SWIFT NORMALASE™ AMPLICON PANEL (SNAP) SARS-CoV-2 ADDITIONAL GENOME COVERAGE

Whole viral genome NGS assay

Highlights

- **99.7% genomic coverage**
- **Up to 1536 UDIs**
- **cDNA-to-sequencer in 3 hours**
- **Obtain genomes from viral titers as low as 10–100 viral copies**

Introduction

The Swift Normalase Amplicon Panel (SNAP) SARS-CoV-2 offers a robust next generation sequencing (NGS) workflow that provides complete genome coverage and subgenomic RNA detection for Illumina® sequencing platforms. This newly improved kit leverages Swift's patented multiplex PCR technology, enabling library construction from cDNA using tiled primer pairs to target the entire 29.9 kb viral genome with a single pool of multiplexed primer pairs. Primers were designed against the NCBI Reference Sequence NC_045512.2 (severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome), and the improved panel demonstrates better coverage uniformity across the viral genome.

SNAP kits utilize multiple overlapping amplicons in a single tube, using a rapid, 2-hour workflow to prepare ready-to-sequence libraries. The PCR1+PCR2 workflow generates robust libraries, even from low viral load samples. The libraries may be quantified with conventional methods such as Thermofisher Qubit® fluorometer or Agilent Bioanalyzer™ machine and normalized by manual pooling or normalized enzymatically with the included Swift Normalase reagents.

SNAP Workflow

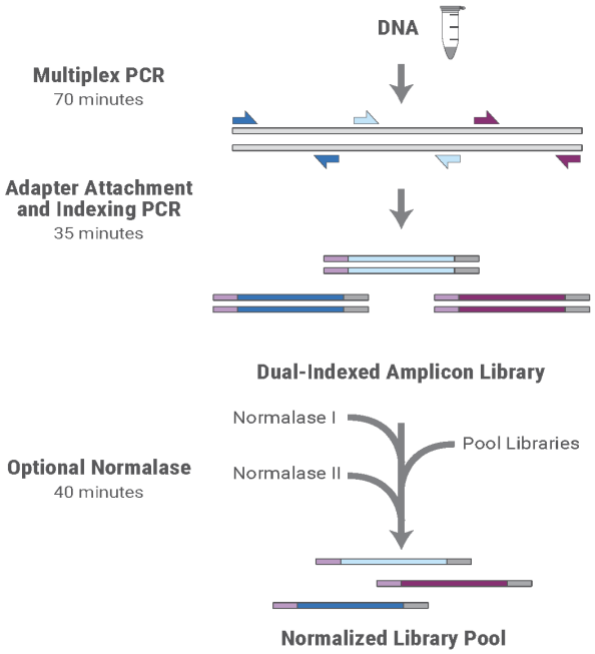


Figure 1. The one-tube workflow prepares normalized libraries from cDNA in 3 hours by replacing qPCR library quantification with Normalase (included in the kit).

Applications and sample types

Research applications: viral surveillance, water-based epidemiology, genomic epidemiology, and variant calling.

Potential sample types: nasopharyngeal/oropharyngeal swabs, sputum, bronchoalveolar lavage (BAL), and stool.

Achieve complete coverage of the SARS-CoV-2 genome

The SNAP SARS-CoV-2 Panel has improved genome coverage at the 5’ end to enable subgenomic RNA detection as well as improved genome coverage at the 3’ end of the genome. In addition, improved primer designs support complete overlapping coverage throughout to attain 99.7% coverage (Figure 2).

Figure 2. Complete coverage of the SARS-CoV-2 genome. Improved primer design shows complete overlapping coverage throughout the entire genome sequence using the new SARS-CoV-2 additional genome coverage panel compared to the original SARS-CoV-2 panel. Genome coverage is shown by blue bars (visualized in IGV (Broad Institute)) where the top blue bar represents the original panel with 98% coverage and the lower blue bar represents the new panel with 99.7% coverage. Please note the improvements in coverage indicated by purple arrows, where primer designs are otherwise consistent across both panels.

