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Version 2

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

DOI

<https://dx.doi.org/10.17504/protocols.io.bi94kh8w>



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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

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

Attachments



[Introduction_QPCR_St...](#) [AB_rt-QPCRguide.pdf](#)

10.3MB



1,011KB

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 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 μ M)	0.5 μ M	1.0	100
	516P (10 μ M)	0.2 μ M	0.4	40
	Template		2	2 x 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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