SWIFT PROTOCOL

Swift Normalase® Amplicon Panel (SNAP) 16S Panel v2 ITS1 Panel

High complexity NGS microbial enrichment assays

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

Protocol for Cat. Nos.:

516G1-96, 16S Panel v2 (96 rxns)
517G1-96, ITS1 Panel (96 rxns)
SN-5X296, Swift Normalase Amplicon Protocol SNAP Core (96 rxns)
SN-5S1A96, SNAP Combinatorial Dual Index Primer Kit (Set 1A, 96 rxns)
SN-5S1B96, SNAP Combinatorial Dual Index Primer Kit (Set 1B, 96 rxns)
SN-5S2A96, SNAP Combinatorial Dual Index Primer Kit (Set 2A, 96 rxns)
SN-5S2B96, SNAP Combinatorial Dual Index Primer Kit (Set 2B, 96 rxns)

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Table of Contents

Support

If you experience problems using this product, 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 Swift Normalase Amplicon Panels (SNAP) for 16S and ITS1 offer a robust NGS workflow that provides optimal coverage and NGS data quality on Illumina® sequencing platforms. This kit leverages Swift's multiplex PCR technology, enabling library construction from DNA using tiled primer pairs to target either (a) all V1-V9 variable regions or (b) ITS1, each with a single pool of multiplexed primer pairs.

Feature	SNAP 16S Panel or ITS1 Panel
Panel Information	17 primers (16S) or 15 primers (ITS1); Average 425 bp amplicon size
Input Material	1 ng bacterial or fungal template DNA
Time	2 hours cDNA-to-Library or 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 do not include magnetic beads
Multiplexing Capability	Up to 384 CDI • Inquire for custom indexing and UDIs
Recommended Depth	>100K reads per library

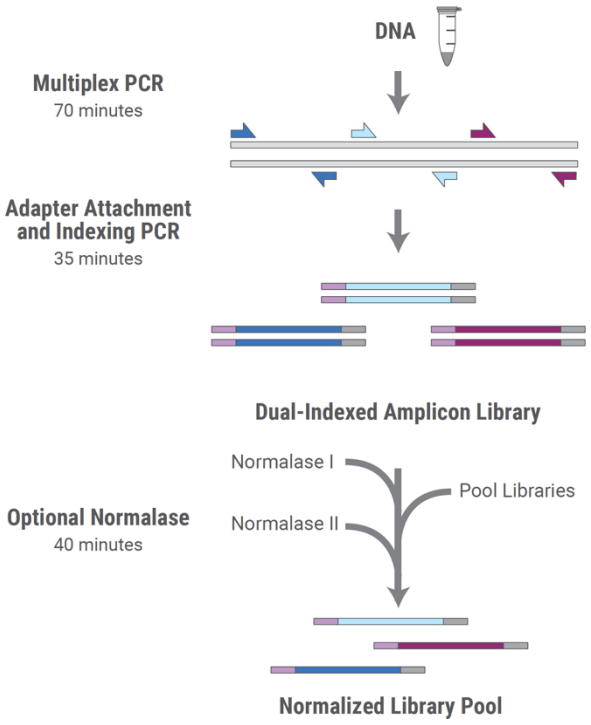
Supported Applications

Metagenomics, Microbiome, Environmental studies, Agricultural and soil microbial health studies, detection and characterization of bacterial and fungal structure in complex samples, custom targets including functional genes like ARGs/AMRs (please inquire).

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 dual indexed adapter sequences, and an optional downstream Normalase step to produce an equal molar library pool.



Kit Contents

These kits contain enough 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	-15 °C to -25 °C
	Reagent G2	317 µl	
	Enzyme G3	1584 µl	
	Pre-PCR TE	1200 µl	
Indexing PCR (Post-PCR box)	Reagent I1	348 µl	
	Enzyme I2	53 µl	
	Enzyme I3	15 µl	
	Enzyme I4	2640 µl	
	Post-PCR TE	4161 µl	
Indexing Box**	SNAP Index D50X	26 µl each	
	SNAP Index S7XX	15 µl each	
Normalase (Post-PCR box)	Buffer S1	454 µl	
	Reagent S2	21 µl	
	Enzyme S3	53 µl	
	Buffer N1	101 µl	
	Enzyme N2	10 µl	
	Reagent X1	21 µl	
Additional reagents	PEG NaCl	20 mL	Room Temp

*Reagent G1 is included in Cat. Nos. 516G1-96 and 517G1-96. Additional Pre-PCR and Post-PCR box reagents and PEG NaCl are included in Cat. No. SN-5X296.

