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TECHNICAL NOTE

Index-first sequencing on the MiSeq™ i100 Series

Faster sequencing times and earlier run quality control metrics

Real-time run management

Early access to demultiplexing information for improved sequencing run planning

High-quality data

Equivalent data performance comparable to read-first sequencing

Fast turnaround times

Various run configurations deliver results faster than read-first sequencing

Introduction

At the core of Illumina next-generation sequencing (NGS) technology is sequencing by synthesis (SBS) chemistry, used across all Illumina sequencing systems. Typically, Illumina systems perform read-first sequencing, in which read 1 is sequenced, followed by index 1, paired-end resynthesis (PER), index 2, and read 2. The MiSeq i100 Series leverages index-first sequencing, in which index 1 is sequenced, followed by index 2, read 1, PER, and read 2 (Figure 1). Index-first sequencing offers faster time to demultiplexing results, enabling real-time run management, and for various run configurations, a faster total run time. With early access to demultiplexing information, users can cancel a run and restart if sample representation is not ideal or plan for subsequent runs as needed. Index-first sequencing is compatible with existing key applications and workflows with no impact on performance or data quality. The MiSeq i100 Series performs index-first sequencing by default, but users have the option to select read-first sequencing during run setup.*

For this technical note, two sequencing run configurations were evaluated: single-read, dual-indexed sequencing with the Respiratory Pathogen ID/AMR Panel and paired-end read, dual-indexed small whole-genome sequencing (sWGS). Results demonstrate the time savings provided by index-first sequencing while maintaining high-quality data, compared to read-first sequencing.

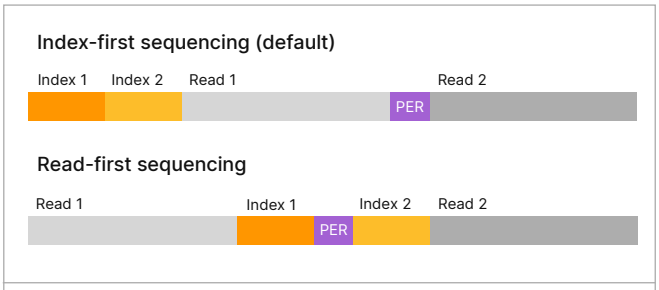

Figure 1: index-first and read-first sequencing on the MiSeq i100 Series
The MiSeq i100 Series features index-first sequencing, which enables early demultiplexing of run data for faster sequencing run times and real-time run management.

Methods

Respiratory Pathogen ID/AMR Panel

Samples were prepared from NATtrol Respiratory Panel 2.1 (RP2.1) Controls (ZeptoMetrix, Catalog no. NATRPC2.1-BIO) following the DNA/RNA Extraction Procedure found in the Respiratory Pathogen ID/AMR Panel User Guide. Libraries were prepared using the Respiratory Pathogen ID/AMR Enrichment Kit Set A (RUO) (96 indexes, 96 samples) (Illumina, Catalog no. 20047050).

Sequencing was performed on the MiSeq i100 Plus System with the MiSeq i100 Series 25M Reagent Kit (300 cycles) (Illumina, Catalog no. 20126568) and the 1 × 147 bp run configuration with 10 bp dual indexing and 1M clusters per sample (12-plex pool). Data analysis was performed using the DRAGEN™ BCL Convert app v4.3.13 for demultiplexing results. Secondary analysis can be performed using the DRAGEN Microbial Enrichment Plus app v1.1.0 for microorganism detection and consensus sequence generation (data not included in this technical note).

Small whole-genome sequencing

Small WGS libraries were prepared from 100 ng commercially available microbial gDNA, using Illumina DNA Prep (Illumina, Catalog no. 20060060).

Sequencing was performed on the MiSeq i100 Plus System with the MiSeq i100 Series 25M Reagent Kit (300 cycles) (Illumina, Catalog no. 20126568) using the 2 × 151 bp run configuration, with 10 bp dual indexing (24-plex pool). Data analysis was performed using the DRAGEN BCL Convert app v4.3.13 for demultiplexing results. Secondary analysis can be performed using the DRAGEN Small Whole-Genome Sequencing app v4.3.13 for reference-based mapping of microbial genomes (data not included in this technical note).

