xGEN™ RESPIRATORY VIRUS AMPLICON PANEL

Comprehensive sequencing coverage in one reaction

Save critical time

Trace variants with confidence

Stocked and ready to ship

ONE PANEL, SIX RESPIRATORY VIRUSES

The IDT xGen NGS Amplicon Sequencing Panels are ready to support the science community in its quest to monitor the evolution and spread of six respiratory viruses including: **Respiratory Syncytial Virus (RSV) A, RSV B, Influenza A H1N1, Influenza A H3N2, Influenza B, and SARS-CoV-2**.

Our uniquely configured, single-tube design creates overlapping amplicons to reliably distinguish viral variants of multiple respiratory viruses—all in one panel. Our differentiated super amplicon technology overcomes amplicon drop out and increases coverage for variable genomes, which is essential for tracing evolving epidemiological patterns.

- **Increase your coverage even in evolving or diverse genomes**—greater than 98.7% coverage of 6 respiratory virus genomes
- **Super amplicons**—coverage possible even if primer dropout occurs
- **Get data from low titers**—as low as 10–100 viral copies
- **Work faster, not harder**—workflow is <2.5 hr for library prep with <1 hr hands-on time
- **Normalize multiplexed samples easily**—optional Normalase™ technology provided
- **Automate your work**—up to 1536 UDI available
- **Customize it**—spike-in more primers to make the panel work for you

Table 1. xGen Respiratory Virus Amplicon Panel features.

Features	Specifications
Design coverage & panel information	1199 amplicons, sized 92–255 bp Primers designed to target RSV A, RSV B, Influenza A H1N1, Influenza A H3N2, Influenza B, & SARS-CoV-2
Input material	1st or 2nd strand cDNA As low as 10–100 viral copies
Time	<2.5 hr viral genomic material-to-library
Multiplexing capability	Up to 1536 UDIs
Compatible with other indexes?	Yes
Recommended depth	200K reads per library, PE150

6-IN-1 COVERAGE

The xGen Respiratory Virus Amplicon Panel is designed with 1199 amplicons ranging in size from 92–255 bp (Table 1). The amplicons are designed in such a way that can result in super amplicons that deliver >98% average coverage for all six genomes sequenced (Figure 1 and Table 2). This panel supports multiplexing up to 1536 UDIs and is compatible with other indexes. Given the high coverage and super amplicon capability, genotypic evolution can be observed in the genomes of RSV A, RSV B, Influenza A H1N1, Influenza A H3N2, Influenza B, and SARS-CoV-2.

