VERSION 2

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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.2

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SoWa RI Anaerobic and Molecular Microbiology (public)
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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>

ATTACHMENTS

[Introduction_QPCR_Strata gene.pdf](#) [AB_rt-QPCRguide.pdf](#)

MATERIALS

MATERIALS

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

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PROTOCOL integer ID:
39964

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

Reagent	Final concentration	1 tube (20 μ l)	plate (20 μ l x 100)
PCR H ₂ O		4.6	460
iQ TM Supermix	1x	10	1000
MgCl ₂ (25 mM)	4.0 mM	0.8 ¹	80
BSA (20 μ g μ l ⁻¹)	0.2 μ g μ l ⁻¹	0.2	20
338F (10 μ M)	0.5 μ M	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 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
- }