

Version 3 ▼

© qPCR: Bacterial SSU rRNA 338F-516P-805R V.3

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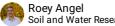
¹Soil and Water Research Infrastructure

1 Works for me

dx.doi.org/10.17504/protocols.io.bi98kh9w

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ABSTRACT

Universal 16S rRNA probe-based-qPCR assay for bacteria. The primers and probe are taken from <u>Yu et al. (2005)</u>.



Yu Y, Lee C, Kim J, Hwang S (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction.

Biotechnology and bioengineering.

http://dx.doi.org/10.1002/bit.20347

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Yu, Y., Lee, C., Kim, J., and Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng 89, 670–679. doi:10.1002/bit.20347.

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

E

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KEYWORDS

qPCR, dual-labelled probe, 16S rRNA gene, bacteria

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PARENT PROTOCOLS

In steps of

RNA-Stable Isotope Probing

MATERIALS

NAME	CATALOG #	VENDOR
iQ™ SYBR® Green Supermix	1708880	BioRad Sciences

Primers and probe

1

Name	Туре	Sequence	Target region ¹
BAC338F	Forward	ACT CCT ACG GGA GGC AG	338-354
BAC516P ²	Probe	TGC CAG CAG CCG CGG TAA TA	516-536
BAC805R	Reverse	GAC TAC CAG GGT ATC TAA TC	785-805

- 1. Relative to E. coli SSU rRNA gene
- 2. The probe must be dual-labelled either with 5'-6-FAM, 3'-BHQ1 or any other valid combination

qPCR mixture

2

Reagent	Final concentration	1 tube (20 μl)	plate (20 µl x 100)
PCR H ₂ O		4.6	460
iQ TM Supermix	1x	10	1000
MgCl ₂ (25 mM)	4.0 mM	0.81	80
BSA (20 μg μl ⁻¹)	0.2 μg μl ⁻¹	0.2	20
338F (10 μM)	0.5 μΜ	1.0	100
805R (10 μM)	0.5 μΜ	1.0	100
516P (10 μM)	0.2 μΜ	0.4	40
Template		2	2 x 100

1 Buffer contains MgCl₂ at final conc. of 3.0 mM

Thermocycler programme

3

```
1. § 95 °C for © 00:05:00
2. x 40 {
2.1 § 95 °C for © 00:00:30
2.2 § 62 °C for © 00:00:30 take snapshot
```

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