Covaris truSHEAR™ Mechanical DNA Shearing for NGS Applications

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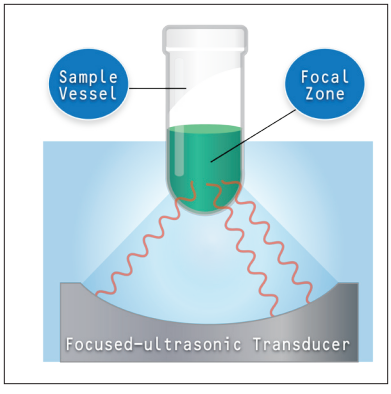
INTRODUCTION

Controlled generation of DNA fragments is a critical step required by all Next-Generation Sequencing (NGS) platforms. Covaris truSHEAR employs Adaptive Focused Acoustics™ (AFA) technology for the controlled mechanical shearing of the phosphodiester backbone of nucleic acids. AFA hydrodynamic shear force-based fragmentation is purely mechanical and designed to be isothermal, ensuring both unbiased fragmentation and high recovery of double-stranded and single–stranded DNA.

Adaptive Focused Acoustics (AFA) technology was exclusively developed by Covaris and is used in all our Focused-ultrasonicator instruments. Our patented approach combines integration of proprietary high performance control electronics, medical-grade transducers, and custom engineered acoustical cuvettes. Together these reproducibly convert focused high frequency acoustic energy to mechanical force delivered within a tightly defined region inside the sample tube. This process (termed as AFA-energetics™) uses controlled bursts of high power acoustic energy to process samples in a temperature-controlled, non-contact, and closed-vessel environment. Uniquely, all AFA Focused-ultrasonicators are calibrated to NIST traceable standards, ensuring highest quality and standardized results.



In this Technical Note, we review how truSHEAR enables simple and cost effective library preparation for NGS by addressing all aspects of the DNA fragmentation workflow.

INPUT

- DNA concentration independent
- Compatible with multiple buffer compositions
- Flexible sample volume
- Flexible throughput

PROCESS

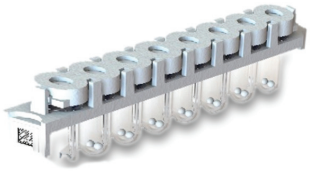
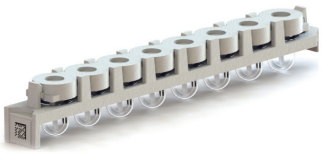
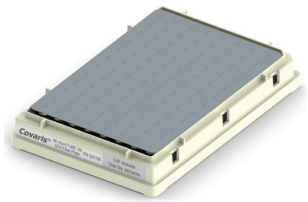
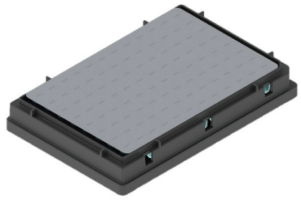
- Tight and tunable fragment sizes
- Highly Reproducible

DATA

- Unbiased fragment generation enables uniform genome coverage and assembly

FLEXIBLE SAMPLE INPUT AND THROUGHPUT

Covaris designed acoustical cuvettes specifically for truSHEAR applications. The sample vial is a key component of the AFA acoustical circuit and is critical for successful isothermal and unbiased mechanical DNA shearing necessary for NGS. The Covaris microTUBE is acoustically engineered and matched to work in combination with Focused-ultrasonicators. The cuvettes are available in single tube, 8 tube strip, and 96 tube plate formats, and are also available for sample volume inputs of 15, 50, and 130 µl to accommodate all commercially available library preparation kit sample requirements.

