DNA Extraction Protocol

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This protocol is to extract DNA from cells collected on a 25 mm diameter, 0.22 μm pore size Supor membrane filter and frozen in 2 mL gasketed bead-beating tubes. The lysis protocol was adapted from references (1-3) and modified to use the Qiagen DNEasy kit instead of phenol:chloroform extraction. The extracted DNA is suitable for qPCR or tag library construction, but may not be suitable for large insert libraries. This method was published in Santoro et al. 2010, EM.

1. Place extraction buffer and SDS solutions under UV light in extraction hood for 10-15 minutes before starting.

2. Set up manifold: Load one Qiagen DNEasy spin column onto the vacuum filter manifold for each sample to be extracted. Make sure manifold is free of contaminating DNA and spacer/adaptors are in place. Hook up vacuum pump to manifold using the upper/top port on manifold.

3. Remove frozen tube containing filter from freezer and place on ice. Add ~30 μL of 0.1 mm glass beads if the tube does not already contain them.

4. Add:

   875 uL Sucrose-EDTA Lysis Buffer (recipe follows)
   100 uL 10% SDS

5. Process samples in bead beater for 1 min.

6. Incubate for 2 min in dry bath/heat block at 99 ºC.

7. Add 25 μL of 20 mg/mL proteinase K (we use NEB).

8. Incubate in hybridization oven @ 55 ºC for 4 hours or overnight.

9. Pipette off the lysis solution into a clean 2 mL centrifuge tube.

10. Discard tube with crushed filter.

11. Add 500 uL 100% ethanol to supernatant and vortex tube to mix.

12. Turn on the vacuum pump.

13. Load samples onto spin columns by pipetting 750 uL of mixture at a time onto the column. Change tips between samples.

14. Turn off pump and break vacuum.

15. Add: 500 uL Buffer AW1 to each column. Wait one minute. Turn on pump to pull wash buffer through the column. Euphotic zone samples may require a second rinse with AW1.

16. Turn off pump and break vacuum.

17. Add: 500 uL Buffer AW2 to each column. Wait one minute. Turn on pump to pull buffer through the column. Transfer columns to clean 2 mL catch tube (provided with kit).

18. Spin columns for 2 minutes at 14,000 *g*.

19. Put spin column membrane in new 1.5 mL micro centrifuge tube, preferable eppendorf safe lock tubes. Label tube with sample name and extraction date.
    a. Add: 50 uL DNAse/RNAse free H_2O **right onto the surface of the membrane** (without touching the membrane).
    b. Let stand at room temperature for 1 minute.
    c. Centrifuge at 8000 rpm for 1 minute.
    d. Repeat steps a-c for a total of 2 x 50 uL = 100 uL elution volume.

20. Use Q-Bit to determine DNA concentration in product. Store archive sample at -80ºC. Record sample location on freezer inventory. Maintain working stock at 4ºC.

Sucrose EDTA Lysis Buffer:

0.75 M Sucrose
20 mM EDTA
400 mM NaCl
50 mM Tris

References

1. **Bernhard, A. E., and K. G. Field.** 2000. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Appl. Environ. Microbiol. **66:**1587-1594.
2. **Fuhrman, J. A., D. E. Comeau, A. Hagstrom, and A. M. Chan.** 1988. Extraction From Natural Planktonic Microorganisms Of DNA Suitable For Molecular Biological Studies. Appl. Environ. Microbiol. **54:**1426-1429.

3. **Massana, R., A. E. Murray, C. M. Preston, and E. F. DeLong.** 1997. Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl. Environ. Microbiol. **63:**50-56.