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Jun 17, 2020

Protocol for DNA extraction from clay-rich subsoils

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1 Works for me dx.doi.org/10.17504/protocols.io.bhmjj44n

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Guerra et al. (2020), *Microorganisms*,*8*(4), 532; **https://doi.org/10.3390/microorganisms8040532**

DOI

dx.doi.org/10.17504/protocols.io.bhmjj44n

PROTOCOL CITATION

Lukas Beule 2020. Protocol for DNA extraction from clay-rich subsoils. **protocols.io** dx.doi.org/10.17504/protocols.io.bhmjj44n

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Guerra et al. (2020), *Microorganisms*,*8*(4), 532; **https://doi.org/10.3390/microorganisms8040532**

CREATED

Jun 17, 2020

LAST MODIFIED

Jun 17, 2020

PROTOCOL INTEGER ID

38283

Protocol source

1 This protocol is the Supplementary File S1 of Guerra et al., *Microorganisms* 2020,*8*(4), 532; **https://doi.org/10.3390/microorganisms8040532**

please cite as Guerra et al. (2020) *Microorganisms*,*8*(4), 532.

Sample preparation

2 Weigh in 200 ± 5 mg freeze-dried soil into a 2-mL tube.

Subsoil DNA extraction 3h

3 1. Homogenize the sample by bead beating using 3 tungsten carbide beads (ø 3 mm) for 1 min at 25 Hz using a swing mill (e.g. MM 400, Retsch, Germany). 3h

2. Add 250 µL PB buffer1 with 0.5% SDS (w/v) and vortex for 10 sec at max speed.

3. Incubate the suspension at 65°C for 10 min and shake the samples every 60 sec for 5 sec.

4. Centrifuge for 1 min at 10,000 rpm and transfer 90 µL of the supernatant into a new 2-mL tube (supernatant will contain bubbles).

5. Add 810 µL ddH2O (1:10 dilution) and subsequently 900 µL phenol2 and shake the samples briefly.

6. Centrifuge for 10 min at 10,000 rpm and transfer 800 µL of the upper phase into a new 2-mL tube.

7. Add 800 µL chloroform:isoamylalcohol (24:1 (v/v)), incubate 10 min on ice and centrifuge for 10 min at 10,000 rpm.
8. Transfer 700 µL of the upper phase into a new 1.5 mL-tube.

9. Add 700 µL chloroform:isoamylalcohol (24:1), incubate 10 min on ice and centrifuge for 10 min at 10,000 rpm.

10. Transfer 600 µL of the upper phase into a new 1.5 mL-tube containing 200 µL 30% PEG 6000 and 100 µL 5M NaCl.

11. Shake the mixture and incubate at room temperature for 20 min.

12. Centrifuge for 15 min at max. speed.

13. Wash the pellet with 500 µL 80% EtOH (v/v) twice and centrifuge for 5 min at max speed in-between each washing before discarding the supernatant. Supernatant should be removed by pipetting.

14. Dry the pellet using vacuum centrifugation at 30°C for 20 minutes or until it is dry under a sterile bench.

15. Add 50 µL TE buffer, vortex or flick the samples to release the pellet from the tube wall and incubate at 42°C for 2h (e.g. in a water bath).

Notes addressing the solutions: 1 PB buffer: 1 M Na2HPO4 and 1 M NaH2PO4, blended to achieve pH 7.2.2 Phenol: redistilled, in TE buffer equilibrated, pH 7.5 to 8.0

Citation: Lukas Beule (06/17/2020). Protocol for DNA extraction from clay-rich subsoils. https://dx.doi.org/10.17504/protocols.io.bhmjj44n