High performance over a wide range of viral copy number

In an interal investigation, 1 to 10 million viral genome copies (Figure 3) were sufficient to generate NGS libraries using the SNAP SARS-CoV-2 panel.

Mixed RNA samples were converted into first strand cDNA and used as input into the SNAP SARS-CoV-2 panel. Libraries were enzymatically normalized to 4 nM using the Normalase workflow provided in the SNAP protocol.

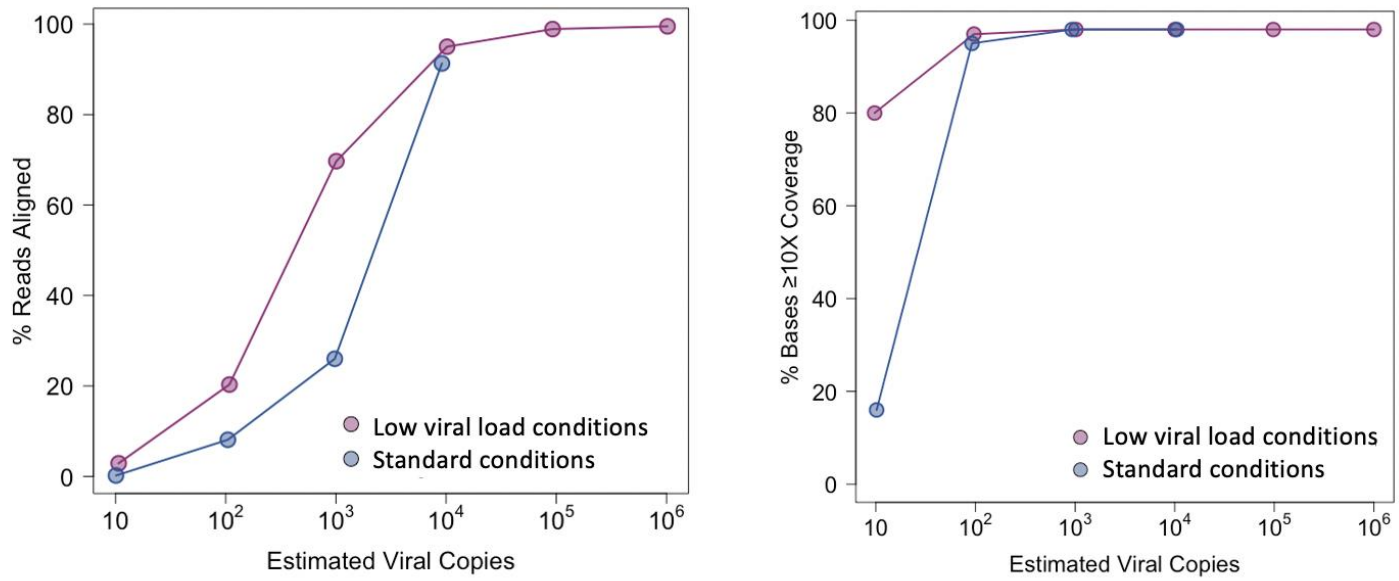


Figure 3. Obtain genomes from as few as 10–100 viral copies. SARS-CoV-2 synthetic template material (Twist Bioscience Cat. No. 102024) was mixed with UHR RNA (Agilent 740000) and converted into first-strand cDNA using the Superscript® IV First-Strand Synthesis System (Thermo Fisher 18091050). cDNA was converted into an NGS library with the Swift SNAP SARS-CoV-2 Kit and sequenced on the Illumina MiniSeq® system at 2 x 150 bp. Resulting data was downsampled to 280k reads per sample.

Complete coverage enables comprehensive analysis

The SNAP SARS-CoV-2 Kit uses overlapping primers to generate 345 amplicons, sized 116–255 bp (average 150 bp), along the length of the 29.9 kb viral genome and obtain 99.7% coverage of the genome (Figure 4). Overlapping primers ensure that variants are detected, even when the mutation is a deletion or a primer dropout occurs. Comprehensive mutation detection is crucial for researching nucleotide variants and improving understanding of virus evolution, transmission, and pathogenesis.

