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Microbial whole-genome sequencing with Illumina DNA PCR-Free Prep, Tagmentation

Fast, flexible library preparation for uniform genomic coverage for microbial species.

Introduction

Next-generation sequencing (NGS) has been established as an important tool for analyzing 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. However, PCR-dependent library preparation for microbial WGS, particularly when combined with in-solution adapter tagging and fragmentation, can introduce bias, leading to uneven coverage across regions of the genome, especially regions with extreme or uneven base composition.

To address this challenge, Illumina DNA PCR-Free Prep, Tagmentation (Illumina DNA PCR-Free) offers a unique combination of On-Bead Tagmentation with a PCR-free workflow (Figure 1). Tagmentation is a transposome-mediated reaction that combines tagging and DNA fragmentation into a single, rapid reaction. By removing PCR amplification steps and using bead-based chemistry, Illumina DNA PCR-Free chemistry provides highly accurate sequence information for sensitive applications, such as microbial WGS.

This application note demonstrates the exceptional performance of Illumina DNA PCR-Free as part of a comprehensive workflow for genome assembly of microbial organisms and presents sequencing results for three different bacterial species with varying GC content.

Methods

Samples

DNA from three bacterial species with differing GC content (Table 1) were obtained from the American Type Culture Collection (ATCC): *B. cereus* strain 971 (ATCC, Catalog no. 14579D-5), *E. coli* strain MG1655 (ATCC, Catalog no. 700926D-5), and *R. sphaeroides* strain ATH 2.4.1 (ATCC, Catalog no. 17023D-5).

Table 1: 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%

Library preparation

Libraries were prepared from 200 ng of input bacterial genomic DNA (gDNA) using Illumina DNA PCR-Free reagents and the standard workflow. For comparison, 600 ng of bacterial gDNA was sheared by Covaris ultrasonication and input into the TruSeq™ DNA PCR-Free workflow. Sonication and the TruSeq DNA PCR-Free workflow were modified to select for an average insert size of 450 bp.

Sequencing

Pooled libraries were sequenced on a MiSeq™ or NextSeq™ 550 Sequencing System with a run configuration of 2 × 150 bp.

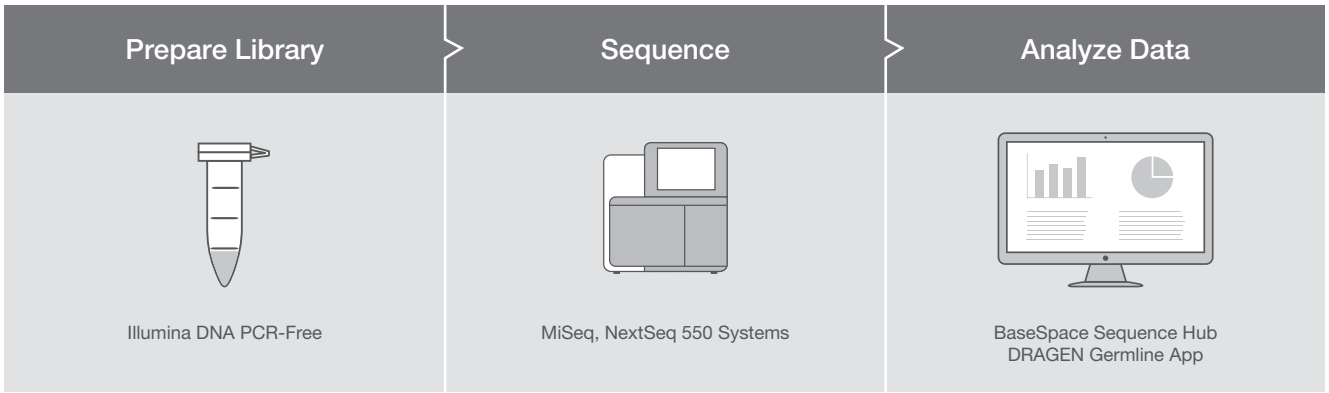

Figure 1: Illumina DNA PCR-Free workflow—Illumina DNA PCR-Free is part of a comprehensive, streamlined workflow for microbial WGS that includes library preparation, sequencing, and data analysis.

Data analysis

After sequencing was complete, data was streamed directly from the instrument into the cloud ecosystem for push-button analysis using DRAGEN™ apps available through BaseSpace™ Sequence Hub. The DRAGEN Germline App v3.4.5 was used for reference alignment.

Results

To evaluate the performance of Illumina DNA PCR-Free for microbial WGS, results were compared to those obtained using TruSeq DNA PCR-Free.

Robust library yield with lower input requirements

Illumina DNA PCR-Free results in equivalent or better total library yield compared to TruSeq DNA PCR-Free for the microbial species tested (Figure 2). Of particular note, library preparation with *B. cereus* gDNA (an AT-rich genome) resulted in significantly higher yield. Importantly, Illumina DNA PCR-Free achieves similar library yields with a lower DNA input requirement, as compared to TruSeq DNA PCR-Free (Table 2).

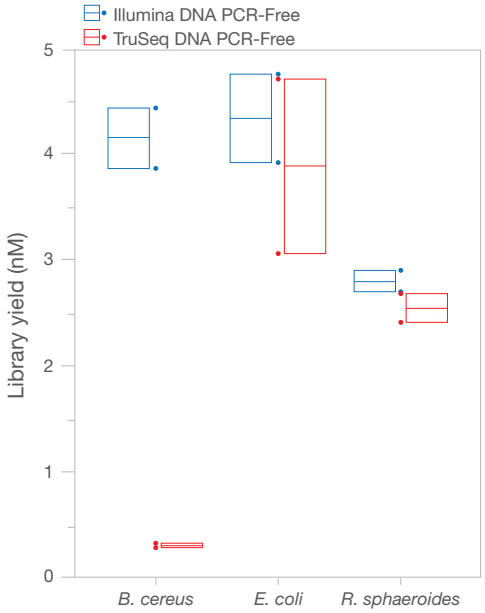

Figure 2: Comparison of library yields across microbial genomes of varying GC content—Illumina DNA PCR-Free generates sequencing libraries with equivalent or better yields, as compared to TruSeq DNA PCR-Free.

