Modified FastDNA® Spin kit for soil (MP Biomedicals) DNA extraction method for deep marine sediments

This method, its development, comparisons with other methods and the history of the problems with DNA extraction from subsurface sediments is described in Webster *et al*. (2003).

Sample handling

Sediment samples, preferably as whole round cores (WRC), for use in molecular diversity studies should be frozen as soon as possible after sampling at -20°C or -80°C and transported frozen back to the laboratory. Sub-sampling in the laboratory should then be carried out in a laminar flow cabinet with a sterile 2-cm diameter stainless steel corer. WRC can be softened by partial thawing if required and the corer can be hammered into the sediment if necessary. Only sediment from the centre of the cores should be used, as studies have shown that the outer layers are more likely to be contaminated than the central section (House et al., 2003). Sub-samples should then be stored frozen before DNA extraction.

Method

Using the FastDNA® Spin kit for soil (MP Biomedicals; cat #6560-200)

1. Add 6x 0.8 g of sediment to lysing matrix E tube.
2. Add 120 µl MT Buffer, 780 µl sodium phosphate buffer and 20 µl polyadenylic acid (10 mg ml^{-1}). Secure tubes in a FastPrep® Instrument and process for 30 sec at speed 5.5.
3. After cell lysis centrifuge lysing matrix E tubes at 13,000 rpm for 8 min.
4. Transfer supernatant to a clean tube (non-stick 1.5 ml tube). Add 250 µl PPS reagent and mix by carefully inverting the tube by hand 10 times.
5. Centrifuge at 13,200 rpm for 5 min to pellet precipitate. Transfer all supernatant to a sterile universal tube and add 1ml binding matrix suspension (note, re-suspend before use).
6. Carefully shake by hand for 2 min to allow binding of DNA to matrix and place tube in a rack for 30 min to allow continued binding and settling of binding matrix.
7. Remove 500 µl of supernatant being careful to avoid settled binding matrix and discard. Resuspend binding matrix in the remaining amount of supernatant. Transfer 700 µl of the mixture to a SPIN™ Filter (as supplied with kit) and centrifuge at 14,000 rpm for 1 min. Empty the catch tube and add the remaining supernatant to SPIN™ Filter and spin again.
8. Add 500 µl SEWS-M wash solution to the SPIN™ Filter and centrifuge at 14,000 rpm for 1 min. Empty the catch tube and replace SPIN™ Filter, centrifuge at 14,000 rpm for 2 min to dry the matrix of residual SEWS-M wash solution.
9. Remove SPIN™ Filter and place in fresh catch tube. Add 100 µl DNase/RNase free water and gently flick matrix on filter membrane to gently re-suspend the binding matrix/DNA for efficient elution of the DNA.
10. Place in a rack for 20-30 min to allow efficient DNA elution. Centrifuge at 14,000 rpm for 2 min to transfer eluted DNA to catch tube.
11. Transfer 6x 100 µl eluted DNA into one Microcon filter (YM-100; Millipore)

and centrifuge at 7,000 rpm for 10 min. Decant Microcon catch tube, wash with 500 µl DNase/RNase free water and centrifuge at 7,000 rpm for 10 min. Repeat washing step with 500 µl DNase/RNase free water.

12. Take out the membrane filter, invert and place in fresh Microcon catch tube. Add 40 µl DNase/RNase free water and centrifuge at 3,000 rpm for 4 min and discard the filter.
13. DNA yield is examined by agarose (1.2% w/v) gel electrophoresis with 10 µl DNA sample and molecular weight markers.

References:

Webster, G., Newberry, C.J., Fry, J.C. & Weightman, A.J. (2003). Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale. *J Microbiol Methods* 55:155-164.