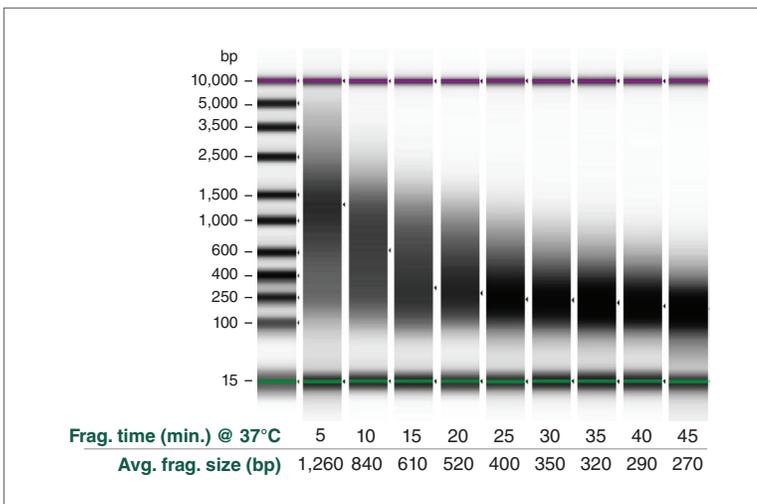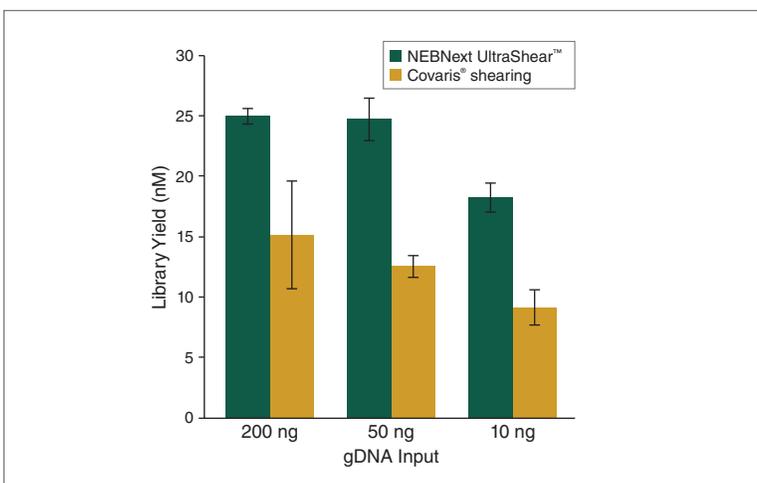# NEBNext UltraShear™

NEB #M7634

FIGURE 1: **NEBNext UltraShear fragments high-quality genomic DNA in a time-dependent manner**


50 ng human DNA (NA12878) 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.

FIGURE 2: **NEBNext UltraShear increases EM-seq™ library yields**

200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris® ME220 (350 bp protocol) followed by EM-seq library preparation. 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 (200 ng = 4 cycles; 50 ng = 6 cycles; 10 ng = 8 cycles).

