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Supplementary material

A lab-made method for extracting DNA from soils

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Soil DNA Extraction Method

Beadbeating (beadbeating breaks open the cells by agitation with the beads in the presence of detergents (SDS)

Add ~250 mg of soil to a 2 ml bead beating tube
Add 1 scoop each of 0.1 and 0.5 zirconium beads and 2.5 mm glass beads. (scoop is approximately 150 µl volume and is manufactured by cutting the bottom off of a 1.5 ml microcentrifuge tube with a razor blade).
Add 1000 ml of Extraction Buffer (EB). Cap the tube tightly.
Bead beat using the bead beating vortexer with tubes attached to the horizontal tube holder (available from Mo Bio or Scientific Instruments) for 10 to 20 min at maximum setting (8+).
Centrifuge for 14,000 g's for 5 minutes to pellet the soil
Remove supernatant with a pipette and transfer to a new 2 ml microcentrifuge tube. You should recover from 700 µl to 850 µl of supernatant.
Note: the supernatants should be clear with a brown coloring which is an indicator of significant humic acids in the solution.

Ammonium Acetate precipitation (ammonium acetate precipitates unwanted humic acids and other impurities while retaining the DNA in the solution phase)
Add 450 µl of 7.5 M of ammonium acetate to each of the 2 ml tubes containing the sample solution
Cap the tubes and vortex for 5 seconds
Place on ice for 5 minutes (the ice aids in the precipitation of contaminants).
Centrifuge for 14,000 g's for 5 minutes pelleting the contaminants

Isopropyl Alcohol Precipitation (isopropyl alcohol precipitates and purifies the DNA in order to concentrate it for the next step)
Using a pipette carefully remove 1000 µl from the supernatant, avoiding the contaminant pellet, and transfer to a new 2 ml tube.
Add 1000 µl of ice cold isopropyl alcohol.
Mix end over end several times and place the tube on ice for at least 10 minutes, or overnight in a freezer at -21° C.
Centrifuge at 14,000 g's for 5 min- the pellets should be brown to dark brown and plainly visible.

Discard the supernatant, upend the tube and drain the remaining liquid onto a paper towel on top of the lab bensch for 5 minutes.
Resuspend the pellet with 50 ul of Sephacryl Wash Buffer and vortex the sample until dissolved. At this stage you can serially resuspend extracts into a single sample if you want to use several sample extracts in your final sample. I routinely extract 3 to 4 samples and resuspend them in a single sample at this stage.

Sephacryl 300 S HR spin column chromatography (prepare Sephacryl column as indicated below)
Load 50 µl of sample on a 400 µl bed volume Sephacryl 300 S HR spin column placed on top of a new 2 ml catch tube.
Centrifuge at 100g's for 2 minutes
Elute the sample by adding 100 to 150 µl of Sephacryl Wash Buffer to the top of the column.
Centrifuge at 100 g's for 2 minutes
The sample is purified.
Note: 100 µl elution volume will result in slightly lower yield but increased purity, 150 µl will result in slightly higher yield but less purity.

Reagents

Extraction Buffer (EB): 1 liter
50 mM TRIS Base 3.02g
50 mM EDTA 9.3 g
500 mM NaCl 14.6g
SDS 20 g (final concentration is 4%)
E water 400 ml
adjust to pH 8.0 with HCl
bring to a total volume of 1 liter with E water

Sephacryl Wash Buffer: 100 ml
100 mM TRIS Base 1.21 g
100 mM NaCl 0.585g
Adjust to pH 8.0 with HCl

7.5 M Ammonium Acetate: 50 ml
Ammonium acetate 28.8g
Add highly purified water to 50 ml mark

Sephacryl Matrix preparation: The Sephacryl Matrix is stored in a solution containing ethanol and microbial preservative which may impact purification and downstream processes if not eliminated. To change the solution remove a desired volumen of Sephacryl Matrix from the storage bottle to a chromatography column or Buchner funnel with a frit or filter paper at the bottom, respectively, blocking the bottom. You may need a column funnel if using a chromatography column. The frit or filter paper allows solution to drain out of the matrix into a catch beaker. Add 10 volumes of Sephacryl Wash Buffer to the column and allow it to flow through. The Sephacryl is now equilibrated into the Sephacryl Wash buffer and is ready to use. Resuspend the Sephacryl in approximately ¼ of the matrix volume and store in the refrigerator for later use, remembering that the solution does not have any antimicrobial agent to keep the microbes from growing. The Matrix should be fine for a week or so.

Sepharyl Column preparation
Take a 600 µl tube and cut off the cap and place on top of a 2 ml centrifuge tube with cap cut off,
Poke a hole in the bottom of the 600 µl tube using a dissecting needle.
Wad up a 1 cm^2 piece of Kimwipe or paper tissue and using a 200 µl pipette tipe with end cut off ram the paper piece down to the bottom of the tube. The paper will serve as a frit to keep the Sephacryl from leaking out upon centrifugation and allow the DNA to flow out. The tube is ready to load with gel filtration matrix

Shake up the Sephacryl 300S HR bottle to completely resuspend the Sephacryl Matrix
With a 1 ml pipette tip with the tip cut off remove 600 ul of resuspension mix and add to top of the 600 µl column.
Centrifuge at 100 g's for 2 minutes.
Place the column on top of a new 2 ml tube for collection
Note: these tubes can be stored for several days in the refrigerator for later use.

Chemical Hygene:
Throw away all spent beadbeating tubes.
Use lab gloves and lab coat when performing all operations- to avoid contaminating extracts and protect against chemicals.