



Version 4

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qPCR: Bacterial SSU rRNA 338F-516P-805R V.4

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Works for me

dx.doi.org/10.17504/protocols.io.bqx5mxq6

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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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Version created by [Roey Angel](#)

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KEYWORDS

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

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MATERIALS TEXT

MATERIALS

 **iQ™ SYBR® Green Supermix BioRad**

Sciences Catalog #1708880

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Universal 16S rRNA probe-based-qPCR assay for bacteria.
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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

A	B	C	D
Reagent	Final concentration	1 tube (20 µl)	plate (20 µl x 100)
PCR H ₂ O		2.2	460
2x TaqMan Fast Advanced Master mix	1x	10	1000
BSA (20 µg µl ⁻¹)	0.4 µg µl ⁻¹	0.4	40
338F	0.5 µM	1.0	100
805R	0.5 µM	1.0	100
516P	0.2 µM	0.4	40
Template		5	5 x 100







 TaqMan[®]; Fast Advanced Master Mix Thermo

Fisher Catalog #4444556

Thermocycler programme 6m 30s

6m 30s

3

1.  **95 °C** for  **00:05:00**
2. x 40 {
 - 2.1  **95 °C** for  **00:00:30**
 - 2.2  **62 °C** for  **00:01:00** take snapshot}