NEBNext® solutions for challenging methods & samples

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NEBNext UltraShear® (NEB# M07634)

NEBNext UltraShear improves libraries for challenging methods & samples compared to other fragmentation methods

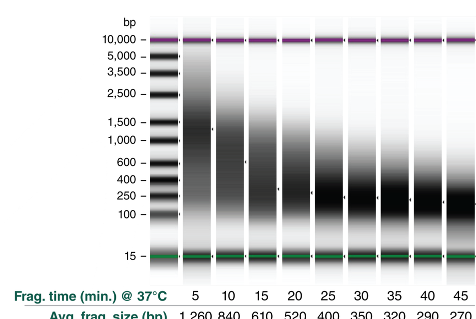
5mC Library Prep:

- Higher library yields
- Improved sequencing metrics
- Improved CpG Coverage

FFPE DNA Library Prep:

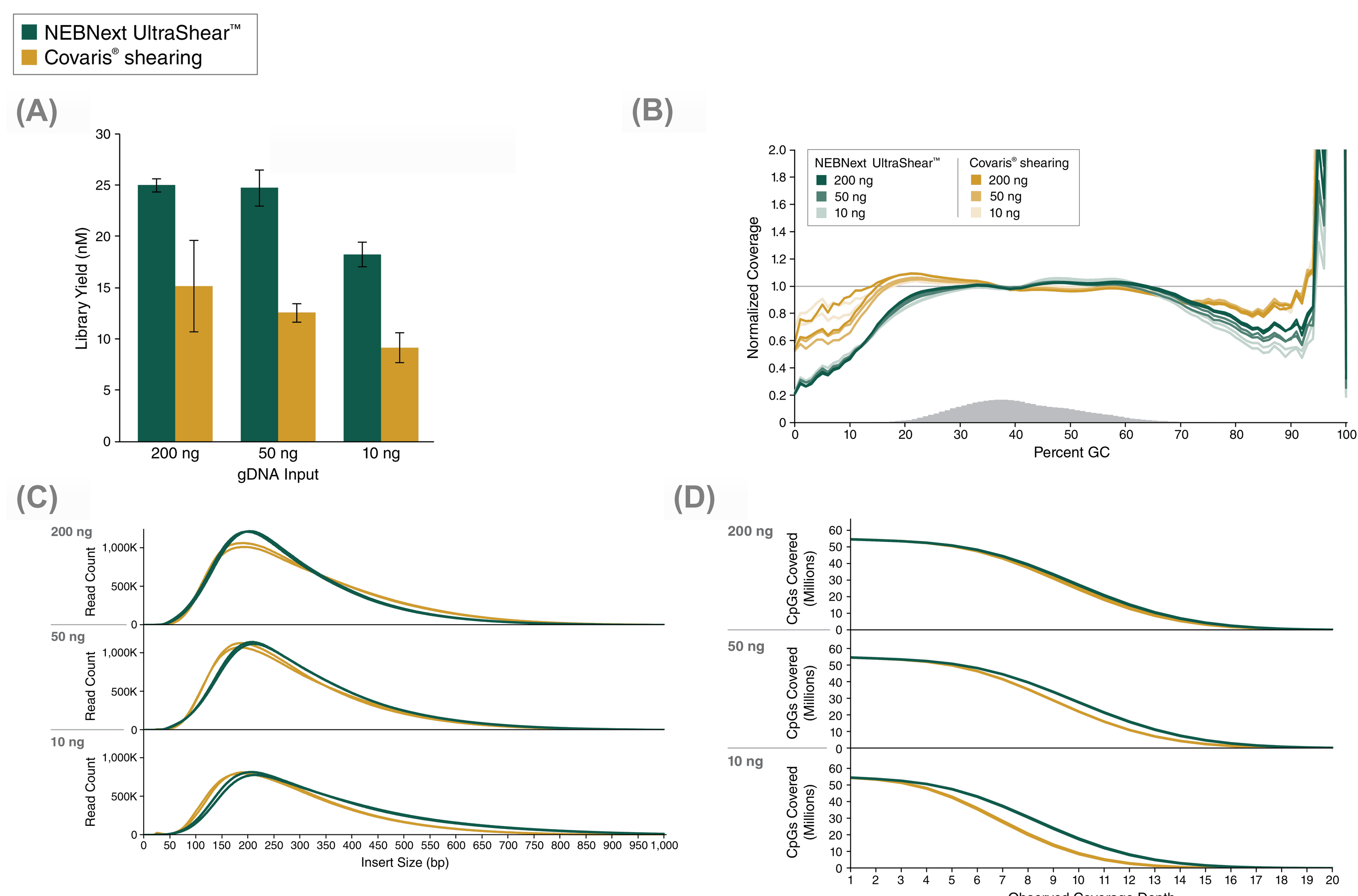
- Improved sequencing metrics
- Reduced artificial mutations

