SWIFT NORMALASE® AMPLICON PANELS (SNAP)
16S v2 Panel
ITS1 Panel
Single pool high complexity NGS microbial enrichment assays

Highlights

- Two multiplexed assays cover all variable regions of the 16S v2 rRNA and ITS1 regions, each available as a single primer pool

- Integrated library normalization enables streamlined library balancing and pooling process without the need to quantify samples

- 16S and ITS1 regions separated into two assays to enable independent balancing of sequencing read depth for each region

- Improved coverage uniformity of the 16S variable regions for increased data quality

- ITS1 primer re-design to increase data output from PE 150 sequencing

Introduction

The Swift Normalase Amplicon Panels (SNAP) for 16S v2 and ITS1 offers a robust NGS workflow that provides optimal coverage and NGS data quality on Illumina® sequencing platforms. These kits leverage Swift's multiplex PCR technology, enabling library construction from DNA using tiled primer pairs to target all V1-V9 variable rRNA regions and the ITS1 region, each with a single pool of multiplexed primer pairs.

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 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.

In addition, the SNAP 16S v2 and ITS1 panels facilitate NGS analysis of complex microbial communities (e.g. bacteria, archaea, fungi) using single primer pools that target the 16S rRNA gene (variable regions 1-9) and ITS1 region. There panels can be customized with additional targets including antibiotic resistance or virulence genes, allowing sub-genera level identification and functional analysis.
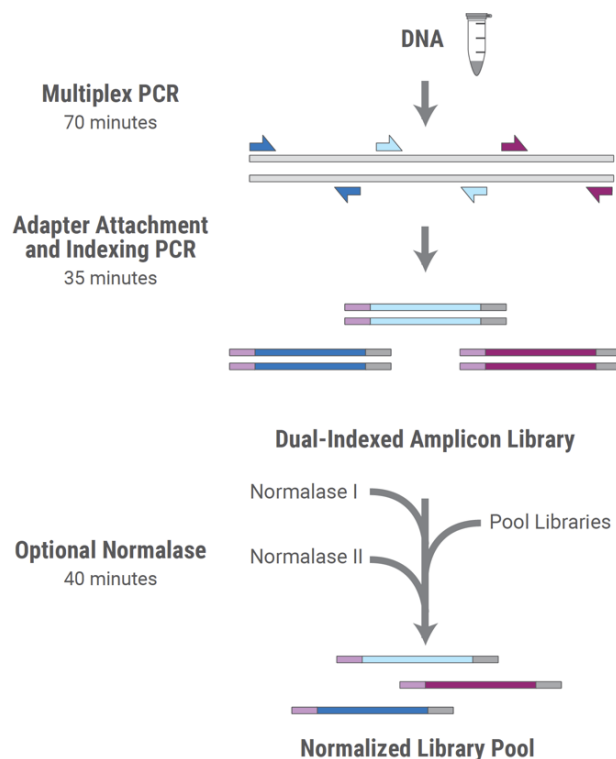
Supported Applications

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).

Specifications

Feature	SNAP 16S v2 Panel and ITS1 Panel
Panel Information	23 primers (16S v2); Average 425 bp amplicon size 15 primers (ITS1); Amplicon size 145-695 bp
Input Material	10 pg for microbial isolates; 1-50 ng for metagenomic samples
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	16S v2: 100K reads per library ITS1: 25K reads per library

* Please inquire with your Swift sales representative or distributor to review a copy of the primer design file.
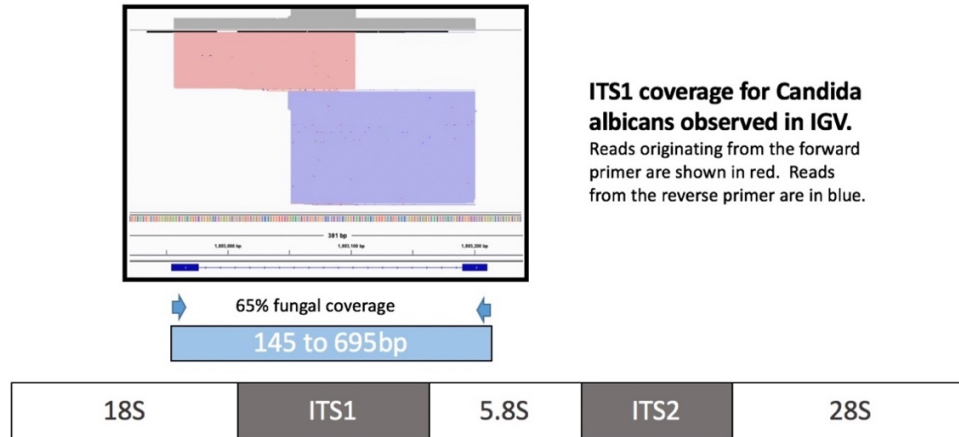
Coverage of All Variable Regions of the 16S rRNA Gene and ITS1 Region

