CleanPlex® NGS Research Panels | Product Sheet

SARS-CoV-2 Emerging Variants Panel Add-on

Optimized SARS-CoV-2 Coverage for Emerging Variants

Highlights

- **Robust Coverage**
  Added primers for enhanced coverage of newly discovered UK and South African variants.
- **Additional Confidence in Variant Calling**
  Optimized for identifying existing and new mutations for surveillance and tracking.
- **Easy Spike-in Workflow**
  Directly add to existing CleanPlex SARS-CoV-2 and FLEX panels for ease of use and flexible future updates.

The SARS-CoV-2 Emerging Variant Panel Add-on is designed to be used with the existing CleanPlex SARS-CoV-2 products to maintain high coverage for variant calling and identification as the virus evolves.

The new variants of concern, such as the UK (B.1.1.7) and South Africa (B.1.351) lineages, contain mutations in the receptor-binding domain (RBD) of the spike protein, which have been shown to significantly increase infection rates and can negatively impact the efficacy of the recently developed vaccines and current COVID-19 qPCR mass testing methods. Thus, a robust detection method and continued screening and surveillance are more critical than ever in our continued global efforts to control this pandemic.

The Add-on primers were generated by cross-referencing the FLEX panel design with selected emerging SARS-CoV-2 variants for even coverage and confident identification of their defining mutations.

Optimized for Variant Surveillance

Critical mutations for the following variants were evaluated for the design and optimization of the Emerging Variant Panel Add-on primers. For mutations that fall on a priming region, additional primers were created when appropriate to upkeep the amplification efficiency for potentially affected amplicons.

Lineage	Name	First Detected
B.1.1.7	20I/501Y.V1	United Kingdom
B.1.351	20H/501.V2	South Africa

SARS-CoV-2 Original, FLEX, and Add-on Designs

When considering which panel works best for your application, the Original and FLEX panels are both top performing panels designed for uniform coverage of the whole SARS-CoV-2 genome.

The **Original panel** utilizes a tiled design, with significant overlap built-in to allow for redundancy and to maintain robust coverage even if a few primers could be affected as the virus evolves.

The **FLEX panel** was designed to add degeneracies in primers at highly polymorphic regions in a preemptive effort for additional confidence in coverage. The FLEX panel also includes a pair of Human RNA primers that serves as a library preparation quality control with negative samples.

Lastly, the **Add-on** primers were designed to supplement the main panels to specifically address newly discovered mutations of interest that could benefit from additional coverage. Although the Original panel continues to perform well in the field, our team continues to stay vigilant and monitor the state of SARS-CoV-2 variants with regular *in silico* and *in vitro* verifications to provide products with the best performance we can offer.

Features	SARS-CoV-2 Original	SARS-CoV-2 FLEX	Emerging Variants Add-on
Full Genome Coverage	✓	✓	-
Degenerate Primer Design		✓	-
Human RNA Control		✓	-
Specific Variants Optimized			✓

Simple Spike-in Workflow

The Emerging Variant Panel Add-on primers can be easily implemented into any CleanPlex SARS-CoV-2 library preparation workflow. The two primer pools from the Add-on kit are added to the master mixes during the multiplex PCR step with no other changes to the overall workflow. This flexibility allows current CleanPlex users to access the most recent updates without having to replace any existing inventory. If additional adjustments are necessary in the future, the Add-on primer pools can be easily revised to include additional primers for superior coverage and performance, as required for your leading research projects.

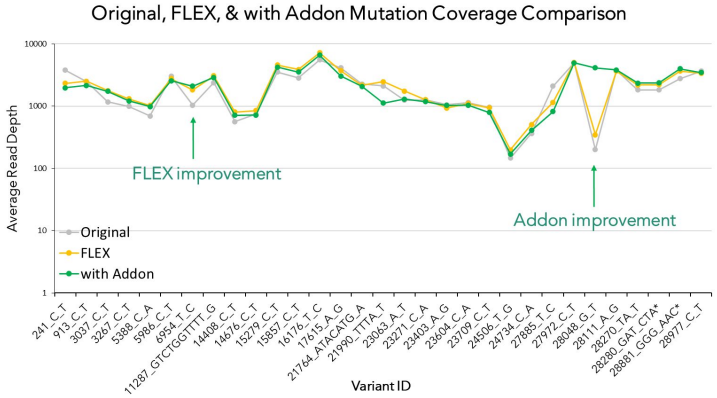
Paragon Genomics, Inc. | 5020 Brandin Ct, FL 2, Fremont CA 94538, USA | +1.650.822.7545
www.paragongenomics.com | techsupport@paragongenomics.com

Coverage of Mutations

The Original SARS-CoV-2 panel, FLEX panel, and the FLEX panel with Add-on primers were tested with Twist synthetic control #14 (UK variant: B.1.1.7_710528), which contains 32 SNPs and 4 deletions[1] (see table below) compared to the NC_045512.2 reference genome. Due to the inherent gaps in the genome from the synthetic control material, clinical sample data was used to confirm one SNP call (NC_045512.2_24914 G_C), which falls into one of these gaps.

