

Comparison of the Agilent HS Small Fragment Kit and Agilent HS NGS Fragment Kit on the Agilent Fragment Analyzer Systems

Authors

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Abstract

The Agilent 5200 Fragment Analyzer system enables easy analysis of sheared genomic DNA (gDNA) smears and libraries with the Agilent HS NGS Fragment kit (1-6000 bp) and the Agilent HS Small Fragment kit. Agilent ProSize data analysis software provides an electropherogram and a digital gel image for visual inspection of smear quality, and automatically reports smear size and concentration. The Agilent 5200 Fragment Analyzer system equipped with the Agilent FA 12-Capillary Array Short, 33 cm, or Ultrashort, 22 cm, facilitates consistent sizing and quantification of gDNA smears with both kits.

Introduction

The quality of next-generation sequencing (NGS) libraries is crucial to successful sequencing results. Production of a high-quality library necessitates quality control analysis at multiple stages throughout preparation. Quality control analysis can be performed on the Agilent 5200 Fragment Analyzer system with the Agilent HS NGS Fragment kit (1-6000 bp) or Agilent HS Small Fragment kit. The Agilent HS NGS Fragment kit sizes larger smears and fragments up to 6,000 bp, while the Agilent HS Small Fragment kit focuses on smaller sizes up to 1,500 bp. The two kits also have similar DNA smear concentration ranges allowing them to be interchangeable for small DNA analysis. We compared the sizing and concentration consistency of different smear preparations on the Agilent 5200 Fragment Analyzer system equipped with an Agilent FA 12-Capillary Array Short, 33 cm (short array) or Ultrashort, 22 cm (ultrashort array) using the described kits.

Experimental

The experiments in this study were done using an Agilent 5200 Fragment Analyzer system and can be replicated with comparable results on Agilent 5300 and 5400 Fragment Analyzer systems.

The human gDNA (Promega, #G304A) was sheared using the Qsonica Q800R2 at 30 % amplitude for 20 minutes. The Agilent 5200 Fragment Analyzer system equipped with the Agilent FA 12-Capillary Array Short, 33 cm (short array) (p/n #A2300-1250-3355) or Agilent FA 12-Capillary Array Ultrashort, 22 cm (ultrashort array) (p/n #A2300-1250-2247) was used to analyze library samples and genomic DNA (gDNA) smears with the Agilent HS NGS Fragment kit (1-6000 bp) (p/n DNF-474) or the Agilent HS Small Fragment kit (p/n DNF-477). The smear analysis function in the ProSize data analysis software was used to determine both the average smear size and concentration of the library and gDNA samples.

Results and discussion

Smear size

Samples were separated with the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit on both the short and ultrashort capillary arrays. The Agilent HS Small Fragment kit has a higher resolution for the smaller sized DNA smears and fragments up to 1,500 bp, while the Agilent HS NGS Fragment kit extends up to 6,000 bp for larger DNA applications (Figure 1 A and D). Samples with notably different smear size ranges were analyzed with the ProSize smear analysis function. Samples #1 and #2 (#2 electropherograms not shown) were small RNA libraries with a narrow base pair range from 100 to 250 bp (Figure 1 B and C), while sample #3 was a gDNA smear with a wide base pair range from 25 to 1,300 bp (Figure 1 E and F). The average smear sizes from the same sample were remarkably close when run on either capillary array with the same analysis kit (Table 1). In addition, sizing was similar between the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit on both the short and ultrashort arrays (Figure 2). The size of the smear range did not affect the consistency of sizing between the Agilent HS Small Fragment and Agilent HS NGS Fragment kits, nor between the short or ultrashort array. These results indicated that smear sizes can be determined with either kit on either Agilent 5200 Fragment Analyzer system array and produce similar smear sizes.

