ZYMO RESEARCH

Microbiomics
Made Simple™

Quick-16S™ Full-Length Library Prep Kit

Simplest 16S library prep for long-read sequencing

Highlights

- The most robust, streamlined full-length 16S library preparation with as little as 30 minutes of hands-on time for 96 samples.
- Optimized for PacBio® sequencing with both SMRTbell® and Kinnex™ library prep kit compatibility while being adaptable to Oxford Nanopore Technologies® (ONT®) platforms.
- 100% automation ready with only a single PCR step, equal volume pooling, & multiplexing capacity up to 384 samples.

Catalog Numbers:
D6450-PS1, D6450-PS2, D6450-PS3, D6450-PS4

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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Product Contents

Quick-16S™ Full-Length Library Prep Kit	D6450 (96 rxns.)	Storage Temp.
Quick-16S™ Full-Length Index Premix Plate[1,2]	13 µL each well	-20°C
ZymoBIOMICS® Microbial Community DNA Standard (50 ng)	10 µL	-20°C
Select-a-Size™ MagBead Concentrate[3]	60 µL	4-8°C
Select-a-Size™ MagBead Buffer[3]	2 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	-

1 Contents of the plate are photosensitive. Protect from light whenever possible.
2 Cat# D6450-PS1, D6450-PS2, D6450-PS3, & D6450-PS4 contain primer sets 1, 2, 3, & 4 respectively.
3 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 –** Normalized[1] and purified microbial DNA, free of PCR inhibitors[2].

- **Full-Length 16S Primer Sequences –** 27f (AGRGTTYGATYMTGGCTCAG, 20 bp) and 1492r (RGYTACCTTGTTACGACTT, 19 bp). Sequencing adapters are included but not shown.

- **Primer Indexes –** Combinational dual indexes (barcodes) to uniquely label samples. Indexed primers are combined with the Polaris™ qPCR Premix (2X) within each well of the *Quick*-16S Full-Length Index Premix Plate.

- **Barcode Sequences –** 10 bp barcodes are available for download by visiting the documentation section of the D6450 Product Page at www.zymoresearch.com.

- **Amplicon Size –** The final amplicon size after 1-Step PCR (targeted amplification and barcode addition) is ~1500 bp.

- **Sequencing Platform –** PacBio® Sequel® II, Sequel® IIe, and Revio® Systems. ONT® PromethION®, GridION®, MinION®, & Flongle®.

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

  Note: Use of a plate format magnetic stand is highly recommended for users processing many samples according to the recommendations of **Appendix A**.

1 Normalization of input DNA to 1ng/µL prior to performing 1-Step PCR is recommended for robustness against potential PCR inhibition. Input DNA normalization is also critical for ensuring similar end PCR product formation which enables equal volume pooling of samples in downstream processing.
2 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.

Product Description

16S rRNA gene sequencing is a routine technique for microbiome composition profiling. Compared to shotgun metagenomics sequencing, 16S sequencing is often more cost-effective, robust, requires less input DNA, and is less impacted by the presence of non-microbial host DNA. Traditional 16S sequencing performed on short-read platforms suffers from the necessity of partial gene sequencing, which can reduce taxonomic resolution. With the advent of long-read sequencing platforms, full-length 16S gene sequencing enables taxonomic profiling with species or even strain level resolution while eliminating region-specific biases.

The ***Quick*-16S™ Full-Length Library Prep Kit** is streamlined, robust, and designed for use with long-read sequencing platforms. Distinguishing features of the kit are described below.

Robust & Streamlined Workflow. The *Quick*-16S™ Full-Length Library Prep Kit utilizes a single qPCR/PCR for combined targeted amplification and sample barcoding. The Polaris™ qPCR Premix (2x) within each reagent plate allows for easy PCR setup at ambient temperatures[1], high fidelity polymerization, and qPCR traceability. Using high quality, normalized microbial DNA enables equal volume pooling of PCR products, and a single-tube cleanup of the pooled library. For samples that are poorly normalized, PCR inhibited, or contain high levels of non-microbial DNA, additional processing may be required (Figure 1).