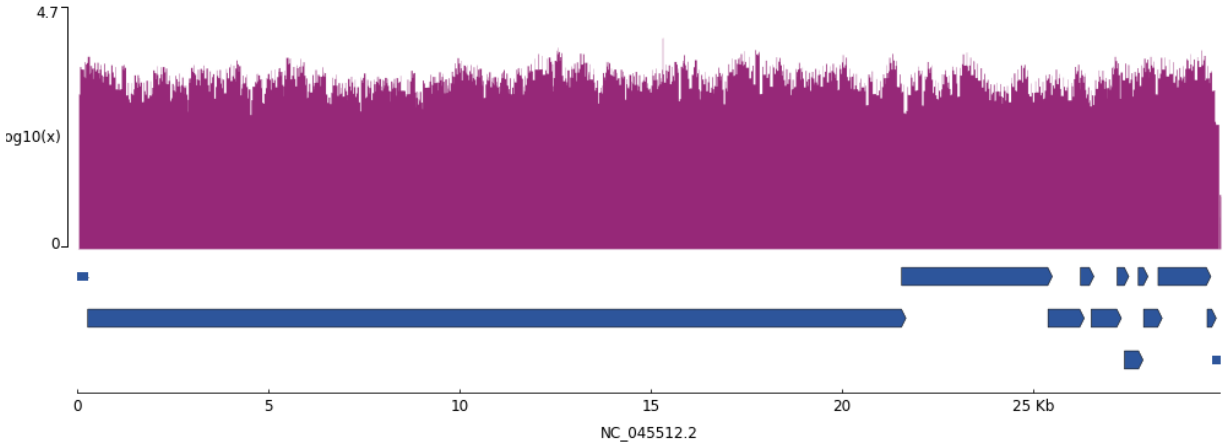
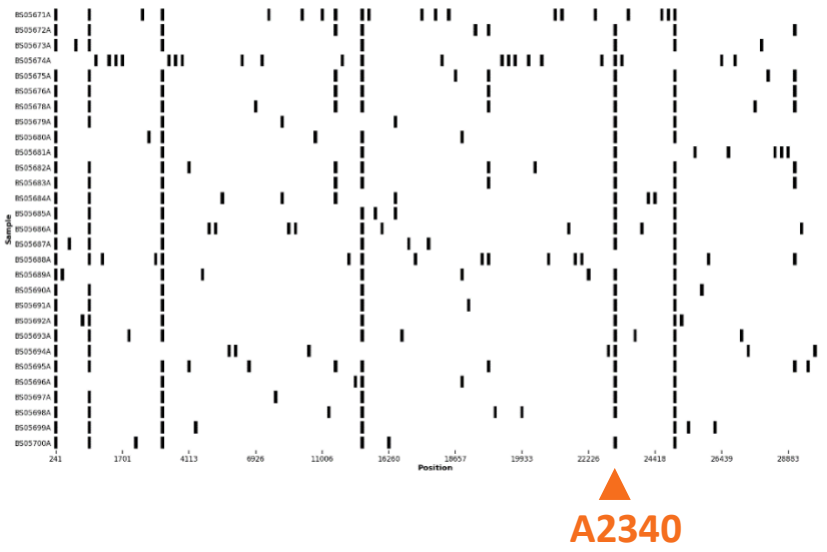


Figure 4. Contiguous coverage of the SARS-CoV-2 genome from position 25 to 29853. 100,000 copies of gamma-irradiated SARS-CoV-2 (BEI, NR-52287) that were originally isolated from an oropharyngeal swab were converted into an NGS library with the Swift SNAP SARS-CoV-2 kit and sequenced 2 x 150 bp on an Illumina MiniSeq system. resulting reads were downsampled to 280k reads per sample for analysis.

