Version 3

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

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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 Yu et al. (2005).

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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PROTOCOL CITATION

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

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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	Type	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_2O		4.6	460
iQ™ Supermix	1x	10	1000
$MgCl_2$ (25 mM)	4.0 mM	0.8[1]	80
BSA (20 µg $µl^{-1}$)	0.2 µg $µl^{-1}$	0.2	20
338F (10 µM)	0.5 µM	1.0	100
805R (10 µM)	0.5 µM	1.0	100
516P (10 µM)	0.2 µM	0.4	40
Template		2	2 x 100

1 Buffer contains $MgCl_2$ 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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