CleanPlex® Research NGS Panels | Product Sheet

CleanPlex® SARS-CoV-2 Research and Surveillance Panel

Targeted Sequencing NGS Panels to Support SARS-CoV-2 Research and Surveillance

Highlights

- **Complete Coverage and Comprehensive Data**
  Sequence the entire SARS-CoV-2 genome with over 99% coverage
- **Ultra-sensitive Detection**
  Detect down to one copy per reaction for degraded or limited sample input
- **Fast, Streamlined Workflow**
  Generate libraries for Illumina® platforms in just 5.5 hours using a simple, four-step protocol
- **Superb Performance**
  Prepare high-quality NGS libraries with excellent coverage uniformity and on-target performance to enable efficient use of sequencing reads and reduce costs

Real Time RT-PCR and antibody-based methods are the main tools for detecting infectious agents, however, such methods can only focus on a limited number of targets and can at times suffer from low assay sensitivity and false negatives results. These methods also do not provide additional information other than a positive or negative diagnosis. The CleanPlex technology is an assay platform for ultra-sensitive and highly-multiplexed PCR-based targeted sequencing tests. This technology provides an easy-to-use, fast, and comprehensive solution for detection, identification, and mutation analysis of infectious pathogens all via a quick and easy workflow.

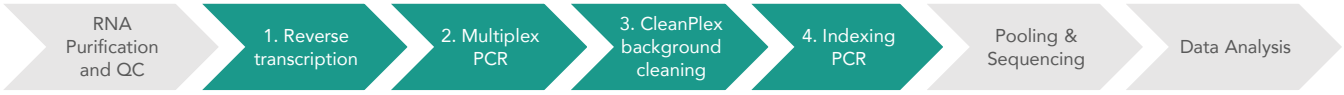
The CleanPlex® SARS-CoV-2 Research and Surveillance Panel was expertly designed from reference sequence MN908947 (NC_045512.2) using a proprietary design pipeline to cover the entire genome. For additional flexibility, the panel is available on two major sequencing platforms including Illumina®. The NGS panel not only allows high sensitivity detection and confirmation of questionable qPCR results, but also enables mutation analysis, tracking, surveillance, and informed infection control through comprehensive sequence information generated.

CleanPlex SARS-CoV-2 Research and Surveillance Panel Specifications for Illumina Platforms

Parameter	Specification
Enrichment Method	Multiplex PCR
Platform	Illumina®
Strain Compatibility	Complete coverage of major strains: MN908947 and MT007544
Cumulative Target Size	29,903 bp
Number of Amplicons	343
Amplicon Size	116 - 196 bp, Median 149bp
Number of Primer Pools	2
Sample Input Requirement	5-11 µL of extracted total RNA or ~50ng purified total RNA
Sample Types	Sputum, nasopharyngeal and oropharyngeal swabs and aspirate, tissue samples, and other methods for viral RNA sampling.
Total Assay Time	5.5 hours
Hands on time	Less than 1 hour
Design Coverage	Complete coverage (except 92 bp at the ends of the genome)
Amplicon Coverage (≥50x)	>95% with 300 copies viral input at 0.2M PE Reads per sample
On-Target Aligned Reads	>98% with 300 copies viral input at 0.2M PE Reads per sample
Total Reads per sample	0.2 to 0.3 M PE per sample with 2 x 150 PE reads

CleanPlex Streamlined Targeted Sequencing Workflow

CleanPlex SARS-CoV-2 Panels offer a simple and streamlined workflow. Starting from purified RNA, the protocol can be completed to generate target-enriched NGS libraries in just 5 hours, with less than 1 hour of hands-on time, using a three-step workflow with minimal tube-to-tube transfers. Libraries generated for Illumina sequencing contain an additional 15min background cleaning step that involves enzymatic digestion of non-specific products for cleaner and higher quality libraries.



CleanPlex Target Enrichment and Library Preparation

5.5 hours of total assay time, with less than 1 hour of hands-on time.

