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DNA extraction with CTAB and chloroform:isoamyl alcohol

Molly A Moynihan[1]

[1]Marine Biological Laboratory

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Molly Moynihan
Marine Biological Laboratory

ABSTRACT

This protocol is based off of Zhou et al. 1996, Kellogg et al. 2019, and Crump et al. 2003.

Zhou, Jizhong, Mary Ann Bruns, and James M. Tiedje. "DNA recovery from soils of diverse composition." *Applied and environmental microbiology* 62.2 (1996): 316-322.

Crump, Byron C., et al. "Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source." *Applied and Environmental Microbiology* 69.4 (2003): 2253-2268.

Kellogg, Colleen TE, et al. "Strong seasonality in arctic estuarine microbial food webs." *Frontiers in microbiology* 10 (2019): 2628.

PROTOCOL CITATION

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Prepare Reagents

1 Prepare DNA extraction buffer:
100mM Tris-HCl (pH 8), 100mM EDTA (pH 8), 100mM sodium phosphate buffer (pH 8), 1.5M NaCl, 1% CTAB

1.1 For a 1000mL (1L) solution of DNA extraction buffer combine:
100mL 1M Tris-HCl (pH 8)
200mL 0.5 M EDTA (pH 8)
100mL 10x sodium phosphate buffer (pH 8)*
300mL 5M NaCl
10g CTAB

*Note: 1M sodium phosphate buffer is made from a mixture of (A) 1M sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$, 156.01g/mol) and (B) 1M sodium phosphate dibasic (Na_2HPO_4, 141.96g/mol). Solutions should be autoclaved.

For pH 8 solution, combine 68mL of A (1M sodium phosphate monobasic dihydrate) with 932mL of B (1M sodium phosphate dibasic).

2 Prepare enzyme stock solutions (aliquot as necessary)

2.1 Combine 200mg of lysozyme and nuclease free water to a final volume of 2mL (100mg/ml). Store aliquots at -20°C.

2.2 Combine 40mg of proteinase-k and nuclease free water to a final volume of 2mL (20mg/ml). Store aliquots at -20°C.

Prepare Samples

3 For sterivex filters, remove casing from filters using pliers. Squeeze at the top of the filter and apply force at the seam of the casing. It is recommended to open the sterivex over a sterilized container (e.g. clean, empty pipette box) as the filter may pop out suddenly.

Using sterile razor blades, cut around the top and bottom of the filter (in the white space) and then cut along the filter to divide the filter into 4-6 strips. Depending on the sample type, 1/2 to the full filter can be used for extraction. Place strips in clean 2mL microcentrifuge tube.

4 For other sample types (e.g. sediment, microbial mat), weight out desired starting sample amount. For 1mL of extraction buffer, recommended mass range: 0.1-0.25g

DNA Extraction

5 Add 1mL of DNA extraction buffer to samples in 2mL microcentrifuge tubes.

6 With samples in buffer, perform 3x freeze thaw cycles. Freeze at -20°C for 20 minutes and thaw at 37°C for 2 minutes.

(Note: in a comparison of -20°C and -80°C freezing temperature, no difference was found in the DNA yield)

7 Add 20µl of lysozyme (100mg/ml) and incubate at 37°C for 30 minutes with a very gentle rotation (200rpm).

8 Add 10µl of proteinase-k (20mg/ml) and incubate at 37°C for 30 minutes with a very gentle rotation (200rpm).

(Good stopping point. Can freeze overnight at -80°C)

9 Add 100µl of filter-sterilized 10% SDS and incubate at 65°C for 2 hours. Invert gently every 25 minutes.

(Can freeze overnight at -80°C. If stopping here, warm again to ~50°C to dissolve SDS before proceeding to next step.)

10 Add chloroform:isoamyl alcohol (24:1) to fill the microcentrifuge tube until full. Mix thoroughly by inverting and/or vortex briefly.

11 Centrifuge for 10minutes at 3,200xg and transfer supernatant to a clean tube.

12 To supernatant (step 11), again add chloroform:isoamyl alcohol (24:1) to fill the microcentrifuge tube. Mix thoroughly by inverting and/or vortex briefly.

Centrifuge for 10minutes at 3,200xg and transfer supernatant to a clean tube.

13

14 Precipitate DNA by adding 0.6x volumes of room temperature 100% isopropanol to the supernatant (step 13). Adjust the amount of isopropanol added to each microcentrifuge tube based on the volume of supernatant. Incubate for 2h in the dark at room temperature.

15 Centrifuge at 20,000xg for 30 minutes and remove supernatant while avoiding DNA pellet. Pellet will be transparent to white in appearance but may be colored for some sulfur-rich sediment samples.

16 Perform 2x ethanol washes using 70% ethanol. Add 1mL 70% ice-cold ethanol, invert several times, centrifuge at 20,000xg for 10 minutes at 4°C. Remove ethanol (supernatant) and repeat for a total of 2 rinses.

17 Dry pellet for 15-30 minutes at room temperature

18 Add 250µl of nuclease free water to pellet. Allow pellet to dissolve by incubating for 1h at 4°C. (Note: reduce elution volume if low yield is expected)

DNA can be stored at -20°C for short term storage or -80°C for long term storage.