

CleanPlex® ACE2&TMPRSS2 Panel

Targeted Sequencing of ACE2 and TMPRSS2 for COVID-19 Susceptibility

Highlights

- **Curated Content and Comprehensive Data**
Interrogates entire *ACE2* & *TMPRSS2* coding and promoter regions plus curated 100+ eQTL targets
- **Fast, Streamlined Workflow**
Generate libraries for Illumina® platforms in just 3 hours using a simple, three-step protocol
- **Superb Performance with Low DNA Input**
Prepare high-quality NGS libraries with excellent coverage uniformity and on-target performance to enable efficient use of sequencing reads and reduce costs

The CleanPlex® ACE2 & TMPRSS2 Panel is a multiplex PCR-based targeted sequencing assay designed to examine two genes associated with COVID-19 susceptibility.

Research has shown that the SARS-CoV-2 virus is internalized via binding of its S protein to the host ACE2 with the help of TMPRSS2. The binding affinity of SARS-CoV-2 with ACE2, along with ACE2 and TMPRSS2 expression levels, are major determinants of viral replication rate and disease severity. This panel targets all exonic and promoter regions of the two genes, and more than 70 expertly curated expression quantitative trait loci (eQTL), shown to regulate their expression.

The CleanPlex ACE2 & TMPRSS2 panel facilitates further investigations in host genome mutations related to the ACE2 binding affinity and expression of *ACE2* and *TMPRSS2*, which would provide additional insights into the individual's viral susceptibility and disease's severity.

CleanPlex Streamlined Workflow

The CleanPlex ACE2 & TMPRSS2 Panel offers a simple and streamlined workflow. Starting from purified and quantified DNA, the multiplex PCR-based protocol can be completed in just 3 hours, with 75 minutes of hands-on time, using a three-step workflow with minimal tube- to-tube transfers. Each step consists of a thermal cycling or incubation condition, followed by "with bead" purification using magnetic beads.



CleanPlex Target Enrichment and Library Preparation

3 hours of total assay time, 75 minutes of hands-on time

CleanPlex® ACE2&TMPRSS2 Panel Specifications

Parameter	Specification
Enrichment Method	Multiplex PCR
Sequencing Platforms	Illumina®
Number of Genes	2
Targets	Full Exon + promoter regions of ACE2 and TMPRSS2 gene & ~70 eQTL
Cumulative Target Size	32.4 Kb (4.3 Kb exonic content)
Variant Types	SNVs, indels ^A
Number of Amplicons	210
Amplicon Size	225 bp on average
Number of Primer Pools	2
Input DNA Requirement	1 – 40 ng per pool (10ng recommended)
Sample Types	Genomic DNA, buccal swabs
Total Assay Time	3 hours
Hands-On Time	75 minutes
Coverage Uniformity (targets with >0.2X mean coverage)	~93%
On-Target Aligned Reads	≥ 95%

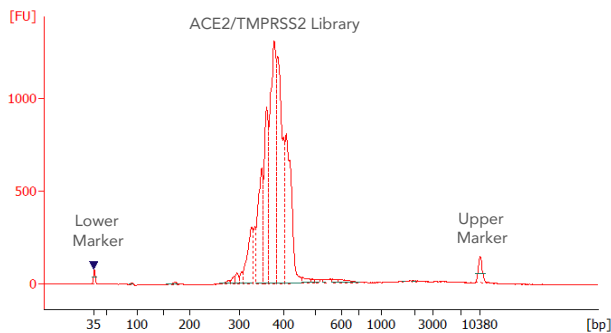
A. SNVs: single nucleotide variations; indels: insertions-deletions

Sensitive Detection with Low Input

The CleanPlex ACE2 & TMPRSS2 Panel allows confident detection of novel or known variants with just 5 ng of DNA.

CleanPlex Background Cleaning Chemistry

The CleanPlex ACE2 & TMPRSS2 panel 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.



Sequencing data of libraries generated with CleanPlex ACE2 & TMPRSS2 Panel show consistently high mapping and on-target rates. The panel is uniquely optimized for superior uniformity resulting in best-in-class target enrichment performance.

Accurate Variant Calls

Three cell lines were sequenced and all expected mutations were called. The table below also shows a subset of calls made in NA12878 that includes point mutations and indels. Variant analysis shows that the CleanPlex ACE2 & TMPRSS2 Panel provides accurate and confident calling for expected homozygous and heterozygous germline mutations.

Cell Line	Expected No. Variants	Detected No. Variants	Concordance
NA12878	72	72	100%
NA12877	62	62	100%
NA24631	20	20	100%

Subset of NA12878 calls			
Chromosome	Position	Mutation	Frequency
chr21	42841988	G_A	49.2%
chr21	42844052	T_G	99.7%
chr22	42859279	CAG_C	99.0%
chr21	42880333	CCGCGGCGCAG_C	98.8%
chrX	15495806	G_GT	50.3%

Recommended Sample Multiplexing for CleanPlex ACE2 & TMPRSS2 Panel

Instrument	Samples per Run ^B
iSeq™ 100 System	38
MiSeq® System (v2 chemistry Nano)	9
MiSeq System (v2 chemistry)	142
MiniSeq® System (High-output)	238
NextSeq® System 550 (Mid Output)	1238

B. Samples per run at an intended average read depth of 500X.

Ordering Information

The CleanPlex ACE2 & TMPRSS2 Panel contains CleanPlex Multiplex PCR Primers and CleanPlex Targeted Library Kit. CleanPlex Indexed PCR Primers and CleanMag® Magnetic Beads are ordered separately to complete the workflow from input DNA to sequencing-ready NGS libraries. For more indexing options, and additional product configurations visit www.paragongenomics.com/store/

Product	SKU
CleanPlex ACE2 & TMPRSS2 Panel (8 reactions)	916120
CleanPlex ACE2 & TMPRSS2 Panel (96 reactions)	916121
CleanPlex Dual-Indexed PCR Primers for Illumina® Set A (96 indexes, 96 reactions)	716006
CleanPlex Dual-Indexed PCR Primers for Illumina® Set B (96 indexes, 96 reactions)	716018
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, visit www.paragongenomics.com/cleanplex_technology/

Curated publications related to ACE2 and TMPRSS2 expression and variants are available upon request by contacting techsupport@paragongenomics.com