Amplicon Shearing Using a Covaris® Focused-ultrasonicator

SUMMARY

The advent of next generation sequencing (NGS) has transformed the utility of genomics particularly in the field of clinical diagnostics. Recent technological advances in high-throughput sequencing applications have aided in the selection of effective personalized therapies based off an individual's genetic makeup. Despite the major advantages provided by DNA sequencing, the generation of quality sequencing data can be significantly affected by a myriad of factors including: quality of input sample, sample mass, and sample preparation methodology used. One of the most significant factors that can affect sequencing is the DNA fragmentation step. Inefficient fragmentation can result in inefficient clustering efficiency thereby producing poor sequencing readouts. In this application note, we describe the use of Covaris Adaptive Focused Acoustics® (AFA®) technology for mechanical DNA shearing of amplicons ranging in size from 2 kb - 400 bp to 400 bp - 150 bp in preparation for massively parallel high-throughput sequencing.

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INTRODUCTION

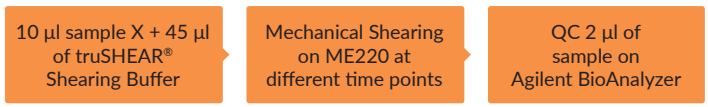
The fragmentation of DNA is a critical step in the preparation of template libraries for next generation sequencing. AFA employs highly controlled bursts of focused high-frequency acoustic energy to efficiently and reproducibly process samples in a temperature-controlled and non-contact environment. Sample processing with AFA ultrasonic energy is accomplished by controlling the creation and collapse of millions of cavitation bubbles within the sample vessel. The Covaris AFA process is conducted under isothermal conditions, ensuring the integrity of the nucleic acid sample is maintained and providing high recovery of double stranded DNA for library preparation. The Covaris AFA mechanical DNA Shearing has been developed from various sources of DNA, and validated protocols have been published and validated when working with genomic DNA (***www.covarisinc.com/resources/protocols***). But when working with shorter DNA lengths such as amplicons, which usually combine a smaller size with a very tight initial fragment size distribution, it is required to fine tune AFA settings to adapt for the ratio between initial and targeted fragment size. In this Application Note, we provide an experimental design for optimizing AFA settings when working with amplicons with for various lengths and to generate randomly fragmented amplicons for library prep.

MATERIALS AND METHODS

DNA Isolated from patient samples were PCR amplified to different amplicon sizes (***Table 1***). Each amplicon was specifically sheared to a specific targeted size (bp) (***Figure 1***). Amplicons were sheared on the ME220 instrument using truSHEAR buffer (PN 520247) to maximize recovery. Covaris 8 microTUBE-50 AFA Fiber H slit strip v2 (PN 520240) were loaded with 55ul volume and Table 2 was followed for method settings. Both rack (PN 500518) and Wave guide (PN 500526) were used as described in the ME220 protocol (PN 010349). To determine the feasibility of fragmenting PCR amplified products using Covaris AFA, we tested multiple amplicon size and target sizes (***Table 1***) and determined the optimal mechanical DNA shearing settings by running a time course for each (***Table 2 for the settings***)

To assess the post-fragment size, we used the Agilent DNA 12,000 chip (Agilent Technologies, CA USA) by following the manufacturer's recommendations.

Workflow



Sample Detail

Amplicon Size Before Shearing	Targeted Amplicon Size After Shearing
322 bp	150 bp
469 bp	180 bp
557 bp	220 bp
718 bp	240 bp
1075 bp	300 bp
2035 bp	375 bp

Table 1. DNA amplicon sizes before and after shearing

AFA Settings

Rack	Rack 8 microTUBE Strip V2 PN 500518
Waveguide	PN 500526
Sample Volume	55 µl
Water Level	5.5
Water Temperature	20C
Peak Power	50 W
Duty Factor	20%
Cycles Per Burst	1000
Duration	Time Course

Table 2. Sample processing settings for shearing amplicons using 8 microTUBE stripV2

Sample ID	Amplitude of Starting Peak	Time in Seconds	Observed Peak in bp
Amplicon 1 Starting Fragment Size 322 bp	344	160	298
	330	180	260
	266	200	245
	200	220	266
	179	240	250
	135	300	206
	50	360	203
	4	700	147

