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Faster detection of respiratory viruses using the MiniSeq™ Rapid Reagent Kit and Illumina RNA Prep with Enrichment

MiniSeq Rapid reagents reduce sequencing run times to < 5 hours, enabling faster detection of respiratory viruses, including SARS-CoV-2.

Introduction

The 2019 outbreak of a novel coronavirus (SARS-CoV-2) that began in Wuhan, China and quickly spread to multiple countries has become a major global health concern. SARS-CoV-2 is a member of the large family of coronaviruses (CoV) that can infect humans and is capable of causing Severe Acute Respiratory Syndrome (SARS). COVID-19, the disease associated with SARS-CoV-2, has resulted in millions of confirmed cases around the world and a rising death toll that has surpassed the SARS epidemic of 2003. This public health emergency of international concern highlights the need for rapid, accurate viral detection. Furthermore, research has shown higher rates of coinfection with SARS-CoV-2 and other viral pathogens than previously reported, which may impact disease management.[1]

Next-generation sequencing (NGS) provides an effective way to screen samples and detect viruses without previous knowledge of the infectious agent.[2] Using targeted resequencing with enrichment allows for highly sensitive detection, without requiring the high read depth needed for shotgun metagenomics sequencing. Additionally, the oligo probes used for target enrichment protocols remain effective, even within highly mutagenic regions, allowing targeting of rapidly evolving viruses, such as RNA viruses. Target enrichment enables applications such as variant analysis for viral evolution or viral surveillance.[3]

In contrast to other methods used for viral detection, such as PCR or quantitative PCR (qPCR), NGS offers numerous advantages, including the ability to:

- Determine the source of infection and route of transmission
- Identify and characterize coinfections and the role of complex disease
- Provide information on strain typing to monitor viral spread
- Screen targets for possible therapeutics

The speed and simplicity of low-throughput, benchtop sequencing systems makes them ideal for fast, targeted workflows, such as viral detection. Indeed, some of the initial sequence data for the SARS-CoV-2 genome was generated using Illumina benchtop systems.[4] However, NGS workflow turnaround times remain a bottleneck for public health efforts to conduct widespread timely screening programs for viral surveillance. For applications that require faster response times, like viral detection and outbreak monitoring, Illumina offers the MiniSeq Rapid Reagent Kit.

MiniSeq Rapid Reagent Kit

The MiniSeq Rapid Reagent Kit enables faster run times for NGS workflows to improve lab efficiency and provide a quicker time to results (Table 1). Multiple innovations to sequencing reagents reduce chemistry and cycle times, optimize reagent usage, and accelerate clustering, purging, and post-run wash steps. The result is a total sequencing time savings of ≥ 60%, compared to MiniSeq standard reagents.

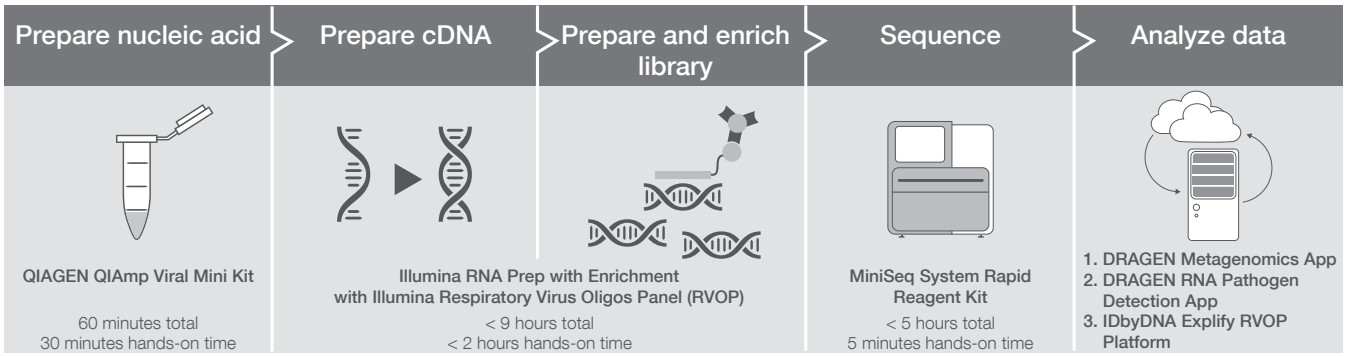

Figure 1: Rapid enrichment workflow for respiratory virus detection—The MiniSeq Rapid Reagent Kit further streamlines the NGS workflow for respiratory virus detection, which integrates sample preparation, library preparation, target enrichment, sequencing on the MiniSeq System, and data analysis.