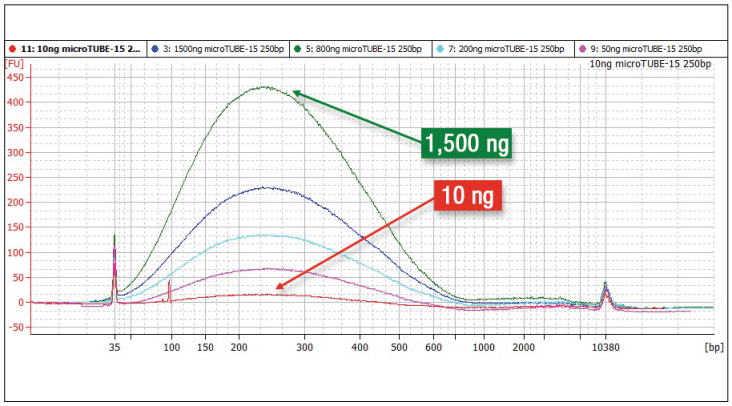
	microTUBE-15 AFA Beads	microTUBE-50 AFA Fiber	microTUBE-130 AFA Fiber
Sample Volume	15 to 20 µl	50 to 60 µl	130 µl
Single tube format	2D barcoded 	2D barcoded 	
8 tube strip format	2D barcoded 	2D barcoded 	
96 plate format	N/A	SBS format –barcoded 	SBS format –barcoded 

DNA CONCENTRATION INDEPENDENT

truSHEAR mechanical shearing is independent of DNA concentration across a broad input range (typically from sub-nanogram to microgram). This eliminates time consuming and expensive steps of precise DNA quantification and DNA normalization of the sample prior to fragmentation. In addition, with AFA, broadly variable DNA concentrations can be processed using the same acoustic settings.

> ***"…The distribution of the fragment lengths was much smaller with the Covaris protocol. Furthermore, the enzymatic fragmentation is not robust and DNA-concentration, enzyme concentration, incubation time and temperature should be tightly controlled. Even then, the yield of the desired fragments is low."***
>
> Van Nieuwerburgh et al. Illumina sequencing of 15 deafness genes using fragmented amplicons. BMC Res Notes. 2014 Aug 9;7:509

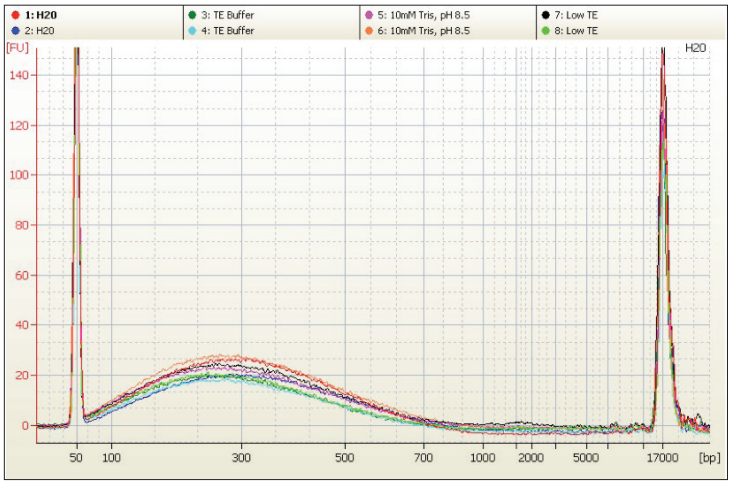
truSHEAR can be used in conjunction with a multitude of low input library preparation solutions. For example, combining low volume microTUBE-15 with sub nanogram DNA input enables streamlined library construction with DNA input as low as 50 pg.[1]



Various input of lambda DNA were diluted in TE buffer and mechanically sheared to 250 bp with a Covaris M220, M220 Holder XTU, and microTUBE-15 using the following settings for all samples: 30 W PIP, 50 cpb, 30% DF, 80 seconds. DNA Inputs: 1,500, 800, 200, 50, and 10 ng

COMPATIBLE WITH MULTIPLE BUFFER COMPOSITIONS

Covaris truSHEAR delivers the same performance with most of the buffers commonly used for DNA purification and NGS library preparation, thus enabling a simpler workflow by eliminating costly and time consuming clean up and/or buffer exchanges which often lead to significant sample loss.