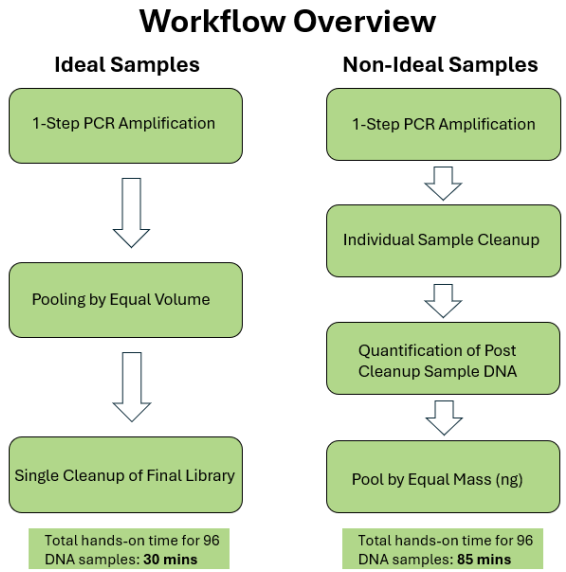

Figure 1. *Quick*-16S™ Full-Length Library Prep Kit Workflow Overview. Total hands-on time calculations are based on the preparation of 96 DNA samples.

1 The *Quick*-16S Full-Length Index Premix Plates use Polaris™ qPCR Premix (2x) which does not require PCR setup to be performed on ice. Additionally, the premixed reagents require only the addition of DNA template.

Low Read Distribution Variation using Equal Volume Pooling.
When sample DNA inputs are free of PCR inhibitors and free of non-microbial DNA, accurate quantification and normalization of DNA inputs enables equal volume pooling of PCR products resulting in low variation of read distribution (Figure 2).

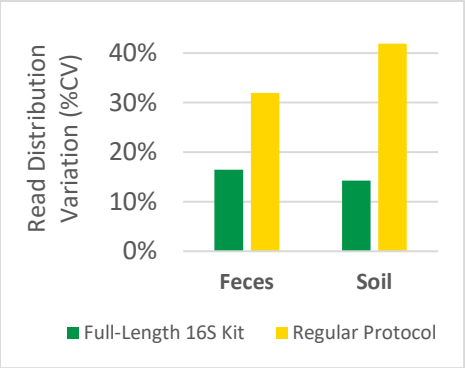

Figure 2. Read Distribution Variation of Full-Length 16S Kit vs. Regular Protocol

DNA from 32 fecal and 10 soil samples was extracted, quantified, and normalized to 1ng/µL. Library prep was performed on the same set of samples according to the Full-Length 16S Library Prep Kit Protocol and the regular PacBio® protocol[1]. Amplicon libraries were processed using the SMRTbell® Prep Kit 3.0 and sequenced on an 8M SMRT cell using the Sequel® IIe instrument. The read distribution variation for each data set is reported as:

$$\frac{\text{Std. Deviation of Sequencing Reads}}{\text{Averaege Sequencing Reads}} \times 100\%$$

Compatibility with both SMRTbell® & Kinnex™ Library Prep Kits. Innovative primer design allows for compatibility of *Quick*-16S™ full-length libraries with both the traditional SMRTbell® Prep Kit 3.0 as well as the Kinnex™ 16S rRNA Kit. For users with larger throughput requirements, the Kinnex™ kit enables concatenation of PCR barcoded 16S amplicons results in a dramatic increase in HiFi Reads (Figure 3). The Kinnex™ kit also scales up the multiplexing capacity of the workflow from 384 PCR barcode combinations to 1,536.

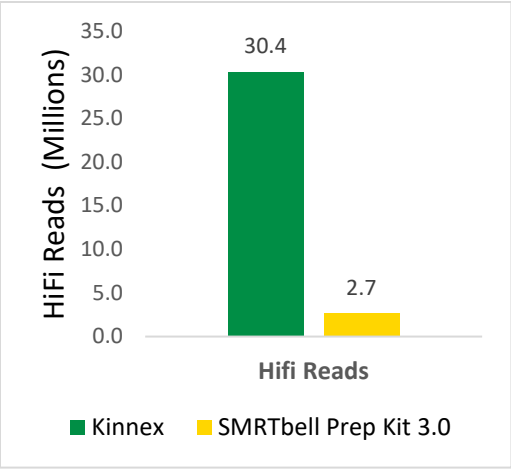

Figure 3. Kinnex™ 16S rRNA Kit vs. SMRTBell® Prep Kit 3.0 HiFi Reads.

