

Resolution Capabilities of the Agilent ZAG DNA Analyzer System with the Agilent ZAG 105 dsDNA Kit

Authors

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Abstract

The Agilent ZAG DNA Analyzer system provides high-throughput analysis of DNA fragments. High reproducibility of fragment sizing was noted on the Agilent ZAG 105 dsDNA kit (1-500 bp) with high precision and accuracy on both the Agilent FA/ZAG 96-Capillary Array Short, 33 cm (short array) and the Agilent FA/ZAG 96-Capillary Array Long, 55 cm (long array). The short array provided a 4 bp separation at the 300 bp size, while the long array displayed a 3 bp separation. The ZAG DNA Analyzer system utilizes a unique gel chemistry, which allows for separation of closely sized DNA fragments.

Introduction

The ZAG DNA Analyzer system is a parallel capillary electrophoresis system for high-throughput sizing of DNA fragments. Ninety-six samples are simultaneously analyzed from a 96-well plate with the ZAG DNA Analyzer system. Ideal for small PCR amplicons and other DNA fragments, the ZAG 105 dsDNA kit (1-500 bp) (ZAG 105 dsDNA kit) has a sizing range of 35 to 500 bp. Both the FA/ZAG 96-Capillary Array Short, 33 cm (short array), and the FA/ZAG 96-Capillary Array Long, 55 cm (long array) are compatible with the ZAG DNA Analyzer system. The short array offers faster separations in 60 minutes, while the long array completes analysis in 80 minutes with increased resolution on the ZAG 105 dsDNA kit. The ability to distinguish between two closely sized fragments is referred to as separation resolution in electrophoresis systems. Resolution of fragments is critical for accurate DNA sizing and identification of extra fragments when studying small PCR products. Traditional agarose gel-based systems lack the ability to resolve fragments close in size. The ZAG DNA Analyzer system utilizes a unique gel chemistry, which allows for separation of closely sized DNA fragments, providing better insight into nucleic acid sample size.

Experimental

gBlocks sized 306, 307, 308, 309, 310, and 311 bp (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, p/n F530S). Digestion of the 200 ng PCR product was completed with HaeIII (Thermo Fisher Scientific, p/n FD0154) or EcoRV (Thermo Fisher Scientific, p/n FD0303) according to manufacturer instructions. To clean up the digested fragments, digests were separated on a 2% agarose gel, and the gel fragment was purified using the Zymoclean gel DNA recovery kit (Zymo Research, p/n D400).

The purified fragment was quantified using a Qubit high sensitivity dsDNA kit (Thermo Fisher Scientific, p/n 32854) and diluted to 250 pg/μL using 1 × TE. The fragments of various lengths (306 to 311 bp, 201 to 205 bp, and 101 to 105 bp) and mixes of the fragments were analyzed on the Agilent ZAG DNA Analyzer system with the Agilent FA/ZAG 96-Capillary Array Short, 33 cm (short array) (p/n A2300-9650-3355), and the Agilent FA/ZAG 96-Capillary Array Long, 55 cm (long array) (p/n A2300-9650-5580) using the Agilent ZAG 105 dsDNA kit (1-500 bp) (p/n ZAG-105-5000). Nine consecutive injections were completed to demonstrate the ability to load 9 × 96 well plates on the ZAG DNA Analyzer system without changes in sample data over the course of analysis.

Results and discussion

Sizing reproducibility

Single fragment reproducibility was analyzed on the ZAG 105 dsDNA kit with the short and long arrays (Figures 1 and 2). The creation of fragments through enzyme restriction sites allows us to unequivocally produce fragments of known base pair sizes. Fragment sizes consisted of 101, 102, 103, 104, 201, 202, 203, 204, 205, 306, 307, 308, 309, 310, and 311 bp. Both arrays displayed excellent precision and accuracy for all the fragments as seen with a low percent CV and percent error, respectively. The fragment sizing was compared between the short and long arrays. In general, the short and long array reported the same size for each fragment with a difference of only 1 bp in some instances (Table 1).



Figure 1. DNA fragments analyzed on the Agilent ZAG DNA Analyzer system with the short array and the Agilent ZAG 105 dsDNA kit (1-500 bp). (A) Average size of each single fragment. (B) Excellent precision and accuracy of the single fragments. *n = 9.

Table 1. Comparison of average single DNA fragment sizes analyzed on the Agilent ZAG DNA Analyzer system with the short and long array on the Agilent ZAG 105 dsDNA kit. *n = 9.

