Technical Overview

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DNA Fragment Sizing with the Agilent ZAG DNA Analyzer System

Introduction

The Agilent ZAG DNA Analyzer system is a parallel capillary electrophoresis system for high-throughput sizing of DNA fragments. Ideal for PCR amplicons and other DNA fragments, there are four ZAG dsDNA kits utilized on the ZAG DNA Analyzer system that cover a sizing range from 35 to 20,000 bp. The ZAG 105 dsDNA kit (1 - 500 bp) provides sizing for small DNA fragments from 35 to 500 bp. The ZAG 110 dsDNA kit (35 - 5000 bp) has a midsize range of 35 to 5,000 bp, and the ZAG 130 dsDNA kit (75 - 20000 bp) covers the largest sizing range of 75 to 20,000 bp. The ZAG 135 dsDNA kit (1 - 1500 bp) is designed for extremely high throughput labs with fragments ranging from 100 to 1,500 bp. The ZAG 135 dsDNA kit utilizes a higher voltage during sample separation which shortens the run time and enables a higher sample throughput over time. Common applications of the ZAG dsDNA kits include analysis of PCR amplicons and restriction digests, genotyping, and microsatellite analysis.

The ZAG DNA Analyzer system analyzes an entire 96-well plate at one time. The instrument holds nine 96-well plates that can be set up in the instrument to run continuously. Total separation times for the kits range from 30 minutes on the ZAG 135 dsDNA kit to one hour on the ZAG 105 dsDNA kit for 96 samples.

This Technical Overview focuses on the performance of the ZAG DNA Analyzer system with the four available kits: ZAG 105 dsDNA kit (1 - 500), ZAG 110 dsDNA kit (35 - 5000 bp), ZAG 130 dsDNA kit (75 - 20000 bp), and ZAG 135 dsDNA kit (1 - 1500 bp). Fragment analysis on each kit explores the sensitivity, accuracy, and precision of sizing. Comparison of the sizing of the fragments between the kits was performed.

Analytical Specifications

The analytical specifications of the ZAG DNA Analyzer dsDNA kits are summarized in Table 1.

Table 1. Specifications of the ZAG DNA Analyzer system dsDNA kits.

ZAG DNA Analyzer System dsDNA Kits				
	Concentration range	Sizing range	Sizing accuracy[1] (%error)	Sizing precision[1] (%CV)
ZAG 105 dsDNA kit (1 - 500 bp)	0.5 to 50 ng/µL	35 to 500 bp	± 5%	2%
ZAG 110 dsDNA kit (35 - 5000 bp)	0.5 to 50 ng/µL	35 to 5,000 bp	± 5%	2%
ZAG 130 dsDNA kit (75 -20000 bp)	0.5 to 50 ng/µL	75 to 20,000 bp	± 10%	5%
ZAG 135 dsDNA kit (1 - 1500 bp)	0.5 to 50 ng/µL	100 to 1,500 bp	± 5%	2%

[1]Results using DNA Ladder or DNA Fragment standards initially prepared in 1X buffer.

Experimental

Methods

A 300 bp fragment (Thermo Fisher, p/n SM1621) was analyzed on all four ZAG kits, including ZAG 105 dsDNA kit (1-500 bp) (p/n ZAG-105-5000), ZAG 110 dsDNA kit (35 - 5000 bp) (p/n ZAG-110-5000), ZAG 130 dsDNA kit (75 - 20000 bp) (p/n ZAG-130-5000), and ZAG 135 dsDNA kit (1 - 1500 bp) (p/n ZAG-135-5000). A 1,000 bp fragment (Thermo Fisher, p/n SM1671) was analyzed on the ZAG 110, 130 and 135 dsDNA kits. A 3,000 bp fragment (Thermo Fisher, p/n SM1711) was analyzed on the ZAG 110 and 130 dsDNA kits.

