200 Fragment Analyzer system (p/n M5310AA) was used with both the Agilent HS NGS Fragment kit (1-6000 bp) (p/n DNF-474)⁴ and the Agilent HS Small Fragment kit (p/n DNF-477)⁵. Analysis on an Agilent 4200 TapeStation system (p/n G2991BA) was performed using the Agilent High Sensitivity D1000 ScreenTape (p/n 5067-5584) and Agilent High Sensitivity D1000 Reagents (p/n 5067-5585)⁶. The average size and concentration of each smear within the range of 200 to 1,000 bp was reported and compared between each instrument.

Table 1. Agilent automated electrophoresis instruments and assay specifications.

Assay Specifications				
	Bioanalyzer High Sensitivity DNA	Fragment Analyzer High Sensitivity NGS	Fragment Analyzer High Sensitivity Small Fragment	TapeStation High Sensitivity D1000
Sizing Range	50 - 7,000 bp	100 - 6,000 bp	50 - 1,500 bp	35 - 1,000 bp
Typical Resolution	50 - 600 bp: 10%	100 - 1,000 bp: ≤5% ²	50 - 900 bp: ≤5% ²	35 - 300 bp: 15%
	600 - 7,000 bp: 20%	1,000 - 6,000 bp: ≤10% ²	900 - 1,500 bp: ≤10% ²	300 - 1,000 bp: 10%
Sizing Precision	5 %CV ¹	2 %CV ¹	2 %CV ^{1,3}	5 %CV ¹
Sizing Accuracy (%error)	±10% ¹	±5% ¹	±5% ^{1,3}	±10% ^{1,4}
Quantitative Precision	50 - 2,000 bp: 15 %CV ¹	15 %CV ¹	15 %CV ¹	15 %CV ¹
	2,000 - 7,000 bp: 10 %CV ¹			
Quantitative Accuracy (%error)	±20% ¹	±25% ¹	±25% ¹	±20% ¹
Quantitative Range	Fragment 5 - 500 pg/μL	Fragment 5 - 500 pg/μL ^{1,3}	Fragment 5 - 500 pg/μL ^{1,3}	10 - 1,000 pg/μL
	Smear 100 - 10,000 pg/μL	Smear 50 - 5,000 pg/μL ³	Smear 100 - 5,000 pg/μL ³	

¹ Determined using ladder as sample

² Determined using short array

³ Determined using DNA samples in 1X TE buffer

⁴ Sizing accuracy for analysis with electronic ladder: ±20%

Results and Discussion

Electropherogram comparison

The Agilent automated electrophoresis instruments including the Bioanalyzer, Fragment Analyzer, and TapeStation systems can each be utilized with different analysis kits for QC of DNA fragments and smears. To demonstrate the equivalency of the systems, NGS libraries were prepared and analyzed with the automated electrophoresis instruments. Each system provides a digital gel image and an electropherogram of the samples. The NGS libraries were displayed as a smear within the size range of 200 to 1,000 bp. Analysis kits were chosen for the instruments that were suited to the sizing and concentration range of the samples. The kits and their respective sizing and quantification specifications are listed in Table 1. Each assay utilizes different gel chemistries, sizing ladders, and electrophoresis settings. Nevertheless, they are all suitable for analysis of the NGS libraries. These differences between the kits contribute to the slightly different electropherogram shapes seen with each, as shown in Figure 1. However, analysis with any of the kits results in a similar-shaped curve between the sizes of 200 and 1,000 bp, with the highest point of the peak at approximately 400 bp, as well as the absence of any artifacts such as primer or adapter dimers.

