Automated NGS Library Construction with Eppendorf epMotion® 5075t/TMX and Covaris® Focused-ultrasonicators

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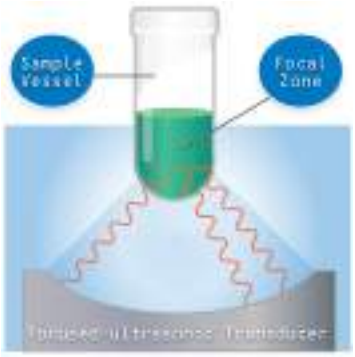
OVERVIEW

Next-Generation Sequencing (NGS) platforms require controlled generation of DNA fragments to produce high-quality sequencing data. Covaris leverages its proprietary and patented Adaptive Focused Acoustics (AFA™) technology to convert focused high-frequency acoustic energy into mechanical force to randomly fragment nucleic acids in an isothermal and non-contact environment.



Covaris combines integration of high performance control electronics, medical-grade transducers, and custom engineered acoustical cuvettes. The technology uses controlled bursts of high power acoustic energy to process samples, resulting in highly tunable randomly fragmented nucleic acids of similar size. Uniquely, all AFA focused-ultrasonicators are calibrated to National Institute of Standards and Technology (NIST) traceable standards, ensuring highest quality and standardized results. These criteria enable robust and reproducible integration into high-throughput workflows and compatibility with liquid handlers to provide the most accurate and precise NGS data.

This technical note has been developed to streamline automated NGS library preparation by integrating DNA fragmentation with Covaris AFA™ technology into Eppendorf ep*Motion*® 5075t/TMX library construction workflows, including **Illumina® TruSeq® Nano DNA** and **TruSeq® DNA PCR-Free protocols**. The Eppendorf line of ep*Motion* automated liquid handling systems are designed to help researchers automate routine pipetting tasks to free up their time. Not only is the ep*Motion* one of the most accurate pipetting stations on the market, by virtue of automation it helps to eliminate manual pipetting errors and maximizes the reproducibility of assay results. ep*Motion* is available in four different formats and with different upgrade options, giving you the flexibility to tailor the system to your specific applications. The unique software makes programming easy and allows you to set-up even complex tasks with minimal training. For more information, please visit **www.eppendorf.com/automation**.

MATERIAL AND METHODS

Covaris® ME220 Focused-ultrasonicator	**Eppendorf ep*Motion*® 5075t/TMX**
	
Focused-ultrasonicator for mechanical DNA shearing	**Automated liquid handling system**

MFR.	Consumables & Accessories	Part Number (PN)
Covaris	8 microTUBE-50 Strip AFA Fiber H Slit V2	520240
	ME220 Rack Loading Station	500523
	ME220 Rack 8 microTUBE Strip V2	500518
	ME220 Waveguide 8 Place	500526
	E-Series Rack 12 Place 8 microTUBE Strip V2	500444
Eppendorf	TM50_8 Dispensing Tool	5280 000.215
	Thermoadapter for PCR Plates, 96-well, Skirted	5075 787.008
	Eppendorf Adapter for Covaris Rack 12 x 8 Strips	5075 751.801
	epT.I.P.S.® *Motion* 50 µL Filtered	0030 014.413
	Eppendorf twin.tec® PCR Plates, LoBind, 96-well, Semi Skirted	0030 129.504
	Eppendorf twin.tec PCR Plates, LoBind, 96-well, Skirted	0030 129.512

SAMPLE INPUT

4ng/µl Human genomic DNA (Promega, Mannheim, Germany) in low TE Buffer (10mM Tris pH 8, 0.1mM EDTA) was used to assess transfer efficiency and shearing on the ME220 Focused-ultrasonicator.