Fragment analysis

To evaluate the reproducibility of the ZAG DNA Analyzer system, DNA fragment dilution series covering the concentration range of the four ZAG dsDNA kit (50 to 0.5 ng/µL) was performed on the ZAG DNA Analyzer system. A stock solution of approximately 50 ng/µL in 1X TE for each fragment was confirmed with the Qubit dsDNA HS Assay kit (Thermo Fisher, p/n Q32851) on the Qubit 4.0 followed by serial dilution in 1X TE down to about 0.4 ng/µL. Each fragment was analyzed with nine consecutive separations.

Results and discussion

ZAG 105 dsDNA kit

The ZAG 105 dsDNA kit is tailored toward smaller DNA fragments from 35 to 500 bp. To demonstrate the consistency of fragment sizing on the ZAG 105 dsDNA kit, a 300 bp fragment was diluted over the concentration range of the kit from 47 to 0.4 ng/µL. The average size throughout the dilution series was 300 bp with precision and accuracy of 0.17% CV and –0.16% error, respectively (Figure 1). Concentration of the fragment did not affect the sizing of the 300 bp fragment.

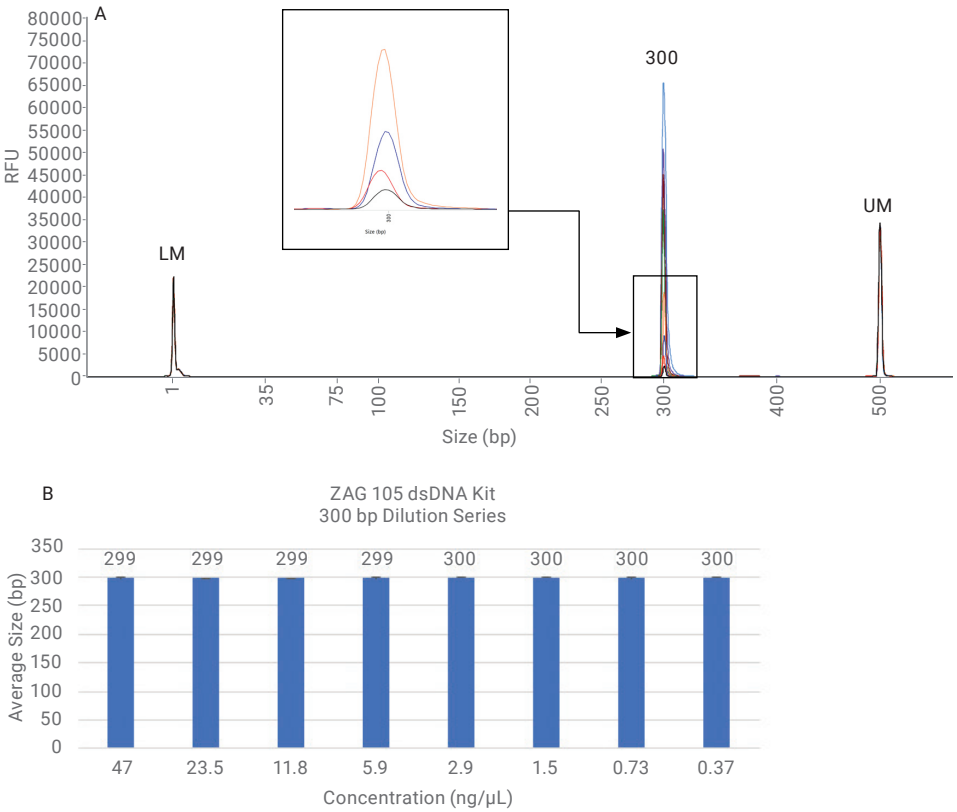


Figure 1. Dilution series (47 to 0.4 ng/µL) of a 300 bp fragment on the ZAG DNA Analyzer system with the ZAG 105 dsDNA kit. (A) Electropherogram overlay, average size over dilution series. (B) Average size at each concentration. LM = lower marker; UM = upper marker, n = 9.