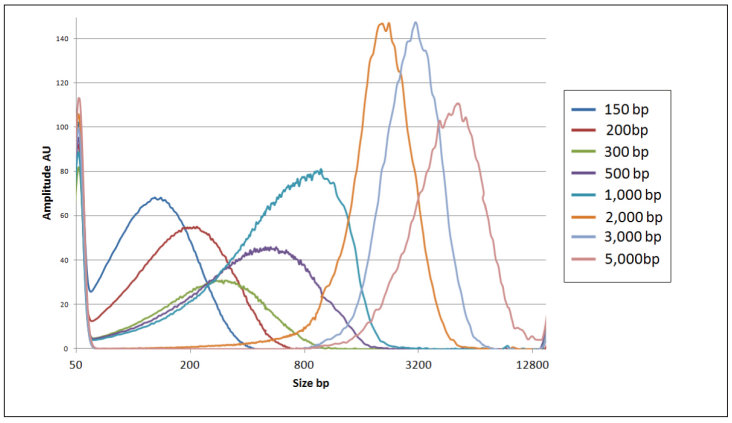
600 ng Lambda DNA was diluted in various buffers and mechanically sheared in duplicate at 250 bp using microTUBE-50 and Covaris M220 and following published Covaris protocols: 75 W PIP, 10% DF, 200 cpb, 130s. A. 1X TE Buffer (10 mM Tris-HCl + 1 mM EDTA, pH 8), B. Low TE (10 mM Tris-HCl + 0.1 mM EDTA, pH 8), C. Ambion Nuclease-free Water D. Tris Buffer (10 mM Tris, pH 8.5)

TIGHT AND TUNABLE FRAGMENT SIZES

truSHEAR mechanical DNA shearing generates fragment size distribution ranging from 150 bp to 5kb using validated pre-optimized protocols. DNA fragments size distributions are uniquely tight, thus enabling workflow without subsequent size selection in some situations. Mechanical DNA Shearing is a very fast process. For example, 96 genomic samples may be fragmented to 300 bp in less than 15 minutes on a Covaris LE220 Focused-ultrasonicator.

"We found that after shearing with the Covaris, a tight size range is attained… negating the need for any size selection with gel electrophoresis, bead-based selection, or specialized equipment"

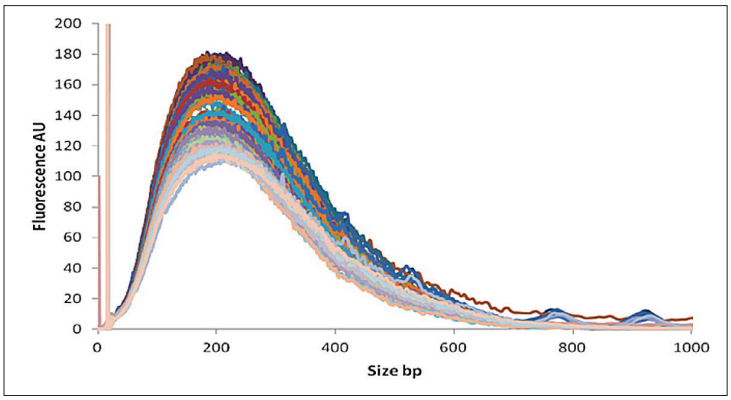
Van Nieuwerburgh et al. Illumina sequencing of 15 deafness genes using fragmented amplicons. BMC Res Notes. 2014 Aug 9;7:509



Legend: Lambda DNA was diluted at 30 µg/nl and mechanically sheared with a Covaris M220 following Covaris published protocols for each fragment sizes.

