

Analysis of DNA fragments using the Agilent 2100 Bioanalyzer

ABSTRACT

During the development of DNA shearing protocols for Covaris Focused-ultrasonicators, the Agilent DNA 12000 kit was used to evaluate the average base pair (peak) size because of the large fragment size range this kit covers, encompassing the 130 bp to 5 kb range achievable with a Covaris system. In the protocols developed by Covaris, the target DNA fragment base pair size is determined using the Agilent DNA 12000 kit to identify the maximum amplitude (Peak) of the electropherogram. When performing DNA analysis using the Agilent High Sensitivity DNA kit, the shape of the curves are quite different in comparison to the DNA 12000 kit results, particularly with DNA fragments 400 base pairs (bp) and larger. This result could be expected, since the two kits employ different gel matrices, causing the same size DNA fragments to migrate differently. When using the High Sensitivity DNA kit, the maximum amplitude method can lead to an overestimation of the average fragment size. When assessing the size of sheared DNA using the Agilent High Sensitivity DNA kit, a smear analysis is the more effective method to determine the average fragment size.

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INTRODUCTION

The ultimate success of Next-Generation Sequencing (NGS) strongly depends on the quality of library preparation. With minor variations, all sequencing platforms share a similar library preparation procedure, which includes DNA fragmentation, followed by fragment sizing and selection, and then platform-specific adaptor ligation. DNA fragmentation can be performed by mechanical shearing, by sonication, or by the use of enzymes. Covaris Focused-ultrasonicators are widely recognized as the industry standard for DNA fragmentation, because they mechanically shear the DNA to randomly generate DNA fragments with an accurate and precise size distribution, which are ideal for NGS library preparation.

The size distribution of the DNA fragments can be analyzed using various methods, including agarose gel electrophoresis, chip-based electrophoresis, and capillary electrophoresis. The Agilent 2100 Bioanalyzer System, an automated on-chip electrophoresis system, is widely used for sizing and quantification of DNA fragments, during NGS library preparation.

Agilent has developed several DNA kits (12000, 7500, and 1000), as well as the High Sensitivity DNA kit, to perform sizing of DNA fragments and smears as a quality control step during library preparation¹. The High Sensitivity DNA kit is especially useful for this purpose, because of the increased sensitivity in the range of pg / μ l of DNA². Each kit has been developed to provide the optimal separation of DNA fragments in a certain size range and rely on gel matrices with different properties to attain this optimal separation. Each kit can be used to provide qualitative information about DNA samples; however, because of the differences in the DNA fragment migration (due to the different properties of the gel matrices employed in each kit) the shape of the electropherograms of sheared DNA may differ. Therefore, caution should be used when comparing results obtained using different DNA analysis kits. When using the Agilent High Sensitivity DNA kit to analyze the average fragment size, it is recommended to perform a smear analysis as a more accurate and precise alternative to using the maximum amplitude of the electropherogram.

MATERIALS AND METHODS

1 μ g of genomic DNA from E. coli was sheared in 50 μ l of low TE buffer in

screw cap microTUBEs using the M220 Focused-ultrasonicator (Covaris PN 500295) according to the published protocols (<http://www.covarisinc.com/supported-protocols.html>). The fragmented DNA was analyzed using the Agilent DNA 12000 kit (PN 5067-1508), and the Agilent High Sensitivity DNA kit (PN 5067-4626) on the Agilent 2100 Bioanalyzer System (Agilent Technologies, Waldbronn, Germany) following the manufacturer's protocols. The DNA fragments were diluted 1:10 in water before loading onto the High Sensitivity chip.

RESULTS & DISCUSSION

Samples sheared with a Covaris M220 Focused-ultrasonicator following the 130 bp protocol and analyzed using the Agilent 2100 Bioanalyzer, resulted in similar average base pair sizes with both the DNA 12000 and High Sensitivity DNA kits. No differences were observed in the shape of the curve, the fragment size range (50 bp to ~300 bp), or maximum peak amplitude (data not shown). When analyzing 200 bp fragments, some differences start to be observed. The peak of the curve with the DNA 12000 kit is close to 200 bp, whereas the peak is closer to 250 bp with the High Sensitivity DNA kit (**Figure 1, A & B**). The fragment size range is identical on both electropherograms (less than 50 bp to ~500 bp); however the peak obtained with the High Sensitivity kit appears to be broader.

