

# Comparison of the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer Systems

## Introduction

DNA fragment analysis is a versatile tool for any molecular biology laboratory, enabling a multitude of applications<sup>1,2,3</sup> including genotyping, PCR amplicon analysis, microsatellite detection and restriction digest assessment. Typically, these analyses are performed using agarose gel electrophoresis. However, this process can be time consuming, labor-intensive and may lack the accuracy and precision often required for higher-throughput laboratories or applications requiring higher sensitivity. To help address these issues, the Agilent automated electrophoresis portfolio includes a suite of instruments that utilize capillary electrophoresis for the analysis of nucleic acids. These instruments include the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer systems.

The ZAG and Fragment Analyzer systems use parallel capillary electrophoresis and fluorescent detection to separate and visualize nucleic acids. Specially formulated gels, markers and ladders are designed for each instrument to enable analysis of a large range of sample sizes and applications. The associated reagent kits and consumables for the ZAG platform are designed for the qualitative analysis of DNA. The Fragment Analyzer system offers reagents and consumables for the qualitative and quantitative analysis of DNA and RNA specimens. Qualitative and quantitative kits both offer accurate and precise sizing, while the quantitative kits also provide the added benefit of reliable concentration analysis. Both instruments operate similarly, with a gel and fluorescent dye mix being pumped through the capillaries. The sample is injected and separates as it proceeds past the capillary window, and a fluorescent light excites the intercalated samples. The emitted fluorescence from the dye bound to the sample is recorded by a CCD detector. Thus, the ZAG and Fragment Analyzer enable real-time detection of nucleic acid separations that are captured as electropherograms.

While the technical principles of the electrophoretic systems are fundamentally equivalent, a number of specific factors differentiate the systems from each other (Table 1). The ZAG DNA Analyzer is a high-throughput instrument, with the ability to hold up to nine, 96-well sample plates and analyze 96 samples at one time. The high-throughput nature and streamlined methods of the ZAG allow for faster run times than the Fragment Analyzer. The ZAG is used for DNA analysis and offers a variety of qualitative kits that cover a broad range of fragment sizes and applications. The Fragment Analyzer product line includes two models offering two throughput ranges. The Agilent 5200 Fragment Analyzer is capable of analyzing 12 samples at a time, while the Agilent 5300 Fragment Analyzer is compatible with an interchangeable array, making it possible to analyze either 48 or 96 samples

simultaneously. Both models can hold up to three, 96-well plates at a time. The Fragment Analyzer systems are compatible with both quantitative and qualitative nucleic acid analysis kits. Analysis of the data generated by both the ZAG and the Fragment Analyzer is easily automated with the Agilent ProSize data analysis software. The electrophoretic results are available in a digital format with both a gel-like image and an electropherogram trace of each sample, along with a peak table that displays size, concentration, percent concentration and molarity. The reported concentration and molarity are considered relative for the qualitative kits, while the quantitative kits provide accurate concentration data. The software also comes with many advanced features that allow for a user to individualize their analysis needs. For example, a flag analysis tool can be

used to quickly determine if a sample fits certain criteria. Flagging can be useful for identifying the presence or absence of fragments of interest in applications such as genotyping<sup>2</sup>.

The qualitative DNA analysis kits offered by the ZAG have the same specifications as the qualitative kits for the Fragment Analyzer (Table 2). Different gels, markers and electrophoresis methods specialized for each kit provide coverage over a broad sizing range from 35–20,000 bp. Depending on the kit and the array length used, separations can be completed in as little as 20 minutes, and can achieve up to a 3 bp resolution for fragments approximately 300 bp or smaller. In this technical overview, the same samples were analyzed on the ZAG and the Fragment Analyzer with a short array to demonstrate the equivalency of the qualitative kits on the systems.

**Table 1.** Comparison of the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer systems.

	ZAG DNA Analyzer	Fragment Analyzer
Nucleic Acid Types	DNA fragments	DNA and RNA smears and fragments
Analysis Kits Available	Qualitative (sizing)	Qualitative (sizing); Quantitative (concentration and sizing)
Number of Samples Per Run	96	12 (5200 model); 48 or 96 (5300 model)
Capillary Array Lengths	Short (33 cm) or long (55 cm)	Ultra-short (22 cm) <sup>*</sup> , short (33 cm), or long (55 cm)
Sample Tray Capacity	9	3

<sup>\*</sup>For use with the 5200 Fragment Analyzer only

**Table 2.** Qualitative kit specifications<sup>\*</sup> of the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer systems.

