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Microbial DNA isolation from 1 gram soil

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Paula Harkes[1], Sven van den Elsen[1], Hans Helder[1]

[1]Wageningen University and Research Centre

Robbert van Himbeeck
Wageningen University & Research

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We use this protocol and it's working

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Disclaimer

Abstract

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

Materials

Plastics:

- Biocomma: CommaPrep™ RNAExtractionColumns,Capped spin columns, Green fixingrings, 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 NH4(Al)SO4 after autoclaving MQ ±20min 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 GuSCN+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 GuHCL+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: 10mMTris-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 ~10.000 x 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 ~20 µg. Loading more might result in loss of DNA.

12 Before the wash step remove waste and perform 1 extra spin step 30 seconds ~10.000 x 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 ~10.000 x 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 °C until further use.