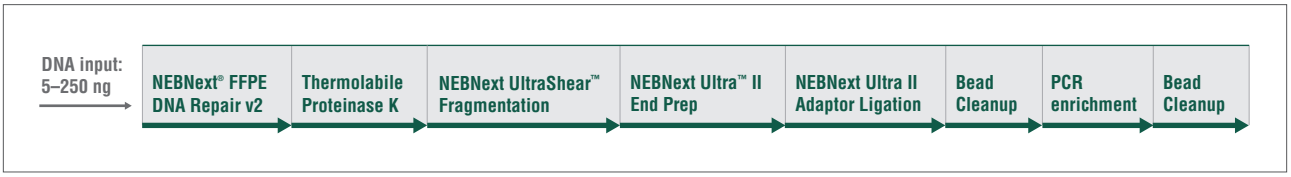
NEBNext UltraShear™ FFPE DNA Library Prep Kit

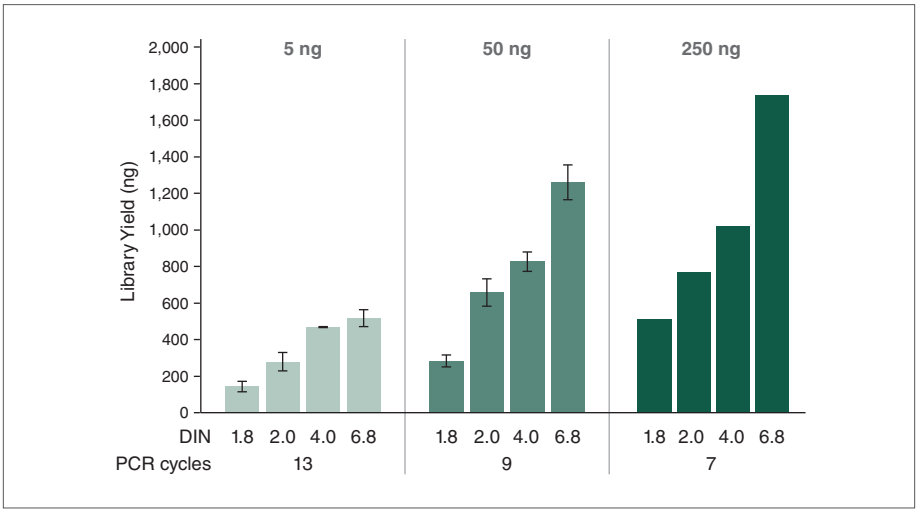
NEB #E6655

FIGURE 1: **NEBNext UltraShear FFPE DNA Library Prep Kit workflow overview**



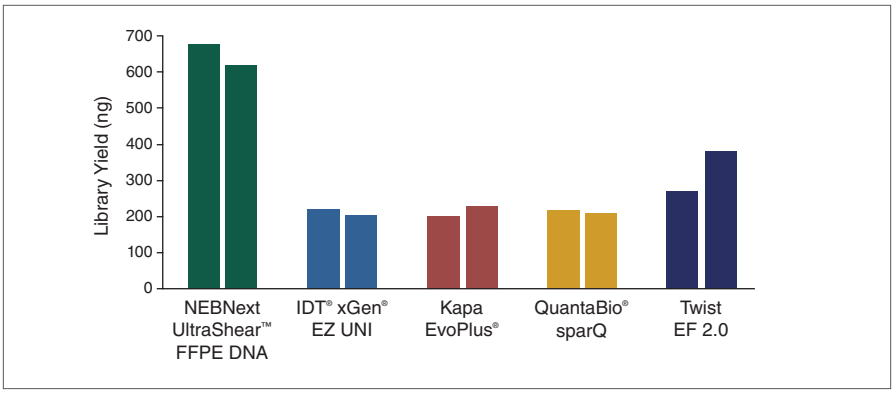
The NEBNext UltraShear FFPE DNA Library Prep Kit has a streamlined workflow with minimal hands-on time across a range of inputs from 5–250 ng. The protocol has been optimized for the user to safely store the reaction after any step in the workflow overnight at -20°C without affecting library yield or quality.