FIGURE 3: **High quality EM-seq libraries produced using NEBNext UltraShear**
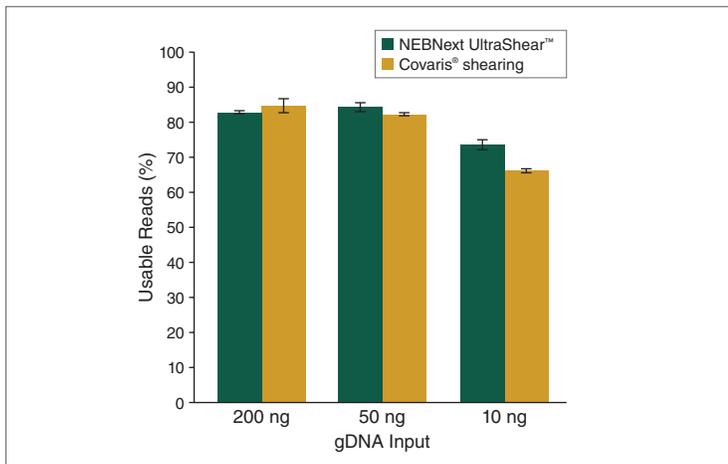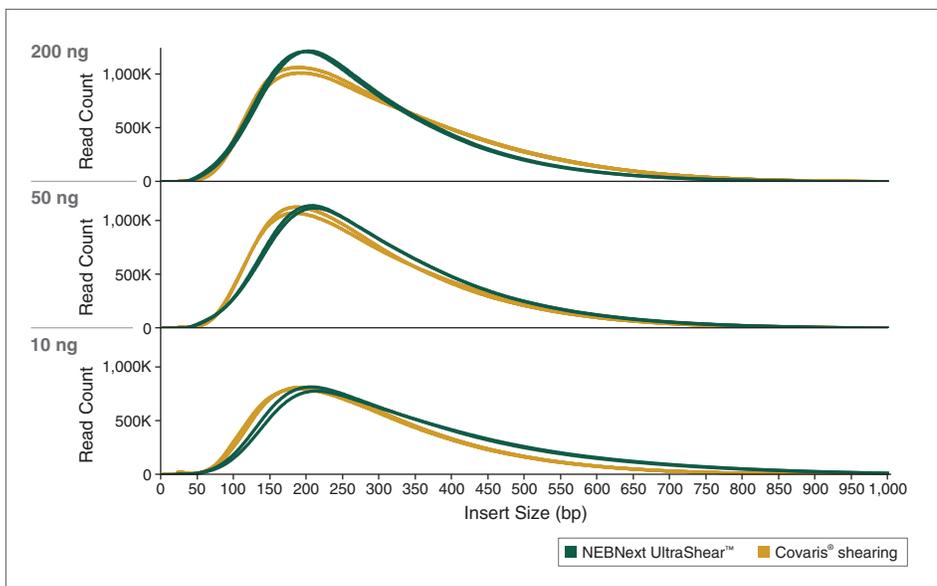
200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) followed by EM-seq library preparation. Technical replicates were generated for each input amount. All libraries were sequenced on the same flowcell of an Illumina® NovaSeq® 6000 (2 x 100 bases). 725 million reads were sampled (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. Percent of usable reads (mappable, proper pairs, MapQ ≥ 10, and non-duplicates reads) were measured for each library and usable reads were averaged for two technical replicates (bars represent error between two technical replicates) for NEBNext UltraShear compared to Covaris for all inputs. The percent usable reads are similar for the 200 ng and 50 ng inputs between the fragmentation methods but improved with NEBNext UltraShear for the 10 ng input.

FIGURE 4: **Fragmentation with NEBNext UltraShear and Covaris generated similar sequenced insert sizes for EM-seq libraries**

