SWIFT PROTOCOL

Swift Normalase® Amplicon Panels (SNAP)

SARS-CoV-2, SARS-CoV-2 Additional Genome Coverage, and SARS-CoV-2 S Gene Panel

Whole viral genome targeted NGS assay

Single-tube Multiplex PCR NGS Library Prep with High-throughput Workflow

Protocol for Cat. Nos.:
COVG1-96, SARS-CoV-2 Panel (primers only, 96 rxns)
COVG1V2-96, SARS-CoV-2 Additional Genome Coverage Panel (primers only, 96 rxns)
SGENE-96, SARS-CoV-2 S Gene Panel (primers only, 96 rxns)

DNA Library Core Kits
Swift Normalase® Amplicon Panel (SNAP) Core Kit (96 rxns)
Swift Normalase® Amplicon Panel (SNAP) Core Kit (4x96 rxns Bundle)

SNAP Indexing Primers
SNAP Set 1A to Set 2B Combinatorial Dual Indexing Primers (96-plex to 384-plex)
SNAP Unique Dual Indexing Primer Plates (96-plex to 384-plex)

See Appendix Section H for details on Product Ordering Information.

Visit swiftbiosci.com/protocols for updates

Swift BIOSCIENCES™

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Support

For additional support please contact Swift at TechSupport@swiftbio.com, or by phone: 734.330.2568 (9:00 am – 5:00 pm ET, Monday through Friday).

Product Information

The SNAP SARS-CoV-2 Kits offer a robust NGS workflow that provides optimal coverage and NGS data quality on Illumina® sequencing platforms. These kits leverage patented multiplex PCR technology, enabling library construction from first- or second-strand cDNA using tiled primer pairs to target the 29.9 kb viral genome or S gene alone 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).

Features	Specifications
Design Coverage and Panel Information	**SARS-CoV-2 Panel** 98.0% (29,313 of 29,903 total bases) 341 amplicons, sized 116-255 bp (average 150 bp)
	SARS-CoV-2 Additional Genome Coverage Panel 99.7% (29,828 of 29,903 total bases) 345 amplicons, sized 116-255 bp (average 150 bp)
	SARS-CoV-2 S gene Panel 100% of S gene (100% of S gene bases) 43 amplicons, sized 140-150 bp (average 146 bp)
Input Material	1st or 2nd strand cDNA Minimum 10 – 100+ viral copies (RT-qPCR Ct value 30-40)
Time	2 hours cDNA-to-Library
	3 hours cDNA-to-Normalized-Library Pool
Components	Included: • Target-specific multiplex primer pool • PCR and library prep reagents Optional: • Combinatorial Dual Indexed Adapters • Swift Normalase Note: Kits does not include RT module or magnetic beads
Multiplexing Capability	Up to 384 CDIs or 384 UDIs – inquire for custom indexing
Recommended Depth	250-500k reads/library for SARS-CoV-2 and SARS-CoV-2 Additional Genome Coverage Panels 0.25M reads/library for SARS-CoV-2 S Gene Panel

*Please inquire with your Swift sales representative or distributor to obtain a copy of the primer design file.

Applications and Sample Types

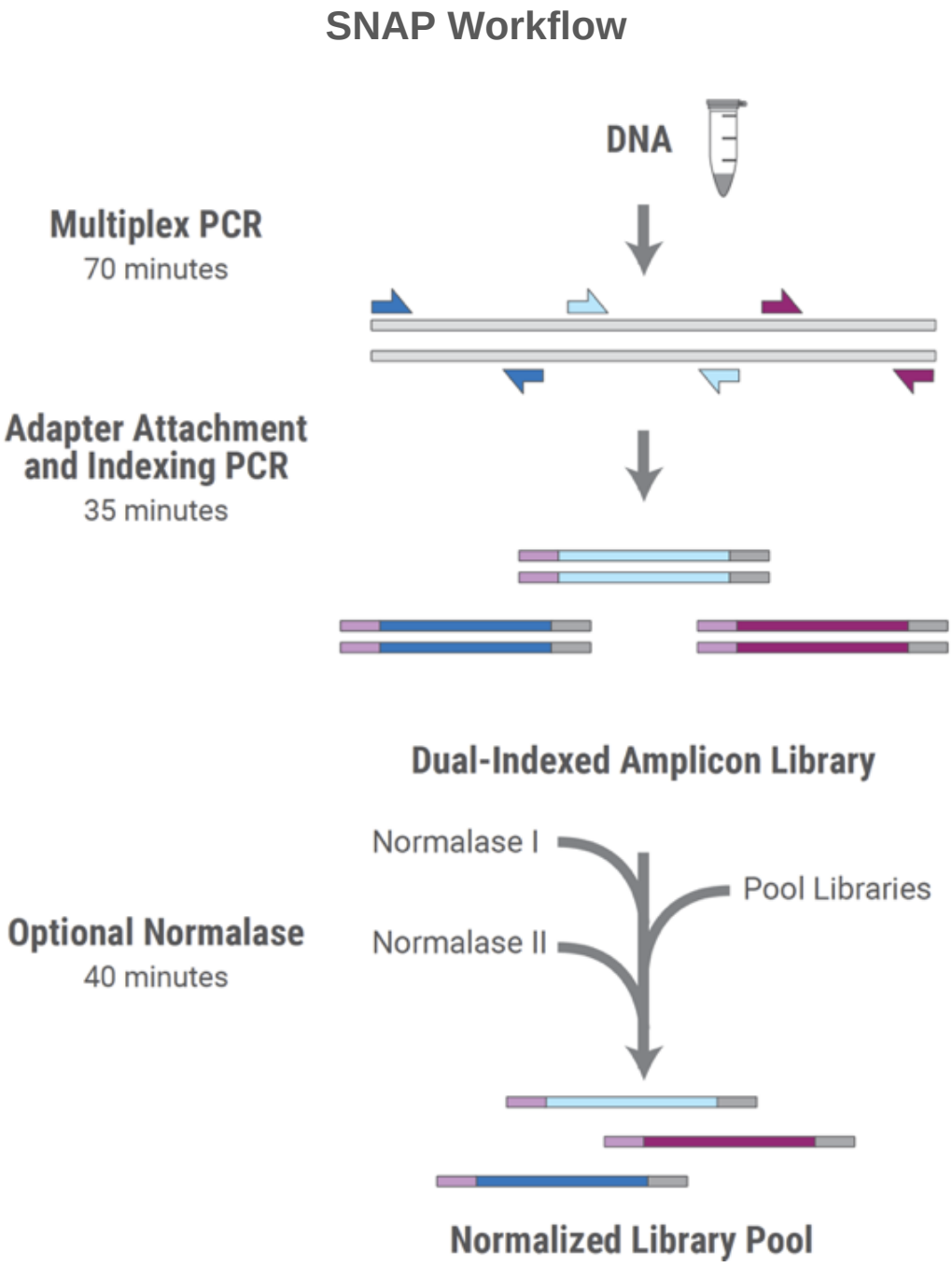
- **Applications:** Detection, Variant Calling, Screening, Epidemiological Studies, Public Health Surveillance, among others.