Table 1: Calculated run times using MiniSeq Rapid reagents[a]

Read length	No indexes	Single index	Dual index
1 × 101 bp	4.6 hours	5.1 hours	5.7 hours
1 × 76 bp	3.7 hours	4.2 hours	4.8 hours
1 × 51 bp	2.8 hours	3.2 hours	3.9 hours
1 × 36 bp	2.3 hours	2.7 hours	3.3 hours

a. Calculations made based on internal experiments demonstrating shorter read lengths under 100 cycles. Times may vary depending on laboratory and system configurations and should be verified by the user.

Rapid, comprehensive RNA enrichment workflow for respiratory virus detection

In response to the rapidly developing COVID-19 pandemic, Illumina continues to deliver NGS solutions to study COVID-19. By combining Illumina RNA Prep with Enrichment, the Respiratory Virus Oligos Panel, and additional DRAGEN™ analysis pipelines, Illumina offers an integrated, comprehensive, and fit-for-purpose solution for detecting and characterizing respiratory viruses, including SARS-CoV-2.[5] With the launch of the MiniSeq Rapid Reagent Kit, Illumina has optimized viral detection workflows by integrating the fastest sequencing times on any Illumina benchtop sequencing system to deliver a rapid, DNA-to-results solution for detection of respiratory viruses, including SARS-CoV-2.

This application note highlights a streamlined workflow for detecting SARS-CoV-2 using Illumina RNA Prep with Enrichment combined with the Illumina Respiratory Virus Oligos Panel v2, rapid sequencing on the MiniSeq System, and simplified data analysis (Figure 1).

Illumina RNA Prep with Enrichment

Illumina RNA Prep with Enrichment uses On-Bead Tagmentation followed by a single hybridization step to provide a rapid workflow for generation of enriched RNA libraries.

Learn more at www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/rna-prep-enrichment.html

Rapid Illumina NGS

Libraries prepared with Illumina RNA Prep with Enrichment and the Respiratory Virus Oligos Panel are well suited to the benchtop MiniSeq, MiSeq™, and NextSeq™ 550 Systems, due to the low read requirements for the panel. However, for users who prioritize speed and minimal time to answer, the MiniSeq System and MiniSeq Rapid Reagent Kit offer the fastest run time (Figure 2).

Software analysis tools for SARS-CoV-2

To support researchers with analysis and sharing of genomic data in relation to the COVID-19 outbreak, Illumina has released a suite of tools to help identify, characterize, and examine SARS-CoV-2 and other respiratory virus coinfections (Table 2). Alternatively, the IDbyDNA Explify RVOP Platform provides simplified analysis and reporting of genomic data. These tools are all available in BaseSpace™ Sequence Hub. DRAGEN pipelines are also on the DRAGEN Server.

Learn more about coronavirus software tools at www.illumina.com/informatics/specialized-bioinformatics-applications/coronavirus-software.html#about

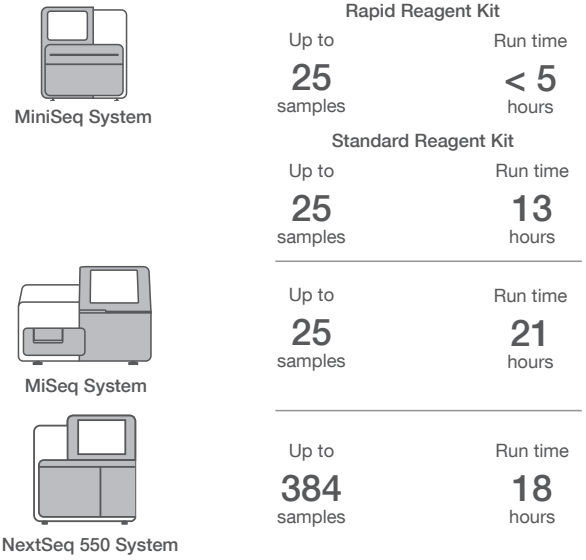

Figure 2: Sequencing system comparison—While Illumina RNA Prep with Enrichment and the Respiratory Virus Oligos Panel v2 are compatible with several benchtop systems, the MiniSeq Rapid Reagent Kit provides significant time savings.

