DATA SHEET

Pan-Coronavirus Panel

Genomic surveillance of known and novel coronaviruses in animal reservoirs

- Use with Illumina RNA Prep with Enrichment library prep, and Illumina NGS systems and analysis software
- Enable flexible strain sequencing with hybrid–capture target enrichment
- Generate comprehensive genomic coverage of known and novel coronaviruses

Introduction

The COVID-19 pandemic has raged across the world since 2019.[1] This onset represents the fourth and most deadly coronavirus outbreak in the past 20 years. Previous outbreaks include two separate epidemics of severe acute respiratory syndrome (SARS) in 2002 and 2003 and Middle East respiratory syndrome (MERS) in 2012. This pattern of coronaviruses emerging from zoonotic sources to create public health crises is expected to repeat in the future.[2,3] It highlights the need for genomic surveillance to identify and monitor coronaviruses in animal hosts. This will help scientists and public health officials better understand coronavirus evolution and pathogenicity with the goal of preventing future pandemics.

To help meet this need, Illumina offers the Pan-Coronavirus Panel. When combined with Illumina RNA Prep with Enrichment, the Pan-Coronavirus Panel allows for detection and sequencing of 225 known human and animal coronaviruses and closely related novel coronaviruses with the potential to become infectious to humans.

Targeted, flexible panel content

Content on the Pan-Coronavirus Panel includes hybrid–capture oligos tiled across reference genomes of multiple coronavirus genera (Table 1) and various known animal hosts (Table 2). The targeted enrichment approach is amenable to capturing mutations and variants throughout the viral genome, enabling sequencing of known and novel coronavirus strains and detection of recombination between strains.

Table 1: Coronavirus genera included in panel

Viral genome	No. genomes
Alphacoronavirus	69
Bafinivirus	1
Betacoronavirus	59
Deltacoronavirus	13
Gammacoronavirus	61
Unclassified coronaviridae	22
Total	225

Table 2: Known host associated coronavirus genomes included in panel

Species	No. genomes
Bat	64
Rat	32
Mink, pangolin, mouse, pig, snake	28
Bird	25
Cow, camel, goat, horse	11
Dolphin, whale, fish	9
Human	7
Dog	7
Cat, ferret	6
Hedgehog	2

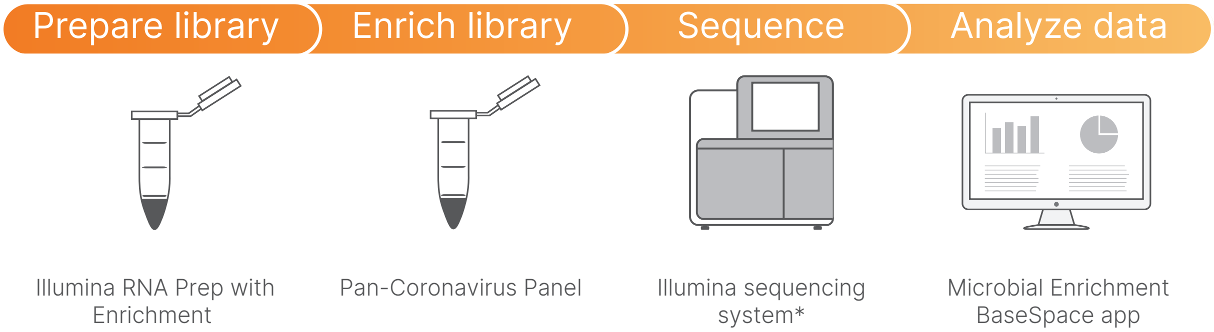

Figure 1: Variant calling workflow—The Pan-Coronavirus Panel is part of an integrated workflow that includes library preparation, sequencing, and simplified data analysis in BaseSpace Sequence Hub.

* Sequencing can be performed on the Illumina MiniSeq, MiSeq, NextSeq 550, NextSeq 1000, or NextSeq 2000 Systems.

