DNA Shearing for Next Generation Sequencing (NGS) with the M220 Focused-ultrasonicator

Introduction

The generation of DNA fragments is a critical sample preparation step required by all Next-Gen Sequencers. The quality and diversity of the final sample library is dependent on this 1st step. It requires a truly random process to generate unbiased libraries as well as an easy and robust method to ensure consistent day-to-day performance.

The M220 Focused-ultrasonicator uses the Adaptive Focused Acoustics® (AFA®) process to apply hydrodynamic shearing forces to the DNA and to randomly fragment it. It is conducted under isothermal conditions ensuring both unbiased fragmentation and high recovery of double-stranded DNA. Coupled with the integrated thermoelectric temperature controller, Covaris' highly efficient and reproducible AFA technology eliminates operator induced variation, and provides standardized results.

Covaris Focused-ultrasonicators are recommended by all the major sequencing platform providers, and are used by leading Genome Centers worldwide such as The Broad Institute of Harvard and MIT (1), Wellcome Trust Sanger Institute (2), and BGI. (1) A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. Fisher et al., 2011. Genome Biology. (2) A large genome center's improvements to the Illumina sequencing system. Quail et al., 2008. Nature Methods.

Versatile Performances

M220 Focused-ultrasonicator generates fragment lengths from 150 bp to 1.5 kb in the microTUBE, and to 2, 3, and 5 kb in the miniTUBE. The sample vial is a key component for a successful DNA shearing and Covaris has developed consumables optimized for this application. Covaris microTUBE™ and miniTUBE™ are acoustically engineered to work in combination with the M220 Focused-ultrasonicator and to reliably deliver high quality DNA fragments at your desired lengths.
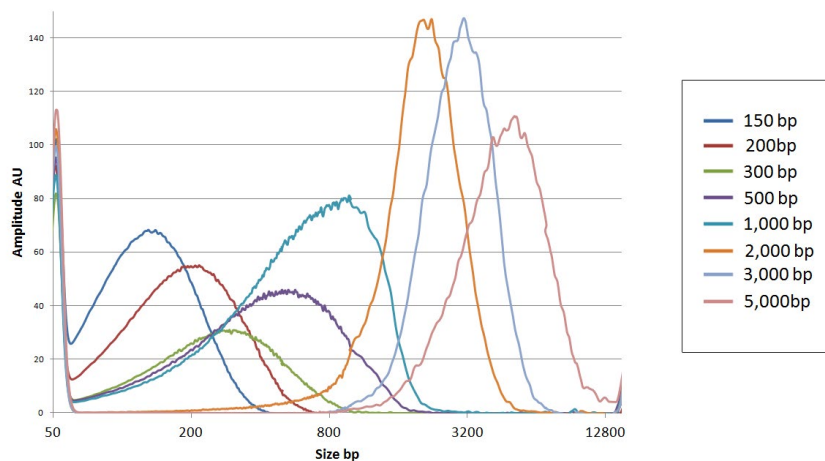

Highly Reproducible

DNA shearing with a M220 Focused-ultrasonicator is highly reproducible. In the figures on the right, electopherograms from replicate samples are overlaid to demonstrate the reproducibility of fragmentation. Replicates samples were processed with the same acoustic settings.


300 bp using Covaris microTUBE (12 replicates)
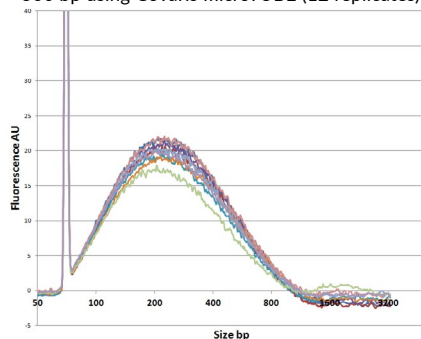
3 kb using Covaris miniTUBE Blue (6 replicates)
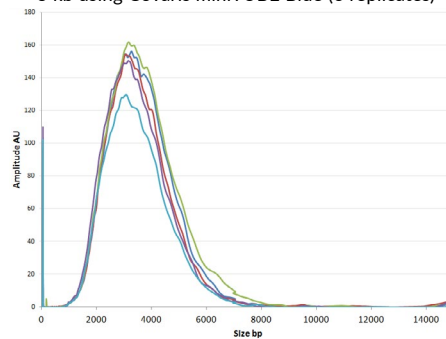
DNA Concentration Independent

DNA mass ranging from 50 ng to 2 µg per tube were sheared with M220 Focused-ultrasonicator and run on a High Sensitivity Agilent Bioanalyzer chip. The same instrument settings were used for all concentrations. DNA Shearing performances are identical, regardless of the DNA concentration.
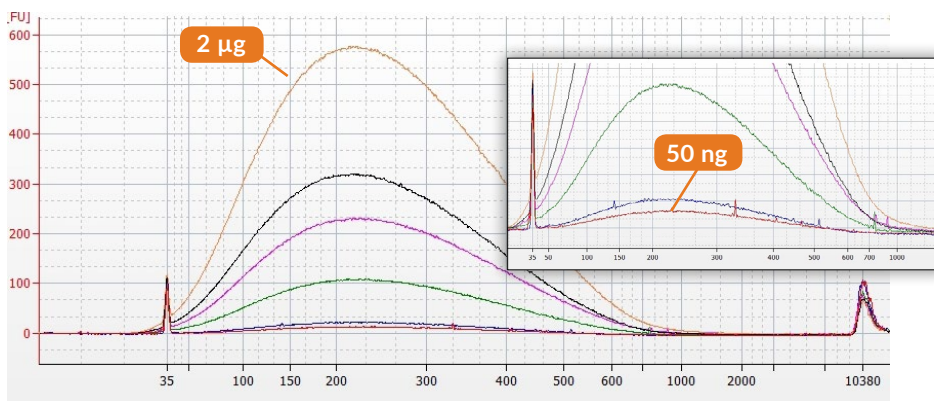
Identical Performances Across 3 Different Genomes with Different GC Contents

