

Soil DNA prep

1. Put 0.5 g of each soil sample into 1.5 ml eppendorf tube with sterile spatula
2. Add 0.5 g 0.5 mm glass beads (sterile) to each
3. Add 600 μ l Extraction buffer S, vortex at high speed 2 min, spin at 15K, 2 min
4. Pour off supernatant (keep) in new eppendorf tube; dispose of pellet
5. Add equal volume of CHCl_3 to supernatant, mix by hand, spin at 8K, 5 min
6. Pipette off supernatant into new eppendorf tube
7. Add equal volume CHCl_3 , mix by hand, spin at 15K, 5 min
8. Pipette off supernatant into new eppendorf tube
9. Add equal volume of -20C isopropanol, mix by hand
10. Store at -20C overnight or longer
11. Spin at 15K, 10 min, dispose of supernatant, wash pellet with 70% EtOH
12. Quick spin and pour off supernatant; allow pellet to dry
13. Add 1-200 μ l TE, pH 8 and resuspend pellet by flicking occasionally
14. Run 15 μ l on 0.8 – 1% agarose gel to check DNA quantity, quality

Extraction buffer S

50 mM Na-phosphate, pH 8
50 mM NaCl
500 mM Tris-HCl, pH 8
5% SDS

Extraction buffer T

500 mM Tris-HCl, pH 8
100 mM NaCl
10% SDS

Equipment/reagents

1 mm glass beads
Extraction buffer
Spatula(s)
Microfuge
Eppendorf tubes 5 per sample
 ClCH_3 ; 91% isopropanol; 70% EtOH; TE pH 8