Microbiomics
Made Simple™

Quick-16S™ Plus NGS Library Prep Kit (V4) - 384 Preps

Fastest, normalization-free 16S library prep

Highlights

- **Fast:** 8 times less hands-on time than conventional 16S library prep protocols.
- **Easy:** Premixed Plate minimizes hands-on time and handling errors.
- **Normalization-free:** 100% automation ready with only a single PCR step and no need for normalization. Just pool by equal volume!

Catalog Numbers:
D6432

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

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Product Contents

Quick-16S™ Plus NGS Library Prep Kit (V4)	D6432 (384 rxns.)	Storage Temp.
V4 Targeted Amplification Plate[1]	8 µl each well	-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[2]	30 µl	-20°C
ZymoBIOMICS™ Microbial Community DNA Standard (50 ng)	10 µl	-20°C
***Select-a-Size*™ MagBead Concentrate**[3]	30 µl	4-8°C
***Select-a-Size*™ MagBead Buffer**[3]	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 Rods	4	-
Instruction Manual	1 pc	-

[1] Protect the plate from light.
[2] Index 2 primer is included for sequencers that require an i5 index sequencing primer.
[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 –** Purified microbial DNA (≤100 ng), free of PCR inhibitors.

- **V4 Primer Sequences (adapters not included) –** 515f (GTGYCAGCMGCCGCGGTAA) and 806r (GGACTACNVGGGTWTCTAAT).

- **Index Sequences –** 10 bp indexes are listed in the MiSeq Sample Sheet Template provided. The template is available for download by visiting the Documentation section of the Product Page at www.zymoresearch.com.

- **Amplicon Size –** The final amplicon size after 1-Step PCR (targeted amplification and barcode addition) is ~388 bp.

- **Sequencing Platform –** Compatible with all Illumina® sequencing platforms using custom sequencing primers. We recommend the MiSeq® Reagent Kit v2 (300-cycle).

- **Equipment Needed** (user provided) **–** Microcentrifuge, plate spinner (centrifuge), 384-well real-time quantitative PCR system. The included plate is compatible with the following Applied Biosystems™ PCR systems: ProFlex, Veriti, VeritiPro™, 9700; QuantStudio 5, 6, 6 Pro, 7, 7 Pro, 12K; ViiA 7; 7900 HT.

- **V4 Targeted Amplification Plate –** Each position on the targeted amplification plate contains 5 µl of Equalase™ qPCR Premix, 1 µl of water, and 1 µl of each uniquely indexed (UDI) 515f and 806r V4 primer. Total reaction volume is 10 µl upon addition of 2 µl DNA as per the protocol.

