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

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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, universal 16s rrna probe, qpcr assay for bacteria, rrna probe, rrna gene, sequencing universal 16, rrna, general bacteria, bacteria, qpcr assay, illumina amplicon, qpcr, primer,

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.

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

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.

10.1128/msystems.00009-15 LINK

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**

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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
	806mod_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		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 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

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. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms.
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10.1128/msystems.00009-15