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Microbial Whole-Genome Sequencing with the Nextera™ DNA Flex Library Preparation Kit

Fast and flexible library prep that provides uniform coverage and improved genome assembly for viruses, bacteria, and other microbial species.

Highlights

- **Fast and Simple Workflow**
  Reduce library preparation time with a low number of steps and minimal hands-on time
- **Optimized Library Prep**
  Obtain robust, consistent results over a wide range of DNA input, even at low DNA input amounts (1 ng)
- **Comprehensive Coverage**
  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), including 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] With the launch of the Nextera DNA Flex Library Preparation Kit, the latest evolution of library prep is poised to advance microbiology genomics research (Figure 1).

Figure 1: Small WGS with the Nextera DNA Flex Library Preparation Kit—The Nextera DNA Flex Library Preparation Kit enables genomic assembly of bacterial species with uniform coverage and high accuracy.

Fast and Simple Workflow

Featuring unique chemistry that integrates multiple pre- and post-library preparation steps, the Nextera DNA Flex Library Preparation Kit delivers the fastest workflow with the fewest number of steps in the Illumina library prep portfolio. In addition to speed and efficiency gains, it offers exceptional flexibility for sample input type, amount, and a wide range of supported applications. The Nextera DNA Flex Library Preparation Kit enables DNA extraction directly from bacterial colonies grown overnight at 37°C on blood agar plates for eight different species, resulting in additional time and cost savings while improving data consistency.[2–4]

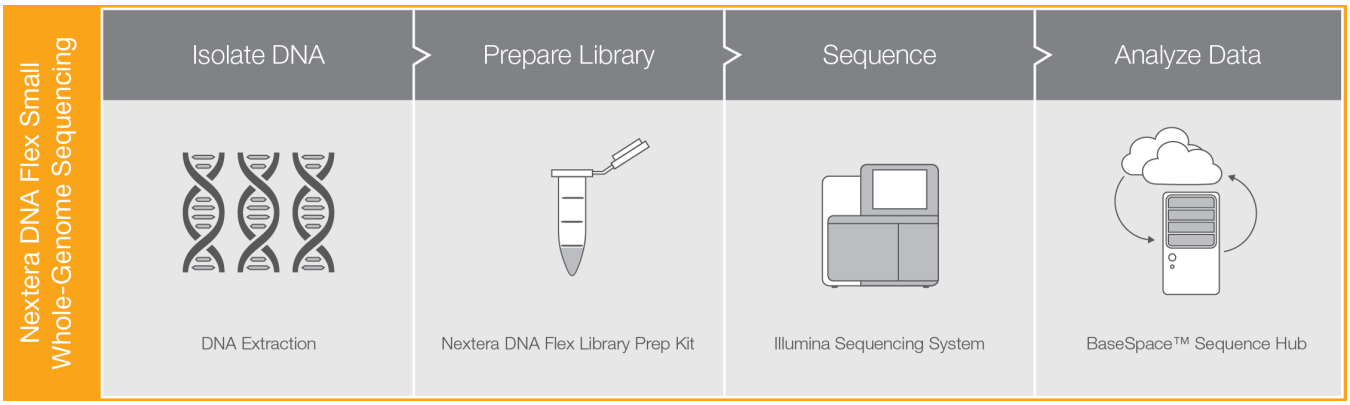


Figure 2: Nextera DNA Flex Library Preparation Workflow—User-friendly Nextera DNA Flex library preparation is part of a streamlined NGS workflow that includes library prep, sequencing, and data analysis.

Compatible with all Illumina sequencing systems, the Nextera DNA Flex Library Preparation Kit delivers the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry, used to generate more than 90% of the world's sequencing data.* As part of an integrated NGS workflow from library prep through sequencing and push-button data analysis in BaseSpace™ Sequence Hub (Figure 2), the Nextera DNA Flex Library Preparation Kit delivers reliable results for small WGS applications.

Optimized Library Prep

A major advance in Illumina library prep chemistry and key feature of the Nextera DNA Flex Library Preparation Kit is On-Bead Tagmentation, which uses bead-linked transposomes (BLTs) to mediate simultaneous DNA fragmentation and the tagging of Illumina sequencing primers (Figure 3).