- **Sample Types**: Nasopharyngeal/Oropharyngeal Swabs, Sputa, Bronchoalveolar Lavage (BAL), Stool, Wastewater, among others.

Swift Normalase® Amplicon Panels (SNAP) Workflow

Swift Normalase Amplicon Panels (SNAP) 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 input quantities. 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.

This protocol includes instructions for a Multiplex PCR step to enrich target sequences, an Indexing PCR step to amplify and add combinatorial or unique dual indexed adapter sequences, and an optional downstream Normalase step to produce an equimolar library pool.



Kit Contents

The SNAP Kits contain sufficient reagents for the preparation of 96 libraries (10% excess volume provided).

Protocol Stage	Component	96 rxns	Storage
Multiplex PCR (Pre-PCR box)	Reagent G1*	211 µL	-20 °C***
	Reagent G2	317 µL	
	Enzyme G3	1,584 all	
	Pre-PCR TE	1,200 µL	
Indexing PCR (Post-PCR box)	Reagent I1	348 µL	
	Enzyme I2	53 µL	
	Enzyme I3	15 µL	
	Enzyme I4	2,640 µL	
	Post-PCR TE	4,161 µL	
Indexing Primer Box**	SNAP CDI D50X	26 µL each	
	SNAP CDI S7XX	15 µL each	
Indexing Primer Plates**	SNAP UDI (pre-mixed pairs)	12 µL per well	
Normalase (Post-PCR box)	Buffer S1	454 µL	
	Reagent S2	21 µL	
	Enzyme S3	53 µL	
	Buffer N1	506 µL	
	Enzyme N2	21 µL	
	Reagent X1	105 µL	
Additional Reagents	PEG NaCl	20 mL	Room Temp

*Reagent G1 is included in Cat. No. COVG1-96, COVG1V2-96, and SGENE-96. Additional Pre-PCR and Post-PCR box reagents and PEG NaCl are included in Cat. Nos. SN-5X296 and SN-5X384.
**Indexing boxes are Cat. Nos. SN-5S1A96, SN-5S1B96, SN-5S2A96 and SN-5S2B96. Indexing Primer Plates are Cat. Nos. SN91384-PLATES, SN91096-1-PLATE, SN91096-2-PLATE, SN91096-3-PLATE, and SN91096-4-PLATE.
***Temperature range -15° C to -25° C.

Storage and Usage Recommendations

- Upon receipt, store the SNAP Library Kit products at -20°C with the exception of the PEG solution, which is stored at room temperature.
- Separate the multiplex PCR Reagents (keep in pre-PCR area), Indexing and Normalase Reagents (keep in post-PCR area).
- To maximize use of enzyme reagents when ready to use, remove enzyme tubes from -20°C storage and place on ice for 10 minutes prior to pipetting. Attempting to pipette enzymes at -20°C may result in shortage of enzyme reagents.
- After thawing reagents on ice, briefly vortex (except enzymes) to mix well, then pulse spin to collect contents before proceeding. Enzyme G3 is the only enzyme that may be vortexed.
- Always add reagents to the master mix in the specified order as stated throughout the Protocol. The indexing primers (SNAP CD and UD Indexes) are the only reagents that are added individually to each sample.