200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) 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 (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and insert sizes were determined using Picard. The read count of inserts at each size (bp) was plotted, illustrating that NEBNext UltraShear and Covaris fragmentation resulted in comparable sequenced insert sizes.
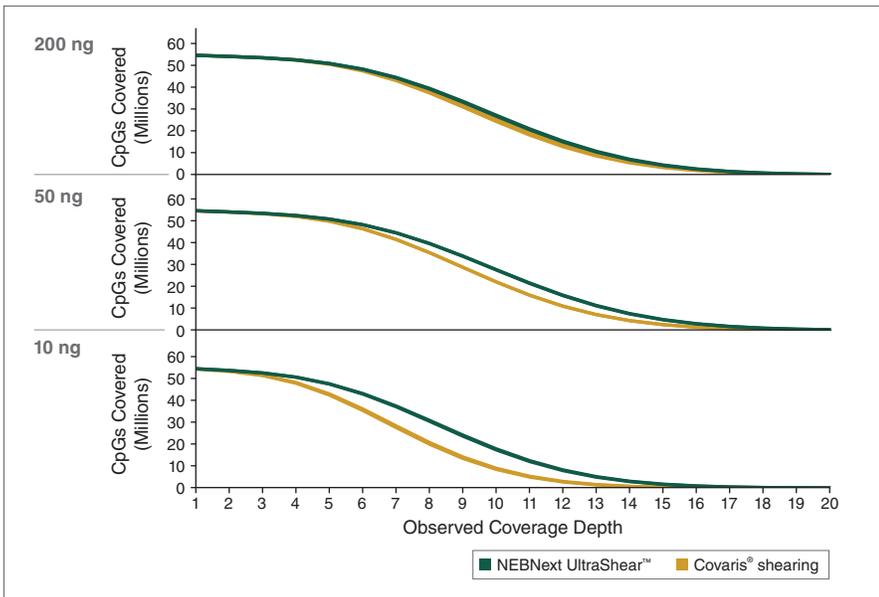
FIGURE 5: EM-seq libraries produced using NEBNext UltraShear have even GC coverage
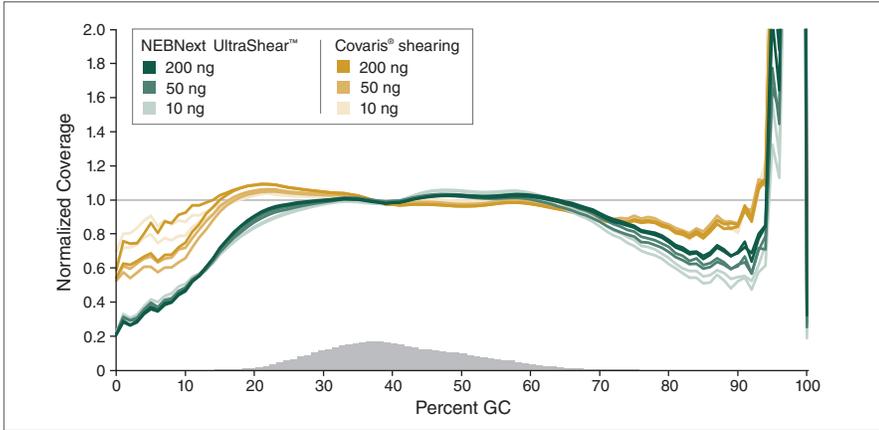
200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350bp protocol) followed by EM-seq library preparation. Technical replicates were generated for each input amount. All libraries were sequenced on the same flowcell of an Illumina NovaSeq 6000 (2 x 100 bases). 725 million reads were sampled (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. 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.
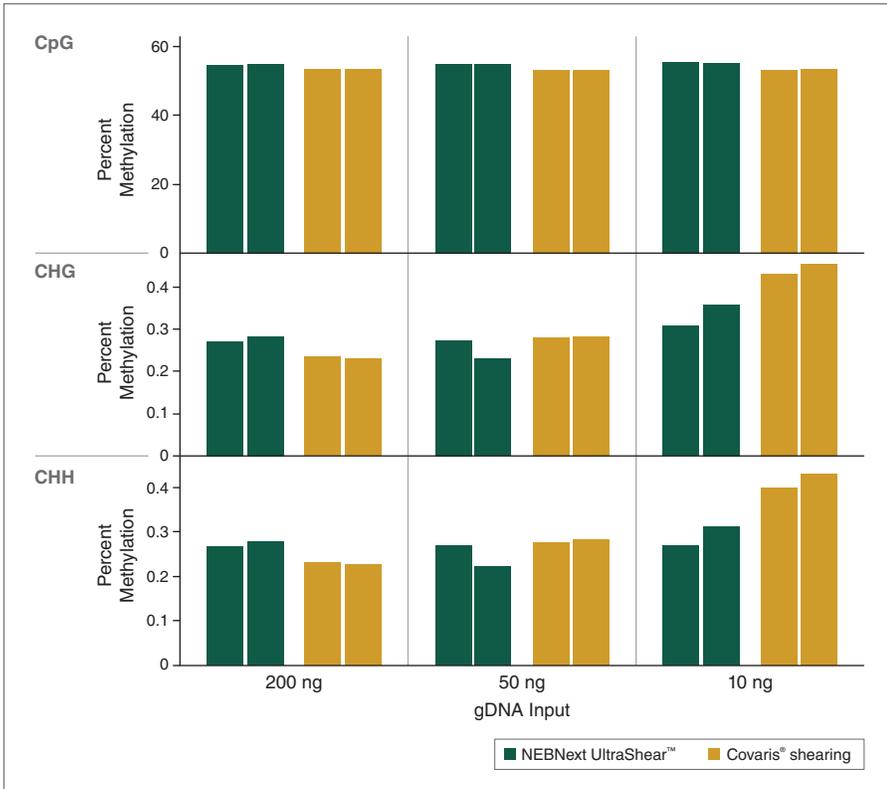
FIGURE 6: Improved CpG coverage in EM-seq libraries produced using NEBNext UltraShear