Concatenated amplicon sequencing of the ZymoBIOMICS® Fecal Reference with TruMatrix™ Technology via the Kinnex™ 16S rRNA kit demonstrates ~11 times more HiFi reads compared to single 16S gene sequencing. The sample processing and data analysis for this comparison were performed and reported by PacBio®. More information regarding the Kinnex™ 16S rRNA Kit and it's applications can be found within the following PacBio® blog:

Watch: Kinnex kits enable full-length 16S and RNA sequencing at scale - PacBio

1 See original PacBio® protocol at the following weblink for additional information: Procedure & checklist - Amplification of bacterial full-length 16S rRNA gene with barcoded primers (pacb.com)

Accurate Microbial Profiling

The *Quick*-16S™ Full-Length Library Prep Kit includes the ZymoBIOMICS® Microbial Community DNA Standard, which is a quantified mixture of genomic DNA extracted from pure cultures of eight bacterial and two fungal strains. The ZymoBIOMICS® Microbial Community DNA Standard serves as positive control to assess biases introduced during library preparation and their effects on accurate microbial profiling. Library prep and sequencing of the standard using the *Quick*-16S™ Full-Length Library Prep Kit reveals minimal introduction of biases and a high degree or consistency between the theoretical and measured microbial profiles as highlighted in Figure 4 below.

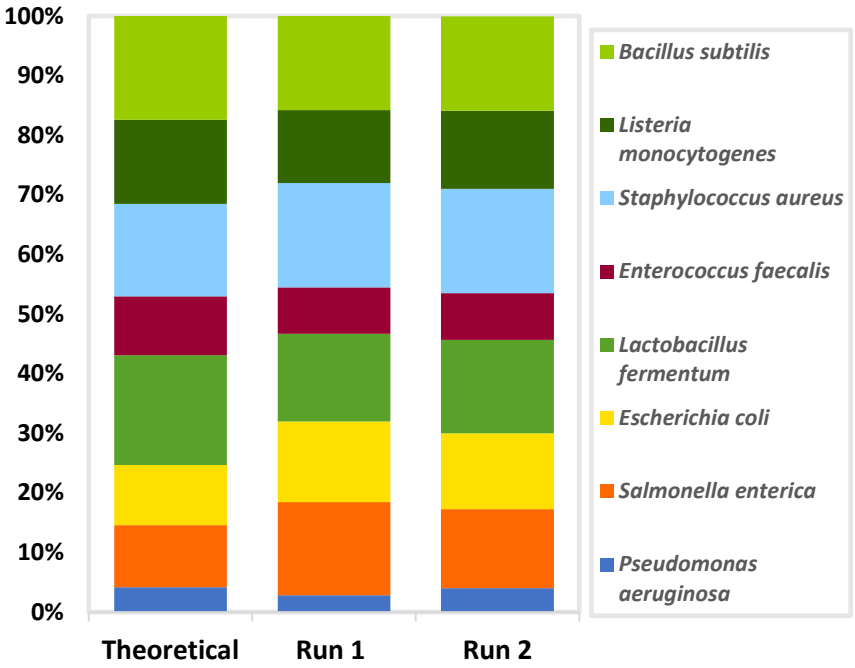

Figure 4. Microbial Profiles of ZymoBIOMICS® Community DNA Standard. The microbial profiles of two independent preparations of the ZymoBIOMICS® Microbial Community DNA Standard. In each run, the standard was normalized to 1 ng/µL, amplified for 25 cycles, and pooled by equal volume alongside other samples to mimic normal usage as a positive control. The pooled libraries for each run were processed using the SMRTbell® Prep Kit 3.0 and sequenced on an 8M SMRT™ cell using the Sequel® IIe instrument.