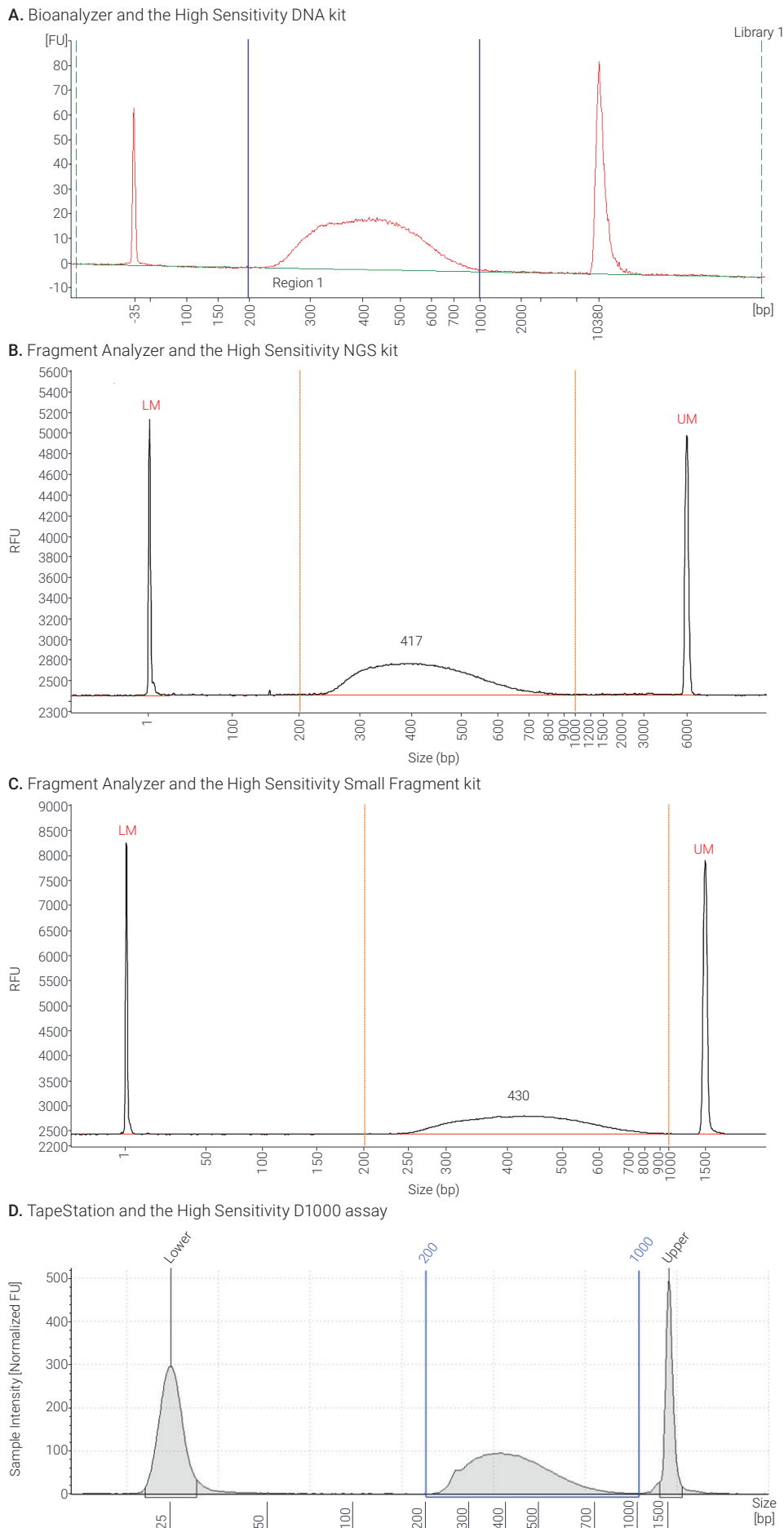


Figure 1. The same NGS library was analyzed on the Agilent automated electrophoresis systems for comparison of the instruments and their respective kits. Shown are the electropherograms from A) the Agilent Bioanalyzer system and the Agilent High Sensitivity DNA kit, B) the Agilent Fragment Analyzer system and the Agilent High Sensitivity NGS kit, C) the Agilent Fragment Analyzer system and the Agilent High Sensitivity Small Fragment kit, and D) the Agilent TapeStation system and the Agilent High Sensitivity D1000 assay. Blue and red vertical lines indicate the smear analysis regions, set at 200 to 1,000 bp. LM: Lower Marker; UM: Upper Marker.

Sizing and quantification comparison

The smear or region functions provided with each system's analysis software are useful tools for determining the average size and quantification of a library. For these libraries, the smear regions were set at 200 to 1,000 bp to include the entire smear region across all instruments, as indicated in Figure 1 by the blue (A, D) and red dashed (B, C) lines. The smear analysis functions allow the user to determine the quantification of only the portion of sample that lies within the smear range, as well as the average size of the sample within the range.

The average size of each library reported by the smear analysis results on each system are comparable (Figure 2). Library 1 displayed an average smear size of 425 bp across all instruments. The mean of the average size of library 2 was 455. The precision, or %CV, was calculated for triplicate replicates of each library. The sizing across each instrument showed excellent precision, with each system displaying less than 1.6 %CV (Figure 2B).

Examination of the average smear concentration over all instruments showed that while all systems gave similar results, the Bioanalyzer and Fragment Analyzer consistently displayed a slightly lower value than the TapeStation (Figure 3). The quantification precision (%CV) and accuracy compared to Qubit (%error) (Figure 3B) for each library are well within each kit's specifications (Table 1). The %CV for each library was less than 15% within each system, and the %error was below 18%.