FIGURE 2: **The NEBNext UltraShear FFPE DNA Library Prep Kit enables robust library preparation from a range of sample input and quality**



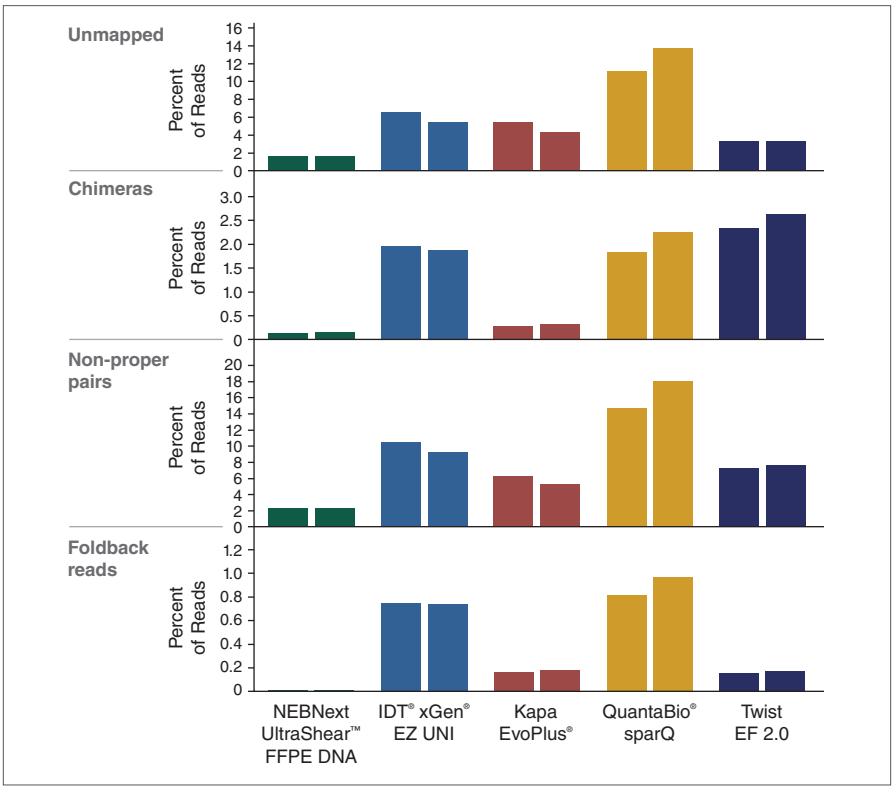
Libraries were prepared from 5, 50, or 250 ng of normal tissue FFPE DNA ranging in quality from DNA Integrity Number (DIN) 1.8 to 6.8, with the indicated PCR cycles. Libraries were prepared in triplicate for 5 ng and 50 ng input and 1 replicate for 250 ng. Each bar represents the average of triplicates with error bars indicating standard deviation for the 5 and 50 ng inputs. Robust library yields were obtained across sample qualities and input amounts. Most target enrichment workflows require 200 ng library for hybrid capture input and sufficient library yield can be obtained using a minimum of 50 ng FFPE DNA with the NEBNext UltraShear FFPE DNA Library Prep Kit.

FIGURE 3: **The NEBNext UltraShear FFPE DNA Library Prep Kit enables higher library yields**