NEBNext UltraShear fragments high-quality gDNA in a time-dependent manner



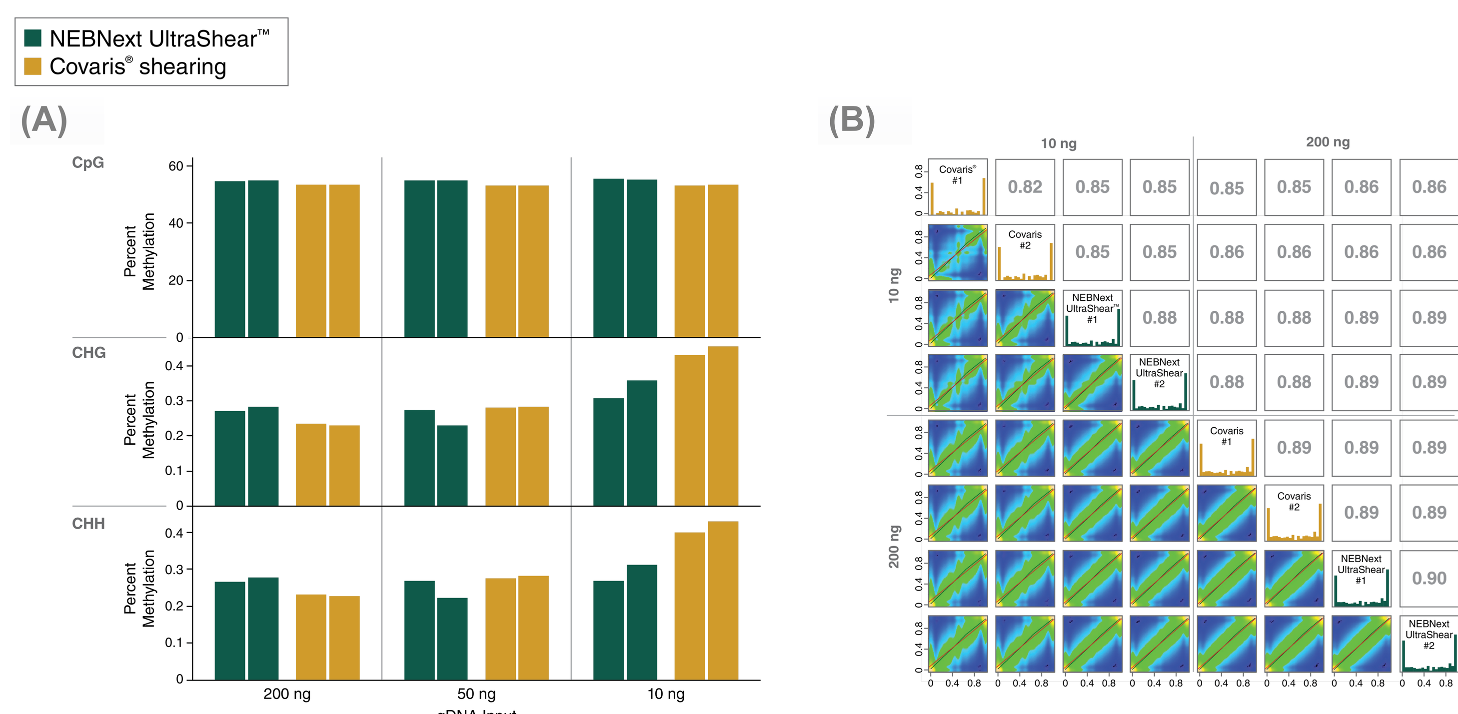
NA12878 DNA was fragmented for 5–45 minutes at 37°C followed by 15 minutes at 65°C. Fragmentation occurs during the 37°C incubation step of NEBNext UltraShear. The average fragmentation size and pattern (High Sensitivity D5000 ScreenTape® on Agilent® TapeStation®) is based on fragmentation time.

