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TECHNICAL NOTE

Faster time to answer for infectious disease identification

Turbo custom recipe for sequencing respiratory pathogens on the MiSeq™ i100 Series

Comprehensive coverage of respiratory viruses, bacteria, fungi, and antimicrobial resistance genes

Optimized sequencing recipe that enables shorter run times while maintaining high quality and yield

Flexible and scalable workflow includes integrated onboard data analysis

Introduction

Accurate and timely identification of respiratory pathogens can be challenging, particularly for mixed or coinfections.[1,2] Next-generation sequencing (NGS) provides an effective way to detect known and emerging respiratory pathogens from various sample types, including those with multiple infectious agents, in a single assay.[3] This technical note highlights a custom sequencing recipe (turbo recipe) on the MiSeq i100 Series that supports an accelerated workflow for respiratory pathogen identification. The MiSeq i100 turbo recipe enables single-end, dual-barcode 100-bp reads to complete in 2.5–3 hours—compared to more than 4–5 hours for the standard sequencing recipe on the MiSeq i100 Series (standard recipe).[4]

The turbo custom recipe was evaluated using the Respiratory Pathogen ID/AMR Enrichment Panel, which targets more than 280 respiratory pathogens, including viruses, bacteria, and fungi, and associated antimicrobial resistance (AMR) markers. With sequencing on the MiSeq i100 Series and analysis with the DRAGEN™ Microbial Enrichment Plus app, the flexible and scalable accelerated workflow[4] (Figure 1) enables the identification of pathogens associated with respiratory tract infections in one day in clinical research settings. With performance consistent with the standard recipe, the time savings achieved with the turbo recipe do not affect sequencing accuracy.

Methods

Sample preparation

Reference material used was a qualitative complex mixture of purified virus particles and bacterial cells supplied in a stabilized, noninfectious state. The NATtrol Respiratory Panel 2.1 (RP2.1) Control (ZeptoMetrix, Catalog no. NATRPC2.1-BIO) consists of two distinct contrived pathogen mixes: RP2.1 Control 1 (12 targeted pathogens) and RP2.1 Control 2 (11 targeted pathogens). RP2.1 Control 1 was used at a 1:10 dilution in 1× PBS before extraction using the ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research, Catalog no. R2002) using the parallel DNA/RNA extraction method.

Library preparation

Library preparation and target enrichment in 3-plex hybridization were carried out using Illumina RNA Prep with Enrichment (Illumina, Catalog no. 20040536) with the Respiratory Pathogen ID/AMR Enrichment Panel (RPIP) Kit (Illumina, Catalog no. 20047050).

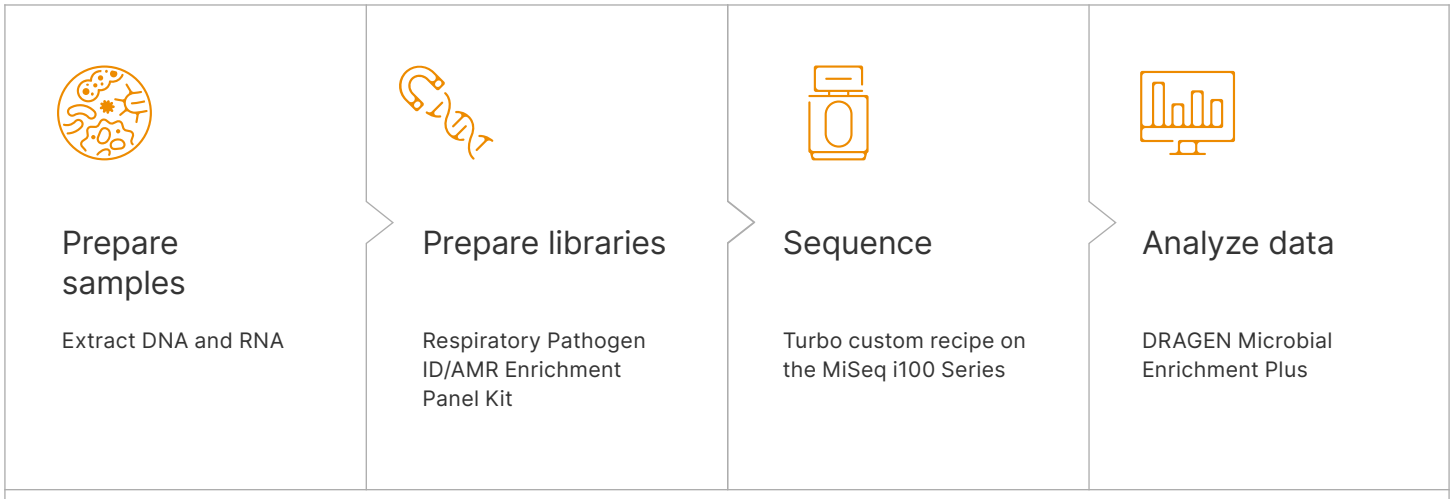

Figure 1: Accelerated workflow for respiratory pathogen detection
The turbo custom recipe on the MiSeq i100 Series accelerates NGS-based respiratory pathogen identification.