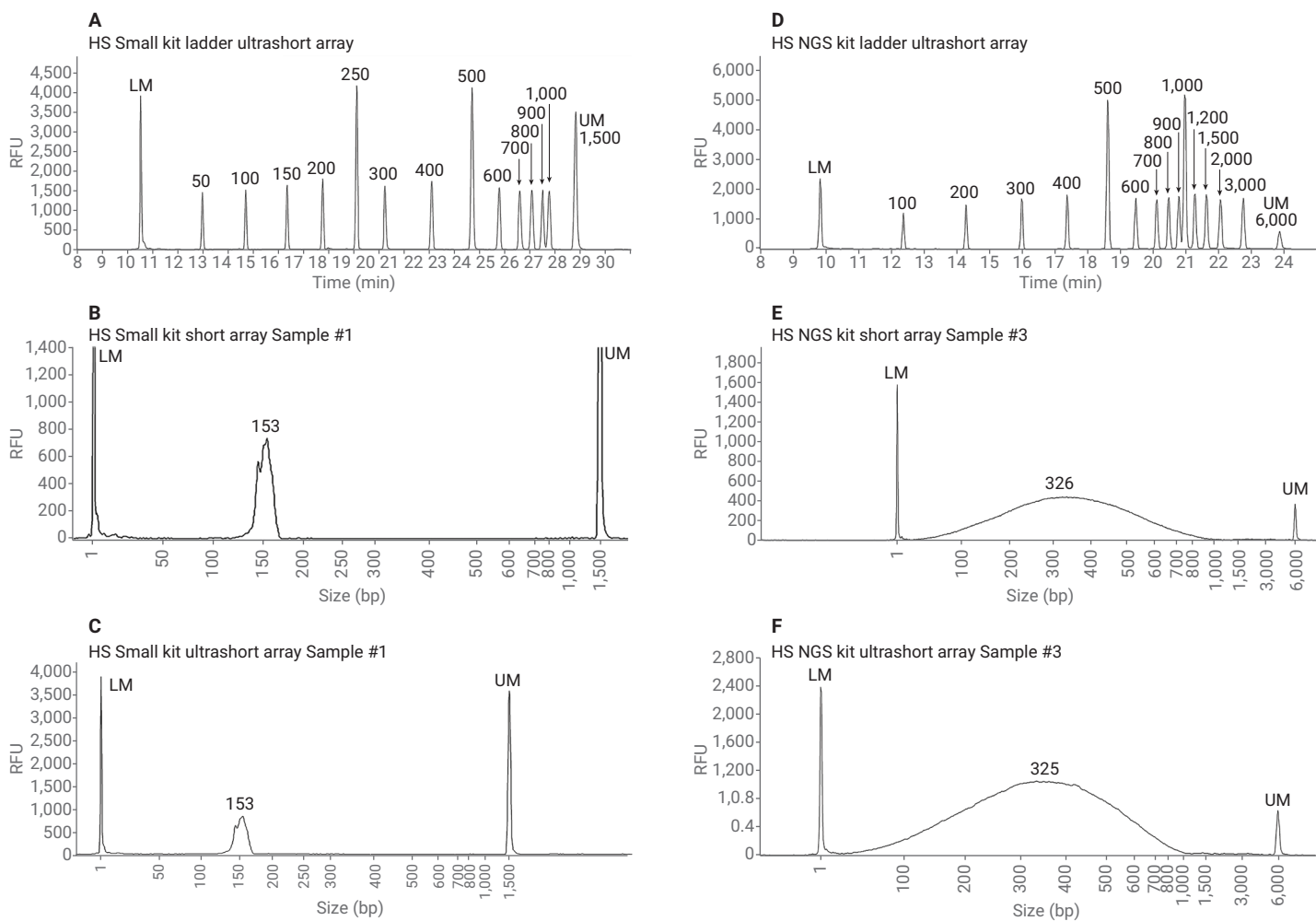


Figure 1. Representative DNA smears separated with the Agilent 5200 Fragment Analyzer system. Agilent HS Small Fragment kit (A–C): (A) Ladder Ultrashort array; (B) Sample #1 Short Array; (C) Sample #1 Ultrashort Array. Agilent HS NGS Fragment kit (D–F): (D) Ladder Ultrashort Array; (E) Sample #3 Short Array; (F) Sample #3 Ultrashort Array. LM = lower marker; UM = upper marker.

Table 1. Smear sizing (bp) on the Agilent 5200 Fragment Analyzer system short and ultrashort array with the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit. *n=3; ^an=5

	Smear range	Average smear size (bp)		
		Short array	Ultrashort array	Size difference
Agilent HS Small Fragment kit Sample #1	100 to 200 bp	153*	153*	0
	100 to 200 bp	150*	150*	0
	25 to 1,300 bp	331 ^a	335 ^a	4
Agilent HS NGS Fragment kit Sample #1	100 to 200 bp	158*	158*	0
	100 to 200 bp	156*	156*	0
	25 to 1,300 bp	326 ^a	325 ^a	1

Smear concentration

Concentration of the smears was reported with the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit on both the short and ultrashort capillary arrays. The smear concentration determined by each kit was similar with both the short or ultrashort array (Table 2). Comparison of the smear concentrations between the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit were similar on both the short and ultrashort array (Figure 3). The DNA smear concentration range for the Agilent HS Small Fragment kit and the Agilent HS NGS Fragment kit was 0.05 to 5 ng/μL and 0.1 to 5 ng/μL input DNA, respectively. The sample concentrations in this study covered the low and high end of the concentration range for both kits. The concentration of the smear did not affect the consistency of the smear quantifications by the Agilent 5200 Fragment Analyzer system. Both the Agilent HS Small Fragment kit and the Agilent HS NGS Fragment kit can be utilized to determine smear concentrations with either the short and ultrashort array.

