Version 4

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General bacteria and archaea 16S-rRNA (515Fmod-806Rmod) for Illumina amplicon sequencing V.4

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

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SUBMIT TO PLOS ONE

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 synthisized with the CS1 and CS2 regions.

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

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.
http://10.1128/msystems.00009-15

DOI

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KEYWORDS

PCR, 16S rRNA, SSU rRNA, Amplicon sequencing, Illumina sequencing, Barcoded sequencing, Targeted metagenomics, Microbiome

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MATERIALS TEXT

STEP MATERIALS

Agarose **Sigma Aldrich Catalog #A9539** Step 6

GeneRuler DNA Ladder Mix **Thermo Fisher Scientific Catalog #SM0331** Step 6

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

TAE buffer (50x), molecular biology grade **Serva, Germany Catalog #4254901** Step 6

Primer: 515Fmod_CS1 **Elisabeth Pharmacon** Step 1

Primer: 806mod_CS2 **Elisabeth Pharmacon** Step 1

DreamTaq Green DNA Polymerase (5 U/µL) **Thermo Fisher Scientific Catalog #EP0712** Step 2

dNTP Set (100 mM each) **Contributed by users Catalog #BR0600601** Step 2

PCR H2O **Top Bio Catalog #P040** Step 2

Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #B14** Step 2

Primers

1

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Name	Direction	Sequence[1]	Target region[2]
515Fmod_CS1	Forward	ACA CTG ACG ACA TGG TTC TAC A**GT GYC AGC MGC CGC CGT AA**	515-533
806Rmod_CS2	Reverse	TAC GGT AGC AGA GAC TTG GTC T**GG 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** .

Do not forget to prepare some additional mixture for the negative (NTC) and positive controls, and to account for pipetting errors.

A	B	C	D
Reagent	**Final. conc.**	**1 tube (25 µl)**	**100 reactions (96-well plate; µl)**
PCR H_2O		17.525	1752.5
10X DreamTaq Green Buffer	1X	2.5	250
dNTP (2 mM each)	0.2 mM	2.5	250
BSA (20 µg $µl^{-1}$)	80 ng $µl^{-1}$	0.1	10
515Fmod-CS1	0.2 µM	0.625	62.5
806Rmod-CS2	0.2 µM	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/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**

3s

3 Vortex and spin down **00:00:03**

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

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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 **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.

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