Table 2: Analysis solutions for respiratory virus detection

Software	Description
	DRAGEN RNA Pathogen Detection Pipeline Accelerates detection of viral pathogens, including SARS-CoV-2, in any DRAGEN RNA-Seq Pipeline run, regardless of application.
	DRAGEN Metagenomics Pipeline Helps drive rapid, simplified species-level detection of common pathogens, including SARS-CoV-2, in shotgun metagenomics studies.
IDbyDNA Explify Platform	**IDbyDNA Explify RVOP Platform** Easy-to-use, clinical analysis and reporting of sequencing data using the Illumina Respiratory Virus Oligos Panel.

Methods

This workflow enriches viral targets from total nucleic acid extraction followed by proven Illumina sequencing and simplified data analysis.

Sample preparation

To demonstrate the performance of the MiniSeq Rapid Reagent Kit in combination with Illumina RNA Prep with Enrichment for detecting SARS-CoV-2, Twist Synthetic SARS-CoV-2 RNA Control 1 (Twist, Catalog no. 102019) was spiked into human saliva RNA (background) and reverse transcribed into cDNA to mimic sequencing results from patient samples.

Library preparation

Sequencing-ready libraries were prepared using cDNA from the spike-in sample with Illumina RNA Prep with Enrichment (Illumina, Catalog no. 20040536) and IDT for Illumina DNA/RNA UD Indexes (Illumina, Catalog no. 20027213). Total RNA input recommended for tagmentation is 10-100 ng. After amplification, samples were enriched

as single-plex reactions using the Respiratory Virus Oligos Panel v2 (Illumina, Catalog no. 20044311), which features ~7800 probes designed to detect respiratory viruses, recent flu strains, and SARS-CoV-2, as well as human probes to act as positive controls in each reaction. After enrichment, the libraries were prepped for sequencing.

Sequencing

Prepared libraries were denatured and diluted to a final loading concentration of 10 pM, according to the MiniSeq System Denature and Dilute Libraries Guide (Document no. 1000000002697 v07) and sequenced on the MiniSeq System at 1 × 76 bp read length using the MiniSeq Rapid Reagent Kit (Illumina, Catalog no. 20044338).

Virus titer, RNA quality, and the number of reads per sample impact the number of virus-specific reads obtained and coverage of the viral genome. As a general guideline, the read recommendation for this workflow is 1M reads per sample. Note that this number can vary and this is only a recommended starting point.

Data analysis

FASTQ sequencing files were input to DRAGEN pipelines for analysis.

Results

To demonstrate the exceptional performance of the MiniSeq Rapid Reagent Kit in accelerating the RNA enrichment workflow for respiratory virus detection, various SARS-CoV-2 control samples were evaluated.

Evaluation of SARS-CoV-2 detection with the MiniSeq Rapid Reagent Kit

A titration experiment was conducted in which Twist Synthetic SARS-CoV-2 RNA Control 1 was spiked into human saliva RNA in diminishing amounts of virus, from 1M down to 0 viral copies. 10 ng total RNA (viral RNA + human saliva RNA) was input for reverse transcription, libraries were prepared and enriched from the resulting cDNA, and sequencing was performed on the MiniSeq System using rapid and standard sequencing reagents, normalized to 1M reads. Comparison of various metrics demonstrated the equivalent performance of the two reagent kits for Q30, reads passing filter (PF), and other parameters and highlights the significant time savings provided with the rapid reagents (Table 3). Analysis with the DRAGEN Metagenomics pipeline shows similar identification of SARS-CoV-2, even at low viral copy numbers, with both reagent kits (Figure 3). However, comparison of SARS-CoV-2 genome coverage shows reduced coverage with MiniSeq Rapid reagents as compared to the standard reagents for the same viral copy numbers (Figure 4).

These results indicate that researchers have two clear options when choosing an NGS viral detection workflow for SARS-CoV-2. For those researchers who prioritize a quick turnaround and faster time to answer, the workflow presented in this application note using the MiniSeq Rapid Reagent Kit is the preferred choice. For researchers who need complete genome coverage for full viral characterization studies, Illumina recommends the workflow presented in the Detection and characterization of respiratory viruses, including SARS-CoV-2, using Illumina RNA Prep with Enrichment application note.

