APPLICATION NOTE

Microbial WGS with Illumina DNA Prep

- Reduce library preparation time with a low number of steps and minimal hands-on time
- Obtain robust, consistent results over a wide range of DNA input, even at low DNA input amounts (1 ng)
- Produce sequencing data with uniform coverage for viruses, bacteria, and other microbial species

Introduction

Whole-genome sequencing (WGS) has been established as an important tool in microbiology for resequencing and *de novo* assembly of small genomes (≤ 5 Mb) such as those of bacteria, viruses, and other microbes. Microbial WGS enables genomic mapping of novel organisms, completing genomes of known organisms and comparing genomes across samples. Nextera™ tagmentation chemistry and the release of Nextera XT DNA Library Prep Kits consolidated DNA fragmentation and adapter tagging steps into a single reaction and eliminated the need for library quantitation before pooling and sequencing.[1] Illumina DNA Prep kits further improve the performance and ease of use of tagmentation library preparation, supporting a wider range of DNA input with consistent library insert size and yield and more uniform coverage. This makes Illumina DNA Prep the ideal choice for microbiology genetics research. (Figure 1).

Fast and simple workflow

Featuring unique chemistry that integrates multiple pre- and post-library preparation steps, Illumina DNA Prep delivers the fastest workflow with the fewest number of steps in the Illumina library prep portfolio.[2] In addition to speed and efficiency gains, it offers exceptional flexibility for sample input type, amount, and a wide range of supported applications. Illumina DNA Prep enables DNA extraction directly from crude lysates of stool or environmental samples[3] and bacterial colonies grown overnight at 37°C on blood agar plates,[4,5] resulting in additional time and cost savings while improving data consistency.[6-8]

Figure 1: Microbial WGS with Illumina DNA Prep—Illumina DNA Prep enables genomic assembly of bacterial species with uniform coverage and high accuracy.

Compatible with all Illumina sequencing systems, Illumina DNA Prep delivers the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry. Push-button data analysis is available in BaseSpace™ Sequence Hub using:

- DRAGEN™ Metagenomics pipeline—Performs taxonomic classification of reads and provides single sample and aggregate reporting (includes Kraken2)[9]
- SPAdes Genome Assembler—Assembles small genomes from standard bacterial data sets[10]
- MetaPHlAn (Metagenomic Phylogenetic Analysis)—Profiles the composition of microbial communities based on unique clade-specific marker genes identified from reference genomes, allowing orders of magnitude speedups and unambiguous taxonomic assignments[11]

As part of an integrated NGS workflow from library prep through sequencing and analysis (Figure 2), Illumina DNA Prep delivers reliable results for small WGS applications.

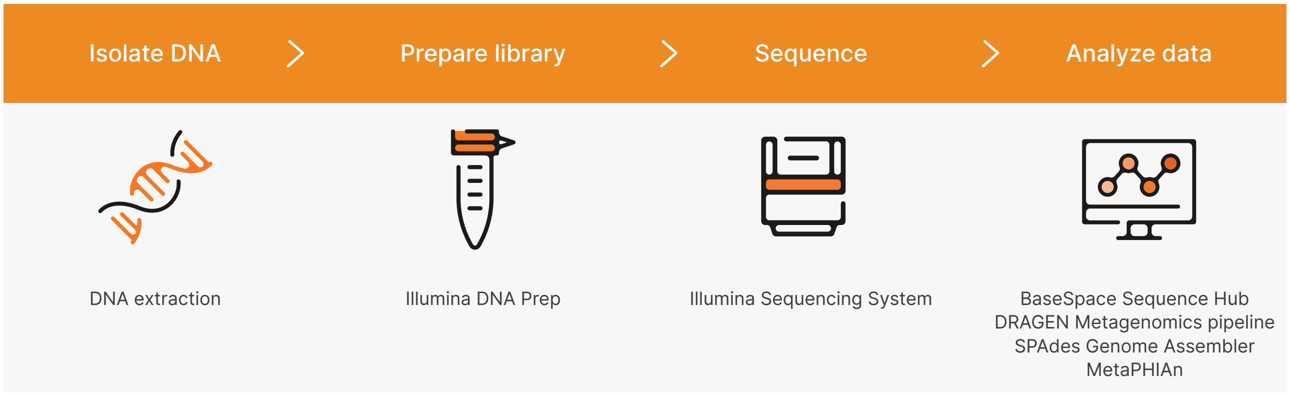

Figure 2: Illumina DNA Prep small genome workflow.

