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

Whole viral genome NGS assay

Highlights

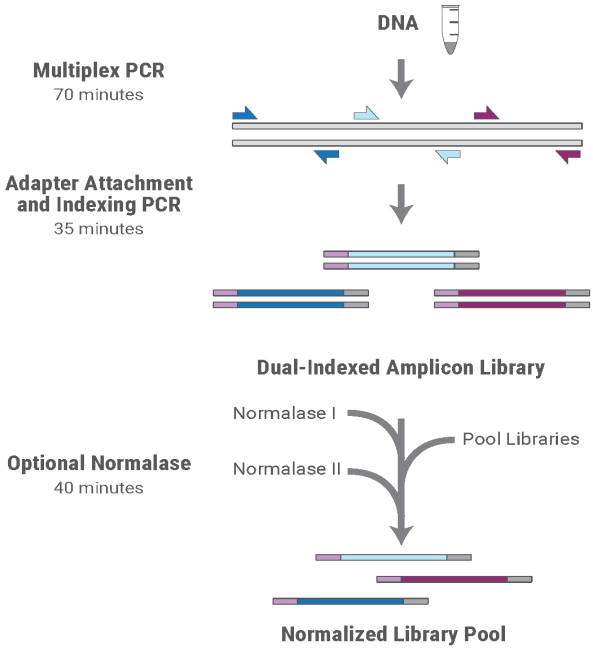
- **Increased genome coverage to 99.7%**
- **Improved coverage uniformity**
- **Detect subgenomic RNAs**
- **Improved performance from low viral load samples**

Introduction

The Swift Normalase Amplicon Panel (SNAP) SARS-CoV-2 offers a robust 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 also 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 Qubit® or Agilent Bioanalyzer and normalized by manual pooling or normalized enzymatically with the included Swift Normalase reagents.

1-Tube, 2-Hour Workflow



Applications and Sample Types

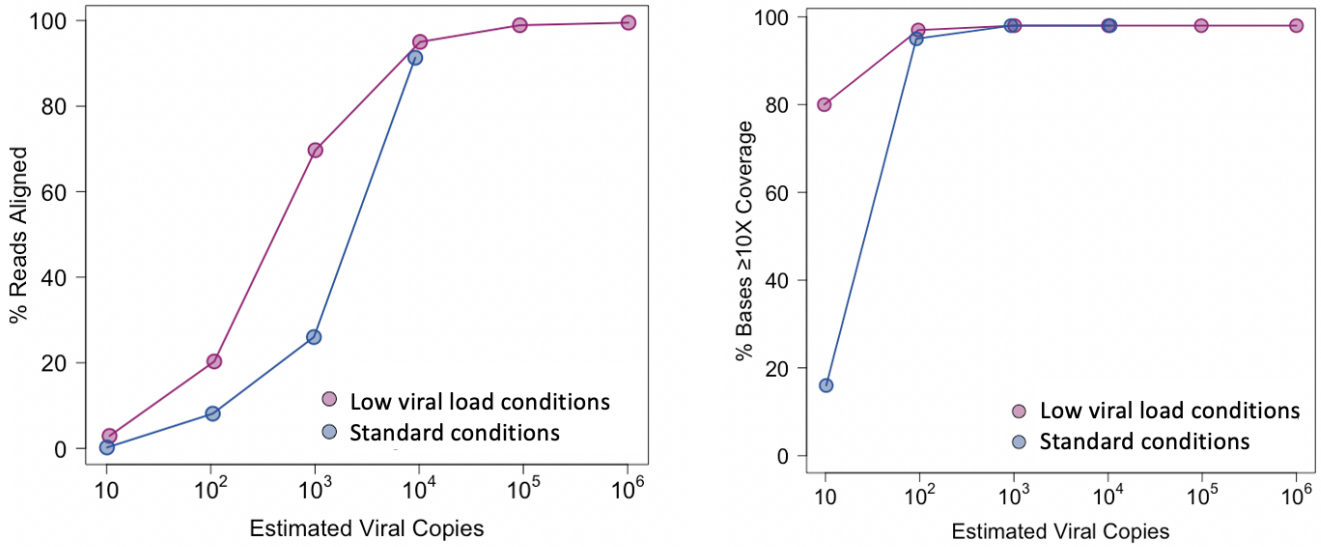
- Applications: Detection, Variant Calling, Screening, Epidemiological Studies, Public Health Surveillance
- Sample Types (not limited to): nasopharyngeal/oropharyngeal swabs, sputa, bronchoalveolar lavage (BAL), stool

