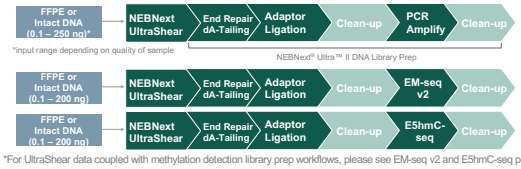
NEBNext enzymatic solutions for challenging samples & workflows

Brittany S. Sexton, Margaret R. Heider, Jian Sun, Louise Williams, Matthew Angel, Bradley W. Langhorst
Pingfang Liu & V K Chaithanya Ponnaluri | New England Biolabs, Inc.

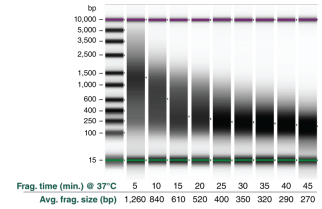
NEBNext UltraShear® (NEB# M7634)

UltraShear fragments FFPE and intact DNA for DNA library prep workflows



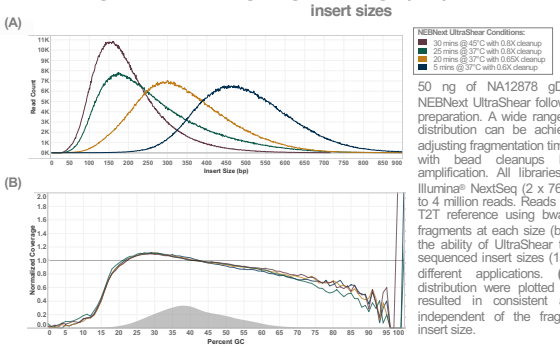
*For UltraShear data coupled with methylation detection library prep workflows, please see EM-seq v2 and E5hmC-seq poster.

UltraShear fragments intact gDNA in a time-dependent manner



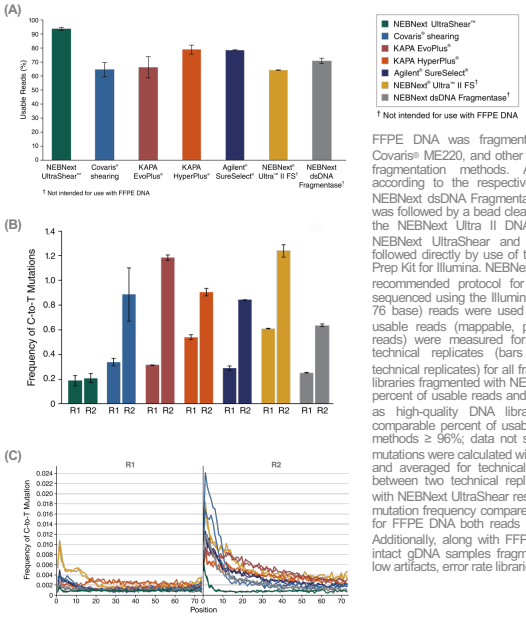
NA12878 DNA was fragmented for 5–45 minutes at 37°C followed by 15 minutes at 65°C. Fragmentation occurs during the 37°C incubation step of NEBNext UltraShear. The average fragmentation size and pattern (High Sensitivity D5000 ScreenTape® on Agilent® TapeStation®) is based on fragmentation time.

UltraShear fragmentation of intact gDNA generates high-quality DNA libraries & tunable sequenced insert sizes



50 ng of NA12878 gDNA was fragmented by NEBNext UltraShear followed by Ultra II DNA library preparation. A wide range of sequenced insert size distribution can be achieved using UltraShear by adjusting fragmentation times and temperatures along with bead cleanups before and after PCR amplification. All libraries were sequenced on an Illumina® NextSeq (2 x 76 bases) and downsampled to 4 million reads. Reads were aligned to the human T2T reference using bwa. **(A)** The read count of fragments at each size (bp) were plotted, illustrating the ability of UltraShear to generate wide range of sequenced insert sizes (150 bp to 450 bp), enabling different applications. **(B)** The GC coverage distribution were plotted across all conditions and resulted in consistent and even GC coverage independent of the fragmentation conditions and insert size.

