NEBNext UltraExpress™ FS DNA Library Prep Kit

NEB #E3340

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

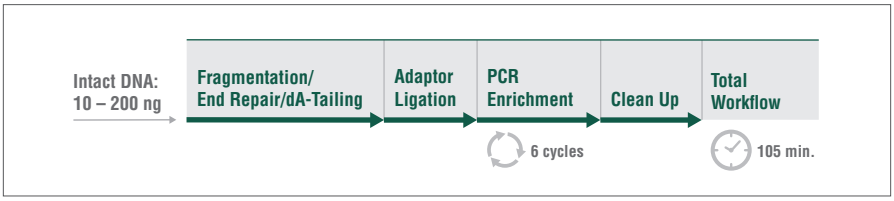
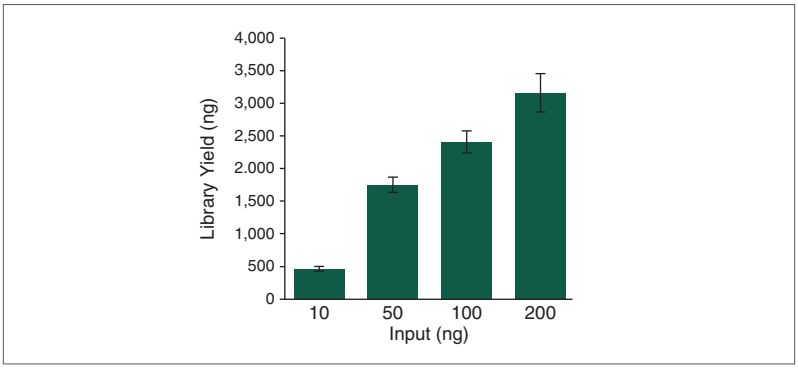
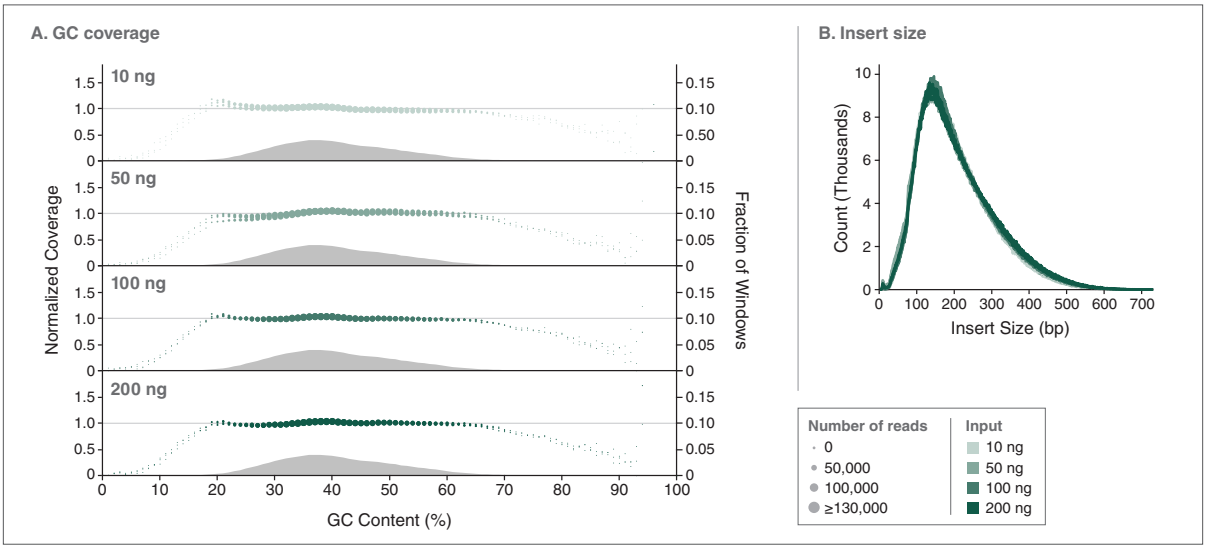


