Version 2

Oct 14, 2020

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

Roey Angel[1], Eva Petrova[1]

[1]Soil and Water Research Infrastructure

1 Works for me dx.doi.org/10.17504/protocols.io.bnf7mbrn

SoWa RI Anaerobic and Molecular Microbiology (public)
Tech. support email: eva.petrova@bc.cas.cz

Roey Angel
Soil and Water Research Infrastructure

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.
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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 (2015). Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys.. mSystems.
https://doi.org/pii:e00009-15

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PROTOCOL CITATION

Roey Angel, Eva Petrova 2020. General bacteria and archaea 16S-rRNA (515Fmod-806Rmod) for Illumina amplicon sequencing. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bnf7mbrn
Version created by Roey Angel

KEYWORDS

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

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CREATED

Oct 14, 2020

LAST MODIFIED

Oct 14, 2020

PROTOCOL INTEGER ID

43231

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Agarose	A9539	Sigma Aldrich
GeneRuler DNA Ladder Mix	SM0331	Thermo Fisher Scientific
DNA Gel Loading Dye (6X)	R0611	Thermo Fisher Scientific
TAE buffer (50x), molecular biology grade	4254901	Serva, Germany
Primer: 515Fmod_CS1		Elisabeth Pharmacon
Primer: 806mod_CS2		Elisabeth Pharmacon
DreamTaq Green DNA Polymerase (5 U/µL)	EP0712	Thermo Fisher Scientific
dNTP Set (100 mM each)	BR0600601	
PCR H2O	P040	Top Bio
Bovine Serum Albumin (BSA)	B14	Thermo Fisher Scientific

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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.

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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
by Elisabeth Pharmacon
View

Primer: 806mod_CS2
by Elisabeth Pharmacon
View

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)
by Thermo Fisher Scientific
Catalog #: EP0712

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dNTP Set (100 mM each)
Catalog #: BR0600601

PCR H2O
by Top Bio
Catalog #: P040

Bovine Serum Albumin (BSA)
by 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.

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Agarose
by Sigma Aldrich
Catalog #: A9539

GeneRuler DNA Ladder Mix
by Thermo Fisher Scientific
Catalog #: SM0331

DNA Gel Loading Dye (6X)
by Thermo Fisher Scientific
Catalog #: R0611

TAE buffer (50x), molecular biology grade
by 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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