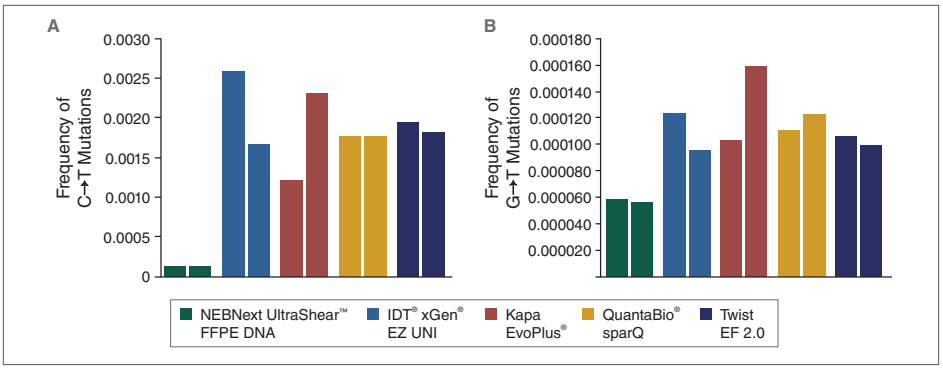
Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, using each vendor's own recommended adaptors (IDT® xGen® EZ UNI, Kapa EvoPlus® Library Prep Kit, QuantaBio® sparQ DNA Library Prep Kit, and Twist Library Preparation EF 2.0 kit). Library yields (total ng) were quantified using the Qubit® High-Sensitivity dsDNA assay (Thermo Fisher Scientific®). The NEBNext UltraShear FFPE DNA Library Prep Kit enables higher library yields, sufficient for target enrichment library input.

FIGURE 4: **The NEBNext UltraShear FFPE DNA Library Prep Kit improves library quality**



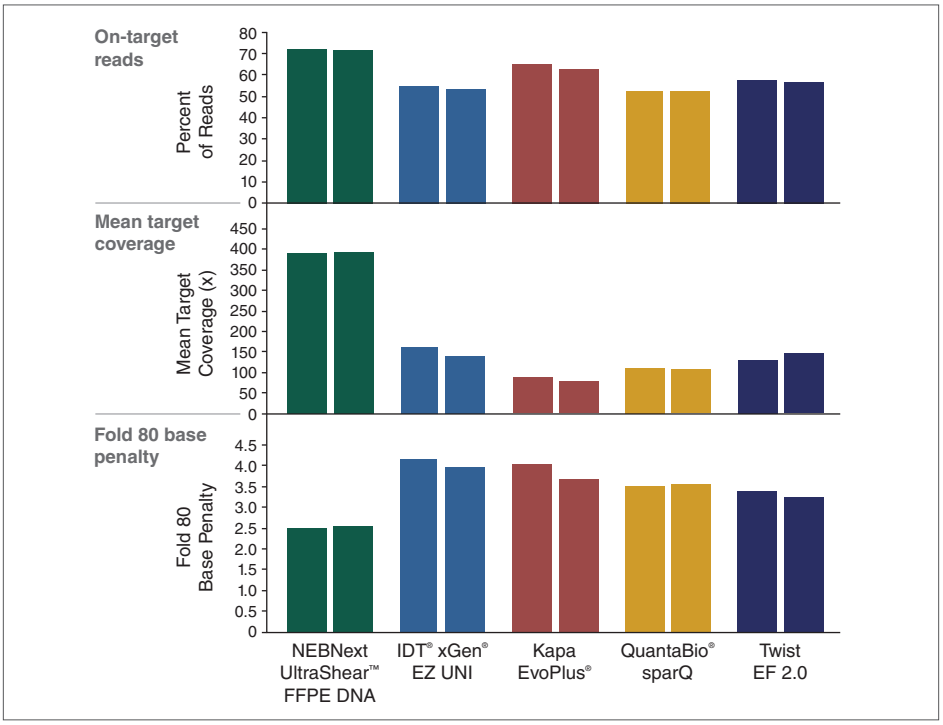
Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, using each vendor's own recommended adaptors (IDT xGen EZ UNI, Kapa EvoPlus Library Prep Kit, QuantaBio sparQ DNA Library Prep Kit, and Twist Library Preparation EF 2.0 kit). Libraries were sequenced on the Illumina® NovaSeq® 6000 (2 x 100 base reads) and downsampled to 5 million paired-end reads. Reads were mapped using Bowtie2 (version 2.3.2.2) to the GRCh38 reference and duplicates marked using Picard MarkDuplicates (version 1.56.0). 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.

