Quick-16S™ NGS Library Prep Kit

Catalog No. D6400

Quick Protocol: Non-Quantitative PCR

Notice

While the *Quick*-16S™ NGS Library Prep Kit is designed for use with a real-time quantitative PCR system, it is possible to use standard thermocycler without quantitative function. Zymo Research does not recommend this option for most applications as certain features are lost, such as direct quantification and control of PCR chimera formation. This Quick Protocol is only recommended for DNA samples that have a high concentration of bacterial DNA, not including host DNA.

The **Select-a-Size DNA Clean & Concentrator MagBead Kit** (Catalog No. D4084) must be purchased separately from the *Quick*-16S™ NGS Library Prep Kit in order to prepare DNA samples with a standard thermocycler without quantitative function.

Protocol

Before Starting

- ✓ **Sample Quantity Requirement.** The PCR conditions and normalization guidelines were designed based on the assumption that there are at least 24 samples in one run; therefore, each run requires a minimum of 24 samples.

- ✓ **Input DNA Guidelines.** All DNA samples should be free of PCR inhibitors. The required concentration of microbial DNA is 5-20 ng/µl, not including host DNA.

- ✓ **Choosing a Targeted Primer Set.** Included in the *Quick*-16S™ NGS Library Prep Kit are two 16S primer sets: the *Quick*-16S™ Primer Set V1-V2 and the *Quick*-16S™ Primer Set V3-V4. Use only one primer set for one PCR / well (i.e., do not mix the two sets into one well). For assistance in choosing a targeted primer set, see the table below.

Primer Set	Region Targeted	Features of the Region
Quick-16S™ Primer Set V1-V2	16S V1-V2	• Better species-level resolution for many human-associate microbes when compared to the V3-V4 region. • Excellent coverage for common genera such as *Methanobrevibacter*, *Bifidobacterium, Propionibacterium, Rickettsia, Chlamydia,* and *Treponema.*
Quick-16S™ Primer Set V3-V4	16S V3-V4	• Broader phylogenetic coverage than the V1-V2 region. • Broad coverage for Archaea. • Improved coverage for *Chloroflexi* and *Candidate Phylum Radiation* (CPR).

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Section 1: Targeted Sequence Amplification

1. Set up a master mix according to the component volumes in the table below:

Component	Volume/Reaction
Quick-16S™ qPCR Premix	10 µl
Quick-16S™ Primer Set V3-V4*	4 µl
ZymoBIOMICS® DNase/RNase Free Water	4 µl
Total	**18 µl**

*Only one *Quick*-16S™ Primer Set should be used during Targeted Sequence Amplification. For more information on the *Quick*-16S™ Primer Sets, please view "Choosing a Targeted Primer Set" on page 1.

2. Add 18 µl of the master mix to each well of a 96-well PCR plate labeled "Targeted Plate." A sample of the plate setup can be found on page 3.
3. Add 2 µl of your DNA samples to individual wells. Include a positive and negative control in the plate. The **ZymoBIOMICS® Microbial Community DNA Standard** (included in this kit) should be used as a positive control.
4. Apply adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner.
5. Place "Targeted Plate" in a thermocycler and run the program shown below:

Temperature	Time	
95°C	10 min	
95°C	30 sec	20 cycles
55°C	30 sec	
72°C	3 min	
Plate read	-	
4°C	Hold	

6. Once the samples have cooled to 4°C, stop the program. Centrifuge "Targeted Plate" in a plate spinner to collect condensation in wells and place plate on ice. Proceed to Section 2 immediately.

Section 2: Reaction Clean-up

1. Add 1 µl of **Reaction Clean-up Solution** to each reaction well in the "Targeted Plate" from Section 1.
2. Apply adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner.
3. Place the plate in a thermocycler and run the program shown below:

Temperature	Time
37°C	15 min
95°C	10 min
4°C	Hold

4. Once the samples have cooled to 4°C, stop the program. Centrifuge the plate in a plate spinner to collect condensation in wells and place plate on ice. Proceed to Section 3, or store plate at ≤ -20°C if necessary for later use.