ZAG Kit	Fragment Analyzer Kit	Sizing Range	Concentration Range	Sizing Precision (%CV)	Sizing Accuracy (% error)	Separation Resolution	Separation Time
ZAG 105 dsDNA Kit (1–500 bp)	dsDNA 905 Reagent Kit (1–500 bp)	35–500 bp	0.5–50 ng/μL	2%	±5%	5–10 bp @ 300 bp	60 min
ZAG 110 dsDNA Kit (35–5000 bp)	dsDNA 915 Reagent Kit (35–5000 bp)	35–5,000 bp	0.5–50 ng/μL	2%	±5%	35–100 bp ≤ 10%; 100–1,000 ≤ 5%; 1,000–5,000 ≤ 10%	50 min
ZAG 130 dsDNA Kit (75–20000 bp)	dsDNA 930 Reagent Kit (75–20000 bp)	75–20,000 bp	0.5–50 ng/μL	5%	±10%	75–1,500 bp ≤ 10%; 1,500–20,000 bp ≤ 15%	30 min
ZAG 135 dsDNA Kit (1–1500 bp)	dsDNA 935 Reagent Kit (1–1500 bp)	100–1,500 bp	0.5–50 ng/μL	2%	±5%	100–1,500 bp ≤ 10%	20 min

<sup>\*</sup>with 33 cm short capillary array

## Experimental

### Fragment preparation: gBlocks

Double-stranded DNA gene fragments, or gBlocks (Integrated DNA Technologies), were designed such that each contained blunt end restriction sites (*HaeIII* and *EcoRV*), allowing for the creation of various known-sized products differing by a single base pair at around 300, 200 and 100 bp. Each gBlock was amplified using Phusion DNA polymerase (Thermo Fisher Scientific, #F530S). Digestion of the 200 ng PCR product was achieved with *HaeIII* (Thermo Fisher Scientific, #FD0154) or *EcoRV* (Thermo Fisher Scientific, #FD0303) according to manufacturer instructions, generating fragments of sizes 101–105, 201–205 and 306–311 bp. To clean up the digested fragments, digests were separated on a 2% agarose gel and the gel fragments were purified using the Zymoclean gel DNA recovery kit (Zymo Research, #D400). The purified fragments were quantified using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, #32854) and diluted to 250 pg/μL using 1x TE.

### Fragment preparation: PCR amplicons

Fragments were prepared from Lambda DNA (Thermo Fisher Scientific, p/n SD0011) with specific primers (IDT, Integrated DNA Technologies) and amplified with Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, p/n F530S) according to manufacturer's instructions. PCR conditions were as follows: initial denaturation (98 °C; 30 seconds), denaturation (98 °C; 10 seconds), annealing (60 °C; 30 seconds),

and extension (72 °C; 15 to 30 seconds per kb) for 30 cycles and final extension (72 °C; 10 minutes). PCR products were digested with FastDigest *MssI* (Thermo Fisher Scientific, p/n FD1344) resulting in blunt end fragments of known size. The digested fragments were separated on a 1% gel and then purified with the Zymoclean Gel DNA Recovery kit (p/n D4007). Fragment concentrations were measured by the Qubit 4.0 using the 1X dsDNA HS Assay kit (Thermo Fisher Scientific, p/n Q33230) and diluted to approximately 8 ng/μL with 1x TE.

### Fragment preparation: NoLimits DNA Fragments

NoLimits DNA Fragments of sizes 500 (p/n SM1641), 1,000 (p/n SM1671), 3,000 (p/n SM1711), 5,000 (p/n SM1731) and 10,000 (p/n SM1751) bp were obtained from Thermo Fisher Scientific. Each fragment concentration was measured by the Qubit 4.0 using the 1X dsDNA HS Assay kit (Thermo Fisher Scientific, p/n Q33230) and diluted to approximately 8 ng/μL with 1x TE.

### Fragment size analysis

The fragments of various lengths were analyzed on the ZAG with the Agilent FA/ZAG 96-Capillary Array Short, 33 cm (short array) (p/n A2300-9650-3355), and with the Fragment Analyzer systems equipped with short arrays for comparison (Table 3). The gBlock samples were analyzed on the 5200 Fragment Analyzer due to limited sample availability. PCR amplicons and No Limits Fragments were analyzed using the 5300 Fragment Analyzer model to enable multiple replicates. Comparable results can be achieved between the 5200, 5300 and 5400 Fragment Analyzer systems. The 5200 Fragment Analyzer used the FA 12-Capillary Array Short, 33 cm (p/n A2300-1250-3355), while the 5300 Fragment Analyzer was also equipped with the FA/ZAG 96-Capillary Array Short, 33 cm. An ultra-short capillary array may be used on the Fragment Analyzer for faster run times. Long capillary arrays are compatible with both the ZAG and the Fragment Analyzers for enhanced separation resolution.

