

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

Fastest, normalization-free ITS rRNA library prep

Highlights

- The most streamlined NGS kit with only 30 minutes of hands-on time for 96 samples.
- 100% automation ready with only a single PCR step and without the need for normalization.
- Real-time PCR enables absolute microbial copy number quantification.

Catalog Numbers:

D6425-PS1, D6425-PS2, D6425-PS3, D6425-PS4



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



tech@zymoresearch.com



www.zymoresearch.com



Toll Free: (888) 882-9682

Table of Contents

Product Contents	01
Specifications	02
Product Description	03
Protocol	05
Before Starting	05
(I) 1-Step PCR	05
(II) Pooling by Equal Volume	07
(III) Final Library Clean-up	07
(IV) Library Quantification	08
Appendices	09-11
Absolute Quantification of Total ITS Copy Number by Real-Time PCR	09
Improving Normalization by Fine-Tuning Pooling Volumes based on qPCR	10
Index Primer Sets	10
Removal of PCR Inhibitors from DNA	10
Primer Trimming Recommendations	10
Composition of ZymoBIOMICS® Microbial Community DNA Standard	11
Illumina MiSeq® Sample Sheet Setup	11
Ordering Information	12
Explore Other Microbiome Products	13
Troubleshooting Guide	14
Notes	15
Guarantee	17

Product Contents

Quick-ITS™ Plus NGS Library Prep Kit (UDI)	D6425 (96 rxns.)	Storage Temp.
ITS Premix Plate¹	13 µl each well	-20°C
ZymoBIOMICS® Microbial Community DNA Standard (50 ng)	10 µl	-20°C
ZymoBIOMICS® 16S/ITS qPCR Standard	10 µl	-20°C
Select-a-Size™ MagBead Concentrate²	30 µl	4-8°C
Select-a-Size™ MagBead Buffer²	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 Protect the plate from light.

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

- **Sample Input** – Purified microbial DNA (≤ 100 ng), free of PCR inhibitors^{1,2}.
- **ITS Primer Sequences (adapters not shown)³** –
ITS3f (GCATCGATGAAGAACGCAG, 19 bp),
ITS4r (TCCTCCGCTTATTGATATGC, 20 bp).
- **Index Primers** – Dual index (barcodes) to uniquely label samples⁴.
- **Barcode Sequences** – 10 bp barcodes are available for download by visiting the Documentation section of the D6425 Product Page at www.zymoresearch.com.
- **Amplicon Size** – The final amplicon size after 1-Step PCR (targeted amplification and barcode addition) is ~480 bp, with an insert size of ~340 bp. Note that the size of ITS regions can vary widely between species.
- **Sequencing Platform** – Illumina MiSeq® without the need to add custom sequencing primers. We recommend the MiSeq® Reagent Kit v3 (600-cycle) or NextSeq 1000/2000® Reagent Kit P1/P2 (600-cycle). For assistance with sample sheet setup, see Appendix F.
- **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.
- **ITS Premix Plate** – Each position on the premix plate contains Equalase™ qPCR Premix, water, and uniquely indexed (UDI) ITS3f and ITS4r primers mixed at their proper ratios for a 10 μ l final reaction volume.

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. See Appendix D for additional information.

2 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.

3 Primers from White, T.J. et al. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. PCR Protocols and Applications, pp.315-322.

4 For projects with more than 96 samples, please see the Guide For >96 Samples which can be found on the D6425 Product Page at www.zymoresearch.com.

Product Description

ITS rRNA gene sequencing is a routine technique for mycobiome (fungal) composition profiling. Compared to shotgun metagenomics sequencing, ITS rRNA gene sequencing is more cost-effective and more robust; it generally requires less input DNA and is less impacted by the presence of non-microbial DNA. However, ITS rRNA gene sequencing has its own challenges. One major challenge is the formation of PCR chimeric sequences, which are artificial sequences resulting from the recombination of two or more PCR templates. Moreover, common ITS library preparation protocols have not been optimized to be cost-effective for large-scale applications.

The **Quick-ITS™ Plus NGS Library Prep Kit (UDI)** is the fastest and simplest library prep method for high-throughput ITS rRNA sequencing. Distinguishing features of the kit are described below.

