Technical Overview

Comparison of Small DNA Fragment Analysis using the Agilent Bioanalyzer and Agilent Fragment Analyzer Systems

Author

Whitney Pike,
Agilent Technologies, Inc.

Introduction

Analysis of DNA fragments is important to many molecular biology workflows involving techniques such as PCR amplification and restriction digestions. While fragment analysis is often performed using manual gel electrophoresis, this analysis can quickly and easily be performed using high-resolution automated electrophoresis instruments, including the Agilent 2100 Bioanalyzer system and the Agilent Fragment Analyzer systems. The Bioanalyzer is a well-established microfluidics-based quality control tool for the analysis of biomolecules, capable of analyzing up to 12 samples at once. The Fragment Analyzer systems are parallel capillary electrophoresis instruments, with three different models that offer a flexible throughput to suit the needs of any laboratory. Both systems are compatible with a variety of sample analysis kits suitable for DNA fragments of varying sizes and concentrations.

Separations from different small fragment samples on each of the automated electrophoresis systems have been detailed in previous Agilent publications. Here, samples are compared across both the Bioanalyzer and Fragment Analyzer analysis kits to demonstrate the equivalency of the systems to analyze small fragments. The kits chosen for this comparison, including the Agilent DNA 1000 kit (p/n 5067-1504) for the Bioanalyzer and the Agilent Small Fragment kit (p/n DNF-476) for the Fragment Analyzer, have a similar sizing range and can provide both sizing and quantitative data (Table 1). Other analysis kits are available for both systems covering expansive sizing and quantitative ranges[1,2]. Additionally, a variety of qualitative kits for the Fragment Analyzer offer a higher input range, but do not provide quantitative data. The sizing and resolution of these Fragment Analyzer kits have been described previously[3, 4].

This technical overview demonstrates that the sizing and quantification data obtained from both the Bioanalyzer and Fragment Analyzer systems are consistent when analyzing a variety of small DNA fragments. The comparison can help provide assurance to researchers that the data obtained by the Agilent automated electrophoresis instruments can be used interchangeably for DNA analysis.

Methods

NoLimits DNA Fragments (Thermo Fisher Scientific) of sizes 150 (p/n SM1601), 500 (p/n SM1641), and 1,000 (p/n SM1671) bp were obtained for this study. Each fragment was diluted to 40 ng/µl in 1X TE, and the concentration confirmed using the Qubit 4.0 and the 1X dsDNA HS Assay Kit (Thermo Fisher Scientific p/n Q33230). Two-fold serial dilutions were prepared from 40 ng/µl to 0.6 ng/µl. The samples were analyzed in triplicate on both the Agilent Bioanalyzer 2100 system and the Agilent 5200 Fragment Analyzer system.

Table 1. Analytical specifications of the Agilent DNA 1000 kit for the Agilent Bioanalyzer system and the Agilent Small Fragment kit for the Agilent Fragment Analyzer.

Analytical Specification	Bioanalyzer system DNA 1000 kit (p/n 5067-1504)	Fragment Analyzer system Small Fragment kit (p/n DNF-476)
Sizing range	25 - 1,000 bp	50 - 1,500 bp[1]
Marker sizes	15 - 1,500 bp	1 - 1,500 bp
Sizing resolution	25 - 1,000 bp: 5 bp	50 - 700 bp ≤ 5%; 700 - 1,500 bp ≤ 10% (ultrashort capillary array)
	100 - 500 bp: 5%	50 - 900 bp ≤ 5%; 900 - 1,500 bp ≤ 10% (short capillary array)
	500 - 1,000 bp: 10%	
Sizing accuracy	± 10%	± 5%[1, 2]
Sizing precision	5 %CV	2 %CV[2]
Quantitative range	0.5 - 50 ng/µL[1]	Fragments: 0.1 - 10 ng/µL[2]
		Smears: 5 - 100 ng/µL[3]
Quantitative accuracy	20%[1]	± 25%[1, 2, 3]
Quantitative precision	25 - 500 bp: 15 %CV[1]	15% CV[2, 3]
	500 - 1,000 bp: 5 % CV[1]	

[1] Determined using the respective DNA ladder as sample
[2] Determined using a 400 bp DNA fragment standard in 1X TE buffer
[3] Determined using sheared gDNA with smear range from 10 - 1,400 bp in 1X TE buffer

To allow for comparison between the systems, analysis kits with similar sizing ranges were chosen for each instrument. Samples that fit within the sizing range of the respective kits were analyzed on the Bioanalyzer using the Agilent DNA 1000 kit (p/n 5067-1504), and on the Fragment Analyzer using the Small Fragment kit (p/n DNF-476). The analytical specifications for each kit are shown in Table 1.