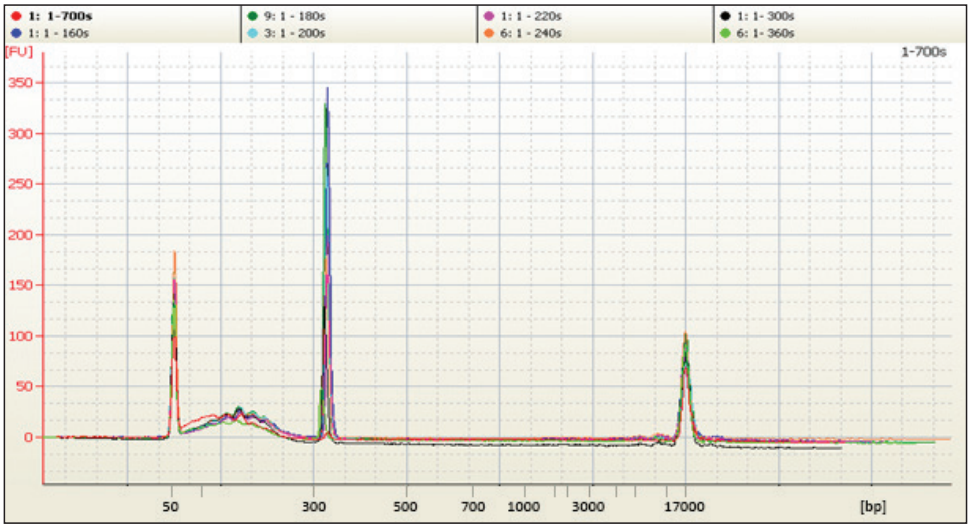


Figure 1. Electropherogram trace file of sheared DNA amplicons to 150 bp target size

Sample ID	Amplitude of Starting Peak	Time in Seconds	Observed Peak in bp
Amplicon 2 Starting Fragment Size 469 bp	209	160	274
	151	180	272
	99	200	256
	91	220	238
	66	240	262
	21	300	205
	4	330	183

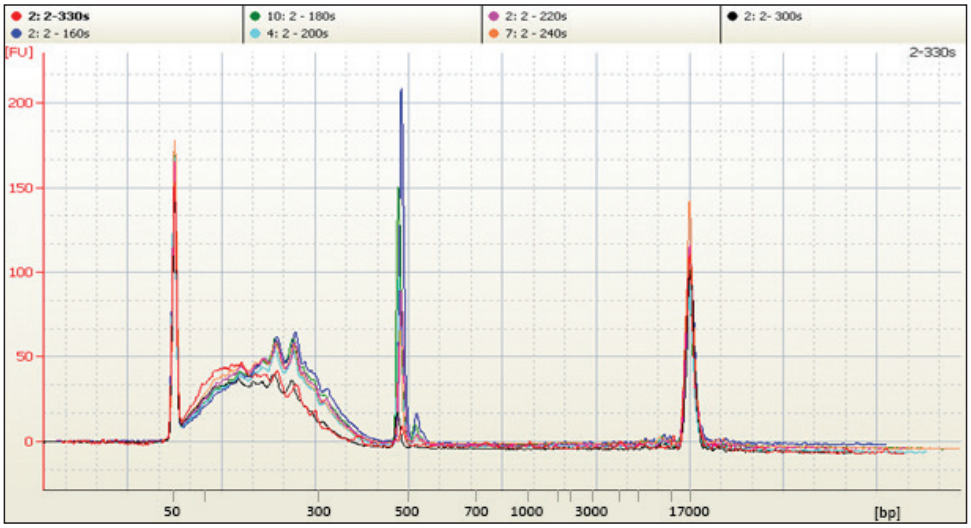


Figure 2. Electropherogram trace file of sheared DNA amplicons to 180 bp target size

Sample ID	Amplitude of Starting Peak	Time in Seconds	Observed Peak in bp
Amplicon 3 Starting Fragment Size 557 bp	45	160	253
	38	180	248
	22.7	200	231
	17	220	228
	5	240	222
	0	260	212