FIGURE 5: **The NEBNext UltraShear FFPE DNA Library Prep Kit reduces sequencing artifacts**



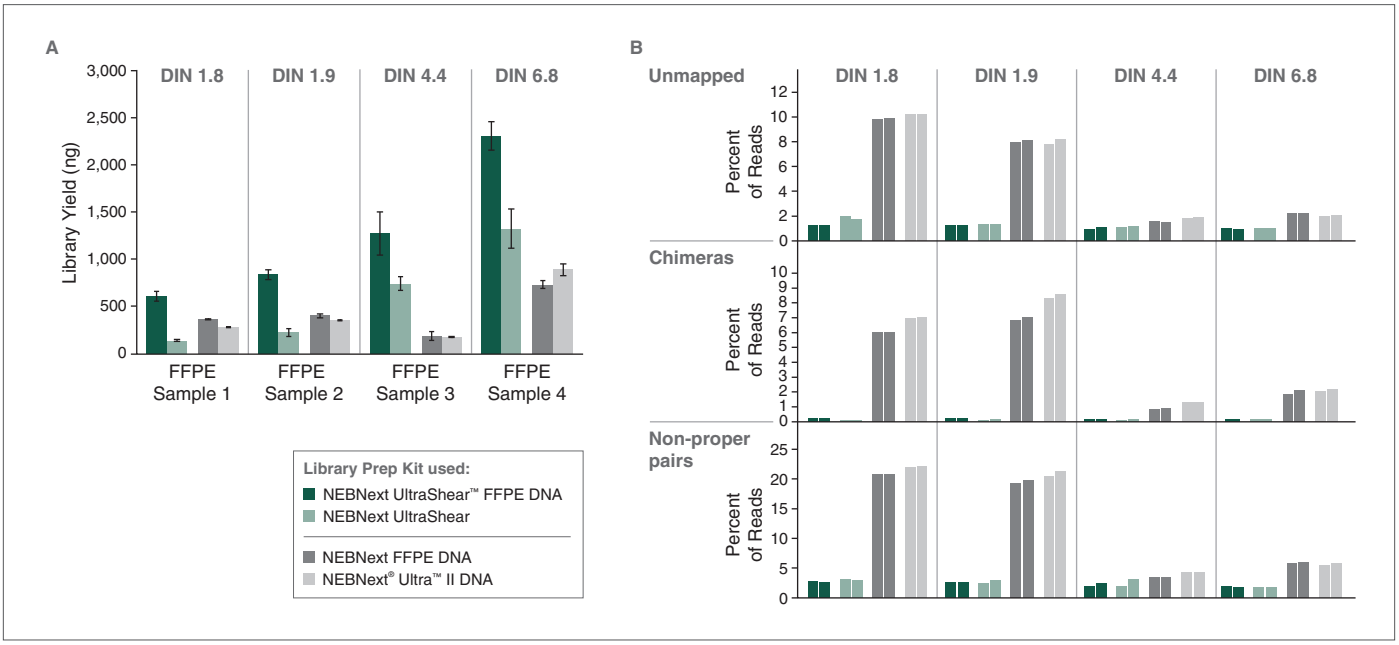
Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, 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). Libraries were sequenced on the Illumina NovaSeq 6000 (2 x 100 base reads) and downsampled to 5 million paired-end reads. Reads were mapped using Bowtie2 (version 2.3.2.2) to the GRCh38 reference and duplicates marked using Picard MarkDuplicates (version 1.56.0). The average frequency of C→T mutations at each C position (A) and G→T mutations at each G position (B) 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.

FIGURE 6: **The NEBNext UltraShear FFPE DNA Library Prep Kit enables superior on-target coverage in hybrid capture sequencing**