Fastest ITS rRNA Workflow. The Quick-ITS™ Plus NGS Library Prep Kit (UDI) utilizes a single qPCR/PCR for combined targeted amplification and barcode addition using specially designed primers. After pooling by equal volume, a single clean-up of the final library is performed, rather than multi-well magnetic bead clean-ups. Additional library quantification analysis such as TapeStation® analysis or gel electrophoresis are not necessary. With this workflow, the hands-on time of ITS library preparation is reduced to only 30 minutes (Figure 1).

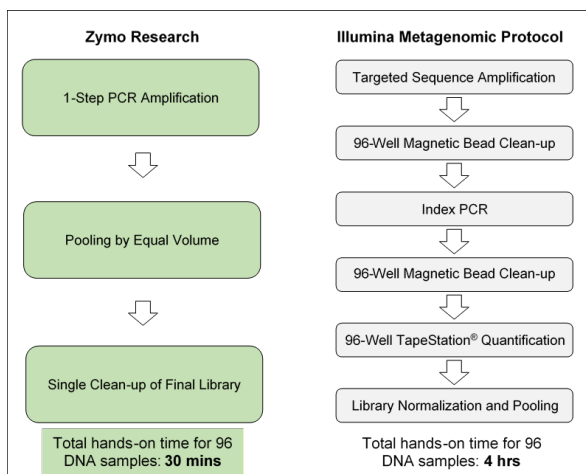


Figure 1. Quick-ITS™ Plus NGS Library Prep Kit workflow versus the Illumina Metagenomic Protocol. Total hands-on time calculations are based on the preparation of 96 DNA samples.

Normalization Free. There is no need to control sample input or PCR cycles. The workflow auto-normalizes and produces similar amounts of reads across all samples regardless of different DNA inputs (Figure 2). Just pool by equal volumes!

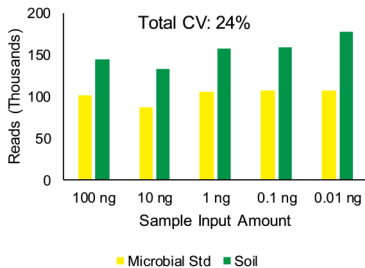


Figure 2. The Quick-ITS™ Plus NGS Library Prep Kit results in similar amounts of reads across different input amounts without normalization. 0.01-100 ng ZymoBIOMICS® Microbial Community Standard and soil DNA were used as inputs. Libraries were pooled by equal volumes (2 µl each) without further normalization and sequenced using the MiSeq® Reagent Kit v3 (600-cycle). The CV (coefficient of variation) is the ratio of the standard deviation to the mean with lower values corresponding to less dispersion around the mean.

High Quality NGS Library. The workflow has been optimized to minimize amplification bias (Figure 3). PCR chimera formation is maintained below 10% without the need to control PCR cycles (Figure 4).

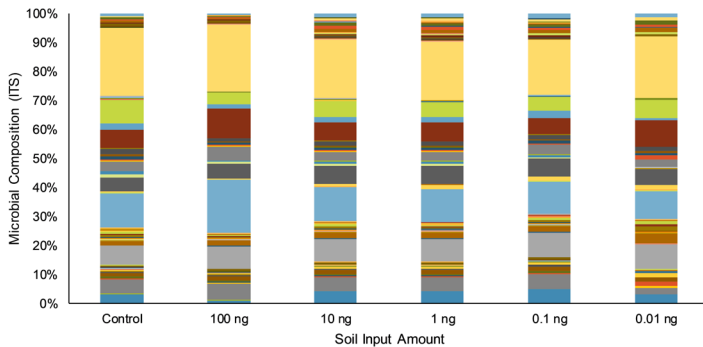


Figure 3. Compatible with wide sample input range. Fungal composition profiles (species level shown) are accurate from 100 ng down to 0.01 ng using soil DNA input and agree with the control (Illumina).

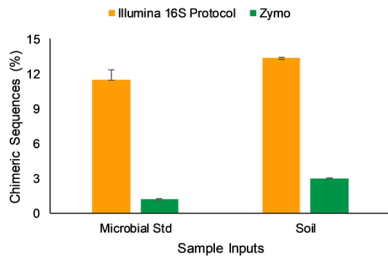


Figure 4. Up to 70% chimera reduction without controlling PCR cycles. Equal amounts of ZymoBIOMICS® Microbial Community Standard and soil DNA were used as inputs and amplified for 42 cycles using 1-step PCR and Equalase™ compared to using the Illumina Metagenomic Protocol. Chimeric sequences were predicted with UCHIME (<https://www.drive5.com/uchime>).

