SARS-CoV-2 amplicon sequencing solutions

Future-proofing with the xGen™ SARS-CoV-2 Amplicon Panel

The discovery and identification of pathogenic genomes are increasingly important in research studies. Next generation sequencing has recently proved its relevance as a thorough research surveillance method analyzing viral materials found in collected wastewater samples. Sequencing also provides researchers insight into the lineages circulating in a population, including SARS-CoV-2 variants (Figure 1).

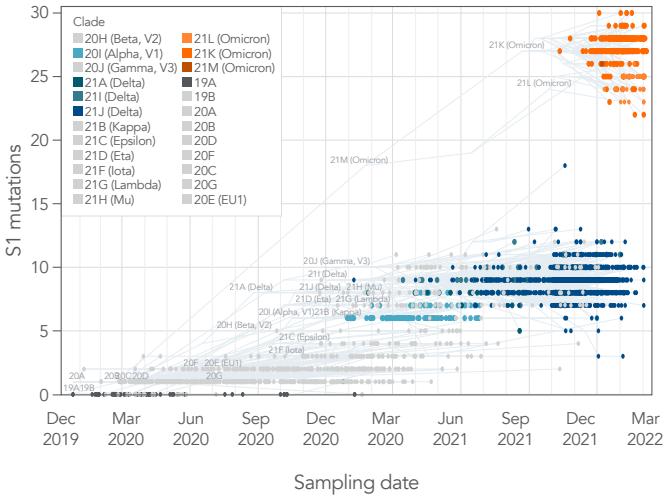

Figure 1. Amongst the SARS-CoV-2 variants, the Omicron variant has more mutations than any known predecessor. This chart shows the number of mutations discovered in the S1 subunit of the spike protein (Nextstrain) [1].

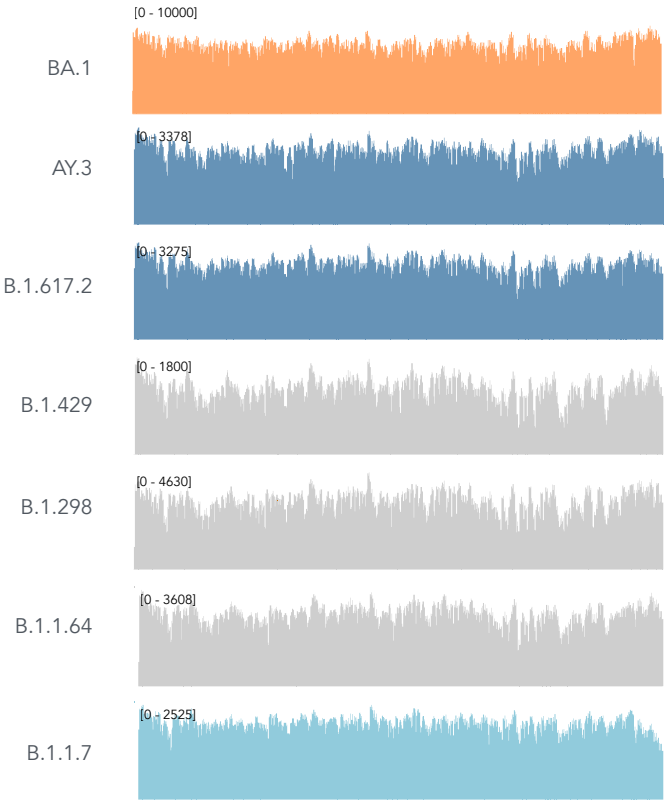

Figure 2. Coverage obtained by Change to IDT scientists from sequencing SARS-CoV-2 variants: BA.1 (Omicron), AY.3 (Delta), B.1.617.2 (Delta), B.1.429, B.1.298, B.1.1.64 (Eplison), B.1.1.7 (Alpha). RNA extracted from nasopharyngeal or oropharyngeal swabs were converted into cDNA using the SuperScript IV Kit (Thermo Fisher Scientific). Libraries were prepared using the xGen SARS-CoV-2 Amplicon Panel and were sequenced (2 x 150 bp) on a MiniSeq® System (Illumina®). The reads were downsampled to 83,000 reads per sample for analysis.