Results and discussion

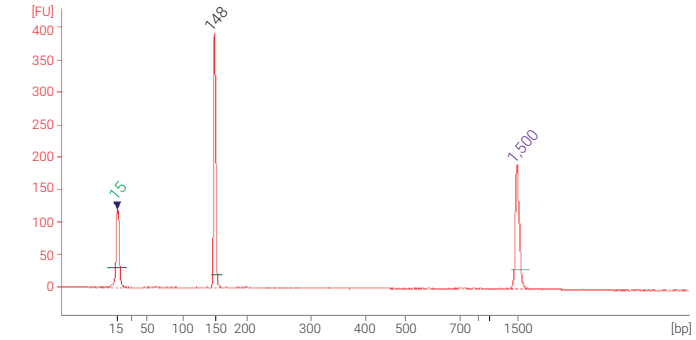
Sizing of multiple DNA fragments across serial dilutions

Serial dilutions of multiple DNA fragments were analyzed across a concentration range that fit the specifications of the respective kits. The fragments were analyzed on the Bioanalyzer DNA 1000 kit from 0.6 to 40 ng/µL. For the Fragment Analyzer Small Fragment kit, the range was from 0.16 to 10 ng/µL. Example electropherograms of 150 (Figure 1), 500 (Figure 2), and 1,000 (Figure 3) bp fragments at 5 ng/µL are shown below for comparison. The concentration of the fragments did not affect the size reported by either system.

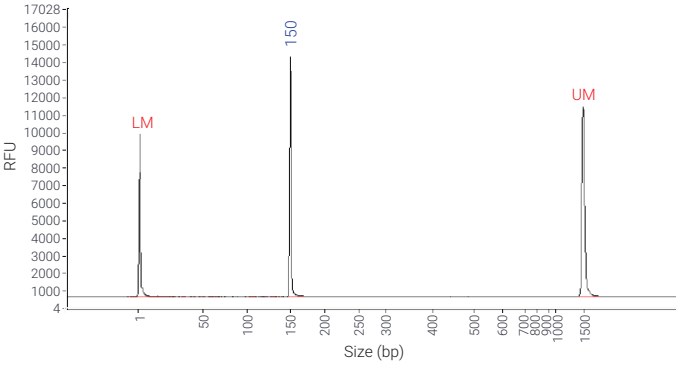
Sizing accuracy was measured by percent error based upon the expected size of each fragment. For example, the 150 bp fragment displayed fragment sizing between 148 and 151 at each concentration tested, with a percent error of less than 1.2% on the Bioanalyzer and less than 0.9% on the Fragment Analyzer (Figure 1). The 500 bp fragment showed an average size of 495 to 500 on the Bioanalyzer, with less than 1% error, and a size of 499 to 500 bp on the Fragment Analyzer, with less than 0.4% error (Figure 2). The 1,000 bp fragment showed an average size between 942 to 981 bp across the different concentrations tested on the Bioanalyzer, with a percent error between 1.9 and 5.8%. On the Fragment Analyzer, this fragment displayed a size of 1,000 to 1,010 bp with a percent error less than 1% (Figure 3). Overall, fragment sizing for each fragment was observed to be highly consistent between the instruments and displayed an excellent sizing accuracy at each concentration tested on both the Bioanalyzer and the Fragment Analyzer.

150 bp fragment

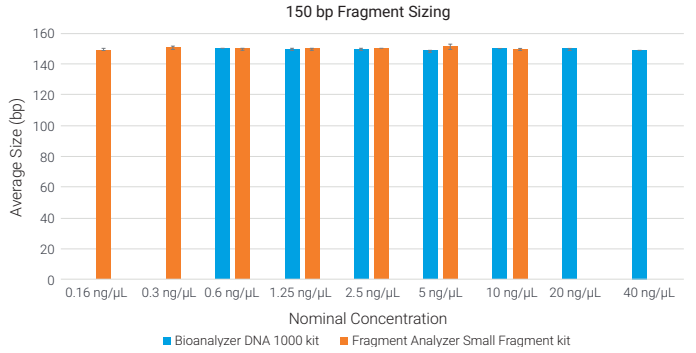
A) Agilent Bioanalyzer system, 150 bp fragment