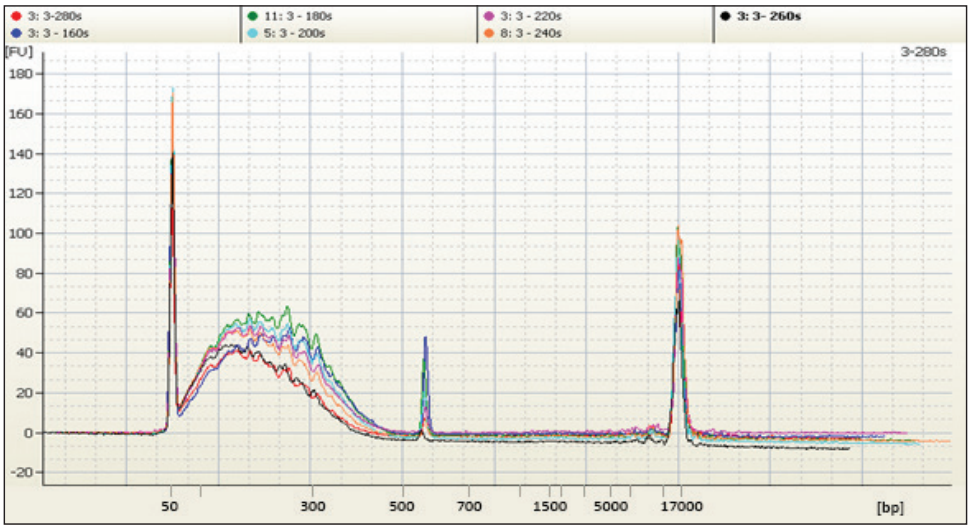


Figure 3. Electropherogram trace file of sheared DNA amplicons to 220 bp target size

Sample ID	Amplitude of Starting Peak	Time in Seconds	Observed Peak in bp
Amplicon 4 Starting Fragment Size 718 bp	450	40	426
	400	45	404
	342	50	387
	228	55	377
	167	60	356
	0	120	353
	0	180	240

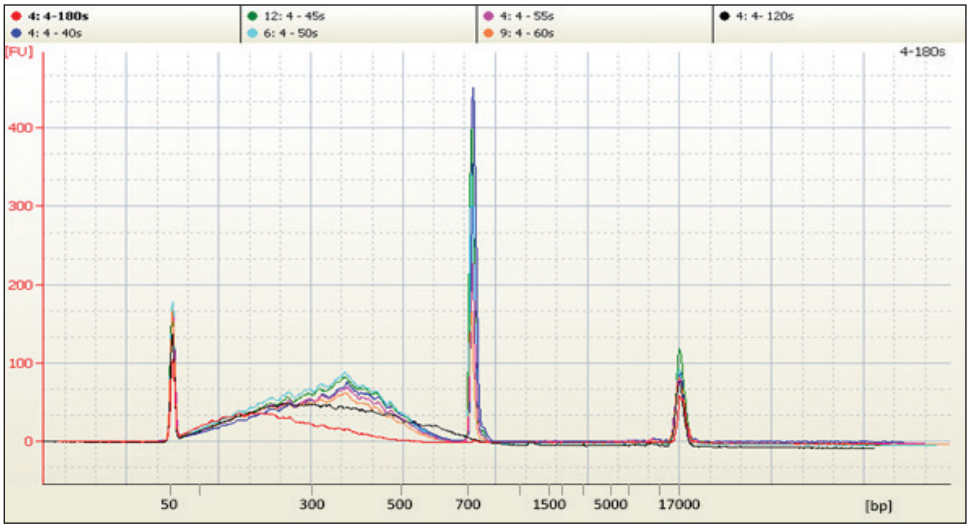


Figure 4. Electropherogram trace file of sheared DNA amplicons to 240 bp target size

Sample ID	Amplitude of Starting Peak	Time in Seconds	Observed Peak in bp
Amplicon 5 Starting Fragment Size 1.7 kb	89.5	40	463
	54.3	45	442
	34	50	414
	20	55	406
	12	60	380
	0	80	327
	0	100	301

