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DATA SHEET

Illumina Rapid Respiratory Virus Enrichment Kit

Streamlined, sensitive detection and characterization of common respiratory viruses

Sensitive detection of respiratory viruses, including influenza and SARS-CoV-2

Comprehensive profiling of multiple viruses in a single sample with target enrichment

Faster respiratory virus surveillance with a streamlined library prep and enrichment workflow

Introduction

Common causes of acute respiratory infections that can result in hospitalization and significant morbidity and mortality worldwide include influenza viruses, respiratory syncytial virus (RSV), and SARS-CoV-2.[1] RSV and SARS-CoV-2 have overlapping disease presentation and routinely cocirculate.[2–4] Continuous monitoring in the form of robust and timely viral surveillance is important to guide public health response and resource allocation.[5,6]

To achieve timely viral surveillance, Illumina offers the Rapid Respiratory Virus Enrichment Kit. This panel enables detection and characterization of ~40 common respiratory viruses, including SARS-CoV-2, influenza A and B viruses, adenovirus, rhinovirus, RSV, and other common respiratory viruses. The Rapid Respiratory Virus Enrichment Kit is part of a streamlined, sample-to-results NGS workflow that enables fast identification of respiratory viruses in clinical research settings (Figure 1).

Save time with a faster workflow

The Rapid Respiratory Virus Enrichment Kit is part of a streamlined solution that offers faster identification of respiratory viruses than the standard Respiratory Virus Enrichment Kit, giving researchers and public health scientists the ability to monitor multiple viruses and variants with a streamlined workflow.

Library preparation

The Rapid Respiratory Virus Enrichment Kit uses a streamlined, 30-minute reaction combining cDNA synthesis and adapter tagging in a single step. This is followed by PCR amplification and then a single hybridization reaction (Figure 2). The entire protocol generates sequencing-ready libraries in ~6.5 hours with ~75 minutes of hands-on time, compared to ~9 hours total assay time with ~2 hours of hands-on time for the Respiratory Virus Enrichment Kit (non-Rapid version).

Sequencing

Enriched libraries are sequenced on the MiSeq™ i100 Plus System, delivering the simplest, fastest benchtop sequencing and proven accuracy.[7] For additional time savings, users can employ the MiSeq i100 turbo custom recipe for single-end, dual barcode 100 bp reads, optimized for rapid pathogen identification. With the MiSeq i100 turbo custom recipe, sequencing can be completed in ~2 hours, enabling completion of the entire workflow in a single working shift.

To learn more, read the Faster time to answer for infectious disease identification technical note