**Table 3.** Fragment types and sizes analyzed on the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer systems

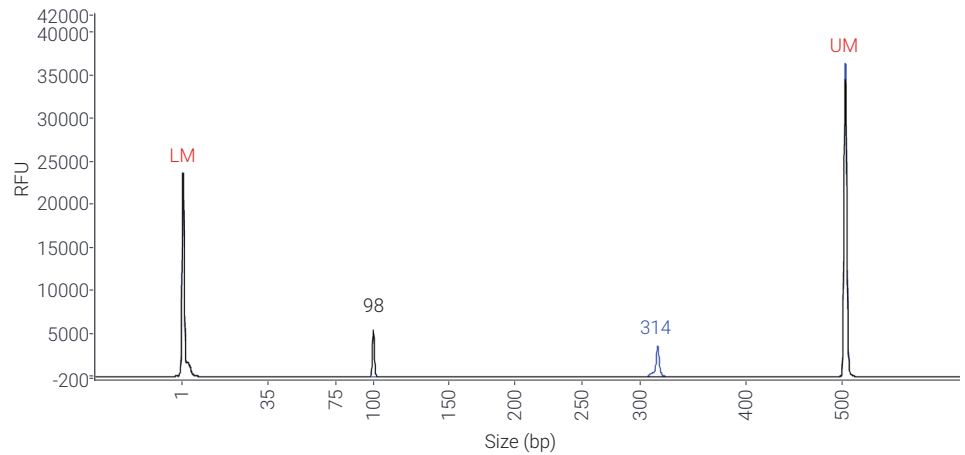
ZAG Kit	Fragment Analyzer Kit	Sample Type Analyzed	Fragment Sizes Analyzed (bp)
ZAG 105 dsDNA Kit (1–500 bp)	dsDNA 905 Reagent Kit (1–500 bp)	gBlock fragments	101–105, 201–205 and 306–311
ZAG 110 dsDNA Kit (35–5000 bp)	dsDNA 915 Reagent Kit (35–5000 bp)	PCR amplicons	400, 425, 1,000, 1,050, 1,100, 3,000 and 4,000
ZAG 130 dsDNA Kit (1–20000 bp)	dsDNA 930 Reagent Kit (1–20000 bp)	NoLimits Fragments	500, 1,000, 2,000, 5,000 and 10,000
ZAG 135 dsDNA Kit (1–1500 bp)	dsDNA 935 Reagent Kit (1–1500 bp)	PCR amplicons	400, 425, 1,000, 1,050, 1,100, 1,350 and 1,425

## Results and discussion

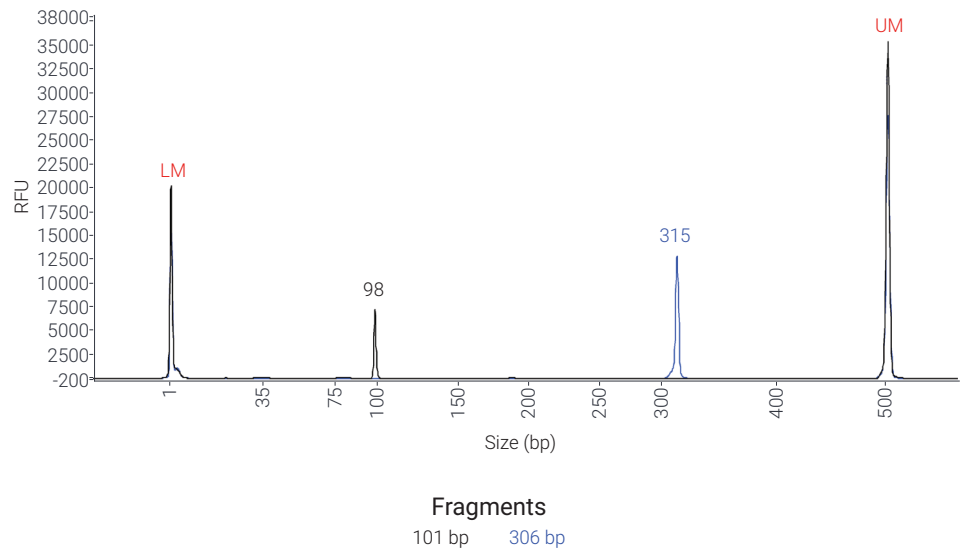
### ZAG 105 and Fragment Analyzer 905 kit comparison

The ZAG 105 and Fragment Analyzer 905 kits provide sizing for small DNA fragments from 35–500 bp. It has previously been shown that each kit, when ran with a long capillary array, can resolve fragments that are 3 bp apart when the fragments are approximately 300 bp or smaller<sup>4,5</sup>. To demonstrate the sizing precision (%CV) and accuracy (percent error) between the ZAG and the Fragment Analyzer, various fragments between the sizes of 100 and 300 bp were analyzed on each system with their corresponding analysis kit and a short capillary array. An electropherogram overlay of a 101 and 306 bp fragment is shown for each instrument to show the similarity of the systems (Figure 1). Each fragment demonstrated excellent precision and accuracy, with both the %CV and the percent error well below each kits' specifications of 2 %CV and  $\pm 5\%$  error (Table 4). Comparison of sizing between the two kits displayed an  $R^2$  value of 0.9999, indicating a high correlation between the two systems (Figure 2). An  $R^2$  value of 1 would mean that the sizes match perfectly between the two instruments.

