VERSION 1

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General bacteria and archaea 16S-rRNA (515Fmod-806R) for Illumina amplicon sequencing V.1

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We use this protocol and it's working

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9870

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ABSTRACT

Universal 16S rRNA probe-based-qPCR assay for bacteria.

The primers target the V4 region of the 16S rRNA gene and were specifically designed for Illumina amplicon sequencing. The original primers were designed by Caporaso *et al.* (2012) and modified by Walters *et al.* (2015). For barcoding, we use the [Fludigm Access Array](#) for barcoding the sample and therefore the primers are synthesized with the CS1 and CS2 regions.

CITATION

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms.. The ISME journal.

LINK

<https://doi.org/10.1038/ismej.2012.8>

CITATION

Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R (2015). Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys.. mSystems.

LINK

<https://doi.org/pii:e00009-15>

MATERIALS

STEP MATERIALS

- ✕ Agarose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9539**
- ✕ GeneRuler DNA Ladder Mix **Thermo Fisher Scientific Catalog #SM0331**
- ✕ DNA Gel Loading Dye (6X) **Thermo Fisher Scientific Catalog #R0611**
- ✕ TAE buffer (50x), molecular biology grade **Serva, Germany Catalog #4254901**
- ✕ Fast Start PCR Master **Roche Catalog #196 10 126**
- ✕ PCR H₂O **Top Bio Catalog #P040**
- ✕ Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14**
- ✕ Primer: 515Fmod_CS1 **Elisabeth Pharmacon**
- ✕ Primer: 806mod_CS2 **Elisabeth Pharmacon**

Primers

1

Name	Direction	Sequence ¹	Target region ²
515Fmod_CS1	Forward	ACA CTG ACG ACA TGG TTC TAC AGT GYC AGC MGC CGC CGT AA	515-533
806mod_CS2	Reverse	TAC GGT AGC AGA GAC TTG GTC TGG ACT ACN VGG GTW TCT AAT	787-806

1. CS + primer sequence (in bold)
2. Relative to *E. coli* SSU rRNA gene

✕ Primer: 515Fmod_CS1 **Elisabeth Pharmacon**

✕ Primer: 806mod_CS2 **Elisabeth Pharmacon**

PCR reaction

2 Prepare the following master mixture **🧊 On ice**.

Don't forget to prepare an additional mixture for the negative (NTC) and positive controls, and to account for pipetting errors.

Reagent	Final. conc.	1 tube (25 µl)	100 reactions (96-well plate; µl)
PCR H ₂ O		9.3	930

Fast Start PCR Master (2x)	1x	12.5	1250
BSA (20 µg/µl)	0.6 µg/µl	0.7	70
515Fmod-CS1 (10 µM)	0.3 µM	0.75	75
806R-CS2 (10 µM)	0.3 µM	0.75	75
Final volume		24	2400

Fast Start PCR Master **Roche Catalog #196 10 126**

PCR H2O **Top Bio Catalog #P040**

Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14**

3 Vortex and spin down 00:00:03

3s

4 Distribute 24 µL of the mixture to each tube and add 1 µL of template DNA or cDNA

PCR reaction

3s

5 Run the following PCR program:

17m 15s

1. 94 °C 00:05:00

2. x 27 {

2.1 94 °C 00:00:45

2.2 50 °C 00:00:45

2.3 72 °C 00:00:45

3. 72 °C 00:10:00

4. 4 °C hold

Evaluate PCR products on an agarose gel

40m

6 Prepare a 1.5% agarose gel by mixing:

🧪 100 mL TAE

🧪 1.5 g agarose

Heat in the microwave until dissolved and pour into a gel frame.
Place solid gel into an electrophoresis bath filled with TAE buffer.

🔗 Agarose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A9539**

🔗 GeneRuler DNA Ladder Mix **Thermo Fisher Scientific Catalog #SM0331**

🔗 DNA Gel Loading Dye (6X) **Thermo Fisher Scientific Catalog #R0611**

🔗 TAE buffer (50x), molecular biology grade **Serva, Germany Catalog #4254901**

- 7 Mix up to 🧪 5 μ L of the PCR reaction sample with 🧪 1 μ L of loading dye and load the sample into a well.
In addition load 🧪 5 μ L of DNA ladder mix (80-10,000 bp) into an empty well, as a marker.
- 8 Run the gel at 110V, 265mA for approx. ⌚ 00:40:00 40m
- 9 Stain gel for at least 40min in an Ethidium bromide TAE bath (or any other DNA stain).
- 10 Visualise the gel using a gel documentation system.