ZYMO RESEARCH

Microbiomics
Made Simple™

Quick-16S/ITS™ NGS Library Prep Sampler

For evaluation of all versions of the *Quick*-16S/ITS™ Plus kits

Highlights

- The included *Quick*-16S/ITS™ **Plus** reactions produce automatically normalized libraries, regardless of sample input, and target a variety of bacterial 16S gene regions in addition to the fungal ITS2 region.
- The included *Quick*-16S™ Full-Length Library Prep Kit reactions offer increased taxonomic resolution for samples sequenced on PacBio® & Oxford Nanopore Technologies® (ONT®) long-read platforms.
- All full-size versions of the kits offered as part of the sampler are 100% automation ready with only a single PCR step, and support multiplexing of 96, 384, or 768 samples.

Catalog Numbers:
D6499

Scan with your smart-phone camera to view the online protocol/video.

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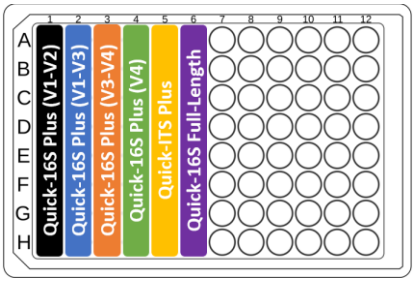
Product Contents

Quick-16S/ITS™ NGS Library Prep Sampler	D6499 (48 rxns.)	Storage Temp.
***Quick*-16S/ITS™ Sampler Premix Plate**[1]	10 µl each well	-20°C
ZymoBIOMICS® Microbial Community DNA Standard (50 ng)	10 µl	-20°C
Read 1 Sequencing Primer	30 µl	-20°C
Read 2 Sequencing Primer	30 µl	-20°C
Index 1 (i7) Sequencing Primer	30 µl	-20°C
Index 2 (i5) Sequencing Primer	30 µl	-20°C
Select-a-Size™ MagBead Concentrate[2]	30 µl	4-8°C
Select-a-Size™ MagBead Buffer[2]	1 ml	4-8°C
DNA Wash Buffer	6 ml	Room Temp.
ZymoBIOMICS® DNase/RNase Free Water	1 ml	Room Temp.
PCR Inactivation Solution	100 µl	Room Temp.
Magnetic Rod	4	-
Instruction Manual	1 pc	-

1 Contents of the plate are photosensitive. Protect from light wherever possible.
2 The Select-a-Size™ MagBead Concentrate and Buffer are shipped at room temperature but should be stored at 4-8°C upon receipt.

Specifications

- ***Quick*-16S/ITS™ Sampler Premix Plate Layout:**



- **Sample Input –** Purified microbial DNA (≤100 ng), free of PCR inhibitors. Note additional requirements for the *Quick*-16S™ Full-Length Library Prep Kit[1].

- **Primer Sequences and Library Info –** For more details including primer barcodes and sample sheets, visit the respective kit protocols/product pages directly:

Target Region (Kit Cat#)	Forward Primer	Reverse Primer	Final Library Size	Sequencing Platform
V1-V2 (D6434)	27f (AGRGTTYGATYMTGGCTCAG)	341r (CTGCWGCCHCCCGTAGG)	~492 bp	Illumina MiSeq/NextSeq
V1-V3 (D6440)	27f (AGRGTTYGATYMTGGCTCAG)	515r (ACCGCGGCTGCTGGCAC)	~650 bp	Illumina MiSeq/NextSeq
V3-V4 (D6421)	341f[2] (CCTACGGGDGGCWGCAG, CCTAYGGGGYGCWGCAG)	806r (GACTACNVGGGTMTCTAATCC)	~606 bp	Illumina MiSeq/NextSeq
V4 (D6430)	515f (GTGYCAGCMGCCGCGGTAA)	806r (GGACTACNVGGGTWTCTAAT)	~388 bp	All Illumina Instruments
ITS2 (D6425)	ITS3f (GCATCGATGAAGAACGCAG)	ITS4r (TCCTCCGCTTATTGATATGC)	~480 bp	Illumina MiSeq/NextSeq
Full-Length (D6450)	27f (AGRGTTYGATYMTGGCTCAG)	1492r (RGYTACCTTGTTACGACTT)	~1500 bp	PacBio/Oxford Nanopore Instruments

- **Equipment Needed** (user provided) **–** Microcentrifuge, plate spinner (centrifuge), 96-well real-time quantitative PCR system (SYBR Green compatible, recommended), or standard PCR system, and 96-well real-time PCR plates.