Variant ID	Ref	Alt	Protein Amino Acid Change
241	C	T	-
913	C	T	QHD43415.1:p.216S
3037	C	T	QHD43415.1:p.924F
3267	C	T	QHD43415.1:p.1001T>I
5388	C	A	QHD43415.1:p.1708A>D
5986	C	T	QHD43415.1:p.1907F
6954	T	C	QHD43415.1:p.2230I>T
14408	C	T	QHD43415.1:p.4715P>L
14676	C	T	QHD43415.1:p.4804P
15279	C	T	QHD43415.1:p.5005H
15857	C	T	QHD43415.1:p.5198T>I
16176	T	C	QHD43415.1:p.5304T
17615	A	G	QHD43415.1:p.5784K>R
23063	A	T	QHD43416.1:p.501N>Y
23271	C	A	QHD43416.1:p.570A>D
23403	A	G	QHD43416.1:p.614D>G
23604	C	A	QHD43416.1:p.681P>H
23709	C	T	QHD43416.1:p.716T>I
24506	T	G	QHD43416.1:p.982S>A
24734	C	Y	QHD43416.1:p.1058-
24914	G	C	QHD43416.1:p.1118D>H
27885	T	C	-
27972	C	T	QHD43422.1:p.27Q>*
28048	G	T	QHD43422.1:p.52R>I
28111	A	G	QHD43422.1:p.73Y>C
28280	G	C	QHD43423.2:p.3D>H
28281	A	T	QHD43423.2:p.3D>V
28282	T	A	QHD43423.2:p.3D>E
28881	G	A	QHD43423.2:p.203R>K
28882	G	A	QHD43423.2:p.203R
28883	G	C	QHD43423.2:p.204G>R
28977	C	T	QHD43423.2:p.235S>F
11287	GTCTGGTTTT	G	QHD43415.1:p.3675-3677SGF>-
21764	ATACATG	A	QHD43416.1:p.68-70IHV>I
21990	TTTA	T	QHD43416.1:p.143-144VY>V
28270	TA	T	-

Variant calling results from this experiment indicate that both the Original and FLEX panels are able to accurately call all 36 mutations. However, with the supplemental Add-on primers, the panel showed improved coverage in a couple of regions. For example, the SNP at position 28048 (see below) showed 10x coverage improvement with the Add-on primers. We also see that the FLEX panel has higher coverage for the amplicon containing the 6954_T_C mutation over the Original panel. These results not only indicate that the Original panel is already robust in capturing new mutations, but also that the FLEX and the Add-on panels do offer improvements for superior performance.



Panels were run in triplicates using a synthetic control (B.1.1.7). Variants were called against the reference genome NC_045512.2. All 36 mutations were covered and accurately called in each panel tested. Improvements of coverage in FLEX and with Add-on Primers are highlighted above.

* Three consecutive SNPs at positions 28280 and 28881 are presented as one mutation each in the plot above.

Ordering Information

The Original SARS-CoV-2 Panel is still available, along with the FLEX panel. For indexing options and additional product configurations, please visit www.paragongenomics.com/store/

Product	SKU
SARS-CoV-2 Emerging Variant Panel Add-on (8 rxn)	918016
SARS-CoV-2 Emerging Variant Panel Add-on (96 rxn)	918017
SARS-CoV-2 Emerging Variant Panel Add-on (384 rxn)	918018

Learn More

To learn more about infectious disease applications and SARS-CoV-2 panels, visit our COVID-19 Applications Page.

Follow the links here to learn more about the Original CleanPlex SARS-CoV-2 and FLEX Panels.

[1] The total of 36 mutations against the reference genome does not include variations in the few bases from 3' and 5' ends of the viral genome that are not covered by the panel.

Paragon Genomics, Inc. | 5020 Brandin Ct, FL 2, Fremont CA 94538, USA | +1.650.822.7545
www.paragongenomics.com | techsupport@paragongenomics.com