ZAG 110 dsDNA kit

The ZAG 110 dsDNA kit sizes fragments from 35 to 5,000 bp. To demonstrate the consistency of fragment sizing on the ZAG 110 dsDNA kit, the 300, 1,000, and 3,000 bp fragments were diluted over the concentration range of the kit from 54 to 0.4 ng/µL. The average size throughout the dilution series of the fragments was 299, 999, and 2,947 bp, respectively (Figure 2). The 300 bp fragment displayed precision and accuracy of 0.4% CV and –0.4% error, along with the 1,000 bp fragment, which showed precision of 0.4% CV and accuracy of –0.1% error. The 3,000 bp followed the same trend with a precision of 1.9% CV and accuracy of 1.8% error. Concentration of the fragments did not affect the sizing of the 300, 1,000, or 3,000 bp fragments.

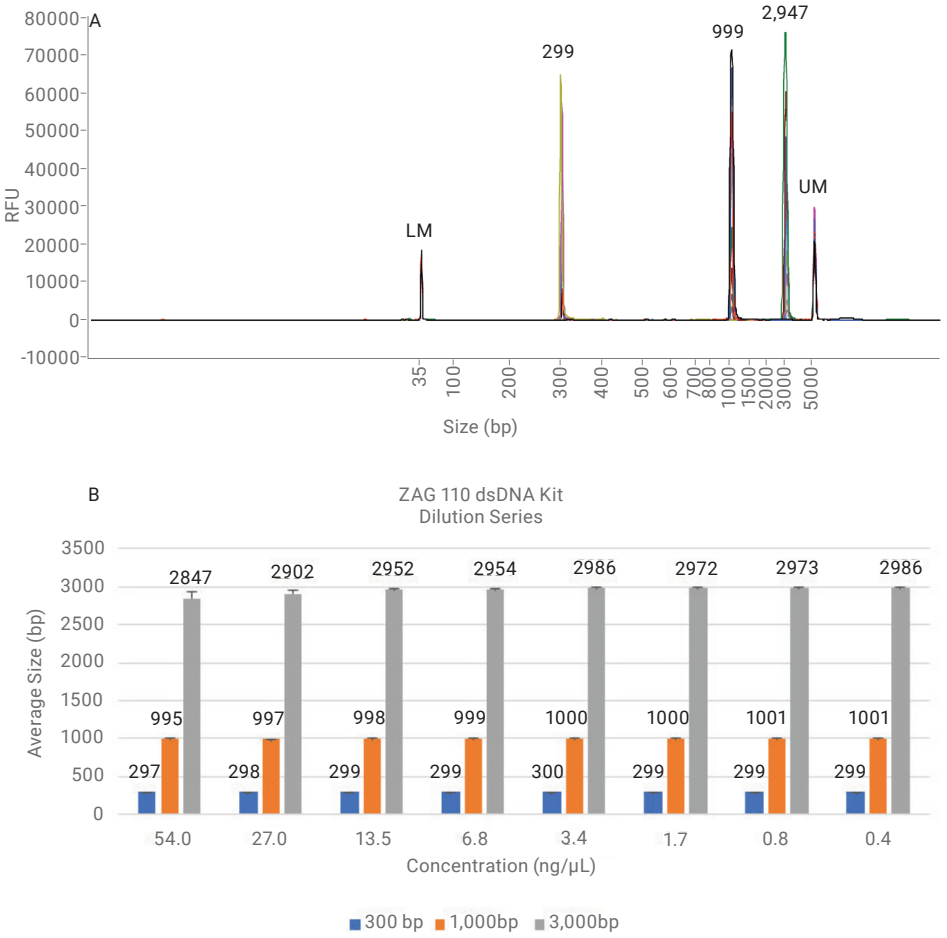


Figure 2. Dilution series (54 to 0.4 ng/µL) of 300, 1,000, and 3,000 bp fragments on the ZAG DNA Analyzer system with the ZAG 110 dsDNA kit. (A) Electropherogram overlay, average size over dilution series. (B) Average size at each concentration. LM = lower marker; UM = upper marker, n = 9.

