# NEBNext UltraExpress™ DNA Library Prep Kit

NEB #E3325

FIGURE 1: **NEBNext UltraExpress DNA Library Prep workflow**

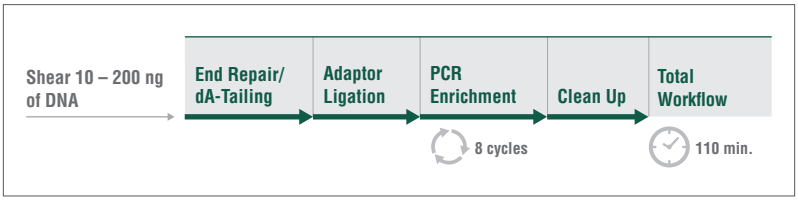
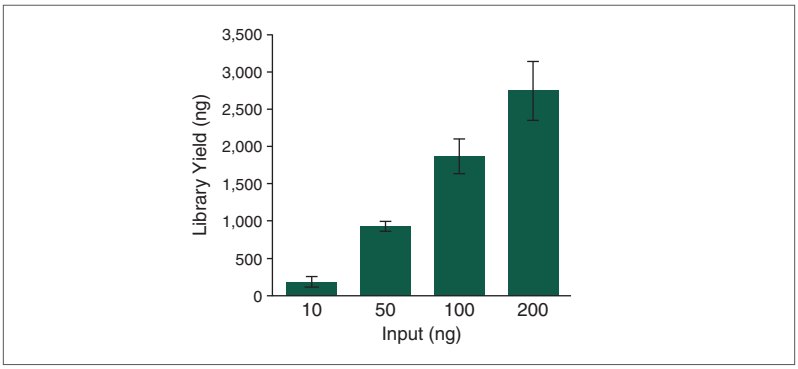
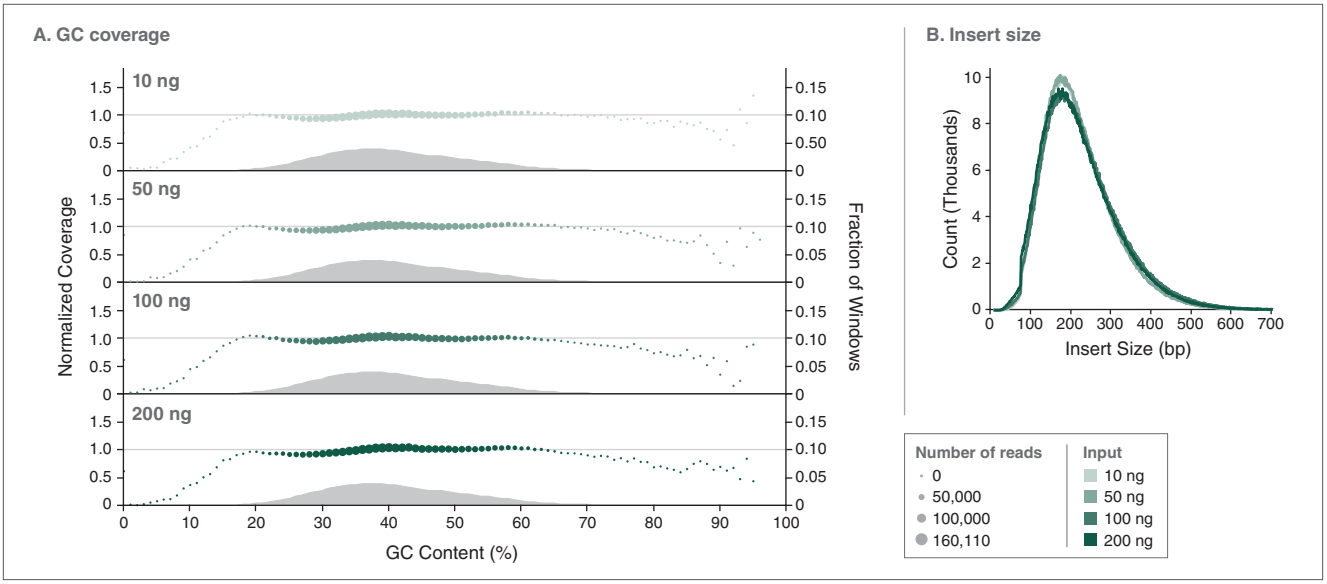



FIGURE 2: **The NEBNext UltraExpress DNA Library Prep Kit provides robust library yields over a wide input range**