E. coli - 50.6% GC

*R. palustris* - 66% GC

V. fischeri - 39% GC
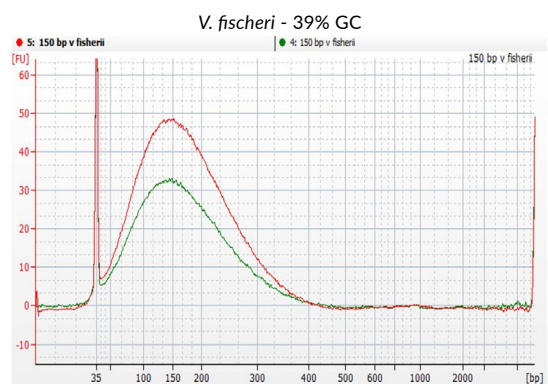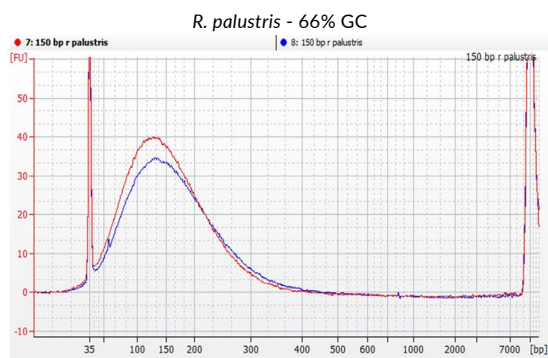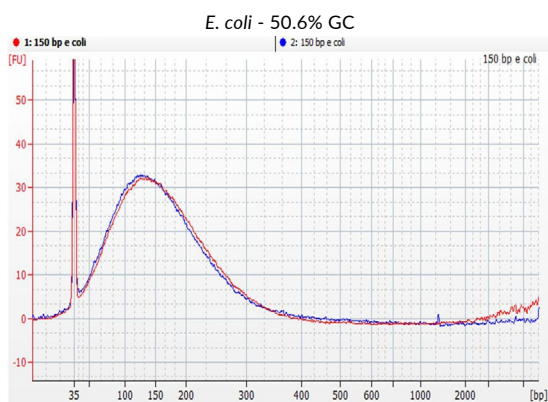
Library Construction and Sequencing

DNA fragmentation of *E. coli* DNA was performed with a M220 Focused-ultrasonicator and libraries were constructed using the Ion Plus Fragment Library Preparation kit. Libraries have been sequenced by EdgeBio (MD, USA) on an Ion Torrent PGM with 314 chip. All libraries yielded more than 40 Mbp of Q20 bases (Ion Torrent advertises 10 Mb per run for 314 chips). After alignment, percentage of genome covered is 100%.

Unbiased DNA Fragmentation

Histogram showing the overall distribution of read depths for each position along *E. coli* genome. The frequency of each read depth is shown on the Y-axis. The read depth is shown on the X-axis. Library has a perfect Gaussian distribution of read depth/frequency.
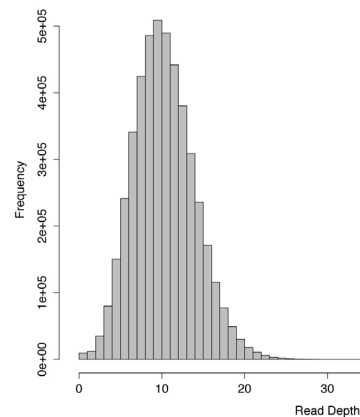
Uniform Coverage

Histogram showing the number of reads mapped at each position across *E. coli* genome. Read depth summed in 25 bp intervals is shown on the Y-axis. Position in base pairs along the genome is shown on the X-axis. Library shows a uniform and consistent coverage.
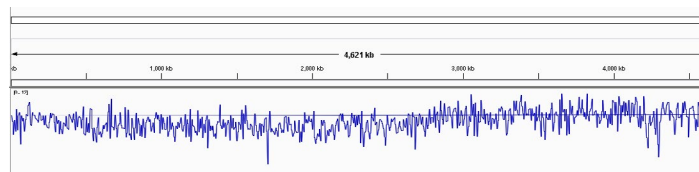
Key Features	Benefits
DNA shearing independent of DNA concentration	• Standardized DNA shearing • Click and shear
DNA shearing independent of base content	
Highly reproducible process	
Truly isothermal process	• AFA-grade fragment library • Standard in major sequencing centers worldwide
No fragmentation bias	
Tight fragment distribution	
Uniform coverage across the genome	• AFA-grade sequencing results • "The Scientist's standard" in all major sequencing centers
Normal distribution of frequency reads vs. coverage	
Same performances regardless of GC/AT percentage	