A. ZAG 105 kit



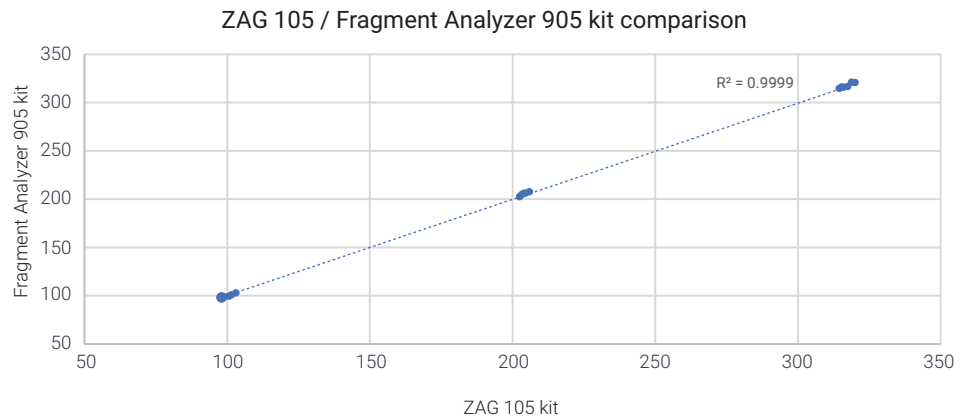
B. Fragment Analyzer 905 kit



**Figure 1.** DNA fragments of known sizes were analyzed on (A) the Agilent ZAG DNA Analyzer system with the ZAG 105 dsDNA kit (1–500 bp) and (B) the Agilent 5200 Fragment Analyzer system with the dsDNA 905 Reagent kit (1–500 bp). Electropherogram overlays display the 101 (black) and 306 (blue) bp fragments. LM: lower marker; UM: upper marker.

**Table 4.** Comparison of the average size of DNA fragments analyzed on the Agilent ZAG DNA Analyzer system with the ZAG 105 dsDNA kit (1–500 bp) (n=9) and the Agilent 5200 Fragment Analyzer system with the dsDNA 905 Reagent kit (1–500 bp) (n=3). Precision and accuracy for each instrument are indicated by the %CV and percent error.

Average fragment size comparison						
Theoretical Size (bp)	ZAG 105			Fragment Analyzer 905		
	Average Size (bp)	%CV	%Error	Average Size (bp)	%CV	%Error
101	98	0.00	2.97	98	0.0	-2.97
102	99	0.00	2.94	99	0.0	-2.94
103	101	0.50	2.27	100	0.6	-3.23
104	101	0.52	2.46	101	0.0	-2.89
105	103	0.00	1.90	103	0.0	-1.91
201	202	0.26	-0.72	203	0.28	0.83
202	203	0.16	-0.55	205	0.28	1.32
203	204	0.22	-0.38	206	0.49	1.48
204	205	0.26	-0.27	206	0.28	1.14
205	206	0.16	-0.43	208	0.28	1.30
306	314	0.17	-2.76	315	0.60	2.86
307	315	0.16	-2.71	316	0.40	3.01
308	316	0.11	-2.56	316	0.93	2.60
309	317	0.17	-2.73	317	1.01	2.51
310	319	0.14	-2.83	321	0.16	3.63
311	320	0.00	-2.89	321	0.30	3.14

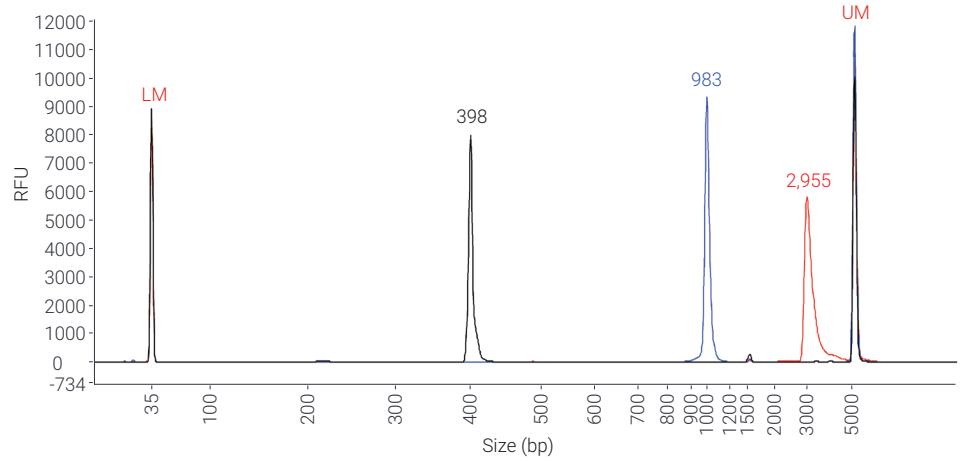


**Figure 2.** The average size of various DNA fragments as measured on the Agilent ZAG DNA Analyzer system with the ZAG 105 dsDNA kit (1–500 bp) (n=9) exhibits excellent correlation to the sizing from the Agilent 5200 Fragment Analyzer system with the dsDNA 905 Reagent kit (1–500 bp) (n=6).

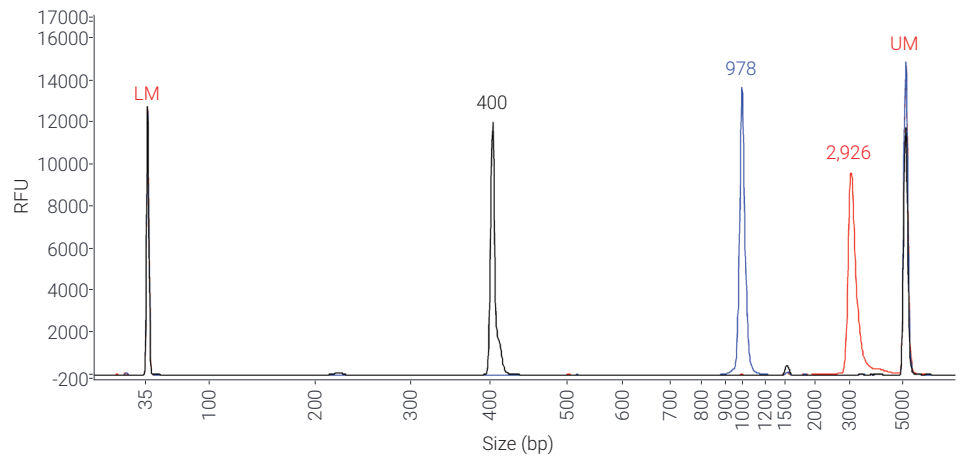
### ZAG 110 and Fragment Analyzer 915 kit comparison