Protocol

Before Starting

- ✓ **Sample Quantity Requirement:** To ensure color balance in index sequencing, a minimum of 9 samples per run is recommended using the index primers
- ✓ **Input DNA Guidelines:** All DNA samples should be free of PCR inhibitors¹. 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.
- ✓ **Preparing More than 96 Samples:** Please utilize the Guide for Preparing More than 96 Samples, which is downloadable from the Documentation section of the D6425 Product Page at www.zymoresearch.com.

Section 1: 1-Step PCR

1. Pierce the foil and transfer 8 µl of premix from the ITS Premix Plate onto a new PCR plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89*
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90*
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91*
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92*
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93*
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94*
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	POS**
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	NEG***

* S89-94 should be reserved for qPCR standards if absolute quantification is desired.

** POS: The ZymoBIOMICS® Microbial Community DNA Standard² (included in kit) as a positive control.

*** NEG: A no template control as a negative control.

2. (Optional): If absolute quantification by real-time PCR is desired, add 2 µl of the serially diluted qPCR standard to the 6 wells highlighted above; S89-S94. Refer to Appendix A for more details.

(Continued on next page.)

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. See Appendix D for additional information.

² Prepare a master mix to include both a positive and negative control.

3. Add 2 μ l of your DNA samples to individual wells. Include a positive and negative control in the plate.
4. Apply an adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner¹.
5. Place plate in a real-time thermocycler² and run the program shown below:

Temperature	Time	} Lid Temp: 105°C Dye: SYBR Green 42 cycles
95°C	10 min	
95°C	30 sec	
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 thermocycler³.
 - a. For example, a sample that is expected to amplify and shows little or no amplification may indicate an error in the reaction setup (See the Troubleshooting Guide).
 - b. The negative control should not amplify before 35 cycles⁴. Earlier amplification of negative control might indicate process contaminations.
 - c. An example of qPCR amplification with controls is shown in Figure 5 below.

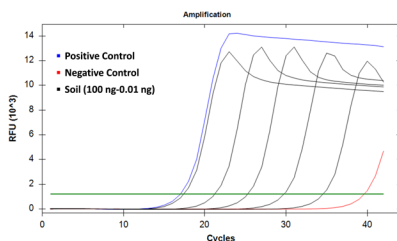


Figure 5. qPCR Amplification Example with Positive and Negative Controls. Serial dilution of soil DNA (black) from 100 to 0.01 ng to were amplified on a Bio-Rad CFX96™ Real-Time PCR Detection System. The positive (blue) amplified at 17.01 and negative (red) amplified at 39.60. Baseline threshold was 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 $\leq -20^{\circ}\text{C}$ for later use.

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 and absolute quantification as shown in Appendix A. A non-quantitative system can be used if quantification is not needed.

3 If real-time PCR was not used, after amplification perform PCR cleanup for a few samples plus positive control. Analyze on a TapeStation® to confirm correct amplicon size (~606 bp).

4 The PCR program runs for 42 cycles, so it is normal to see some amplification from the negative control. The negative control should be sequenced together with other samples. If appropriate for your project, the taxa from the negative control can be subtracted from the analysis.

Section 2: Pooling by Equal Volume

Add 50 µl of **PCR Inactivation Solution** into a new microcentrifuge tube. Pool equal volumes (5 µl¹) of PCR products from each well of the plate from **Section 1** into the tube² and mix well. Skip the wells of S89-S94 if they are used for qPCR standards. Proceed to **Section 3**.

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 rack³ 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 supernatant⁴.
6. While the beads are still on the magnetic rack, add 1 ml of **DNA Wash Buffer**. Remove and discard the supernatant. Repeat this step.
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.

(Continued on next page.)

¹ If pooling >96 samples, use 2 µl instead to accommodate all samples.

² There is no need for additional normalization procedures. To obtain better normalization and adjust pooling volumes, See Appendix B.

³ Alternatively, the provided Magnetic Rod can be used.

⁴ Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid behind.