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

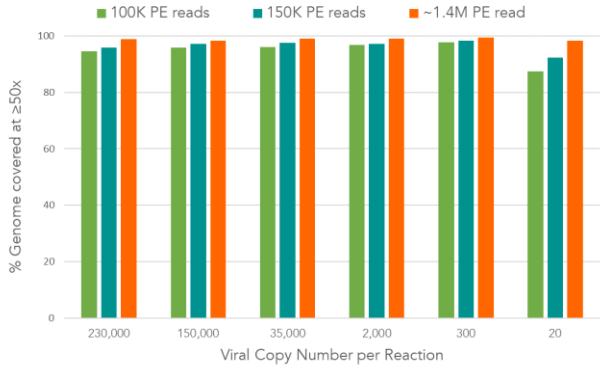
PARAGON GENOMICS

High Performance Translates to Cost-Effective Sequencing and Confident Analysis

Specifications	20 copies	300 copies
% Uniformity (0.2x Mean)	87%	97%
% Mapping Rate	84%	98%
% On-Target Rate	93%	99%

CleanPlex Panels exhibit highly uniform coverage even with low template input and ultra high multiplexing. Without the need for deep sequencing to capture all targets, high sequencing performance allows for more cost-effective sequencing with more samples pooled per chip for higher throughput.

Genomic Coverage (min 50x) vs Viral Copy Input at Various Sequencing Depths



Using nasopharyngeal swab samples from COVID-19 patients spanning 4 to 40,000 copies/µL (5.5 µL input/reaction) libraries were prepared and sequenced at ~0.7 million cluster reads per sample. The plot above shows *in silico* down sampling data indicating high coverage even at low reads and copy numbers. At the original sequencing depth, >98% of the amplicons are covered at >50X, more than sufficient for mutation monitoring and phylogenetic analysis. For strain confirmation of samples with as little as 4 copies/uL of viral RNA, just 0.1M PE reads is sufficient.

Base Position	Reference base	Alternative Base	Call Frequency
19065	T	C	99.4%
22303	T	G	99.0%
26144	G	T	99.3%
29749	ACGATCGAGTG	A	99.5%

CleanPlex Libraries were created using Twist's control synthetic SARS-CoV-2 MT007544 strain RNA, and variant calling was performed against reference genome NC_045512.2 (MN908947). As shown in the table above, all four expected variants (3 SNVs and 1 deletion) were confidently detected with 99% calling frequency.

Recommended Sample Multiplexing for CleanPlex SARS-CoV-2 Research and Surveillance Panel

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 Micro)	155
MiSeq System (v2 chemistry)	583
NextSeq™ 550 System (mid-output)	5053

A. Calculated based on an intended average read depth of 75x per amplicon 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. Additional reads might be needed to achieve deeper coverage per amplicon and more completeness of genome coverage for low viral copy number detection or low percentage variant calling.

Ordering Information

The CleanPlex SARS-CoV-2 Research and Surveillance panel contains CleanPlex Multiplex PCR Primers and CleanPlex Targeted Library Kit with RT reagents. CleanPlex Indexed PCR Primers and CleanMag® Magnetic Beads are ordered separately to complete the workflow from input RNA to sequencing-ready NGS libraries. For more indexing options and additional product configurations, please visit www.paragongenomics.com/store/

Product	SKU
CleanPlex SARS-CoV-2 Panel (8 reactions)	918010
CleanPlex SARS-CoV-2 Panel (96 reactions)	918011
CleanPlex Dual-Indexed PCR Primers for Illumina® Set A (12 x 8 Index, 96 reactions)	716033
CleanPlex Dual-Indexed PCR Primers for Illumina® Set B (12 x 8 Index, 96 reactions)	716034
CleanPlex Dual-Indexed PCR Primers for Illumina® Set C (12 x 8 Index, 96 reactions)	716035
CleanPlex Dual-Indexed PCR Primers for Illumina® Set D (12 x 8 Index, 96 reactions)	716036
CleanMag Magnetic Beads (1 mL)	718001
CleanMag Magnetic Beads (5 mL)	718002
CleanMag Magnetic Beads (60 mL)	718003

Learn More

To learn more about NGS applications for Infectious Diseases, please visit www.paragongenomics.com/applications/infectious_disease/

To learn more about CleanPlex Technology, please visit www.paragongenomics.com/cleanplex_technology/

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