200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) was fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) 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 (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. 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.
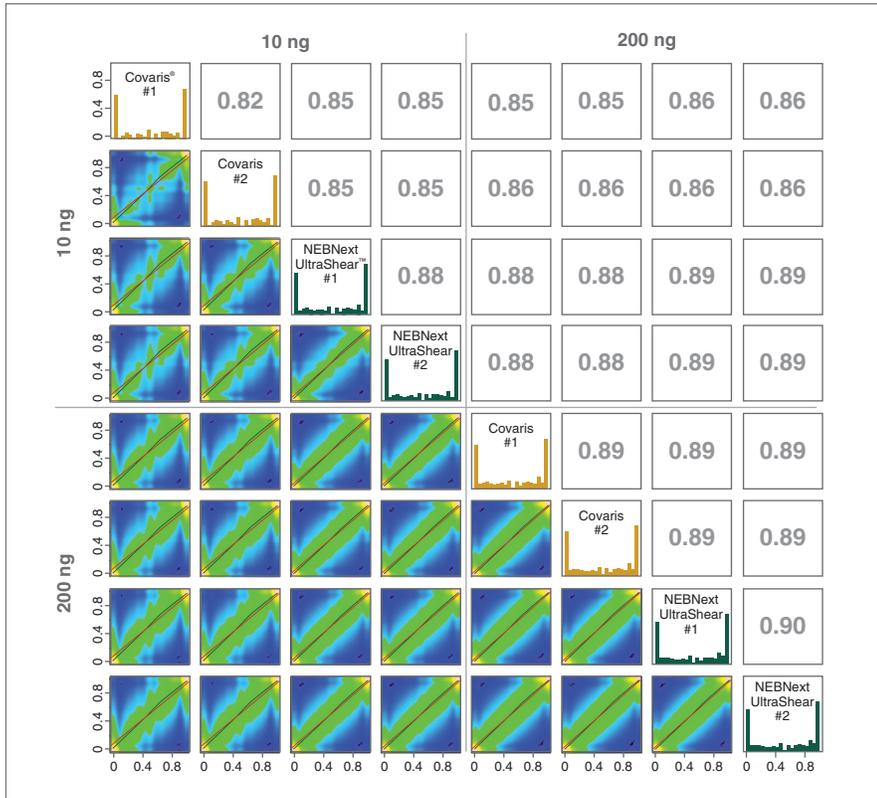
FIGURE 7: **Similar total methylation is detected by EM-seq with NEBNext UltraShear and Covaris shearing**

200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) 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 (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. The percent of aggregated methylation in all contexts are similar for EM-seq libraries fragmented by NEBNext UltraShear or Covaris. The spike-in control DNAs used in the EM-seq workflow had expected methylation: CpG methylated pUC19 had > 97% methylated Cs in CpG context and < 1.5% methylated Cs in other contexts; unmethylated Lambda had < 0.5% methylated Cs in all contexts (data not shown).

FIGURE 8: **Human CpG Methylation is highly correlated for EM-seq libraries generated using NEBNext UltraShear and Covaris shearing**

200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) 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 (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. CpG methylation correlations were generated for 10 ng and 200 ng input libraries using methylKit. Approximately 53 million CpGs were common to all libraries at a 1X sequencing depth. There is high correlation between CpG methylation of NEBNext UltraShear and Covaris EM-seq libraries for 200 and 10 ng inputs.