Comparison of Average Size* (bp)	
Short Array	Long Array
98	99
99	99
101	101
101	102
103	104
202	202
203	204
204	204
205	205
206	206
314	315
315	316
316	317
317	318
319	319
320	320

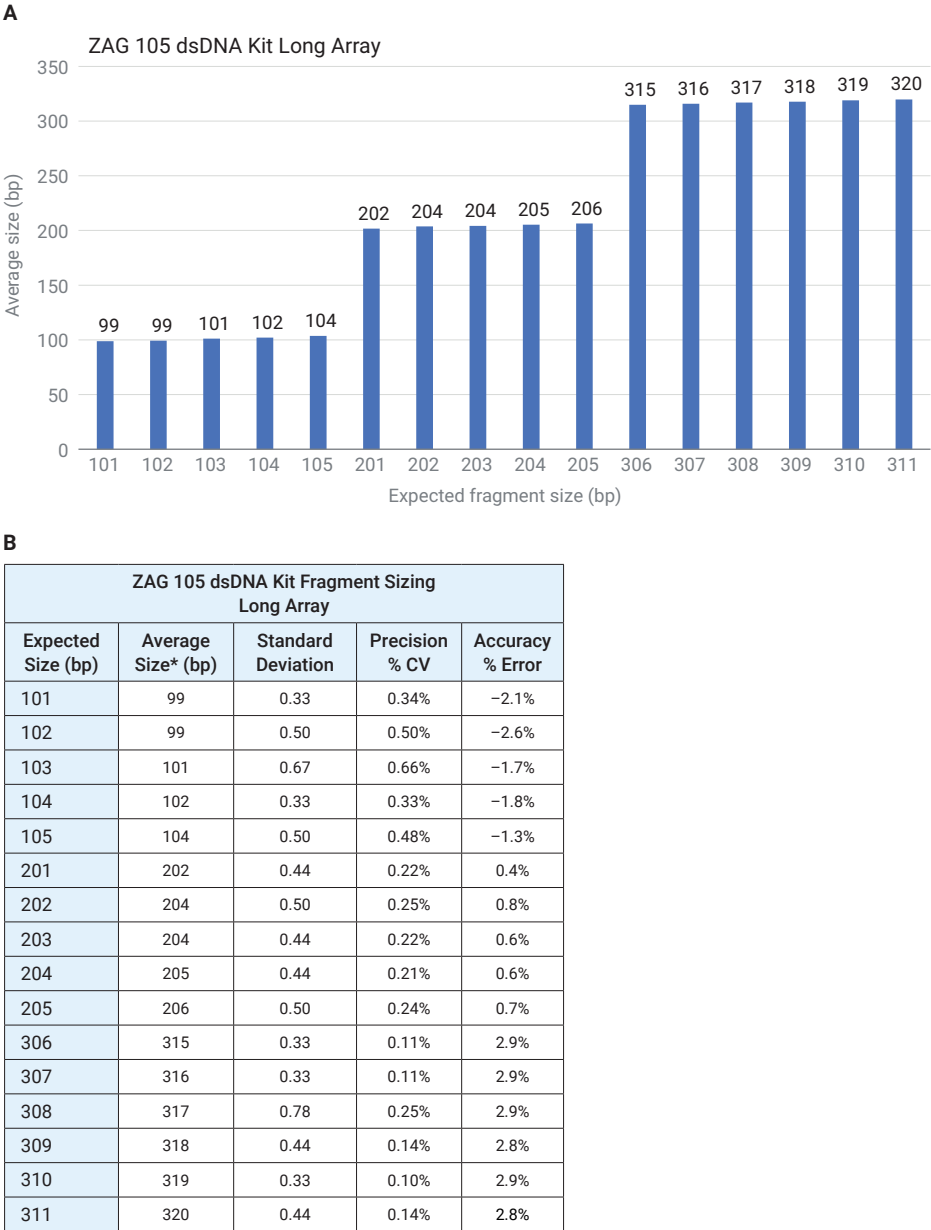


Figure 2. DNA fragments analyzed on the Agilent ZAG DNA Analyzer system with the long array and the Agilent ZAG 105 dsDNA kit (1-500 bp). (A) Average size of each single fragment. (B) Excellent precision and accuracy of the single fragments. *n = 9.

Separation and resolution

Resolution and separation are often used interchangeably when describing electropherograms. However, in liquid chromatography applications, resolution of an elution is a quantitative measure of how well two elution peaks can be differentiated. It is defined as the difference in retention times of the two peaks, divided by the combined full width or width at half maximum for each elution peak. Both equations are commonly used for calculating resolution (Figure 3). Peaks are usually successfully differentiated when resolution is greater than one. Equation two is used for resolution specifications for the ZAG DNA Analyzer system.