**Indexing boxes are Cat. Nos. SN-5S1A96, SN-5S1B96, SN-5S2A96 and SN-5S2B96.

Storage and Usage Recommendations

Upon receipt, store the kit 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) and 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, NOT in a cryocooler, for at least 10 minutes. Attempting to pipette enzymes at -20 °C may result in a shortage of enzyme reagents. After thawing reagents, briefly vortex (except the enzymes) to mix them well. Enzyme G3 is the only enzyme that may be vortexed. Spin all tubes in a microfuge to collect contents prior to opening. Always add reagents to the master mix in the specified order as stated throughout the Protocol. The indexing primers (SNAP Indexes) are the only reagents that are added individually to each sample.

Materials and Equipment Not Included

- SPRIselect beads (Beckman Coulter, Cat. No. B23317/B23318/B23319) or Agencourt AMPure XP beads (Beckman Coulter, Cat. No. A63880/A63881/A63882)
- Invitrogen DynaMag, Agencourt SPRIPlate or similar magnetic rack for magnetic bead clean-ups
- Qubit, Nanodrop, or similar input DNA quantification assay
- qPCR-, electrophoretic-, or fluorometric-based library quantification assay for Illumina libraries
- Microcentrifuge
- Programmable thermocycler operating within manufacturer's specifications
- 0.2 mL PCR tubes or 96-well plate
- Aerosol-resistant tips and pipettes ranging from 1-1000 µL
- 200-proof/absolute ethanol (molecular biology grade) and nuclease-free water for preparation of 80% ethanol

Starting Material Recommendations

It is recommended that input DNA concentration be determined using Qubit, or a similar fluorometric method. Sample purity should be determined by spectrophotometry (260/280). This kit has been tested with purified microbial DNA isolates as low as 10 pg. However, many samples contain an abundance of host or other non-microbial DNA and microbial DNA content is a minor fraction of the sample. In this case a minimum of 1 ng and up to 50 ng is recommended as the amount of amplifiable microbial DNA content cannot be determined. When considering input amounts, please also take into account the expected complexity of your sample and sensitivity required. If your sample does not produce yields and you suspect sample impurities to be an inhibitory factor, a bead-based cleanup may improve amplification.

- Use as low as 10 pg for microbial isolates, and 1-50 ng for metagenomic samples
- Input DNA should be re-suspended in 10 µl of Pre-PCR TE buffer.

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.
- Avoiding Cross-Contamination: physically separate the laboratory space, equipment, and supplies where pre-PCR and post-PCR processes are performed, including appropriate reagent boxes for Multiplex PCR and Indexing PCR. Clean both the pre-PCR and post-PCR 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 and dispose of pipette tips and other disposables in sealed plastic bags. Move samples to post-PCR area before opening tubes. 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.

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 Thermocycler Program	**Lid heating ON**		
	30 sec	98 °C	
	10 sec	98 °C	**4 cycles**
	5 min	63 °C	
	1 min	65 °C	
	10 sec	98 °C	**14 cycles**
	1 min	64 °C	
	1 min	65 °C	
	Hold	4 °C	

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 DNA 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.

Component	Volume (1 Reaction)
• Reagent G1*	2 µl
• Reagent G2	3 µl
• Enzyme G3	15µl
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 DNA 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 in the post-PCR area with the following components. **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.

Component	Volume (1 Reaction)
• Reagent I1	3.3 µl
• Enzyme I2	0.5 µl
• Enzyme I3	0.1 µl
• Enzyme I4	25 µl
Reaction Mix	**28.9 µl**

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.

Indexing PCR Thermocycler Program	**Lid heating ON (105 °C)**		
	20 min	37 °C	
	30 sec	98 °C	
	10 sec	98 °C	**7 cycles***
	30 sec	60 °C	
	1 min	66 °C	
	Hold	4 °C	

***The PCR cycle number can be increased for samples that may give low yields.**

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).
17. Add a unique combination of 2 µl SNAP Index D50X + 1.7 µl SNAP Index S7XX to each sample.
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 42.5 µl (ratio: 0.85) 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.

Sequencing Recommendations

SNAP libraries may be sequenced using paired-end sequencing on Illumina instruments. We recommend using 2 x 150 paired-end reads. 2 x 300 paired-end read sequencing is an option for the 16S Panel v2 and ITS1 Panels. The depth of coverage required will depend on the application.