FIGURE 9: **Genomic Coverage and CpG methylation at key genomic features are similar between NEBNext UltraShear and Covaris shearing for EM-seq libraries**
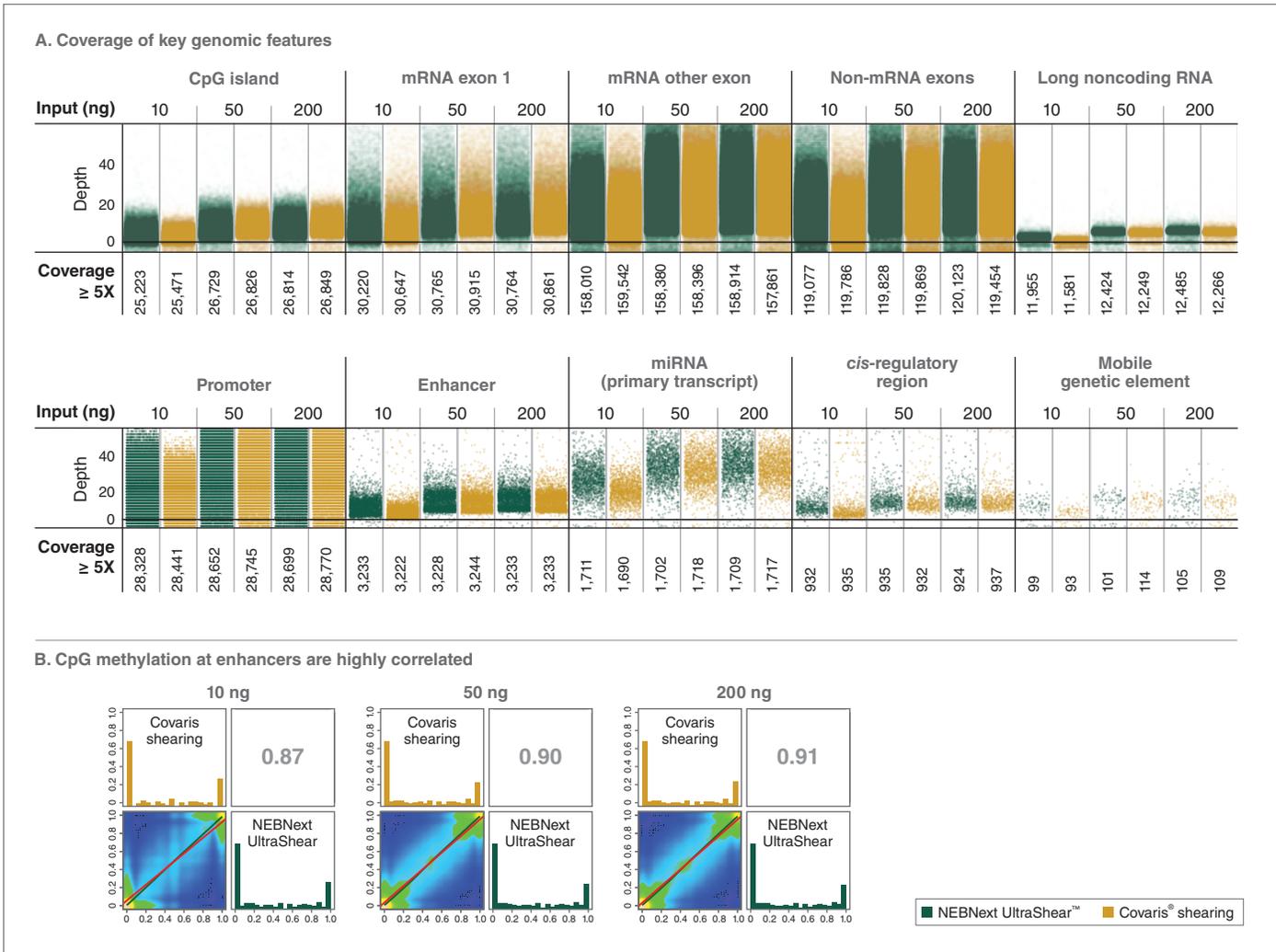
A. Coverage of key genomic features