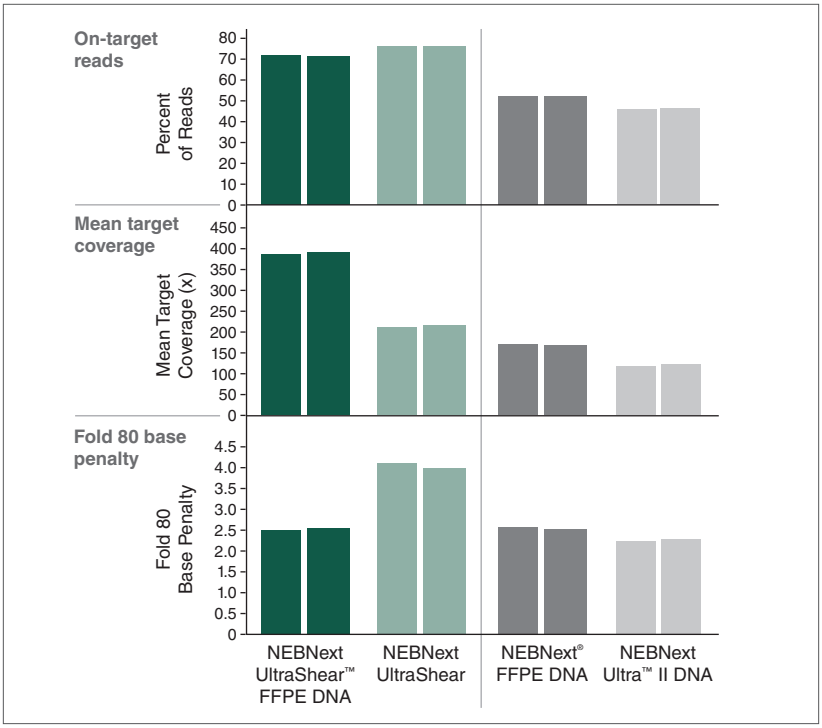
Libraries were prepared in duplicate from 100 ng of low quality, normal tissue FFPE DNA (DIN 1.8) and 9 PCR cycles, using the NEBNext UltraShear FFPE DNA Library Prep Kit. Results were compared to other enzymatic fragmentation-based library prep kits that have been validated for use with FFPE samples, 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) 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.

FIGURE 7: **The NEBNext UltraShear FFPE DNA Library Prep Kit enables superior yield and library quality**



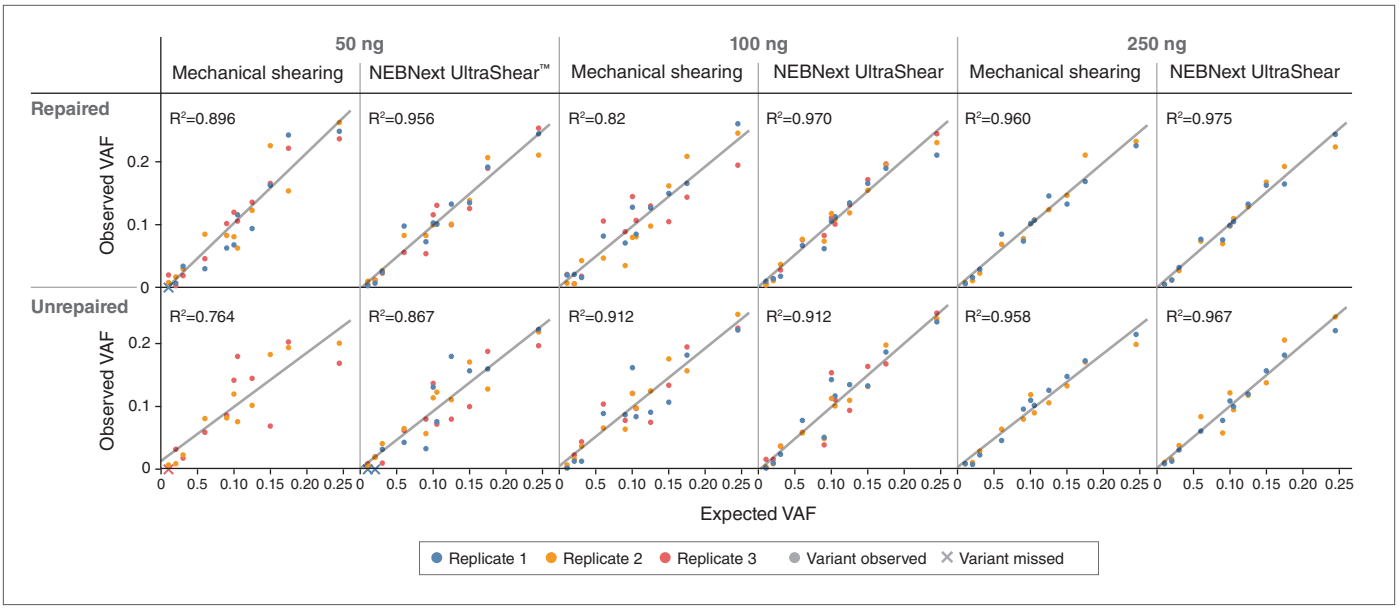
Libraries were prepared in duplicate from 50 ng of unsheared FFPE DNA ranging in quality from DIN 1.8 to DIN 6.8 using either the NEBNext UltraShear FFPE DNA Library Prep Kit or NEBNext UltraShear with the NEBNext Ultra™ II DNA Library Prep Kit. The NEBNext FFPE DNA Library Prep Kit or the NEBNext Ultra 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. 9 PCR cycles were used for all libraries, and final library yield was quantified using the Qubit High-Sensitivity dsDNA Assay (Thermo Fisher Scientific). (A) The highest library yield for all qualities of FFPE DNA was obtained when the full NEBNext UltraShear FFPE DNA Library Prep Kit was used compared to kits lacking the combination of NEBNext FFPE DNA Repair v2, NEBNext UltraShear fragmentation, and the NEBNext MSTC™ FFPE Master Mix. (B) Libraries were sequenced on the Illumina NextSeq® 500 (2 x 76 base reads). 1 million paired-end reads were mapped using Bowtie2 (version 2.3.2.2) to the GRCh38 reference and duplicates marked using Picard MarkDuplicates (version 1.56.0). Library quality metrics were assessed using Picard Alignment Summary Metrics (version 1.56.0). The NEBNext UltraShear FFPE DNA Library Prep Kit enables high quality sequencing data while maintaining the highest library yield.