Part of an integrated workflow

The Pan-Coronavirus Panel is part of an integrated viral sequencing solution (Figure 1). Libraries are prepared using Illumina RNA Prep with Enrichment and Illumina DNA/RNA UD Indexes. After enrichment with the Pan-Coronavirus Panel, libraries are run on an Illumina next-generation sequencing (NGS) system. With a recommended read length of 2 × 150 bp and a depth of 0.5–1M reads, the panel is ideal for use with benchtop systems such as the MiniSeq™, MiSeq™, NextSeq™ 550, NextSeq 1000, and NextSeq 2000 Systems. Push-button analysis can be performed on the DRAGEN™ Microbial Enrichment app, available at no cost in BaseSpace™ Sequence Hub.

Increased genome coverage with Pan-Coronavirus Panel enrichment

To demonstrate the exceptional performance of the Pan-Coronavirus Panel, five synthetic viral samples (Table 3) were spiked into Universal Human Reference RNA (Agilent Technologies, Catalog no. 74000). Libraries were prepared in triplicate using Illumina RNA Prep with Enrichment, (L) Tagmentation (Illumina, Catalog no. 20040537) and IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (Illumina, Catalog no. 20027214).[*] Enrichment was performed with the Pan-Coronavirus Panel by pooling replicates into 3-plex hybridization reactions. Enriched and unenriched control libraries were sequenced on the NextSeq 550 System with a read length of 2 × 150 bp.

Enrichment with the Pan-Coronavirus Panel increased the number of Coronaviridae reads > 100-fold across libraries tested, as compared to unenriched controls (Figure 2). Enrichment with the panel also resulted in exceptional viral genome coverage (Figure 3A). While a read length of 2 × 74 bp provides sufficient coverage for some viral genomes, a 2 × 150 bp read length provides superior coverage for more complex genomes. To achieve the best results with most viral genomes, Illumina recommends the longer 2 × 150 bp read length (Figure 3B). This combination of longer read length and increased sequencing depth improves coverage for some coronavirus strains.

Table 3: Coronavirus control samples for validation

Sample	Genera
SARS-CoV-2 (Twist Bioscience, Catalog no. 102019)	Betacoronavirus (2b)
SARS-CoV-2 (BA.2) (Twist Bioscience, Catalog no. 105346)	Betacoronavirus (2b)
HCoV-OC43 (Twist Bioscience, Catalog no. 103013)	Betacoronavirus (2a)
HCoV-229E (Twist Bioscience, Catalog no. 103011)	Alphacoronavirus (1b)
HCoV-NL63 (Twist Bioscience, Catalog no. 103012)	Alphacoronavirus (1b)

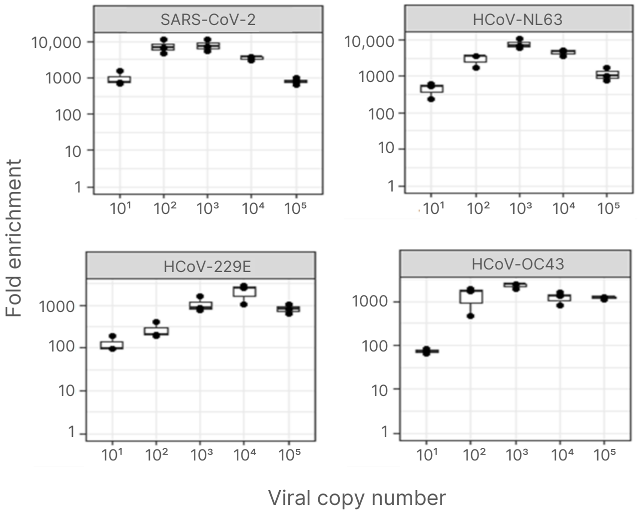

Figure 2: Enrichment of Coronaviridae reads—The Pan-Coronavirus Panel successfully enriched Coronaviridae reads in all libraries tested across a range of viral copy number input. Fold enrichment was calculated as the ratio of Coronaviridae reads in enriched/unenriched libraries. Data was downsampled to 1M clusters and trimmed to 74 bp. Fold enrichment is reduced in higher viral titers because unenriched libraries perform better with high viral titers, however, enriched performance remains strong.

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[*] Catalog no. 20027214 is now obsolete, replaced by Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples), Catalog no. 20091656.