Sequencing

Prepared libraries were sequenced on the MiSeq i100 Plus System (Illumina, Catalog no. 20115695) at 1 × 101 bp read length using the turbo custom recipe and the standard recipe for the MiSeq i100 Series 25M Reagent Kit (100 cycles) (Illumina, Catalog no. 20126567), MiSeq i100 Plus 50M Reagent Kit (100 cycles) (Illumina, Catalog no. 20141595), and MiSeq i100 Plus 100M Reagent Kit (100 cycles) (Illumina, Catalog no. 20141598). All runs were configured with the default index-first read configuration.

Data analysis

Demultiplexed FASTQ sequencing files were downsampled to 1M clusters using the FASTQ toolkit and analyzed using the DRAGEN Microbial Enrichment Plus app (v1.1.1) onboard the MiSeq i100 Series. Results were used with DRAGEN Microbial Enrichment Plus cloud software to verify concordance of onboard workflows.

Results

Faster run times with high accuracy and yield

The MiSeq i100 turbo custom recipe achieves ~40% time savings during sequencing for 100-cycle versions of the 25M, 50M, and 100M reagent kits, relative to the standard recipe. Time savings was realized in all aspects of the sequencing by synthesis (SBS) recipe, including chemistry, fluidics, and imaging steps, with the goal of maintain high accuracy and yield. For the turbo recipe, sequencing completed in ~2.5 hours and ~3 hours for the 25M and 100M kits, respectively (Figure 2). Onboard DRAGEN Microbial Enrichment Plus analysis of Respiratory Pathogen ID/AMR Enrichment Panel samples completed in under 30 minutes. The full workflow including prerun checks and onboard DRAGEN Microbial Enrichment Plus analysis for the turbo recipe in a single-end, dual-barcode 100-bp reads completes in under three hours for the 25M kit (Figure 2).

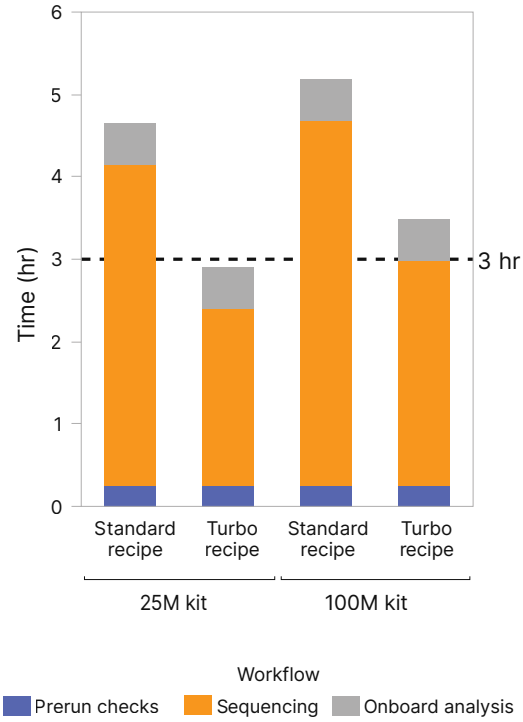

Figure 2: Faster sequencing workflow with the MiSeq i100 turbo custom recipe
Comparison of workflow times for the MiSeq i100 turbo recipe relative to the standard recipe for 25M flow cell and 100M flow cell 100-cycle kits with single-end, dual-indexed 100-bp reads in the default index-first configuration.

Control samples run with the turbo and standard sequencing recipes showed excellent concordance of primary metrics. Quality and yield were maintained with the turbo recipe. (Figure 3). From DRAGEN Microbial Enrichment Plus analysis, the percentage of post-quality filtered reads exhibits high concordance between the turbo recipe and standard recipe, indicating a high fidelity of reads is retained for secondary analysis (Figure 4).