Protocol

Before Starting

- ✓ **Preparing More than 96 Samples.** Users who wish to process more than 96 samples will need to purchase additional primer sets. See **Appendix C** for more information**.**
- ✓ **Input DNA Guidelines.** All DNA samples should be free of PCR inhibitors[1] and ideally contain pure *microbial* DNA. Additionally, all DNA samples should be normalized to equal concentration (1ng/µL recommended).
- ✓ **Quantification Guidelines.** Use of a fluorescent quantification method (Qubit®) for input DNA normalization is recommended. Spectroscopic quantification methods (Nanodrop®) can be used for higher biomass samples but are often inaccurate when measuring concentrations below 10 ng/µL.
- ✓ **Non-Ideal Sample Types.** For very low biomass and/or high host DNA containing sample types that make it difficult or impossible to normalize *microbial* DNA to 1 ng/µL, (i.e. skin swabs, tissue samples, wastewater, etc.) see **Appendix A** for additional processing instructions.

Section 1: 1-Step PCR with Polaris™ qPCR Premix

1. Pierce the foil and transfer 8 µL of premix to a new plate for each well of the ***Quick*-16S™ Full-Length Index Premix 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***

** POS: Normalized **ZymoBIOMICS® Microbial Community DNA Standard**.
*** NEG: A no template control as a negative control.

(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. Add 5 µL of the **ZymoBIOMICS® Microbial Community DNA Standard** to 20 µL[1] of water and mix well by pipetting or vortexing. This will serve as the positive control in the plate layout above.

3. Add 2 µL of sample and positive control DNA to their appropriate wells. Add 2 µL of water to serve as an NTC for the negative control.

4. Apply an adhesive PCR plate seal and mix the plate on a plate shaker. Briefly centrifuge in a plate spinner to collect liquid at the bottom of each well.

5. Place plate into thermal cycler (qPCR recommended) and run the program shown below[2]:

Temperature	Time	
95°C	3 min	Lid Temp: 105°C Dye: SYBR Green
95°C	30 sec	25 cycles
62°C	30 sec	
72°C	1 min	
Plate read	-	
4°C	Hold	

Once the samples have cooled to 4°C, stop the program.

6. (Optional): Analyze qPCR amplification to assess equal product formation and expected performance of controls. Samples demonstrating greater than a 3 Ct deviation from the average can be considered **PCR outliers** not fit for equal volume pooling. See **Appendix B** for instructions on how to process PCR Outliers for optimal end read distributions.[3]

   Note: PCR Outlier identification can be expedited by inputting **Section 1** Ct data into the "Outlier Sample Identification" worksheet of the Supplemental Protocol Workbook. This document is available for download on the D6450 product page at http://www.zymoresearch.com.

7. Centrifuge plate in a plate spinner to collect condensation in wells and place plate on ice. Proceed to **Section 2**, the **Supplemental Protocol** (pg. 10), or store plate at ≤-20°C for later use.

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1 & 2: If following the **Low Microbial DNA** protocol outlined in **Appendix A**, normalize positive control to 0.1 ng/µL by using 245 µL of water in step 2 and use 30 cycles instead of 25 cycles in Step 5.
3 Alternatively, read variations may be ignored in favor of pooling all samples by equal volume in **Section 2**.

Section 2: Pooling by Equal Volume

In a fresh microcentrifuge tube, add 50 µL[1] of **PCR Inactivation Solution.** Pool equal volumes (5 µL)[2] of PCR product from each well of the PCR plate (including positive control) into the tube. Mix well and proceed to **Section 3**.

Section 3: Pooled 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 shaking or pipetting 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[3] 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.[4]
6. While the beads are still on the magnetic rack, add 1 mL of **DNA Wash Buffer**. Incubate for 30 seconds before removing and discarding the supernatant. Repeat this step.
7. While the beads are still on the magnetic rack, aspirate out any residual **DNA Wash Buffer** with a 10 µL pipette tip.
8. Remove the 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 & 2: If using multiple primer sets concurrently, add 50 µL of **PCR Inactivation Solution** per primer set of 96 samples. Additionally, pooling volume may be reduced to 2 µL from each sample.
3 Alternatively, the provided Magnetic Rods 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 50 µL[1] of **ZymoBIOMICS® DNase/RNase Free Water** to the beads and vortex or pipette mix thoroughly. Incubate at room temperature for 2 minutes.

10. Place the sample on a magnetic rack and incubate for 1 minute at room temperature, or until the magnetic beads have fully separated from eluate.