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1 The *Quick*-16S™ Full-Length Library Prep Kit requires pure microbial input DNA normalized to 1 ng/µl. Please see the D6450 Kit Protocol for more information.
2 The forward primer 341f is a mixture of the two sequences listed.

Product Description

The ***Quick*-16S/ITS™ NGS Library Prep Sampler** provides 8 reactions from each of our *Quick*-16S/ITS™ Plus library prep kits, targeting various hypervariable regions across the bacterial/archaeal 16S rRNA gene, as well as the fungal ITS2 region. Additionally provided are 8 reactions of the *Quick*-16S™ Full-Length Library Prep Kit, designed for sequencing of the entire 16S rRNA gene on PacBio® & Oxford Nanopore® long read sequencing platforms, to provide users unparalleled taxonomic resolution.

The ***Quick*-16S/ITS™ Plus** kits are the fastest and simplest library prep method for short read, high-throughput 16S/ITS sequencing. Each kit utilizes a single qPCR/PCR step for combined targeted amplification and barcode addition, using specially designed primers. The uniquely formulated Equalase™ qPCR Premix enables equal PCR product formation regardless of input DNA, which in turn allows equal volume pooling of libraries without the need for further normalization. A single-tube clean-up of the pooled libraries prior to Qubit® quantification completes the library prep as TapeStation® or gel electrophoresis is not necessary. All reaction components are delivered premixed in plate format, and only require transfer to a new PCR plate before DNA addition. With this workflow, the hands-on time of 16S/ITS library preparation is reduced to only 30 minutes (Figure 1). For additional information including kit-specific performance data, please see the respective product page protocols for each *Quick*-16S/ITS™ Plus kit.

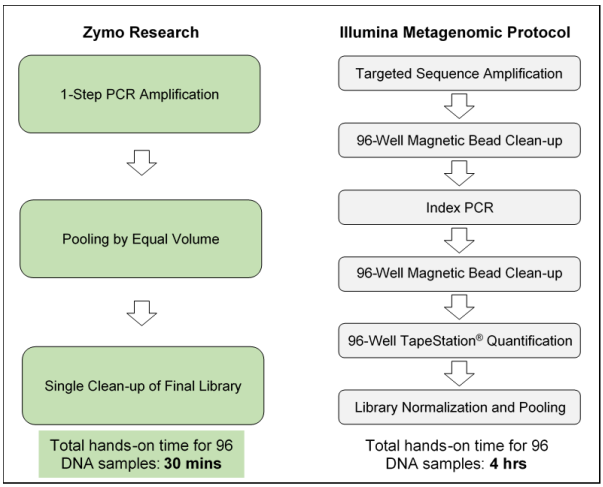

Figure 1. *Quick*-16S/ITS™ Plus NGS Library Prep Kit workflow versus the Illumina Metagenomic Protocol. Total hands-on time calculations are based on the preparation of 96 DNA samples.

Protocols

Reference the protocols below in conjunction with the ***Quick*-16S/ITS™ Sampler Premix Plate Layout** provided on the **Specifications** page to prepare 8 samples using the desired library prep kit reactions.

It is highly recommended to prepare 6 user-provided samples, the provided ZymoBIOMICS® Microbial Community DNA Standard, and a negative control to validate library preparation integrity and any resultant data.