The SNAP 16S v2 and ITS1 Panels facilitate NGS analysis of complex microbial communities (e.g. bacteria, archaea, fungi) using primer pools that target the 16S rRNA gene (variable regions 1-9) and ITS1 region. In addition, these panels can be customized with additional targets including antibiotic resistance or virulence genes, allowing sub-genera level identification and functional analysis.
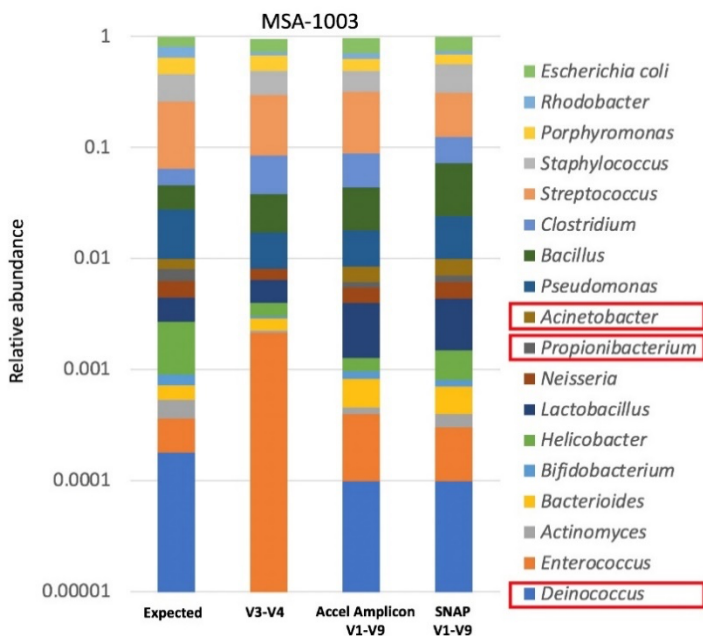
16S Panel v2 (primers), Swift Cat. No. 516G1-96
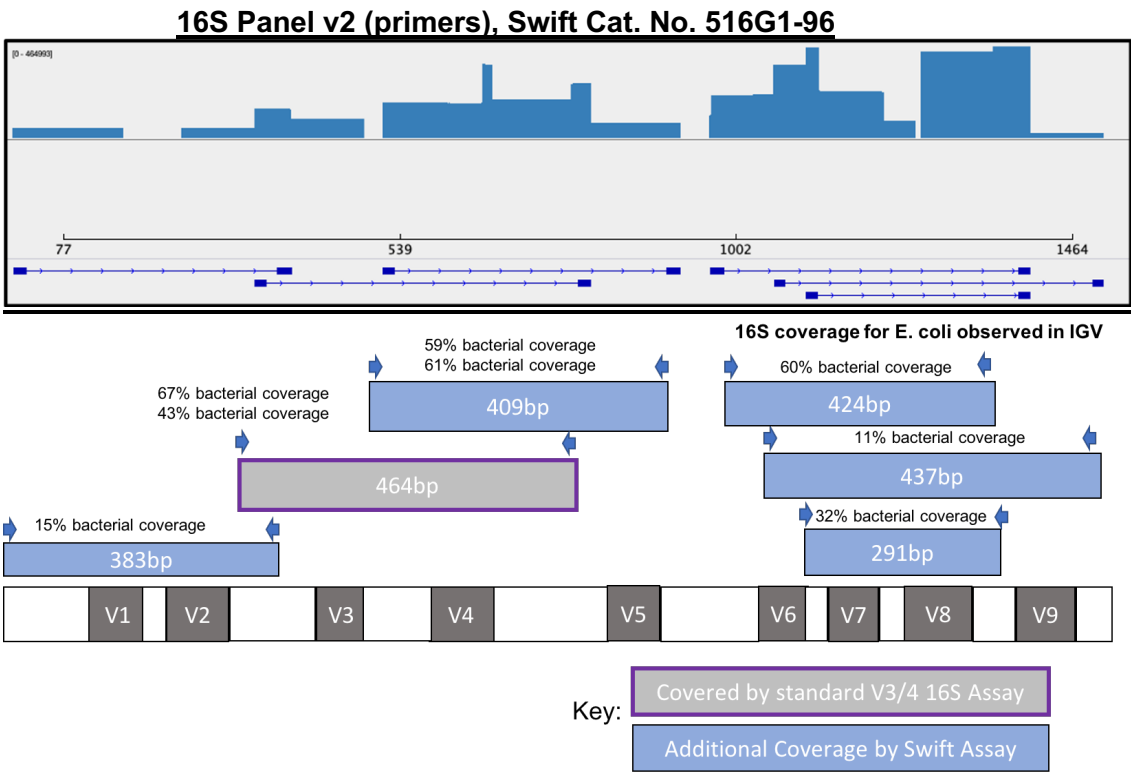
Sequencing read coverage (IGV Sashimi plot) and illustration of multiplexed primer coverage of all nine variable regions of 16S rRNA.

