DATA SHEET

Viral Surveillance Panel

Streamlined whole-genome sequencing of high-impact viruses using hybrid–capture enrichment

- Coverage of 66 viruses identified as high-risk to public health
- Targeted enrichment for RNA and DNA viral pathogens
- Compatible with a range of host and environmental sample types

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Monitoring viral threats to public health

The 2019 SARS-CoV-2 outbreak and 2022 monkeypox virus outbreak have demonstrated the critical need for a pathogen early warning system and tools for monitoring and evaluating outbreaks. Next-generation sequencing (NGS) provides an effective approach for screening samples and detecting viruses without requiring previous knowledge of the infectious agent. The detailed information provided by NGS enables important characterization and monitoring applications, including:

- Reflexive sequencing of known positive samples during outbreaks
- Tracking sources of infection and routes of transmission
- Monitoring for viral evolution and antiviral resistance

The Viral Surveillance Panel enables NGS detection of 66 viral genomes, including viruses identified as important risks to public health by the World Health Organization (WHO) (Table 1).[1] The panel uses a hybrid–capture target enrichment workflow that allows for sequencing of various sample types without the need for the high sample read depth that is required for shotgun metagenomics sequencing. Compared to other targeted resequencing methods, such as amplicon sequencing, hybrid–capture also provides more uniform coverage across genomes, substantially larger probe panels, and a greater ability to identify mutations and related sequences making the Viral Surveillance Panel ideal for outbreak surveillance.

Integrated, comprehensive NGS workflow

The Viral Surveillance Panel workflow enriches for viral genomes from a range of host and environmental samples, including wastewater.[2] The library preparation sequencing steps can be completed in two days on benchtop sequencing systems (Figure 1).

Table 1: Included on the Viral Surveillance Panel.[1]

Adenovirus	Hepatitis B virus	Parechovirus
Aichivirus	Hepatitis C virus	Parvovirus
Astrovirus	Hepatitis E virus	Poliovirus
Chapare virus	Human Immunodeficiency Virus 1	Polyomavirus
Chikungunya virus	Human Immunodeficiency Virus 2	Respiratory syncytial virus
Coronavirus-229E	Influenza A virus	Rhinovirus
Coronavirus-HKU1	Influenza B virus	Rift Valley fever virus
Coronavirus-OC43	Japanese encephalitis virus	Rotavirus
Coronavirus-NL63	Junin virus	Rubella virus
Coxsackievirus	Kyasanur Forest disease virus	Sabia virus
Crimean-congo haemorrhagic fever virus	Lassa fever virus	Salivirus
Dengue virus 1	Lujo hemorrphagic fever virus	Sapovirus
Dengue virus 2	Machupo virus	SARS-COV
Dengue virus 3	Marburg virus	SARS-COV-2
Dengue virus 4	MERS-CoV	Tick-borne encephalitis virus
Eastern equine encephalitis virus	Metapneumovirus	Torque Teno virus
Ebola virus	Monkeypox virus	Variola virus
Enterovirus	Nipah virus	Venezuelan equine encephalitis virus
Guanarito virus	Norovirus	West Nile virus
Hantavirus	Omsk hemorrhagic fever virus	Western equine encephalitis virus
Hendra henipavirus	Oncolytic human papillomavirus	Yellow fever virus
Hepatitis A virus	Parainfluenza virus	Zika virus