Improved library yields & sequencing metrics in EM-seq™ libraries produced using NEBNext UltraShear



NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) was fragmented by either NEBNext UltraShear or Covaris® ME220 followed by EM-seq library preparation. Technical replicates were generated for each input amount. All libraries were sequenced on the same flowcell of an Illumina® NovaSeq® 6000 (2 x 100 bases). 725 million reads were sampled from each library for methylation analysis. **(A)** Library yields were quantified using Agilent TapeStation with the High Sensitivity D1000 ScreenTape. EM-seq libraries fragmented by NEBNext UltraShear have higher yields than Covaris for the same number of PCR cycles for each input. **(B)** The read count of inserts at each size (bp) was plotted, illustrating that NEBNext UltraShear and Covaris fragmentation resulted in comparable sequenced insert sizes. **(C)** The GC distribution for NEBNext UltraShear and Covaris and across inputs for EM-seq libraries were plotted. The GC distributions are similar in the 30% to 60% GC, which contains most of the genome. **(D)** NEBNext UltraShear and Covaris fragmentation used ahead of the EM-seq workflow yielded a similar number of CpGs (~54 million) at minimum 1X coverage. At minimum 10X coverage, more CpGs are identified when NEBNext UltraShear is used, due to improved library diversity and coverage evenness.

Similar methylation is detected by EM-seq with NEBNext UltraShear & Covaris



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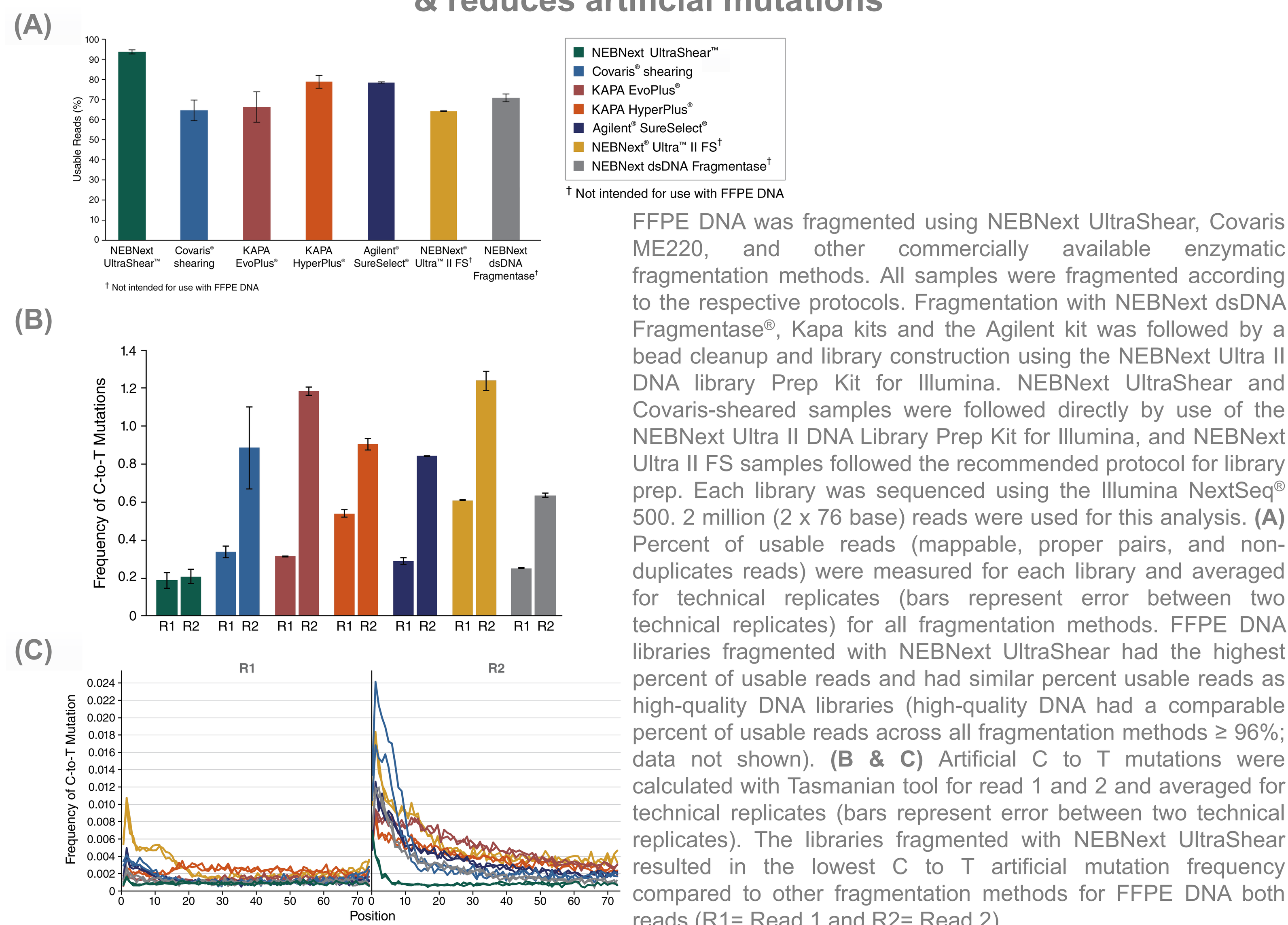
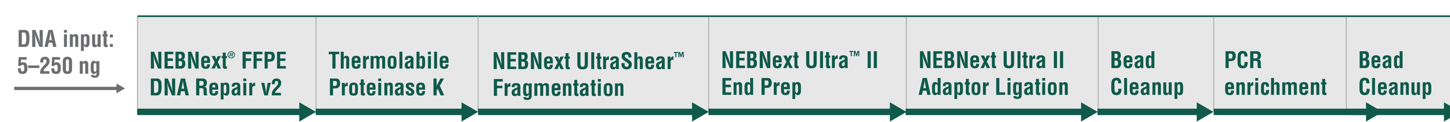
NEBNext UltraShear with FFPE DNA improves usable reads & reduces artificial mutations



