VERSION 4

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MANUSCRIPT CITATION:

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 status: Working

We use this protocol and it's working

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

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ABSTRACT

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

The primers and probe are taken from [Yu et al. \(2005\)](#).

CITATION

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*.

LINK

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

MATERIALS

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iQ™ SYBR® Green Supermix **BioRad Sciences Catalog #1708880**

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PROTOCOL integer ID:
45789

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

Primers and probe

1

Name	Type	Sequence	Target region
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

A	B	C	D
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

3

6m 30s

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
- }