Optimized library prep

A major advance in Illumina library prep chemistry and key feature of Illumina DNA Prep is on-bead tagmentation, which uses bead-linked transposomes (BLTs) to mediate simultaneous DNA fragmentation and tagging of Illumina sequencing primers (Figure 3).

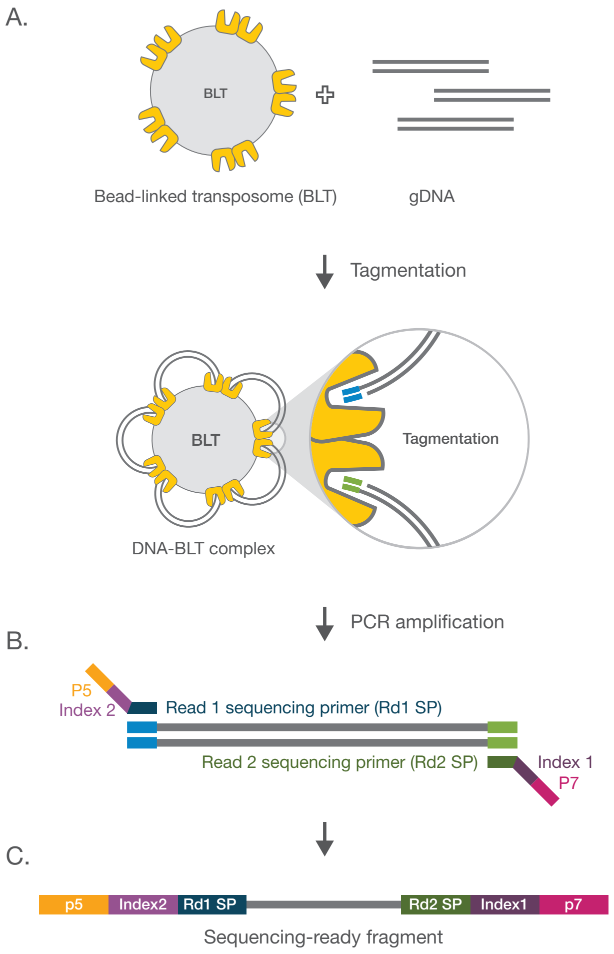

Figure 3: Illumina bead-linked transposome chemistry—(A) Bead-linked transposomes mediate the simultaneous fragmentation of gDNA and addition of sequencing primers. (B) Reduced-cycle PCR amplifies sequencing-ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

On-bead tagmentation provides several significant advantages:

- Eliminates the need for quantitation of the initial DNA sample saving time and costs associated with DNA quantitation and normalization reagents, kits, and equipment
- Eliminates the need for DNA fragmentation, saving time and costs associated with separate shearing instruments or enzymatic kits
- Eliminates the need for individual library quantitation and normalization before pooling and sequencing

On-bead tagmentation produces libraries with consistent insert sizes (~350 bp) over a wide DNA input range (1–500 ng) (Figure 4), offering increased flexibility for varying sample types, including precious samples. This chemistry delivers robust performance with DNA input amounts down to 1 ng. With ≥ 100 ng DNA input, the on-bead tagmentation reaction becomes saturated, leading to consistent, normalized yields (Figure 5). This normalized input range offers significant flexibility in the amount of input DNA used with Illumina DNA Prep.

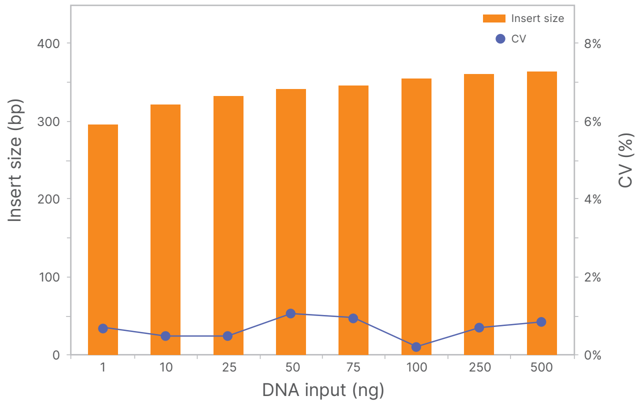

Figure 4: Uniform and consistent insert sizes—On-bead tagmentation delivers consistent insert sizes regardless of DNA input amount. From 1–500 ng DNA input, the total coefficient of variance (CV) is 6.09%. Libraries were produced with *E. coli* replicate samples using Illumina DNA Prep and run on a MiSeq™ System (2 × 76 bp).