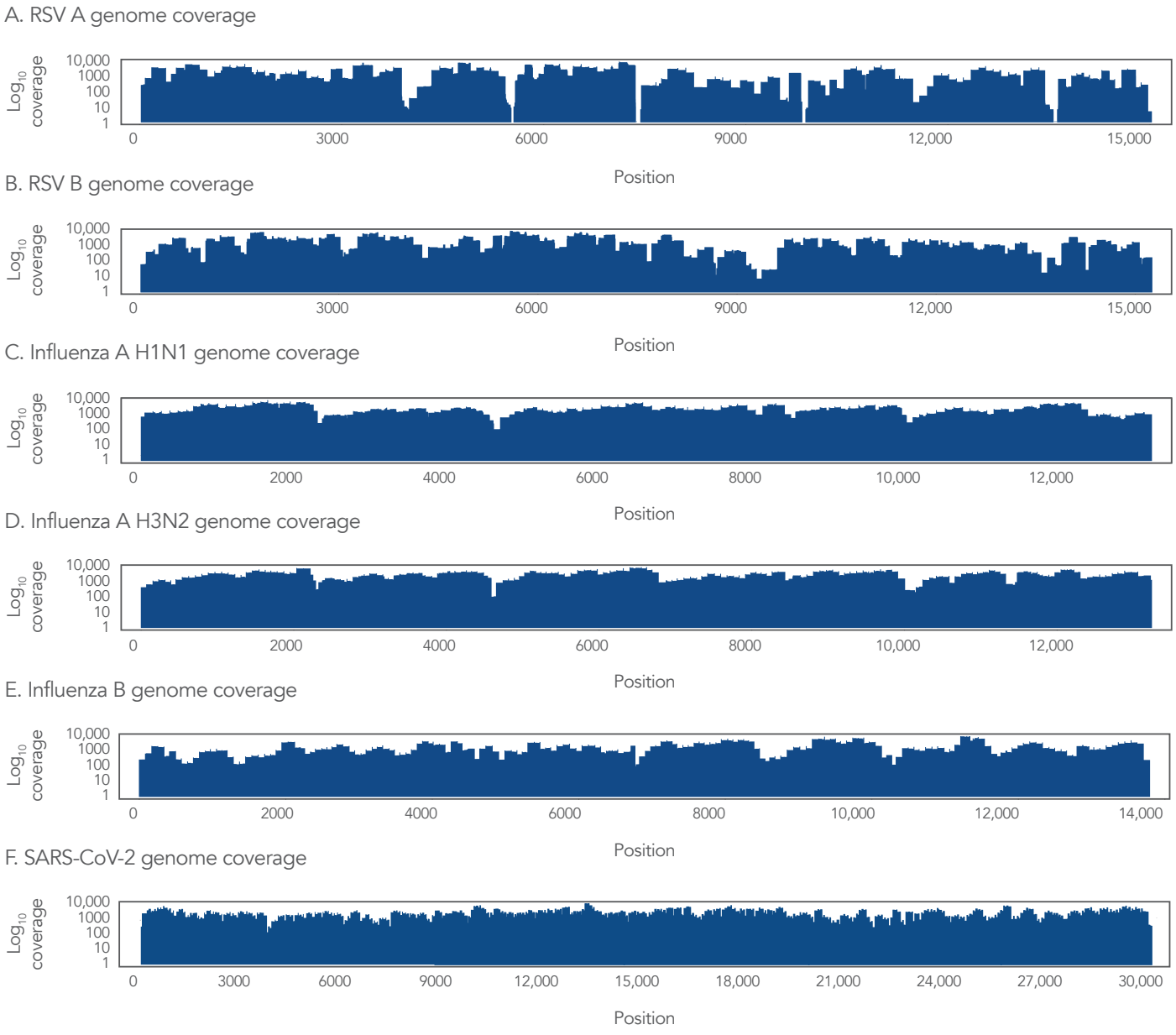

Figure 1. The xGen Respiratory Virus Amplicon Panel provides a high level of coverage for various respiratory virus genomes even when the sample has variations. Example data for one replicate per respiratory virus genome are shown in panels A–F. (**A**) RSV A coverage. 5 µL of BEI NR-43976 was spiked into reverse transcriptase (RT) reaction. The input genome, JX069800, has 96.25% ID to the designed primers. (**B**) RSV B coverage. 5 µL of BEI NR-48831 was spiked into the RT. The input genome, AF013254, has 96.7% ID to the designed primers. (**C**) Influenza A H1N1 coverage. 50K copies of Twist RNA control 103001 was spiked into RT. The input genome, A/California/7/2009, matches the designed primers. (**D**) Influenza A H3N2 coverage. 50K copies of Twist RNA control 103002 was spiked into the RT. The input genome, A/New York/392/2004, matches the designed primers. (**E**) Influenza B coverage. 5 µL of BEI NR-10048 was spiked into the RT. The input genome, B/Malaysia/2506/2004, matches the designed primers. (**F**) SARS-CoV-2 coverage. 50K copies of BEI NR-52287 was spiked into the RT. The input genome, USA-WA1/2020, matches the designed primers. For all libraries, cDNA was created using the SuperScript™ IV Kit (Thermo Fisher Scientific) with 10 ng of universal human reference (UHR) RNA. The resulting NGS libraries generated with the xGen Respiratory Virus Amplicon Panels were sequenced on a MiniSeq™ (Illumina®) with 150 bp paired-end (PE150) sequencing. Resulting reads were downsampled to 1M reads per sample for analysis.