Table 3: MiniSeq reagent kit performance comparison[a]

Parameter	MiniSeq Standard Reagents	MiniSeq Rapid Reagents
Read length	2 × 76 bp	1 × 76 bp
Run time	13 hours	5 hours
Q30	> 85%	Equivalent to standard
Reads PF	25M (max)	Equivalent to standard
Demux	> 90%, 384 plex	Equivalent to standard
Minimal loading concentration	1.4 pM	1.6 pM
Sensitivity/limit of detection	< 5 viral copies	Equivalent to standard
Genome coverage at 1000 viral copies	~ 90%	~ 70%
Carryover contamination	< 0.05%	Equivalent to standard

a. Demonstrated at 20M reads minimum, but up to 32M reads is possible.

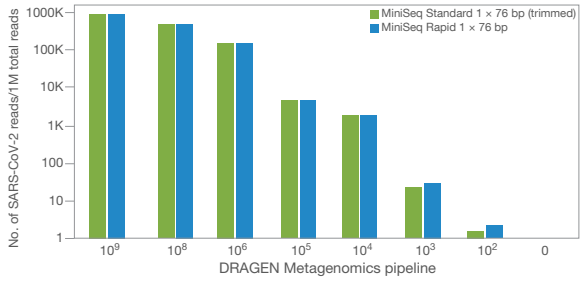

Figure 3: Highly sensitive SARS-CoV-2 detection—Using MiniSeq Rapid Reagents (blue) resulted in equivalent SARS-CoV-2 detection as a function of viral copy number, as compared to standard reagents (green). Analysis with the DRAGEN Metagenomics pipeline is shown here to measure limit of detection. Other analysis tools that can be used include the Dragen RNA Pathogen Detection pipeline and the IDbyDNA Explify RVOP Platform.

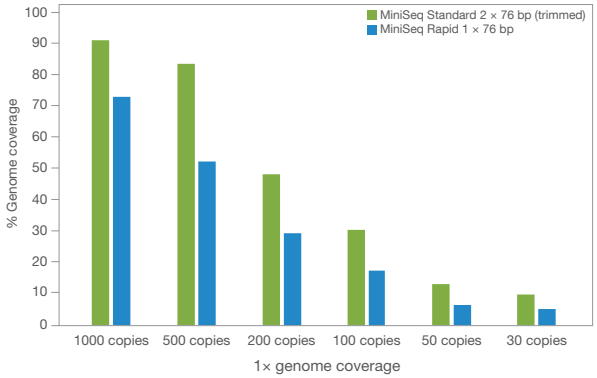

Figure 4: Reduced SARS-CoV-2 genome coverage—Using MiniSeq Rapid Reagents (blue) resulted in reduced SARS-CoV-2 genome coverage as a function of viral copy number, as compared to standard reagents (green).

Summary

Rapid identification and characterization of emerging viruses is central to improving public health. There is an increasing need for methods that can rapidly detect emerging viruses to accelerate containment efforts and prevent spread. Illumina is continuing to improve its solutions to deliver faster answers to support public health efforts. The MiniSeq Rapid Reagent Kit offers the fastest turnaround in the Illumina portfolio for a mid-throughput enrichment approach, enabling rapid detection of viral outbreaks, such as COVID-19. For those researchers who prioritize a quick turnaround and faster time to answer, the workflow presented in this application note using the MiniSeq Rapid Reagent Kit is the preferred choice.

Learn more

Learn more about using NGS to detect respiratory viruses, including SARS-CoV-2, on other Illumina sequencing systems at www.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/illumina-rna-enrichment-coronavirus-app-note-470-2020-005/illumina-rna-enrichment-coronavirus-app-note-470-2020-005.pdf

Learn more about the Illumina SARS-CoV-2 NGS Data Toolkit at www.illumina.com/informatics/specialized-bioinformatics-applications/coronavirus-software.html

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2. Bulcha B. Review on viral metagenomics and its future perspective in zoonotic and arboviral disease surveillance. *J Biol Agric Healthcare*. 2017;7(21):35–41.
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5. Illumina (2020) Detection and characterization of respiratory viruses, including SARS-CoV-2, using Illumina RNA Prep with Enrichment. Accessed August 20, 2020.

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