CleanPlex® Respiratory Virus Research Panel V2

Targeted Sequencing NGS Panel to Support SARS-CoV-2 and Respiratory Virus Research

Highlights

- **Strategically Designed Content**
  RSV and Influenza A and B subtype detection and complete SARS-CoV-2 genome sequencing.
- **Ultra-sensitive Detection**
  Multiplex PCR based amplification for sensitive detection.
- **Fast and Streamlined Workflow**
  Generate sequencing-ready libraries in just 6 hours using a rapid, four-step protocol from extracted RNA to sequence ready libraries.

The CleanPlex® Respiratory Virus Research Panel v2 combines SARS-CoV-2 whole genome amplification with 149 strategically designed primers to cover characteristic regions that span multiple gene segments for each Influenza A subtype H1N1, H1N2, H3N2, Influenza B, and respiratory syncytial virus (RSV) types A and B. The increased target regions yield a higher coverage, resulting in higher sensitivity and making this panel more suitable for high-throughput screening strategies such as testing multi-sample pools, than qPCR methods with only 1 to 2 targets per subtype. The respiratory virus primers were expertly designed in consideration of over 45,000 viral genome sequences to generate highly specific, but also universally conserved targets for each subtype to allow confident detection throughout flu seasons, regardless of changes in strain prevalence.

Built upon the CleanPlex SARS-CoV-2 Research and Surveillance Panel, the Respiratory Virus Research Panel v2 addresses the need to assay the SARS-CoV-2 and other respiratory viruses concurrently during the overlapping flu season and the COVID-19 pandemic. The panel utilizes the CleanPlex multiplex PCR-based targeted sequencing technology to provide an easy-to-use, fast, and comprehensive solution for detection and differentiation of these common viruses, influenza and RSV subtyping, and SARS-CoV-2 mutation analysis, tracking, and surveillance for informed infection control through comprehensive sequence information.

CleanPlex Respiratory Virus Research Panel v2 Specifications

Parameter	Specification
Enrichment Method	Multiplex PCR
Sequencing Platforms	Illumina®
Amplicon Size	105 – 196 bp (150 bp Average)
Number of Primer Pools	2
Sample Input Requirement	5-11 µL (or ~50ng) of extracted total RNA
Sample Types	Sputum, nasopharyngeal and oropharyngeal swabs and aspirate, tissue samples, and other methods for viral RNA sampling.
Total Assay Time	6 hours
Hands-On Time	Less than 1 hour
Amplicon Coverage (≥30x)	> 92% with 10,000 copies viral input at less than 0.1M PE reads per sample
On-Target Aligned Reads	> 95%

Viruses	Selective Covered Regions	Amplicon Count
SARS-CoV-2	Complete coverage (except 92 bp at the ends of the genome)	172 (pool 1) 171 (pool 2)
Influenza A H1N1	HA, M1, NA, NP, NS1, PA, PB1, PB2	18 (pool 1)
Influenza A H1N2	HA, M1, M2, NA, NP, NS1, PB1, PB2	17 (pool 1)
Influenza A H3N2	HA, M1, M2, NA, NP, NS1, PA, PB1, PB2	31 (pool 1)
Influenza B	HA, M1, NA, NS1, PB1, PB2	32 (pool 1)
RSV A	G, F, M, N, NS1, NS2, L, SH, M2-1	21 (pool 1)
RSV B	G, F, M, N, NS1, NS2, P, L, SH, M2-1	30 (pool 1)

CleanPlex Streamlined Targeted Sequencing Workflow

CleanPlex Respiratory Virus Research Panel v2 offers a simple and streamlined workflow. Starting from extracted RNA, the protocol includes cDNA synthesis by reverse transcription (RT), followed by the multiplex PCR-based CleanPlex workflow. The entire workflow requires just 6 hours with minimal hands-on time to generate target-enriched NGS libraries, and is easily automatable on liquid handling platforms.

CleanPlex Target Enrichment and Library Preparation

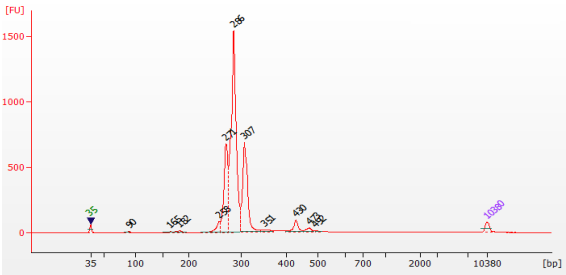
6 hours of total assay time, 60 minutes of hands-on time

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PARAGON GENOMICS

CleanPlex® Background Cleaning Chemistry

The CleanPlex Respiratory Virus Research Panel v2 is powered by Paragon Genomics' CleanPlex technology, which uses a proprietary multiplex PCR background cleaning chemistry to effectively remove non-specific PCR products, resulting in best-in-class target enrichment performance and efficient use of sequencing reads. The cleaning technology effectively tackles the nonspecific products formed by primers that do not find their targets in a sample. The figure below shows the library product fragment analysis trace generated with influenza A H1N1 control samples.