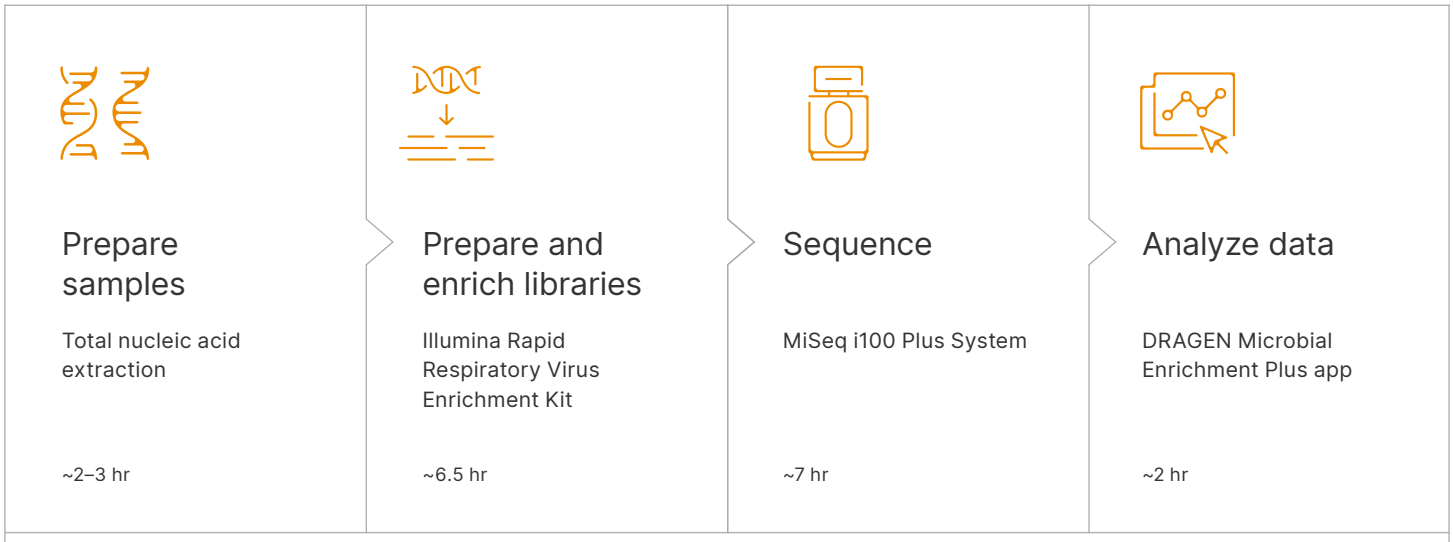

Figure 1: Rapid Respiratory Virus Enrichment Kit workflow
The streamlined NGS workflow integrates sample preparation, library preparation and enrichment with the Illumina Rapid Respiratory Virus Enrichment Kit, sequencing on the MiSeq i100 Plus System, and analysis with the DRAGEN Microbial Enrichment Plus app.

Data analysis

FASTQ files are analyzed using the DRAGEN™ Microbial Enrichment Plus app either onboard the MiSeq i100 Plus System or in BaseSpace™ Sequence Hub. The app delivers easy-to-use, powerful secondary analysis of Illumina sequencing data with workflows for sample quality control (QC), viral WGS (whole-genome sequencing), and pathogen detection and quantification.

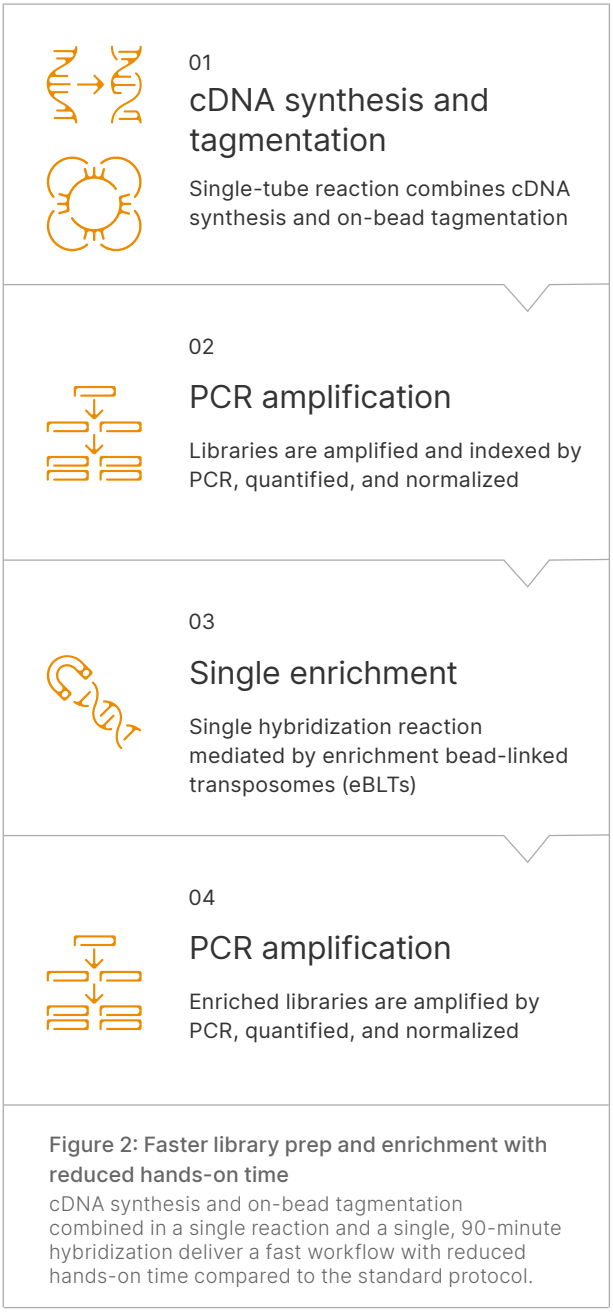

Figure 2: Faster library prep and enrichment with reduced hands-on time
cDNA synthesis and on-bead tagmentation combined in a single reaction and a single, 90-minute hybridization deliver a fast workflow with reduced hands-on time compared to the standard protocol.