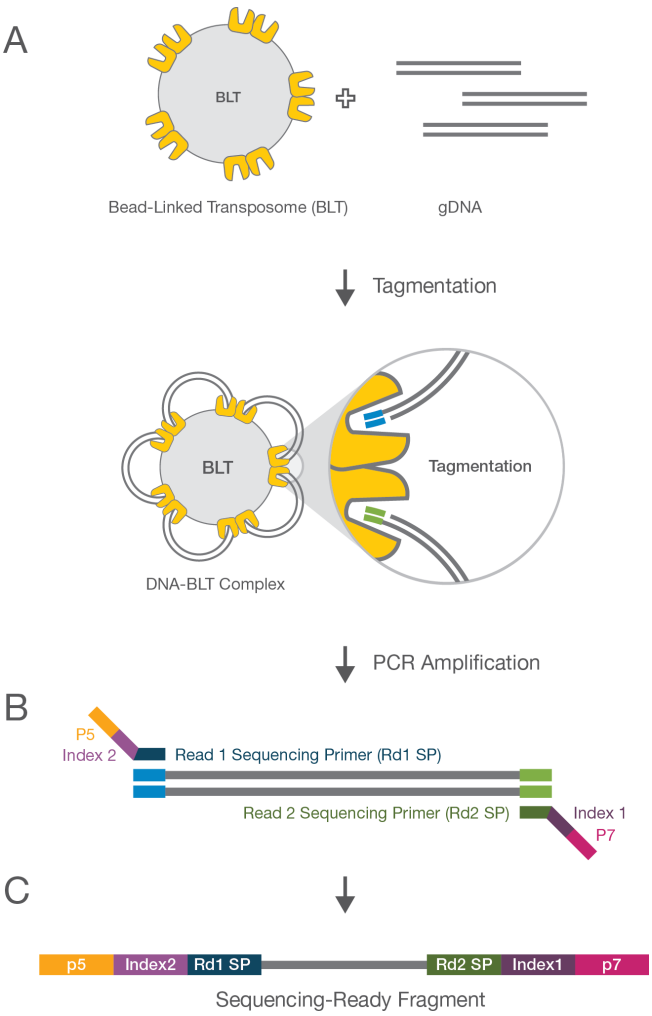


Figure 3: Nextera On-Bead Tagmentation Chemistry—(A) BLTs mediate tagmentation. (B) Reduced-cycle PCR amplifies sequencing ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

On-Bead Tagmentation provides several significant advantages:

- Eliminates the need for quantitation of the initial DNA sample, across a wide DNA input range (100–500 ng), 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, across a wide DNA input range (100–500 ng), before pooling and sequencing.

On-Bead Tagmentation produces libraries with consistent insert sizes (~ 350 bp) over a wide DNA input range (Figure 4). This wide range of DNA input (1–500 ng) offers increased flexibility for varying sample types, including precious samples. This new 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 for Nextera DNA Flex library prep.

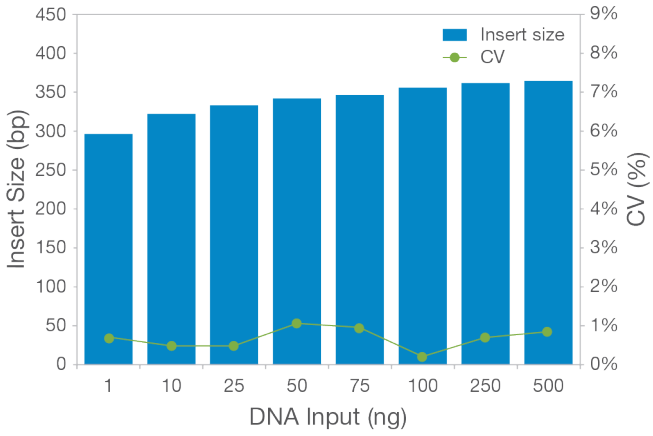


Figure 4: Uniform and Consistent Insert Sizes—On-Bead Tagmentation delivers consistent insert sizes over a wide range of DNA input. Libraries were prepared with *E. coli* replicate samples using Nextera DNA Flex Kit. Sequencing was performed on a MiSeq™ System (2 × 76 bp run).