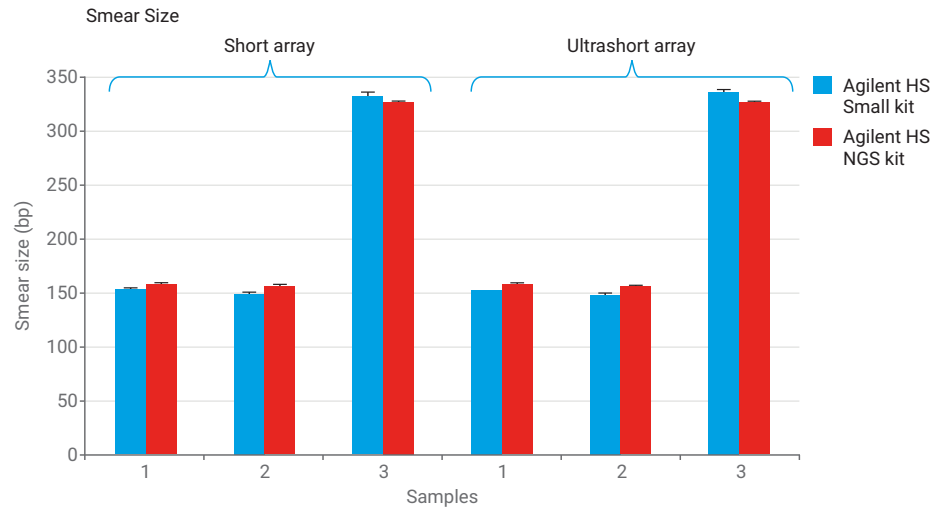


Figure 2. DNA smear size (bp) on the Agilent 5200 Fragment Analyzer system. Comparison of the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit on the short and ultrashort array.

Table 2. DNA smear concentrations (ng/μL) from the Agilent 5200 Fragment Analyzer system short and ultrashort array with the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit. *n=3; ^an=5

	Smear range	Average smear concentration (ng/μL)		
		Short array	Ultrashort array	Concentration difference
Agilent HS Small Fragment kit	100 to 200 bp	0.45*	0.44*	0.01
	100 to 200 bp	0.78*	0.77*	0.01
	25 to 1,300 bp	3.40 ^a	3.38 ^a	0.02
Agilent HS NGS Fragment kit	100 to 200 bp	0.61*	0.63*	0.02
	100 to 200 bp	1.17*	1.19*	0.02
	25 to 1,300 bp	3.55 ^a	3.59 ^a	0.04

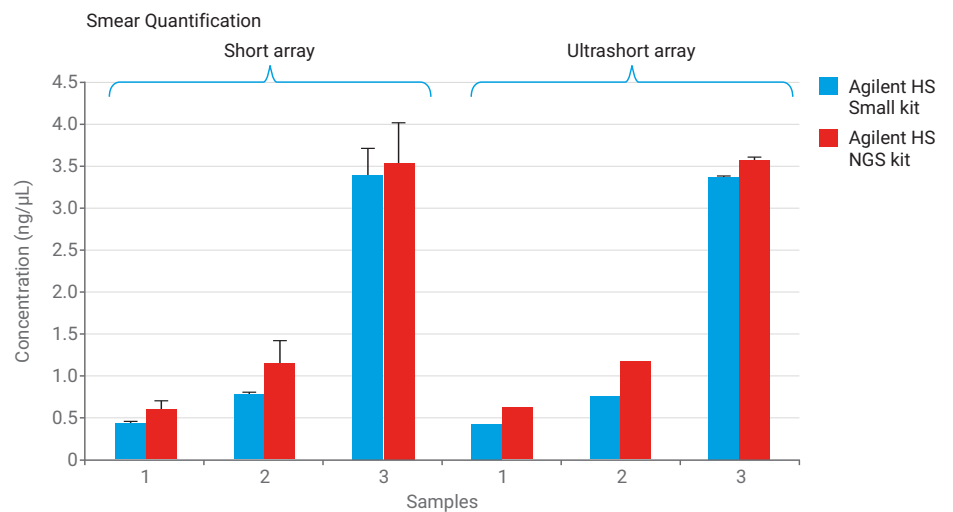


Figure 3. DNA smear quantification (ng/μL) by the Agilent 5200 Fragment Analyzer system. Comparison of the Agilent HS Small Fragment kit and the Agilent HS NGS Fragment kit on the short and ultrashort array.

Conclusions

The smear size and concentration analysis using the Agilent 5200 Fragment Analyzer system short and ultrashort arrays separated with the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit (1-6000 bp) are summarized in Table 3. The library sizing and quantification remained consistent between the short and ultrashort arrays and both kits. The ultrashort array offered the convenience of a shortened run time, while reporting comparable smear size and concentration to the short array.

Table 3. Summary of smear analysis with the Agilent HS Small Fragment kit and Agilent HS NGS Fragment kit.

Agilent Fragment Analyzer application kit	Run times		Comments
	Short array	Ultrashort array	
Agilent HS Small Fragment kit (50-1500 bp)	40 minutes	30 minutes	<ul style="list-style-type: none"> • Consistent library sizing between arrays and to Agilent HS NGS Fragment kit • Consistent library concentrations between arrays
Agilent HS NGS Fragment kit (1-6000 bp)	45 minutes	25 minutes	<ul style="list-style-type: none"> • Consistent library sizing between arrays and to Agilent HS Small Fragment kit • Consistent library concentrations between arrays

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