

## Optimized SARS-CoV-2 Coverage for Emerging Variants of Interest and Concern

### Highlights

- Robust Coverage**  
 Added primers for enhanced coverage of newly discovered variants and their sub-lineages such as Alpha, Beta, Delta, Mu, and Omicron (covers all characteristic mutations of BA.1, BA.2, and BA.3)
- Additional Confidence in Variant Calling**  
 Optimized for identifying existing and new mutations for surveillance and tracking, especially with “super-amplicon” formation in base panels
- Easy Spike-in Workflow**  
 Directly add to existing CleanPlex SARS-CoV-2 FLEX panel for ease of use and flexible future updates

The new variants of importance and concern, such as the delta variant (B.1.617.2 and AY lineages), mu variant (B.1.621) and most recently, the omicron variant (B.1.1.529) contain several mutations in the receptor-binding domain (RBD) of the spike protein, which have been shown to significantly increase transmission rates and can negatively impact the efficacy of the vaccines and current COVID-19 qPCR mass testing methods. Particularly with omicron, prominent PCR diagnostic tests have been shown to fail to detect the virus. 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 SARS-CoV-2 Emerging Variant Panel Add-on v2 (EVA v2) primers were optimized from EVA v1 to maintain even coverage and confident identification of the defining mutations of each variant. It has been designed to be used with the existing CleanPlex SARS-CoV-2 full genome sequencing products and is compatible with both Illumina and Ion Torrent sequencers.

### Optimized for Variant Surveillance

Critical identifying mutations of various emerging variants were evaluated for the design and optimization of the Emerging Variant Panel Add-on primers, then fortified with this v2 release to cover the most recent variants. This update has been confirmed *in silico* to cover all defining and characteristic mutations of the sub-lineages of omicron: BA.1, BA.2, and BA.3. For mutations that fall on a priming region, additional primers were generated when appropriate to upkeep the amplification efficiency for potentially affected amplicons. Please refer to the table on the lower right for specifications of the variants considered.

### SARS-CoV-2 Original, FLEX, and Add-on v2 Designs

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

The **Original panel** utilizes a tiled 2-pool design, with significant amplicon overlap built-in for redundancy and to maintain robust coverage even if a few primers could be affected as the virus evolves. “Super amplicons” can be formed in situations in which a forward primer from one amplicon and reverse primer from a down stream neighboring amplicon form a long amplicon. In situations when an inner primer’s performance might suffer from a mutation, these super amplicons have been shown to recover reads and salvage coverage.

The **FLEX panel** was designed with 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. Version 2 encompasses all original EVA primers and includes additional primers optimized for improved coverage of Beta, Delta, Mu, and Omicron variants.

Although the Original panel continues to perform well in the field, our team stays vigilant and monitors 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	EVA	EVA v2
Full Genome Coverage	✓	✓	-	-
Degenerate Primer Design		✓	-	-
Human RNA Control		✓	-	-
Specific Variants Optimized			Alpha, Beta	Alpha, Beta, Delta, Mu, Omicron

Lineage	Greek Name	Name	First Detected
B.1.1.7	Alpha	20I/501Y.V1	United Kingdom
B.1.351	Beta	20H/501.V2	South Africa
B.1.617.2 and AY lineages	Delta	20A	India
B.1.621	Mu	21H	Colombia
B.1.1.529	Omicron	21K	South Africa

Paragon Genomics, Inc. | 3521 Investment Blvd Suite 1, Hayward CA 94545, USA | +1.650.822.7545  
 www.paragongenomics.com | techsupport@paragongenomics.com

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## Coverage of Mutations and Identification of Sub-lineages

The active monitoring and characterization of all the lineages of SARS-CoV-2 is a critical tool for more specific diagnosis, informed prediction, and timely and appropriate treatment. To better understand the virus genetic diversity and its dynamic dispersion patterns, EVA v2 has been confirmed *in silico* to cover all defining and characteristic mutations of the sub-lineages of omicron: BA.1, BA.2, and BA.3.

## Simple Spike-in Workflow

The Emerging Variant Panel Add-on v2 primers can be easily implemented into any CleanPlex SARS-CoV-2 library preparation workflow. The Add-on v2 primers follow the same process as the original Add-on product: the primers simply need to be 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 primer pool can be easily adjusted, as required for your leading research projects.

## Ordering Information

The Original SARS-CoV-2 and FLEX panels will remain available for purchase. The Emerging Variant Panel Add v2 replaces the original version with the follow SKU options. For indexing options and additional product configurations, please visit [www.paragongenomics.com/store/](http://www.paragongenomics.com/store/)

Product	SKU
SARS-CoV-2 Emerging Variant Panel Add-on v2 (8 rxn)	918022
SARS-CoV-2 Emerging Variant Panel Add-on v2 (96 rxn)	918023
SARS-CoV-2 Emerging Variant Panel Add-on v2 (384 rxn)	918024
CleanPlex SARS-CoV-2 Panel (8 rxn)	918010
CleanPlex SARS-CoV-2 Panel (96 rxn)	918011
CleanPlex SARS-CoV-2 Panel (384 rxn)	918012
CleanPlex SARS-CoV-2 FLEX Panel (8 rxn)	918013
CleanPlex SARS-CoV-2 FLEX Panel (96 rxn)	918014
CleanPlex SARS-CoV-2 FLEX Panel (384 rxn)	918015
CleanPlex Plated Dual-Indexed PCR Primers for Illumina® Set A,B,C,D (12 x 8 Indexes, 96 rxn)	716033- 716036
CleanPlex Plated Single-Indexed PCR Primers v2 for Ion Torrent™ Set C, D, E, F (12 x 8 Indexes, 192 rxn)	716053 - 716056

## 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.

To read more about recent work that has been done using the Paragon SARS product line, please visit our [publications page](#).