- **V4 UDI Plate Layout –**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	ZB0001	ZB0193	ZB0009	ZB0201	ZB0017	ZB0209	ZB0025	ZB0217	ZB0033	ZB0225	ZB0041	ZB0233	ZB0049	ZB0241	ZB0057	ZB0249	ZB0065	ZB0257	ZB0073	ZB0265	ZB0081	ZB0273	ZB0089	ZB0281
B	ZB0097	ZB0289	ZB0105	ZB0297	ZB0113	ZB0305	ZB0121	ZB0313	ZB0129	ZB0321	ZB0137	ZB0329	ZB0145	ZB0337	ZB0153	ZB0345	ZB0161	ZB0353	ZB0169	ZB0361	ZB0177	ZB0369	ZB0185	ZB0377
C	ZB0002	ZB0194	ZB0010	ZB0202	ZB0018	ZB0210	ZB0026	ZB0218	ZB0034	ZB0226	ZB0042	ZB0234	ZB0050	ZB0242	ZB0058	ZB0250	ZB0066	ZB0258	ZB0074	ZB0266	ZB0082	ZB0274	ZB0090	ZB0282
D	ZB0098	ZB0290	ZB0106	ZB0298	ZB0114	ZB0306	ZB0122	ZB0314	ZB0130	ZB0322	ZB0138	ZB0330	ZB0146	ZB0338	ZB0154	ZB0346	ZB0162	ZB0354	ZB0170	ZB0362	ZB0178	ZB0370	ZB0186	ZB0378
E	ZB0003	ZB0195	ZB0011	ZB0203	ZB0019	ZB0211	ZB0027	ZB0219	ZB0035	ZB0227	ZB0043	ZB0235	ZB0051	ZB0243	ZB0059	ZB0251	ZB0067	ZB0259	ZB0075	ZB0267	ZB0083	ZB0275	ZB0091	ZB0283
F	ZB0099	ZB0291	ZB0107	ZB0299	ZB0115	ZB0307	ZB0123	ZB0315	ZB0131	ZB0323	ZB0139	ZB0331	ZB0147	ZB0339	ZB0155	ZB0347	ZB0163	ZB0355	ZB0171	ZB0363	ZB0179	ZB0371	ZB0187	ZB0379
G	ZB0004	ZB0196	ZB0012	ZB0204	ZB0020	ZB0212	ZB0028	ZB0220	ZB0036	ZB0228	ZB0044	ZB0236	ZB0052	ZB0244	ZB0060	ZB0252	ZB0068	ZB0260	ZB0076	ZB0268	ZB0084	ZB0276	ZB0092	ZB0284
H	ZB0100	ZB0292	ZB0108	ZB0300	ZB0116	ZB0308	ZB0124	ZB0316	ZB0132	ZB0324	ZB0140	ZB0332	ZB0148	ZB0340	ZB0156	ZB0348	ZB0164	ZB0356	ZB0172	ZB0364	ZB0180	ZB0372	ZB0188	ZB0380
I	ZB0005	ZB0197	ZB0013	ZB0205	ZB0021	ZB0213	ZB0029	ZB0221	ZB0037	ZB0229	ZB0045	ZB0237	ZB0053	ZB0245	ZB0061	ZB0253	ZB0069	ZB0261	ZB0077	ZB0269	ZB0085	ZB0277	ZB0093	ZB0285
J	ZB0101	ZB0293	ZB0109	ZB0301	ZB0117	ZB0309	ZB0125	ZB0317	ZB0133	ZB0325	ZB0141	ZB0333	ZB0149	ZB0341	ZB0157	ZB0349	ZB0165	ZB0357	ZB0173	ZB0365	ZB0181	ZB0373	ZB0189	ZB0381
K	ZB0006	ZB0198	ZB0014	ZB0206	ZB0022	ZB0214	ZB0030	ZB0222	ZB0038	ZB0230	ZB0046	ZB0238	ZB0054	ZB0246	ZB0062	ZB0254	ZB0070	ZB0262	ZB0078	ZB0270	ZB0086	ZB0278	ZB0094	ZB0286
L	ZB0102	ZB0294	ZB0110	ZB0302	ZB0118	ZB0310	ZB0126	ZB0318	ZB0134	ZB0326	ZB0142	ZB0334	ZB0150	ZB0342	ZB0158	ZB0350	ZB0166	ZB0358	ZB0174	ZB0366	ZB0182	ZB0374	ZB0190	ZB0382
M	ZB0007	ZB0199	ZB0015	ZB0207	ZB0023	ZB0215	ZB0031	ZB0223	ZB0039	ZB0231	ZB0047	ZB0239	ZB0055	ZB0247	ZB0063	ZB0255	ZB0071	ZB0263	ZB0079	ZB0271	ZB0087	ZB0279	ZB0095	ZB0287
N	ZB0103	ZB0295	ZB0111	ZB0303	ZB0119	ZB0311	ZB0127	ZB0319	ZB0135	ZB0327	ZB0143	ZB0335	ZB0151	ZB0343	ZB0159	ZB0351	ZB0167	ZB0359	ZB0175	ZB0367	ZB0183	ZB0375	ZB0191	ZB0383
O	ZB0008	ZB0200	ZB0016	ZB0208	ZB0024	ZB0216	ZB0032	ZB0224	ZB0040	ZB0232	ZB0048	ZB0240	ZB0056	ZB0248	ZB0064	ZB0256	ZB0072	ZB0264	ZB0080	ZB0272	ZB0088	ZB0280	ZB0096	ZB0288
P	ZB0104	ZB0296	ZB0112	ZB0304	ZB0120	ZB0312	ZB0128	ZB0320	ZB0136	ZB0328	ZB0144	ZB0336	ZB0152	ZB0344	ZB0160	ZB0352	ZB0168	ZB0360	ZB0176	ZB0368	ZB0184	ZB0376	ZB0192	ZB0384

