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

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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.

Protocol status: Working
We use this protocol and it's working

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Keywords: qPCR, dual-labelled probe, 16S rRNA gene, bacteria, qpcr assay for bacteria, qpcr assay, rrna probe, qpcr, 16s rrna, rrna, probe,

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.

http://dx.doi.org/10.1002/bit.20347 LINK

Materials

MATERIALS

iQ™ SYBR® Green Supermix **Bio-Rad Laboratories Catalog #**1708880

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 × 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
   }

Citations

Yu Y, Lee C, Kim J, Hwang S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction
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