* Targeted sequencing methods that use custom primers, eg, the Illumina Custom Enrichment Panel v2, are not compatible with index-first sequencing and require users to select read-first sequencing during run setup.

Results

Faster run times with index-first sequencing

Respiratory Pathogen ID/AMR Panel and sWGS libraries were sequenced on the MiSeq i100 Plus System using index-first and read-first sequencing protocols. The total run time was reduced for Respiratory Pathogen ID/AMR Panel libraries with index-first sequencing due to PER not being necessary, and the time to demultiplexing results was reduced for both libraries types and run configurations (Figure 2).

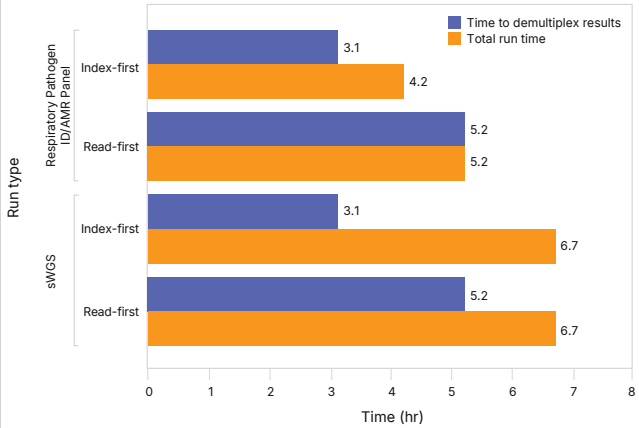

Figure 2: Comparison of sequencing run times
Index-first sequencing on the MiSeq i100 Plus System results in faster total run time (orange bars) for sequencing of Respiratory Pathogen ID/AMR Panel libraries (single reads) and faster time to demultiplexing results (blue bars) for both libraries (single reads and paired-end reads) compared to read-first sequencing.

High-quality run metrics with index-first sequencing

Sequencing run metrics were evaluated, including percent bases above Q30 and clusters passing filter. Index-first and read-first sequencing on the MiSeq i100 Plus System delivered high-quality data with both library types and run configurations (Figure 3).

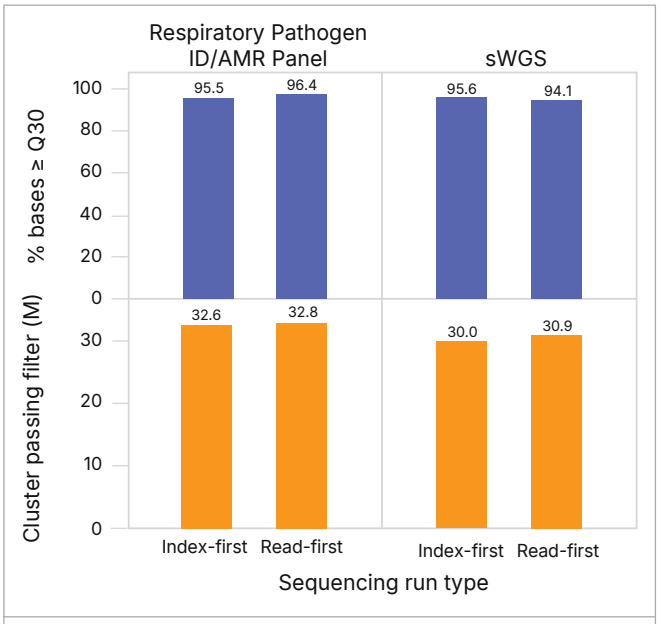

Figure 3: Comparison of sequencing run metrics
Index-first sequencing on the MiSeq i100 Plus System results in high-quality data that are comparable with read-first sequencing for both Respiratory Pathogen ID/AMR Panel and sWGS libraries, indicated by % bases ≥ Q30 (blue bars) and clusters passing filter (orange bars).

