illumina®

Microbial Whole-Genome Sequencing with the iSeq™ 100 Sequencing System

Fast and efficient sequencing that provides uniform coverage and genome assembly for microbial species.

Highlights

- **Streamlined Workflow**
  Access a comprehensive workflow from DNA to data
- **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 microbes

Introduction

Next-generation sequencing (NGS) has been established as an important tool in microbiology research for analysis of small genomes (≤ 5 Mb), including bacteria, viruses, and other microbes. Microbial NGS, including whole-genome sequencing (WGS) and targeted resequencing, enables mapping and *de novo* assembly of novel organisms, completing genomes of known organisms, and comparing genomes across samples.

The development of Nextera™ chemistry shortened and simplified library preparation by consolidating DNA fragmentation and adapter tagging steps into a single reaction (termed tagmentation) and eliminating the need for library quantitation before pooling and sequencing.[1] The Nextera DNA Flex Library Preparation Kit represents the next step in the evolution of Illumina library prep. In addition to speed and efficiency gains in the workflow, Nextera DNA Flex offers exceptional flexibility for sample input type and amount and robust, consistent preparation of sequencing-ready libraries.

Figure 1: The iSeq 100 System—The iSeq 100 System harnesses the power of NGS in the most affordable, compact benchtop sequencing system in the Illumina portfolio.

The latest innovation in NGS is poised to advance microbiology genomics research. The compact iSeq 100 System (Figure 1) combines complementary metal-oxide–semiconductor (CMOS) technology with proven Illumina sequencing by synthesis (SBS) chemistry to deliver high-accuracy data with fast time to results. The iSeq 100 System is part of a streamlined NGS workflow for targeted and whole-genome microbial sequencing (Figure 2).

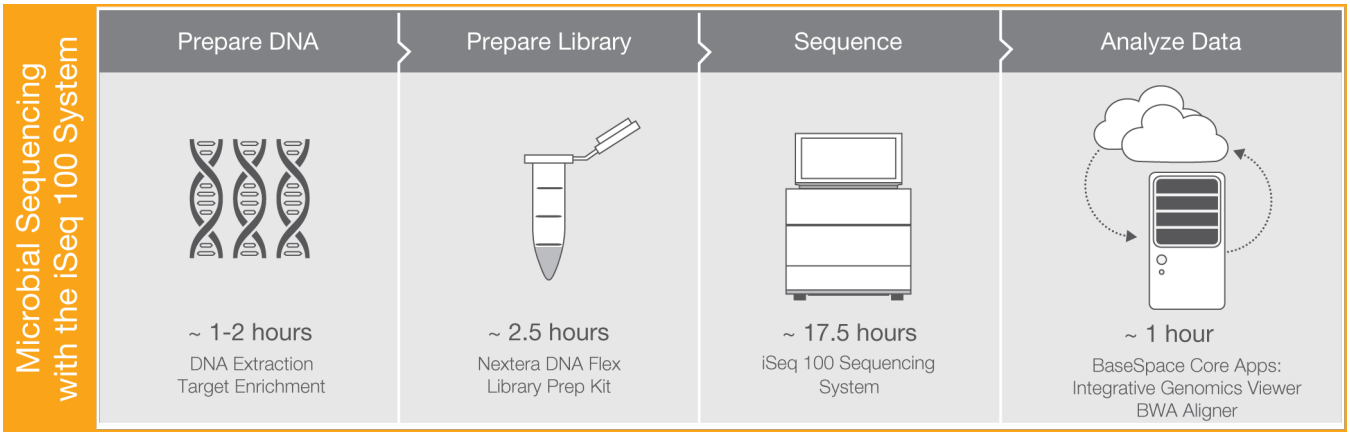

Figure 2: Microbial Sequencing Workflow—Microbial sequencing on the iSeq 100 System is part of a streamlined, comprehensive NGS workflow that includes Nextera DNA Flex library preparation, sequencing, and data analysis.

Simple, Integrated Workflow

Microbial sequencing on the iSeq 100 System is part of an integrated NGS workflow that includes library preparation with the Nextera™ DNA Flex Library Preparation Kit, proven Illumina sequencing, and push-button data analysis in BaseSpace™ Sequence Hub (Figure 2). The entire workflow proceeds from DNA to data in less than 24 hours.