Primer Plate A
Primer Plate B
Primer Plate C
Primer Plate D

Product Description

16S rRNA gene sequencing is a routine technique for microbiome composition profiling. Compared to shotgun metagenomics sequencing, 16S 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, 16S rRNA gene sequencing has its own challenges. Common 16S library preparation protocols have not been optimized to be cost-effective for large-scale applications.

The ***Quick*-16S™ Plus NGS Library Prep Kit (V4)** is the fastest and simplest library prep method for high-throughput 16S rRNA sequencing. Distinguishing features of the kit are described below.

Fastest 16S Workflow. The *Quick*-16S™ Plus NGS Library Prep Kit (V4) 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 is not necessary. The addition of custom sequencing primers allows the library to be sequenced on any Illumina 300 cycle kit and ensures there is sufficient overlap between paired reads. With this workflow, the hands-on time of 16S library preparation is reduced to only 30 minutes (Figure 1).

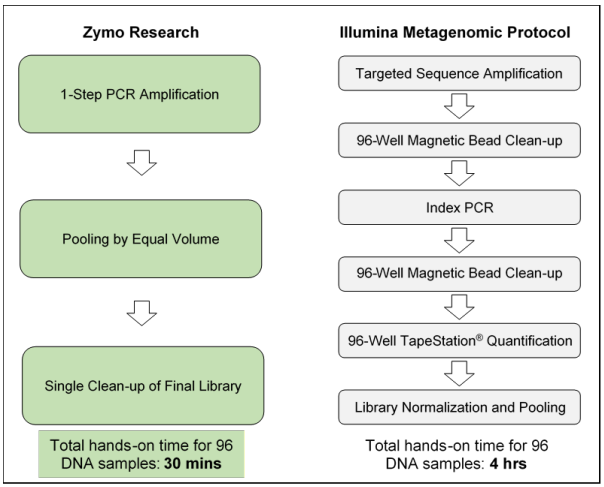

Figure 1. *Quick*-16S™ Plus NGS Library Prep Kit (V4) workflow versus the Illumina Metagenomic Protocol. Total hands-on time calculations are based on the preparation of 96 DNA samples.

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

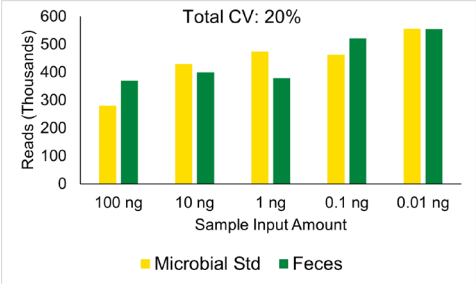

Figure 2. The *Quick*-16S™ Plus NGS Library Prep Kit (V4) results in similar amounts of reads across different input amounts without normalization. 0.01-100 ng of ZymoBIOMICS™ Microbial Community DNA Standard and fecal DNA were used as inputs. Libraries were pooled by equal volumes (2 µl each) without further normalization and sequenced using the MiSeq® Reagent Kit v2 (300-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).