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 complete overlapping coverage throughout to attain 99.7% coverage. 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

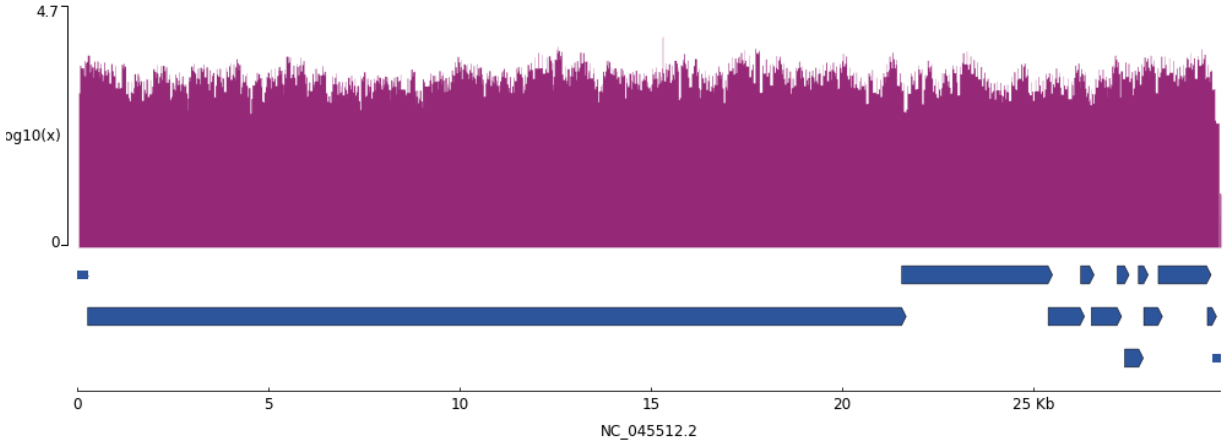
The new SNAP SARS-CoV-2 panel was tested with inputs ranging from 10 to 1 million viral genome copies mixed with 50 ng of Universal Human Reference (UHR) RNA to simulate host background. Mixed RNA samples were converted into first strand cDNA and used as input into the SNAP SARS-CoV-2 workflow, using both the standard thermocycling conditions and the low viral load thermocycling conditions. Libraries were enzymatically normalized to 4nM using the Normalase workflow provided in the SNAP protocol prior to sequencing.



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 processed with the Swift SNAP SARS-CoV-2 Kit, sequenced with Illumina® MiniSeq® 2 x 150 bp chemistry and down sampled to 1 million reads per sample. These plots illustrate the percentage of reads aligned to the NC_045512.2 reference (left) and the percentage of SARS-CoV-2 genomic bases covered at >10X depth (right) vs. viral copy number. As shown by comparison of the low viral load conditions to the standard conditions (purple vs. blue), improved performance (% alignment and % bases ≥10X coverage) was observed with adjusted cycling conditions for low viral load samples. See protocol for further details.