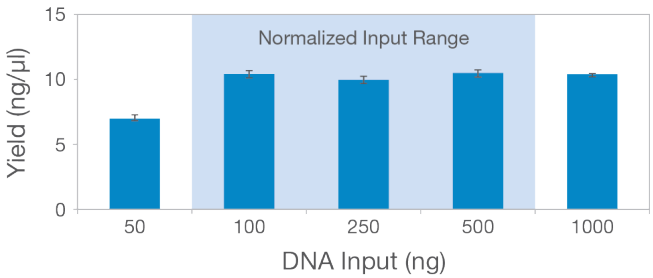


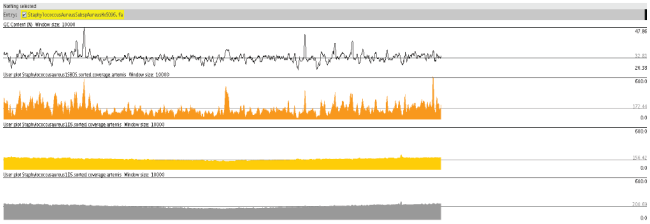
Figure 5: Tagmented and Normalized Libraries—Beads become saturated at or near 100 ng, leading to normalized yield of tagmented DNA. Libraries were prepared with Human-NA12878 samples (Coriell Institute) using the Nextera DNA Flex Kit. Sequencing was performed on a MiSeq System (2 × 76 bp).

*Data calculations on file. Illumina, Inc., 2015.

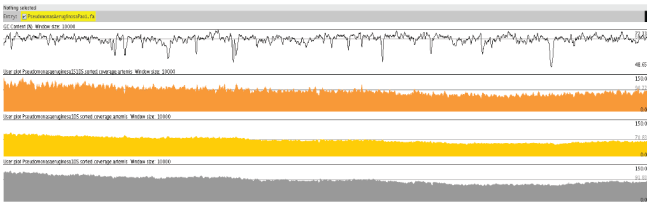
Comprehensive Coverage

To demonstrate the improved accuracy of Nextera DNA Flex library prep in the genome assembly of microbial organisms, varying amounts of input genomic DNA from up to eight different bacterial species were prepared with either the Nextera DNA Flex Library Preparation Kit or the Nextera XT DNA Library Preparation Kit. Libraries were sequenced using paired-end 2 × 150 bp reads on the NextSeq™ 550 System.

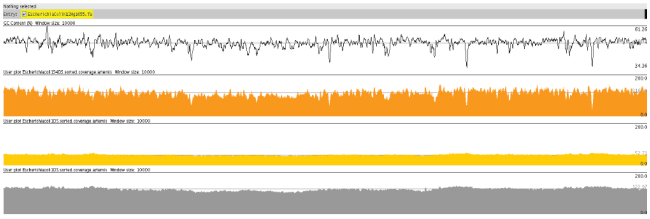
Staphylococcus aureus



Pseudomonas aeruginosa



Escherichia coli



Klebsiella pneumoniae

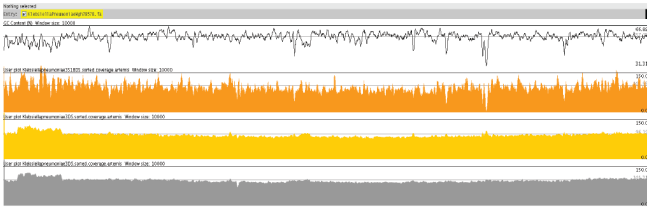


Figure 6: Improved Uniformity of Coverage —The Nextera DNA Flex Library Preparation Kit achieves greater uniformity of coverage across different Gram-positive and Gram-negative bacterial species, as compared to the Nextera XT DNA Library Prep Kit.

The Nextera DNA Flex Library Preparation 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, particularly at low DNA input amounts (Figure 6). Two additional measurements of genome assembly quality were used for comparison. N50 is defined as the minimum contig length needed to cover 50% of the genome.[5] 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 Nextera DNA Flex Library Preparation 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. Together, these results support the superior performance of the Nextera DNA Flex Library Preparation Kit for microbial genome assembly, especially at low DNA input amounts.