Materials and Equipment Not Included

- Reverse Transcriptase (RT) Module – See below
- SPRIselect beads (Beckman Coulter, Cat. Nos. B23317/B23318/B23319) or Agencourt AMPure XP beads (Beckman Coulter, Cat. Nos. A63880/A63881/A63882)
- Permagen Magnetic Separator (Cat. No. MSR812), Agencourt SPRIPlate or similar magnetic rack for magnetic bead clean-ups
- Qubit®, Nanodrop, or other similar input RNA quantification assay
- qPCR-, electrophoretic-, or fluorometric-based library quantification assay for Illumina® libraries
- Microcentrifuge
- Vortex
- Programmable thermocycler
- 0.2 mL PCR tubes or 96-well plates
- Aerosol-resistant tips and pipettes ranging from 1-1,000 µL
- 200-proof/absolute ethanol and nuclease-free water (both molecular biology grade) for preparation of fresh 80% ethanol
- Pipette tips (i.e., 8-channel or 12-channel), 8-tube strips, an un-skirted 96 well plate or plate puncher for pre-piercing the foil seal if using the single use UD indexing plates.

Reverse Transcriptase and Starting Material Recommendations

The Swift SARS-CoV-2 amplicon panels support first- or second-strand cDNA as input. Select a cDNA synthesis module that supports two-step RT-PCR, includes random primers, and has a processivity of >1 kb.

The amplicon panels have been validated with synthetic SARS-CoV-2 genome (Twist Biosciences Cat. No. 102024). As few as 10-100 viral genome copies were mixed with 50 ng of Universal Human Reference RNA (Agilent 740000) and used as input into the Superscript® IV First-Strand Synthesis System (Thermo Fisher Cat. No. 18091050). The manufacturer's protocol was followed as written using the random primers and associated specifications, with a single modification of increasing the RT incubation step (50 ºC) from 10 min to a minimum of 30 min. Optional host gDNA/RNA removal steps were not performed. The optional RNase H step was not performed and 10 µL of the resulting cDNA was used directly as input into the amplicon panel.

Tips and Techniques

Assemble all reagent master mixes and reactions ON ICE and scale volumes as appropriate, using 5% excess volume to compensate for pipetting loss. Calculate the total volume of the master mixes and prepare them in advance to ensure the magnetic beads do not over-dry during size selection steps while awaiting completion of master mix assembly. Neglecting to store master mixes and reagents on ice prior to incubations reduces yields and performance of this product.

This workflow, like any amplicon enrichment technology, poses a risk of contamination of surfaces and other samples following the amplification step. Please use caution when opening your sample tubes following the Multiplex PCR step. To reduce the risk of RNA and library contamination, physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed. Follow the instructions below to avoid cross-contamination:

- Clean lab areas using 0.5% sodium hypochlorite (10% bleach).
- Use barrier pipette tips to avoid exposure to potential contaminants.
- Always change tips between each sample.

Prepare SNAP Libraries

Multiplex PCR Step

1. Load the Multiplex PCR program and allow the block to reach 98 °C before loading samples (**confirm lid heating is turned ON and is set to reach 105 °C**).

Pre-Program Thermocycler		
Multiplex PCR	98 °C, 30 sec	
	98 °C, 10 sec	4 cycles
	61 °C, 5 min	
	65 °C, 1 min	
	98 °C, 10 sec	18 cycles*
	64 °C, 1 min	
	65 °C, 1 min	
	4 °C, Hold	

* The PCR cycle number can be increased for samples that may give low yields. See the **Low Viral Low Input Recommendations** for adjusted thermocycling programs (Appendix, Section C page 14).

2. Gently nutate Enzyme G3 at Room Temperature for 5 minutes, or until all solutes appear to be in solution. Place back on ice for remainder of use.

3. Load 10 µL of cDNA sample directly into each PCR tube.

4. Keep all tubes on ice during assembly of the master mix and the reaction until placed in thermocycler.

Panel-Specific Multiplex PCR Reaction Mix

Before mixing, calculate the total volume of the master mix based on the number of reactions required with appropriate overage for pipetting. Vortex components G1 and G2 and pulse-spin tubes to collect contents. Make the Multiplex PCR Reaction Mix. Keep prepared master mix on ice until ready to use.

Reagents	Volume per Sample
• Reagent G1*	2 µL
• Reagent G2	3 µL
• Enzyme G3	15 µL
Reaction Mix	**20 µL**

* Reagent G1 is the panel-specific set of amplification primers.

5. Mix the master mix well and then add 20 µL of the Multiplex PCR Reaction Mix to each 10 µL input cDNA sample on ice. Mix well, then place in the thermocycler and run the program.

IMPORTANT: Move samples to post-PCR area before opening tubes.

6. Near the completion of the thermocycler run, prepare the Indexing Reaction Mix (below) in the post-PCR area. Assemble this reaction mix on ice and keep cold until adding it to samples in the Indexing Step. All components except indexes may be master-mixed when running multiple samples in parallel.

Indexing PCR Step

Before mixing, calculate the total volume of the master mix based on the number of reactions of choice, with appropriate overage for pipetting. We recommend preparing at least 10 reactions at any one time to maintain a volume of Enzyme I3 that can be accurately pipetted. Keep prepared master mix on ice.

Reagents	Volume per Sample
• Reagent I1	3.3 µL
• Enzyme I2	0.5 µL
• Enzyme I3	0.1 µL
• Enzyme I4	25 µL
Reaction Mix	**28.9 µL**

IMPORTANT: Keep prepared master mix ON ICE during Size Selection and Clean-Up Step 1.

