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We use this protocol and it's working

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Zymo Protocol

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ABSTRACT

Zymo Automation

PROTOCOL integer ID:
90156

Keywords: metagenomics,
Nanopore sequencing, WGS

1-Step PCR

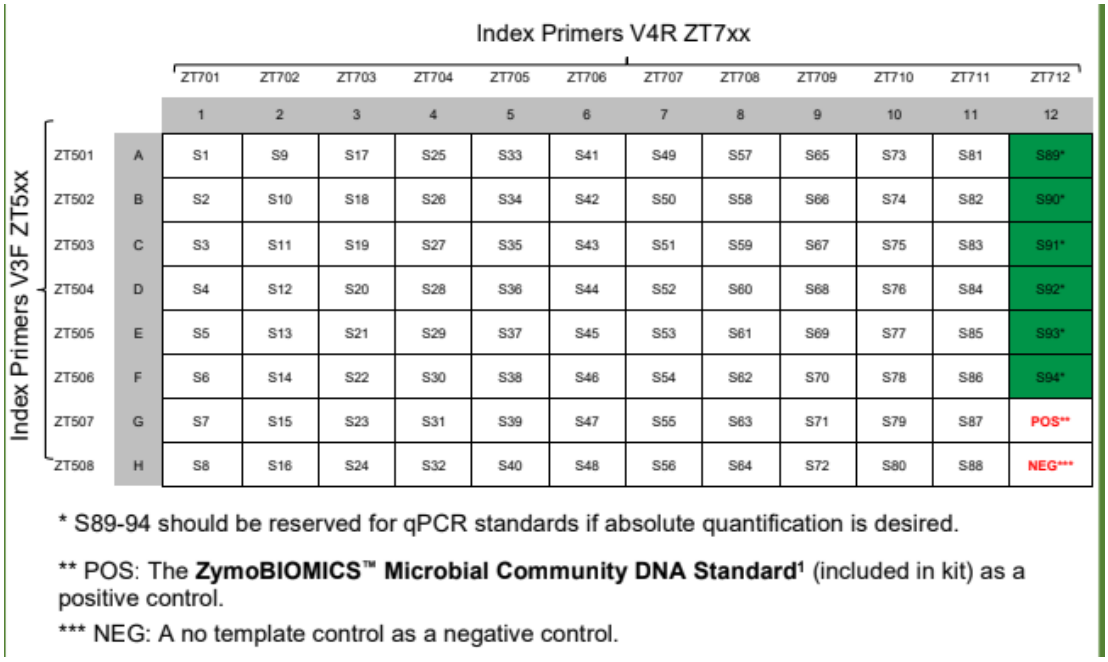
1 Set up a master mix according to the table below:

Component	Volume/Reaction
Equalase™ qPCR Premix	10 µl
ZymoBIOMICS™ DNase/RNase Free Water	4 µl
Total	**14 µl**

2 For each reaction, add 14 µl of the master mix to the appropriate wells of a 96-well real-time PCR plate. A sample of the plate setup can be found on the next page and on the Plate Setup Guide.

3 Index Primer Addition:

a. If using V3-V4 Index Primer Sets 1, 2, 3, or 4, pierce the foil and add 4 µl of the appropriate Index Primer V4R ZT7XX and Index Primer V3F ZT5XX combination to the proper wells of the PCR plate as indicated in the diagram below.
b. If using V3-V4 Index Primer Set 5, add 2 µl of i7 index primer and 2 µl of i5 index primer from the appropriate tubes.



Index Primers V4R ZT7xx

Index Primers V3F ZT5xx		ZT701	ZT702	ZT703	ZT704	ZT705	ZT706	ZT707	ZT708	ZT709	ZT710	ZT711	ZT712
		1	2	3	4	5	6	7	8	9	10	11	12
ZT501	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89*
ZT502	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90*
ZT503	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91*
ZT504	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92*
ZT505	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93*
ZT506	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94*
ZT507	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	POS**
ZT508	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 Standard**[1] (included in kit) as a positive control.

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

4 (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.