Table 2. xGen Respiratory Virus Amplicon Panel NGS metrics.*

	RSV A	RSV B	Influenza A H1N1	Influenza A H3N2	Influenza B	SARS-CoV-2
% mapping	86%	93.60%	99.20%	99.40%	99.50%	98.50%
% on-target (base)	97.20%	96.70%	96.50%	96.30%	96.60%	97.20%
% base uniformity	67.40%	70.10%	93.20%	93.10%	77.80%	92.90%
% genome >10x coverage	94.50%	98.90%	100%	100%	100%	100%

*Sequencing metrics are shown for the libraries in Figure 1.

xGEN AMPLICON SEQUENCING TECHNOLOGY—SUPER AMPLICONS COVER THE GENOME

xGen Amplicon Technology from IDT enables single-tube multiplexed PCR even when contiguous target coverage of overlapping amplicons is needed, unlike other methods that require multiple tubes for target enrichment. In this methodology, primers are designed to compensate for changes in the target genomic DNA. If one of the primers cannot anneal to a location due to a mutation, then neighboring primers can compensate for the loss, and amplify the region as a longer amplicon—a super amplicon—that provide you complete coverage of novel variants and identification of genotypic evolution.

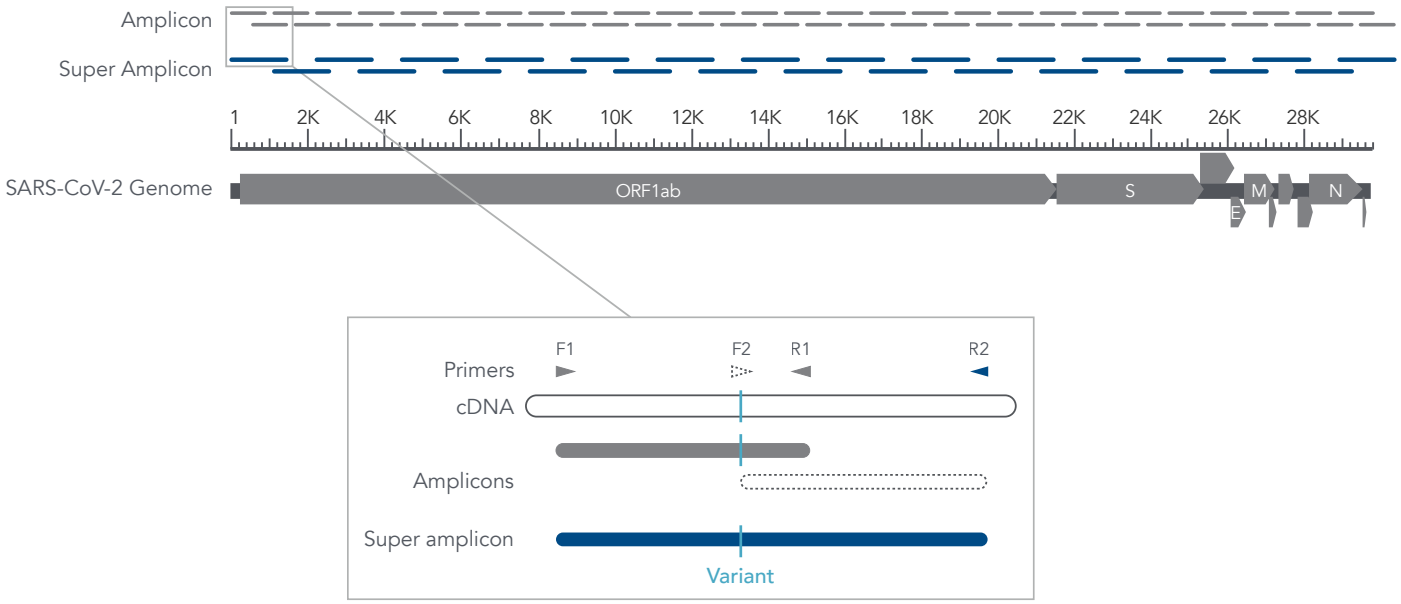

Figure 2. xGen Amplicon Sequencing Panels are a one-tube solution for coverage. With the xGen amplicon sequencing single-tube workflow, there is high coverage even when an individual primer (such as primer F2) anneals less efficiently due to a genomic mutation in its binding site. Super amplicons are created when the nearby primer pair, F1 and R2, amplify the area including the variant.

DNA-TO-SEQUENCER IN 2.5 HOURS

Multiplex PCR
70 minutes

Adapter attachment & indexing PCR
35 minutes

Library normalization with Normalase™ technology (optional)
40 minutes

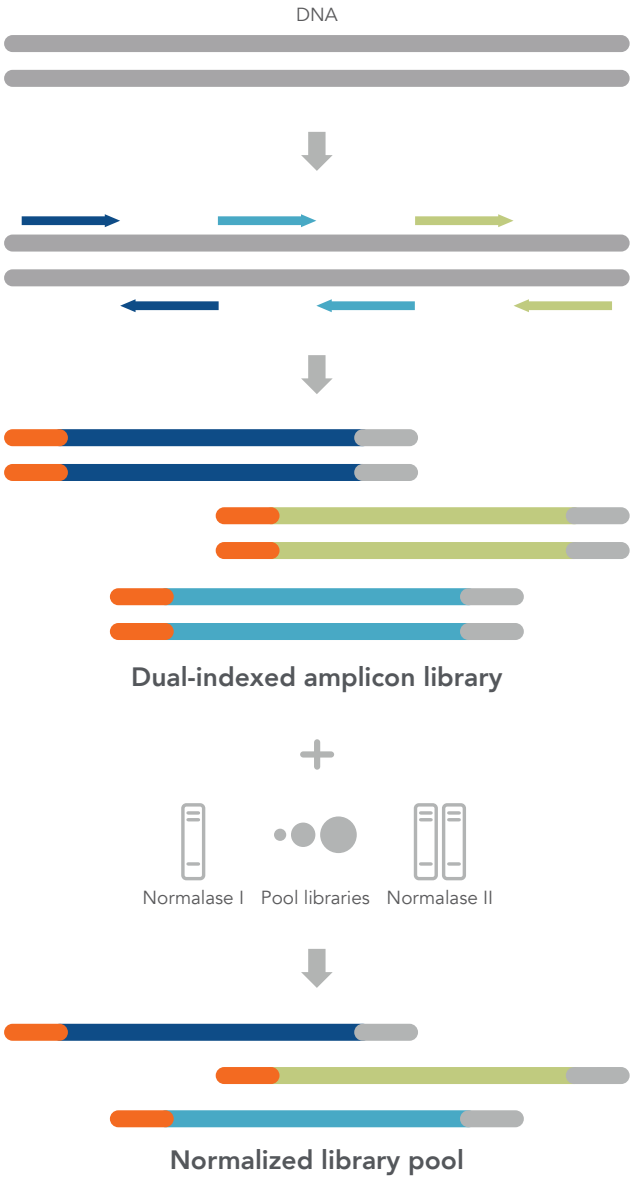

Figure 3. xGen Amplicon Panel workflow. A dual-index library is prepared from viral samples in three main steps: 1) multiplex PCR, 2) adapter attachment with indexing PCR, and 3) an optional Normalase step to produce equimolar library pools.

ORDERING INFORMATION

Product	Catalog #
xGen Amplicon Respiratory Virus Panel 96rxn	10017901
xGen Amplicon UDI Primers Plate 1	10009847
xGen Amplicon Core 96rxn	10009827

> FOR MORE INFORMATION, VISIT **WWW.IDTDNA.COM/NGS**