Covaris Shearing Guide for 500 Base Pairs using the 96 AFA-TUBE TPX Plate

Introduction

Library preparation for high-throughput whole-genome sequencing (WGS) requires an automation-ready workflow and larger insert sizes than targeted sequencing. The AFA-TUBE TPX™ product line, developed to meet high-throughput demands, is designed to adhere to automation microtiter plate standards (ANSI/SLAS 4-2004 (R2012)) and is available in an 8-strip or 96-well plate format.

This application note provides additional support for the current AFA-TUBE TPX shearing protocol to achieve 500 base pair (bp) DNA fragments using Adaptive Focused Acoustics® (AFA®) on the LE220-plus Focused-ultrasonicator. This protocol is optimized for the 500 cycle Illumina® sequencing chemistry used in WGS, and is also compatible with PCR-free library construction, including the NxSeq® AmpFREE Low DNA Library Kit (Lucigen®). Here, we present two variations of sample preparation techniques utilizing the Covaris truSHEAR™ buffer, which yield comparable, qualitative results. This protocol is ideal for high-throughput analyses and enables flexibility in sample elution methods.

Materials

Instruments and Consumables

- Covaris LE220-plus Focused-ultrasonicator (PN 500569)
- Covaris 96 AFA-TUBE TPX Plate (PN 520291)
- Covaris Rack 96 AFA-TUBE TPX Plate (PN 500684)
- Covaris truSHEAR Buffer (PN 520248)
- Promega® Human Mixed Genomic DNA (PN G3041)
- Sigma® Tris-EDTA 100X pH 8.0 (PN 1002206243)

Methods

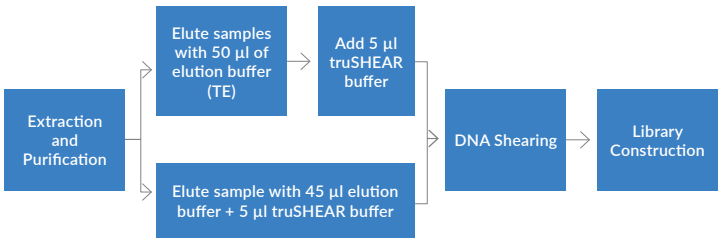
This method is optimized for 50 to 55 µL solution of genomic DNA in TE buffer using a concentration of up to 100 ng/µL. The following settings are developed with human genomic DNA (Promega). Samples are sheared in a Covaris 96 AFA-TUBE TPX Plate. DNA fragment analysis is performed using the Agilent® Bioanalyzer® HS DNA Chip. Fragment distributions are then analyzed via Covaris truANALYZE software.

Table 1 outlines the specific instrument settings and consumables used for this 500 bp shearing protocol. DNA peak size is obtained by calculating the average of the modes (N=16) of the DNA distributions (***Figure 1***). Samples are prepared using truSHEAR as outlined in the workflow shown in ***Scheme 1***:

- The first condition requires the combination of elution buffer and truSHEAR buffer prior to DNA elution
- The second condition requires elution with the desired buffer first and then addition of truSHEAR buffer to eluted samples

Consumable	96 AFA-TUBE TPX Plate (PN 520249) 
Rack	Rack 96 AFA-TUBE TPX Plate (PN 500588)
Plate Definition	"LE220plus_520249 96 AFA-TUBE TPX Plate -2.2 mm offset"
Instrument	LE220-plus
Dithering	1mm Y-dither at 20mm/s
Temperature (°C)	20
Sample Volume (µl)	50 and 55
Target BP (Mode)	500 bp
Peak Incident Power (W)	250
Duty Factor (%)	25
Cycles per Burst	50
Treatment Time (s)	120

Table 1. AFA Treatment Settings for shearing DNA to 500 bp using the 96 AFA-TUBE TPX Plate with the LE220-plus.



Scheme 1. Sample preparation workflow.

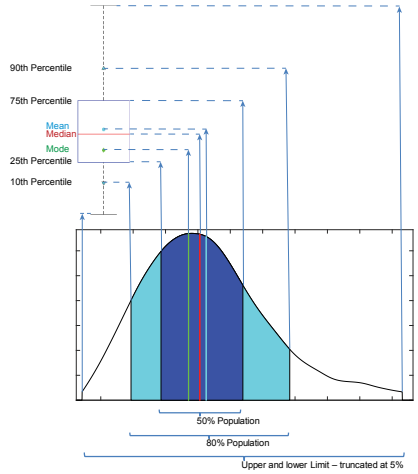


Figure 1. Correlation between DNA shearing electropherogram distribution profile and boxplots.

