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Microbial DNA isolation from 1 gram soil

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We use this protocol and it's working

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Abstract

This protocol describes the extraction of microbial DNA from 1 gram of soil.



Materials

Plastics:

- Biocomma: CommaPrep™ RNAExtractionColumns, Capped spin columns, Green fixing rings, 2mL, 800 µL, capacity ~20 µg (Catnr: RP20-A-N)
- Corning 15 mL centrifuge tube
- General lab plastics (e.g. 1.5 mL tubes and pipet tips)

Chemicals and solutions:

- 1.5gr of silicon carbide powder (grit 46), e.g. A14470.36
- bead solution C0: 181 mM disodium phosphate, 121 mM guanidinium thiocyanate | *After autoclaving cover bottle with aluminium foil to keep it from light | Solution C0 is a solution that disperse soil particles, dissolve humic acids and protect nucleic acids from degradation*
- lysis buffer C1: 150mM NaCl, 4% (w/v) SDS, 0.5 M Tris (pH not adjusted) | *Autoclave | Solution C1 contains SDS, an anionic detergent that breaks down the cell membrane*
- Solution C2: 1.33M ammonium acetate | *Autoclave | Solution C2 precipitates non-DNA organic and inorganic matter that can inhibit the PCR reaction*
- Solution C3: 120 mM ammonium aluminum sulfatedodecahydrate | *Add $\text{NH}_4(\text{Al})\text{SO}_4$ after autoclaving MQ $\pm 20\text{min}$ 121°C to prevent precipitation | Solution C3 is another inhibition (humic acids) removing solution*
- binding solution C4a (Toxic!): an aqueous solution of 5M guanidinium thiocyanate and 30mM Tris-HCl (pH: 8.0) with 9% (v/v) isopropanol | *Autoclave $\text{GuSCN} + \text{Tris-HCl}$ in Endvolume-9%. After autoclaving add the additional isopropanol and cover bottle with aluminium foil to keep it from light. | Isopropanol is added after autoclaving*
- binding solution C4b (Toxic!): an aqueous solution of 5M guanidinium hydrochloride and 30mM Tris-HCl (pH: 8.0) with 9% (v/v) isopropanol | *Autoclave $\text{GuHCl} + \text{Tris-HCl}$ in Endvolume-9%. After autoclaving add the additional isopropanol and cover bottle with aluminium foil to keep it from light.*
- Washing solution C5: 10mM Tris-HCl - pH: 6.5, 100mM NaCl, and absolute EtOH final v/v 50% | *After autoclaving add an equal volume of 99.9% ethanol | After autoclaving add an equal volume of 99.9% ethanol | Solution C5 is an ethanol based wash solution that removes residual salt, humic acid and other contaminants while leaving the DNA bound to the filter*
- Elution buffer C6: 10mM Tris-HCl, pH8.0



Safety warnings

- ⚠ Wear gloves at all times. Due to toxicity, handling of solution C4a or C4b should be performed in the fume hood.



Sample preparation

- 1 Weigh 1 gram of thoroughly mixed soil, transfer it to a 15 mL bead tube, and add 1.5 gram of silicon carbide powder (grit 46).

DNA extraction

- 2 Add 3 mL of bead solution C0, 0.24 mL of lysis buffer C1. If solution C1 is precipitated, heat solution to max. 60 °C until the precipitate has dissolved before use.
- 3 Bead beat the tubes for 10 minutes in a paint shaker or a vortex.
- 4 Centrifuge the tubes for 2-5 minutes (2,500 x g) at 4°C to separate the soil particles from the lysate.
- 5 Transfer 2 mL of the upper aqueous phase to a new 15 mL tube, and add 1 mL of solution C2 and hand mix ~1 minute.
- 6 Centrifuge the tubes for 2-5 minutes (2,500 x g) at 4°C to separate the precipitate from the nucleic acids.
- 7 Transfer 2.6 mL of the upper aqueous phase to a new 15 mL tube, and add 0.8 mL of Solution C3. Shake 1 minute by hand to mix. If you want a break you can incubate the samples longer than 5 minutes in 4°C (max. 12 hours) there is no significant loss of DNA yields or purity seen.
- 8 Centrifuge the tubes for 2-5 minutes (2,500 x g) at 4°C to separate the precipitate from the nucleic acids.
- 9 Avoiding the pellet, transfer a fixed volume (eg. 1 mL) of supernatant into a clean 1.5-15 mL tube (depending on volume).

DNA purification

- 10 Add 2 volumes binding solution C4a **OR** C4b at RT to the supernatant, and shake by hand to mix. Spin few seconds to pellet the toxic droplets.





- 11 Load your samples on Biocomma spin columns and put on a vacuum manifold and turn on the vacuum system. Alternatively, load your samples on the spin columns and spin 30 seconds $\sim 10.000 \times g$.
Tip: If the sample volume exceeds the volume of the spin filter repeat this loading process again.
Note: The capacity of a Biocomma spin filter is $\sim 20 \mu g$. Loading more might result in loss of DNA.
- 12 Before the wash step remove waste and perform 1 extra spin step 30 seconds $\sim 10.000 \times g$ to dry the pellet.
- 13 Add 0.5 mL of washing solution C5 to the pall plate or spin filter column. You may repeat this step up to 3X more to obtain a cleaner sample.
- 14 Before the final elution step remove waste and perform 1 extra spin step 30 seconds $\sim 10.000 \times g$ to dry the pellet.
- 15 Air-dry the filter for 5 minutes, put a collection plate underneath the vacuum manifold and add 0.2 mL of elution buffer C6 to the pall plate or column.
- 16 Collect the eluate (0.2 mL) and store it at $-20^\circ C$ until further use.