B) Agilent Fragment Analyzer system, 150 bp fragment



C) Sizing comparison

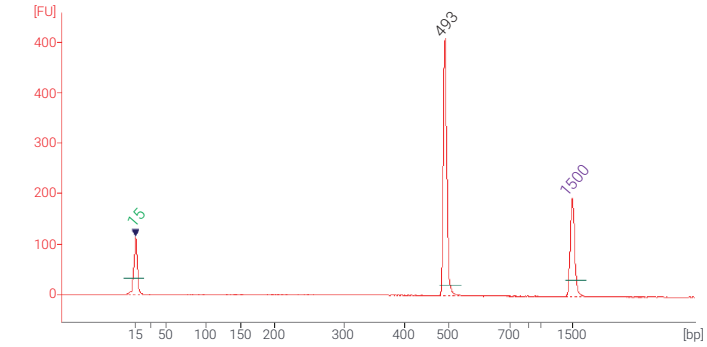


Nominal Concentration	Average Size (bp)		% Error	
	Bioanalyzer DNA 1000 kit	Fragment Analyzer Small Fragment kit	Bioanalyzer DNA 1000 kit	Fragment Analyzer Small Fragment kit
0.16 ng/µL	NA	149	NA	0.44
0.3 ng/µL	NA	151	NA	-0.44
0.6 ng/µL	150	150	0.00	0.22
1.25 ng/µL	149	150	0.44	0.22
2.5 ng/µL	149	150	0.44	0.00
5 ng/µL	148	151	1.11	-0.89
10 ng/µL	150	149	0.00	0.44
20 ng/µL	150	NA	0.22	NA
40 ng/µL	149	NA	0.67	NA

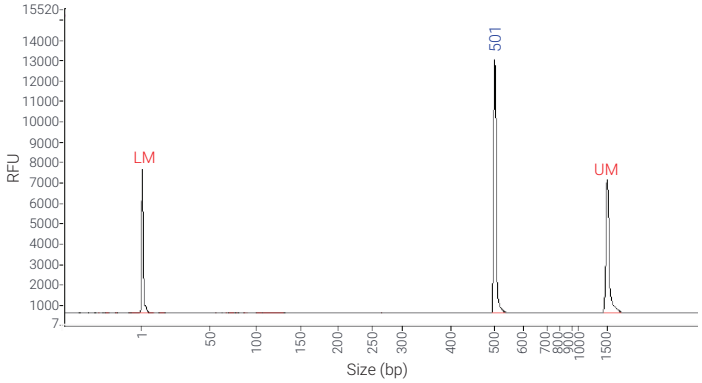
Figure 1. A 150 bp DNA fragment was analyzed on A) the Agilent Bioanalyzer system with the DNA 1000 kit and B) the Agilent Fragment Analyzer system with the Small Fragment kit for comparison. Example electropherograms of the fragment at a concentration of 5 ng/uL are shown. C) The reported size of the 150 bp fragment across a serial dilution was compared between the two systems. The average size and percent error are shown. n = 3 replicates per concentration.

500 bp fragment

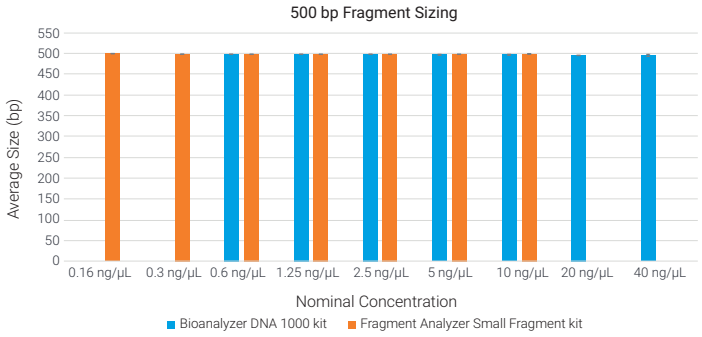
A) Agilent Bioanalyzer system, 500 bp fragment



