# 16S Bacteria 338F-516P-805R BSA

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Jul 30, 2020
1 Works for me
dx.doi.org/10.17504/protocols.io.qckdsuw

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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.

ATTACHMENTS

Introduction_QPCR_Strata gene.pdf
AB_rt-QPCRguide.pdf

DOI

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

PROTOCOL CITATION

Roey Angel, Eva Petrova 2020. 16S Bacteria 338F-516P-805R BSA. **protocols.io** dx.doi.org/10.17504/protocols.io.qckdsuw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

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

CREATED

May 24, 2018

LAST MODIFIED

Jul 30, 2020

PROTOCOL INTEGER ID

12396

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| iQ™ SYBR® Green Supermix | 1708880 | BioRad Sciences |

## 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* 16S 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 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**
   }



**Citation:** Roey Angel, Eva Petrova (07/30/2020). 16S Bacteria 338F-516P-805R BSA. https://dx.doi.org/10.17504/protocols.io.qckdsuw