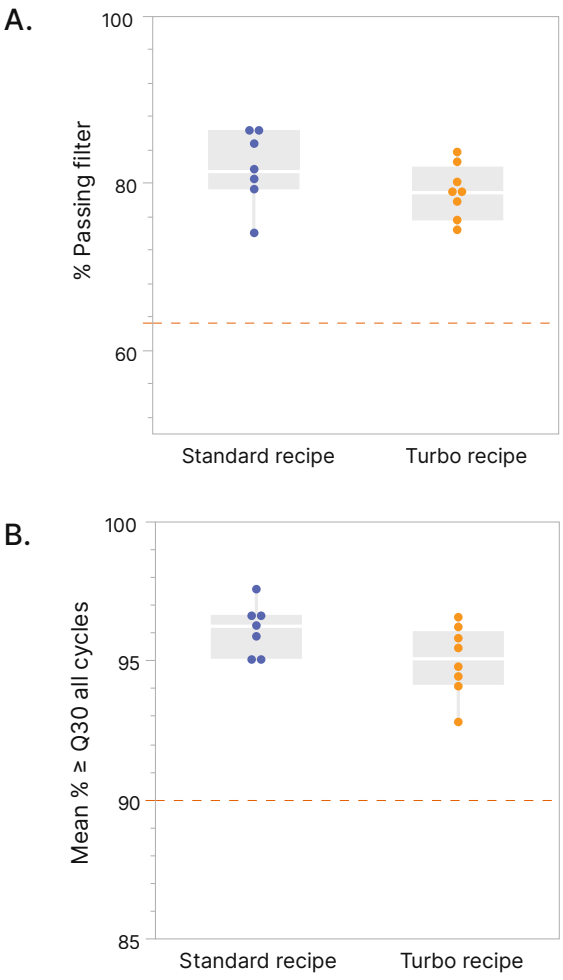

Figure 3: Concordance of primary metrics between the MiSeq i100 turbo and standard recipes

Comparison of (A) percent reads passing filter and (B) mean percent of reads ≥ Q30 for the two sequencing recipes, aggregated from runs with MiSeq i100 25M, 50M, and 100M 100-cycle reagent kits.

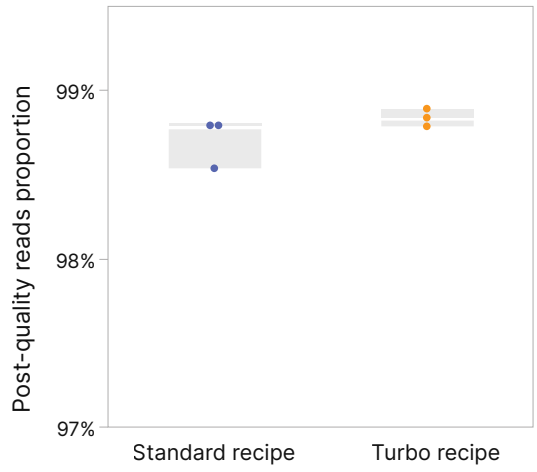

Figure 4: High-fidelity reads with the MiSeq i100 turbo custom recipe

Comparison of the proportion of post-quality reads output from the DRAGEN Microbial Enrichment Plus app for three runs each of the turbo recipe and standard recipe for 25M 100-cycle reagent kits.

High analytical performance for respiratory pathogen detection

The turbo custom recipe achieves significant time savings without sacrificing assay performance. The Respiratory Pathogen ID/AMR Enrichment Panel Kit targets 11 of the 12 pathogens in RP2.1 Control 1 and all were detected in both the turbo recipe and standard recipe runs (Table 1). Adenovirus type 31 is also included in the RP2.1 Control 1 mix but is not targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit and is not expected to be reported.

The mean RPKM (reads per kilobase of targeted sequence per million quality-filtered reads) across replicates, as reported by the DRAGEN Microbial Enrichment Plus app, were evaluated for each targeted virus and bacteria. RPKM normalizes the targeted read count across pathogens and samples by accounting for differences in targeted sequence length and sequencing depth. RPKM reported per pathogen within the RP2.1 Control 1 showed high correlation between the turbo recipe and standard recipe sequencing runs (Figure 5).