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Traditional sequencing methods have struggled to maintain full genomic coverage of novel variants due to the increasing number of primer dropouts found in some of the more recent SARS-CoV-2 variants (Omicron and Delta) (Figure 2). Because of this, researchers have had to seek out updated primer pool designs and spike-ins to account for these primer dropouts. The challenge becomes maintaining assay performance without expending additional time and resources in continually revalidating new assays and sequencing workflows.

With IDT's proprietary amplicon chemistry, researchers can help to eliminate primer pool redesigns and assay revalidation by using a solution that leverages the power of super amplicons.

Traditional amplicon sequencing methods use a two-tube, multiplex PCR chemistry that requires additional workflow steps that cannot detect super amplicons, making this method less efficient and prone to primer dropouts when a mutation is detected. The xGen SARS-CoV-2 Amplicon Panel is a single-tube, multiplex PCR solution that uses proprietary amplicon chemistry to identify super amplicons and mutations within 2 hours, while maintaining genomic coverage.

KEY BENEFITS:

- Obtain 99.7% genomic coverage
- cDNA-to-library in less than 2 hours
- Single-tube multiplex PCR chemistry—super amplicon generation
- Limit assay redesigns and spike-ins for novel mutations

FULL GENOMIC COVERAGE–OMICRON

Using a single, consistent primer set, the xGen SARS-CoV-2 Amplicon Panel covers multiple lineages, including sub-lineages of Omicron, while maintaining genomic coverage (Figure 3). The primer set has remained unchanged since its release in 2020, preventing the need for frequent changes to the original primer design.