For the 16S Panel v2 and ITS1 Panel, >100,000 paired end reads per library are recommended. 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®**	
v2 Nano	v2 Micro	v2	v3	Mid-Output	High-Output
20	80	300	500*	160	500*

* 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.

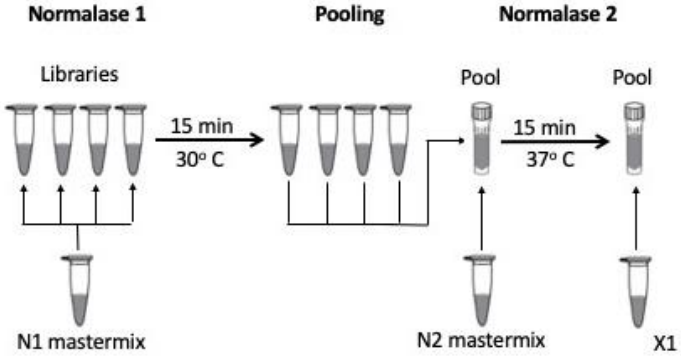
Introduction to Normalase

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 equal molar library pool. Most samples processed with the 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/ul and nM is available. For the 16S v2 and ITS1 Panels, please use a finished library size of 475 bp (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 library fraction
2. **Library Pooling** of samples for multiplexed sequencing
3. **Normalase II** to enzymatically generate an equimolar library pool



Workflow schematic: *Normalase I Mastermix is added to samples and incubated at 30ºC for 15 minutes. Sample pooling is then performed and then the Normalase II Mastermix is added to the pool and incubated at 37ºC for 15 minutes. Reagent X1 inactivates the reaction and a final equal molar 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 pM to achieve a 1000-1200 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 for each library after Indexing PCR:

- Spot check library yields using either fluorometric methods (ie: Qubit) or electrophoretic methods (ie: Bioanalyzer).
- A Normalase workflow modification can be performed that requires a 6 nM threshold to obtain a 2nM 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 2nM (instead of 4nM), 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

Libraries can be stored 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 100,000 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.

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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

2. 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.

3. Add 1 µl of Normalase II Master Mix multiplied by the total number of libraries within each prepared pool.
4. Mix well by vortexing for 5 seconds, and spin down the library pools in a microfuge.
5. 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 (1.5 mL screw cap microfuge tube)
Hold at 95 ºC 2 min at 95 ºC with lid kept at 95 ºC Hold at 4 ºC	2 min at 95 ºC

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 equal molar. 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

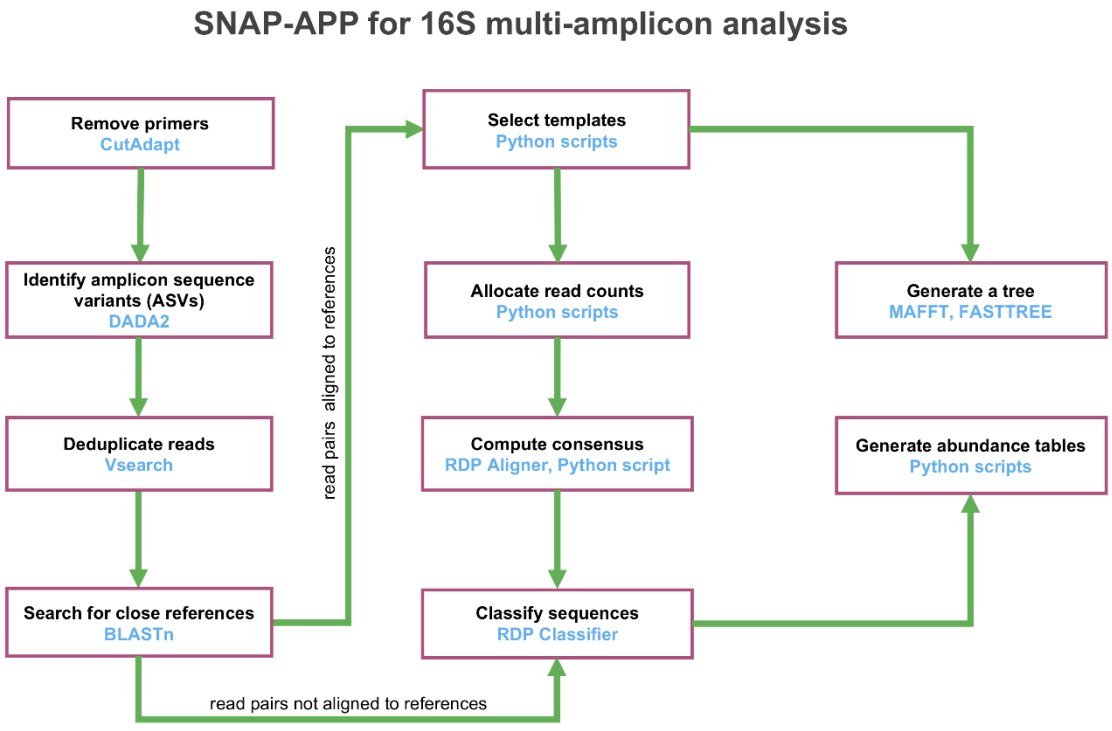
To ensure optimal sequencing results, perform a qPCR quantification on your final Normalase pool(s). Final library pools are ssDNA and cannot be quantified by dsDNA-based fluorometric methods or fragment analysis. If you do not have a qPCR assay, validate a commercially available kit by calibrating your 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.