The ZAG 110 and Fragment Analyzer 915 kits are used for sizing of DNA between 35–5,000 bp. To demonstrate the analysis consistency between the two instruments, various PCR fragments ranging in size from 400–4,000 bp were analyzed with both methods. Electropherogram overlays of multiple fragments are shown for the ZAG (Figure 3A) and the Fragment Analyzer (Figure 3B). Across the sizing range, all fragments displayed excellent sizing, with less than 1.4 %CV and 2.7 percent error, well within the kit specifications (Table 5). An  $R^2$  value of 0.9984 indicates excellent sizing correlation between the ZAG 110 and the Fragment Analyzer 915 kits (Figure 4).

#### A. ZAG 110 kit



#### B. Fragment Analyzer 915 kit

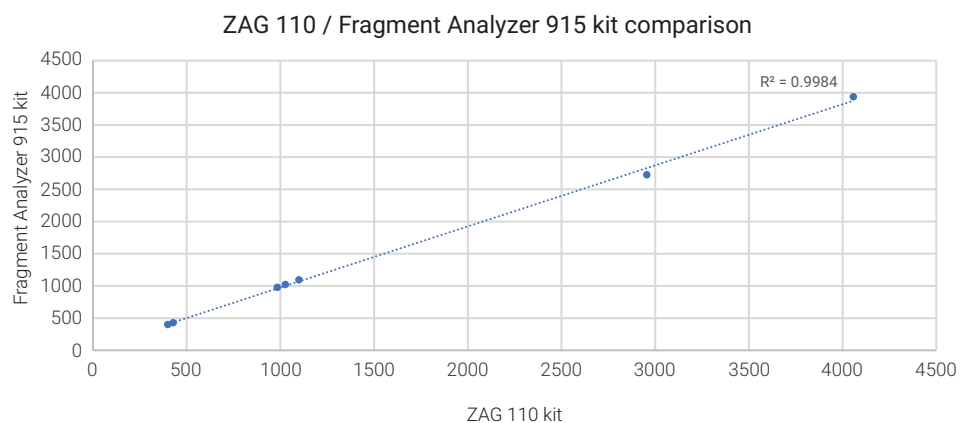


Fragments  
400 bp    1,000 bp    3,000 bp

**Figure 3.** DNA fragments of known sizes were analyzed on (A) the Agilent ZAG DNA Analyzer system with the ZAG 110 dsDNA kit (35–5000 bp) and (B) the Agilent 5300 Fragment Analyzer system with the dsDNA 915 Reagent kit (35–5000 bp). Electropherogram overlays display the 400 (black), 1,000 (blue), and 3,000 (red) bp fragments. LM: lower marker; UM: upper marker.

**Table 5.** Comparison of the average size of DNA fragments analyzed on the Agilent ZAG DNA Analyzer system with the ZAG 110 dsDNA kit (35–5000 bp) (n=6) and the Agilent 5300 Fragment Analyzer system with the dsDNA 915 Reagent kit (35–5000 bp) (n=6). Precision and accuracy for each instrument are indicated by the %CV and percent error.

Average fragment size comparison						
Theoretical Size (bp)	ZAG 110			Fragment Analyzer 915		
	Average Size (bp)	%CV	%Error	Average Size (bp)	%CV	%Error
400	398	0.26	0.42	400	0.20	0.11
425	428	0.25	-0.59	428	0.33	-0.71
1,000	983	0.74	1.68	978	0.80	2.16
1,050	1,026	0.79	2.27	1,022	0.94	2.70
1,100	1,099	0.45	0.11	1,095	0.76	0.44
3,000	2,955	0.68	1.51	2,926	0.51	2.47
4,000	4,057	0.39	-1.43	3,939	1.34	1.54

