Protocol: Quantifying species abundance using 16S Sanger Sequencing and CASEU

Jack Reddan

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Contents

1 Culture Collection

1.1 Overview

This section describes collecting cultures for which you wish to quantify species abundances. In addition to any experimental mixtures, pure cultures of community members are needed for the analysis.

1.2 Materials

- Pure and mixed liquid cultures
- 2-mL microcentrifuge tubes

1.3 Method

1. In a 2-mL microcentrifuge tube, Collect 1 ODmL of liquid culture for all mixtures and pure cultures. Spin-down and remove supernatant.
2. Either store pellets at $-20°C$, or continue to gDNA Isolation.

2 gDNA Isolation

2.1 Overview

This section describes how to isolate gDNA to be used for 16S rDNA amplification. These steps are for the QIAGEN DNeasy Blood & Tissue Kit. If you happen use a different kit, please follow its instructions, and then proceed to 16S rDNA Amplification.

2.2 Materials

- QIAGEN DNeasy Blood & Tissue kit
- 100% ethanol
- Cell pellets from cultures collected in Culture Collection

2.3 Method

Follow the subsequent instruction in parallel for all samples to be processed.

1. Resuspend pellet in 180 μL of buffer ATL, and add 20 μL of Proteinase K. Mix thoroughly by vortexing. Incubate at 56°C for 1 hour, and vortex every 15 minutes.
2. Vortex for 15s, then add 200 μL of buffer AL and mix thoroughly by vortexing. Immediately add 200 μL of 100% ethanol and, again, mix thoroughly by vortexing.

3. Pipet the mixture from step 2 into a DNeasy Mini spin column placed in a 2-mL collection tube. Centrifuge at $\geq$ 6000 x g for 1 min. Afterwards, discard flow-through and collection tube.

4. Place DNeasy Mini spin column into a new 2-mL collection tube and add 500 μL of buffer AW1. Centrifuge at $\geq$ 6000 x g for 1 min. Afterwards, discard flow-through and collection tube.

5. Place DNeasy Mini spin column into a new 2-mL collection tube and add 500 μL of buffer AW2. Centrifuge at 20000 x g for 3 min. Afterwards, discard flow-through and collection tube.

6. Place DNeasy Mini spin column into a clean 1.5-mL microcentrifuge tube. Add 200 μL of buffer AE and incubate at room tempurature for 1 min. Centrifuge at $\geq$ 6000 x g for 1 min to elute.

7. Check gDNA concentration on the nano spectrophotometer. Then store at $-20°$C or continue to 16S rDNA Amplification.

3 16S rDNA Amplification

3.1 Overview

This section describes how to amplify the 16S rDNA using Phusion polymerase for Sanger sequencing. If you are using a different polymerase, please follow its specific instructions and proceed to Sanger Sequencing.

3.2 Materials

- ddH_2O
- Phusion polymerase
- 5X High Fidelity (HF) reaction buffer
- dNTPs
- 27F forward primer
- 1492R reverse primer
- gDNA template from gDNA Isolation

3.3 Method

Follow the subsequent instruction in parallel for all gDNA samples collected in gDNA Isolation.

1. Mix the ddH_2O, dNTPs, forward/reverse primers, 5X HF buffer, and polymerase according to Appendix A for $N + 1$ reactions, where N is the number of gDNA samples to be processed.

2. Pipet 38 μL of the PCR master mix into 200-μL PCR tubes. Then add 2 μL of template gDNA, at a concentration of 100 ng/μL, to the mix for each sample.

3. Place reactions into the thermocycler and run the PCR program specified in Appendix B.

4. Once the run is complete, check for synthesis of the desired product using gel electrophoresis.

5. If the proper product was amplified, either store the reaction at $-20°$ C or proceed to 16S rDNA Purification

4 16S rDNA Purification

4.1 Overview

4.2 Materials

4.3 Methods

5 Sanger Sequencing

5.1 Overview

5.2 Materials

5.3 Method

6 Appendix

A PCR Master Mix

B PCR Program

References