To determine the separation capabilities of the ZAG DNA Analyzer system with the different length arrays, mixes of two fragments differing by a known number of base pairs were analyzed with the ZAG 105 dsDNA kit. The ZAG 105 dsDNA kit was specifically designed for accurate sizing of fragments less than 500 bp, and utilizes a specially formulated gel that enhances separation of similarly sized fragments. Common applications

of this kit include genotyping, the analysis of PCR amplicons, and microsatellites/simple sequence repeats.

The creation of fragments through enzyme restriction sites allows us to unequivocally produce fragments of known base pair sizes. A series of known fragment sizes were mixed and analyzed with the ZAG 105 dsDNA kit. The 100 bp series included: 101/102, 101/103, 101/104, and 101/105 bp. The 200 bp series included: 201/202, 201/203, 201/204, and 201/205 bp. The 300 bp series included: 306/307, 306/308, 306/309, 306/310, and 306/311 bp.

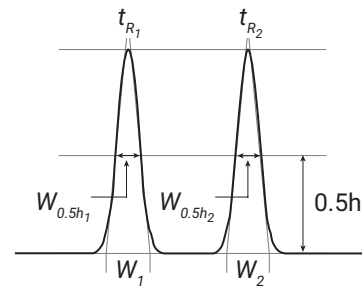
Fragment mixes of known sizes (101/104 bp, 201/204 bp, 306/309, and 306/310 bp) were compared on the short and long array. The short array (Figure 4) demonstrated a 3 bp separation for the 101/104 and 201/204 bp mix and a 4 bp separation for the 306/310 bp mix as two partially resolved peaks. The long array (Figure 5) performed an almost complete baseline resolution of the 101/104 bp fragments while displaying two partially separated peaks for the 201/204 and 306/309 bp fragment mixes. All three mixes had a 3 bp separation with the long array.

Equation 1.

$$R = \frac{t_{R_2} - t_{R_1}}{1/2 (W_1 + W_2)}$$

Equation 2.

$$R = 1.18 \left(\frac{t_{R_2} - t_{R_1}}{W_{0.5h_1} + W_{0.5h_2}} \right)$$



t_{R_1}, t_{R_2} = Retention time for each peak ($t_{R_1} < t_{R_2}$)

$W_{0.5h_1}, W_{0.5h_2}$ = Full width at half maximum (FWHM) of each peak

W_1, W_2 = Width of each peak

Figure 3. Equations and diagram for calculating resolution (R).