11. Transfer supernatant to a clean microcentrifuge tube. Proceed to **Section 4** or store pooled, cleaned libraries at 4°C for up to a week or -20°C for up to a year.

Section 4: Library Quantification & Downstream Processing

Characterizing Post-cleanup Pooled Library

Quantify the pooled library using a Qubit® dsDNA HS Assay Kit. Use a TapeStation®, Fragment Analyzer®, or similar method to verify that final libraries are ~1500bp. The final library can be stored at 4°C for up to a week or -20°C for up to a year.

Downstream Processing via SMRTbell® Prep Kit 3.0

Dilute the pooled library to achieve a 5-10ng/µL concentration in a 50 µL volume[2]. Transfer 46µL of library to a 0.2 mL PCR strip tube and proceed beginning at Step 2.1 of the SMRTbell prep kit 3.0 protocol.

Downstream Processing via Kinnex™ 16S rRNA Kit

The pooled library is ready for direct use beginning at step 3.1.1 of the Kinnex 16S rRNA Kit Protocol. If the pooled library is highly concentrated, it may require dilution to enable accurate addition of 35ng of library to the master mix in Step 3.2.1.

Downstream Processing via ONT® Ligation Sequencing Kit V14

The pooled library can now be used directly by preparing 180ng of product in a 49 µL volume as described by Section 3 of each platform-specific Ligation Sequencing Amplicons V14 (SQK-LSK114) Protocol.

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1 Increase the elution volume by 50 µL for every additional set of ≤ 96 samples prepped (i.e. A 240 sample pool should be eluted in 150 µL). Alternatively, if prepping less than 48 samples, elute in as low as 30 µL.
2 This concentration range ensures that the total library input introduced into the SMRTbell Prep Kit protocol comfortably exceeds the recommended minimum input of 150 ng. If necessary, lower concentrations can be used provided they still meet or exceed the 150 ng minimum input threshold.

Supplemental Protocol: Processing Section 1 PCR Outliers

*Identification of PCR Outliers may be expedited by inputting **Section 1** PCR data into the "Outlier Sample Identification" worksheet of the Supplemental Protocol Workbook. This document is available for download on the Quick-16S™ Full-Length Library Prep Kit (D6450) product page at http://www.zymoresearch.com*

1. Identify the outlier PCR reactions demonstrating **late** or **no** amplification from the **Section 1** PCR plate and transfer their full volume (10 µL) to a new PCR plate[1] labeled "**Outlier Samples**". (Note: In the absence of any **late** or **no** amplification outliers, proceed directly to step 4 below).

2. Seal and spin down the **Outlier Sample Plate** and perform additional cycling according to the following protocol:

Temperature	Time	
95°C	3 min	Lid Temp: 105°C Dye: SYBR Green
95°C	30 sec	5 cycles
62°C	30 sec	
72°C	1 min	
Plate read	-	
4°C	Hold	

3. 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.

4. Identify and transfer any outlier reactions demonstrating **early** amplification from the **Section 1** PCR plate to the **Outlier Sample Plate.**

5. The **Outlier Sample Plate** may now proceed to **Supplemental Protocol: Individual Reaction Cleanup** while the remaining samples of the **Section 1** PCR plate can proceed to **Section 2** of the main protocol. Either or both plates may be stored at ≤-20°C for later use.

1 PCR tube-strips may be used in place of a new PCR plate when working with few samples or if preferrable for ease of use with magnetic racks.

Supplemental Protocol: Individual Reaction Cleanup

When working with 16 or more samples, consider aliquoting the prepared cleanup reagents (magbeads, wash buffer, and water) into 8-tube strips for ease of use with a multichannel pipette.

6. Add 10 µL of **ZymoBIOMICS® DNase/RNase Free Water** to each sample.
7. 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**.[1] Resuspend the magnetic particles by vigorously shaking until homogenous.
8. Add 16 µL of **Select-a-Size™ MagBeads** to each well and mix by pipette or by sealing and shaking plate until beads and sample are homogenized. Incubate 5 minutes at room temperature.
9. Place the samples on a magnetic rack and incubate until the magnetic beads have fully separated from solution.
10. While the beads are magnetized, remove and discard the supernatant. Add 50 µL of **DNA Wash Buffer** to each well and incubate 30 seconds.
11. Carefully remove and discard the supernatant. Be sure to aspirate out any residual buffer with a 10 µL pipette tip if necessary.[2]
12. Elute each sample in 20 µL of **ZymoBIOMICS® DNase/RNase Free Water**.
13. Remove samples from magnetic rack and pipette mix vigorously to fully resuspend and homogenize the beads.
14. Incubate at room temperature for 5 minutes before placing the samples on the magnetic rack until beads are fully cleared from solution.
15. Transfer the eluted DNA of each sample to a new PCR plate.