FIGURE 2: **The NEBNext UltraExpress FS DNA Library Prep Kit provides robust library yields over a wide input range**



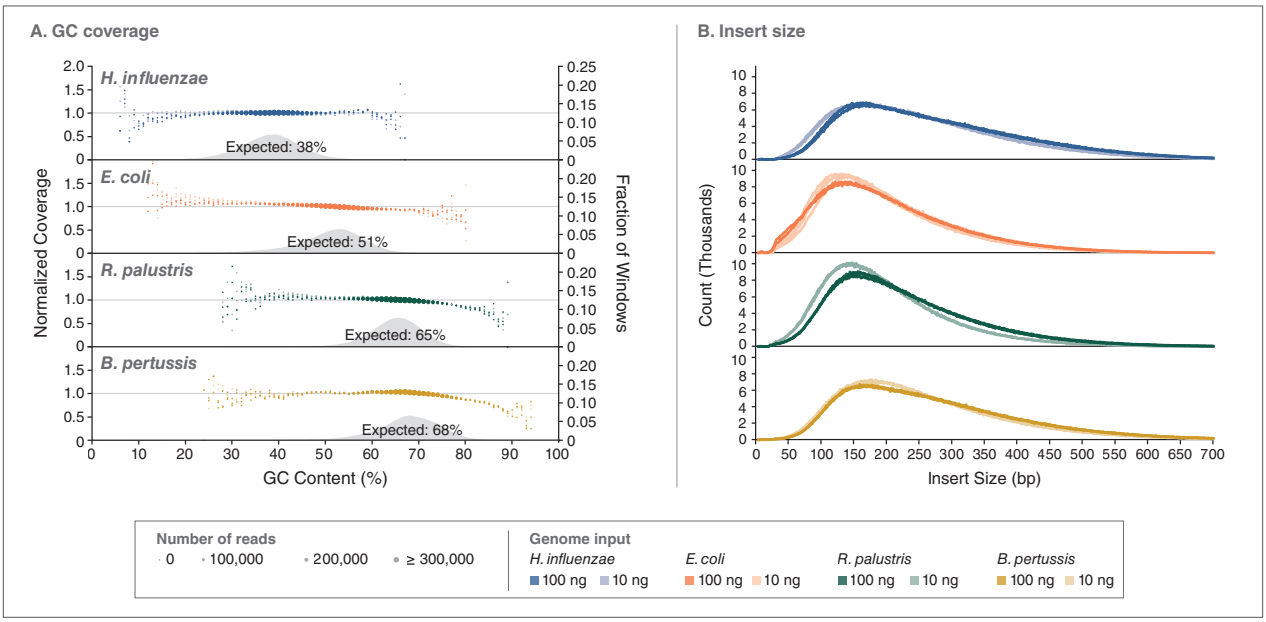
Libraries were prepared in triplicate from 10, 50, 100 and 200 ng of a 9:1 Human NA19240 genomic DNA (Coriell Institute for Medical Research) and *Escherichia coli* gDNA (Lofstrand Labs Limited) mixed sample, using the NEBNext UltraExpress FS DNA single-protocol workflow (e.g., same adaptor amount and 6 PCR cycles for all input amounts). 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 FS DNA Library Prep Kit produces libraries with uniform GC coverage and insert size from a range of input amounts**