ITS1 coverage for Candida albicans observed in IGV. Reads originating from the forward primer are shown in red. Reads from the reverse primer are in blue.

65% fungal coverage

145 to 695bp

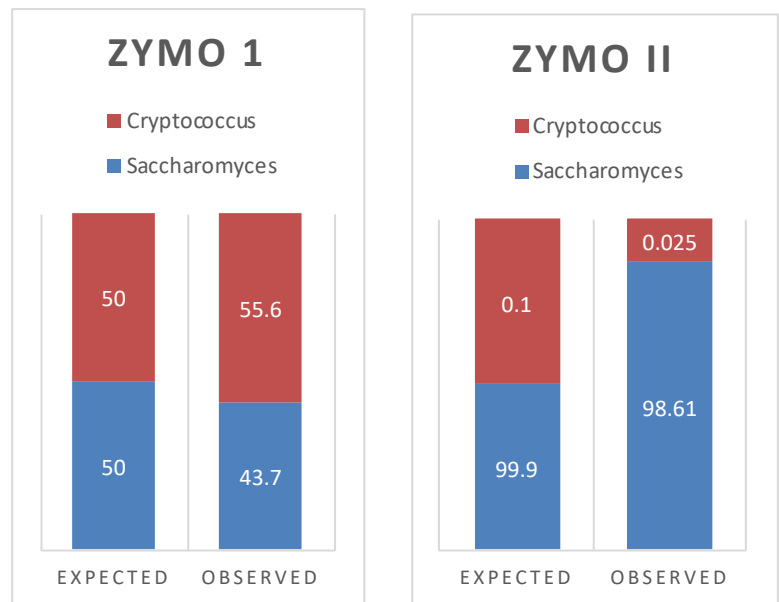| 18S	ITS1	5.8S	ITS2	28S

Sequencing read coverage (IGV) and illustration of multiplexed primer coverage of ITS1 region from C. albicans.

Panels Provide Superior Representation of Diverse Microbial Communities

The 16S v2 panel covering V1-V9 regions of 16S rRNA (Swift Cat. No. 516G1-96) provides accurate representation of each genus in a commercially available standard (MSA-1003) compared to libraries interrogating the V3-V4 region alone. The Swift Accel-Amplicon and 16S v2 SNAP panels performed equally. Strains were present at levels from 0.02% to 18% in MSA-1003. Organisms marked in red were not detected with sole use of the V3-V4 region.

The panel covering the ITS1 region (Swift Cat. No. 517G1-96) provides accurate representation of each genus in two commercially available standards (Zymo I- ZymoBIOMICS Microbial Community standard and Zymo II- ZymoBIOMICS Microbial Community standard II (Log distribution)). Fungal strains were present at levels from 0.1% to 99.9% in Zymo II. The ITS1 SNAP panel performance resulted in expected representation of the fungal species in a bacterial background.

Consistent Performance with Varying Biomass, Sample Type, & Read Length
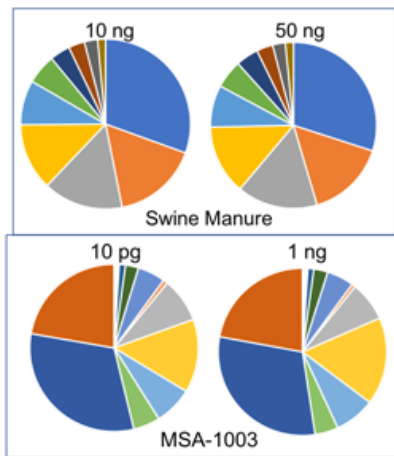

| Sample	Accel-Amplicon V1-V9 2x150 PE sequencing		16S SNAP V1-V9 2x150 PE sequencing		Accel-Amplicon V1-V9 2x300 PE sequencing	
	Shannon Species Diversity	% Reads PF Classified to Genus	Shannon Species Diversity	% Reads PF Classified to Genus	Shannon Species Diversity	% Reads PF Classified to Genus
Manure 1	2.84	94.1%	2.72	95.2%	2.84	92.5%
Manure 2	2.87	94.6%	2.80	92.2%	2.85	92.8%
Manure 3	2.71	94.6%	2.92	92.5%	2.69	92.6%
Manure 4	2.74	95.0%	2.98	93.8%	2.70	93.6%