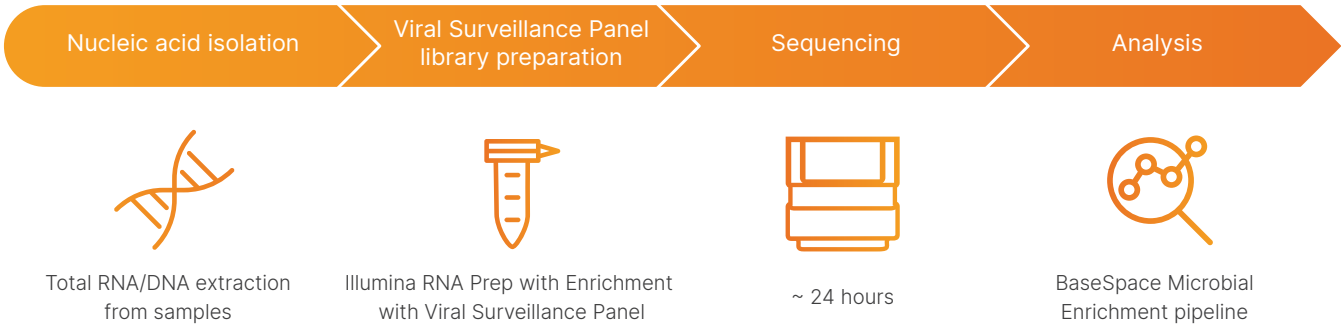

Figure 1: Viral Surveillance Panel workflow—In a streamlined, comprehensive workflow, libraries are prepared from environmental or host samples, sequenced on any Illumina sequencing system, and analyzed in the BaseSpace Microbial Enrichment pipeline for viral detection, whole-genome consensus generation, read mapping to viral best hits, and strain typing. Sequencing time varies with sample read depth and sequencing system used.

Library preparation

The Viral Surveillance Panel follows the same library preparation protocol as the Illumina Respiratory Virus Oligo Panel.[3] Illumina RNA Prep with Enrichment uses on-bead tagmentation followed by a single hybridization step to provide a rapid workflow for generating enriched libraries. Illumina RNA Prep with Enrichment provides:

- Rapid, automation-compatible workflow that can be completed in approximately two days with minimal hands-on time
- Flexible sample input amount ranging from 10 ng to 100 ng total nucleic acid
- Scalable throughput that supports multiplexing of up to 384 samples in a single run

Sequencing

The lower read depth requirements for VSP-enriched libraries allow for multiple sequencing system options, including the benchtop MiniSeq™, MiSeq™, and NextSeq™ 550, NextSeq 1000, and NextSeq 2000 systems. Virus titer, nucleic acid sample quality, sample read depth, and the number of reads per sample impact the number of virus-specific reads and sequence coverage obtained. The general sequencing read depth recommendation for good quality samples is a minimum of 2M paired-end reads per sample with a read length of 149 bp. The recommended sample read depth also varies with sample type. For more complex samples, such as wastewater, a minimum of 4M paired reads are recommended per sample.

Data analysis

The Viral Surveillance Panel is compatible with the Microbial Enrichment secondary analysis pipeline, available on the BaseSpace™ Sequence Hub. The Microbial Enrichment pipeline provides contig assembly, consensus sequences, and genome coverage for viral genomes featured in the panel.

Performance

Target enrichment

Hybrid–capture target enrichment for the Viral Surveillance Panel is performed with the Illumina RNA Prep with Enrichment kit. Compared to shotgun metagenomic sequencing, where all RNA/DNA is sequenced, targeted hybrid–capture reduces unnecessary sequencing of host and nontargeted microbes, reducing costs and allowing for broad sequencing of viral genomes on benchtop sequencing systems (Figure 2).

Whole-genome sequencing (WGS) of multiple viruses at once allows for viral surveillance and analysis of viral evolution. The target enrichment probes in the Viral Surveillance Panel provide uniform coverage of whole virus genomes (Figure 3). Also, the oligo probes used for hybrid–capture protocols remain effective within mutated regions, allowing for the capture of rapidly evolving viruses, such as RNA viruses.

