VERSION 3

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General bacteria and archaea 16S-rRNA (515Fmod-806Rmod) for Illumina amplicon sequencing V.3

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We use this protocol and it's working

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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

[10.1128/msystems.00009-15](https://doi.org/10.1128/msystems.00009-15)

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**
- ✂ Primer: 515Fmod_CS1 **Elisabeth Pharmacon**
- ✂ Primer: 806mod_CS2 **Elisabeth Pharmacon**
- ✂ DreamTaq Green DNA Polymerase (5 U/μL) **Thermo Fisher Scientific Catalog #EP0712**
- ✂ dNTP Set (100 mM each) **Contributed by users Catalog #BR0600601**
- ✂ PCR H2O **Top Bio Catalog #P040**
- ✂ Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14**

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
806Rmod_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 μ l)	100 reactions (96-well plate; μ l)
PCR H ₂ O		17.525	1752.5
10X DreamTaq Green Buffer	1X	2.5	250
dNTP (2 mM each)	0.2 mM	2.5	250
BSA (20 μ g/ μ l)	80 ng μ l ⁻¹	0.1	10
515Fmod-CS1 (10 μ M)	0.2 μ M	0.625	62.5
806Rmod-CS2 (10 μ M)	0.2 μ M	0.625	62.5
DreamTaq Green DNA Polymerase	0.625 U	0.125	12.5
Final volume		24	2400

DreamTaq Green DNA Polymerase (5 U/ μ L) **Thermo Fisher Scientific Catalog #EP0712**

dNTP Set (100 mM each) **Contributed by users Catalog #BR0600601**

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 μ L of the mixture to each tube and add 1 μ L of template DNA or cDNA

PCR reaction

3s

5 Run the following PCR program:

17m 15s

1. 94 °C 00:05:00
2. x 28 {
 - 2.1 94 °C 00:00:45

- 2.2 52 °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 µL of the PCR reaction sample with 1 µL of loading dye and load the sample into a well.

In addition load 5 µL 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.