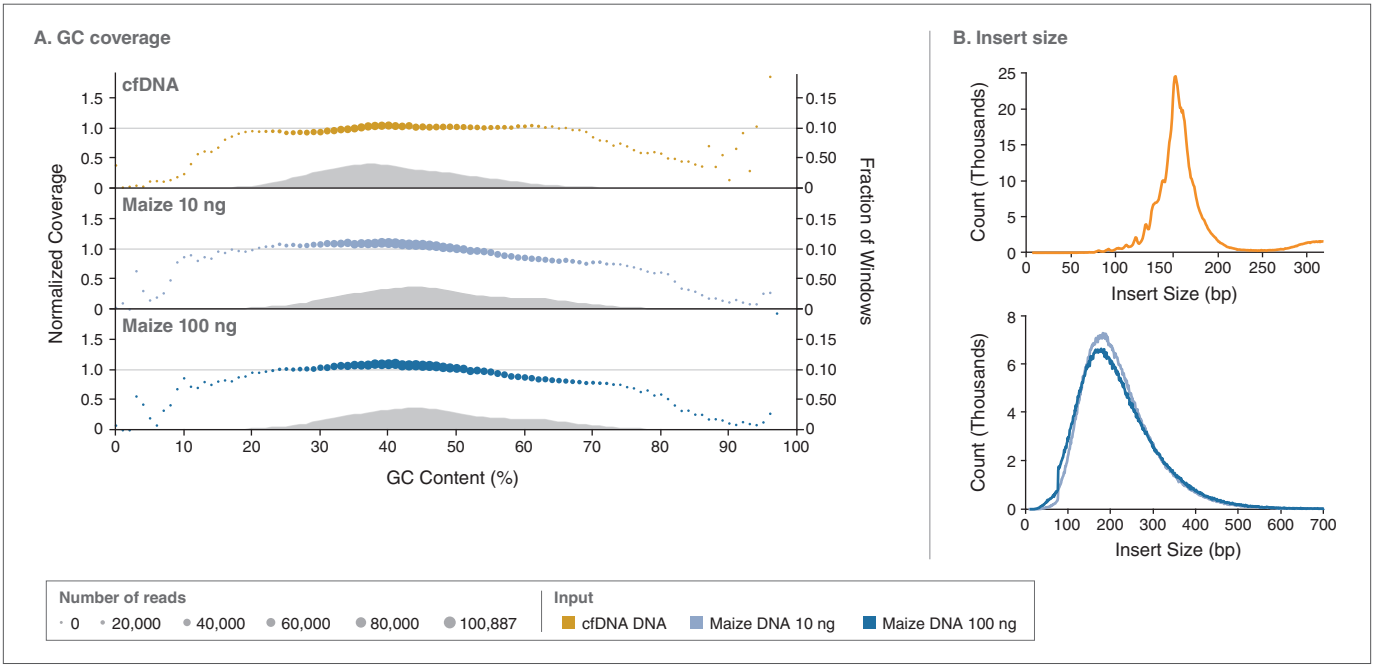

Libraries were prepared from 10, 50, 100 or 200 ng of Human NA19240 genomic DNA (Coriell Institute for Medical Research) using the same adaptor amount and 8 PCR cycles. Yields exceeded the minimum requirement (40 ng) for a single Illumina® NovaSeq® 6000 run to achieve whole genome sequencing with at least 30X coverage.

FIGURE 3: **The NEBNext UltraExpress DNA Library Prep Kit produces libraries with uniform GC coverage and insert size from a range of input amounts**




Libraries were prepared from 10, 50, 100 or 200 ng of Human NA19240 genomic DNA (Coriell Institute for Medical Research) using the same adaptor amount and 8 PCR cycles. Libraries were pooled and sequenced on an Illumina MiSeq® (2 x 75 bases). Data showed consistent GC coverage (A) and insert size (B). 2 million paired-end reads from each library were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1) and mapped to the GRCh38 reference (bowtie2 v2.4.5), and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v 1.56.0). In (A), the horizontal grey line indicates the expected normalized coverage of 1.0, and the dots in shades of green represent read numbers at each GC%. The grey area plot is a histogram representing the distribution of GC content in 100 bp windows of the reference genome.