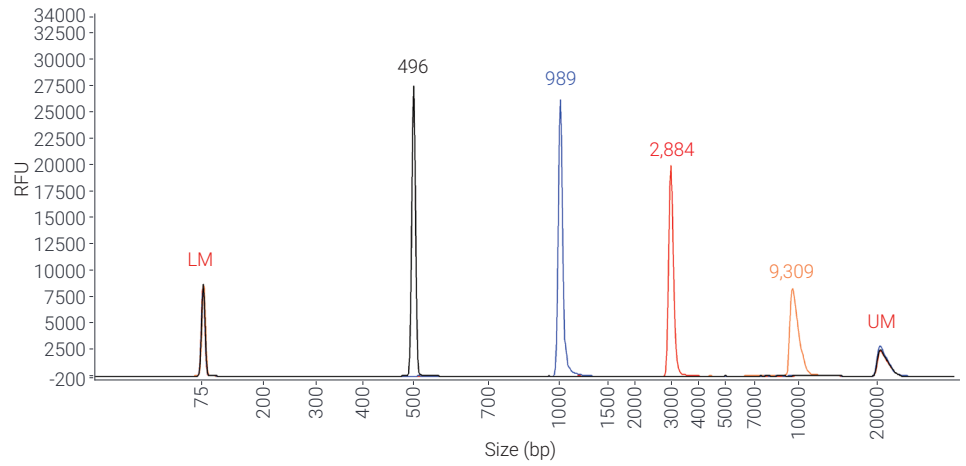


**Figure 4.** The average size of various DNA fragments as measured on the Agilent ZAG DNA Analyzer system with the ZAG 110 dsDNA kit (35–5000 bp) (n=6) exhibits excellent correlation to the sizing from the Agilent 5300 Fragment Analyzer system with the dsDNA 915 Reagent kit (35–5000 bp) (n=6).

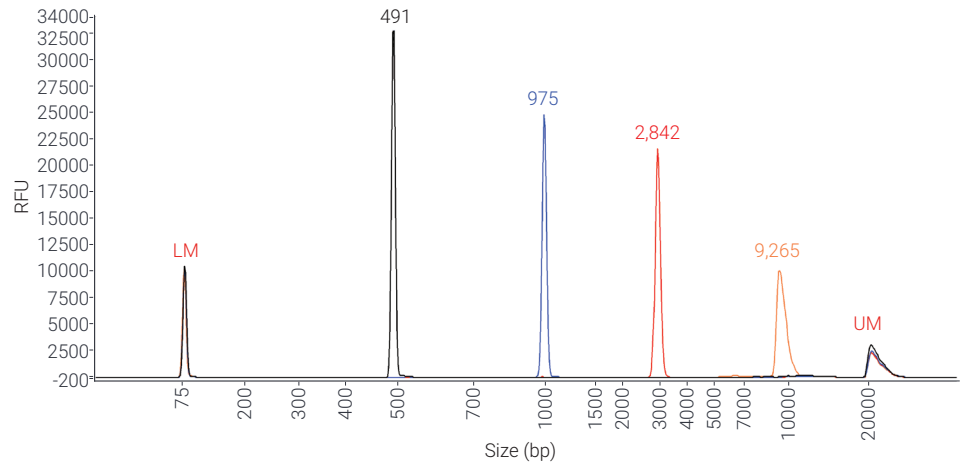
### ZAG 130 and Fragment Analyzer 930 kit comparison

The ZAG 130 and Fragment Analyzer 930 kits have the largest sizing range of the qualitative kits, from 75–20,000 bp. To demonstrate the consistency of sizing between the ZAG and the Fragment Analyzer, a variety of NoLimits DNA Fragments between 500 and 10,000 bp were analyzed on both systems. Overlays of the 500, 1,000, 3,000 and 10,000 bp fragments on both the ZAG and the Fragment Analyzer are shown to highlight the similarity of the electropherograms and fragment sizing across the expansive sizing range of the kits (Figure 5). The sizing provided by the two systems show a perfect linear correlation, as indicated by an  $R^2$  value of 1 (Figure 6). Additionally, each system displayed excellent precision and accuracy across the sizing range, with less than 2 %CV and percent error less than 7.5, well within the kit specifications of 5% CV and 10% error (Table 6).

**A. ZAG 130 kit**



**B. Fragment Analyzer 930 kit**



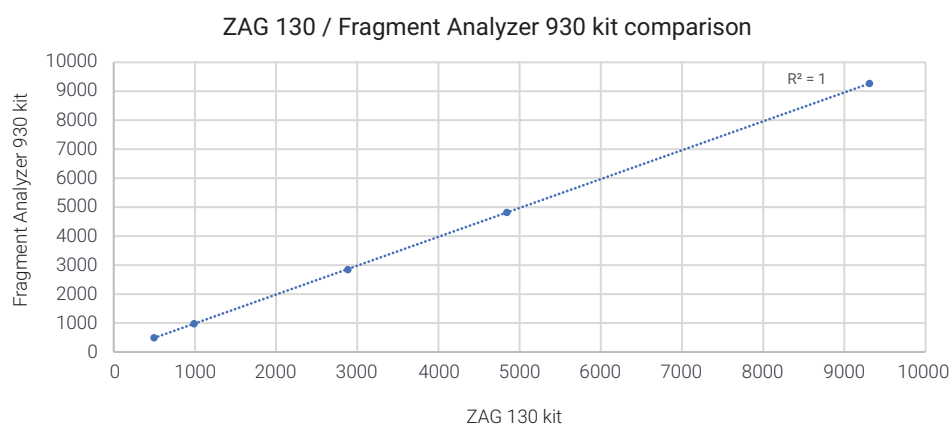
**Fragments**  
 500 bp    1,000 bp    3,000 bp    10,000 bp

**Figure 5.** DNA fragments of known sizes were analyzed on (A) the Agilent ZAG DNA Analyzer system with the ZAG 130 dsDNA Kit (75–20,000 bp) and (B) the Agilent 5300 Fragment Analyzer system with the dsDNA 930 Reagent Kit (75–20,000 bp). Electropherogram overlays display the 500 (black), 1,000 (blue), 3,000 (red) and 10,000 bp fragments (yellow). LM: lower marker; UM: upper marker.



