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General bacteria and archaea 16S-rRNA (515Fmod-806Rmod) for Illumina amplicon sequencing V.2

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Roey Angel[1], Eva Petrova[1]

[1]Soil and Water Research Infrastructure

Anaerobic and Molecular Microbiology Lab, Biology Centre CAS
Tech. support email: **eva.petrova@bc.cas.cz**

Roey Angel
Soil and Water Research Infrastructure

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Protocol status: Working
We use this protocol and it's working

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

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.

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 **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) **Catalog #**BR0600601
- PCR H2O **Top Bio** **Catalog #**P040
- Bovine Serum Albumin (BSA) **Thermo Fisher Scientific** **Catalog #**B14

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Primers

1

	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 .

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_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)	80 ng $µl^{-1}$	0.1	10
	515Fmod-CS1 (10 µM)	0.2 µM	0.625	62.5
	806Rmod-CS2 (10 µM)	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) **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 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 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.

Citations

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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. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys.
10.1128/msystems.00009-15