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1 When 60 or more samples require cleanup, prepare and mix both tubes of Select-a-Size™ Magbead Buffer and Concentrate to provide enough magbeads for all samples.
2 To prevent drying out beads when processing many samples, remove and discard wash buffer from no more than 4 columns of the plate (36 samples) at a time before proceeding to step 12 and immediately adding 20 µL of water to each of those samples.

Supplemental Protocol: Pooling Outlier & Main Protocol Samples

The steps below require the use of the Supplemental Protocol Workbook to perform necessary pooling calculations. This document is available for download on the Quick-16S™ Full-Length Library Prep Kit (D6450) product page at http://www.zymoresearch.com.

16. Quantify the eluted DNA of each outlier sample and input the concentration values into the highlighted cells under step 1 of the Supplemental Protocol Workbook: **Pooling Calculations** worksheet.

17. Adjust the pooling mass in step 2 of the worksheet until the pooling volumes reported in section 3 fall within the recommended range of 1-19 µL.[1]

18. In a 1.5 mL microcentrifuge tube, pool the appropriate volumes of each outlier sample as indicated in step 3 of the worksheet.

19. Thoroughly mix the outlier sample pool by vortexing or pipette mixing before quantifying the pool using a Qubit® dsDNA HS Assay Kit.

    Note: If processing samples according to **Appendix A,** this is your final library which may proceed to **Section 4** for downstream processing.

20. In step 4 of the worksheet, input the pool concentration for both the Outlier Sample pool and the Main Protocol sample pool measured in **Section 4**.

21. Input the number of samples pooled during Section 2 of the main protocol into the appropriate cell in section 4 of the worksheet. Note that the number of samples pooled for the Outlier Sample pool is already calculated from section 1 of the worksheet.

22. Normalize the **per-sample** concentrations of the Main Protocol & Outlier Sample pools by diluting the higher concentration pool according to the specified volumes in section 5 of the worksheet.

23. Combine an equal volume (at least 30 µL) of the normalized Outlier Sample and Main Protocol pools in a 1.5 mL microcentrifuge tube. This combined pool is the final library and is now ready for storage or downstream processing as described in **Section 4**.

1 Severe concentration differences between samples may result in inability to generate pooling volumes within the recommended range. In such cases, users are advised to either dilute highly concentrated samples or simply pool all available volume from lower concentration samples.

Appendices

Appendix A: Processing Non-Ideal Samples

The main protocol has been optimized for sample types containing high levels of pure microbial DNA such as fecal, soil, and pure culture samples. Non-ideal sample types are defined as those containing high levels of non-microbial host DNA, very dilute microbial DNA, or a combination of the two. Please refer to the table and flowchart below to determine the most appropriate processing for non-ideal samples.

Non-Ideal Sample Workflow

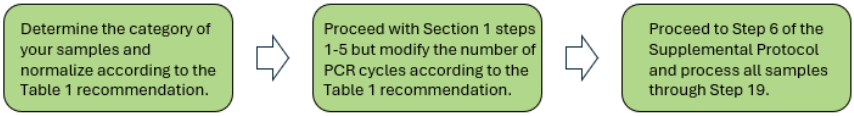


Table 1 – Processing Recommendations for Non-Ideal Samples

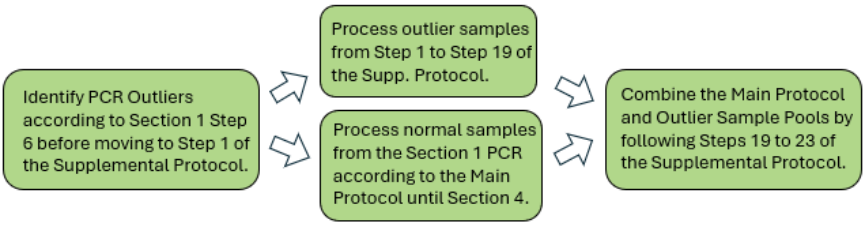
Sample Category	Example of DNA Sample Sources	Input DNA Normalization	Recommended Cycle Number
High Host DNA	Intestinal tissue, whole insect	No normalization	25 Cycles
Low Microbial DNA	Water samples, surface swabs	0.1 ng/µL when possible[1]	30 Cycles
Low Microbial DNA & High Host	Skin swabs, cheek swabs	No normalization	30 Cycles

