

# qPCR: Bacterial SSU rRNA 338F-516P-805R V.2

Roey Angel<sup>1</sup>, Eva Petrova<sup>1</sup>

<sup>1</sup>Soil and Water Research Infrastructure

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Works for me

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SoWa RI Anaerobic and Molecular Microbiology (public)  
Tech. support email: [eva.petrova@bc.cas.cz](mailto:eva.petrova@bc.cas.cz)



Roey Angel

Soil and Water Research Infrastructure



## 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.

## ATTACHMENTS

[Introduction\\_QPCR\\_Strata](#) [AB\\_rt-QPCRguide.pdf](#)  
[gene.pdf](#)

## DOI

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## PROTOCOL CITATION

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

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.

## KEYWORDS

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

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MATERIALS

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

## Primers and probe

1

Name	Type	Sequence	Target region <sup>1</sup>
BAC338F	Forward	ACT CCT ACG GGA GGC AG	338-354
BAC516P <sup>2</sup>	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 <sub>2</sub> O		4.6	460
iQ™ Supermix	1x	10	1000
MgCl <sub>2</sub> (25 mM)	4.0 mM	0.8 <sup>1</sup>	80
BSA (20 µg µl <sup>-1</sup> )	0.2 µg µl <sup>-1</sup>	0.2	20
<b>338F</b> (10 µM)	0.5 µM	1.0	100
<b>805R</b> (10 µM)	0.5 µM	1.0	100
<b>516P</b> (10 µM)	0.2 µM	0.4	40
Template		2	2 x 100

1 Buffer contains MgCl<sub>2</sub> at final conc. of 3.0 mM

## Thermocycler programme

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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}