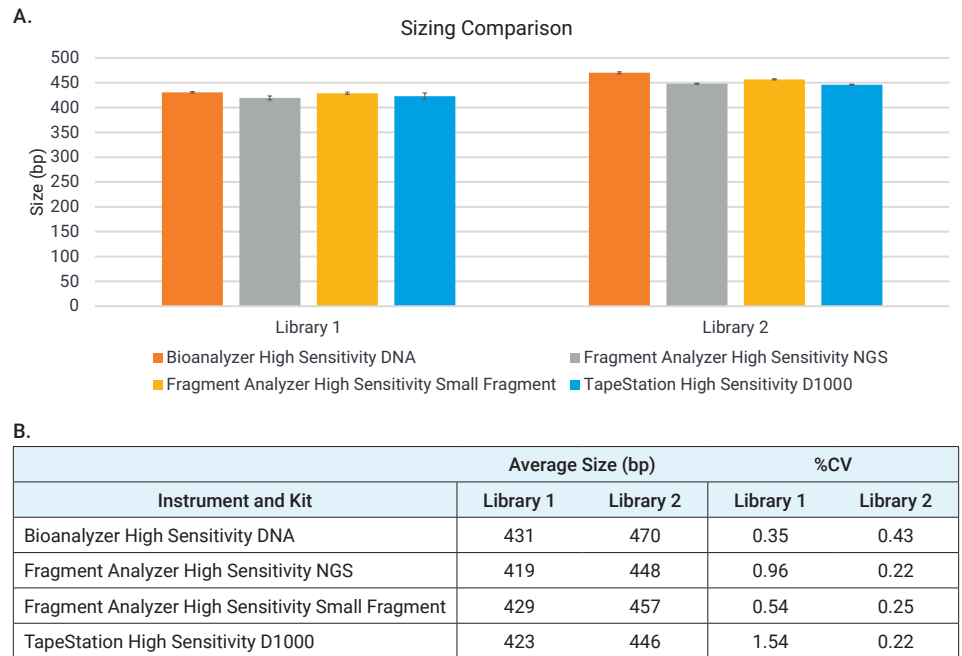


Figure 2. Sizing comparison. The average size of two NGS libraries reported by the Agilent Bioanalyzer, Agilent Fragment Analyzer, and Agilent TapeStation systems. Error bars represent standard deviation. n = 3 replicates per instrument.

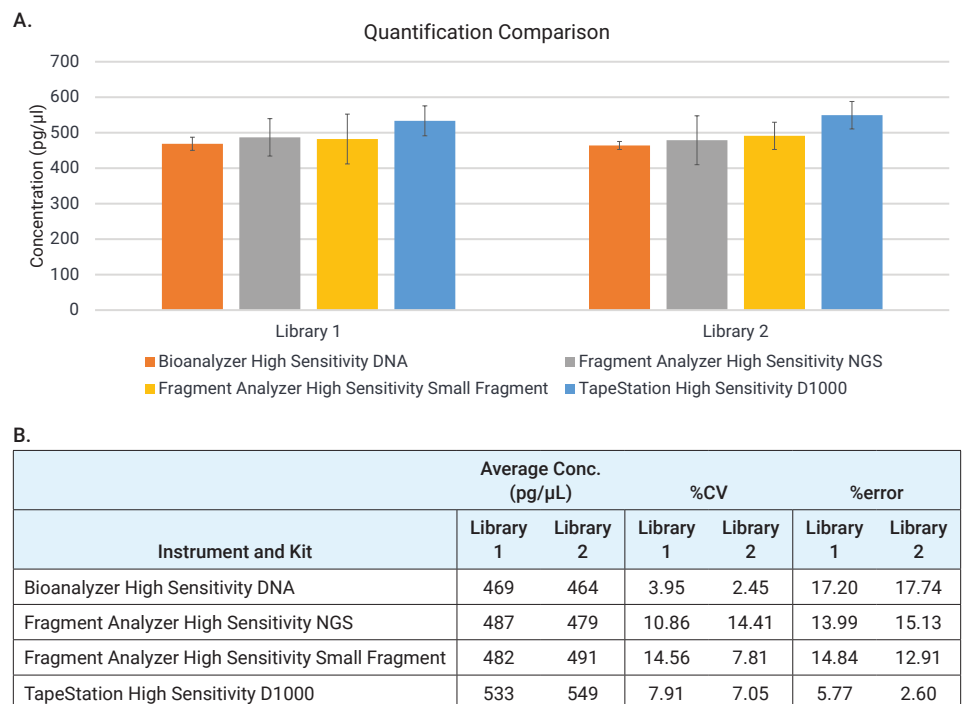


Figure 3. Quantification comparison. The average concentration of two NGS libraries reported by the Agilent Bioanalyzer, Agilent Fragment Analyzer, and Agilent TapeStation systems. Error bars represent standard deviation. %error nominal to Qubit. n = 3 replicates per instrument.

Summary

The Agilent automated electrophoresis instruments, including the Agilent Bioanalyzer, Agilent Fragment Analyzer, and Agilent TapeStation systems, are important tools in any molecular biology lab, providing sizing and quantification data for a variety of nucleic acid samples that is essential to downstream applications. In this technical overview, the performance of each system in the evaluation of the sample quality during NGS library preparation workflows was demonstrated by comparing two final NGS libraries. Each system reported a similar average smear size and concentration of the libraries, demonstrating the equivalency of the Agilent automated electrophoresis systems to analyze DNA smears. Since the performance across the platforms is observed to be equivalent, researchers can have confidence in selecting any of the systems based on factors important to their laboratory's needs, such as sample throughput or speed.

References

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