ZAG 130 dsDNA kit

The ZAG 130 dsDNA kit has the largest fragment sizing range of 35 to 20,000 bp. To demonstrate the consistency of fragment sizing on the ZAG 130 dsDNA kit, the 300, 1,000, and 3,000 bp fragments were diluted over the concentration range of the kit from 54 to 0.4 ng/µL. The average size throughout the dilution series of the fragments was 298, 988, and 2,901 bp, respectively (Figure 3). The 300 bp fragment displayed precision and accuracy of 0.7% CV and –0.6% error, along with the 1,000 bp fragment which gave a precision of 0.9% CV and accuracy of –1.2% error. The 3,000 bp followed the same trend with a precision of 3.5% CV and accuracy of 3.3% error, well within the kit specifications for accuracy and precision (Table 1). Concentration of the fragments did not affect the sizing of the 300, 1,000 or 3,000 bp fragments.

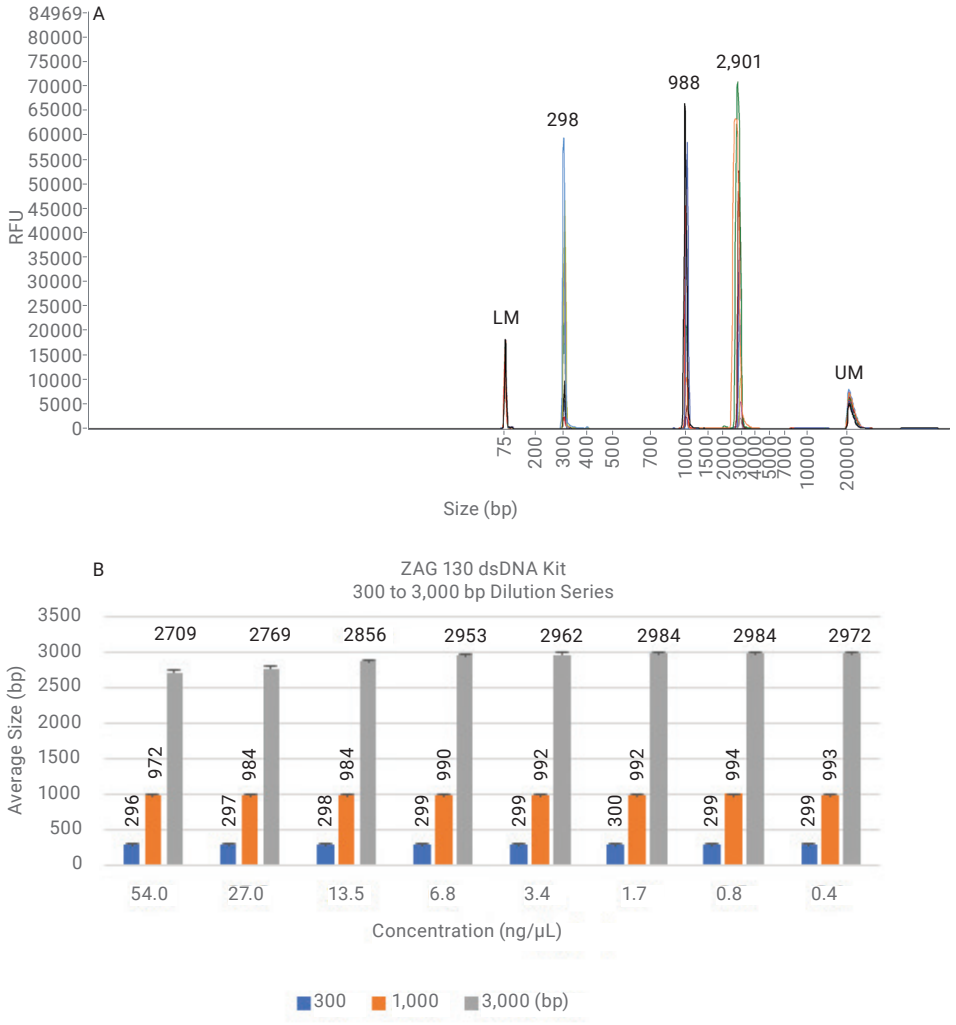