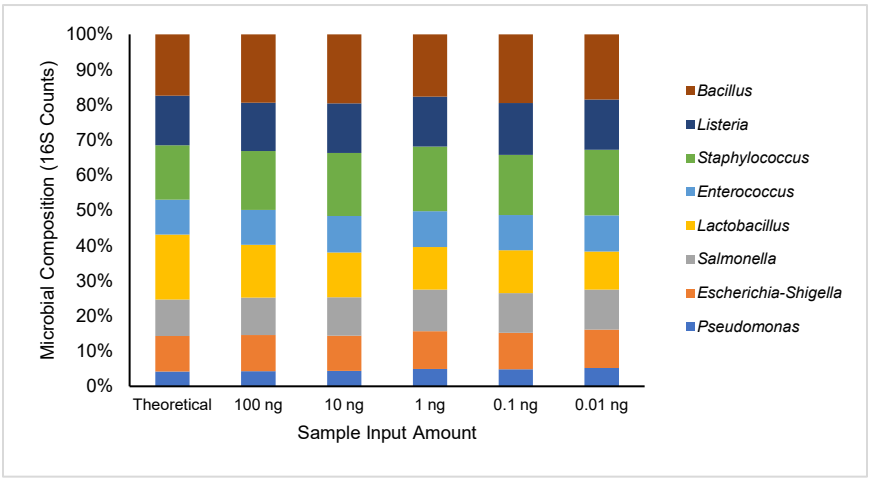

Figure 3. Benchmarked performance with ZymoBIOMICS™ Microbial Community DNA Standard. Bacterial composition profiles are accurate with inputs from 100 ng down to 0.01 ng.

Protocol

Before Starting

- ✓ **Sample Quantity Requirement.** To ensure color balance in index sequencing, a minimum of 9 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 optimal performance and robustness against potential PCR inhibition.
- ✓ **Sequencing Primer Requirement.** This kit utilizes custom sequencing primers that must be spiked into your Illumina cartridge prior to sequencing.

Section 1: 1-Step PCR

1. Add 2 µl of your DNA samples into the individual wells of the V4 Targeted Amplification Plate. Include a positive and negative control in the plate.

2. Apply an adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner[2].

3. Place plate in a real-time thermocycler[3] and run the program shown below:

Temperature	Time	
95°C	10 min	
95°C	30 sec	42 cycles[4]
55°C	30 sec	
72°C	3 min	
Plate read	-	
4°C	Hold	

(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.
[2] PCR reactions can be pipette mixed if a plate shaker is not available.
[3] 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.
[4] The number of cycles can be adjusted to further reduce library prep time. See **Appendix B** for more details.

4. Monitor and QC the library preparation when running the reaction on a real-time thermocycler.[1]

   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 cycles.[2] Earlier amplification of negative control may indicate process contaminations.

   c. An example of qPCR amplification with controls is shown in Figure 5 below.

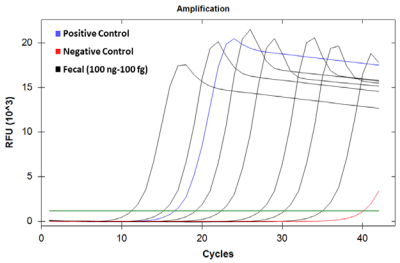

Figure 5. qPCR Amplification Example with Positive and Negative Controls. Serial dilutions of fecal DNA (black) from 100 ng to 100 fg were amplified on a Bio-Rad CFX96™ Real-Time PCR Detection System. The positive (blue) amplified at 16.59 and negative (red) amplified at 40.12. Baseline threshold was set at 1200 RFU.

5. 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. The plate will be stable at -20°C for up to a month.

Section 2: Pooling by Equal Volume

Add 50 µl of **PCR Inactivation Solution** into a new microcentrifuge tube.[3] Pool equal volumes (2-8 µl) of PCR products from each well of the plate from **Section 1** into the tube[4] and mix well. If the total pooled library exceeds 500 µl, proceed to **Section 3** with a maximum of 500 µl of the well-mixed library.

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[1] 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 (~388 bp).

[2] 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.

[3] If pooling from 384-well plate, a multi-channel pipette may be used to pool into 8-strip tubes pre-filled with 7 µl of **PCR Inactivation Solution** before consolidating into a microcentrifuge tube.

[4] There is no need for additional normalization procedures.

Section 3: Final Library Clean-up

1. Equilibrate the ***Select-a-Size*™ MagBeads** to room temperature (15-30°C). Resuspend 30 µl of the ***Select-a-Size*™ Magbead Concentrate** in 1 ml of the ***Select-a-Size*™ Magbead Buffer.**[1] 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[2] 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.[3]

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.

9. Add 10-100 µl[4] 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 room temperature, or until the magnetic beads have fully separated from eluate.