Sequencing Data Analysis

Multiple Variable-Region Aware Read Classification Tool

Multi V-region 16S NGS data generated with the SNAP 16S Panel v2 may be processed and analyzed by an open-source tool, the 16S SNAP APP, published by Swift Biosciences at https://github.com/swiftbiosciences/snapp. Details for Fastq read processing using 16S SNAPP can be found in the README file included at the Github repository and in the Technical Note.



Indexed Adapter Sequences

During the Indexing PCR step in the protocol, you must use a unique indexing primer combination D50X/S7XX (combinatorial dual indexes) to label each library. Libraries made with uniquely indexed adapters may be pooled prior to cluster generation and co-sequenced on the same Illumina flow cell. The full-length adapter sequences of the combinatorial dual indices are below. The underlined text indicates the location of the index sequences, as detailed in the tables below.

Combinatorial Dual Indexing

P5 TruSeq Adapter (D501-D508):

5' AATGATACGGCGACCACCGAGATCTACACXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT

P7 TruSeq Adapter (S701-S748):

5' GATCGGAAGAGCACACGTCTGAACTCCAGTCACXXXXXXXXATCTCGTATGCCGTCTTCTGCTTG

Dual Index	i5 Sequence for MiSeq, NovaSeq, HiSeq 2500	i5 Sequence for MiniSeq, NextSeq, HiSeq 4000
Index D501	TATAGCCT	AGGCTATA
Index D502	ATAGAGGC	GCCTCTAT
Index D503	CCTATCCT	AGGATAGG
Index D504	GGCTCTGA	TCAGAGCC
Index D505	AGGCGAAG	CTTCGCCT
Index D506	TAATCTTA	TAAGATTA
Index D507	CAGGACGT	ACGTCCTG
Index D508	GTACTGAC	GTCAGTAC

Set S1A Index #	i7 Index Sequence
S701	CAACACAG
S702	ACACCTCA
S703	ACCATAGG
S704	CAGGTAAG
S705	AACGCACA
S706	TAGTCTCG
S707	CAGTCACA
S708	CCAACACT
S709	ACATGCCA
S710	ATTCCGCT
S711	CAAGGTAC
S712	CCATGAAC

Set S1B Index #	i7 Index Sequence
S713	TCAGCCTT
S714	CAGTGCTT
S715	CTCGAACA
S716	ACAGTTCG
S717	ATCCTTCC
S718	CGAAGTCA
S719	CTCTATCG
S720	ACTCTCCA
S721	TCCTCATG
S722	AACAACCG
S723	CTCGTTCT
S724	TCAGTAGG

Set S2A Index #	i7 Index Sequence
S725	GCTTCACA
S726	CGATGTTT
S727	TTAGGCAT
S728	ACAGTGGT
S729	GCCAATGT
S730	CAGATCTG
S731	ACTTGATG
S732	TAGCTTGT
S733	TGGTTGTT
S734	TGTACCTT
S735	TCTGCTGT
S736	TTGGAGGT

Set S2B Index #	i7 Index Sequence
S737	TCGAGCGT
S738	TGATACGT
S739	TGCATAGT
S740	TGCGATCT
S741	TTCCTGCT
S742	TACAGGAT
S743	TGTGGTTG
S744	TTCCATTG
S745	TAACGCTG
S746	TTGGTATG
S747	TGAACTGG
S748	TACTTCGG

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.

Revision History

Document #	Revision	Date	Description of Change
PRT-029	Version 1	5/20/2020	Initial release.
PRT-029	Version 2	7/10/20	Amended Kit Contents, Introduction to Normalase, Calculator and Indexing tables.

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