Using the same protocol and cycling conditions, input quantities of 10 pg to 50 ng with both MSA-1003 (bottom left) and swine manure (top left) gave consistent and expected sequencing results in terms of sensitivity and relative abundance. The Accel-Amplicon 16S+ITS and 16S v2 SNAP products gave similar results (top). When comparing 2x150 and 2x300 PE reads, a comparable number of species were identified from swine manure samples (right).

16S SNAP APP: Multiple Variable-Region Aware Read Classification Tool

Multi V-region 16S NGS data generated with the 16S Panels 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 SNAP APP can be found in the README file included at the Github repository and in the Technical Note. For ITS1 data analysis support, please contact us at techsupport@swiftbio.com
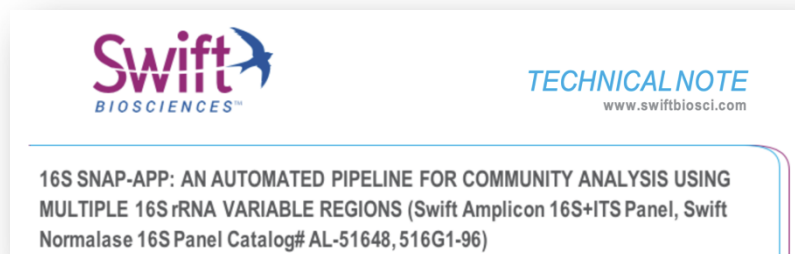
SNAP-APP for 16S multi-amplicon analysis
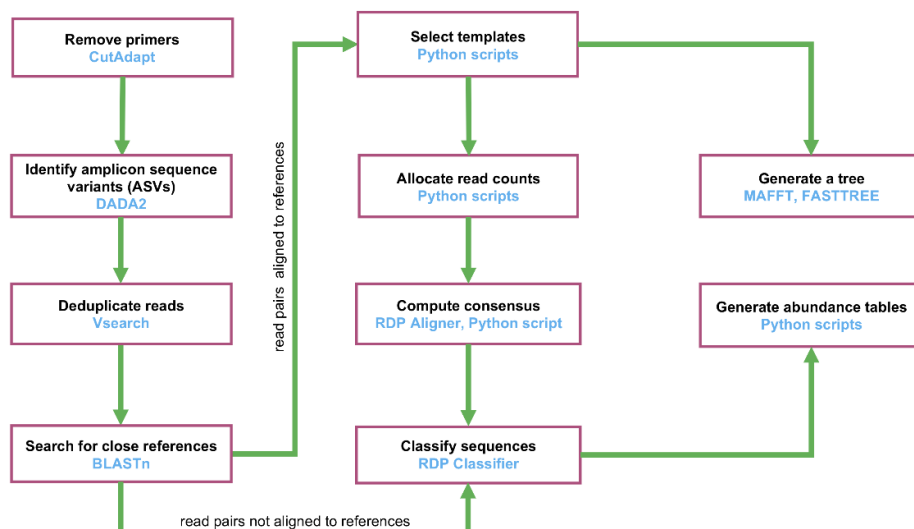
Ordering Information

Workflow Component	Product Name	Catalog Number
Primer Pool	16S v2 Panel (primers only)	516G1-96
	ITS1 Panel (primers only)	517G1-96
SNAP Core	Swift Normalase Amplicon Protocol SNAP Core (96 rxns, no indexing)	SN-5X296
Indexing Primers*	SNAP Combinatorial Dual Index Primer Kit (Set 1A, 96 rxns)	SN-5S1A96
	SNAP Combinatorial Dual Index Primer Kit (Set 1B, 96 rxns)	SN-5S1B96
	SNAP Combinatorial Dual Index Primer Kit (Set 2A, 96 rxns)	SN-5S2A96
	SNAP Combinatorial Dual Index Primer Kit (Set 2B, 96 rxns)	SN-5S2B96

* Un kit complet est composé de Primer pool (16S/ITS1) + SNAP Core kit + Index au choix parmi la liste

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