Quick-16S™ Plus NGS Library Prep Kit (V1-V2)

Barcodes: UDI indexing, up to 96 samples

Compatibility: Illumina MiSeq®, MiSeq i100®, NextSeq 1000/2000®

Requires Custom Sequencing Primers: No

Quick-16S™ Plus NGS Library Prep Kit (V1-V3)

Barcodes: UDI indexing, up to 384 samples

Compatibility: Illumina MiSeq®, MiSeq i100®, NextSeq 1000/2000®

Requires Custom Sequencing Primers: No

Quick-16S™ Plus NGS Library Prep Kit (V3-V4, UDI)

Barcodes: UDI indexing, up to 768 samples

Compatibility: Illumina MiSeq®, MiSeq i100®, NextSeq 1000/2000®

Requires Custom Sequencing Primers: No

Quick-16S™ Plus NGS Library Prep Kit (V4) – 96 Preps

Barcodes: UDI indexing, up to 384 samples

Compatibility: All Illumina Platforms

Requires Custom Sequencing Primers: **Yes**

Quick-ITS™ Plus NGS Library Prep Kit (UDI)

Barcodes: UDI indexing, up to 384 samples

Compatibility: Illumina MiSeq®, MiSeq i100®, NextSeq 1000/2000®

Requires Custom Sequencing Primers: No

Quick-16S™ Full-Length Library Prep Kit

Barcodes: CDI indexing, up to 384 samples

Compatibility: PacBio® and ONT® Platforms

Requires added platform-specific prep: **Yes**

Appendix A: Library Multiplexing Guide

The following protocol is provided for users who wish to run multiple *Quick*-16S/ITS™ **Plus** Kit regions in parallel.

Before Starting

- ✓ **Sample Quantity Requirement.** To ensure color balance in index sequencing, a minimum of 8 samples per run is recommended.
- ✓ **Input DNA Guidelines.** All DNA samples should be free of PCR inhibitors[1]. The 1-Step PCR reaction can accommodate DNA inputs of up to 100 ng but reducing inputs to ≤10 ng is recommended for robustness against potential PCR inhibition.
- ✓ **Short Read Libraries Only.** The following protocol does not apply to the *Quick*-16S™ Full-Length reactions as they must be prepared for long-read sequencing according to the D6450 Kit protocol.

Section 1: 1-Step PCR

1. Pierce the foil and transfer 8 µl of premix from the *Quick*-16S/ITS™ Sampler Premix Plate to a new PCR plate.
2. For each unique region being run, add 2 µl of the included ZymoBIOMICS® DNA Community Standard as a positive control and 2 µl of water as a negative control.
3. Add 2 µl of your sample DNA to each of the 6 remaining reactions for each library prep region (see the example plate layout below).

	1	2	3	4	5	6	7
A	Sample1	Sample1	Sample1	Sample1	Sample1		
B	Sample2	Sample2	Sample2	Sample2	Sample2		
C	Sample3	Sample3	Sample3	Sample3	Sample3		V1-V2
D	Sample4	Sample4	Sample4	Sample4	Sample4		V1-V3
E	Sample5	Sample5	Sample5	Sample5	Sample5		V3-V4
F	Sample6	Sample6	Sample6	Sample6	Sample6		V4
G	POS	POS	POS	POS	POS		ITS
H	NEG	NEG	NEG	NEG	NEG		

1 DNA that contains potent PCR inhibitors such as polyphenolics, humic/fulvic acids, tannins, melanin, etc. can be quickly cleaned using the OneStep™ PCR Inhibitor Removal Kit (D6031).

4. Apply an adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner[1].

5. Place plate in a real-time thermocycler[2] and run the program shown below:

Temperature	Time	
95°C	10 min	Lid Temp: 105°C Dye: SYBR Green
95°C	30 sec	42 cycles
55°C	30 sec	
72°C	3 min	
Plate read	-	
4°C	Hold	

6. Monitor and QC the library preparation when running the reaction on a real-time thermocycler[3].

   a. For example, a sample that is expected to amplify and shows little or no amplification may indicate an error in the reaction setup.
   b. The negative control should not amplify before 35 cycles[4]. Earlier amplification of negative control may indicate process contamination.
   c. An example of qPCR amplification with controls is shown in Figure 5 below.