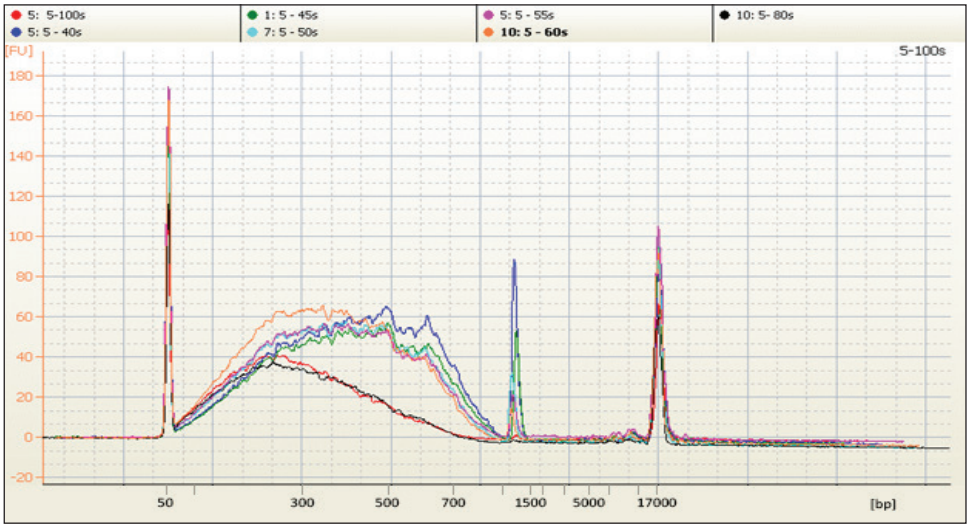


Figure 5. Electropherogram trace file of sheared DNA amplicons to 300 bp target size

Sample ID	Amplitude of Starting Peak	Time in Seconds	Observed Peak in bp
Amplicon 6 Starting Fragment Size 2.0 kb	2	40	437
	0	45	427
	0	50	376

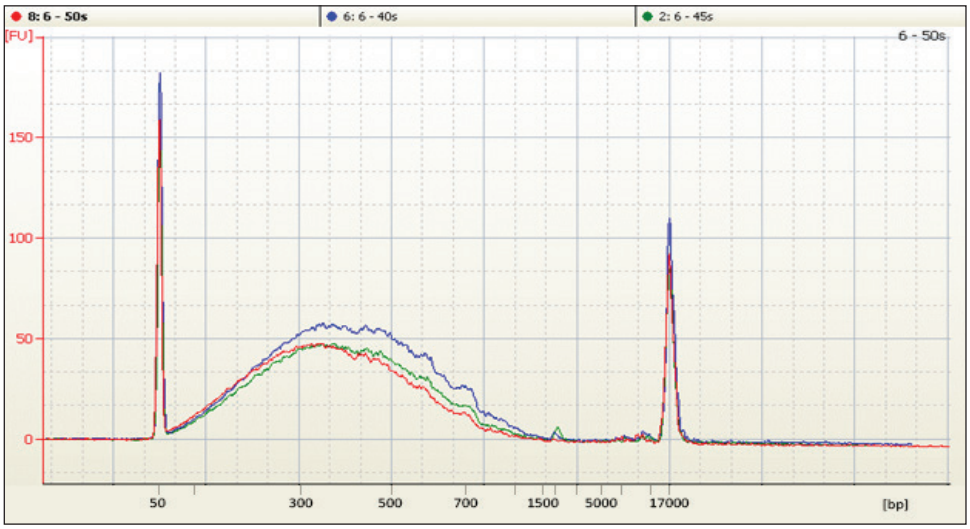


Figure 6. Electropherogram trace file of sheared DNA amplicons to 375 bp target size

RESULTS

Mechanical DNA fragmentation method was evaluated for shearing amplicons. Using AFA, samples were robustly and reproducibly sheared to shorter fragment sizes. The size distributions were consistently moving towards smaller sizes as shearing was done. DNA amplicons were fragmented in a relatively sequence–independent manner. Figures 7 through 12 show the electropherograms for Initial amplicon peak before shearing and final amplicon peak after shearing.

Bioanalyzer Traces

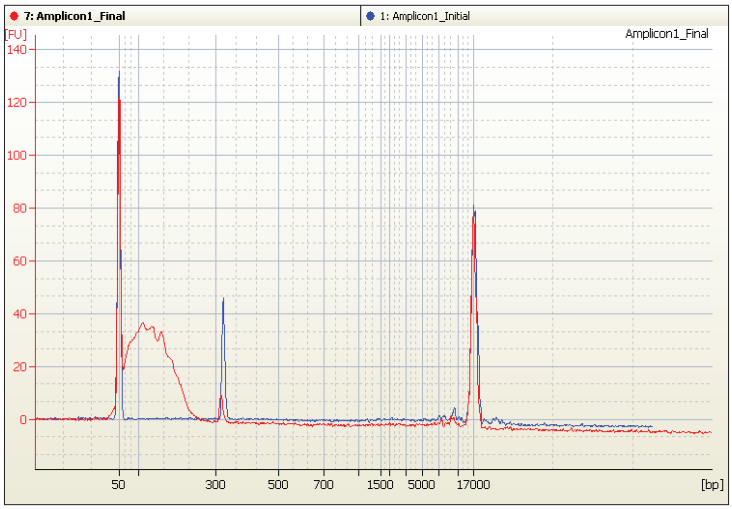


Figure 7. Shearing Amplicon 1 for 700 sec reduces the initial peak height 45.7 to final peak height 7.0