Highly concordant demultiplexing results

Demultiplexing metrics were consistent across run configurations on the MiSeq i100 Plus System, with high sample correlation between index-first and read-first sequencing for both the Respiratory Pathogen ID/AMR Panel and sWGS (Table 1 and Figure 4).

Table 1: Comparison of demultiplexing metrics

Library	Run type	% reads identified	CV	Min %	Max %
Respiratory Pathogen ID/AMR Panel (12-plex pool)	Index-first sequencing	97.1	0.20	5.3	11.5
	Read-first sequencing	96.3	0.21	5.3	11.6
sWGS (24-plex pool)	Index-first sequencing	96.8	3.1	3.1	4.8
	Read-first sequencing	96.7	3.3	3.3	4.8

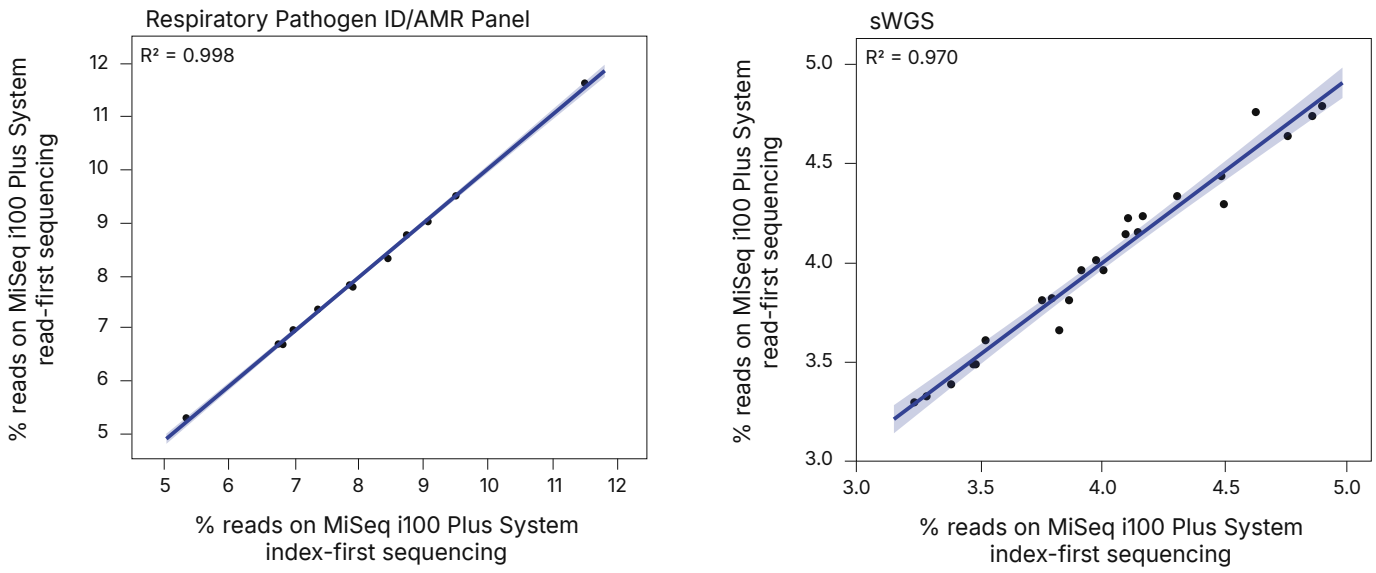

Figure 4: Concordance of demultiplexing metrics
Index-first sequencing on the MiSeq i100 Plus System results in demultiplexing metrics that are highly consistent with read-first sequencing for both Respiratory Pathogen ID/AMR Panel and sWGS libraries.

Summary

The MiSeq i100 Series introduces index-first sequencing to Illumina NGS technology. Offering faster time to demultiplexing results, index-first sequencing enables real-time run management. This technical note demonstrates the high-quality, equivalent data provided by index-first sequencing, compared to read-first sequencing.

Learn more

MiSeq i100 Series

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