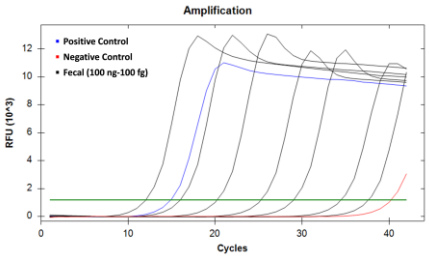

Figure 5. qPCR Amplification Example with Positive and Negative Controls. Serial dilutions of fecal DNA (black) from 100 ng to 100 fg were amplified on a Bio-Rad CFX96™ Real-Time PCR Detection System. The positive (blue) amplified at 14.93 and negative (red) amplified at 40.42. Baseline threshold was set at 1200 RFU.

7. Once the samples have cooled to 4°C, stop the program. Centrifuge plate in a plate spinner to collect condensation in wells and place plate on ice. Proceed to Section 2, or store plate at ≤-20°C for later use.

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1 PCR reactions can be pipette mixed if a plate shaker is not available.
2 A real-time thermocycler is recommended as it enables QC of the library prep of all wells.. A non-quantitative system can be used if absolute quantification is not needed.
3 If real-time PCR was not used, a combination of PCR cleanup and TapeStation® analysis can be used to confirm correct amplicon size.
4 It is normal to see some amplification from the negative control. If desired, the negative control(s) may be omitted from pooling in **Section 2** to preserve reads for the remaining samples.

Section 2: Multiple Region Pooling

Add 50 µl of **PCR Inactivation Solution** into a new microcentrifuge tube. Depending on the 16S/ITS region prepared, use the table below to pool PCR products from each well of the **Section 1** plate into the tube. Mix pooled libraries well and proceed with **Section 3**.

Target Region	Pooling Volume (µl)
V1-V2	2
V1-V3	4.8
V3-V4	4
V4	2
ITS2	2

Section 3: Final Library Clean-up

1. Equilibrate the **Select-a-Size MagBead Buffer** to room temperature (15-30°C). Add 30 µl of **Select-a-Size MagBead Concentrate** to the 1 ml Select-a-Size MagBead Buffer. Resuspend the magnetic particles by vigorously shaking until homogenous.
2. Add **Select-a-Size™ MagBeads** to the pooled library from Section 2 at a ratio of 0.8x volume. For example, add 400 µl of Select-a-Size™ MagBeads to 500 µl of the pooled library and PCR Inactivation Solution mixture.
3. Mix thoroughly by pipetting or vortexing until homogenous. Incubate for 5 minutes at room temperature.
4. Place the sample on a magnetic rack[1] and incubate for 3-10 minutes at room temperature, or until the magnetic beads have fully separated from solution.
5. Once the beads have cleared from solution, remove and discard the supernatant[2].
6. While the beads are still on the magnetic rack, add 1ml of **DNA Wash Buffer**. Remove and discard the supernatant. Repeat this step.

1 Alternatively, the provided Magnetic Rod can be used.
2 Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid behind.

7. While the beads are still on the magnetic rack, aspirate out any residual buffer with a 10 µl pipette tip.
8. Remove tube from the magnetic rack and keep the cap open for 3 minutes at room temperature to dry the beads.

Section 4: Library Quantification

Use a fluorescence-based method (Qubit® dsDNA HS Assay Kit recommended) to quantify the final cleaned libraries. Using the average size of the 16S regions prepared, convert ng/µl to nM using the equation below.

$$\frac{concentration\ in\ ng/ul}{660\ g/mol\ x\ average\ library\ size\ in\ bp}\ x\ 10^6\ =\ concentration\ in\ nM$$

DNA Fragment Analysis (Not Required)

If a fragment analyzer (e.g. TapeStation®) is used to analyze the final library, there may be a distinct band corresponding to the library. Because of the protocol design, some library products have run through additional PCR cycles and may not fully reanneal. This has no bearing on sequencing performance, as the libraries will be denatured before loading onto the sequencing instrument.

This is your final 16S/ITS library

The pooled library DNA is now ready for use or storage at ≤-20ºC. Refer to platform-specific guidelines for preparation for sequencing. **If sequencing 16S V4 Libraries, custom sequencing primers are necessary. Please refer to the D6430 Protocol or your Illumina instrument's manual for guidance on how to prepare custom sequencing primers.**

Illumina Loading Parameters:

For the MiSeq® Reagent Kit v3 (600-cycle), a final library loading concentration of 10-12pM[1] with 15% PhiX spike-in is recommended. For the NextSeq® Reagent Kit P1/P2 (600-cycle), a final library loading concentration of 750 pM using onboard denature and dilute with 40% PhiX spike-in is recommended.