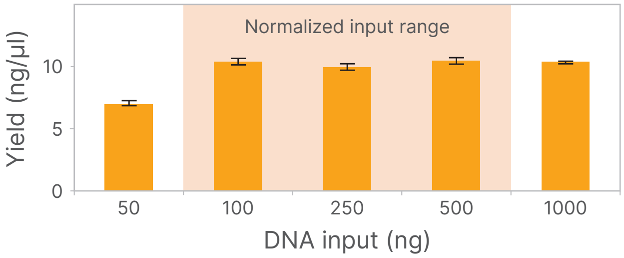

Figure 5: Tagmented and normalized libraries—Beads become saturated at or over 100 ng, leading to a normalized yield of tagmented DNA and eliminating the need for downstream library normalization steps. Libraries produced with Human-NA12878 samples (Coriell Institute) and run on a MiSeq System (2 × 76 bp).

Comprehensive coverage

Illumina DNA Prep Kit achieves greater uniformity of coverage across different Gram-positive and Gram-negative bacterial species, as compared to the Nextera XT DNA Library Prep Kit, even at input amounts as low as 1 ng (Figure 6). Two additional measurements of genome assembly quality were used for comparison: N50 and the number of contigs. N50 represents the "contig length where 50% of the entire assembly is contained in contigs of this length or longer."[12] Generally, higher N50 values, ie, longer contigs on average, are indicative of better genome assembly. By extension, fewer contigs in an assembly is another indicator of quality, as a fewer number of longer contigs will result in higher accuracy than a higher number of smaller contigs. Libraries prepared from eight bacterial species with the Illumina DNA Prep Kit have higher N50 values (Figure 7) and fewer total numbers of contigs (Figure 8) compared to libraries prepared with the Nextera XT DNA Library Prep Kit.

Additionally, customer publications have demonstrated the superior performance of Illumina DNA Prep over Nextera XT DNA Library Prep for microbial genome assembly.[13-15]