HIGHLY REPRODUCIBLE

truSHEAR mechanical DNA Shearing on a Covaris AFA Focused-ultrasonicator is a highly reproducible process. Batches of up to 96 samples can be processed with a fragment size coefficient of variation of less than 3%. All Covaris Focused-ultrasonicators have unique high-performance electronics, which enables them to be calibrated to NIST traceable standards. This enables DNA Shearing protocols to be easily transferred from instrument-to-instrument.



Legend: Lambda DNA was diluted at 30 ng/µl and mechanically sheared in a Covaris E220 following Covaris published protocol. 96 samples were processed in a 96 microTUBE Plate (PN 520078) and analyzed with Caliper GX 1k

UNBIASED FRAGMENT GENERATION FOR UNIFORM GENOME COVERAGE

truSHEAR mechanical shearing fragments DNA in a completely random fashion regardless of AT or GC content. A completely random fragmentation is fundamental to obtaining uniform genome coverage and high complexity libraries. Uniform genome coverage is a key element to get actionable data for NGS projects.

"…technologies that require restriction enzymes or transposase missed several SNVs largely because of the lack of coverage."

Comparison of Custom Capture for Targeted Next-Generation DNA Sequencing. Samorodnitsky, Eric et al. The Journal of Molecular Diagnostics , Volume 17 , Issue 1 , 64-75

"…In this study, tagmentation generated greater coverage nonuniformity compared to mechanical DNA shearing by sonication. Importantly, we showed this depth bias had a direct and negative effect on genotype calling. Algorithm and primer-related issues were responsible for only a minority of the errors observed in this study. In contrast, allele coverage bias was the principal cause of genotyping errors found in transposase-treated samples"

Lan et al. Impact of three Illumina library construction methods on GC bias and HLA genotype calling, Hum Immunol. 2015 Mar;76(2-3):166-75

" Although the specific effect seems to vary slightly from genome to genome, there is a general trend for TSF, KP , and KF preparation methods to behave nearly identically, whereas XT requires a deeper average sequencing depth to capture an equivalent fraction of the genome with reads."

Notes: TSF (Illumina TruSeq PCR Free), KP (Kapa Hyper Prep), and KF (Kapa Hyper Prep PCR Free) are all based on mechanical DNA shearing with Covaris truSHEAR. XT (illumina Nextera XT) is based on enzymatic tagmentation.

Jones et al. Library preparation methodology can influence genomic and functional predictions in human microbiome research, http://www.ncbi.nlm.nih.gov/pubmed/26512100 Proceedings of the National Academy of Sciences of the United States of America. 2015 Nov 10;112(45):14024-9.

CONCLUSION

truSHEAR mechanical DNA shearing uniquely enables a simple, robust implementation of DNA fragmentation for NGS library preparation without compromising library quality, quantity, complexity, or loss of precious samples. truSHEAR is well proven in laboratories throughout the world and is considered the Gold Standard for NGS applications with thousands of peer reviewed publications.

truSHEAR mechanical DNA shearing has a significant impact on every stage of the library preparation process. Upstream, truSHEAR compatibility with a wide range of DNA input mass or quality enables easy operation without unnecessary DNA pretreatments or QC. Downstream, truSHEAR mechanical DNA shearing delivers the highest quality DNA fragments and its impact is reflected on key NGS metrics such as library complexity and coverage, ultimately impacting genotyping or variant calling.

truSHEAR process is designed for seamless integration with all NGS workflows. It is extremely robust, truly random, and concentration independent. Optimized settings are easily transferable across instrument-to-instrument, operator-to-operator, and site-to-site. The workflow can be adapted to any throughput, from single tube to batch processing in a format compatible with all liquid robotic handling instruments used in the library preparation process.

REFERENCE

1. Application Note : A Practical Protocol for Library Preparation of Samples Sheared in the Covaris® microTUBE-15 using Rubicon's ThruPLEX® DNA-seq Kit. John Paul Jerome, Sean Carey, Hamid Khoja, Guillaume Durin, and Kamran Shazand.

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