UltraShear fragmentation of FFPE DNA improves usable reads & reduces artificial mutations



FFPE DNA was fragmented using NEBNext UltraShear, Covaris® ME220, and other commercially available enzymatic fragmentation methods. All samples were fragmented according to the respective protocols. Fragmentation with NEBNext dsDNA Fragmentase, Kapa kits and the Agilent kit was followed by a bead cleanup and library construction using the NEBNext Ultra II DNA library Prep Kit for Illumina®. NEBNext UltraShear and Covaris-sheared samples were followed directly by use of the NEBNext Ultra II DNA Library Prep Kit for Illumina. NEBNext Ultra II FS samples followed the recommended protocol for library prep. Each library was sequenced using the Illumina NextSeq® 500. Two million (2 x 76 base) reads were used for this analysis. **(A)** Percent of usable reads (mappable, proper pairs, and non-duplicates reads) were measured for each library and averaged for technical replicates (bars represent error between two technical replicates) for all fragmentation methods. FFPE DNA libraries fragmented with NEBNext UltraShear had the highest percent of usable reads and had similar percent usable reads as high-quality DNA libraries (high-quality DNA had a comparable percent of usable reads across all fragmentation methods ≥ 96%; data not shown). **(B & C)** Artificial C to T mutations were calculated with Tasmanian tool for read 1 and 2 and averaged for technical replicates (bars represent error between two technical replicates). The libraries fragmented with NEBNext UltraShear resulted in the lowest C to T artificial mutation frequency compared to other fragmentation methods for FFPE DNA both reads (R1= Read 1 and R2= Read 2). Additionally, along with FFPE gDNA it has been shown that intact gDNA samples fragmented with UltraShear generates low artifacts, error rate libraries (Lawson et al. *Nature*, 2025[1]).

Conclusions

- NEBNext UltraShear enzymatic fragmentation is optimized for use with intact gDNA and FFPE DNA samples for both Ultra II DNA library prep and methylation detection library prep workflows (e.g. EM-seq & E5hmC-seq).
- UltraShear is a time-dependent fragmentation method that can be tuned to generate varying sequenced insert sizes and high-quality libraries for intact gDNA samples.
- UltraShear works on a wide range of input amounts (0.1 – 250 ng).
- FFPE and intact gDNA (Lawson et al. *Nature*, 2025[1]) fragmented with UltraShear generates low artifacts & error rate libraries.

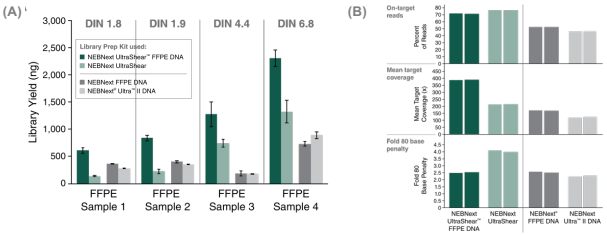
References: 1. Lawson, A.R.J., Abascal, F., Nicola, P.A. *et al.* Somatic mutation and selection at population scale. *Nature* **647**, 411–420 (2025). https://doi.org/10.1038/s41586-025-09584-w

NEBNext UltraShear® FFPE DNA Library Prep Kit (NEB# E6655)

A library prep workflow designed to improve the data output and sequencing accuracy from FFPE DNA independent of sample quality

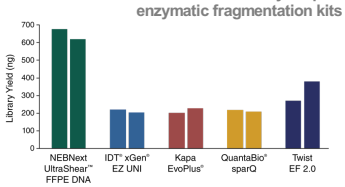
* New enzyme mixes optimized for FFPE library prep

Use of NEBNext FFPE DNA Repair v2 upstream of NEBNext UltraShear improves library yield and coverage



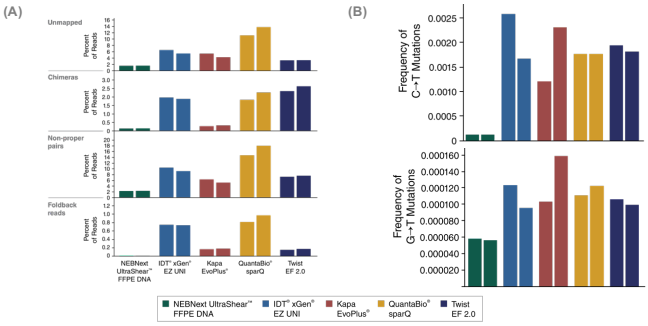
Libraries were prepared in duplicate from 50 ng of unsheared FFPE DNA ranging in quality from DIN 1.8 to DIN 6.8 using either the NEBNext UltraShear FFPE DNA Library Prep Kit or NEBNext UltraShear with the NEBNext Ultra™ II DNA Library Prep Kit. The NEBNext FFPE DNA Library Prep Kit or the NEBNext Ultra II DNA Library Prep Kit were used to prepare libraries from the same FFPE DNA samples sheared to 350 bp with the Covaris® ME220 instrument. UMI-containing adaptors and 9 PCR cycles were used for all libraries. (A) Final library yield was quantified using the Qubit High-Sensitivity dsDNA Assay. The highest library yield is obtained when combining FFPE DNA Repair v2 with UltraShear fragmentation. (B) FFPE DNA (DIN 1.8) libraries were then captured using a custom panel designed with Twist Bioscience and sequenced on the NovaSeq6000 to 15 million paired-end reads. Target enrichment quality metrics were obtained using Picard HS Metrics (v. 2.18.29). The improved yield obtained with FFPE DNA Repair v2 and UltraShear (NEBNext UltraShear FFPE DNA Library Prep Kit) translates to improved complexity (higher mean target coverage) and improved coverage uniformity (lower Fold-80 base penalty).