1 Optimal loading concentration may vary by instrument. Adjust final library loading concentrations as needed to reach a target cluster density of 700 K/mm^2.

Ordering Information

Product Description	Catalog No.	Size / Format
Quick-16S™ Plus NGS Library Prep Kit (V3-V4)	D6421	96 reactions (768 indexes total, across 8 x 96 plates)
Quick-ITS™ Plus NGS Library Prep Kit	D6425	96 reactions (384 indexes total, across 4 x 96 plates)
Quick-16S™ Plus NGS Library Prep Kit (V4)	D6430	96 reactions (384 indexes total, across 4 x 96 plates)
Quick-16S™ Plus NGS Library Prep Kit (V1-V2)	D6434	96 reactions (96 indexes total, across 1 x 96 plate)
Quick-16S™ Plus NGS Library Prep Kit (V1-V3)	D6440	96 reactions (384 indexes total, across 4 x 96 plates)
Quick-16S™ Full-Length Library Prep Kit	D6450	96 reactions (384 indexes total, across 4 x 96 plates)

Individual Kit Components	Catalog No.	Amount
ZymoBIOMICS® DNase/RNase Free Water	D4302-5-10	10 ml
ZymoBIOMICS® Microbial Community DNA Standard (200 ng)	D6305	200 ng
ZymoBIOMICS® Microbial Community DNA Standard (2000 ng)	D6306	2000 ng

Complete Your Workflow

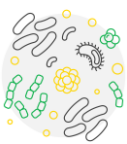
✓ To collect and transport samples at ambient temperatures:

DNA/RNA Shield™ and Collection Devices		
R1100	DNA/RNA Shield™ Reagent	50 mL, 250 mL
R1200	DNA/RNA Shield™ Reagent (2x Concentrate)	25 mL, 125 mL
R1101	DNA/RNA Shield™ Fecal Collection Tube	10 pack
R1150	DNA/RNA Shield™ Blood Collection Tube	50 pack
R1160	DNA/RNA Shield™ SafeCollect Swab Collection Kit	1 mL, 2 mL
R1211	DNA/RNA Shield™ SafeCollect Saliva Collection Kit	2 mL

✓ Unbiased and inhibitor-free DNA and RNA extraction (high-throughput and automatable) for microbial profiling:

ZymoBIOMICS® DNA and RNA Kits		
D4300	ZymoBIOMICS® DNA Miniprep Kit	50 preps
D4301	ZymoBIOMICS® DNA Microprep Kit	50 preps
D4302	ZymoBIOMICS® 96 MagBead DNA Kit	2 x 96 preps
R2001	ZymoBIOMICS® RNA Miniprep Kit	50 preps
R2137	ZymoBIOMICS® MagBead RNA Kit	96 preps
R2002	ZymoBIOMICS® DNA/RNA Miniprep Kit	50 preps
R2135	ZymoBIOMICS® MagBead DNA/RNA Kit	96 preps

✓ Microbial standards and references for profiling quality control, benchmarking, positive controls, and to assess performance of entire microbiomics/metagenomic workflows:



ZymoBIOMICS® Standards and Reference Materials		
D6300	ZymoBIOMICS® Microbial Community Standard	10 preps
D6305	ZymoBIOMICS® Microbial Community DNA Standard	200 ng
D6320	ZymoBIOMICS® Spike-In Control (High Microbial Load)	25 preps
D6321	ZymoBIOMICS® Spike-In Control II (Low Microbial Load)	25 preps
D6323	ZymoBIOMICS® Fecal Reference *with TruMatrix™ Technology*	10 preps
D6331	ZymoBIOMICS® Gut Microbiome Standard	10 preps
D6332	ZymoBIOMICS® Oral Microbiome Standard	10 preps

Notes

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Integrity of kit components is guaranteed for up to one year from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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