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16S and 18S rRNA mock community construction

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We use this protocol and it's working

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Abstract

Mock communities are defined mixtures of cells or DNA for methods development, testing, or validation. Here we present mock communities based on previously used 16S and 18S rRNA gene sequences from diverse marine microorganisms. Their use as controls for amplicon sequencing enables detection of biased or aberrant runs.

Attachments

CSV 16S Sequences.csv 43KB

CSV 18S Sequences.csv 30KB

XLSX 18S Mock Community R... 15KB

XLSX 16S Mock Community R... 17KB

Materials

- Stock plasmids containing individual 16S or 18S sequences (see spreadsheet)
- PCR reagents: NEB OneTaq 2X Master Mix with Standard Buffer (Cat no. M0482)
- PCR and gel clean up kits: Zymo DNA Clean & Concentrator (D4013), NucleoSpin Gel and PCR Clean-up XS kit (Cat. no. 740611), Zymoclean Gel DNA Recovery Kit (Cat. no. D4001)
- Qubit dsDNA, High Sensitivity (Q32851 or Q32854)

The kits listed here simply describe the ones used for the creation of our mock communities and may be subsituted for others that serve the same role.

Obtain plasmids

1 The stock plasmids were synthesized by Twist with the Kan High Copy vector and rehydrated to 40 ng/µL in low-EDTA TE Buffer. Use 1:10 dilutions of the stock plasmids (~4 ng/µL) for the first PCR.

First PCR of inserts

2 Set up 25 µL PCR reactions with M13 primers and NEB OneTaq 2X Master Mix with Standard Buffer:

	Reagent	Volume (µL)
	10 uM M13 Forward Primer	1
	10 uM M13 Reverse Primer	1
	Master Mix	12.5
	Template DNA	1
	Water	10.5

3 Run PCR with the following conditions:

	Step	Temp (°C)	Time
	Initial Denaturation	94	30 seconds
	25 cycles	94	30 seconds
		55	1 minute
		68	2 minutes
	Final Extension	68	5 minutes

4 Run PCR products on a gel to confirm successful amplification. Most samples are cleaned up with the Zymo DNA Clean-up and Concentrator Kit following the manufacturer's instructions. Perform the final elution in 50 µL of elution buffer and prepare 1:10 dilutions in 50 µL with molecular-grade H_2O for the next PCR.

18S Sample 14 (3323) and the archael sequences (6445, 6446, 6447) are gel extracted with either the
Macherey-Nagel NucleoSpin Gel and PCR Clean-up XS kit (Cat. no. 740611) or
Zymoclean Gel DNA Recovery Kit (Cat. no. D4001)

Second PCR of inserts

5 Set up PCR reactions as previously described with the following primers and annealing temperatures depending on the sequence type:

	Type	Forward Primer	Reverse Primer	Annealing Temp (°C)
	16S Bacteria	27F	1492R	55
	16S Archaea	20F	1392R	54
	18S	EukA	EukB	54

	Primer	Sequence (5' - 3')
	27F	AGAGTTTGATCMTGGCTCAG
	1492R	TACGGYTACCTTGTTACGACTT
	20F	AGTTTGATCMTGGCTCAG
	1392R	ACGGGCGGTGTGTRC
	EukA	AACCTGGTTGATCCTGCCAGT
	EukB	GATCCTTCTGCAGGTTCACCTAC

27F/1492R: Heuer, Holger, et al. "Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients." *Applied and environmental microbiology* 63.8 (1997): 3233-3241.

20F/1392R: Lane, D. J. "16S/23S rRNA sequencing." *Nucleic acid techniques in bacterial systematics* (1991).

EukA/EukB: Medlin, Linda, et al. "The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions." *Gene* 71.2 (1988): 491-499.

6 Perform clean-ups again with the Zymo DNA Clean-up and Concentrator Kit. Perform the final elution in 50 µL. Here the archael sequences are gel extracted again.

Quantification, dilution, and mixing

7 Quantify the cleaned-up PCR product in duplicate with the Qubit dsDNA High Sensitivity (HS) assay kit.

7.1 (optional) Perform sanger sequencing with the second PCR primers for validation. This was performed with the construction of our mock communities.

8 Using the recipe spreadsheets, enter the concentrations, prepare dilutions, and mix accordingly. The expected ratios are also included in these spreadsheets. The final mock communities are also then serially diluted to approximately 0.01 ng/µL.

9 (optional) Confirm successful amplication following the PCR conditions and primers used for amplicon library prepartion with both the diluted PCR product and mixtures. This was performed with the construction of our mock communities.

Protocol references

Parada AE, Needham DM, Fuhrman JA. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18:1403-1414.

Yeh Y-C, McNichol J, Needham DM, Fichot EB, Berdjeb L,Fuhrman JA. 2021. Comprehensive single-PCR 16S and 18S rRNA community analysis validated with mock communities, and estimation of sequencing bias against 18S. Environ Microbiol 23:3240-3250.

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