The High Sensitivity and 12000 DNA kits were developed to resolve different DNA size ranges, 50-7000bp and 100-12000bp respectively. Each kit exhibits the optimal separation performance for the targeted DNA fragment range and provides less separation in other size ranges, by utilizing different gel matrices for the separation of the DNA fragments. Therefore, the shape of the curve looks different when comparing results of DNA analyzed with the two different kits.

By analyzing DNA fragments sheared to an average of 300 bp, the differences in the two kits begin to be more apparent. The maximum amplitude of the DNA curve analyzed with the 12000 DNA kit is 300 bp, however the curve apex observed with the High Sensitivity kit is closer to the 400 bp (**Figure 1, C & D**). While the peak size and shape of the curve begin to show differences, the fragment size distribution in both electropherograms (50 bp to ~1000) remains comparable.

FIGURE 1:

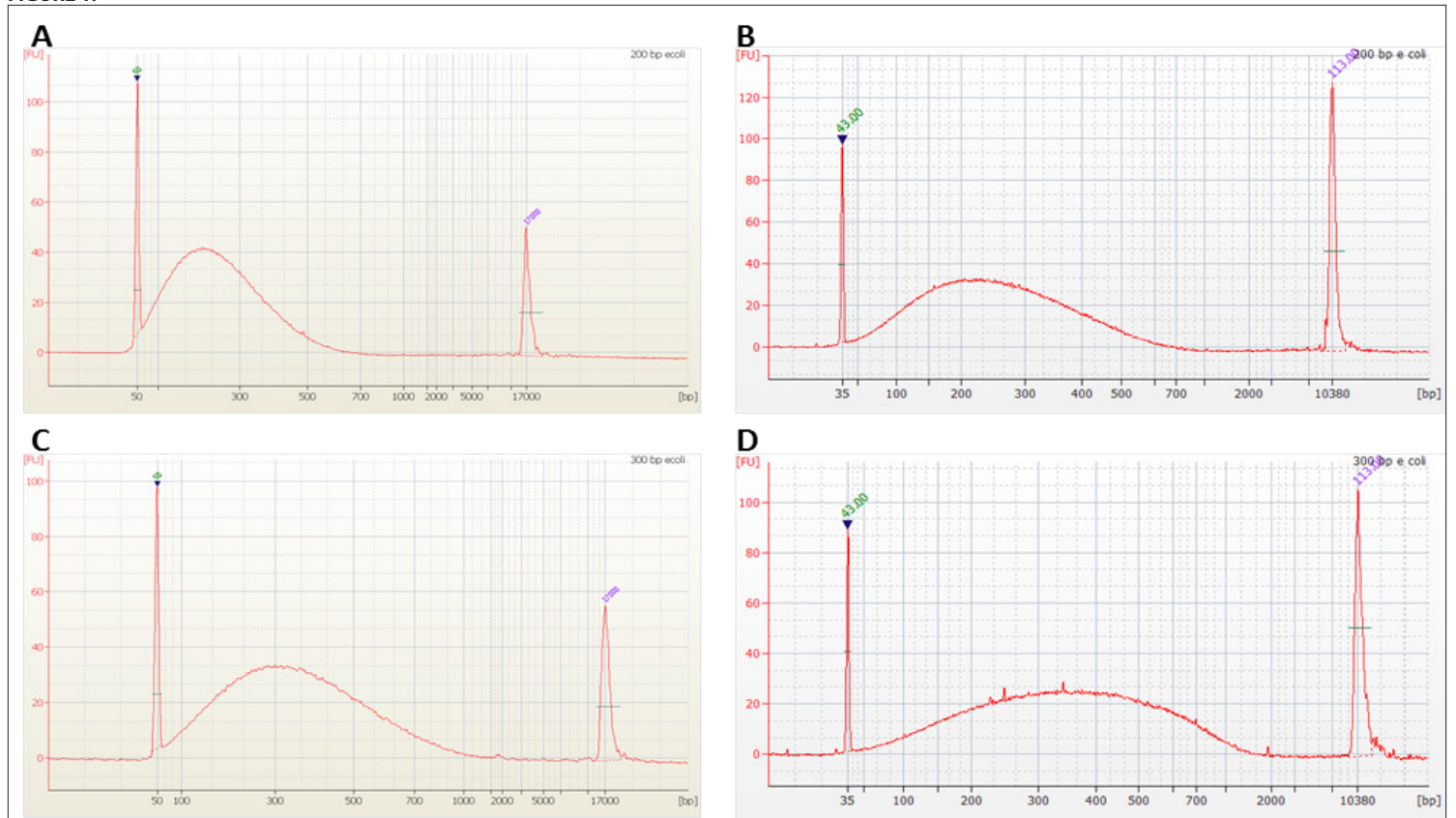


FIGURE 1: Electropherograms of DNA sheared to 200 bp (A&B) or 300 bp (C&D) with a M220 Focused-ultrasonicator and run on an Agilent 2100 using the (A&C) DNA 12000 or (B&D) High Sensitivity Kit with 1 ug or 0.1 ug of sheared DNA respectively.