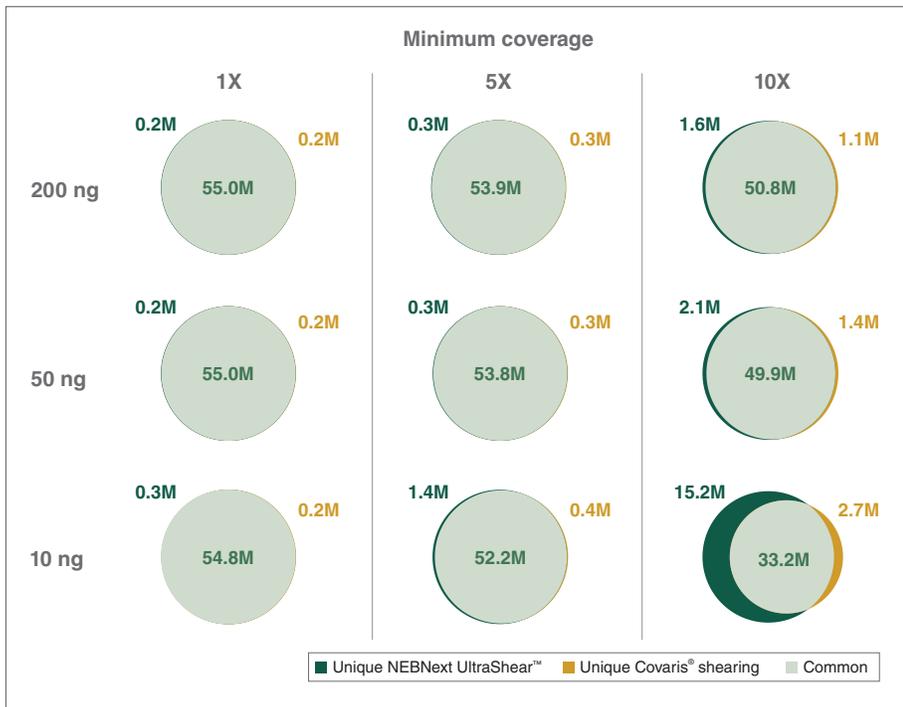**B. CpG methylation at enhancers are highly correlated**

200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) 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 (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel.

A. Coverage of NEBNext UltraShear and Covaris-sheared EM-seq libraries at key genomic features for all inputs is shown. NEBNext UltraShear EM-seq libraries show an improvement in coverage for the 10 ng input at some genomic features such as enhancers. Coverage of various genomic feature types is represented with one point per region. The vertical position is defined by the average coverage of the feature. Points are staggered horizontally for display. Features from NCBI's RefSeq, the Eukaryotic Promoter Database, UCSC Table Browser, and Dfam are shown, and the features covered at ≥ 5X average depth are indicated.

B. Individual CpG methylation in RefSeq annotated enhancer regions is highly correlated between NEBNext UltraShear and Covaris for EM-seq libraries for all inputs. MethylKit correlation figures were produced using 1X minimum CpG coverage shared between fragmentation methods within each input. ~94,000 CpGs are represented in each correlation plot.

FIGURE 10: **EM-seq libraries produced using NEBNext UltraShear and Covaris share a high number of CpGs**


200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) 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 (seqtk) from each library for methylation analysis. Reads were adapter trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. The number of unique and common CpGs were identified for NEBNext UltraShear and Covaris shearing for EM-seq libraries at 1X, 5X and 10X minimum. The technical replicates are combined for each sample and coverage for each input amount are shown. NEBNext UltraShear has 15.2 million (29.7%) more unique CpGs covered at the 10X minimum coverage for the 10 ng inputs.