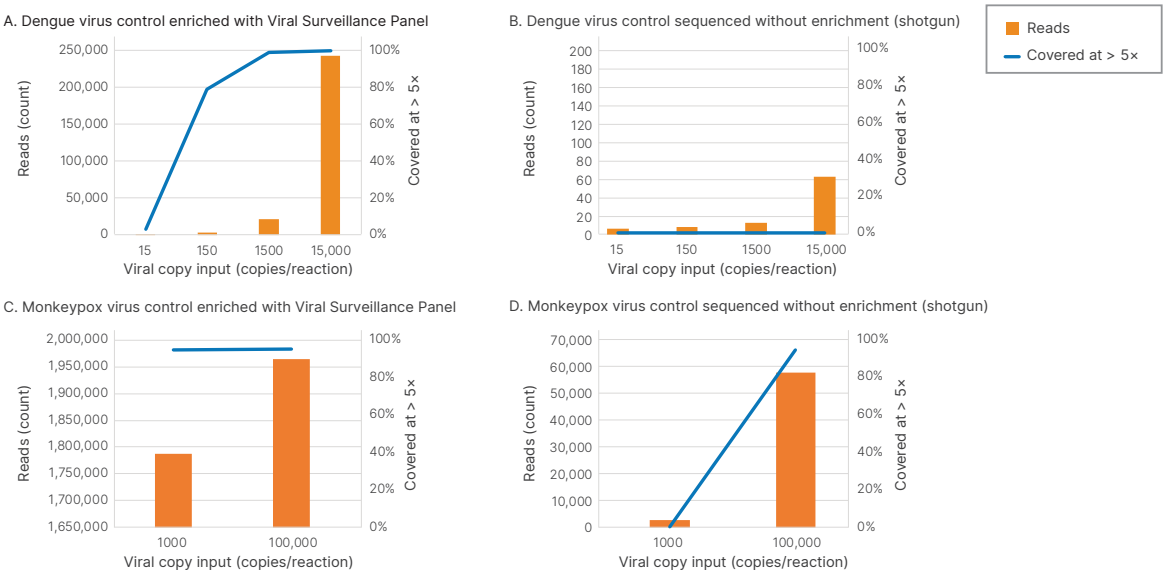

Figure 2: Read counts and viral genome coverage gains using Viral Surveillance Panel—Performance of Viral Surveillance Panel and sequencing without enrichment compared using commercially available viral controls. (A) Dengue virus control mixed into 10 ng human RNA background and enriched with Viral Surveillance Panel, (B) Dengue virus control mixed into 10 ng human RNA background and sequenced without enrichment, (C) Monkeypox virus control mixed into 10 ng human RNA and 10 ng human DNA background and enriched with Viral Surveillance Panel, (D) Monkeypox virus control sequenced without enrichment mixed into 10 ng human RNA and 10 ng human DNA background and sequenced without enrichment. Samples were sequenced and data normalized to 2M paired-end reads at 2 × 149 bp.

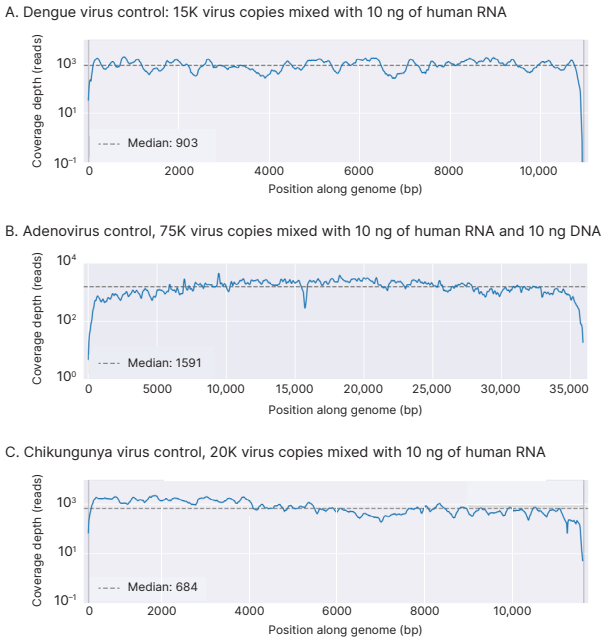

Figure 3: Uniform viral genome following enrichment with the Viral Surveillance Panel—Virus controls were prepared by mixing virus controls at known copy numbers with 10 ng human RNA/DNA mix. Libraries were prepared and sequenced following the Viral Surveillance Panel workflow.