9. Add 10-100 μl of **ZymoBIOMICS® DNase/RNase Free Water** to the beads and pipette mix thoroughly. Incubate at room temperature for 2 minutes.
10. Place the sample on a magnetic rack and incubate for 1 minute at RT, or until the magnetic beads have fully separated from eluate
11. Transfer supernatant to a clean microcentrifuge tube. Proceed to **Section 4.**

Section 4: Library Quantification

Use a fluorescence-based method (Qubit® dsDNA HS Assay Kit recommended) to quantify the final library. Using a final amplicon size of 480 bp, convert ng/ μl to nM using the equation below.

$$\frac{\text{concentration in ng/}\mu\text{l}}{660 \text{ g/mol} \times \text{average library size in bp}} \times 10^6 = \text{concentration in nM}$$

For example: 20 ng/ μl DNA of the final library is equivalent to 63.13 nM.

If preferred, a qPCR-based method for quantification may be used¹.

DNA Fragment Analysis (Not Required)

If a fragment analyzer (e.g. TapeStation®) is used to analyze the final library, there may be a lack of a tight band at ~480 bp. Because of the library prep design, some library products have run through additional PCR cycles and might not anneal well. They are perfectly fine for sequencing, which already denatures double-stranded DNA into single strands.

This is your final 16S library.

The ultra-pure pooled library DNA is now ready for use or storage at $\leq 20^\circ\text{C}$. Refer to platform-specific guidelines for preparation for sequencing. No custom sequencing primers are needed.

Illumina Loading Parameters:

For the MiSeq® Reagent Kit v3 (600-cycle), a final library loading concentration of 10-12 pM² 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 If pooling fewer than 10 samples, use 10 μl for elution.

² Optimal loading concentration may vary by instrument. Adjust final library loading concentrations as needed to reach a target cluster density of 700 K/mm².

Appendices

Appendix A: Absolute Quantification of Total ITS Copy Number by Real-Time PCR

Absolute quantification is determined with a real-time PCR standard curve using serially diluted amounts of the ZymoBIOMICS® 16S/ITS qPCR Standard.

1. Dilute the ZymoBIOMICS® 16S/ITS qPCR Standard (7.5×10^6 copies/ μ l) to 7.5×10^6 copies/ μ l, 7.5×10^4 copies/ μ l and 7.5×10^2 copies/ μ l.
2. Add 2 μ l of the standard and its dilutions to six wells (each in duplicate in positions S89-S94; see plate layout on Page 6) on the same plate that contains unknown samples for library prep.
3. After 1-Step PCR, plot the concentration (ITS copies/ μ l) of the standards against the Ct values to produce a standard curve. See Figure 6 and 7 for an example.
4. Calculate the total ITS copy number of unknown samples based on their Ct value with the standard curve accordingly¹.

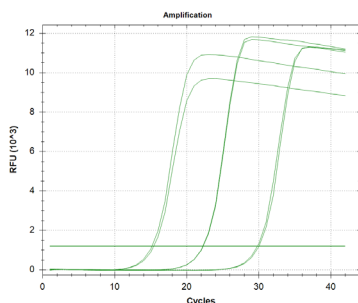


Figure 6. qPCR of Diluted Amounts of 16S/ITS Standard. The standard was amplified on a Bio-Rad CFX96™ Real-Time PCR Detection System. Baseline threshold was set at 1200 RFU.

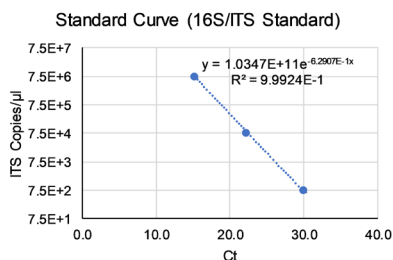


Figure 7. Absolute Quantification using a Standard Curve. Ct can be correlated to the total ITS copies based on the regression.

¹ Excel template for the Absolute Quantification of Total ITS Copy Number is available for download by visiting the Documentation section of the D6425 Product Page at www.zymoresearch.com.

Appendix B: Improving Read Distribution by Fine-Tuning Pooling Volumes based on qPCR

Typically, pooling samples by equal volumes will result in sufficient normalization and similar number of reads per sample. To obtain even better normalization, the pooling volumes can be adjusted based on Ct values obtained.