B) Agilent Fragment Analyzer system, 500 bp fragment



C) Sizing comparison, 500 bp fragment

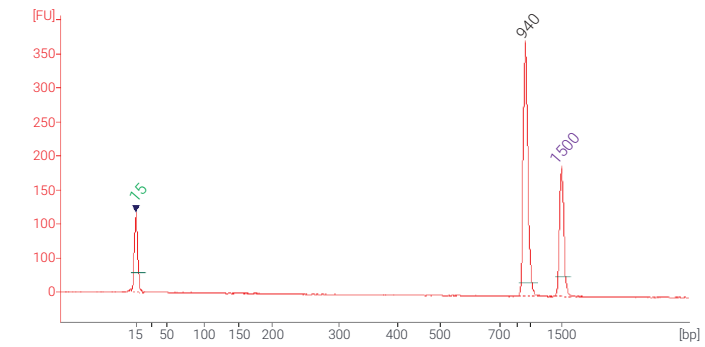


Nominal Concentration	Average Size (bp)		% Error	
	Bioanalyzer DNA 1000 kit	Fragment Analyzer Small Fragment kit	Bioanalyzer DNA 1000 kit	Fragment Analyzer Small Fragment kit
0.16 ng/µL	NA	498	NA	0.33
0.3 ng/µL	NA	500	NA	0.07
0.6 ng/µL	500	500	0.00	0.07
1.25 ng/µL	498	500	0.40	0.07
2.5 ng/µL	498	499	0.40	0.27
5 ng/µL	496	500	0.73	0.00
10 ng/µL	498	499	0.47	0.20
20 ng/µL	496	NA	0.80	NA
40 ng/µL	495	NA	0.93	NA

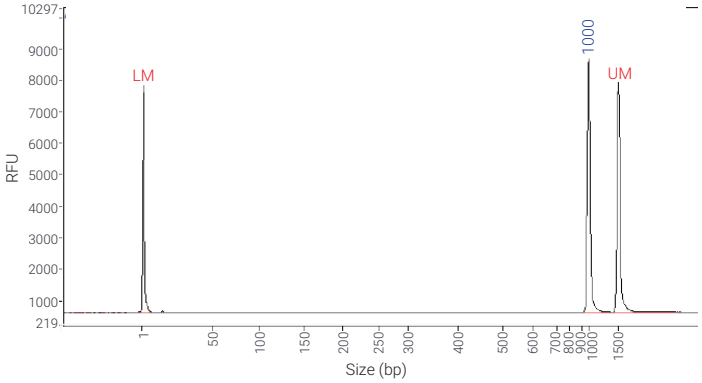
Figure 2. A 500 bp DNA fragment was analyzed on A) the Agilent Bioanalyzer system with the DNA 1000 kit and B) the Agilent Fragment Analyzer system with the Small Fragment kit for comparison. Example electropherograms of the fragment at a concentration of 5 ng/uL are shown. C) The reported size of the 500 bp fragment across a serial dilution was compared between the two systems. The average size and percent error are shown. n = 3 replicates per concentration.

1,000 bp fragment

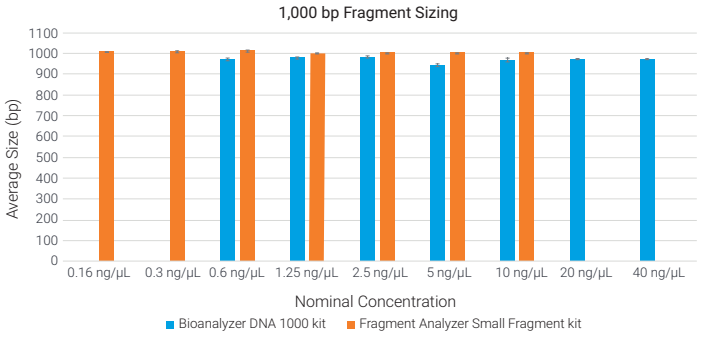
A) Agilent Bioanalyzer system, 1,000 bp fragment



B) Agilent Fragment Analyzer system, 1,000 bp fragment



C) Sizing comparison, 1,000 bp fragment



Nominal Concentration	Average Size (bp)		% Error	
	Bioanalyzer DNA 1000 kit	Fragment Analyzer Small Fragment kit	Bioanalyzer DNA 1000 kit	Fragment Analyzer Small Fragment kit
0.16 ng/µL	NA	1,007	NA	-0.70
0.3 ng/µL	NA	1,005	NA	-0.50
0.6 ng/µL	969	1,010	3.10	-0.95
1.25 ng/µL	978	1,000	2.23	0.00
2.5 ng/µL	981	1,000	1.90	-0.03
5 ng/µL	942	1,000	5.80	-0.03
10 ng/µL	965	1,001	3.47	-0.07
20 ng/µL	970	NA	3.03	NA
40 ng/µL	970	NA	3.03	NA