FIGURE 8: **The NEBNext UltraShear FFPE DNA Library Prep Kit workflow enables superior on-target coverage in hybrid capture sequencing**



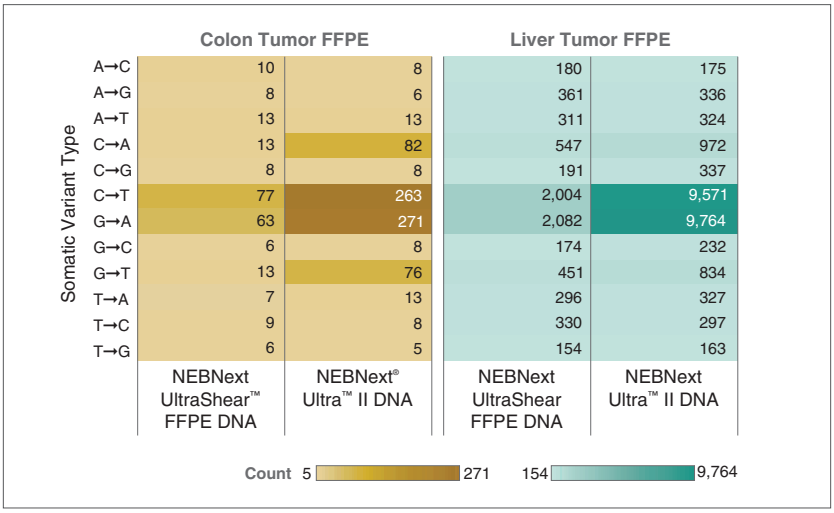
Libraries were prepared in duplicate from 100 ng of unsheared FFPE DNA of DIN 1.8 using either the NEBNext UltraShear FFPE DNA Library Prep Kit or NEBNext UltraShear with the NEBNext Ultra II DNA Library Prep Kit. The NEBNext FFPE DNA Library Prep Kit or the NEBNext Ultra II DNA Library Prep Kit were used to prepare libraries from the same FFPE DNA samples sheared to 350 bp with the Covaris ME220 instrument. 9 PCR cycles were used for all libraries and final library yield was quantified using the Qubit High-Sensitivity dsDNA Assay (Thermo Fisher Scientific). 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) 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 with the NEBNext UltraShear FFPE DNA Library Prep Kit in whole genome sequencing (WGS) libraries (shown in Figure 7) correlates to improved coverage, on-target rate, and coverage uniformity in target enrichment libraries.