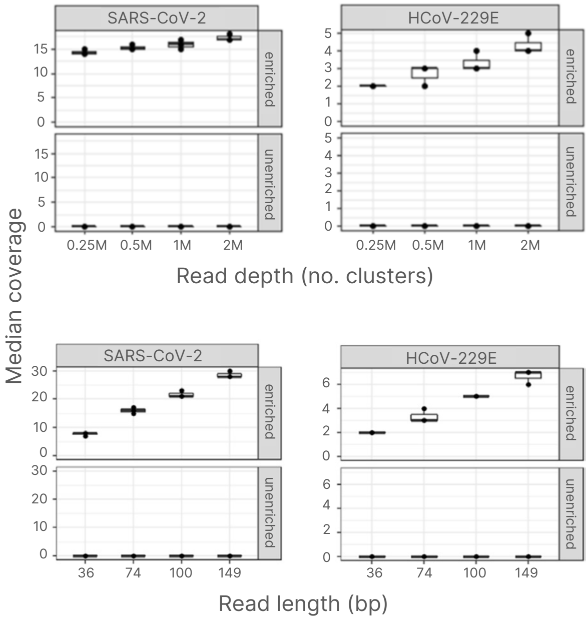

Figure 3: Improved coronavirus genome coverage—Median coverage of synthetic viral RNA controls (SARS-CoV-2 and HCoV-229E) in 10 ng UHR after enrichment with the Pan-Coronavirus Panel. Increased read depth (top) and read length (bottom) resulted in improved genome coverage. Read lengths were trimmed to 36, 74, 100, and 149 bp.

Sequencing of strains not in panel

The panel was designed before the emergence of the Omicron BA.2 variant of SARS-CoV-2, yet enrichment and sequencing resulted in comprehensive genomic coverage of the Omicron BA.2 strain (Figure 4). This demonstrates that the Pan-Coronavirus Panel can be used to sequence novel coronavirus strains not included in the panel.

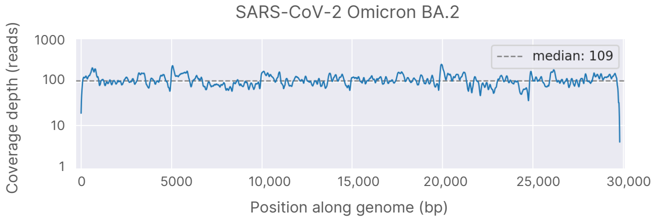

Figure 3: Comprehensive genome coverage of Omicron BA.2 strain—Enrichment with the Pan-Coronavirus Panel resulted in near 100% coverage of the Omicron BA.2 SARS-CoV-2 variant.

Summary

The COVID-19 pandemic and past coronavirus outbreaks highlight the need for genomic surveillance to identify and monitor coronaviruses in animal hosts. Such efforts may help to prevent future pandemics. The Pan-Coronavirus Panel is part of an integrated solution that includes Illumina RNA Prep with Enrichment, a benchtop NGS system, and push-button analysis in BaseSpace Sequence Hub. It enables sequencing of known and novel coronavirus strains with comprehensive genome coverage, even with low viral copy numbers and high host background, at relatively low sequencing depths. Flexibility built into the panel means that novel strains not included in the initial design can also be sequenced with significant coverage. The Pan-Coronavirus Panel is an essential tool in the arsenal to fight future viral outbreaks.

Learn more

Pan-Coronavirus Panel

Illumina RNA Prep with Enrichment

Illumina RNA Prep with Enrichment protocol checklist

Ordering information

Product	Catalog no.
Pan-Coronavirus Panel (96 reactions)	20088155
Illumina RNA Prep with Enrichment, (L) Tagmentation (16 samples)	20040536
Illumina RNA Prep with Enrichment, (L) Tagmentation (96 samples)	20040537
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples) 20091654	20027213
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples) 20091656	20027214
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples) 20091658	20042666
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples) 20091660	20042667

References

1. World Health Organization. WHO Director-General's statement on IHR Emergency Committee on Novel Coronavirus (2019-nCoV). Published January 30, 2020. Accessed September 9, 2022.
2. Morens DM, Taubenberger JK, Fauci AS. A Centenary Tale of Two Pandemics: The 1918 Influenza Pandemic and COVID-19, Part II. *Am J Public Health*. 2021;111(7):1267-1272. doi: 10.2105/AJPH.2021.306326.
3. Rubin R. The Search for a Single Vaccine Against Coronaviruses Yet to Come. *JAMA*. 2021;326(2):118-120. doi: 10.1001/jama.2021.9477.

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