**Table 6.** Comparison of the average size of DNA fragments analyzed on the Agilent ZAG DNA Analyzer system with the ZAG 130 dsDNA Kit (75–20000 bp) (n=24) and the Agilent 5300 Fragment Analyzer system with the dsDNA 930 Reagent Kit (75–20000 bp) (n=24). Precision and accuracy for each instrument are indicated by the %CV and percent error.

Average fragment size comparison						
	ZAG 130			Fragment Analyzer 930		
Theoretical Size (bp)	Average Size (bp)	%CV	%Error	Average Size (bp)	%CV	%Error
500	496	0.83	0.78	491	0.69	1.84
1,000	989	1.09	1.07	975	0.35	2.49
3,000	2,884	1.10	3.86	2,842	1.49	5.25
5,000	4,843	1.10	3.15	4,817	1.47	3.66
10,000	9,309	0.99	6.91	9,265	1.59	7.35

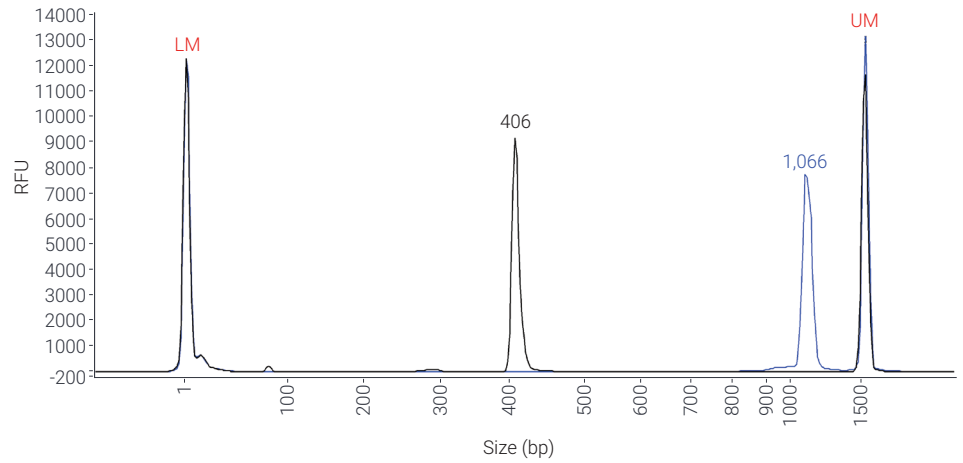


**Figure 6.** The average size of various DNA fragments as measured on the Agilent ZAG DNA Analyzer system with the ZAG 130 dsDNA Kit (75–20000 bp) (n=24) exhibits excellent correlation to the sizing from the Agilent 5300 Fragment Analyzer system with the dsDNA 930 Reagent Kit (75–20000 bp) (n=24).

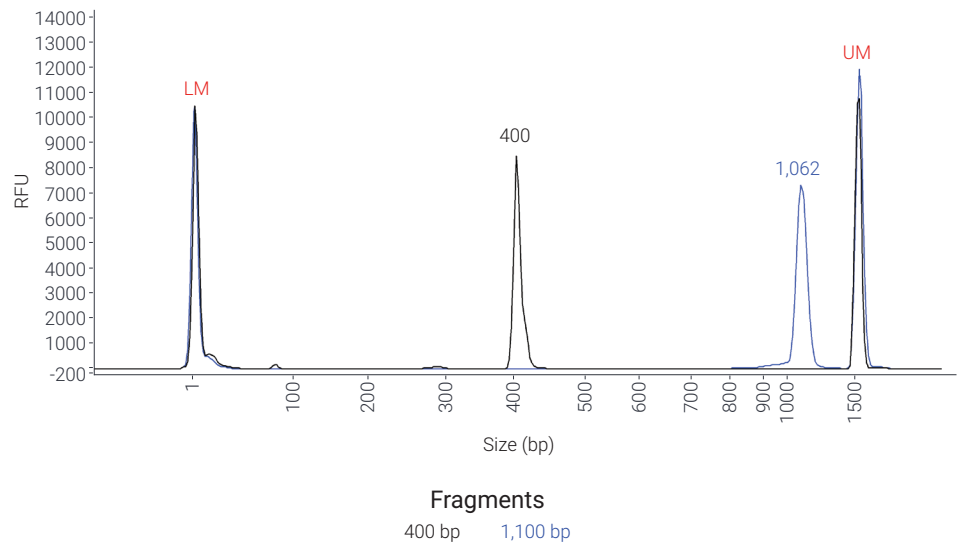
### ZAG 135 and Fragment Analyzer 935 kit comparison

The ZAG 135 and Fragment Analyzer 935 kits separate samples from 100–1,500 bp in 20 minutes. Rapid analysis of up to 96 wells simultaneously makes them the ideal kits for ultrahigh throughput laboratories<sup>2</sup>. PCR amplicons ranging in size from 400–1,425 bp were analyzed on both the ZAG and the Fragment Analyzer, and showed excellent congruity between systems (Figure 7). Both kits displayed accuracy and precision well within the specified %CV and percent error of each kit (Table 7). Additionally, the fragments displayed an excellent sizing correlation between instruments, as indicated by the R<sup>2</sup> value of 0.9999 (Figure 8).