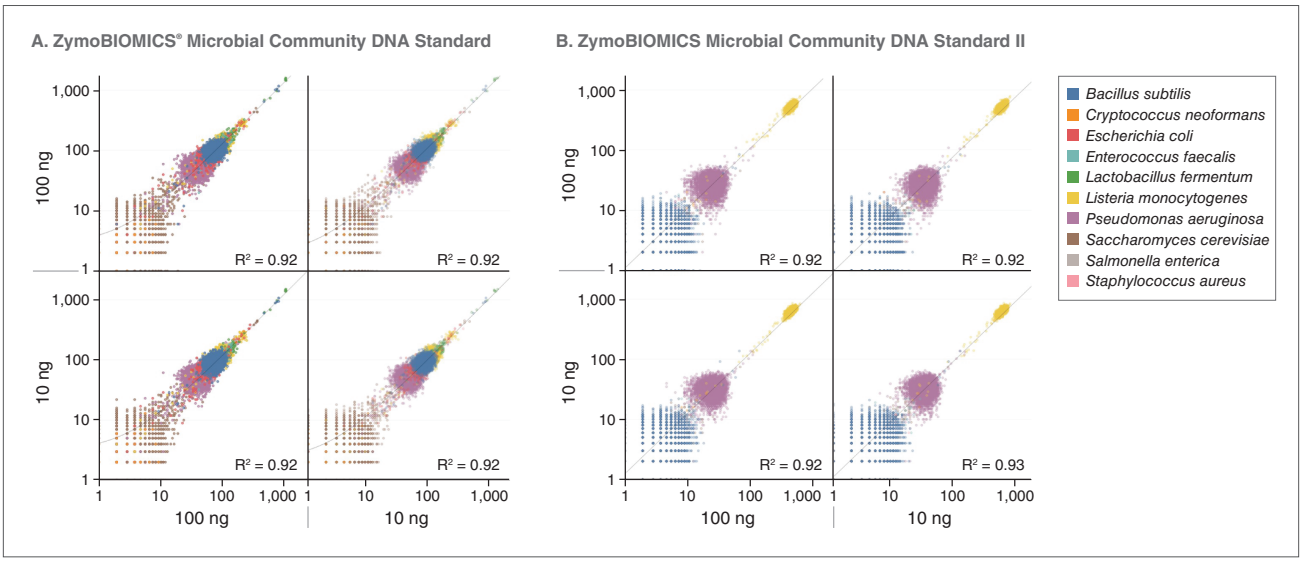
Libraries were prepared from 10, 50, 100 and 200 ng of a 9:1 Human NA19240 genomic DNA (Coriell Institute for Medical Research) and *Escherichia coli* gDNA (Lofstrand Labs Limited) mixed sample, using the NEBNext UltraExpress FS DNA single-protocol workflow (e.g., same adaptor amount and 6 PCR cycles for all input amounts). Libraries were pooled and sequenced on an Illumina NextSeq® 500/550 (2 x 75 bases). Data showed consistent (A) GC coverage and (B) insert size. 2 million paired-end reads from each library were sampled (seqtk v1.0), adaptor-trimmed (seqprep v0.1) and mapped to a composite reference containing *GRCh38* and *E. coli* MG1655 contigs (bowtie2 v2.5.0). GC coverage and insert size distributions were calculated using Picard's CollectGCBiasMetrics and Picard CollectInsertSizeMetrics (v1.56.0); Picard CollectGCBiasMetrics. (1.56) was run on human autosomes only due to the even copy number assumption of the tool. 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 FS DNA Library Prep Kit produces representative GC coverage and insert size peaks for microbial genomic DNA over a broad range of GC composition**