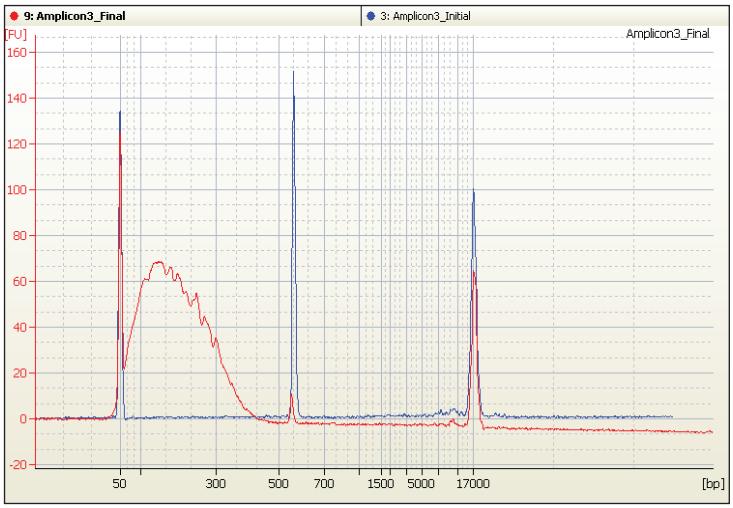


Figure 9. Shearing Amplicon 3 for 260 sec reduces the initail peak height 151.0 to final peak height 10.0

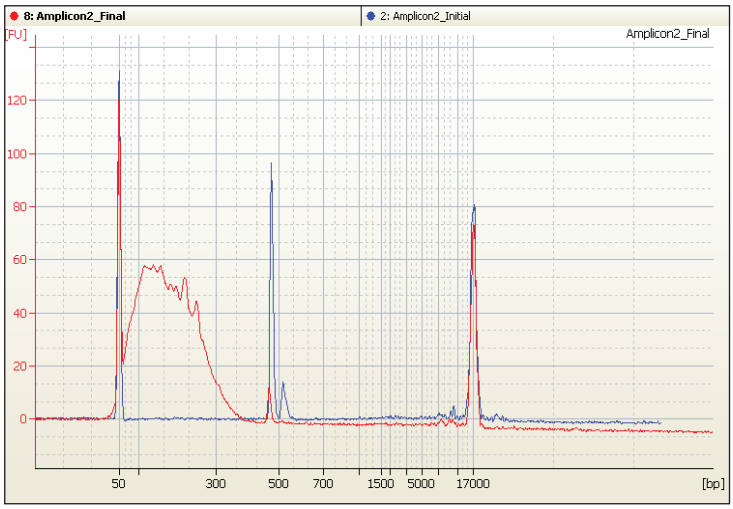


Figure 8. Shearing Amplicon 2 for 330 sec reduces the initail double peak height (96.4,14) to final peak height 10.0

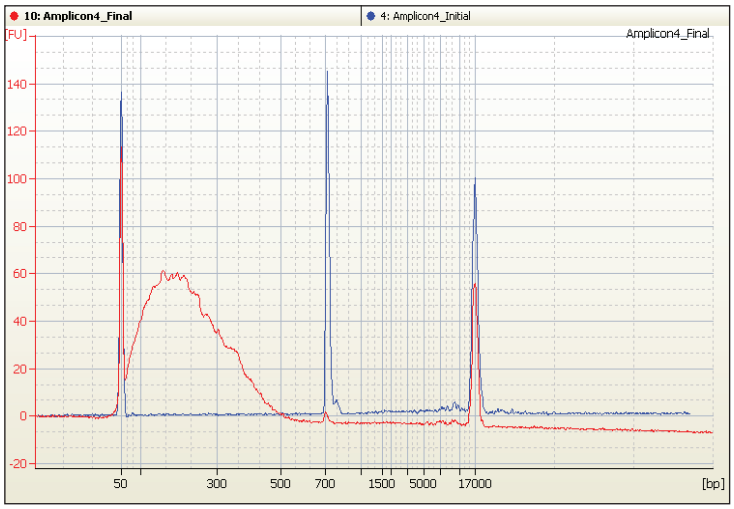


Figure 10. Shearing Amplicon 4 for 180 sec reduces the initail peak height 144.0 to final peak height 0.5