Wastewater surveillance

Surveillance for viral sequences in wastewater provides a regional indicator of communal spread of viral pathogens, giving public health professionals valuable information for response planning. The Viral Surveillance Panel can be used with these samples to enable early detection and identification of viral genomes in wastewater at lower concentrations than shotgun sequencing (Table 2).

Summary

The Viral Surveillance Panel provides an optimized, complete workflow for viral outbreak detection and monitoring. It includes hybrid–capture probes for 66 whole RNA and DNA virus genomes that have been identified as high risks to public health.[1] The hybrid–capture target enrichment reduces the need for high sample read depth by focusing on target sequences. This reduces costs and increases throughput capabilities. The workflow is also compatible with a range of sample types and applications, including wastewater surveillance for regional

Table 2: Viruses detected in wastewater using Viral Surveillance Panel or shotgun sequencing.[a]

	Viral Surveillance Panel	Shotgun sequencing	Viral Surveillance Panel	Shotgun sequencing
Virus identified	Genome covered ≥ 5× (%)		Reads (count)	
Astrovirus	98.9	0	122525	7
JC polyomavirus	98.9	0	29749	0
BK polyomavirus	97.8	0	29318	5
hCoV-OC43	87.3	0	23352	8
Aichivirus A	95.1	0	16919	4
Norovirus GII	90.0	0	7873	0
Coxsackievirus A19	65.2	0	7195	0
Norovirus GII.P7_GII.6	69.7	0	2572	0
Norwalk-like virus	57.3	0	1191	0
Norovirus GI strain	51.2	0	859	0

a. Samples were collected by researchers at Colorado State University and purified total nucleic acids shipped to Illumina for testing. Libraries were prepared and sequenced using 100 ng total nucleic acids

presence of viruses. Finally, the Viral Surveillance Panel is compatible with the free Microbial Enrichment Analysis pipeline on BaseSpace Sequence Hub. This NGS workflow provides public health organizations and researchers with an advanced alternative to expensive, complicated shotgun sequencing.

Learn more

Viral Surveillance Panel, illumina.com/products/by-type/sequencing-kits/library-prep-kits/viral-surveillance-panel.html

Illumina RNA Prep with Enrichment, illumina.com/products/by-type/sequencing-kits/library-prep-kits/rna-prep-enrichment.html

BaseSpace applications, illumina.com/products/by-type/informatics-products/basespace-sequence-hub/apps.html

Illumina sequencing platforms, illumina.com/systems/sequencing-platforms.html

Ordering information

Product	Catalog no.
Viral Surveillance Panel (96 samples)	20088154
Viral Surveillance Panel with Illumina RNA Prep with Enrichment Indexes Set A (96 samples)	20087932
Viral Surveillance Panel with Illumina RNA Prep with Enrichment Indexes Set B (96 samples)	20087929

References

1. Bloom DE, Cadarette D. Infectious Disease Threats in the Twenty-First Century: Strengthening the Global Response. *Front Immunol.* 2019;10:549. Published 2019 Mar 28. doi:10.3389/fimmu.2019.00549
2. McClary-Gutierrez JS, Aanderud ZT, Al-Faliti M, et al. Standardizing data reporting in the research community to enhance the utility of open data for SARS-CoV-2 wastewater surveillance. *Environ Sci (Camb).* 2021;9:10.1039/d1ew00235j. doi:10.1039/d1ew00235j
3. Illumina. Illumina RNA Prep with Enrichment (L) Tagmentation: Reference guide. support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/illumina_prep/RNA/illumina-rna-prep-reference-guide-1000000124435-03.pdf. Published 2021. Accessed September 13, 2022.

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M-GL-01240 v1.0