FIGURE 11: NEBNext UltraShear with FFPE DNA improves usable reads
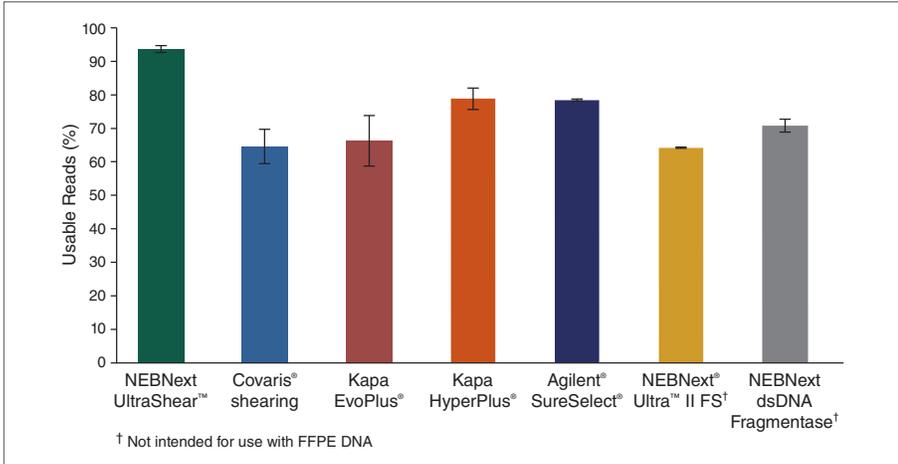
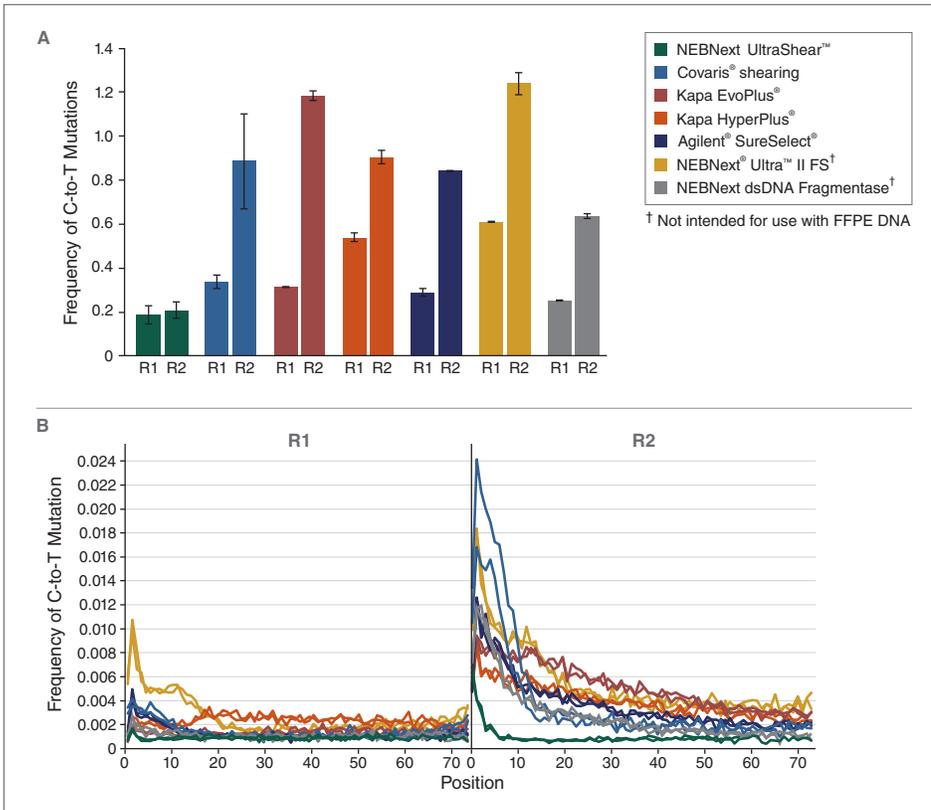† Not intended for use with FFPE DNA

FFPE DNA was fragmented using NEBNext UltraShear (15 minutes at 37°C), Covaris ME220, Kapa EvoPlus® Kit, Kapa HyperPlus® Kit, Agilent SureSelect® Enzymatic Fragmentation Kit, NEBNext dsDNA Fragmentase® or NEBNext Ultra II FS. 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. Reads were aligned to GRCh38 with Bowtie2 and analyzed using samtools flagstats and Picard CollectAlighmentSummaryMetrics. Percent of usable reads (mappable, proper pairs, and non-duplicates reads) were measured for each library and usable reads were 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).