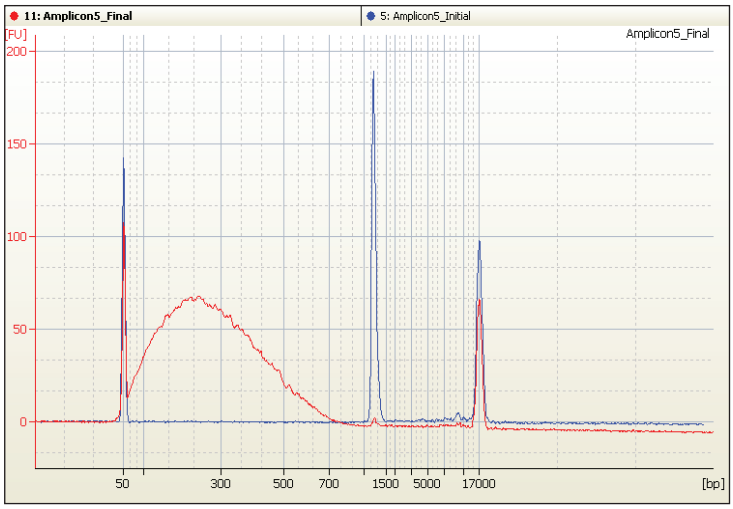


Figure 11. Shearing Amplicon 5 for 100 sec reduces the initial peak height 189.0 to final peak height 0.75

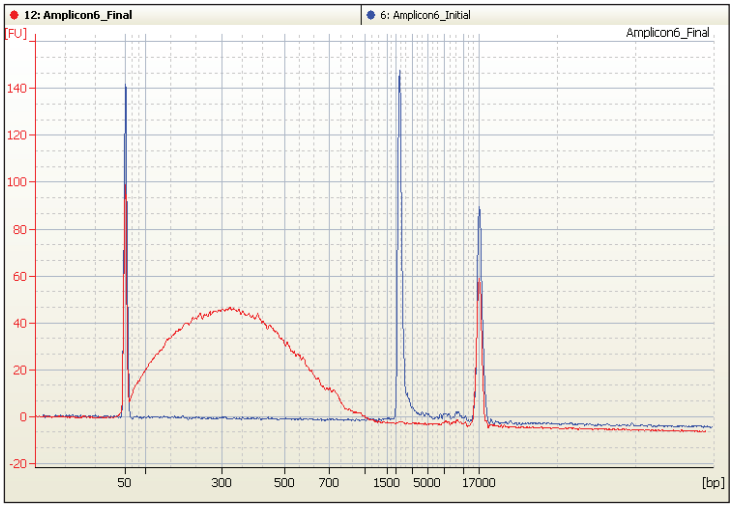


Figure 12. Shearing Amplicon 6 for 50 sec reduces the initail peak height 148.0 to final peak height 0.0

Amplicon Shearing

Sample ID	Amplicon Size Before Shearing	Targeted Amplicon Size After Shearing	Actual Mean Size After Shearing	% of Initial Amplicon Left After Shearing	Optimum Shearing Time in Seconds
Amplicon 1	322 bp	150 bp	147	0.09%	700 sec
Amplicon 2	469 bp	180 bp	183	0.01%	330 sec
Amplicon 3	557 bp	220 bp	212	0.01%	260 sec
Amplicon 4	718 bp	240 bp	240	0%	180 sec
Amplicon 5	1075 bp	300 bp	301	0%	100 sec
Amplicon 6	2035 bp	375 bp	376	0%	50 sec

Table 3. Shearing efficiency analysis of varying amplicon sizes

CONCLUSION

In summary, we have demonstrated the use of Covaris AFA to fragment amplicons to the desired size for next generation sequencing applications. We showed that the ME220 can consistently produce fragment sizes required for preparing sequencing libraries from PCR amplified products. Depending on the treatment time, each initial amplicon was sheared to a target peak. We could achieve the desired fragment size with minimal initial amplicon left over. Experimental method and test settings can be easily transferred to any of the other Covaris instruments by optimizing time and other factors.

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