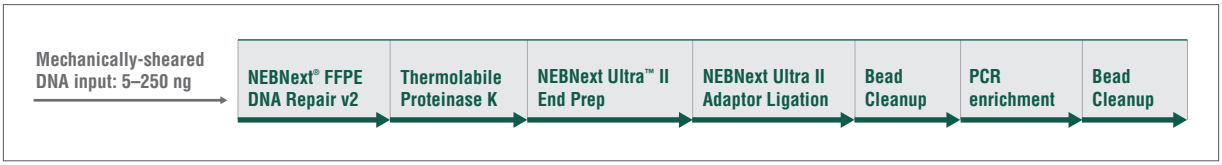
NEBNext® FFPE DNA Library Prep Kit

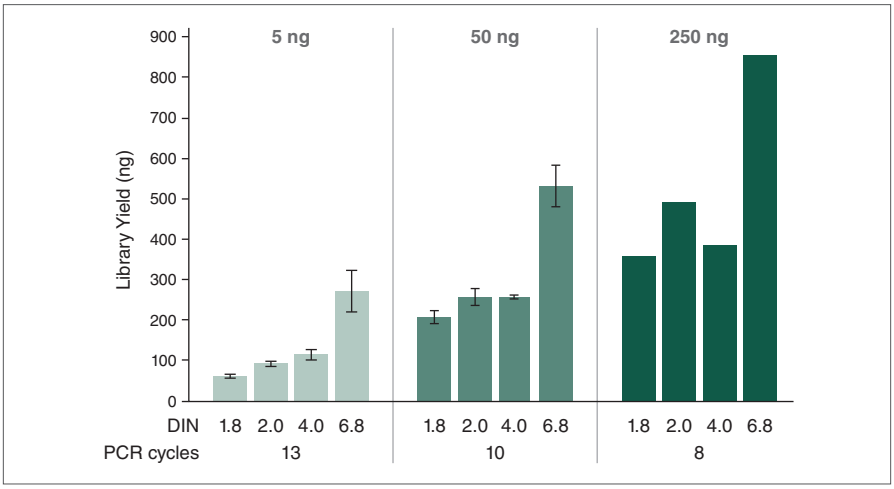
NEB #E6650

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



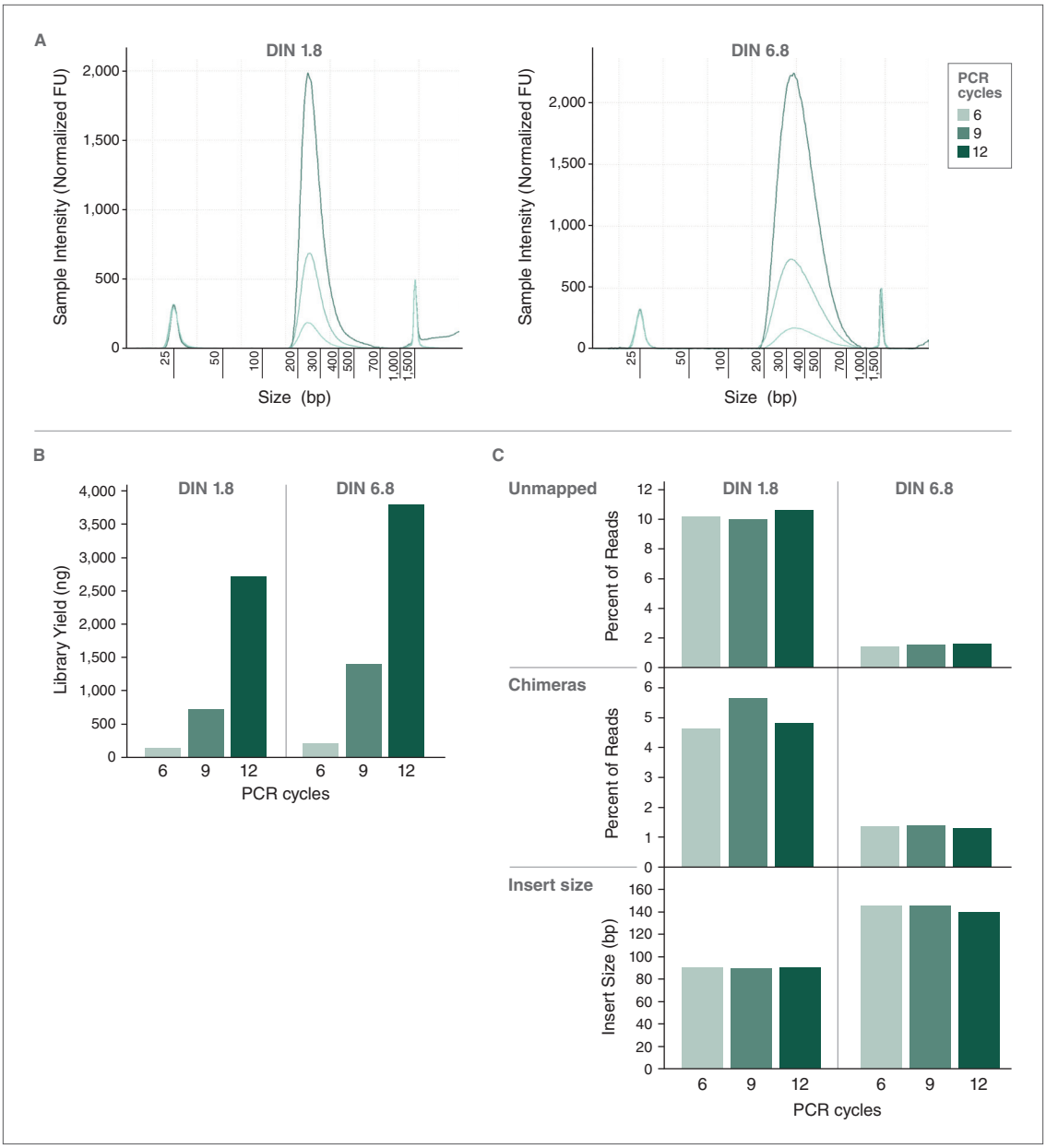
The NEBNext FFPE DNA Library Prep kit has a streamlined workflow with minimal hands-on time. The protocol has been optimized for the user to safely store the reaction after any step in the workflow overnight at -20°C without affecting library yield or quality.