The differences between the electropherograms from the two kits of DNA sheared to a 400 bp target size are even more significant. The maximum curve amplitude of fragments analyzed with the DNA 12000 kit is approximately 400 bp, compared to nearly 600 bp with the Agilent High DNA Sensitivity kit (**Figure 2, A & B**). Also, the shapes of the curves are again very different due to the different gel matrix properties used by each kit. The electropherograms of 500 bp DNA fragments show similar differences to the analysis of 400 bp fragments. The maximum curve amplitude with the DNA 12000 kit is around 500 bp, while the High Sensitivity DNA kit is closer to 700 bp (**Figure 2, C & D**). However, the fragment size distributions remain identical for both size ranges.

The DNA smear analysis algorithm, included in the Agilent Bioanalyzer 2100 Expert software, was designed to analyze the broad distribution of bands that occur with fragmented genomic DNA. In this analysis, the peak area is divided into multiple regions and the software automatically determines the average size in bp, percentage of the total DNA analyzed, molarity, concentration etc. for each defined region to determine the average DNA fragment size. As shown in **Table 1**: Using the smear analysis, similar results are obtained with both the DNA 12000 and the High Sensitivity DNA kits, confirming the sheared DNA samples are identical, despite the objective differences in the shape of the electropherograms.

The difference in the shape of the electropherograms obtained with the Agilent Bioanalyzer is explained by differences in optimal DNA fragment size separation range and gel matrix properties of the High Sensitivity DNA and 12000 DNA kits.

The Agilent Bioanalyzer 2100 Expert software creates a standard curve of the DNA migration time versus DNA fragment size with a DNA ladder containing fragments of known size and concentration. From the migration times measured for each fragment in the sample, the size of the respective fragment is calculated (**Figure 3**). Both kits use a specific ladder to create this standard curve and employ gel matrices with different properties causing the same size DNA fragments to migrate differently. Therefore, the electropherograms created with different Bioanalyzer kits are not directly comparable.

Migration of the smaller DNA ladder fragments is similar in both gel matrixes; hence the shape of electropherograms for 150, 200, and even 300 bp are quite similar. Differences in the shape of the curves start to occur with larger DNA fragments, for example 500 bp where ladder peaks exhibit different migration patterns. However, the average size range of fragments remains the same, regardless of the kit type.

FIGURE 2:

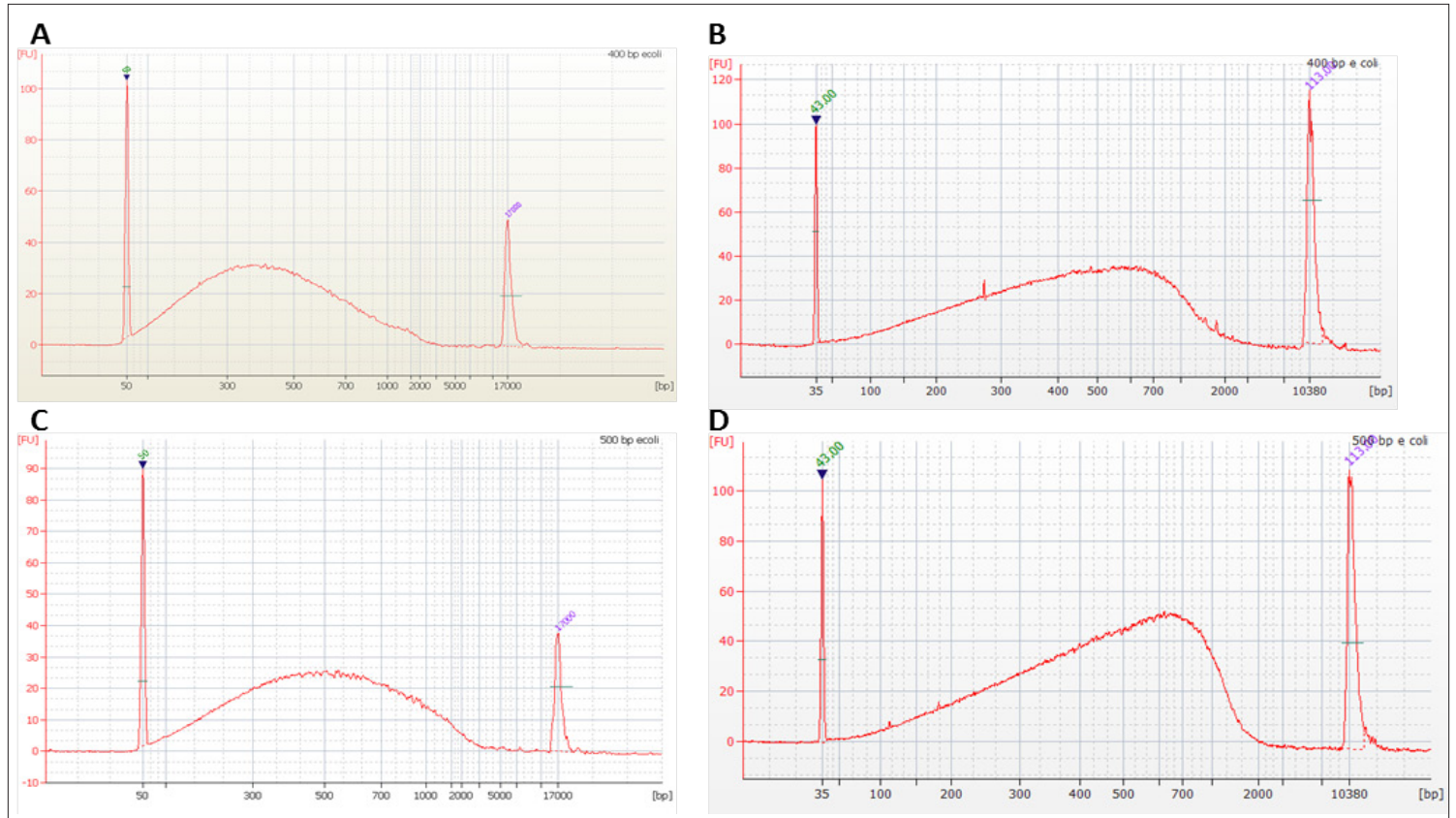


FIGURE 2: Electropherograms of DNA sheared to 400 bp (A&B) or 500 bp (C&D) with a M220 Focused-ultrasonicator and run on an Agilent 2100 using the (A&C) DNA 12000 or (B&D) High Sensitivity Kit with 1 ug or 0.1 ug of sheared DNA respectively.

CONCLUSION

The maximum curve amplitude can be reliably used for the determination of the fragment size with the Agilent DNA 12000 kit. The results of our experiments show that using the same strategy with the High Sensitivity DNA kit, especially for fragments larger than 300 bp, leads to the

overestimation of the fragment size; therefore, using DNA smear analysis is a more accurate and precise manner to determine the average DNA fragment size with the High Sensitivity DNA kit. Further, caution should be used when comparing Bioanalyzer electropherograms obtained using different DNA analysis kits, as the same size DNA fragments will migrate differently through the different gel matrices.

TABLE 1: Results of DNA smear analysis performed on DNA sheared to different target sizes with an M220 Focused-ultrasonicator and analyzed with the Agilent 12000 and High Sensitivity DNA kits.

Target DNA Fragment Size	DNA Size Distribution		DNA 12000 kit		High Sensitivity DNA kit	
	From (bp)	To (bp)	Ave. Size (bp)	% Total DNA	Ave. Size (bp)	% Total DNA
200 bp	100	300	203	62	206	58
300 bp	100	500	296	77	303	76
400 bp	100	900	414	92	429	89
500 bp	200	1000	502	83	509	79

FIGURE 3:

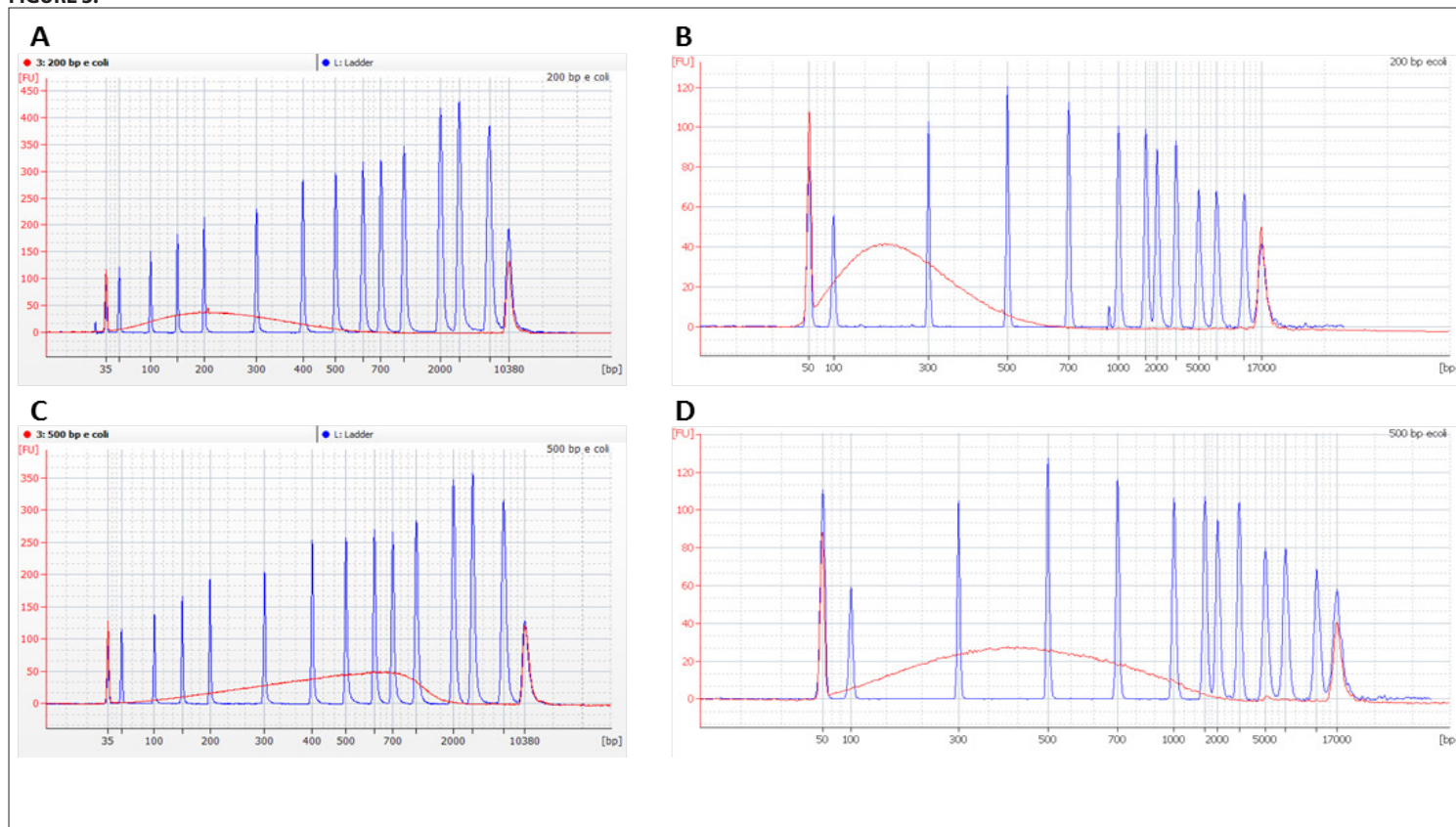


FIGURE 3: Electropherograms of DNA sheared to 200 bp (A&B) or 500 bp (C&D) with a M220 Focused-ultrasonicator and run on an Agilent 2100 using the (A&C) DNA 12000 or (B&D) High Sensitivity Kit with 1 ug or 0.1 ug of sheared DNA respectively. The DNA ladders for the respective kits are overlaid to demonstrate the differences in the migration of DNA fragments in each gel matrix.

REFERENCES:

¹"Improving sample quality for target enrichment and next-gen sequencing with the Agilent High Sensitivity DNA kit and the Agilent SureSelect Target Enrichment Platform" Agilent Technologies Application Note, publication number 5990-5008EN, 2010.

²"Performance characteristics of the High Sensitivity kit for the Agilent 2100 Bioanalyzer" Agilent Technical Note, publication number 5990-4417EN, 2009.