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Bacterial eDNA Collection, Purification, and amplification with fresh water

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We use this protocol and it's working

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Abstract

A protocol to extract and purify bacterial eDNA from fresh-water lakes was determined using lysis by alkali and ethanol precipitation. This was confirmed through the use of 16S forward and reverse primers to amplify with PCR and visualize as bands through agarose gel electrophoresis.

Solutions for Alkaline Lysis Protocol

1 [Note, we can make a 1L solution of the buffer minus lysozyme. Then, when we're ready to extract a batch, we aliquot smaller volumes and then add the lysozyme. E.g. for 50 ml of buffer solution, we can add 200 mg of lysozyme.]

To make 1L of lysis buffer without lysozyme:
20 ml of 1M Tris-Cl (final concentration of 20mM Tris-Cl in buffer)
2 ml of 0.5M EDTA (final concentration of 2mM EDTA in buffer)
12ml Triton® X-100 (final concentration 1.2% Triton® X-100 in buffer).

To make lysozyme buffer:
200 mg of lysozyme powder/granules
Fill up to 50ml of the lysis buffer described above

*Adjust amount of each solution depending on amount of samples
Solution I (50mls) Enzymatic Lysis Buffer
20 mM Tris-HCl (pH 8) buffer
2 mM sodium EDTA
1.2% Triton X-100 detergent
20 mg/mL lysozyme

Solution II (50mls)

0.2 NaOH	0.85mls (850µl)
1% SDS (diluted from 20% stock)	2.5mls (2500µl)
H2O	46.65mls

Solution III (50mls)

Potassium acetate	14.7g
Glacial acetic acid	5.75mls
H2O	to 50mls

Water Collection

2
1. Collect 50 ml tube of water from fresh-water lake wearing gloves
2. Mark collection spot with GIS or compass coordinates
3. Put weigh paper into tube and store in refrigerator for 24 hrs

Lysis by Alkali

3 **Make Solutions**
1. Make the needed amount of each solution (especially solution I, add lysozyme) to account for all of samples being analyzed

Incubation

1. Remove weigh paper from 50ml tube and cut into thin 0.5 cm x 2.5 cm strips; put 3-5 strips into each 2ml microcentrifuge tube
2. Add 100µl of ice-cold Solution I and 20µl proteinase K; vortex vigorously
3. Incubate 30 min, 55o F

After Incubation

1. Add 200µl of freshly prepared Solution II. Close the tube tightly, and mix the contents by inverting the tube rapidly. (Make sure the entire surface of the tube comes in contact with Solution II) Do not vortex. Store the tube on ice for 2 min.
2. Add 150µl of ice-cold Solution III. Close the tube and vortex it gently for 10 sec to disperse Solution III through the viscous bacterial lysate. Store the tube on ice for 5 min.
3. Centrifuge at 12,000 x g for 5 min in microcentrifuge. Transfer supernatant to a fresh tube with a pipette.
4. Add 450µl of phenol: chloroform. Mix by vortexing. Centrifuge at 12,000 x g for 2 min in microcentrifuge. Transfer aqueous (upper) phase to a fresh tube.
5. Add 450µl of chloroform. Mix by vortexing. Centrifuge at 12,000 x g for 2 min in microcentrifuge. Transfer aqueous (upper) phase to a fresh tube.

Ethanol Precipitation

4 **Precipitation and Resuspension of DNA**

1. Precipitate the DNA sample in ice cold 95% ethanol (2:1, ethanol:sample). Mix by vortexing. Place tubes on ice for 10 min.
2. Centrifuge at maximum speed (14,000 x g) for 5 min. Remove the tube from the centrifuge carefully to avoid dislodging the pellet.
3. Carefully pour out supernatant, and invert the open tube on a paper towel.

NanoDrop Spectrophotometer

5 **Ethanol Evaporation**

1. Allow the pellet to dry in the air for 30 min (or until ethanol has evaporated), best overnight
2. Resuspend the plasmid DNA in 50µl of Tris-Cl (pH 8.0) containing DNAse-free pancreatic RNAse (20µg/ml). Vortex briefly.
3. Use a NanoDrop Spectrophotometer to measure concentration and purity of each sample. Use Tris-Cl (pH 8.0) as the blank
4. Store the DNA in the fridge (4oC or -20oC).

PCR

6 **Prepare for PCR**

Master mix (16S)

1. 17.5µl MQ water
2. 70µl Econo Taq Buffer

3. 1.25µl forward primer
4. 1.25µl reverse primer
5. 1µl DNA

Agarose Gel Electrophoresis

7 **0.1% Agarose gel**

1. Combine 0.6g agarose and 56 ml 0.5xTAE buffer
2. Heat in microwave for 45 sec, check every 15 sec to stir
3. Let cool for 3 min
4. Add 1µl ethidium bromide and hand stir for 5 min
5. Pour solution into mold and insert combs, let solidify for 15 min
6. Pipette 3µl samples (with 1µl dye) and ladder into well
7. Run gel at 110V ~1 hr (dye 75% across gel)
8. Visualize gel under UV, take a picture of banding