11. Transfer supernatant to a clean microcentrifuge tube. Proceed to **Section 4**.

[1] Once the concentrate and buffer have been mixed, the mix can be stored at 4°C for a maximum of 3 months.
[2] Alternatively, the provided Magnetic Rods can be used.
[3] Avoid aspirating any beads when removing the supernatant. To best prevent this, leave 2-5 µl of liquid behind.
[4] If pooling fewer than 10 samples, use 10 µl for elution.

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 388 bp, convert ng/µl to nM using the equation below.

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

If preferred, a qPCR-based method for quantification such as the KAPA® Library Quantification kit may be used.

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 ~388 bp. Because of the library prep design, some library products have run through additional PCR cycles and might not anneal well. This is perfectly fine for sequencing. Double-stranded DNA will be denatured into single strands before loading onto the sequencer.

This is your final 16S library

The ultra-pure pooled library DNA is now ready for use or storage at ≤-20ºC. Refer to platform-specific guidelines for preparation for sequencing.

Illumina MiSeq® Setup:

The MiSeq® Reagent Kit v2 (300 Cycle) with 15% PhiX spike-in is recommended. See Appendices D for assistance with sample sheet setup. Remember to set the index size to 10 bp. Proceed to **Section 5.**

Section 5: Denaturation and Dilution

Using the recommended loading concentrations below, denature and dilute your final library according to the Illumina® Denature and Dilute Protocol for your specific sequencing platform. A 15% PhiX spike-in is recommended across all Illumina® Platforms.

Recommended final library loading concentrations:

Illumina® Platform	Loading Concentration
MiSeq v2	4 pM
MiSeq v3	8 pM
MiniSeq	1 pM
NovaSeq	150 pM

The final loading concentration may need to be further optimized for your specific machine to achieve optimal cluster densities.

Proceed to **Section 6.**

Section 6: Spiking Custom Primers into Illumina Reagent Cartridge

1. Refer to **Appendix E** for the primer positions.
2. Using a pipette tip, pierce the foil at the appropriate position on the cartridge.
3. Using a Pasteur pipette, aspirate the contents of the cartridge well and transfer into an empty microcentrifuge tube.
4. Add the appropriate volume of 100 µM custom primer into the tube. Vortex and quick spin.
5. Using a P1000, transfer the contents of the tube back to its original position on the cartridge.
6. Repeat for all the necessary primers.
7. Load the final library into the proper position.
8. Look at the bottom of the cartridge and make sure that there are no bubbles in the reagent wells.
9. Your cartridge is now ready for sequencing.

Appendix A: Removal of PCR Inhibitors from Starting DNA

The input DNA samples for the ***Quick*-16S™ Plus NGS Library Prep Kit (V4)** 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 **D6030** Product Page at www.zymoresearch.com.

Appendix B: Adjusting PCR Cycles

If samples, excluding the no template control, consistently plateau before 42 cycles, the PCR cycles can be adjusted to further reduce library prep time. Using previous data that is representative of your samples, determine the Ct which all samples, excluding the no template control, plateau. If samples plateau before 25 cycles, set the PCR program to 25 cycles. If the samples plateau before 30 cycles, set the PCR program to 30 cycles. If the samples plateau before 35 cycles, set the PCR program to 35 cycles.

Appendix C: Sequencing Recommendations for Other Platforms

For sequencing platforms not listed, we recommend diluting your final library to a concentration lower than the recommended concentration in the standard Illumina protocol for your specific platform to prevent over clustering. Increase the loading concentration until you achieve optimal cluster densities. We recommend 15% PhiX spike-in across all platforms and reagent kits. Final library concentrations and PhiX spike-in percentages may be further adjusted to uniquely optimize cluster density and sequencing read quality respectively.

Appendix D: Illumina MiSeq® Sample Sheet Setup

A template for the Illumina MiSeq® sample sheet is available for download 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 for use with the Illumina MiSeq®.