Comprehensive mutation profiles from challenging specimens

Department of Pathology investigators at NYU Grossman School of Medicine use Swift's SNAP SARS-CoV-2 Kit to identify mutation profiles from specimens with qRT-PCR C_t values ranging from 16 to 42. Following presence/absence assessment using qRT-PCR, excess cDNA was used as input into the Swift SNAP workflow to establish an NGS-based mutation profile for public health surveillance. In the data shown, a total of 29 nasopharyngeal swab specimens were processed with the Swift SNAP SARS-CoV-2 Kit by NYU Langone Health and sequenced on an Illumina MiSeq system to 50,000 reads per sample (Figure 5). The location of the A23403G/D614G mutation, a key variant of interest, is highlighted and was detected in 26 of the 29 sequenced libraries.



"Swift has been a valued research partner, and we *look forward to working with them to continually improve the ability of amplicon-based methods to achieve greater coverage in fewer reads, which would enable us to achieve good genome coverage for low viral load samples."*

– Adriana Heguy, PhD, Professor of Pathology at NYU Langone Health, NYU Grossman School of Medicine.

Figure 5. Detect variants. Sequencing data was aligned to the NC_045512.2 reference using BWA, and variants were called using GATK Haplotype Caller. Variants with allele fractions ≥ 0.5 are shown.

Specifications

Features	Specifications
Design coverage	99.7% (29,828 of 29,903 total bases)
Panel information	345 amplicons, sized 116–255 bp (average 150 bp)
Input material	1st or 2nd strand cDNA Minimum 10–100+ viral copies (qRT-PCR Ct value 30–40)
Time	2 hours: cDNA to library 3 hours: cDNA to normalized library pool
Components	Included in the kit: • Target-specific multiplex primer pool • PCR and library prep reagents • Swift Normalase Sold separately: • Combinatorial Dual Indexes (CDIs) • Unique Dual Indexes (UDIs) Not included: RT module or magnetic beads
Multiplexing capability	Up to 384 CDIs Up to 1536 UDIs
Compatible with other indexes	Yes
Recommended depth	Variant calling: 250–500k reads per library

Ordering information

Workflow component	Product name	Catalog number
Primer pools	SARS-CoV-2 Additional Genome Coverage Panel (96 rxns)	COVG1V2-96
SNAP Core	Swift Normalase Amplicon Panels (SNAP) Core Kit (96 rxns)	SN-5X296
	Swift Normalase Amplicon Panels (SNAP) Core Kit (4x96 rxns Bundle)	SN-5X384
CDI primers	SNAP Set S1AB-S2AB Combinatorial Dual Indexing Primers (384-plex, 4x96 rxns Bundle)	SN-5S0384
	SNAP Set 1A Combinatorial Dual Indexing Primers (96-plex, 96 rxns)	SN-5S1A96
	SNAP Set 1B Combinatorial Dual Indexing Primers (96-plex, 96 rxns)	SN-5S1B96
	SNAP Set 2A Combinatorial Dual Indexing Primers (96-plex, 96 rxns)	SN-5S2A96
	SNAP Set 2B Combinatorial Dual Indexing Primers (96-plex, 96 rxns)	SN-5S2B96
UDI primers	SNAP Unique Dual Indexing Primer Plate (384-plex, 4x96 rxns Bundle, SU001-SU384)	SN91384-PLATES
	SNAP Unique Dual Indexing Primer Plate (96-plex, 96 rxns SU001-SU096)	SN91096-1-PLATE
	SNAP Unique Dual Indexing Primer Plate (96-plex, 96 rxns SU097-SU192)	SN91096-2-PLATE
	SNAP Unique Dual Indexing Primer Plate (96-plex, 96 rxns SU193-SU288)	SN91096-3-PLATE
	SNAP Unique Dual Indexing Primer Plate (96-plex, 96 rxns SU289-SU384)	SN91096-4-PLATE
	SNAP Unique Dual Indexing Primer Plate (384-plex, 4x96 rxns Bundle, SU385-SU768)	SN91384-B-PLATES
	SNAP Unique Dual Indexing Primer Plate (384-plex, 4x96 rxns Bundle, SU769-SU1152)	SN91384-C-PLATES
	SNAP Unique Dual Indexing Primer Plate (384-plex, 4x96 rxns Bundle, SU1153-SU1536)	SN91384-D-PLATES

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