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



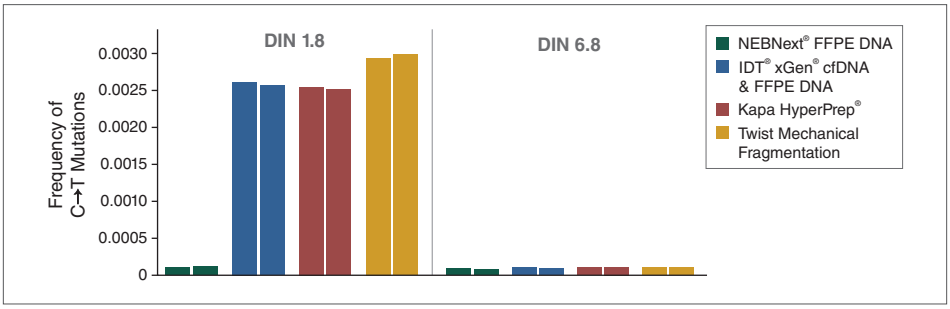
Libraries were prepared from 5, 50 or 250 ng of Covaris®-sheared normal tissue FFPE DNA ranging in quality from DNA Integrity Number (DIN) 1.8 to 6.8 with the indicated PCR cycles. Libraries were made in triplicate for 5 and 50 ng input and 1 replicate for 250 ng. Each bar represents the average of triplicates 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 FFPE DNA Library Prep Kit.

FIGURE 3: **The NEBNext FFPE DNA Library Prep Kit enables flexibility in PCR cycle numbers without compromising library yield or quality**



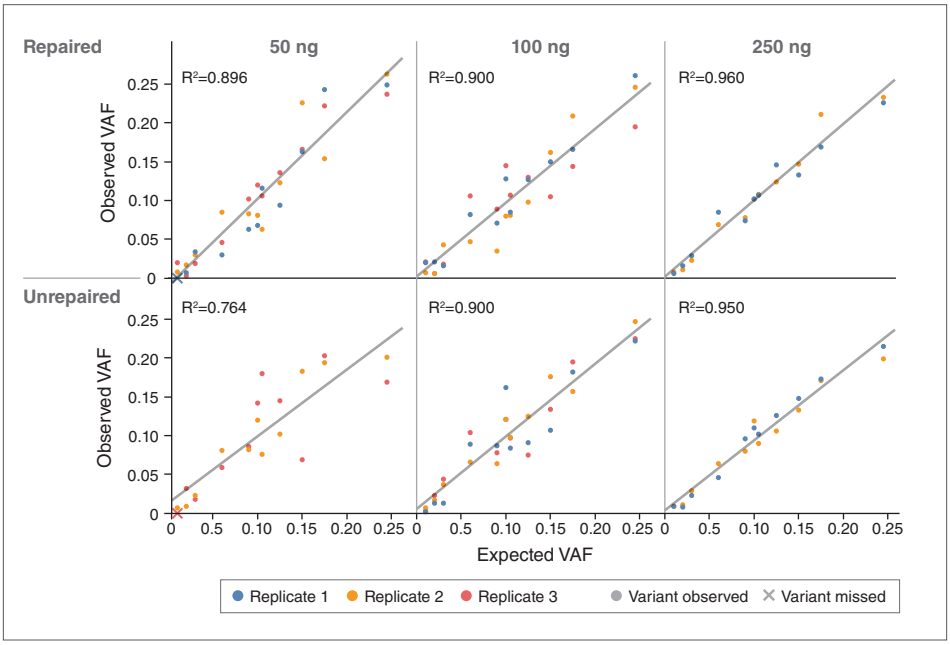
Libraries were prepared from 100 ng of Covaris-sheared normal tissue FFPE DNA of either low (DIN 1.8) or high (DIN 6.8) quality using 6, 9 or 12 PCR cycles. Despite using a high number of PCR cycles, the library yield continues to increase as demonstrated by both the library profile on the Agilent® HSD1000 TapeStation® (A) or quantification by Qubit® HS dsDNA assay (Thermo Fisher Scientific®) (B). Library quality metrics are maintained across all cycle numbers (C).