High-quality data

To demonstrate the performance of the Rapid Respiratory Virus Enrichment Kit, NATtrol Respiratory Panel 2.1 (RP2.1) Controls (ZeptoMetrix, Catalog no. NATRPC2.1-BIO) were procured and diluted in a background of stool pool (DNA Genotek, Catalog no. OMR-200) or ZymoBIOMICS Fecal Reference with TruMatrix Technology (Zymo Research, Catalog no. D6323) at ratios of 1:5 (Table 1). Enriched libraries were prepared with Illumina Rapid RNA Prep with Enrichment with the Respiratory Virus Enrichment Kit and sequenced across two runs on the MiSeq i100 Plus System (Illumina, Catalog no. 20115695) using a 25M flow cell with a run configuration of 2 × 150 bp. Resulting data was downsampled to 1M clusters (2M paired-end (PE) reads) and analyzed using the DRAGEN Microbial Enrichment Plus app. Shotgun metagenomics libraries were sequenced and analyzed as a nonenriched control for comparison.

Table 1: Respiratory controls used for evaluation

Control	Dilution	TNA	Back-ground (10 ng)	Sample name
RPctl1	1:5	2 µl	8 µl	RPctl1-SPDG
				RPctl1-Zfec
RPctl2	1:5	2 µl	8 µl	RPctl2-SPDG
				RPctl2-Zfec

TNA, total nucleic acid; SPDG, stool pool DNA Genotek; Zfec, ZymoBIOMICS Fecal Reference.

Sequencing metrics pass QC thresholds

Both runs resulted in an average % Q30 > 90% and percent reads passing filter (PF) ≥ 70%, indicating high-quality reads and consistent instrument loading concentrations. The total number of PE reads obtained exceeds the 50M specification of the flow cell (Table 2). These metrics support high-confidence downstream analysis.

Table 2: Sequencing metrics pass QC thresholds

Run	No. of libraries	Avg Q30	% PF	Total reads	PF reads	% Reads identified (PF)	% Reads undetermined	CV	Min	Max
Run 1	42	94.35%	74.67%	79,073,280	59,302,052	96.30%	3.66%	0.20	0.73	3.56
Run 2	42	93.63%	73.25%	79,073,280	60,329,280	94.90%	5.07%	0.45	0.56	6.02

Respiratory virus detection and coverage

Evaluation of viral detection and genome coverage showed the exceptional performance of the Rapid Respiratory Virus Enrichment Kit with most of the expected viral targets detected with varying levels of coverage (Table 3, Figure 3).

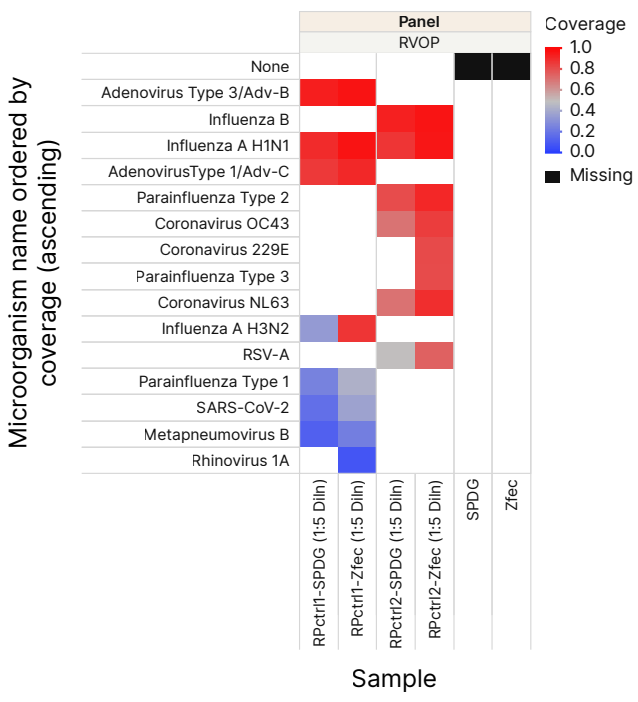

Figure 3: Virus coverage across samples
Viruses detected with the Rapid Respiratory Virus Enrichment Kit were ordered by ascending coverage.