FIGURE 9: **The NEBNext UltraShear FFPE DNA Library Prep Kit detects expected variants in formalin-compromised reference standard DNA**



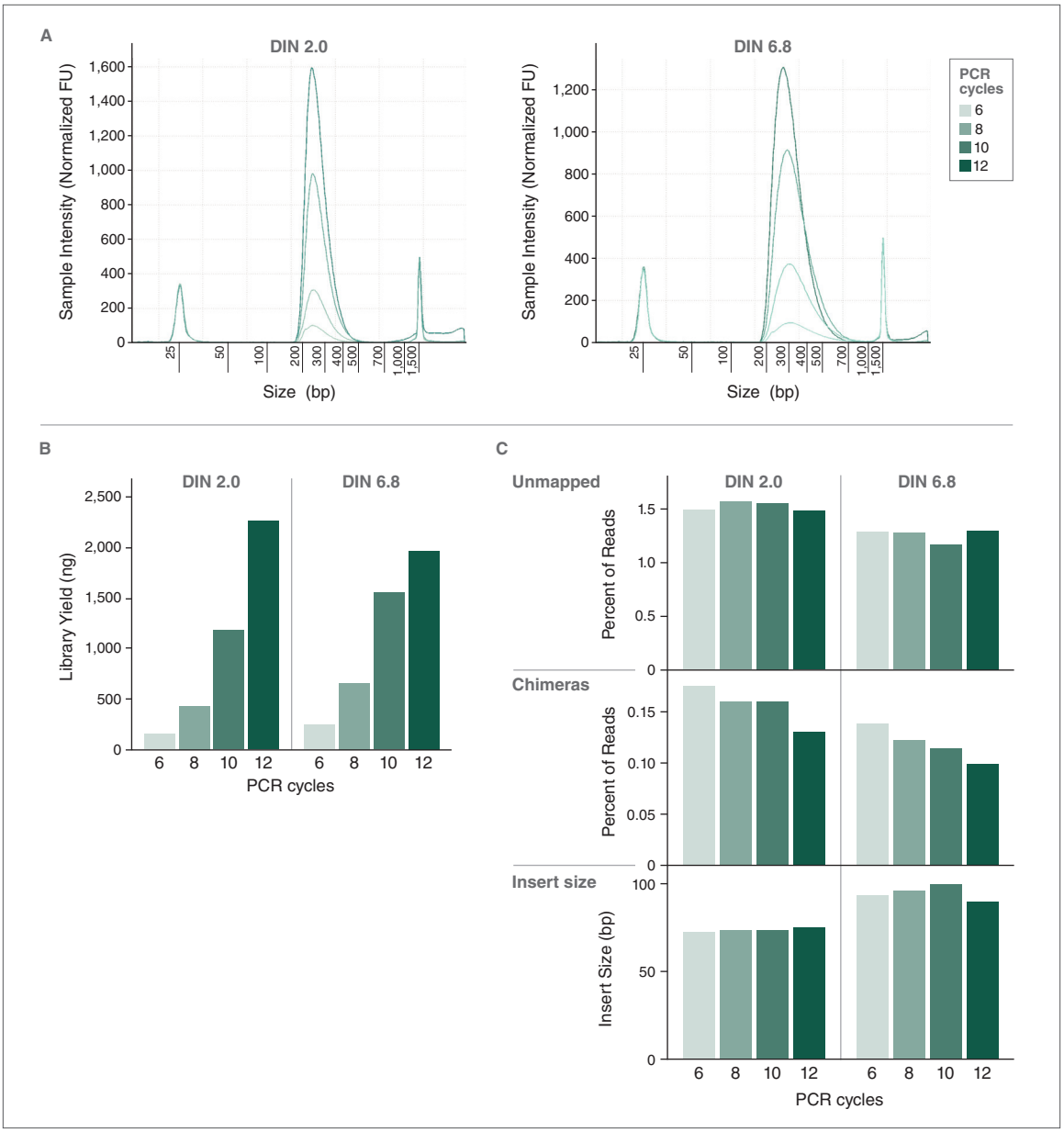
Libraries were prepared in triplicate from 50, 100, or 250 ng of formalin-compromised reference standard DNA (severe) (Horizon Discovery HD803) by enzymatic fragmentation library prep using either the NEBNext UltraShear FFPE DNA Library Prep Kit or NEBNext UltraShear with the NEBNext Ultra II DNA Library Prep Kit. Alternatively, the same DNA and input amounts were prepared using Covaris shearing (ME220) and the NEBNext FFPE DNA Library Prep Kit or the NEBNext Ultra II DNA Library Prep Kit. The NEBNext UltraShear FFPE DNA Library Prep Kit contains NEBNext FFPE DNA Repair v2, the NEBNext UltraShear Enzymatic Fragmentation Mix, and the NEBNext MSTC FFPE Master Mix. The full library yield was used in target enrichment with a custom cancer panel (Twist Bioscience) as a 6-plex capture reaction. Libraries were sequenced on the Illumina NovaSeq 6000 (2 x 100 base reads). Libraries were downsampled to 22 million paired-end reads, mapped using Bowtie2 (version 2.3.2.2) to the T2T reference, and duplicates marked using Picard MarkDuplicates (version 1.56.0.). Variant allele frequencies (VAF) were calculated from Mpileup using Samtools (version 1.16.1) and plotted against the expected VAF in the Horizon reference DNA. Only the NEBNext UltraShear FFPE DNA Library Prep Kit detects all variants at all input amounts. The combination of the NEBNext FFPE DNA Repair v2 mix and the NEBNext UltraShear enzymatic fragmentation mix improves the correlation of expected to observed VAF in low input target enrichment libraries (50 ng), further indicating the benefit of the yield and coverage obtained with the NEBNext UltraShear FFPE DNA Library Prep Kit workflow.