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, and NEBNext Ultra II FS samples followed the recommended protocol for library prep. Each library was sequenced using the Illumina NextSeq® 500. 2 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).

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

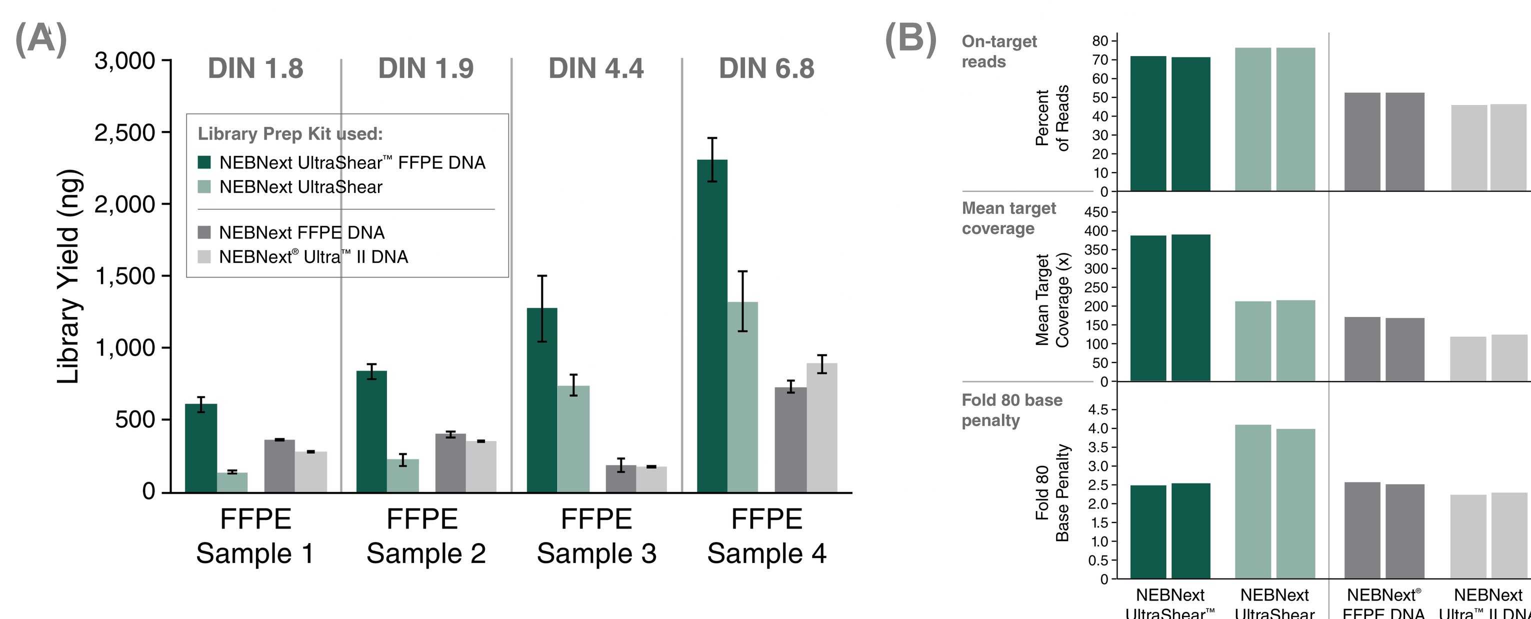
A library prep workflow designed to improve the data output and sequencing accuracy from FFPE DNA independent of sample quality