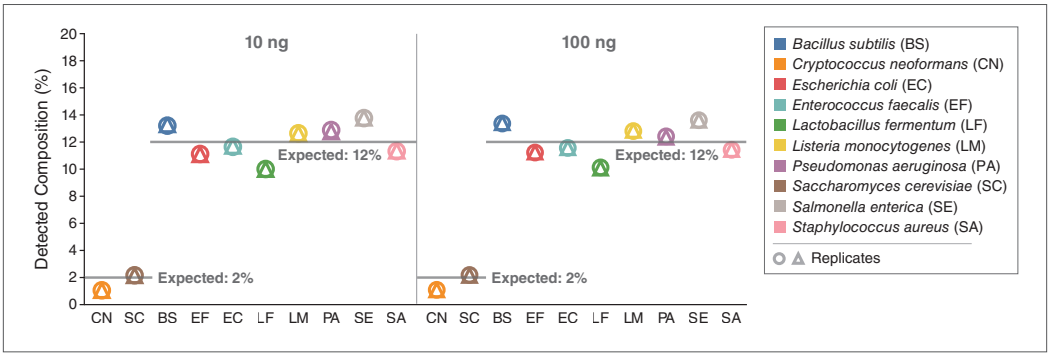
Libraries were prepared using the NEBNext UltraExpress FS DNA protocol for 10 ng and 100 ng of genomic DNA from *Haemophilus influenzae*, *Escherichia coli*, *Rhodopseudomonas palustris* and *Bordetella pertussis*. Data showed (A) representative GC coverage and (B) insert size peaks across samples with genome GC contents of 38%-68% GC. Libraries were pooled and sequenced on an Illumina NextSeq 500/550 (2 x 75 bases). 2 million paired-end reads from each library were sampled (seqtk v1.0), adaptor-trimmed (seqprep v0.1), and aligned to their respective reference genomes (bowtie2 v.2.5.0). GC coverage and insert size distributions were calculated using Picard's CollectGCBiasMetrics and Picard CollectInsertSizeMetrics (v1.56.0). 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 for the 10 and 100 ng inputs.

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



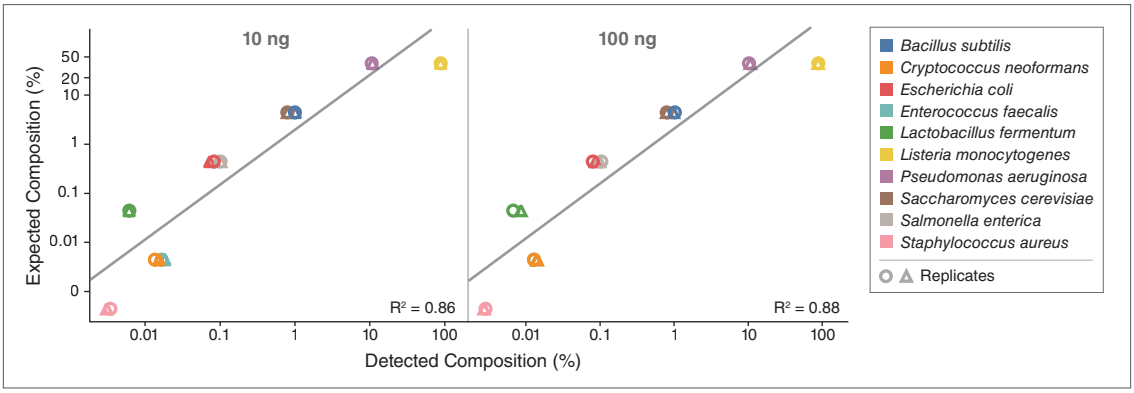
Libraries were prepared using the NEBNext UltraExpress FS DNA protocol from (A) 10 ng and 100 ng of the ZymoBIOMICS® Microbial Community DNA Standard (Zymo Research®, Catalog #D6306), and (B) 10 ng and 100 ng of the ZymoBIOMICS Microbial Community DNA Standard II (Log Distribution) (Zymo Research, Catalog #D6311). Libraries were pooled and sequenced on an Illumina® MiSeq® (2 x 75 bases). 750,000 paired-end reads from each library were sampled (seqtk v1.3), adaptor-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 FS DNA Library Prep Kit generates libraries representative of input DNA**



Libraries were prepared using using the NEBNext UltraExpress FS DNA 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). 750,000 paired-end reads from each library were sampled (seqtk v1.3), adaptor-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 FS DNA Library Prep Kit generates libraries representative of input DNA even with complex mixtures across a log range**



Libraries were prepared using the NEBNext UltraExpress FS DNA 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). 750,000 paired-end reads from each library were sampled (seqtk v1.3), adaptor-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 R2 = 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%.

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