FIGURE 4: **The NEBNext UltraExpress DNA Library Prep Kit produces representative GC coverage and insert size for a range of sample types**

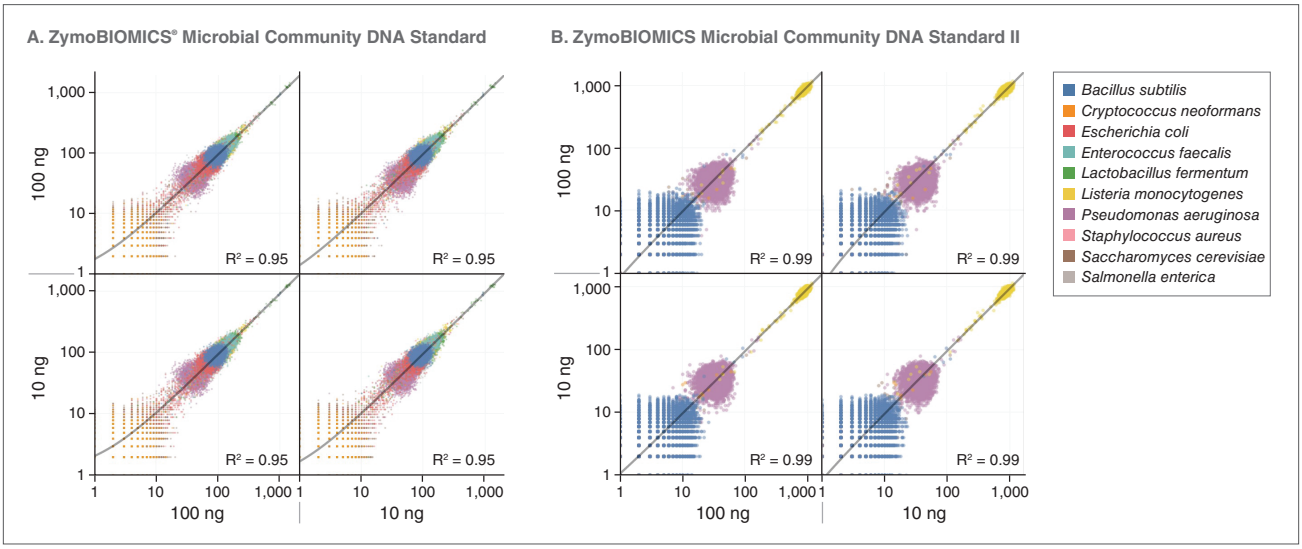



Libraries were prepared using a single protocol from cell-free DNA (cfDNA, 12 ng) without shearing and Maize DNA (10 ng and 100 ng) sheared to 200 bp (Covaris® ME220). Libraries were pooled and sequenced on an Illumina MiSeq (2 x 75 bases). 1.4 million paired-end reads from each library were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1), and aligned to either the GRCh38 reference (human) or Zea mays reference genome (maize) (bowtie2 v2.4.5).

A. All sample types showed representative GC coverage. High- and low-input Maize DNA generated consistent GC coverage. The horizontal grey line indicates the expected normalized coverage of 1.0, and the colored dots represent read numbers at each GC%. The grey area plot is a histogram representing the distribution of GC content in 100 bp windows of the reference genome.

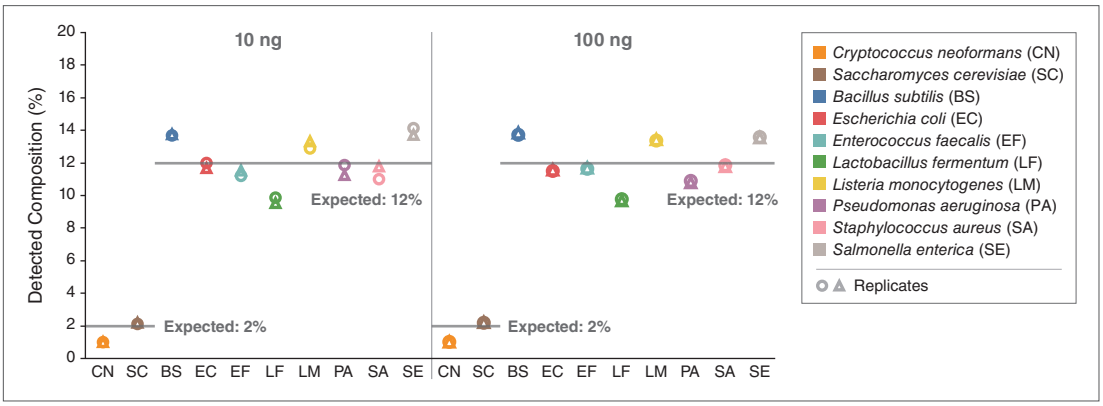
B. cfDNA insert size had the characteristic fragmentation pattern with 167 bp peak size and periodicity feature of nucleosome-bound DNA in shorter fragments. 10 ng and 100 ng Maize DNA showed consistent insert size, as expected with the shearing protocol used.