Size Selection and Clean-Up Step 1

7. Ensure beads and samples are at room temperature. Briefly vortex beads to homogenize before use.

8. Add **30 µL (ratio: 1.0)** of magnetic beads to each 30 µL sample. Mix by vortexing. Pulse-spin the samples in a microfuge. Ensure no bead-sample suspension droplets are left on the sides of the tube.

9. Incubate the samples for 5 minutes at room temperature off the magnet.

10. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).

11. While leaving your sample on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 µL may be left behind). Leave tubes on the magnet.

12. Add 180 µL of freshly prepared 80% ethanol solution to the pellet while it is still on the magnet. Use care not to disturb the pellet. Incubate for 30 seconds, and then carefully remove the ethanol solution.

13. Repeat, for a second wash with the ethanol solution.

14. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube with a small volume tip.

15. Resuspend each bead pellet in 17.4 µL Post-PCR TE Buffer. Proceed to the Indexing PCR Step.

IMPORTANT: Continue working in the post-PCR area. Keep samples at room temperature. At no time should 'with bead' samples be stored on ice, as this affects binding to magnetic beads.

16. Load the Indexing PCR program and allow the block to reach 37 °C before loading samples (**confirm lid heating is turned ON and is set to reach 105 °C**).

Thermocycler Program		
Indexing PCR	37 °C, 20 min	
	98 °C, 30 sec	
	98 °C, 10 sec	9 cycles*
	60 °C, 30 sec	
	66 °C, 1 min	
	4 °C, Hold	

* The PCR cycle number can be increased for samples that may give low yields. See the **Low Viral Low Input Recommendations** for adjusted thermocycling programs (See Appendix, Section C page 14).

17. Add a unique combination of 2 µL SNAP CD Index D50X + 1.7 µL SNAP CD Index S7XX to each sample
    OR
    Add 3.7 µL of a pre-mixed SNAP UD Indexing primer pair to each sample if using the single use plates (See Appendix, Section F page 16 for UDI plate usage guidelines).

18. Add 28.9 µL of the cold Indexing PCR Reaction Mix to each sample and mix thoroughly (total volume 50 µL).

19. Place in the thermocycler and run the program.

Size Selection and Clean-Up Step 2

20. Ensure PEG NaCl solution is at room temperature. Briefly vortex the PEG NaCl solution to homogenize before use.

21. Add 32.5 µL (ratio: 0.65) of PEG NaCl solution to each 50 µL sample. Mix by vortexing. Ensure no bead-sample suspension droplets are left on the sides of the tube.

22. Incubate the samples for 5 minutes at room temperature off the magnet.

23. Pulse-spin the samples in a microfuge. Place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (≈ 5 minutes).

24. While leaving your sample on the magnet, remove and discard the supernatant without disturbing the pellet (approximately 5 µL may be left behind). Leave tubes on the magnet.

25. Add 180 µL of freshly prepared ethanol solution to the pellet while it is still on the magnet. Use care not to disturb the pellet. Incubate for 30 seconds and then carefully remove the ethanol solution.

26. Repeat, for a second wash with the ethanol solution.

27. Pulse-spin the samples in a microfuge, place back onto the magnet and remove any residual ethanol solution from the bottom of the tube with a small volume tip.

28. Proceed immediately to add 20 µL of Post-PCR TE buffer and resuspend the pellet, mixing well by pipetting up and down until homogenous. Incubate at room temperature for 2 minutes off the magnet. Then place the sample back on the magnet and transfer the clean 20 µL library eluate to a fresh tube. Ensure that eluate

does not contain magnetic beads (indicated by brown coloration in eluate). If magnetic beads are present, pipette eluate into a new tube, place on magnet, and transfer eluate again.

NOTE: Libraries are now completed and ready to sequence. Please proceed with quantifying and pooling libraries, using either conventional fluorometric (i.e., Qubit) or electrophoretic (i.e., Bioanalyzer) methods, or proceed to Normalase below (reagents are included).

Library Quantification

Accurate library quantification is essential to properly load the sequencing instrument. Libraries can be quantified using fluorometric-, electrophoretic-, or qPCR-based methods and normalized manually. Alternatively, libraries can be enzymatically normalized following the Normalase protocol below. Note, for optimal normalization using Normalase, a minimum of 12 nM yield is needed per sample. If library yields are below 12 nM, increase the number of PCR cycles to pass the 12 nM threshold or switch to the 6 nM threshold Normalase protocol described below.

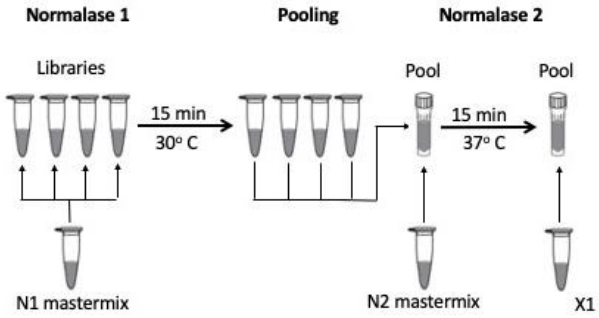
Introduction to Normalase

This guide provides instructions for optional enzymatic normalization of multiplexed SNAP next generation sequencing (NGS) libraries for equimolar pools and balanced sample representation in sequencing. The protocol is designed for SNAP libraries that produce consistent amplified library yields of ≥12 nM following Indexing PCR, and it generates an equimolar library pool. Most samples processed with SNAP protocol produce amplified library yields of 12 nM or greater; however, if there's concern that all samples won't reach 12 nM, adjusting Normalase chemistry to only require a minimum of 6 nM can alternatively be performed.