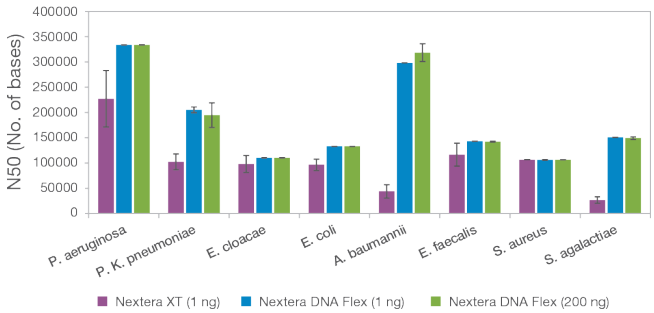


Figure 7: Comparison of Contig Length by Library Prep Kit —Libraries prepared with the Nextera DNA Flex Library Preparation Kit from eight different bacterial species and sequenced using paired-end 2 × 150 bp reads on the NextSeq 550 System result in higher quality genome assemblies, compared to libraries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

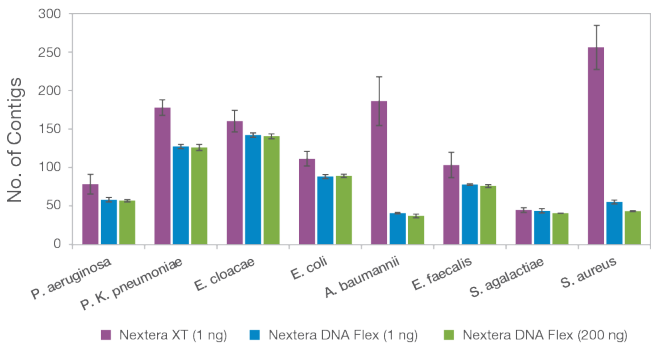


Figure 8: Comparison of the Number of Contigs by Library Prep Kit —Libraries prepared with the Nextera DNA Flex Library Preparation Kit from eight different bacterial species and sequenced using paired-end 2 × 150 bp reads on the NextSeq 550 System result in fewer numbers of contigs, resulting in higher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

Summary

The Nextera DNA Flex Library Preparation Kit features an innovative workflow that combines DNA extraction, quantitation, fragmentation, and library normalization to deliver the fastest and most 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. With improved performance over the Nextera XT DNA Library Prep Kit in coverage uniformity and genome assembly for Gram-positive and Gram-negative bacterial species, the Nextera DNA Flex Library Preparation Kit is the ideal solution for small WGS.

Ordering Information

Product	Catalog No.
Nextera DNA Flex Library Prep Kit (24 samples)	20018704
Nextera DNA Flex Library Prep Kit (96 samples)	20018705
Nextera DNA CD Indexes (24 indexes, 24 samples)	20018707
Nextera DNA CD Indexes (96 indexes, 96 samples)	20018708

Learn More

To learn more about the Nextera DNA Flex Library Preparation Kit, visit www.illumina.com/nextera-dna-flex.

References

1. Illumina. Nextera XT DNA Library Preparation Kit Data Sheet. www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_nextera_xt_dna_sample_prep.pdf. Accessed September 2017.
2. Rubin BE, Sanders JG, Hampton-Marcell J, Owens SM, Gilbert JA, Moreau CS. DNA extraction protocols cause differences in 16S rRNA amplicon sequencing efficiency but not in community profile composition or structure. *MicrobiologyOpen*. 2014;3(6):910–921.
3. van Tongeren SP, Degener JE, Harmsen HJM. Comparison of three rapid and easy bacterial DNA extraction methods for use with quantitative real-time PCR. *Eur J Clin Microbiol Infect Dis*. 2011;30(9):1053–1061.
4. Vesty A, Biswas K, Taylor MW, Gear K, Douglas RG. Evaluating the impact of DNA extraction method on the representation of human oral bacterial and fungal communities. *PLoS One*. 2017;12(1):e0169877.
5. Miller JR, Koren S, Sutton G. Assembly algorithms for next-generation sequencing data. *Genomics*. 2010;95(6):315–327.

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