AUTOMATION WORKFLOW AND SETUP

This workflow is designed for the construction of 8/16 or 24 Illumina sequencing ready libraries using the ep*Motion*. The first stage of library preparation, fragmentation of DNA with Covaris Focused-ultrasonicators, is incorporated into this automated method as sub-method 1 & 2. Transfer steps are completely automated and controlled by the epBlue-Software.

ep*Motion* Sub-method 1 ▼	Transfer of input DNA from Eppendorf twin.tec PCR plates into 8 microTUBE-50 strip
Covaris ▼	DNA fragmentation using the Covaris ME220 Focused-ultrasonicator
ep*Motion* Sub-method 2 ▼	Transfer of fragmented DNA into 8 microTUBE-50 strip into Eppendorf twin.tec semi-skirted PCR plates
ep*Motion* ▼	NGS Library construction of Illumina libraries following Eppendorf protocols

ep*Motion* Sub-method 1

Strips were filled using the 50 µl multichannel tool with optimized parameters. Samples were transferred from twin.tec PCR plates, LoBind, 96-well, skirted into 8 microTUBE-50 strips positioned in a rack adapter, following the deck layout in Figure 2. After transfer the samples were removed from the rack adapter for shearing on the ME220.

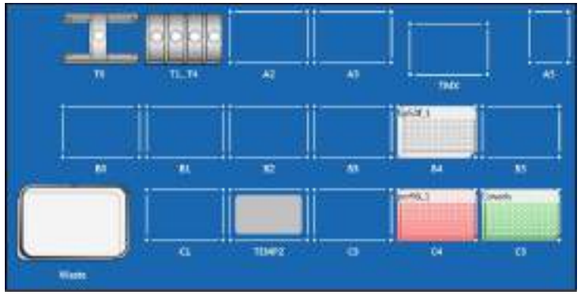


FIGURE 2: ep*Motion* deck layout for sub-method 1. Position B4 contains 50 µl Filtertips, C4 holds the skirted PCR plate containing DNA and C5 contains the rack adapter with 8 microTUBE-50 strips.

The Covaris 8 microTUBE-50 strips were loaded into the E-series Rack and onto the Eppendorf adapter for use with the ep*Motion*, as shown in Figure 1.

FIGURE 1: ep*Motion* deck layout with Covaris 8 microTUBE-50 strips in the rack and adapter at position C5.

DNA shearing using the ME220 Focused-ultrasonicator

Mechanical DNA shearing settings were based on Covaris standard parameters for fragmentation to achieve 200bp (Table 1).

Target BP (Peak)	200 bp
Peak Incident Power (W)	50
Duty Factor (%)	30
Cycles per Burst	1000
Treatment Time (s)	125
Water Temperature (°C)	9
Water Level	5.5

TABLE 1: Settings for shearing using an 8 microTUBE–50 Strip AFA Fiber H Slit V2 with the ME220.

ep*Motion* Sub-method 2

Fragmented DNA in the 8 microTUBE-50 strips were secured in the rack adapter and emptied using the 50 µl multichannel tool with optimized settings. 52.5 µl of sheared DNA was transferred into a semi-skirted twin.tec PCR LoBind 96-well PCR plate on a thermoadapter, following the deck layout in Figure 3.

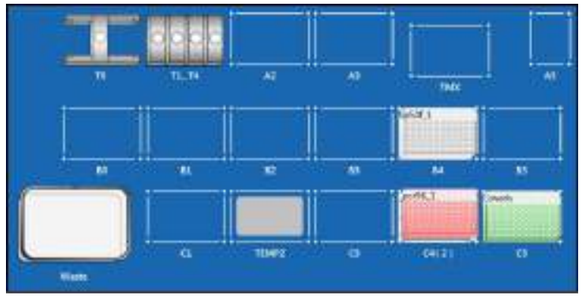


FIGURE 3: ep*Motion* deck layout for sub-method 2. Position B4 contains 50 µl Filtertips, C4 holds the thermoadapter PCR 96 + PCR Plate and C5 contains the rack adapter with 8 microTUBE-50 strips with fragmented DNA.