* New enzyme mixes optimized for FFPE library prep

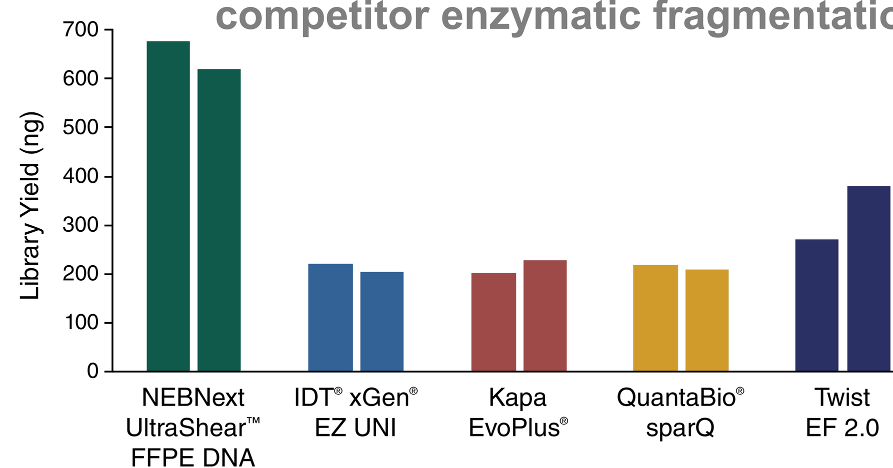
- New and more efficient enzymatic DNA repair using NEBNext FFPE DNA Repair v2
- New NEBNext UltraShear enzymatic fragmentation mix optimized for use with FFPE DNA
- New NEBNext MSTC™ FFPE PCR Master Mix to achieve high yields for target enrichment
- 5 – 250 ng input of FFPE DNA required, validated on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

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



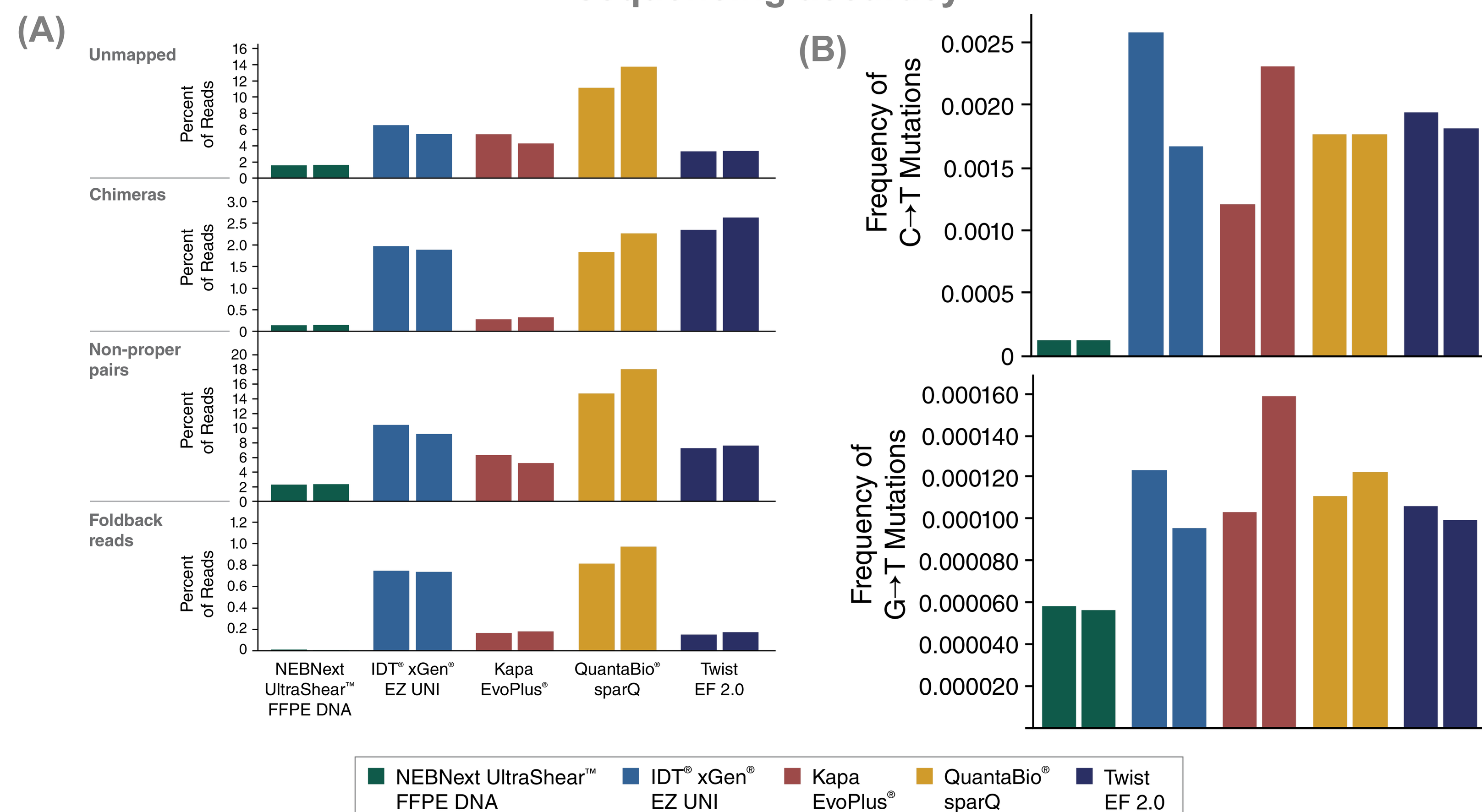
Libraries were prepared in duplicate from 50 ng of unsheared FFPE DNA ranging in quality from DIN 1.8 to DIN 6.8 using either the NEBNext UltraShear FFPE DNA Library Prep Kit or NEBNext UltraShear with the NEBNext Ultra II DNA Library Prep Kit. The NEBNext FFPE DNA Library Prep Kit or the NEBNext Ultra 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, and final library yield was quantified using the Qubit High-Sensitivity dsDNA Assay (A). (B) FFPE DNA 1.8 libraries were then captured using a custom panel designed with Twist Bioscience and sequenced on the NovaSeq6000 to 15M PE 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