Uniform coverage across genomes with varying GC content

To assess coverage performance across bacterial species with a range of low, medium, and high GC content, normalized coverage data from Illumina DNA PCR-Free and TruSeq DNA PCR-Free were plotted against reference genome content by GC percentage. Both kits show even coverage levels across all microbial species tested, regardless of GC content (Figure 3, Table 2). For comparison, genome coverage was also evaluated with Illumina DNA Prep (includes PCR). Results show comparable performance with some biases at extremes of GC content (Figure 3). However, Illumina DNA Prep performs better than library prep kits with in-solution tagmentation.

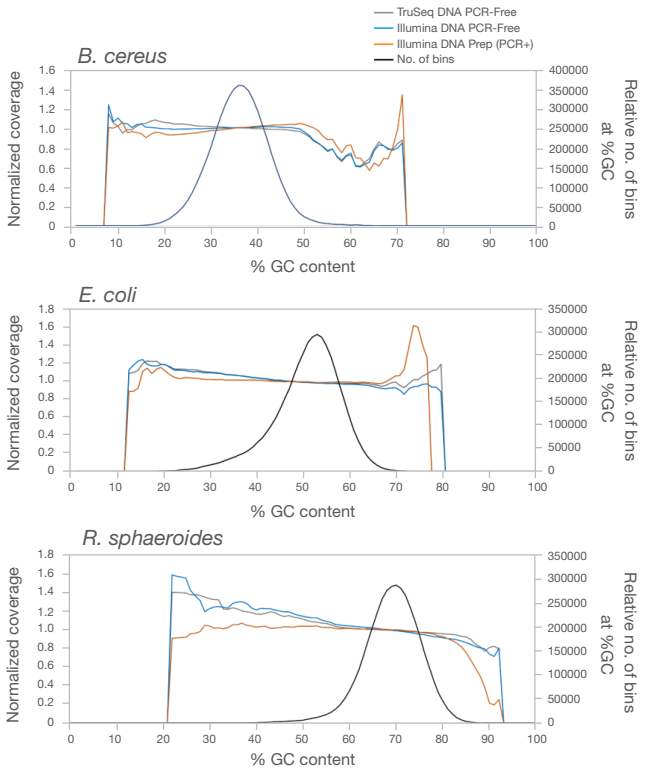

Figure 3: Comparison of read coverage across microbial genomes of varying GC content—Illumina DNA PCR-Free (blue line) provides consistent and comparable read coverage across microbial genomes of varying GC content, as compared to TruSeq PCR-Free (gray line) and Illumina DNA Prep (orange line). The bell curve trace (black line) shows the actual GC composition of each microbial species.

Table 2: Genome build metrics

	B. cereus		*E. coli*		*R. sphaeroides*	
Library preparation	Illumina DNA PCR-Free	TruSeq DNA PCR-Free	Illumina DNA PCR-Free	TruSeq DNA PCR-Free	Illumina DNA PCR-Free	TruSeq DNA PCR-Free
Input amount	200 ng	600 ng	200 ng	600 ng	200 ng	600 ng
Paired-end (PE) reads subsampled	1M	1M	1M	1M	1M	1M
Yield	4.5 nM	0.35 nM	3.9 nM	3.1 nM	2.7 nM	2.4 nM
Average alignment coverage over genome	52.2	51.0	61.1	62.5	58.7	59.9
Uniformity of coverage (% > 0.2 mean) over genome	98.9%	99.0%	99.3%	99.4%	96.9%	96.9%
Percent mapped	96.3%	93.1%	97.2%	97.8%	92.4%	93.0%

Figure 4: Comparison of PCR-Free workflows—Illumina DNA PCR-Free delivers a rapid total assay time of 90 minutes from tagmentation through library clean-up, compared to > 7 hours total assay time for TruSeq DNA PCR-Free.

On-Bead Tagmentation and PCR-free protocol

Illumina DNA PCR-Free provides a unique and powerful combination of benefits from On-Bead Tagmentation and PCR-free chemistry. On-Bead Tagmentation uses bead-linked transposomes to perform a more uniform tagmentation reaction compared to in-solution tagmentation. After the bead-linked transposomes are saturated with DNA, no additional tagmentation can occur, delivering robust insert size control and normalized yields from DNA input amounts above 150 ng.[1,2] This minimizes quantification steps both before and after library prep. Normalized libraries can be pooled by volume, thus avoiding time-consuming quantification of individual libraries. By eliminating quantification and PCR steps, Illumina DNA PCR-Free offers significant time savings, as compared to TruSeq DNA PCR-Free (Figure 4).

Summary

Illumina DNA PCR-Free offers a unique combination of benefits from On-Bead Tagmentation and PCR-Free chemistry steps. Illumina DNA PCR-Free delivers exceptional ease of use, uniform coverage, and high-accuracy data for microbial WGS, even for challenging genomic regions with extreme GC content.

Learn more

To learn more about Illumina DNA PCR-Free, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep.html

References

1. Illumina (2018). Nextera DNA Flex Library Preparation Kit. Accessed April 10, 2020.
2. Bruinsma S, Burgess J, Schlingman D, Czyz A, Morrell N, et al. Bead-linked transposomes enable a normalization-free workflow for NGS library preparation. *BMC Genomics*. 2018;19(1):722.

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