A simple calculator for converting between ng/µL and nM is available. For the SNAP SARS-CoV-2 Panels, please use a finished library size of 285bp (for use in the Base Pair Length column).

The workflow consists of three steps for libraries amplified to a minimum yield of 12 nM during the adapter attachment and Indexing PCR Step:

1. **Normalase I** to enzymatically select a 4 nM (or 2 nM if using ≥6 nM option) library fraction
2. **Library Pooling** of samples for multiplexed sequencing
3. **Normalase II** to enzymatically generate an equimolar library pool



Workflow schematic: *Normalase I Master Mix is added to samples and incubated at 30ºC for 15 minutes. Sample pooling is then performed and then the Normalase II Master Mix is added to the pool and incubated at 37ºC for 15 minutes. Reagent X1 inactivates the reaction and a final equimolar pool is produced.*

Notes Regarding Normalase Specification

The Normalase product specification is defined by cluster density of the Normalase pool when loaded on a MiSeq v2 flow cell at 12 nM to achieve a 1,000-1,200 K/mm^2 cluster density and CV ≤ 15% within a pool. Across Illumina platforms, library types, and insert sizes, the optimization of loading concentration may be required to achieve the optimal number of reads supported by the flow cell of choice.

Normalase I: Enzymatic Selection

If you are concerned that the 12 nM threshold has not been met after Indexing PCR:

- Spot check library yields using either fluorometric methods (i.e., Qubit) or electrophoretic methods (i.e., Bioanalyzer).
- A Normalase workflow modification can be performed that requires a 6 nM threshold to obtain a 2 nM Normalase pool (see below).

1. Pre-set a thermocycler program as listed below.

Thermocycler Program
15 min at 30 °C with open lid or lid heating OFF

2. Prepare the Normalase I Master Mix as listed in the table below. The mix can be prepared at room temperature and stored on ice until use if prepared in advance. Ensure that it is thoroughly mixed by moderate vortexing followed by a pulse spin to collect contents prior to use. For libraries with lower yields ≥ 6 nM, or for a final pool of 2 nM (instead of 4 nM), please use half of the specified volume of Reagent S2 and add an equal volume of TE, thus reducing concentration by two-fold, then proceed as written.

Reagent	Per Library	24 Libraries	96 Libraries
• Buffer S1	4.3 µL	103.2 µL	412.8 µL
• Reagent S2	0.2 µL	4.8 µL	19.2 µL
• Enzyme S3	0.5 µL	12 µL	48 µL
Total Volume	**5 µl**	**120 µl**	**480 µl**

IMPORTANT: The Normalase I Master Mix should be built for a minimum of 10 reactions to ensure pipetting accuracy.

3. Using a calibrated P10 pipette, add 5 µL of Normalase I Master Mix into each 20 µL library eluate at room temperature and thoroughly mix by moderate vortexing for 5 seconds.

4. Spin down the sample tube in a microfuge. Place in the thermocycler and run the program.

---- SAFE STOPPING POINT ----

Store freshly prepared libraries at -20 °C post-Normalase I.

Equal Volume Library Pooling

Sufficient Normalase II reagents are supplied so this step can be repeated to enable various re-pooling combinations as only 5 µL of post-Normalase I library (out of 25 µL volume) is used for pooling. Also note that stability of normalized pools (after Normalase II) is limited with a storage time of four weeks since the resulting normalized pools contain single-stranded DNA. Therefore, if re-sequencing is required after four weeks, for best results re-pool the Normalase I libraries and repeat Normalase II and inactivation.

NOTE: If you are pooling < 5 libraries, contact tech support at TechSupport@swiftbio.com for low-plex pooling recommendations.

NOTE: If pooling 5 µL per sample does not generate a normalized pool of sufficient volume for instrument loading, contact tech support at TechSupport@swiftbio.com for high sample volume pooling recommendations.

IMPORTANT: Consider your desired number of reads for each sample and only pool those samples together that have the same required depth. For example, samples each requiring 50,000 reads can be pooled together whereas samples requiring 1 million reads should be combined in a separate pool. Thus, you can adjust your ratio of pools when loading the instrument to achieve the desired sequence depth for each pool.

1. Following the Normalase I incubation, generate a library pool (or pools) by placing 5 µL of each individual library into a 0.2 mL PCR tube if pooling 30 libraries or less (achieves up to a final volume of 186 µL). Alternatively, use a 1.5 mL screw cap microfuge tube, particularly when pooling greater than 30 libraries as the volume will exceed the PCR tube maximum volume.

   To ensure even pooling, use of a calibrated P10 pipette will produce the best results.

2. Thoroughly mix, spin the library pools in a microfuge and proceed to the Normalase II reaction.

Normalase II: Enzymatic Normalization

1. Pre-set a thermocycler program as listed below. Alternatively, if using a 1.5 mL screw cap microfuge tube, set a heat block at 37 °C.

Thermocycler Program	Heat Block (1.5 mL screw cap microfuge tube)
15 min at 37 °C with open lid or lid heating OFF	15 min at 37 °C

IMPORTANT: Pre-mix Normalase II Master Mix (listed in the table below). The master mix can be stored on ice until use, and then added to pools at room temperature.