Figure 3. A 1,000 bp DNA fragment was analyzed on A) the Agilent Bioanalyzer system with the DNA 1000 kit and B) the Agilent Fragment Analyzer system with the Small Fragment kit for comparison. Example electropherograms of the fragment at a concentration of 5 ng/uL are shown. C) The reported size of the 1,000 bp fragment across a serial dilution was compared between the two systems. The average size and percent error are shown. n = 3 replicates per concentration.

Quantification of DNA fragments

To compare the quantification of fragments between each system, the three fragments were analyzed at the same concentration in triplicate with both the DNA 1000 kit for the Bioanalyzer and the Small Fragment kit for the Fragment Analyzer. As shown in Figure 4, the reported concentrations of the 150, 500, and 1,000 bp fragments were similar between the two instruments. The 150 bp fragment displayed an average concentration of 1.35 ng/µL on the Bioanalyzer and 1.23 ng/µL on the Fragment Analyzer, a 9.8% difference between the two systems. The 500 bp fragment quantified at 1.41 ng/µL on the Bioanalyzer and 1.49 ng/µL on the Fragment Analyzer, with a percent difference of 5.1%. The 1,000 bp fragment showed the closest correlation, at 1.10 ng/µL on the Bioanalyzer and 1.08 ng/µL on the Fragment Analyzer, only a 2.1% difference in quantification between the two systems.

Conclusion

In this technical overview, the Agilent 2100 Bioanalyzer system and Agilent Fragment Analyzer systems were compared using similar analysis kits for each system. Both the DNA 1000 kit for the Bioanalyzer and the Small Fragment kit for the Fragment Analyzer yielded comparable sizing and quantification data across a variety of small DNA fragments. Each sample tested exhibited very similar sizing and quantification between the instruments and displayed an excellent sizing accuracy. Additionally, the reported size was not affected by concentration on either system. Any of the DNA fragment analysis kits for the Bioanalyzer and Fragment Analyzer can be used for quality control of small fragment samples, allowing researchers to seamlessly move between instruments and have confidence in their sample analyses.

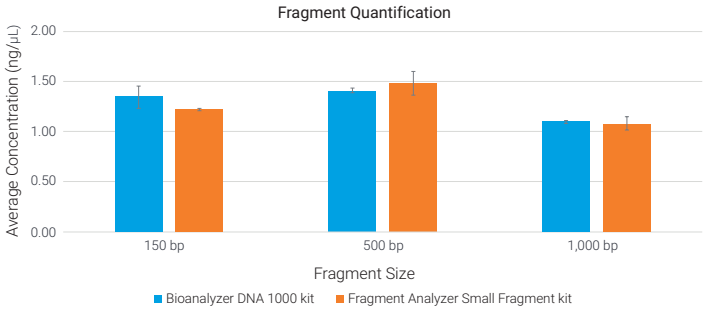


Figure 4. The 150, 500, and 1,000 bp DNA fragments were analyzed at a nominal concentration of 1.25 ng/µl on the Agilent Bioanalyzer system with the DNA 1000 kit and the Agilent Fragment Analyzer system with the Small Fragment kit for comparison. The average reported concentration of each fragment is shown. Error bars represent standard deviation. n = 3 replicates per system.

References

1. Agilent DNA Kits for the Agilent 2100 Bioanalyzer System. *Agilent Technologies data sheet*, publication number 5991-7888EN, **2019**.
2. Agilent Fragment Analyzer Consumables. *Agilent Technologies data sheet*, publication number 5994-0407EN, **2021**.
3. Comparison of the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer Systems. *Agilent Technologies technical overview*, publication number 5994-3650EN, **2021**.
4. Separation Resolution Capabilities of the Agilent ZAG 135 and 110 dsDNA Kits and the Agilent Fragment Analyzer dsDNA 935 and 915 Reagent Kits. *Agilent Technologies technical overview*, publication number 5994-3638, **2021**.

www.agilent.com.genomics/automated-electrophoresis

PR7000-8781

Published in the USA, June 20, 2022
5994-5024EN

Agilent
Trusted Answers