NEBNext UltraShear™: Novel Enzymatic Fragmentation for Challenging Samples and Methods.

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Next Generation Sequencing (NGS) requires fragmentation of DNA molecules upstream of sequencing. Current methods for fragmentation include mechanical shearing and enzymatic fragmentation for NGS library preparation. Mechanical shearing requires costly instruments, can be difficult to automate for high throughput labs, and results in sample loss. In comparison, enzymatic fragmentation methods do not require expensive instruments and are automation friendly. However, current enzymatic fragmentation methods on the market can remove DNA modifications such as methylation, cement DNA damage resulting from formalin fixation in final libraries and can introduce sequencing artifacts or bias. Therefore, while many fragmentation methods are commercially available, challenges remain for sequencing samples such as FFPE DNA or for detecting DNA modification (e.g., 5-methylcytosine).

We have developed NEBNext UltraShear, a novel enzymatic fragmentation method, that can be used upstream of library preparation for DNA methylation assessment or FFPE DNA. NEBNext UltraShear is quick and robust, taking as little as 20 minutes. It is automation-friendly and can be used to generate DNA fragments ranging from as small as 50 bp up to over 1000 bp.

Here, we demonstrate the use of the novel enzymatic fragmentation with FFPE DNA library preparation for WGS. Sequencing metrics greatly improved with our novel method compared to mechanically sheared DNA and other enzymatic fragmentation methods. Additionally, FFPE damage derived mutations (typically C to T mutations) were reduced.

NEBNext UltraShear was also used upstream of the NEBNext® Enzymatic Methyl-Seq (EM-Seq™) workflow for methylome analysis. This fragmentation method does not erase the methylation marks in genomic DNA. These libraries showed improvements in library yield, library complexity and other sequencing metrics over libraries generated with mechanically sheared DNA.

Fragmenting DNA to defined sizes for diverse applications and sample types remains a challenging aspect of NGS library preparation. This new, robust enzymatic fragmentation method overcomes the limitations of some traditional mechanical and other enzymatic fragmentation methods and improves the library preparation and sequencing metrics for both DNA modification assessment studies and FFPE DNA.