NEBNext UltraShear FFPE Library Prep Kit enables higher yields than competitor enzymatic fragmentation kits for low quality FFPE DNA



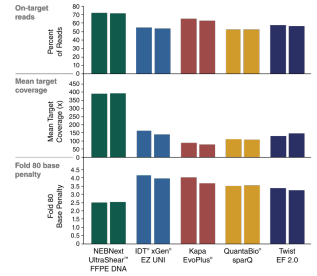
The NEBNext UltraShear FFPE DNA Library Prep Kit enables higher library yields than competitor library prep kits. Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, using each vendor's own recommended adaptors Note: kits from Agilent® and Watchmaker Genomics were not available for purchase.

Enzymatic Fragmentation and DNA repair improve library quality and sequencing accuracy



The NEBNext UltraShear FFPE DNA Library Prep Kit improves library quality and sequencing accuracy compared to competitor library prep kits. (A) Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, using each vendor's own recommended adaptors. Libraries were sequenced on the Illumina® NovaSeq® 6000 (2 x 100 base reads) and library quality metrics were assessed using Picard Alignment Summary Metrics (version 1.56.0). The level of foldback reads was calculated using Seq_frag_remap (version 0.2). The NEBNext UltraShear FFPE DNA Library Prep Kit improves library quality by reducing the percentage of unmapped, chimeric, non-properly paired, and foldback reads. (B) The average frequency of C→T mutations at each C position (top) and G→T mutations at each G position (bottom) in Read 1 and 2 was calculated for two technical replicates using Tasmanian (version 1.0.7). C→T mutations arising from cytosine deamination and G→T mutations arising from oxidative damage in low quality FFPE DNA are effectively repaired by the NEBNext FFPE DNA Repair v2 Mix in the NEBNext UltraShear FFPE DNA Library Prep Kit. Other kits show a high level of C→T and G→T artifacts in low quality FFPE DNA due to a lack of DNA damage repair.

NEBNext UltraShear FFPE DNA Library Prep enables high on-target coverage in hybrid capture



Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, with each vendor's own recommended adaptors (IDT xGen EZ UNI, Kapa EvoPlus Library Prep Kit, QuantaBio sparQ DNA Library Prep Kit, and Twist Library Preparation EF 2.0 kit). The full library yield was used in singleplex target enrichment with a custom cancer panel (Twist Bioscience) and libraries were sequenced on the Illumina NovaSeq 6000 (2 x 100 base reads). Fifteen million paired-end reads were trimmed with Fastp (version 0.20.0) and mapped with BWA mem (version 0.7.17) to the T2T reference. Duplicates were marked using Picard MarkDuplicates (version 2.20.6) with UMI. Target enrichment quality metrics were assessed using Picard HS Metrics (version 2.18.29). The improved yield, coverage, and fraction of usable reads observed in NEBNext UltraShear FFPE DNA Library Prep Kit whole genome sequencing (WGS) libraries correlates to improved coverage, on-target rate, and coverage uniformity in target enrichment libraries.

Conclusions

- New and more efficient enzymatic DNA repair using NEBNext® FFPE DNA Repair v2
- New NEBNext UltraShear enzymatic fragmentation mix optimized for use with FFPE DNA
- New NEBNext MSTC FFPE PCR Master Mix to achieve high yields for target enrichment
- 5 – 250 ng input of FFPE DNA required, validated on FFPE with DIN 1.5 to 6
- Compatible with high quality DNA for convenience in processing with matched high-quality DNA

We thank Dora Posfai, Kristen Augulewicz, and Harry Bell for their technical support and members of NEBNext for productive discussions.