Viral coverage correlates with abundance

Reads per kilobase of transcript per million reads mapped (RPKM) is a common means to normalize NGS data that combines the depth of coverage with the length of the target region. This permits more accurate comparisons across different pathogens, and for a single pathogen over time. Coverage of the detected viruses ranged from detection (≤ 20%) to surveillance (20–60%) and full genome coverage (≥ 60%) (Figure 4). As expected, coverage increased with abundance, with similar trends for both controls and backgrounds.

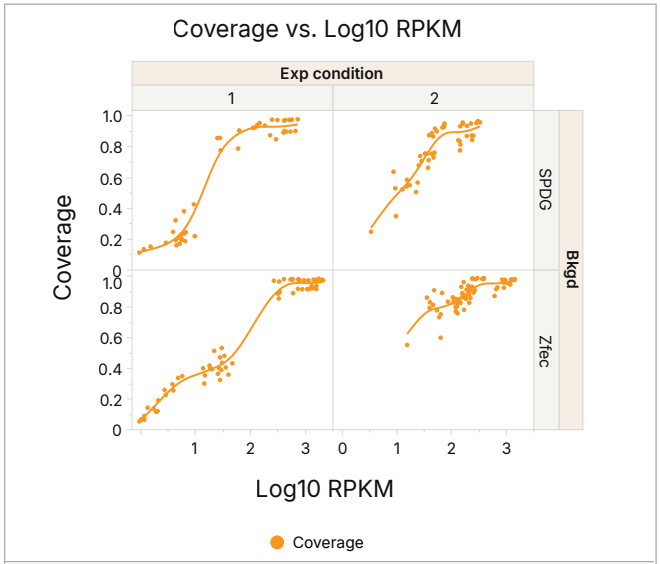

Figure 4: Viral coverage correlates with abundance
Viruses detected with the Rapid Respiratory Virus Enrichment Kit show increasing coverage with increasing reads per kilobase of transcript per million reads mapped (RPKM) values for both controls and backgrounds.

Table 3: Detection of respiratory viruses with the Rapid Respiratory Virus Enrichment Kit

Organism	Strain	RPctl1-SPDG	RPctl1-Zfec
Adenovirus Type 1/AdV-C	N/A	Detected	Detected
Adenovirus Type 3/AdV-B	N/A	Detected	Detected
Adenovirus Type 31/AdV-A[a]	N/A	Not detected	Not detected
Influenza A 2009 H1N1pdm	A/NY/02/2009	Detected	Detected
Influenza A H3N2	A/Brisbane/10/07	Detected	Detected
Metapneumovirus 8	Peru6-2003	Detected	Detected
Parainfluenza Type 1	N/A	Detected	Detected
Parainfluenza Type 4	N/A	Not detected	Not detected
Rhinovirus 1A	N/A	Not detected	Detected
SARS-CoV-2	USA-WA1/2020	Detected	Detected
Coronavirus 229E	N/A	Not detected	Detected
Coronavirus HKU-1[b]	Recombinant	Not detected	Not detected
Coronavirus NL63	N/A	Detected	Detected
Coronavirus OC43	N/A	Detected	Detected
Influenza A H1N1	A/New Caledonia/20/99	Detected	Detected
Influenza B	B/Florida/02/06	Detected	Detected
Parainfluenza Type 2	N/A	Detected	Detected
Parainfluenza Type 3	N/A	Not detected	Detected
RSV-A	N/A	Detected	Detected

a. Adenovirus A is not included in the Respiratory Virus Enrichment Kit, so it was not expected to be detected.
b. HKU-1 is a recombinant strain consisting only of a short sequence of the viral genome; therefore it is not expected to be detected with this panel.
N/A, not applicable

Summary

The identification and characterization of respiratory pathogens is central to improving public health. NGS is a powerful method for simultaneous, broad-range detection of respiratory viruses. The Rapid Respiratory Virus Enrichment Kit targets ~40 common respiratory viruses, including SARS-CoV-2 and influenza A and B. By delivering a streamlined workflow with reduced turnaround time and fewer manual steps than the standard workflow, the Rapid Respiratory Virus Enrichment Kit accelerates viral surveillance with reduced hands-on time.

Learn more →

Illumina Rapid Respiratory Virus Enrichment Kit

Ordering information

Product	Catalog no.
Illumina Rapid Respiratory Virus Enrichment Kit (96 samples)	20158827
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

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