5 Add 2 µl of your DNA samples to individual wells. Include a positive and negative control in the plate.

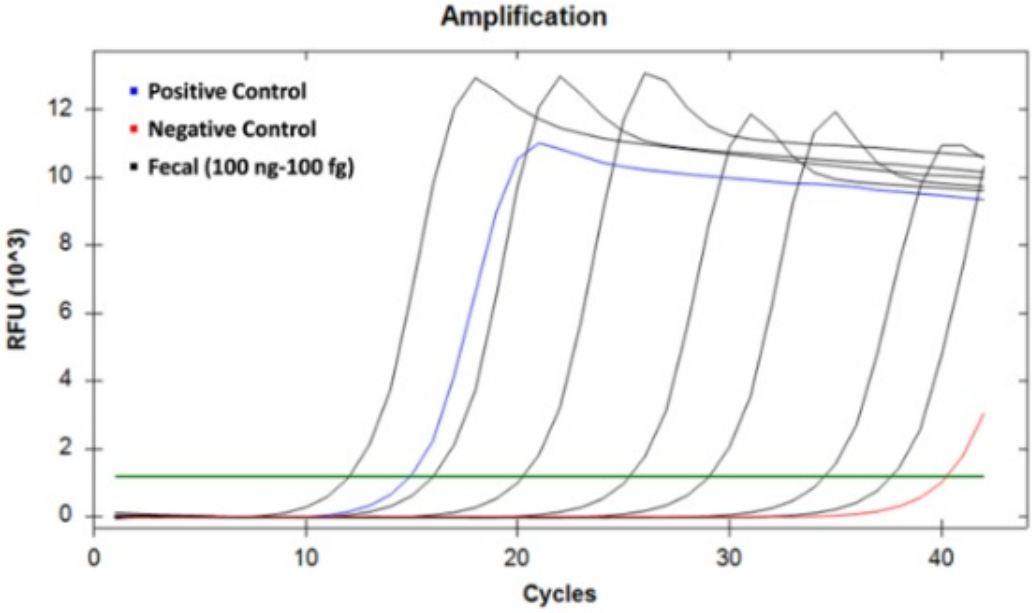
6 Apply an adhesive PCR plate seal. Mix the plate on a plate shaker and centrifuge in a plate spinner.

7 Place plate in a real-time thermocycler1 and run the program shown below:

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

8 Monitor and QC the library preparation when running the reaction on a real-time thermocycler.

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. Earlier amplification of negative control may indicate process contaminations.
c. An example of qPCR amplification with controls is shown in Figure 5 below.



9 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 step 10, or store plate at ≤-20°C

for later use.

10 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 1-Step PCR Section into the tube and mix well. Skip the wells of S89-S94 if they are used for qPCR standards. Proceed to Final Library Clean-up Section.

Final Library Clean-up

11 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.

12 Add Select-a-Size^{TM} MagBeads to the pooled library from Step 10 at a ratio of 0.8x volume. For example, add 400 µl of Select-a-SizeTM MagBeads to 500 µl of the pooled library and PCR Inactivation Solution mixture.

13 Mix thoroughly by pipetting or vortexing until homogenous. Incubate for 5 minutes at room temperature.

14 Place the sample on a magnetic rack and incubate for 3-10 minutes at room temperature, or until the magnetic beads have fully separated from solution. Once the beads have cleared from solution, remove and discard the supernatant.

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

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

17 Add 10-100 µl1 of ZymoBIOMICS^{TM} DNase/RNase Free Water to the beads and pipette mix thoroughly. Incubate at room temperature for 2 minutes.

18 Place the sample on a magnetic rack and incubate for 1 minute at RT, or until the magnetic beads have fully separated from eluate. Transfer supernatant to a clean microcentrifuge tube. Proceed to
Section 4.

Library Quantification

19 Use a fluorescence-based method (Qubit® dsDNA HS Assay Kit recommended) to quantify the final library. Using a final amplicon size of 606 bp, convert ng/µl to nM using the equation below.

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

For example: 20 ng/µl DNA of the final library is equivalent to 50.0 nM.
If preferred, a qPCR-based method for quantification such as the KAPA® Library Quantification kit may be used.