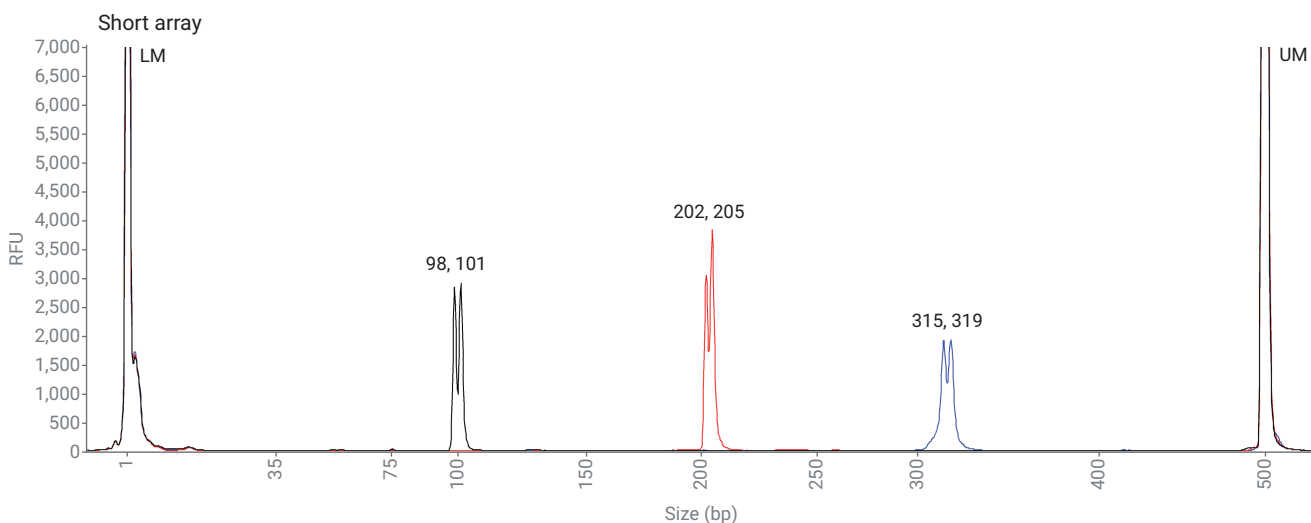


Figure 4. Separation of fragment mixes on the Agilent ZAG DNA Analyzer system with the Agilent ZAG 105 dsDNA kit (1-500 bp) and the short array. Known sizes are 101/104 bp, 201/204 bp, and 306/310 bp with a 3, 3, and 4 bp difference, respectively. Overlay shows average sizes. LM = lower marker; UM = upper marker; n = 9.

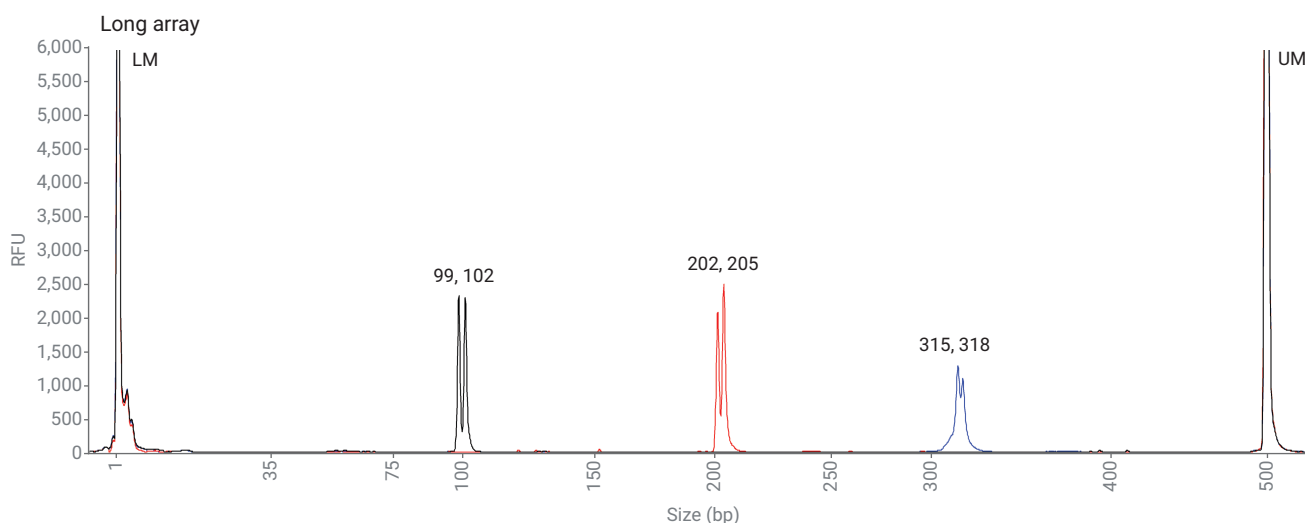


Figure 5. Separation of fragment mixes on the Agilent ZAG system with the Agilent ZAG 105 dsDNA kit (1-500 bp) and the long array. Known sizes are 101/104 bp, 201/204 bp, and 306/309 bp with a 3, 3, and 3 bp difference, respectively. Overlay shows average sizes. LM = lower marker; UM = upper marker; n = 9.

Table 2 summarizes the minimum base pair difference required to see two partially separated peaks for the ZAG 105 dsDNA kit with the short and long arrays.

Conclusion

The Agilent ZAG DNA Analyzer system is ideal for high-throughput analyzes of DNA fragments. The Agilent ZAG 105 dsDNA kit (1-500 bp) displays excellent precision and accuracy for single fragments with both the short and long arrays. The long array is capable of 3 bp separation at the 100, 200, and 300 bp sizing range, while the short array separates 3 bp at the 100 and 200 bp length, with a 4 bp separation at 300 bp. The ZAG DNA Analyzer system and the ZAG 105 dsDNA kit (1-500 bp) provides reliable fragment sizing and exceptional resolution of DNA fragments.

Table 2. Base pair separation on the Agilent ZAG DNA Analyzer system with the Agilent ZAG 105 dsDNA kit (1-500 bp) on the short and long array. n = 9. *Includes partially resolved peaks.

Base Pair Separation*		
	Short Array (33 cm)	Long Array (55 cm)
101 bp Mix	3	3
201 bp Mix	3	3
306 bp Mix	4	3

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