Reagents*	Per Library	24 Libraries	96 Libraries
• Buffer N1	0.96 µL	23.04 µL	92.16 µL
• Enzyme N2	0.04 µL	0.96 µL	3.84 µL
Total Volume	**1 µL**	**24 µL**	**96 µL**

*It is recommended to prepare Normalase II master mix for 24 samples even if you are processing less than 24 samples in order to avoid pipetting extremely low volumes; for best results use a calibrated P2 pipet for adding Enzyme N2. Although sufficient reagents are supplied for up to 5 repeated Normalase II reactions per sample, repeatedly processing a lower number of samples will result in significant loss of Normalase II reagents.

1. Add 1 µL of Normalase II Master Mix multiplied by the total number of libraries within each prepared pool.

2. Mix well by vortexing for 5 seconds and spin down the library pools in a microfuge.

3. Place the library pools in the thermocycler and run the program or place the 1.5 mL screw cap microfuge tubes into the 37 °C heat block.

Normalase Inactivation

1. Following the Normalase II reaction, pre-set a thermocycler program as listed below.

Thermocycler Program	Heat Block (1.5mL screw cap microfuge tube)
Hold at 95 °C 2 min at 95 °C with lid kept at 95 °C Hold at 4 °C	2 min at 95 °C

2. Add 0.2 µL of Reagent X1 multiplied by the total number of libraries within each prepared pool, see examples below:

Reagent	Per Library	24-Plex Pool	96-Plex Pool
• Reagent X1	0.2 µL	4.8 µL	19.2 µL

3. Place the library pools in the thermocycler and advance the program or place the 1.5 mL screw cap microfuge tubes into the heat block. If using a 1.5 mL screw cap microfuge tube, set a heat block at 95 °C to incubate your library pools, being careful not to incubate the samples longer than 2 minutes.
4. Your final multiplexed library pools are now equimolar. Proceed to qPCR quantification of your Normalase pool and sequencing. It is not necessary to perform an additional purification step.

Quantification and Calibration of Normalase Pools

To ensure optimal sequencing results, perform a qPCR quantification on your final Normalase pool(s). Final library pools are ssDNA and cannot be quantified by dsDNA-based fluorometric methods or fragment analysis. If you do not have a qPCR assay, validate a commercially available kit by calibrating your qPCR results and sequencer loading concentrations before proceeding (for example KAPA Library Quantification Kit, Cat. No. KK4828).

The Normalase 4 nM formulation may not conform to your qPCR assay quantification due to the lack of precision across different qPCR assays and laboratory practices. Using your validated qPCR assay that reproducibly predicts an optimal number of reads on your sequencing instrument, load your final pool based on your qPCR results. Across other Illumina platforms, library types, and insert sizes, optimization of loading concentration may be required to achieve the optimal number of reads supported by the flow cell of choice. If you have chosen the 6 nM to 2 nM option but require a higher pool concentration for your sequencer, perform a 2.0X SPRI to concentrate pool and then proceed to qPCR quantification and loading.

Appendix

Section A: Sequencing Recommendations

SNAP libraries may be sequenced using paired-end sequencing on Illumina instruments. We recommend using 2 x 150 paired-end reads. The depth of coverage required will depend on the application. Be sure to use either 8 (CDI) or 10 (UDI) sequencing cycles for each index read.

For the SARS-CoV-2 Panel, >50,000 reads per library are recommended for presence/absence (+/-) confirmation whereas 250-500k reads per library are recommended for variant calling. Accordingly, the following table shows examples of the number of libraries that can be multiplexed to achieve this depth per sequencing run:

		Libraries Per Sequencing Run					
		MiSeq®				MiniSeq®	
Panel	**Application**	**v2 Nano**	**v2 Micro**	**v2**	**v3**	Mid-Output	High-Output
SARS-CoV-2 Panels	+/- Confirmation	40	160	600*	1,000*	320	1,000*
	Variant Calling	2	8	30	50	16	50
S gene	Variant Calling	40	160	384*	384*	320	384*

* Note: custom indexing primers are required to multiplex >384 per run. Please inquire for compatibility or to order custom Normalase indexing primers.

Due to the complexity of the libraries, no PhiX spike-in is required on MiSeq or MiniSeq instruments. The NextSeq550 may be sensitive to low complexity and PhiX or another suitable high-complexity library spike-in may be required. Contact Illumina technical support for further information regarding sequencing instrument compatibility with low-complexity sequences.

Section B: Sequencing Data Analysis

Dockerized Data Analysis Workflow

For customers using the SNAP SARS-CoV-2 Panel Kits who are comfortable with command line tools and want a ready-to-use variant calling analysis workflow they can run on a local Linux machine, Swift offers a full variant calling workflow with all tools and reference files pre-installed and configured in a Docker image available for download.