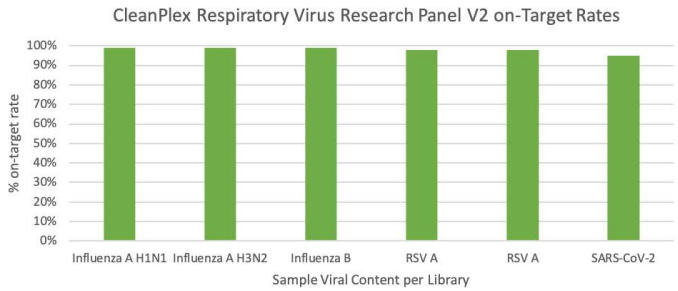
Specificity and High on-Target Rates

CleanPlex amplicon-based target enrichment method allows highly specific amplification, resulting in high on-target rates and reduced overall sequencing depth per sample. The specificity table below shows the percentage of amplicons with >30X coverage in libraries prepared from each virus control samples with sequencing data down-sampled to only 50,000 cluster reads per sample. The on-target rates for each sample is also shown in the histogram below. The data demonstrate high coverage and on target rates for detection at even at low sequencing depths.

Sample Viral Content for each Library

% Amplicon Covered >30X	H1N1 RNA only	H3N2 RNA only	Influ B RNA only	RSV A RNA only	RSV B RNA only	SARS-CoV-2 RNA only
H1N1 Reads	100%	0%	0%	0%	0%	0%
H3N2 Reads	0%	97%	0%	0%	0%	0%
Influ B Reads	0%	0%	97%	0%	0%	0%
RSV A Reads	0%	0%	0%	100%	0%	0%
RSV B Reads	0%	0%	0%	0%	100%	14%*
SARS-CoV-2 Reads	0%	0%	0%	0%	0%	92%

* The off target reads in SARS-CoV-2 samples was confirmed to be from RSV B sample cross-contamination.



Recommended Sample Multiplexing for CleanPlex Respiratory Virus Research Panel v2

Detection of positive samples can be achieved with a single-pool workflow, and libraries do not require deep sequencing for viral identification and differentiation. For applications that require deeper sequencing, such as mutation analysis or very low copy count, we suggest two pool workflow and deeper sequencing to achieve higher coverage of the targets and deeper coverage per target. Fewer total reads are required to sequence influenza-only or RSV-only samples as there are fewer target specific amplicons for these two viruses than for SARS-CoV-2.

Instrument	Samples per Run [A]
iSeq™ 100 System	155
MiniSeq™ System (mid-output)	311
MiniSeq System (high-output)	972
MiSeq® System (v2 chemistry Nano)	39
MiSeq System (v2 chemistry)	583
NextSeq™ 550 System (mid-output)	5053

A. Calculated based on an intended average read depth of 75x per amplicon of SARS-CoV-2 full genome coverage or about 50,000, 2x 150bp, paired-end reads per sample when using both pools for viral detection. If only one pool is used for streamlined detection workflow, 2x more samples can be added per run. For applications that require higher coverage, we suggest 1000x read depth per amplicon, or 0.7M PE reads per sample to ensure full coverage of SARS-CoV-2 genome.

Ordering Information

The CleanPlex Respiratory Virus Research Panel v2 is ready for early access customers. CleanPlex® Indexed PCR Primers and CleanMag® Magnetic Beads are ordered separately to complete the workflow from input RNA to sequencing-ready NGS libraries. Please contact sales@paragongenomics.com for more information.

Product	SKU
CleanPlex® Respiratory Virus Research Panel v2 (8 Rxns)	918303
CleanPlex® Respiratory Virus Research Panel v2 (96 Rxns)	918304
CleanPlex Plated Unique Dual-Indexed PCR Primers for Illumina® Set C 96-well (96 indexes, 96 reactions)	716037
CleanPlex Dual-Indexed PCR Primers for Illumina® Set A (96 indexes, 96 reactions)	716006
CleanMag Magnetic Beads (20 mL)	718005
CleanMag Magnetic Beads (60 mL)	718003

Learn More

To learn more about infectious disease applications and SARS-CoV-2 panel, visit https://www.paragongenomics.com/applications/infectious-disease/coronavirus-research/

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