Technical Note:

For high-throughput users working with non-ideal sample types, we recommend the usage of the n6 Tec IconPCR™ instrument which eliminates the complications non-ideal sample types via auto-normalizing PCR.

Appendix B: Processing Section 1 PCR Outliers

The following workflow diagram illustrates how to proceed with sample processing in situations where a portion of the samples demonstrate outlier PCR performance as described by the criteria in Section 1 Step 6 of the main protocol.



1 When working with a mixed group of samples where some are above and some are below the 0.1 ng/µL threshold, dilution of the higher concentration samples is recommended to reduce PCR chimera formation over the course of 30 PCR cycles.

Appendix C: Index Primer Sets

To accommodate sequencing projects of various sizes, Zymo Research offers additional, non-overlapping primer sets that support combinational barcoding of 96 samples per primer set. For projects that require indexes for >96 samples, refer to Cat# D6450-PS2, D6450-PS3, & D6450-PS4 that contain primer plates 2, 3, & 4 respectively. The "Guide for Preparing More than 96 Samples" document is available for download by visiting the Documentation section of the *Quick-16S*™ Full-Length Library Prep Kit (D6450) Product Page at www.zymoresearch.com.

Appendix D: Removal of PCR Inhibitors from Starting DNA

The sample input DNA for the *Quick-16S*™ Full-Length 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 OneStep™ PCR Inhibitor Removal Kit (D6031) Product Page at www.zymoresearch.com.

Appendix E: SMRTLink Run Design Setup

Kinnex-16S rRNA Kit Libraries

For Kinnex-16S libraries prepared as described in **Section 4**, select "MAS-Seq 16S rRNA" as the application type from the "Application" drop-down menu when creating a run design. In the "Barcoded Sample Options" drop-down menu[1], select "MAS SMRTbell barcoded adapters (v2)" as the index primer set. If opting to include automatic Read Segmentation analysis, select "MAS-Seq Adapter v2 (MAS12) as the Segmentation Adapter Set. Final amplicon-level demultiplexing should be performed using the "Kinnex 16S 384-plex primers" index primer set.

SMRTbell Prep Kit 3.0 Libraries

For SPK 3.0 libraries prepared as described in **Section 4**, select "Full-Length 16S rRNA Sequencing" as the application type from the "Application" drop-down menu when creating a run design. In the "Barcoded Sample Options" drop-down menu[1], select "Kinnex 16S 384-plex primers" as the index primer set.

Appendix F: Sample Sheet Setup

PacBio® Sequel II/IIe/Revio® sample sheet templates are available for download in both 96 and 384 sample formats by visiting the Documentation section of the 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.

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1 On the Revio platform, the "Barcoded Sample Options" information is shown under the "Samples" section.

Ordering Information

Product Description	Catalog No.	Size / Format
***Quick-16S*™ Full-Length Library Prep Kit**	D6450-PS1	96 rxns. / Primer Set 1
	D6450-PS2	96 rxns. / Primer Set 2
	D6450-PS3	96 rxns. / Primer Set 3
	D6450-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 DNA Standard (200 ng)	D6305	200 ng
ZymoBIOMICS® Microbial Community DNA Standard (2000 ng)	D6306	2000 ng

Cleanup Components	Catalog No.	Amount
Select-a-Size™ DNA Clean & Concentrator Kit	D4080	25 preps
Select-a-Size™ DNA Clean & Concentrator MagBead Kit	D4084 D4085	10 mL 50 mL

Explore Other Microbiome Products

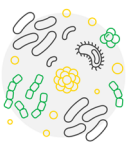
✓ To collect and transport samples at ambient temperatures:

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

✓ 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

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