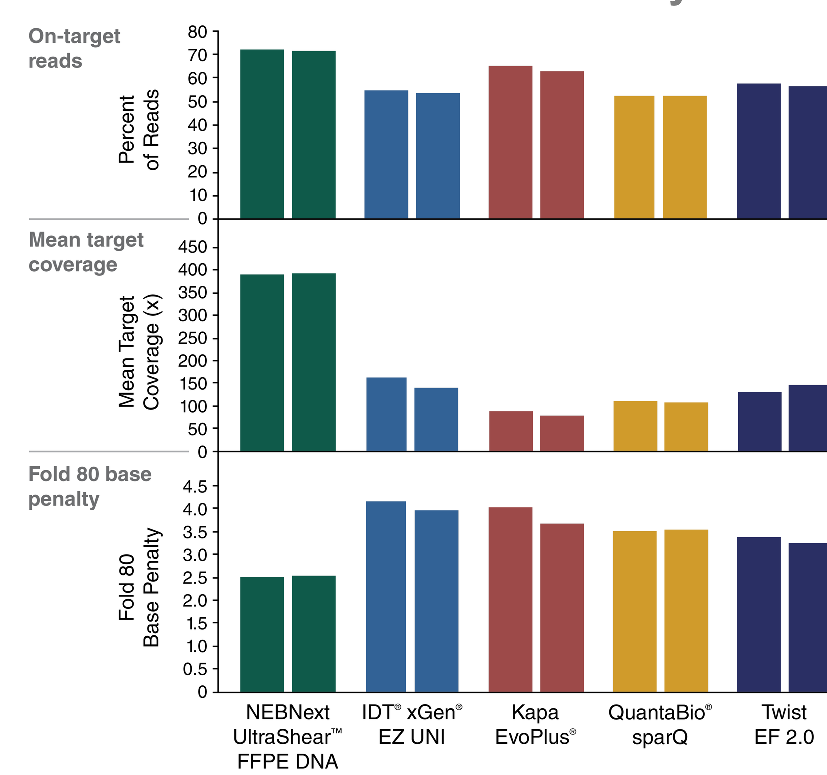
The NEBNext UltraShear FFPE DNA Library Prep Kit enables higher library yields than competitor library prep kits. Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared 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 (left) and G→T mutations at each G position (right) 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



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). 15 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). 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.