Total soil DNA extraction and purification:

This method can be used to extract and purify total nucleic acids from soil/sediment samples, plant parts, bacterial cultures and fungal mycelia. The method below details the approach for soil or sediment.

1. 0.5 g sieved soil is added to 2 ml screw-capped microcentrifuge tubes. 0.5 ml of modified CTAB (hexadecyltrimethylammonium bromide) extraction buffer (equal volumes of 10% CTAB in 0.7 M NaCl with 240 mM potassium phosphate buffer [pH 8.0]) is added and tubes are vortexed briefly.

2. Tubes are then incubated at 70^{o}C for 10 min in a waterbath.

3. After incubation 0.5 g each of 0.1 mm glass and 0.5 mm zirconia/silica beads are added. 0.5 ml phenol:chloroform:isoamylalcohol (25:24:1) is then added and tubes are then shaken in the FastPrep Instrument (Qbiogene) at 5.5 m/s for 30 s.

4. Following bead-beating, tubes are centrifuged at 16000 x g for 5 min at 4^{o}C. The aqueous layer is removed and combined with an equal volume of chloroform:isoamylalcohol (24:1). Tubes are mixed well and centrifuged at 16000 x g for 5 min at room temperature. The aqueous layer is removed into a clean 1.5 ml microcentrifuge.

   (Note: if DNA yield is expected to be low you can increase efficiency by performing this second organic extraction in a pre-spun Phase-Lock gel (Heavy) tube - Eppendorf).

5. If the extract is a light yellow/brown colour this indicates residual humic acid contamination requiring a further purification step (7), but if humic contamination is not obvious (a feint yellow tint is OK) then continue from (9).

6. Incubate the extract with 50 μl of lysozyme solution (100 mg/ml) for 30 min at 37°C.

7. Centrifuged tubes at 16000 x g for 5 min at 4°C.

8. Remove supernatant and purify using a Gene Clean Turbo Spin Kit (BIO 101). Most of this procedure is according to manufacturer's instructions, with a few exceptions. The binding buffer used is our own (4.5M guanidine isothiocyanate, 0.5M potassium acetate [pH 5.0]). Use one volume of this buffer per volume of DNA extract. DNA is eluted in a final volume of 50 μl.

   (Note: In our hands this buffer gives a greater yield than the GNOMIC binding buffer that comes with the kit).

9. Store extracted DNA at -20^{o}C for future use. For long term storage store at -80^{o}C.