Version 1

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# qPCR: Bacterial SSU rRNA 338F-516P-805R V.1

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1 Works for me dx.doi.org/10.17504/protocols.io.bqwymxfw

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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.

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PROTOCOL CITATION

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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



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MATERIALS TEXT

MATERIALS

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

TaqMan™ Fast Advanced Master Mix **Thermo Fisher Scientific Catalog #4444556**

Step 2

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## 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



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2

| A | B | C | D |
|---|---|---|---|
| **Reagent** | **Final concentration** | **1 tube (20 µl)** | **plate (20 µl x 100)** |
| PCR H2O | | 2.2 | 220 |
| 2x TaqMan Fast Advanced Master mix | 1x | 10 | 1000 |
| BSA (20 µg/µl) | 0.4mg/ml | 0.4 | 40 |
| 338F | 0.5 µM | 1.0 | 100 |
| **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 Scientific Catalog #4444556**

Thermocycler programme 1h 30m

3 1h 30m

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**
   }



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