Figure 3. Dilution series (54 to 0.4 ng/µL) of 300, 1,000, and 3,000 bp fragments on the ZAG DNA Analyzer system with the ZAG 130 dsDNA kit. (A) Electropherogram overlay, average size over dilution series. (B) Average size at each concentration. LM = lower marker; UM = upper marker, n = 9.

ZAG 135 dsDNA kit

The ZAG 135 dsDNA kit sizes fragments from 100 to 1,500 bp with complete separations in approximately 20 minutes for rapid sample analysis. To demonstrate the consistency of fragment sizing on the ZAG 135 dsDNA kit, the 300 and 1,000 bp fragments were diluted over the concentration range of the kit from 54 to 0.4 ng/µL. The average size throughout the dilution series of the fragments was 299 and 997 bp, respectively (Figure 4). The 300 bp fragment displayed precision and accuracy of 0.5% CV and –0.5% error, along with the 1,000 bp fragment, which showed a precision of 0.4% CV and accuracy of –0.3% error. Concentration of the fragments did not affect the sizing of the 300 or 1,000 bp fragments.

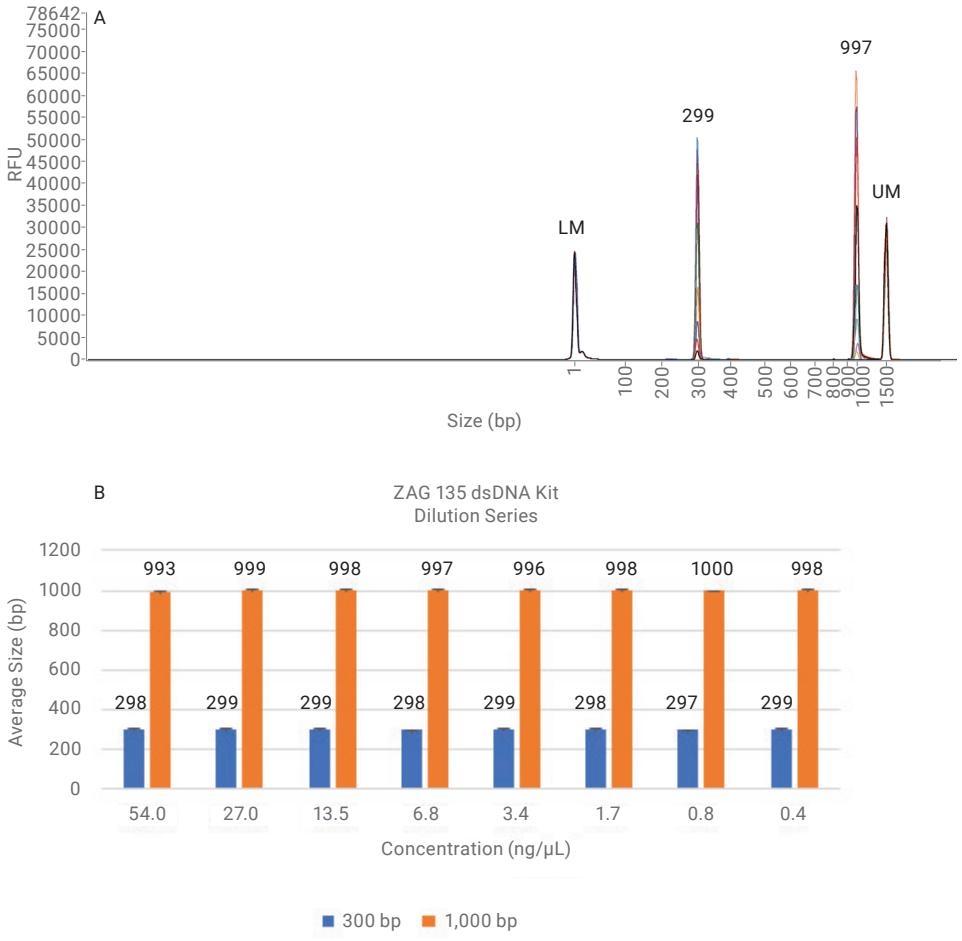
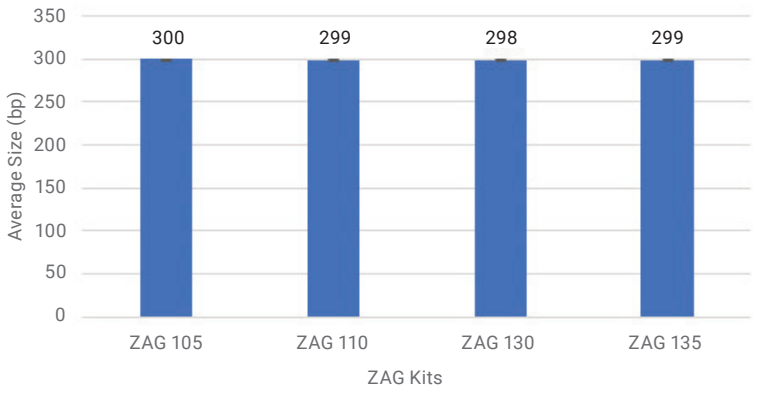