Features

- One easy install: Docker image file can be downloaded and loaded onto a Linux server running the Docker daemon
- Easy-to-run analysis workflow includes a run script which handles all Docker commands, requiring only the input FASTQ files and the panel master file as arguments

Requirements

- Linux server with Docker installed
- At least 8GB of RAM (>= 32GB recommended)

Analysis Overview

1. Adapter trimming and filtering out of reads < 30 bases long (Trimmomatic)
2. Alignment (BWA MEM)

3. Primer trimming (Primerclip)
4. On-target and coverage metrics calculation and reporting
5. Variant calling (GATK Haplotype Caller)

Follow this Download Link for the analysis workflow: https://ws.onehub.com/folders/apetm9gs
For more detailed information about primer trimming, please review the Primerclip Technical Note at: https://swiftbiosci.com/wp-content/uploads/2019/11/TEC-005-PRIMERCLIP-A-TOOL-FOR-TRIMMING-PRIMER-SEQUENCES-USING-COMMAND-LINE-OR-GALAXY-Rev-1.pdf

Section C: Low Viral Load Input Recommendations

If your samples have a low viral load, please use the following adjustments to cycle number for both the Multiplex PCR and Indexing PCR thermocycling programs, as shown below in **blue underlined text**.

Multiplex PCR Step

1. Load the Multiplex PCR program and allow the block to reach 98 °C before loading samples (**confirm lid heating is turned ON and is set to reach 105 °C**).

Pre-Program Thermocycler		
Multiplex PCR	98 °C, 30 sec	
	98 °C, 10 sec	**4 cycles**
	61 °C, 5 min	
	65 °C, 1 min	
	98 °C, 10 sec	**24 cycles**
	64 °C, 1 min	
	65 °C, 1 min	
	4 °C, Hold	

Then proceed as written.

Indexing PCR Step

16. Load the Indexing PCR program and allow the block to reach 37 °C before loading samples (**confirm lid heating is turned ON and is set to reach 105 °C**).

Thermocycler Program		
Indexing PCR	37 °C, 20 min	
	98 °C, 30 sec	
	98 °C, 10 sec	**5 cycles**
	60 °C, 30 sec	
	66 °C, 1 min	
	4 °C, Hold	

Then proceed as written.

Section D: Indexing Primer Sequences

The full-length indexing primer sequences are below, where the underlined text indicates the location of the index sequences which are 8b for CDI and 10b for UDI. These sequences represent the adapter sequences following completion of the **Indexing PCR** step.

Index 1 (i7) Adapter:
5' – GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXX(XX)ATCTCGTATGCCGTCTTCTGCTTG – 3'

Index 2 (i5) Adapter:
5' – AATGATACGGCGACCACCGAGATCTACACYYYYYYYY(YY)ACACTCTTTCCCTACACGACGCTCTTCCGATCT – 3'

Please refer to the accompanying **Swift Index Master List** available at https://swiftbiosci.com/protocols/ for index sequences for preparing your Illumina sequencing sample sheet on your instrument of choice. Please contact TechSupport@swiftbio.com if you would like assistance confirming compatibility of your own primers with the SNAP workflow, or your local sales representative or distributor to inquire about the purchase of custom Swift Normalase Indexing Primers that use your own index sequences.

Section E: Swift 384 UDI Plate Specification and Dimensions

This product is dispensed in a 96-well plate. Physical specifications are below:

Plate Dimension	**Low-Profile 96-Well Skirted Plates**
Length at base plane	127.76 mm
Width at base plane	85.48 mm
Height overall	16.06 mm
Well depth	14.81 mm
Well diameter at opening	5.46 mm
Well diameter at bottom of conical section	2.64 mm
Well volume	200 µL
Well spacing	9.00 mm
Well angle	17.5°
Well offset	
Left edge to well A1	14.38 mm
Top edge to well A1	11.24 mm
Left edge to H12	113.38 mm
Top edge to H12	74.24 mm

Section F: Plate Usage Guidelines

Prior to piercing the foil and pipetting out the necessary indexes, please be sure to thaw the plate at room temperature, vortex briefly and centrifuge for one minute to spin down the primer reagents to the bottom of the plate wells.

Carefully pre-pierce the foil seal for the intended well(s) prior to pipetting the primer mix out of the plate to add to your reaction(s). Pre-piercing the foil avoids accidental clogging of pipette tips used for liquid pipetting as well as the introduction of foil into the reaction. In addition, pre-piercing the foil reduces the resistance of multi-channel pipettors which can result in undesired movement of the plate that may cause cross-contamination of reagents. The foil may

be pre-pierced with pipette tips (i.e., 8-channel or 12-channel), 8-tube strips, an un-skirted 96 well plate or plate puncher.

During the **Indexing PCR** step, use 3.7 μL of a unique indexing primer pair (SU001-SU384 UDIs) to amplify and index each library, where the UDI primer pair must be added individually to each sample.

Libraries made with uniquely indexed adapters may be pooled prior to cluster generation, subjected to Normalase chemistry, and co-sequenced on the same Illumina flow cell.