Sample volume weighing

The most accurate assessment of the amount of liquid transferred could be made during the filling process. The 8 microTUBE-50 strip was weighed on a Sartorius Cubis Analytical MSE3245-000-DU Balance (Satorius Weighting Technology GmbH, Göttingen, Germany) before and after being filled by the ep*Motion* 5075t/TMX. The weight change was recorded as an average change and converted to microliters per microTUBE for each strip.

RESULTS

	Strip 1	Strip 2	Strip 3	Average	CV (%)
DNA Volume Filled per microTUBE (µl)	54.1	53.9	53.8	53.9	0.31
Average DNA Fragment Peak Size (bp)	204	203	204	204	0.29

TABLE 2: Average fill volume transferred using the ep*Motion* and average sheared DNA fragment sizes for 3 microTUBE-50 strips.

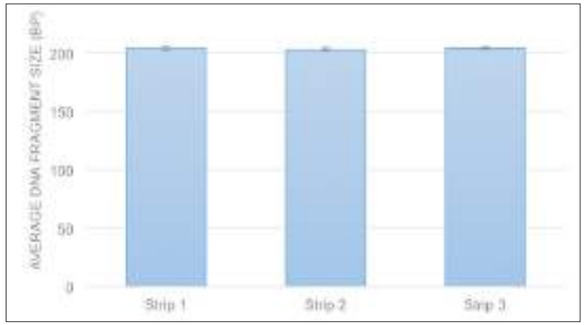


FIGURE 4: Average DNA fragment size and standard deviation for each 8 microTUBE-50 strip. Target size is 200bp.

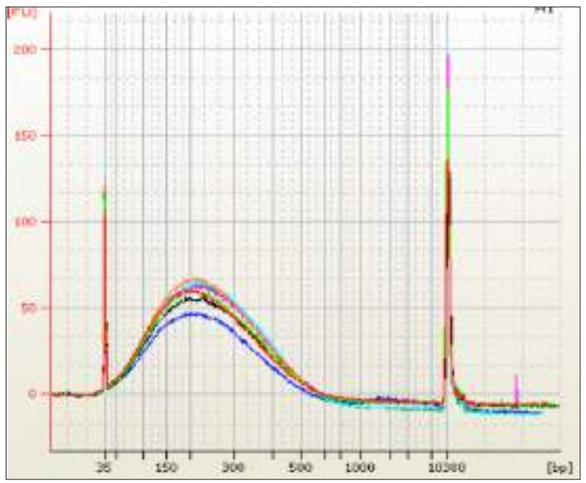


FIGURE 5: Overlay of 8 samples in one 8 microTUBE-50 strip fragmented to 200bp using the ME220, measured by Agilent Bioanalyzer High Sensitivity DNA Chip.

The results show DNA transfer was highly consistent, with a coefficient of variation (cv) of 0.31% (Table 2). Furthermore sample recovery was efficient, with at least 52.5 µl of sample recovered from each microTUBE, as required for downstream processing with Illumina® TruSeq® Nano DNA and TruSeq® DNA PCR-Free protocols.

Fragmentation of DNA was also highly reproducible, with a fragment size cv of 0.29%. Tight fragment size distributions were observed with >95% of fragments within the 50 to 500bp size range.

CONCLUSION

The Covaris ME220 Focused-ultrasonicator combined with the Eppendorf ep*Motion* 5075t/TMX liquid handler enables automated sample transfer both before and after mechanical DNA fragmentation. Importantly the results show highly accurate volume transfer, and subsequent tightly controlled DNA fragmentation - critical for producing high quality NGS data.

This technical note provides users with a fully automated solution for integrating DNA fragmentation using Covaris batch processing instruments, such as the ME220, with the ep*Motion* 5075t/TMX liquid handling system for automated NGS library preparation workflows. After executing the described method, the DNA is directly ready for downstream processing.

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