First, adjust the fluorescence threshold/cutoff to a certain value so that the positive control¹ has a Ct of 17. Use the following equation to calculate the pooling volume of each well based on its Ct: $\text{Volume } (\mu\text{l}) = 0.00104x^2 - 0.0965x + 3.736$; $x = \text{Ct}$. For example, the pooling volumes for Ct values of 15, 20, and 30 are 2.52 μl , 2.22 μl , and 1.78 μl respectively. This strategy can further reduce the CV (coefficient of variation) of reads assigned to samples by 3-6%.

Appendix C: Index Primer Sets

To accommodate sequencing projects of various sizes, Zymo Research offers primer sets that can uniquely barcode more than 96 samples. The barcodes of each index primer are distinct from one another by at least 5 bp to boost the accuracy of demultiplexing. For projects that require indexes for >96 samples, refer to Cat# D6425-PS2, D6425-PS3, & D6425-PS4 that contain primer sets 2, 3, & 4 respectively. The Guide for >96 Samples is available for download by visiting the Documentation section of the D6425 Product Page at www.zymoresearch.com.

Appendix D: Removal of PCR Inhibitors from DNA

The input DNA samples for the *Quick-ITS™* Plus NGS Library Prep Kit must be free of PCR inhibitors such as polyphenolics, humic/fulvic acids, tannins, melanin, etc. To further remove PCR inhibitors from purified DNA samples, Zymo Research recommends performing a one-step cleanup with the OneStep™ PCR Inhibitor Removal Kit. Additional information can be found by visiting the D6031 Product Page at www.zymoresearch.com.

Appendix E: Primer Trimming Recommendations

The first base 'G' in the ITS3f primer is shared with the Illumina adapter. For the primer trimming, use the following sequence, CATCGATGAAGAACGCAG, to remove the forward primer from read1. For example, when using cutadapt to remove primers, use the following command:

```
cutadapt -g ^CCTAYGGGDBGWCWGCAG -G ^GACTACNVGGGTMTCTAATCC  
-o trim_R1.fastq.gz -p trim_R2.fastq.gz R1.fastq.gz R2.fastq.gz
```

1 The positive control is the sample that uses 2 μl of the ZymoBIOMICS® Microbial Community DNA Standard.

Appendix F: Composition of ZymoBIOMICS® Microbial Community DNA Standard (50 ng)

The **ZymoBIOMICS® Microbial Community DNA Standard (50 ng)** is a mixture of genomic DNA extracted from pure cultures of eight bacterial and two fungal strains. Genomic DNA from each culture is quantified before mixing. The ZymoBIOMICS® Microbial Community DNA Standard allows for assessment of bias from library preparation, sequencing, and bioinformatics analysis. More information about the standard can be found below. For more detail, see D6305 Instruction Manual.

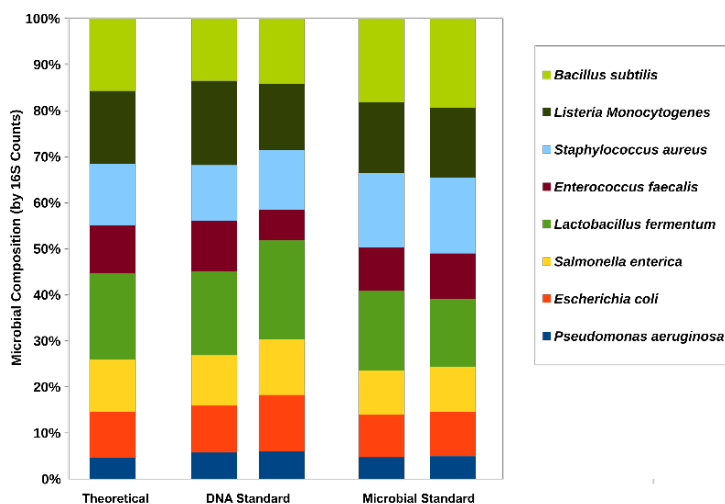


Figure 8. Accurate composition for reliable use to evaluate 16S rRNA sequencing. Characterization of the microbial composition of the two ZymoBIOMICS® standards by 16S rRNA gene targeted sequencing. The measured composition of the two standards agrees with the theoretical/designed composition. "DNA Standard" represents ZymoBIOMICS® Microbial Community DNA Standard and "Microbial Standard" represents ZymoBIOMICS® Microbial Community Standard. 16S composition by 16S rRNA gene targeted sequencing was calculated based on counting the amount of 16S raw reads mapped to each genome.