Figure 4. Dilution series (54 to 0.4 ng/µL) of 300 and 1,000 bp fragments on the ZAG DNA Analyzer system with the ZAG 135 dsDNA kit. (A) Electropherogram overlay, average size over dilution series. (B) Average size at each concentration. LM = lower marker; UM = upper marker, n = 9.

Comparison between ZAG dsDNA kits

Sizing of fragments between the four ZAG dsDNA kits was investigated to demonstrate reliability of fragment sizing regardless of the kit. Sizing of the 300 bp fragment throughout the dilution series varied from 298 to 300 bp, with excellent precision and accuracy across all four kits (Figure 5). The 1,000 bp fragment sizing varied from 988 to 999 bp, with high precision and accuracy across the ZAG 110, 130, and 135 dsDNA kits (Figure 6).

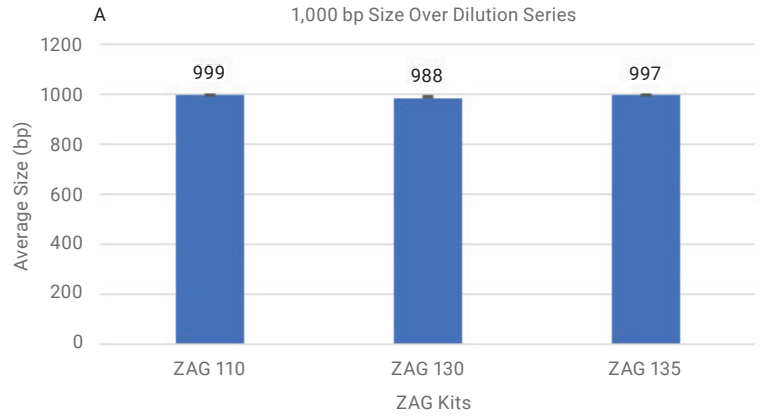
A



B	300 bp Size Throughput Dilution Series with ZAG dsDNA Kits			
	ZAG 105	**ZAG 110**	**ZAG 130**	**ZAG 135**
Average size* (bp)	300	299	298	299
Standard deviation	0.5	1.2	2.0	1.5
Precision %CV	0.17%	0.40%	0.67%	0.49%
Accuracy %error	- 0.16%	- 0.40%	- 0.56%	- 0.48%

Figure 5. Comparison of the average size of a 300 bp fragment throughout a dilution series (54 to 0.4 ng/µL) on the ZAG DNA Analyzer system with all four ZAG dsDNA kits. (A) Average size for each kit. (B) Statistics including average size, standard deviation, precision, and accuracy. *n = 9.