## FIGURE 5: **The NEBNext UltraExpress DNA Library Prep Kit provides robust library complexity**



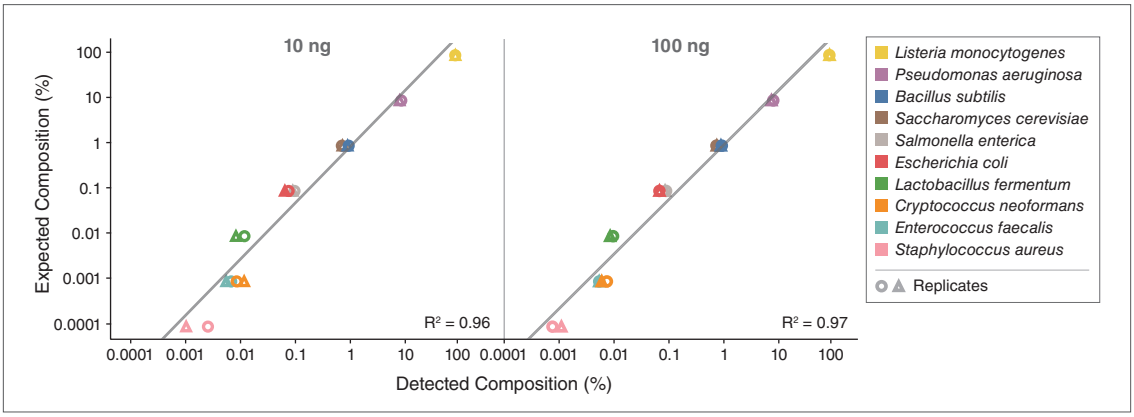

Libraries were prepared using a single protocol from (A) 10 ng and 100 ng of the ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research®, Catalog #D6306), and (B) 10 ng and 100 ng of the ZymoBIOMICS Microbial Community DNA Standard II (Log Distribution) (Zymo Research, Catalog #D6311). Libraries were pooled and sequenced on an Illumina MiSeq (2 x 75 bases). 1.4 million paired-end reads from each library were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1) and aligned to a composite reference genome (bowtie2 v2.4.5). 1,000 bp windows of constituent genomes were counted (bedtools 2.30.0) and compared across replicates and input levels. High correlation was observed between replicates and between inputs.

## FIGURE 6: **The NEBNext UltraExpress DNA Library Prep Kit generates libraries representative of input DNA**




Libraries were prepared using a single protocol from 10 ng and 100 ng of the ZymoBIOMICS Microbial Community DNA Standard (Zymo Research #D6306). Libraries were pooled and sequenced on an Illumina MiSeq (2 x 75 bases). 1.4 million reads from each library were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1), and aligned to a composite reference genome (bowtie2 v2.4.5). 1,000 bp windows of constituent genomes were counted (bedtools 2.30.0) and compared across expected and detected composition for both input levels. The detection of specific microbial gDNA was consistent with the expected composition. Expected composition: *Cryptococcus neoformans* 2%, *Saccharomyces cerevisiae* 2%, *Bacillus subtilis* 12%, *Escherichia coli* 12%, *Enterococcus faecalis* 12%, *Lactobacillus fermentum* 12%, *Listeria monocytogenes* 12%, *Pseudomonas aeruginosa* 12%, *Staphylococcus aureus* 12% *and Salmonella enterica* 12%.

FIGURE 7: **The NEBNext UltraExpress DNA Library Prep Kit generates libraries representative of input DNA even with complex mixtures across a log range**




Libraries were prepared using a single protocol from 10 ng and 100 ng of the ZymoBIOMICS Microbial Community DNA Standard II (Log Distribution) (Zymo Research, Catalog # D6311). Libraries were pooled and sequenced on an Illumina MiSeq (2 x 75 bases). 1.4 million reads from each library were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1), and aligned to a composite reference genome (bowtie2 v2.4.5). 1,000 bp windows of constituent genomes were counted (bedtools 2.30.0) and compared across expected and detected composition for both input levels. Consistent correlation between expected and detected composition was noted ($R^2 = 0.96$ for 10 ng and $R^2 = 0.97$ for 100 ng). Expected composition: *Listeria monocytogenes* 89.1%, *Pseudomonas aeruginosa* 8.9%, *Bacillus subtilis* 0.89%, Saccharomyces cerevisiae 0.89%, *Escherichia coli* 0.089%, *Salmonella enterica* 0.089%, *Lactobacillus fermentum* 0.0089%, *Enterococcus faecalis* 0.00089%, *Cryptococcus neoformans* 0.00089% and *Staphylococcus aureus* 0.000089%.