Appendix G: Illumina MiSeq® Sample Sheet Setup

A template for the Illumina MiSeq® sample sheet is available for download by visiting the Documentation section of the D6425 Product Page at www.zymoresearch.com. Fill in the project and sample information in the highlighted fields, then save the file in comma-separated values (CSV) format for use with the Illumina MiSeq®.

Ordering Information

Product Description	Catalog No.	Size / Format
Quick-ITS™ Plus NGS Library Prep Kit (UDI)	D6425-PS1	96 rxns. / Primer Set 1
	D6425-PS2	96 rxns. / Primer Set 2
	D6425-PS3	96 rxns. / Primer Set 3
	D6425-PS4	96 rxns. / Primer Set 4

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

Explore Other Microbiome Products

- ✓ 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

- ✓ Ultra-streamlined workflows offering the fastest targeted 16S library prep available, with only 30 minutes of hands-on time and no tedious normalization required:



Quick-16S/ITS™ Plus NGS Library Prep Kits

D6421	Quick-16S™ Plus NGS Library Prep Kit (V3-V4)	768 Indexes
D6425	Quick-ITS™ Plus NGS Library Prep Kit (ITS2)	384 Indexes
D6430	Quick-16S™ Plus NGS Library Prep Kit (V4)	384 Indexes
D6434	Quick-16S™ Plus NGS Library Prep Kit (V1-V2)	96 Indexes
D6440	Quick-16S™ Plus NGS Library Prep Kit (V1-V3)	96 Indexes

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Background Contamination	<p>Workspace contamination:</p> <ul style="list-style-type: none"> - Clean workspace, microcentrifuge, and pipettes with 10% bleach routinely to avoid contamination. - Use of kit in exposed environment without proper filtration can lead to back ground contamination. Check pipettes, pipette tips, microcentrifuge tubes, workspace, etc. for contamination - Make sure all reagent tubes and bottles are properly sealed for storage. Use of these outside a clean room or hood can result in contamination.
Loss of Volume during PCR	<p>Adhesive seal:</p> <ul style="list-style-type: none"> - A loosened adhesive seal on the PCR plate can lead to sample evaporation. Ensure that the plate seal is secure on every well during targeted sequence amplification. <p>Lid pressure:</p> <ul style="list-style-type: none"> - Inconsistent lid pressure. Ensure that the lid pressure on the real-time quantitative PCR instrument is consistent over the PCR plate according to the manufacturer's recommendation.
Unexpected or No Amplification of DNA Sample During PCR Program in Section 1	<p>Sample with high microbial DNA concentration:</p> <ul style="list-style-type: none"> - Reaction setup error. A sample that is expected to amplify but shows little or no amplification during the PCR program in Section 1 may indicate an error in the reaction setup. Use a new aliquot of the sample and repeat Section 1. <p>Sample with low microbial DNA concentration:</p> <ul style="list-style-type: none"> - Check negative control. A sample with little microbial DNA may not amplify before the negative control. Either use more concentrated DNA or use more DNA volume during reaction setup. <p>Abnormal qPCR curves:</p> <ul style="list-style-type: none"> - Proceed as normal. Abnormal qPCR amplification curves may occur and this is normal performance. This is usually a slight dip in RFU (forming a small "hump").
Diminished Amplicon Bands in Library Analysis	<p>No single amplicon peak and/or high background:</p> <ul style="list-style-type: none"> - Proceed as normal. There may be a lack of a single band and/or high background if using TapeStation® or similar methods to determine amplicon size. This is normal and is part of the library prep design. Do not use this sizing and quantification data. To properly quantify library, use a fluorescence-based method and calculation in Section 4.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

This image shows a single page of white paper with horizontal blue or grey ruling lines. The lines are evenly spaced and run across the width of the page, leaving small margins at the top and bottom. There are no vertical margin lines, text, or other markings on the page.

[illegible]



**100% satisfaction guarantee on all Zymo Research products,
or your money back.**

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

[™] Trademarks of Zymo Research Corporation, [®] Registered Trademarks of Zymo Research Corporation
Other trademarks: Bio-Rad CFX96[™] Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.), MiSeq[®] (Illumina, Inc.), TapeStation[®] (Agilent Technologies, Inc.), Qubit[®] (Thermo Fisher Scientific), Kapa[®] (Roche), TruMatrix[™] (The BioCollective).

*The **BEAUTY** of **SCIENCE** is to Make Things **SIMPLE**®*