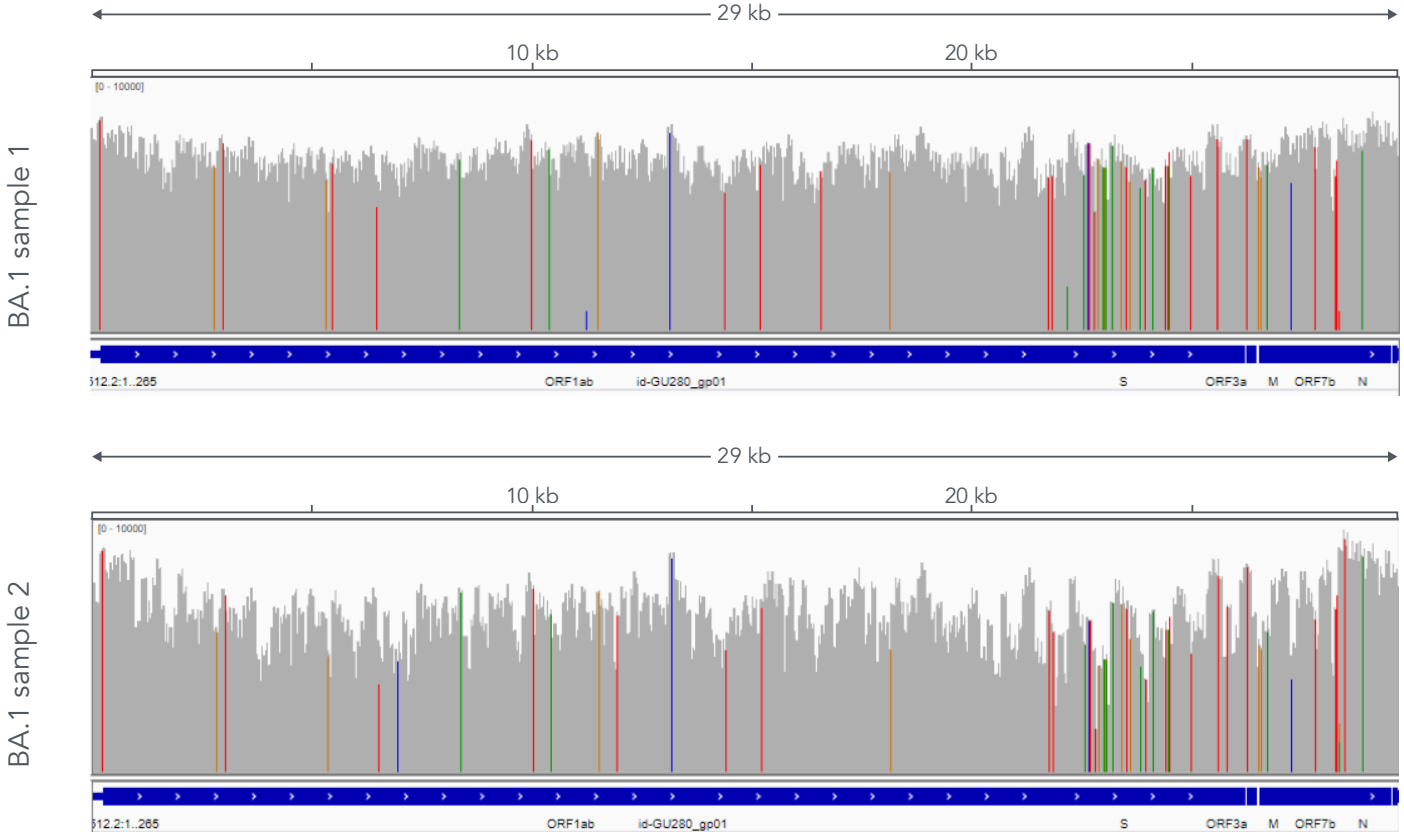

Figure 3. RNA was extracted by IDT scientists from nasopharyngeal swabs and converted into cDNA using the SuperScript IV Kit (Thermo Fisher Scientific). The resulting library generated with the xGen SARS-CoV-2 Amplicon Panel was sequenced (2 x 150 bp) on a MiniSeq® System (Illumina®). The resulting reads were downsampled to 83,000 reads per sample to analyze the two BA.1 lineages. Ct values are 13.6 and 19.9. A total of 93 biological research samples that exhibited S gene dropout by qPCR were analyzed, and 90 samples were identified as Omicron variants.

"In the first Omicron sample we detected, we sequenced 99.9% of the genome, and a highly-robust consensus sequence was generated. In examining the alignments, we were able to identify so-called 'super amplicons' that improve coverage of regions that have mutations in primer sites for shorter amplicons. Overall, we have been very pleased with the performance of the IDT kit and primer set during the constantly evolving pandemic."

Kevin Kunstman and Stefan Green, Rush University Medical Center

Table 1. Overview of the xGen SARS-CoV-2 Amplicon Panel.

Features	Specifications
Recommended CT value of input	<33
Panel information	345 amplicons, sized 116–255 bp (average 150 bp)
Recommended read length	2 x 150
Input material	1st or 2nd strand cDNA Minimum 10–100+ viral copies (RT-qPCR Ct value 30–40)
Recommended sequencing depth	Identification: 50 k reads per library Variant calling: 1 M reads per library
Multiplexing capabilities	Up to 384 CDIs or 1,536 UDIs
Workflow time	~2 hours (+1 hour with Normalase™)
Recommended Illumina® platforms	NovaSeq®, MiSeq®, MiniSeq®, NextSeq® 500/550/1000/2000
Components provided	Target-specific multiplex primer pool, PCR and library prep reagents, Normalase technology, combinatorial dual indexed adapters

LEARN MORE ABOUT OUR SARS-COV-2 RESEARCH SOLUTIONS

There are multiple factors to take into consideration when determining the best NGS approach for your SARS-CoV-2 research needs. Download this 6-page **brochure** to explore amplicon sequencing and hybridization capture options that may be right for you.

REFERENCES

1. Nextstrain data, https://nextstrain.org/ncov/gisaid/global

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/SARS-CoV-2AMP**.

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