FIGURE 10: **The NEBNext UltraShear FFPE DNA Library Prep Kit reduces false positive variant calls in patient FFPE samples**



Libraries were prepared from 100 ng of moderate quality colon FFPE DNA (DIN 4.4) and poor quality liver FFPE DNA (DIN 1.5) (average of 2 technical replicates shown) using the NEBNext UltraShear FFPE DNA Library Prep Kit or the NEBNext Ultra II DNA Library Prep Kit and 10 PCR cycles. Libraries were captured using a custom cancer panel (Twist Bioscience) and sequenced on the Illumina NovaSeq 6000 platform with 2 x 100 base reads. All fastq files were downsampled to 40 million paired-end reads. Reads were trimmed with Fastp (version 0.20.0) and mapped with BWA mem (version 0.7.17) to the T2T reference. Picard MarkDuplicates was processed (version 2.20.6) with UMI and Fgbio (version 0.8.1) was used to obtain UMI consensus sequence reads. Somatic variant calling was performed with Strelka2 (version 2.9.10) and variant calls are plotted according to substitution type with color scale independent to each sample. The NEBNext UltraShear FFPE DNA Library Prep Kit reduces false positive variant calls deriving from cytosine deamination (C→T/G→A) and oxidative damage (G→T/C→A) compared to the NEBNext Ultra II DNA Library Prep Kit (lacking NEBNext FFPE DNA Repair v2 and NEBNext UltraShear fragmentation).

FIGURE 11: **The NEBNext UltraShear FFPE DNA Library Prep Kit enables flexibility in PCR cycle numbers without compromising library yield or quality**



Libraries were prepared from 100 ng of FFPE DNA of either low (DIN 2.0) or high (DIN 6.8) quality using the NEBNext UltraShear FFPE DNA Library Prep Kit and 6, 8, 10 or 12 PCR cycles. Despite using a high number of PCR cycles, the library yield continues to increase as demonstrated by both the library profile on the Agilent® HSD1000 TapeStation® (A) or quantification by Qubit High-Sensitivity dsDNA assay (Thermo Fisher Scientific) (B). Library quality metrics are maintained across all cycle numbers (C).

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