Table 1: Summary of detection of organisms across the turbo custom recipe and standard recipe runs

NATtrol RP2.1 Control 1 Panel ID	Reported pathogen name	Detection summary across three runs	
		Standard recipe	Turbo recipe
Adenovirus type 1	Human adenovirus C	3/3	3/3
Adenovirus type 3	Human adenovirus B	3/3	3/3
Adenovirus type 31[a]	(Not targeted or reported)	–	–
Chlamydia pneumoniae CWL-029	*Chlamydia pneumoniae*	3/3	3/3
Influenza A 2009 H1N1 pdm A/NY/02/2009	Influenza A virus (H1N1)	3/3	3/3
Influenza A H3N2 A/Brisbane/10/07	Influenza A virus (H3N2)	3/3	3/3
Metapneumovirus B Peru6-2003	Human metapneumovirus (HMPV)	3/3	3/3
Mycoplasma pneumoniae M129	*Mycoplasma pneumoniae*	3/3	3/3
Parainfluenza type 1	Human parainfluenza virus 1 (HPIV-1)	3/3	3/3
Parainfluenza type 4	Human parainfluenza virus 4 (HPIV-4)	3/3	3/3
Rhinovirus 1A	Human rhinovirus A (HRV-A)	3/3	3/3
SARS-CoV-2 USA-WA 1/2020	SARS-CoV-2	3/3	3/3

a. Adenovirus type 31 is included in the RP2.1 Control 1 mix but is not targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit and not expected to be detected.

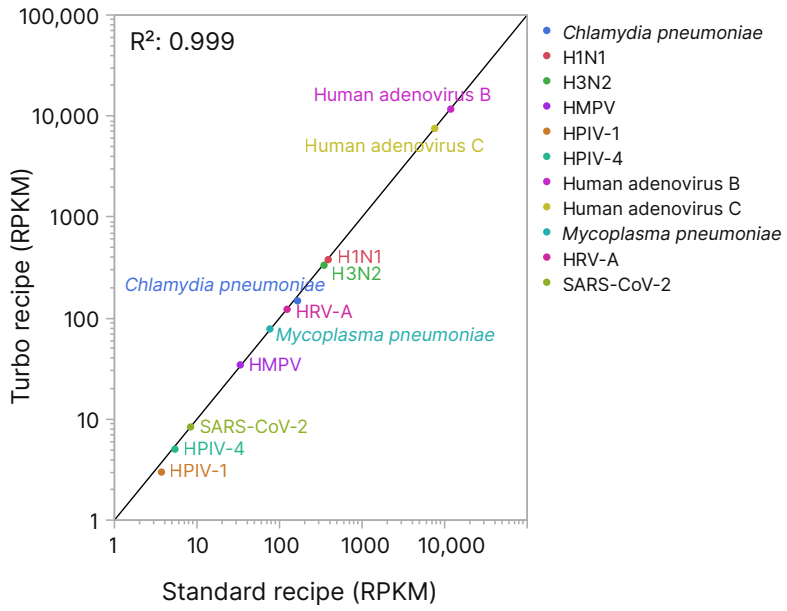

Figure 5: High correlation of pathogen detection with the MiSeq i100 turbo recipe and the standard recipe
Correlation of RPKM (reads per kilobase of targeted sequence per million quality-filtered reads) of the 11 pathogens in the NATtrol RP2.1 Control 1 that are targeted by the Respiratory Pathogen ID/AMR Enrichment Panel, averaged from three runs each of the turbo custom recipe and standard recipe for 25M 100-cycle reagent kits.

Summary

A turbo custom recipe on the MiSeq i100 Series enables accelerated sequencing for respiratory pathogen identification, reducing sequencing time by 40% while maintaining high-quality sequencing performance. Results from respiratory pathogen quality controls were highly concordant between the turbo recipe–enabled workflow and the standard MiSeq i100 sequencing recipe.

Contact Illumina technical support (techsupport@illumina.com) for access to the turbo custom recipes for the MiSeq i100 Series 25M, 50M, and 100M 100-cycle reagent kits.

Learn more →

MiSeq i100 Series

Respiratory Pathogen ID/AMR Enrichment Panel Kit

References

1. Georgakopoulou VE. Insights from respiratory virus co-infections. *World J Virol*. 2024;13(4):98600. doi:10.5501/wjv.v13.i4.98600
2. Li X, Pan L, Li Z, et al. Etiology and clinical characteristics of pathogen co-detection in pediatric Mycoplasma pneumoniae pneumonia: A multicenter retrospective study. *Int J Infect Dis*. 2026;162:108230. doi:10.1016/j.ijid.2025.108230
3. Zhao M, Shi Y, Zhang C, et al. Diagnostic value of metagenomic next-generation sequencing using bronchoalveolar lavage fluid samples for pathogen detection in children with severe or refractory pneumonia. *Microbiol Spectr*. 2025;13(3):e0108724. doi:10.1128/spectrum.01087-24
4. Illumina. Simplest, fastest. For every lab. MiSeq i100 Series brochure. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/miseq-i100-brochure-m-gl-02245/miseq-i100-brochure-m-gl-02245.pdf. Published 2025. Accessed March 4, 2026.

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