Complete Coverage Enables Comprehensive Analysis

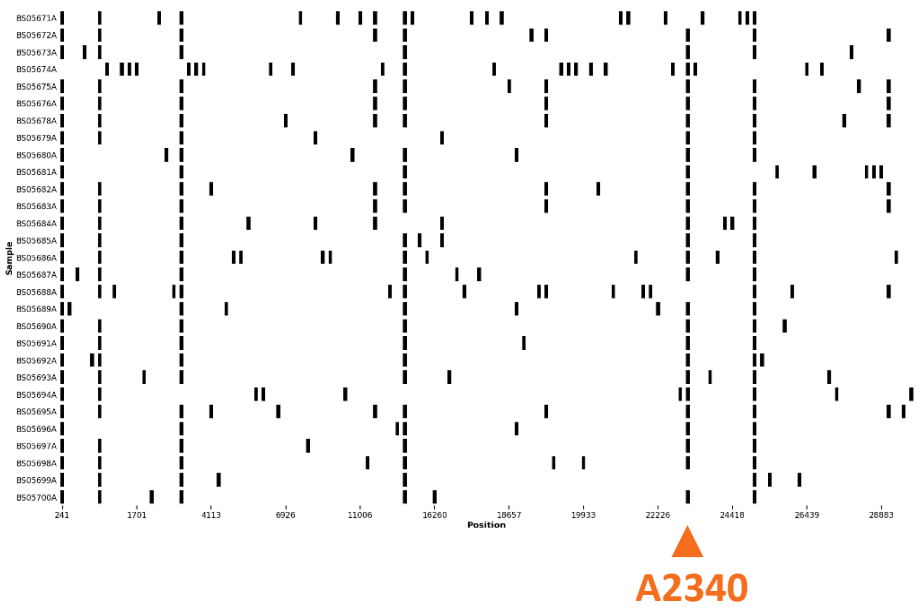
The SNAP SARS-CoV-2 Kit uses a single-tube multiplex primer pool to generate amplicons along the length of the 29.9 kb viral genome. Sequencing the full-length SARS-CoV-2 genome is important as it enables detection of known key variants of interest as well as discovery of novel mutations. Confident mutation detection is crucial for tracking nucleotide variants and improving our understanding of virus evolution, transmission, and pathogenesis.



Genome coverage from 100,000 equivalents of gamma-irradiated SARS-CoV-2 infected cell lysate from BEI (NR-52287). Processed with Swift SNAP SARS-CoV-2 kit and sequenced on Illumina MiniSeq demonstrates contiguous coverage across 99.7% of the sarscov2 genome, from position 25 to 29853.

Establish Comprehensive Mutation Profiles from Challenging Specimens

Department of Pathology investigators at NYU Grossman School of Medicine are using Swift's SNAP SARS-CoV-2 Kit to identify mutation profiles from specimens with qRT-PCR Ct 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.



"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

A total of 29 nasopharyngeal swab specimens were processed with the Swift SNAP SARS-CoV-2 Kit by NYU Langone Health and sequenced with Illumina® MiSeq® to 50,000 reads. Sequencing data was aligned to the NC_045512.2 reference using BWA and variants were called using GATK Haplotype Caller. Variants with allele fractions of 0.5 or greater are shown. The location of the A23403G/D614G mutation, a key variant of interest is highlighted and was detected in 26 of the 29 sequenced libraries.

Specifications

Feature	Specification
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 Provided	• Target-specific multiplex primer pool • PCR and library prep reagents • Swift Normalase • Combinatorial Dual Indexed Adapters Note: Kits does not include RT module or magnetic beads
Multiplexing Capability	Up to 384 CDI Inquire for custom indexing and UDIs
Recommended Depth	50K reads/library (+/-) detection; 1M reads per library (variant calling)

*Please inquire with your Swift sales representative or distributor to review the primer design file.

Ordering Information

Workflow Component	Product Name	Reactions	Catalog No.
Primer Pools	SNAP SARS-CoV-2 w/ Additional Genome Coverage	96	COVG1V2-96
SNAP Core	Swift Normalase Amplicon Core Kit	96, No indexing	SN-5X296
Indexing Primers*	SNAP Combinatorial Dual Index Primer Kit	96, Set 1A	SN-5S1A96
	SNAP Combinatorial Dual Index Primer Kit	96, Set 1B	SN-5S1B96
	SNAP Combinatorial Dual Index Primer Kit	96, Set 2A	SN-5S2A96
	SNAP Combinatorial Dual Index Primer Kit	96, Set 2B	SN-5S2B96

Please inquire for custom index primer compatibility (UDIs, etc.).

Un kit complet est composé de Primer pools + SNAP Core kit + Index au choix parmi la liste

Nous contacter

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Instrumentation : 01 30 85 92 88 - instrum@ozyme.fr