A



B	1,000 bp Size Throughput Dilution Series with ZAG dsDNA Kits		
	ZAG 110	**ZAG 130**	**ZAG 135**
Average size* (bp)	999	988	997
Standard deviation	3.8	8.9	4.4
Precision %CV	0.38%	0.91%	0.44%
Accuracy %error	- 0.10%	- 1.23%	- 0.26%

Figure 6. Comparison of the average size of a 1,000 bp fragment throughout a dilution series (54 to 0.4 ng/µL) on the ZAG DNA Analyzer system with three ZAG dsDNA kits. (A) Average size for each kit. (B) Statistics including average size, standard deviation, precision, and accuracy. *n = 9.

The 1,000 bp fragment is outside the sizing range of 35 to 500 bp for the ZAG 105 dsDNA kit and therefore was not included in the comparison. Sizing of the 3,000 bp fragment throughout the dilution series varied from 2,947 to 2,901 bp, with excellent precision and accuracy for the ZAG 110 and 130 kits, respectively (Figure 7). The 3,000 bp fragment is outside the sizing range of the ZAG 105 and 135 dsDNA kits and therefore was not included in the comparison. In general, reliable fragment sizing can be achieved on any of the four ZAG dsDNA kits if the fragment falls within the sizing range of that particular kit.

Conclusion

Sizing reproducibility is important for DNA fragments and PCR amplicons. All ZAG dsDNA kits provided reliable sizing of DNA fragments with excellent precision and accuracy. Comparison of various fragment sizes between the ZAG dsDNA kits was consistent. Reliable fragment sizing can be achieved on any of the ZAG dsDNA kits if the fragment falls within the sizing range of that particular kit.

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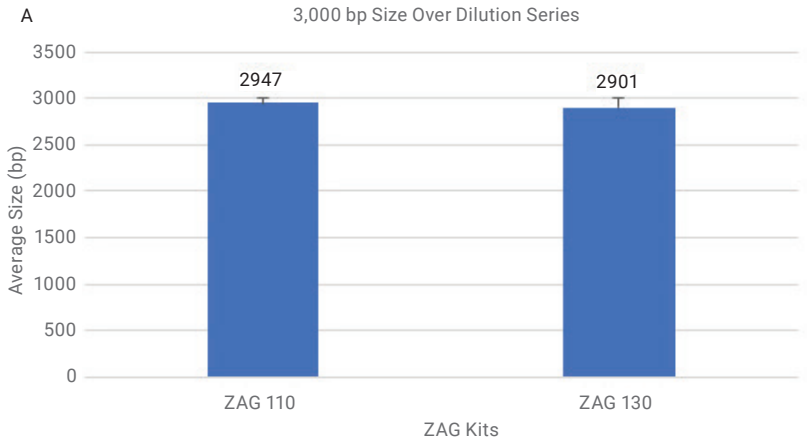

B

3,000 bp Size Throughput Dilution Series with ZAG dsDNA Kits

	ZAG 110	ZAG 130
Average size* (bp)	2,947	2,901
Standard deviation	55.2	102.6
Precision %CV	1.9%	3.3%
Accuracy %error	1.8%	3.5%

Figure 7. Comparison of the average size of a 3,000 bp fragment throughout a dilution series (50 to 0.4 ng/µL) on the ZAG DNA Analyzer system with two ZAG dsDNA kits. (A) Average size for each kit. (B) Statistics including average size, standard deviation, precision, and accuracy. *n = 9.

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