APPLICATION NOTE

Microbial whole-genome sequencing with the iSeq™ 100 System

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

- Access a comprehensive, streamlined DNA-to-data workflow
- Obtain robust, consistent results over a wide range of DNA input amounts
- Produce sequencing data with uniform coverage for viruses, bacteria, and other microbes

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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.

Innovations in library prep chemistry consolidated DNA fragmentation and adapter tagging steps into a single reaction (termed tagmentation), simplifying workflows and eliminating the need for library quantification before pooling and sequencing. Illumina DNA Prep represents the next step in the evolution of Illumina library preparation. In addition to speed and efficiency gains in the workflow, Illumina DNA Prep offers exceptional flexibility for sample input type and amount and robust, consistent preparation of sequencing-ready libraries.

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 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.

Simple, integrated workflow

Microbial sequencing on the iSeq 100 System is part of an integrated NGS workflow that includes library preparation with the Illumina DNA Prep, 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 a 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).

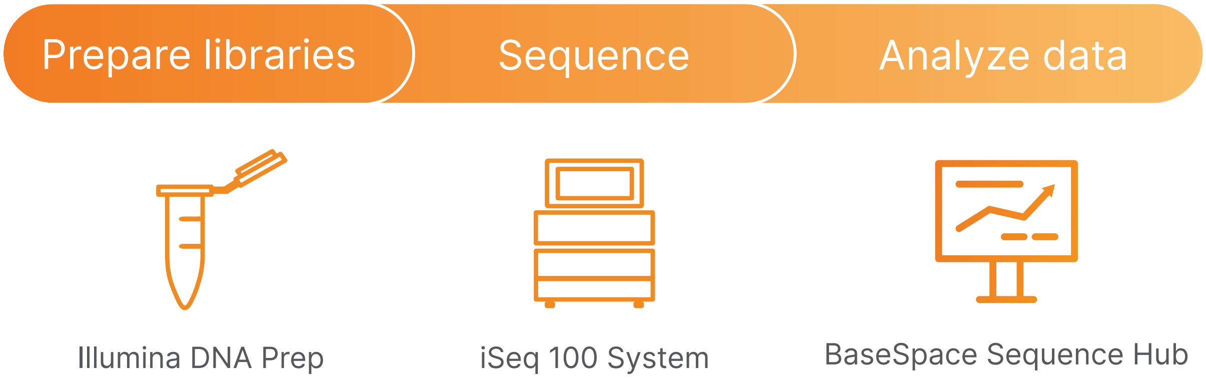

Figure 2: Microbial genome sequencing workflow—Illumina offers a streamlined, comprehensive workflow for microbial WGS that includes library preparation with Illumina DNA Prep, sequencing on the iSeq 100 System, and analysis with BaseSpace core apps.

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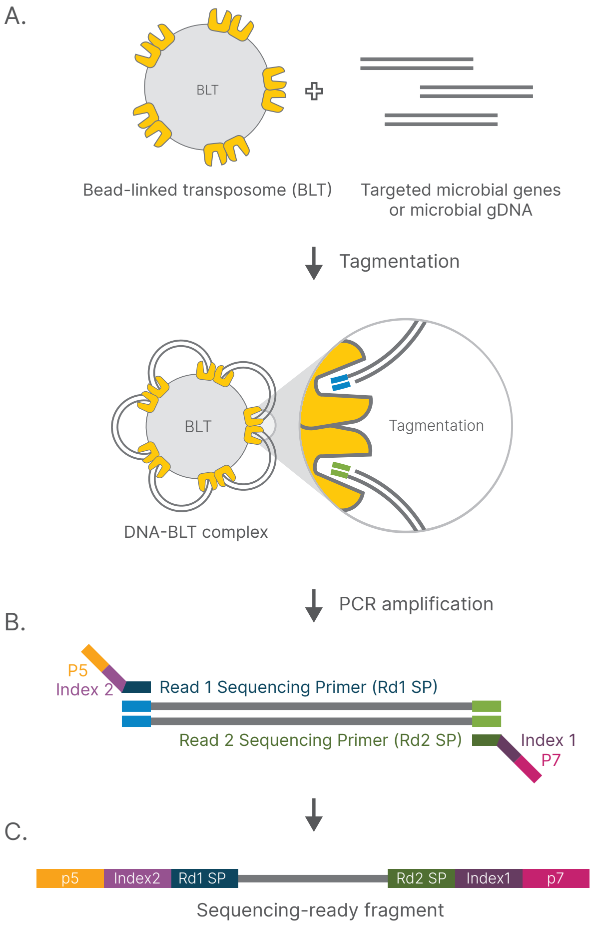

Figure 3: On-bead tagmentation chemistry—(A) BLTs mediate tagmentation, (B) reduced-cycle PCR amplifies sequencing-ready DNA fragments and adds indexes and adapters, (C) sequencing-ready fragments are washed and pooled.

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 set up. Clonal amplification, sequencing, and data analysis are consolidated onto the iSeq 100 System. 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 setup time.

The iSeq 100 System harnesses proven Illumina SBS chemistry, 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 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 DRAGEN™ Metagenomics Pipeline (Table 1).

Table 1: Apps for microbial sequencing data analysis

Data analysis app	Learn more
DRAGEN Metagenomics Pipeline	Link
CosmosID Metagenomics	Link
MetaPhlAn	Link
SPAdes Genome Assembler	Link
Prokka Genome Annotation	Link

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 Illumina DNA Prep. 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.

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Table 2: GC content of sequenced microbial genomes

	B. cereus	*E. coli*	*R. sphaeroides*
Genome size	~ 5.4 Mb	~ 4.8 Mb	~ 4.1 Mb
% GC content	~ 35%	~ 51%	~ 69%

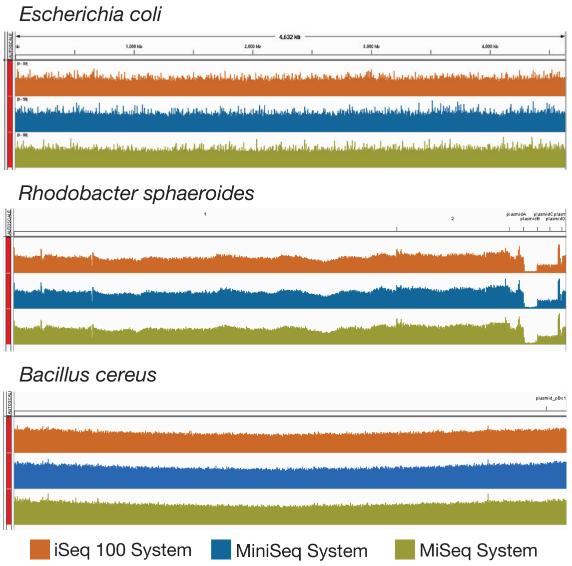

Figure 4: Uniform coverage—The iSeq 100 System delivers similar uniformity of coverage across three different bacterial species, as compared to the MiniSeq and MiSeq Systems.

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 Illumina DNA Prep, 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.
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
Sequencing system	Catalog no.
iSeq 100 System	20021532
Sequencing reagents	Catalog no.
iSeq 100i1 Reagents (300 cycles single kit)	22021533
iSeq 100i1 Reagents (300 cycles quad kit)	20021534

Learn more

iSeq 100 System

Illumina DNA Prep

Microbial WGS

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