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).

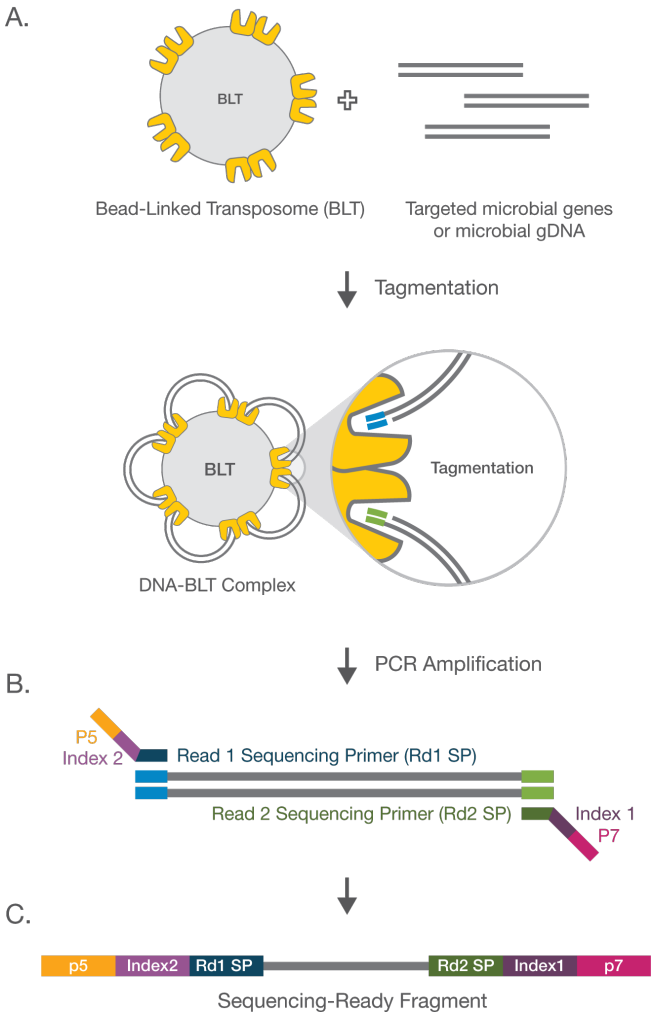

Figure 3: Nextera On-Bead Tagmentation Chemistry—(A) BLTs mediate tagmentation. (B) Reduced-cycle PCR amplifies sequencing ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

To learn more about Nextera DNA Flex and On-Bead Tagmentation, read the Nextera DNA Flex Library Preparation Kit Data Sheet at www.illumina.com/nextera-dna-flex

Sequencing on the iSeq 100 System

After preparation, libraries are loaded into a prefilled reagent cartridge for sequencing on the iSeq 100 System. Starting a run on the iSeq 100 System is as easy as load and go with less than five minutes of setup. The iSeq 100 System integrates clonal amplification, sequencing, and data analysis into a single instrument. The intuitive user interface provides guidance through every step of the run setup and run initiation processes, allowing researchers to perform various sequencing applications with minimal user training and minimal set up time.

The iSeq 100 System harnesses proven Illumina SBS chemistry, used to generate more than 90% of the world's sequencing data.[2] Illumina SBS chemistry is used in all Illumina sequencing systems, enabling researchers to compare data across systems and scale their studies to higher throughput systems.

Easy, Flexible Data Analysis

The iSeq 100 System offers several data analysis options, including onboard and cloud-based data analysis. The Local Run Manager software, an onboard analysis software, features modular architecture to support current and future assays. Local Run Manager software supports planning sequencing runs, tracking libraries and runs with audit trails, and integration with onboard data analysis modules.

Alternatively, sequence data can be instantly transferred, analyzed, and stored securely in BaseSpace Sequence Hub, the Illumina genomics computing environment. BaseSpace Sequence Hub features a rich ecosystem of commercial and open-source apps for downstream data analysis, including the Integrative Genomics Viewer and BWA Aligner apps (Table 1).