Section G: Plate Layout

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU001	SU009	SU017	SU025	SU033	SU041	SU049	SU057	SU065	SU073	SU081	SU089
B	SU002	SU010	SU018	SU026	SU034	SU042	SU050	SU058	SU066	SU074	SU082	SU090
C	SU003	SU011	SU019	SU027	SU035	SU043	SU051	SU059	SU067	SU075	SU083	SU091
D	SU004	SU012	SU020	SU028	SU036	SU044	SU052	SU060	SU068	SU076	SU084	SU092
E	SU005	SU013	SU021	SU029	SU037	SU045	SU053	SU061	SU069	SU077	SU085	SU093
F	SU006	SU014	SU022	SU030	SU038	SU046	SU054	SU062	SU070	SU078	SU086	SU094
G	SU007	SU015	SU023	SU031	SU039	SU047	SU055	SU063	SU071	SU079	SU087	SU095
H	SU008	SU016	SU024	SU032	SU040	SU048	SU056	SU064	SU072	SU080	SU088	SU096

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU097	SU105	SU113	SU121	SU129	SU137	SU145	SU153	SU161	SU169	SU177	SU185
B	SU098	SU106	SU114	SU122	SU130	SU138	SU146	SU154	SU162	SU170	SU178	SU186
C	SU099	SU107	SU115	SU123	SU131	SU139	SU147	SU155	SU163	SU171	SU179	SU187
D	SU100	SU108	SU116	SU124	SU132	SU140	SU148	SU156	SU164	SU172	SU180	SU188
E	SU101	SU109	SU117	SU125	SU133	SU141	SU149	SU157	SU165	SU173	SU181	SU189
F	SU102	SU110	SU118	SU126	SU134	SU142	SU150	SU158	SU166	SU174	SU182	SU190
G	SU103	SU111	SU119	SU127	SU135	SU143	SU151	SU159	SU167	SU175	SU183	SU191
H	SU104	SU112	SU120	SU128	SU136	SU144	SU152	SU160	SU168	SU176	SU184	SU192

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU193	SU201	SU209	SU217	SU225	SU233	SU241	SU249	SU257	SU265	SU273	SU281
B	SU194	SU202	SU210	SU218	SU226	SU234	SU242	SU250	SU258	SU266	SU274	SU282
C	SU195	SU203	SU211	SU219	SU227	SU235	SU243	SU251	SU259	SU267	SU275	SU283
D	SU196	SU204	SU212	SU220	SU228	SU236	SU244	SU252	SU260	SU268	SU276	SU284
E	SU197	SU205	SU213	SU221	SU229	SU237	SU245	SU253	SU261	SU269	SU277	SU285
F	SU198	SU206	SU214	SU222	SU230	SU238	SU246	SU254	SU262	SU270	SU278	SU286
G	SU199	SU207	SU215	SU223	SU231	SU239	SU247	SU255	SU263	SU271	SU279	SU287
H	SU200	SU208	SU216	SU224	SU232	SU240	SU248	SU256	SU264	SU272	SU280	SU288

Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	SU289	SU297	SU305	SU313	SU321	SU329	SU337	SU345	SU353	SU361	SU369	SU377
B	SU290	SU298	SU306	SU314	SU322	SU330	SU338	SU346	SU354	SU362	SU370	SU378
C	SU291	SU299	SU307	SU315	SU323	SU331	SU339	SU347	SU355	SU363	SU371	SU379
D	SU292	SU300	SU308	SU316	SU324	SU332	SU340	SU348	SU356	SU364	SU372	SU380
E	SU293	SU301	SU309	SU317	SU325	SU333	SU341	SU349	SU357	SU365	SU373	SU381
F	SU294	SU302	SU310	SU318	SU326	SU334	SU342	SU350	SU358	SU366	SU374	SU382
G	SU295	SU303	SU311	SU319	SU327	SU335	SU343	SU351	SU359	SU367	SU375	SU383
H	SU296	SU304	SU312	SU320	SU328	SU336	SU344	SU352	SU360	SU368	SU376	SU384

Section H: Product Ordering Information

Workflow Component	Product Name	Catalog Number
SNAP Core Kits	Swift Normalase® Amplicon Panels (SNAP) Core Kit (96 rxns, no indexing)	SN-5X296
	Swift Normalase® Amplicon Panels (SNAP) Core Kit (4x96 rxns Bundle, no indexing)	SN-5X384
SNAP Panel (Primer Specific)	SARS-CoV-2 Panel (primers only, 96 rxns)	COVG1-96
	SARS-CoV-2 Additional Genome Coverage Panel (primers only, 96 rxns)	COVG1-96
	SARS-CoV-2 S Gene Panel (primers only, 96 rxns)	SGENE-96
SNAP CDI Primers	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
	SNAP Set S1AB-S2AB Combinatorial Dual Indexing Primers (384-plex, 4x96 rxns Bundle)	SN-5S0384
SNAP UDI Primer Plates	SNAP Unique Dual Indexing Primer Plates (384-plex, 4x96 rxns Bundle)	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

Support

For additional support please contact Swift at TechSupport@swiftbio.com, or by phone: 734.330.2568 (9:00 am – 5:00 pm ET, Monday through Friday).

Revision History

Document #	Revision	Date	Description of Change
PRT-028	Version 1	5/20/2020	Initial release.
PRT-028	Version 2	7/10/2020	Amended Kit Contents, Introduction to Normalase, Calculator and Indexing tables.
PRT-028	Version 3	8/24/2020	Added panel COVG1V2-96 Cat No. and New Product Information.
PRT-028	Version 4	8/31/2020	Amended multiplex PCR annealing temperature and cleanup step 2 bead ratio; Added PCR cycle number option for low viral loads.
PRT-028	Version 5	10/28/2020	Addition of Swift 384 UDIs and new index information strategy
PRT-028	Version 6	01/18/2021	Addition of SARS-CoV-2 S Gene Panel and reformatting.
PRT-028	Version 7	02/10/2021	Corrected minor spelling errors.
PRT-028	Version 8	04/14/2021	Updated recommendations to sequencing depth.

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