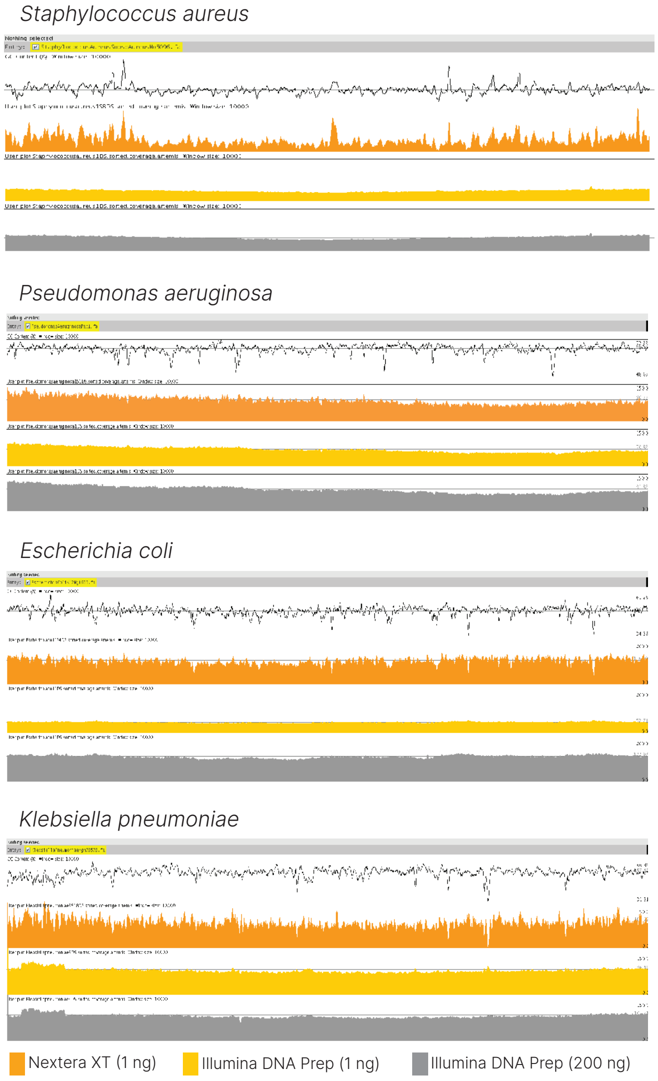

Figure 6: Improved uniformity of coverage—Varying amounts of input genomic DNA from up to eight different bacterial species were prepared with both Illumina DNA Prep and the Nextera XT DNA Library Preparation Kit. Libraries were sequenced using paired-end 2 × 150 bp reads on the NextSeq 550 System. Results demonstrate the greater uniformity of coverage across different Gram-positive and Gram-negative bacterial species achieved with Illumina DNA Prep compared to the Nextera XT DNA Library Prep Kit.

Studies demonstrate equivalent performance of Illumina DNA Prep for microbial WGS on the NextSeq™ 550 System and the NextSeq 2000 System.[16]

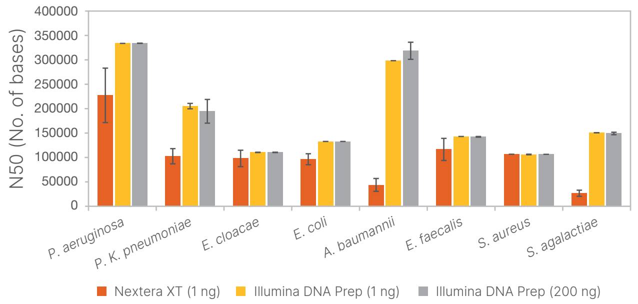

Figure 7: Comparison of contig length by library prep kit—Libraries prepared with Illumina DNA Prep from eight different bacterial species and sequenced using paired-end 2 × 150 bp reads on the NextSeq 550 System result in higher quality genome assemblies compared to libraries prepared with the Nextera XT DNA Library Prep Kit, as measured by N50.

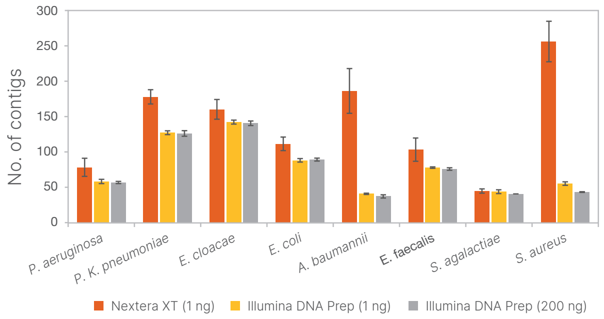

Figure 8: Comparison of the number of contigs by library prep kit—Libraries prepared with Illumina DNA Prep from eight different bacterial species and sequenced using paired-end 2 × 150 bp reads on the NextSeq 550 System result in fewer numbers of contigs, resulting in higher quality genome assemblies compared to libraries prepared with the Nextera XT DNA Library Prep Kit.

Summary

Illumina DNA Prep features an innovative workflow that combines DNA extraction, quantitation, fragmentation, and library normalization to deliver the most robust and flexible library prep workflow in the Illumina portfolio. On-bead tagmentation chemistry enables support for a wide range of DNA input amounts, various sample types, and a broad range of applications. Illumina DNA Prep is the ideal solution for microbial WGS, demonstrating better coverage uniformity and genome assembly for Gram-positive and Gram-negative bacterial species as compared to the Nextera XT DNA Library Prep Kit.

Ordering information

Product	Catalog no.
Illumina DNA Prep, (M) Tagmentation (24 Samples, IPB)	20060060
Illumina DNA Prep, (M) Tagmentation (96 Samples, IPB)	20060059
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)	20091660
Nextera DNA CD Indexes (96 indexes, 96 samples)	20018708

Learn more

Illumina DNA Prep

Illumina DNA Prep performance for microbial WGS has been demonstarted across various Illumina sequencing systems:

- Small whole-genome sequencing on the NextSeq 1000 and NextSeq 2000 Systems
- Microbial whole-genome sequencing with the iSeq™ 100 Sequencing System
- Microbial whole-genome sequencing with Illumina DNA PCR-Free Prep, Tagmentation

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