FIGURE 4: **NEBNext FFPE DNA Library Prep Kit reduces damage-derived sequencing artifacts**



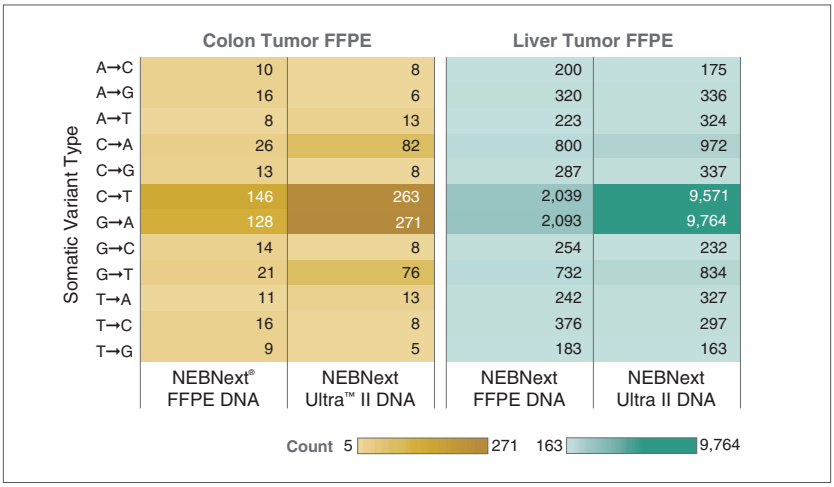
Libraries were prepared from 50 ng of Covaris-sheared normal tissue FFPE DNA of either low (DIN 1.8) or high (DIN 6.8) quality using the NEBNext FFPE DNA Library Prep Kit and other library prep kits as shown. Libraries were sequenced on the Illumina® NextSeq® 500 (2 x 76 bases). Libraries were downsampled to 600,000 paired-end reads, mapped using bowtie2 (version 2.3.2.2) to the GrCh38 reference, and duplicates marked using Picard MarkDuplicate (version 1.56.0). The average frequency of C→T mutations at each C position in Read 2 was calculated for two technical replicates using Tasmanian (version 1.0.7). C→T mutations arising from cytosine deamination damage in low quality FFPE DNA are effectively repaired by the NEBNext FFPE DNA Repair v2 mix included in the NEBNext FFPE DNA Library Prep Kit. Other kits show a high level of C→T artifacts in low quality FFPE DNA (DIN 1.8) due to a lack of DNA damage repair.

FIGURE 5: **The NEBNext 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 Covaris-sheared formalin-compromised reference standard DNA (severe) (Horizon Discovery HD803) using either the NEBNext FFPE DNA Library Prep Kit or NEBNext Ultra™ II DNA Library Prep Kit. The full library yield was used in a target enrichment workflow with a custom cancer panel (Twist Bioscience®) as a 6-plex capture reaction. Libraries were sequenced on the Illumina NovaSeq® 6000 (2 x 100 bases). All fastq files were downsampled to 22 million paired-end reads, mapped using BWA mem (version 0.7.17) 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. A variant with frequency 0.88% was missed at 50 ng input for 1/3 replicates but all variants were detected in all replicates at 100 and 250 ng input. The NEBNext FFPE DNA Library Prep kit improves the correlation of expected to observed VAF in low input libraries (50 ng) compared to standard library prep (NEBNext Ultra II DNA) indicating the benefit of the yield and coverage obtained with the NEBNext FFPE DNA Library Prep Kit including repair with NEBNext FFPE DNA Repair v2.

FIGURE 6: **The NEBNext FFPE DNA Library Prep Kit reduces false positive variant calls in patient FFPE samples arising from DNA damage**



Libraries were prepared from 100 ng of Covaris-sheared moderate quality colon FFPE DNA (DIN 4.4) and poor quality liver FFPE DNA (DIN 1.5) in duplicate (average shown) using the NEBNext FFPE DNA Library Prep Kit or the NEBNext Ultra II DNA Library Prep Kit and 10 PCR cycles. Libraries were captured using a singleplex capture reaction and a custom cancer panel (Twist Bioscience) and sequenced on the Illumina NovaSeq 6000 (2 x 100 bases). All fastq files were downsampled to 40 million paired-end reads. Paired 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 information and fgbio (version 0.8.1) was used to obtain UMI consensus sequence reads. The final bam files with UMI-based consensus reads were used for somatic variant calling with Strelka2 (version 2.9.10). The total number of somatic variant calls are plotted according to substitution type and color scale is independent to each FFPE DNA sample with the darker shade indicating a higher number of variant calls. The NEBNext 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).