Table 1: BaseSpace Apps for Microbial Sequencing Data Analysis

BaseSpace App	Description
Integrative Genomics Viewer	The Integrative Genomics Viewer (IGV) app displays alignments and variants from multiple samples for performing complex variant analysis.
BWA Aligner	The BWA Aligner app aligns samples (FASTQ files) to a reference genome using the Burrows-Wheeler Aligner maximal exact match (BWA-MEM) algorithm.

Comprehensive Coverage

To demonstrate the comparable performance of the iSeq 100 System to other sequencing systems in the Illumina portfolio in the genome assembly of microbial organisms, input genomic DNA from three different bacterial species with varying GC content (Table 2) were prepared with the Nextera DNA Flex Library Preparation Kit. Libraries were sequenced using paired-end 2 × 151 bp reads on the iSeq 100 System, MiniSeq™ System, and MiSeq™ System.

The iSeq 100 System delivers similar uniformity of coverage across different bacterial species, as compared to the MiniSeq and MiSeq Systems (Figure 4). These results support the exceptional performance of the iSeq 100 System for targeted and whole-genome microbial sequencing.

Table 2: GC Content of Sequenced Microbial Genomes

	B. cereus	*E. coli*	*R. sphaeroides*
Genome Size	~ 5.4 Mb	~ 4.6 Mb	~ 4.1 Mb
% GC Content	~ 35%	~ 51%	~ 69%

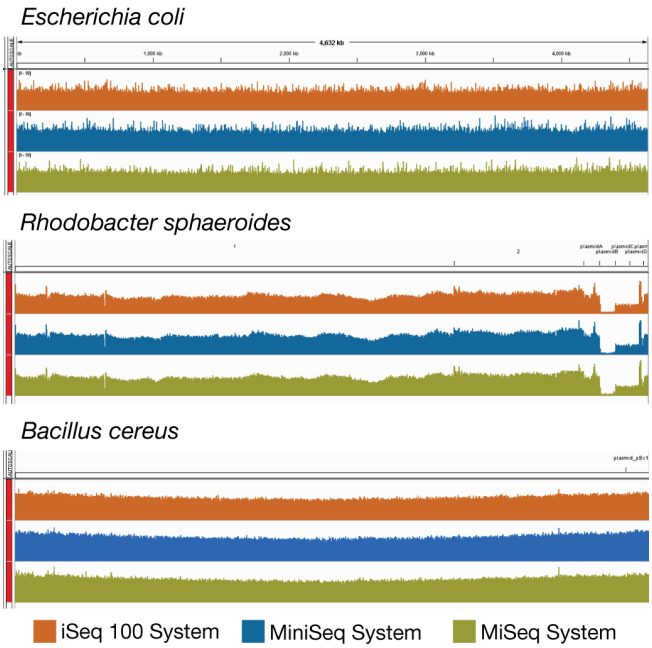

Figure 4: Consistent Uniformity of Coverage —The iSeq 100 System delivers similar uniformity of coverage across different three bacterial species, as compared to the MiniSeq and MiSeq Systems.

Summary

The iSeq 100 System is part of a fully supported solution for targeted and whole-genome microbial sequencing that includes simplified library preparation with the Nextera DNA Flex Library Preparation Kit, sequencing, and user-friendly data analysis. The iSeq 100 System delivers the same data quality as larger benchtop sequencers in a smaller footprint with faster run times, making it an ideal, cost-effective solution for small-scale microbiology NGS applications.

Ordering Information

Library Prep	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
Sequencing System	**Catalog No.**
iSeq 100 System	20021532
Sequencing Reagents	**Catalog No.**
iSeq 100 i1 Reagents (300 cycles single kit)	20021533
iSeq 100 i1 Reagents (300 cycles quad kit)	20021534

Learn More

To learn more about the iSeq 100 System, visit www.illumina.com/systems/sequencing-platforms/iseq.html

To learn more about microbial whole-genome sequencing, visit www.illumina.com/microbiology.html

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. Data calculations on file. Illumina, Inc., 2017.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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