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Section 3: Barcode Addition

1. Set up a master mix according to the component volumes in the table below:

Component	Volume/Reaction
Quick-16S™ qPCR Premix	10 µl
ZymoBIOMICS® DNase/RNase Free Water	4 µl
Total	**14 µl**

2. Add 14 µl of the master mix to each well of a new 96-well real-time PCR plate labeled "Barcoded Plate."
3. From **Index Primer Set A**, add 2 µl of the **Index Primer ZA7xx** and 2 µl of the **Index Primer ZA5xx** to the proper wells as indicated in the diagram below:

Index Primers ZA7xx

Index Primers ZA5xx		ZA701	ZA702	ZA703	ZA704	ZA705	ZA706	ZA707	ZA708	ZA709	ZA710	ZA711	ZA712
		1	2	3	4	5	6	7	8	9	10	11	12
ZA501	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
ZA502	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
ZA503	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
ZA504	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
ZA505	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
ZA506	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
ZA507	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	POS*
ZA508	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	NEG**

* POS: The **ZymoBIOMICS® Microbial Community DNA Standard** should be used as a positive control.

** NEG: A no template control should be used as a negative control.

4. Pipette mix and transfer 2 µl of the DNA samples from the "Targeted Plate" collected at the end of Section 2 to the corresponding wells of the "Barcoded Plate."
5. Apply adhesive PCR plate seal. Mix the plate on plate shaker and centrifuge in a plate spinner.
6. Place "Barcoded Plate" in a thermocycler and run the program shown below:

Temperature	Time	
95°C	10 min	
95°C	30 sec	5 cycles
55°C	30 sec	
72°C	3 min	
Plate read	-	
4°C	Hold	

7. Proceed to Section 4, or store plate at ≤ -20°C if necessary for later use.

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Section 4: Select-a-Size DNA Clean & Concentrator MagBead Kit

The **Select-a-Size DNA Clean & Concentrator MagBead Kit** (Catalog No. D4084) must be purchased separately from the *Quick*-16S™ NGS Library Prep Kit when using a non-quantitative PCR system. Clean each reaction from **Section 3** according to the Select-a-Size DNA Clean & Concentrator MagBead Kit protocol using left-sided size selection to retain DNA fragments ≥ 200bp. The protocol is also shown below:

1. Resuspend the magnetic particles of the **Select-a-Size MagBeads** by vigorously shaking until homogenous.
2. Add 16 µl of **Select-a-Size MagBeads** to each reaction well in the "Barcoded Plate" collected at the end of **Section 3**.
3. Mix thoroughly by pipetting or vortexing until homogenous. Incubate for 5 minutes at room temperature.
4. Place the samples on a magnetic plate 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.
6. While the beads are still on the magnetic rack, add 200 µl of **DNA Wash Buffer**. Remove and discard the supernatant. Repeat this step.
7. While the beads are still on the magnetic plate, aspirate out any residual buffer with a 10 µl pipette tip.
8. Remove samples from the magnetic plate. Incubate for 3 minutes at room temperature to remove all traces of buffer.
9. Add 25 µl of **DNA Elution Buffer** to the beads and pipette mix thoroughly. Incubate at room temperature for 2 minutes.
10. Place the sample on a magnetic plate for 1 minute to separate the magnetic beads from eluate.
11. Transfer supernatant to a new 96-well plate.
12. Proceed to Section 5, or store plate at ≤ -20°C if necessary for later use.

Section 5: Sample Quantification and Normalization

1. Quantify each cleaned reaction from **Section 4** using an appropriate quantification method such as Qubit® or TapeStation®.
2. Normalize samples based on equal molarity and pool together in a 1.5 ml microcentrifuge tube placed on ice.

The pooled library DNA is now ready for use.