A. ZAG 135 kit



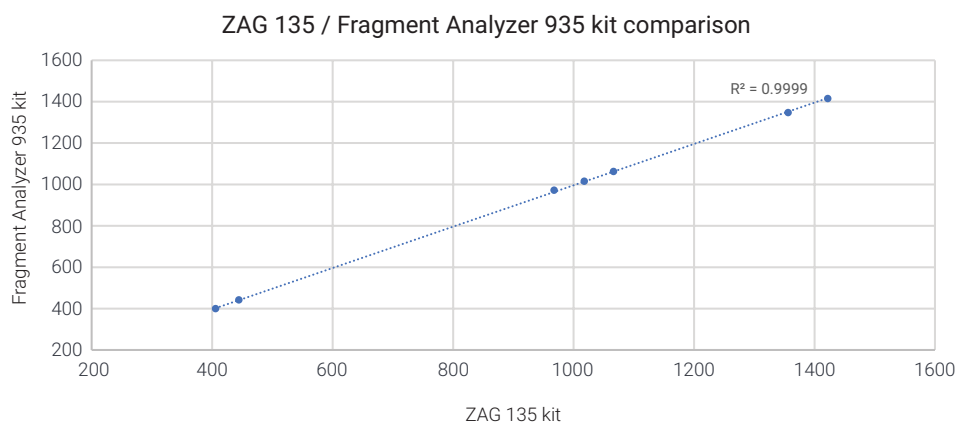
B. Fragment Analyzer 935 kit



**Figure 7.** DNA fragments of known sizes were analyzed on (A) the Agilent ZAG DNA Analyzer system with the ZAG 135 dsDNA kit (1–1500 bp) and (B) the Agilent 5300 Fragment Analyzer system with the dsDNA 935 Reagent kit (1–1500 bp). Electropherogram overlays display the 400 (black) and 1,100 (blue) bp fragments. LM: lower marker; UM: upper marker.

**Table 7.** Comparison of the average size of DNA fragments analyzed on the Agilent ZAG DNA Analyzer system with the ZAG 135 dsDNA kit (1–1500 bp) (n=6) and the Agilent 5300 Fragment Analyzer system with the dsDNA 935 Reagent kit (1–1500 bp) (n=6). Precision and accuracy for each instrument are indicated by the %CV and percent error.

Average fragment size comparison						
Theoretical Size (bp)	ZAG 135			Fragment Analyzer 935		
	Average Size (bp)	%CV	%Error	Average Size (bp)	%CV	%Error
400	406	0.92	-1.42	400	0.31	0.07
425	435	1.04	-2.35	429	0.66	-1.04
1,000	968	1.61	3.25	972	0.73	2.78
1,050	1,018	1.43	3.08	1,015	1.01	3.30
1,100	1,066	0.93	3.08	1,062	1.07	3.42
1,350	1,356	0.70	-0.44	1,347	0.83	0.21
1,425	1,422	0.68	0.22	1,415	0.29	0.72



**Figure 8.** The average size of various DNA fragments as measured on the Agilent ZAG DNA Analyzer system with the ZAG 135 dsDNA kit (1–1500 bp) (n=6) exhibits excellent correlation to the sizing from the Agilent 5300 Fragment Analyzer system with the dsDNA 935 Reagent kit (1–1500 bp) (n=6).

## Conclusion

Both the Agilent ZAG DNA Analyzer and Agilent Fragment Analyzer systems provide high resolution fragment analysis with automated size assessment. The ZAG is an ultrahigh throughput instrument for qualitative analysis of DNA fragments. Alternately, the Fragment Analyzer has low to high throughput capabilities and is compatible with quantitative and qualitative kits for both DNA and RNA analysis. Both systems provide reliable and accurate sizing analysis of DNA fragments with the qualitative ZAG and Fragment Analyzer kits, enabling researchers to confidently choose the platform that best suits their laboratory's needs for sample throughput and application flexibility requirements.

## References

1. Research Citation Collection (Fragment Analyzer systems). *Agilent Technologies application compendium*, publication number 5994-2351EN, **2020**.
2. Genotyping with the Agilent ZAG DNA Analyzer System. *Agilent Technologies application note*, publication number 5994-1186EN, **2019**.
3. Arabidopsis thaliana Ecotypes Differentiated by Simple Sequence Repeats. *Agilent Technologies application note*, publication number 5994-2458EN, **2020**.
4. Resolution Capabilities of the Agilent ZAG DNA Analyzer System with the Agilent ZAG 105 dsDNA Kit. *Agilent Technologies application note*, publication number 5994-1211EN, **2019**.
5. Highly Resolved Separation of DNA Fragments on the Agilent 5200 Fragment Analyzer System. *Agilent Technologies application note*, publication number 5994-0517EN, **2019**.

[www.agilent.com/genomics/automated-electrophoresis](http://www.agilent.com/genomics/automated-electrophoresis)

For Research Use Only. Not for use in diagnostic procedures.  
PR7000-8038

This information is subject to change without notice.

© Agilent Technologies, Inc. 2021  
Printed in the USA, July 1, 2021  
5994-3650EN