Appendix E: Illumina Sequencing Primer Positions

Sequencing Kit	Illumina Primer (name)	Cartridge Position	Vol of Custom Primer Spike In (µl)
MiniSeq High Output (300 cycles)	Read 1	24	1.65
	Read 2	25	1.83
	Index 1 and Index 2	28	2.46
MiniSeq Mid Output (300 cycles)	Read 1	24	1.65
	Read 2	25	1.83
	Index 1 and Index 2	28	2.46
MiSeq v2 and v3	Read 1	12	3.4
	Index 1	13	3.4
	Read 2	14	3.4
NextSeq 500/550 High Output (300 Cycles)	Read 1	20	5.19
	Read 2	21	5.94
	Index 1 and Index 2	22	8.49
NextSeq 500/550 Mid Output (300 Cycles)	Read 1	20	3.99
	Read 2	21	4.56
	Index 1 and Index 2	22	6.27
NovaSeq v1.0 SP (300 Cycles)	Read 1	24	12
	Index 1	23	15
	Read 2	13	6
NovaSeq v1.0 S1 and S2 (300 Cycles)	Read 1	24	12
	Index 1	23	15
	Read 2	13	6
NovaSeq v1.0 S4 (300 Cycles)	Read 1	24	21.9
	Index 1	23	15
	Read 2	13	10.5
NovaSeq v1.5 SP (300 Cycles)	Read 1	24	12
	Index 1 and Index 2	23	15
	Read 2	13	6
NovaSeq v1.5 S1 and S2 (300 Cycles)	Read 1	24	12
	Index 1 and Index 2	23	15
	Read 2	13	6
NovaSeq v1.5 S4 (300 Cycles)	Read 1	24	21.9
	Index 1 and Index 2	23	15
	Read 2	13	10.5

*For reagent kits that list both Index 1 and Index 2, the primers should be spiked into the same position.

For example: For the MiniSeq High Output Kit, 2.46 µl of Index 1 Sequencing Primer and 2.46 µl of Index 2 Sequencing Primer should be spiked into cartridge position 28.

Explore Other Microbiome Products

✓ To collect and transport samples at ambient temperatures:

DNA/RNA Shield™ and Collection Devices	
1X Reagent #R1100	For sample lysis and stabilization of DNA/RNA
2X Concentrate #R1200	Reagent concentrate (2X) for use with liquids at 1:1 ratio
Fecal Collection Tube #R1101	15 mL container (prefilled with 9 mL DNA/RNA Shield™). Direct collection of up to 1g or 1 mL stool
Collection Tube w/ Swab #R1106	12 x 80 mm screwcap container filled with 1 mL DNA/RNA Shield™ and sterile swab for specimen collection

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

ZymoBIOMICS™ DNA and RNA Kits	
DNA Miniprep #D4300	Up to 25 µg DNA
DNA Microprep #D4301	Up to 5 µg DNA
MagBead DNA #D4302	Automatable (Tecan, Hamilton, Kingfisher, etc.)
96-Well DNA #D4309	Spin-plate
DNA/RNA Miniprep Kit #R2002	Up to 100 µg DNA/RNA

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

ZymoBIOMICS™ Standards and Reference Materials	
Microbial Community Standard #D6300	Contains 8 bacteria and 2 yeasts for QC and method optimization
Microbial Community DNA Standard #D6305	Contains 8 bacteria and 2 yeasts DNA for bioinformatics optimization
Gut Microbiome Standard #D6331	Contains 21 different human gut strains for method benchmarking
Fecal Reference with TruMatrix™ Technology #D6323	Contains real human fecal material for benchmarking and improved data reproducibility

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Background Contamination	Workspace contamination: - Clean workspace, microcentrifuge, and pipettes with 10% bleach routinely to avoid contamination. - Use of kit in exposed environment without proper filtration can lead to background 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	Adhesive seal: - 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. Lid pressure: - 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	Sample with high microbial DNA concentration: - 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**. - Sample may contain high levels of PCR inhibitors. See Appendix A on how to remove these and repeat **Section 1**. Additionally, samples may be diluted to lower concentration (<10 ng/µl) to see if that improves amplification efficiency. Sample with low microbial DNA concentration: - 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. Abnormal qPCR curves: - 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	No single amplicon peak and/or high background: - 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

Notes

Notes

Notes

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