Results

Electropherograms are obtained following execution of the 500 bp shearing protocol. ***Figures 2A & 2B*** illustrate the DNA distributions for each sample. Data indicates repeatability within each sample preparation protocol, both of which present nearly identical electropherogram profiles demonstrating low %CVs and optimal target size. ***Table 2*** summaries results for each protocol.

Sample Volume	truSHEAR Volume	Shearing Volume	Average Mode	CV	Total Replicates
45 µl	5 µl	50 µl	460 bp	11%	16
50 µl	5 µl	55 µl	523 bp	8%	16

Table 2. Summary of shearing protocol results for 50 and 55 µL reaction volumes.

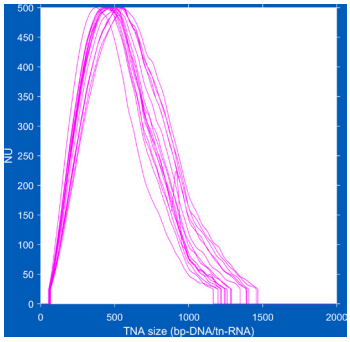


Figure 2A. Superposition of electropherograms of DNA sheared to 500 bp on LE220-plus using the 50 µl protocol (50 µl elution/truSHEAR buffer).

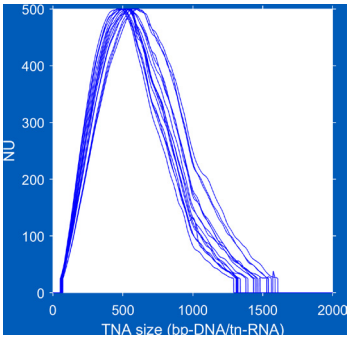


Figure 2B. Superposition of electropherograms of DNA sheared to 500 bp on LE220-plus using 55 µl protocol (5 µl truSHEAR + 50 µl TE/ hgDNA).

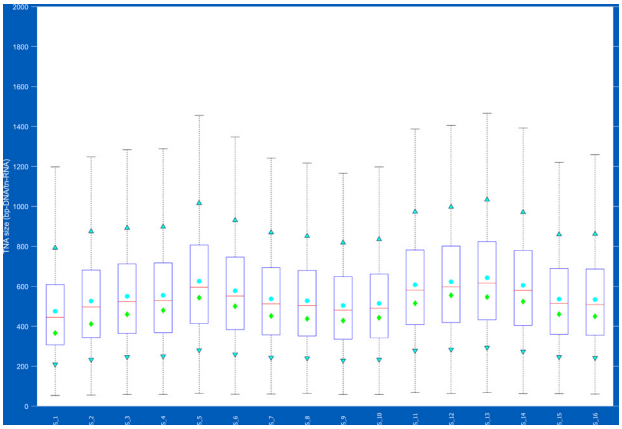


Figure 3. Boxplot showing means (circle), medians (line), modes (diamond), and variances for samples sheared to 500 bp on LE220-plus using 50 µl protocol (50 µl elution/truSHEAR buffer).

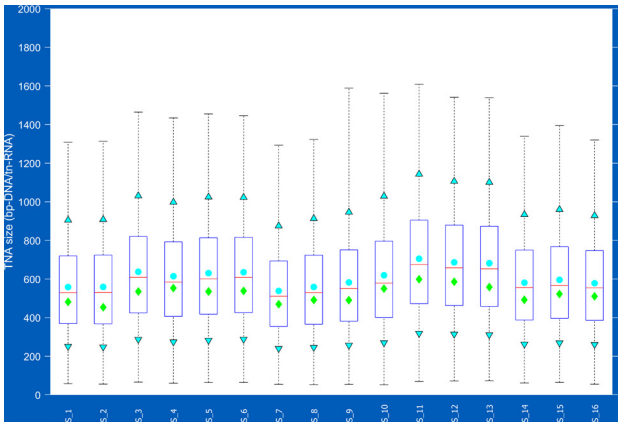


Figure 4. Boxplot showing means, medians, modes, and variances for samples sheared to 500 bp on LE220-plus using the 55 µl protocol (5 µl truSHEAR + 50 µl TE/ hgDNA).

Conclusion

This application note delivers a reproducible and robust shearing protocol targeting 500 bp DNA fragments while also providing modifiable elution parameters. The developed protocols have undergone rigorous quality measures to provide coefficient of variation (CV) values below 12%. These protocols utilize the 96 AFA-TUBE TPX Plate on the Covaris LE220-plus Focused-ultrasonicator and are exemplary for high throughput shearing applications and Next-Generation Sequencing library preparation.

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