FIGURE 12: NEBNext UltraShear improves coverage uniformity for FFPE DNA
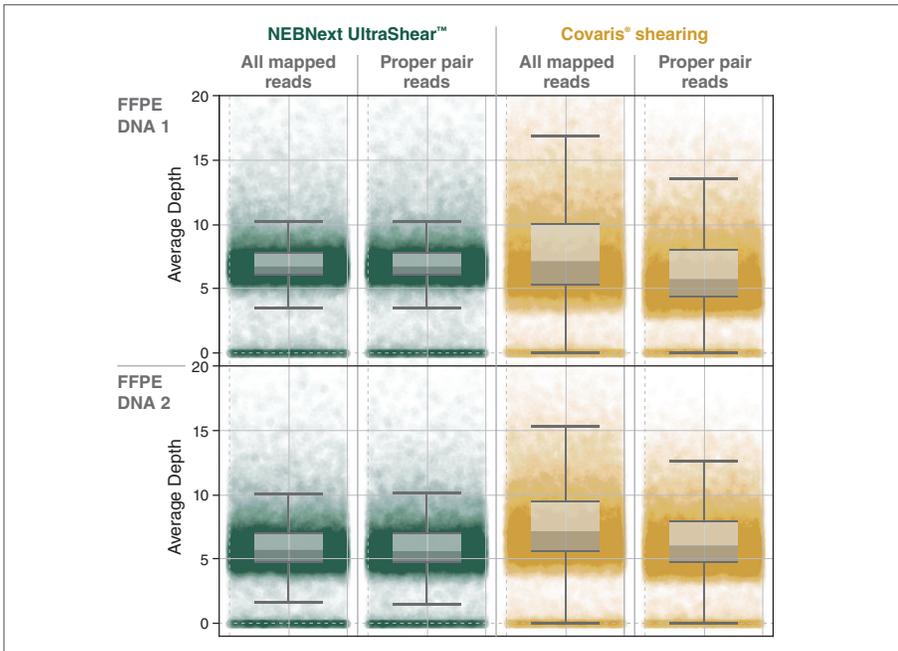
FFPE DNA was fragmented using NEBNext UltraShear (5 minutes at 37°C) or Covaris ME220 350 bp protocol. DNA fragmentation was followed by library construction using the NEBNext Ultra II DNA Library Prep Kit for Illumina. The full library yield was used for singleplex capture using a custom cancer hotspot panel (Twist Bioscience®). Each library was sequenced using the Illumina NovaSeq 2000, down-sampled to 2 x 400 million reads and mapped to genome CHM13_v1_0 with BWA (Version: 0.7.17-r1188). UMIs were processed with fgbio (Version: 1.3.0). Mosdepth (Version: 0.2.6) was used to calculate genome coverage with window size 10,000 bp on original UMI-based markduplicated bam (all), as well as on bam excluding improper paired reads (Proper Pairs). A random selection of 10% of total data points are displayed here. NEBNext UltraShear provides more even coverage, which is not affected by removal of improper paired reads.

FIGURE 13: **NEBNext UltraShear with FFPE DNA reduces artificial mutations**

FFPE DNA was fragmented using NEBNext UltraShear (15 minutes at 37°C), Covaris ME220, Kapa EvoPlus Kit, Kapa HyperPlus Kit, Agilent SureSelect Enzymatic Fragmentation Kit, NEBNext dsDNA Fragmentase or NEBNext Ultra II FS. All samples were fragmented according to the recommended protocols. Fragmentation with NEBNext dsDNA Fragmentase, Kapa® kits and the Agilent kit was followed by a bead cleanup and 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. Reads were aligned to GRCh38 with Bowtie2. 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).

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