DNA extraction and genomic DNA cleanup protocol for soil and other environmental samples

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ABSTRACT

This is a spin column based environmental DNA extraction protocol that has been validated with mineral soil and forest floor samples. The protocol starts with disruption and lysis in a chaotropic solution, followed by two PCR inhibitor removal steps, binding of DNA to a silica membrane, and is finished with wash steps. Guanidine carryover, which impedes PCR, is a common feature of these kinds of methods, so DNA cleanup is often required. A cost-effective magnetic bead clean-up step is recommended for samples with low 260/230 ratios, and a published protocol is provided for the magnetic bead solution. Overall, this protocol can reduce extraction costs to <$2 (CAD) per sample. The protocol can also be adapted to a high-throughput magnetic bead based setup.

SAFETY WARNINGS

Read all safety data sheets for all reagents before beginning. Wear appropriate personal protective equipment.

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Protocol status: Working
We use this protocol and it's working

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Reagents

1

Procure the following reagents before beginning.

	A	B	C
	Chemical name	**CAS**	**Molecular weight (g/mol)**
	Sodium phosphate, Na3PO4	7784-26-1	453.33
	Guanidium isothiocyanate, GITC	77-86-1	121.136
	Sodium chloride, NaCl	7647-14-5	58.44
	Tris base	7601-54-9	163.94
	Sodium dodecyl sulfate, 10%	151-21-3	288.38
	Ammonium acetate	67-63-0	60.10
	Aluminium ammonium sulfate dodecahydrate	64-17-5	46.07
	Guanidine hydrochloride	631-61-8	77.08
	Calcium chloride dihydrate	593-84-0	118.16
	Ethanol, 100%	50-01-1	95.53
	Isopropanol, 100%	10035-04-8	147.01

Table 1: Reagents required for extraction procedure.

Materials

2 Procure the following materials before beginning.

- 2 mL screw cap tubes
- 1.5 mL microcentrifuge tubes
- 2 mL microcentrifuge tubes OR 2 mL collection tubes (lidless)
- Vortex tube adapter (e.g. Scientific Industries SI-HP524 or other bead-based homogenizer)
- Silica spin columns (Epoch life sciences, cat. no. 1250F-250)
- 1.0 mm and 0.7 mm zirconia beads (cat. no. 11079110zx and 11079107zx, respectively, BioSpec

Products)
- Magnetic stand for 1.5-2mL tubes (e.g. Promega cat. no. Z5342)
- Pipette tips (1000, 200, 100, 20 and 10 uL)

Note

*The quality of the spin column is critical, a plastic 'frit' is required for support, and the column should have at least 4 silica layers. The epoch columns listed above have been tested with the protocol and perform fairly. Spare columns from Qiagen/Mobio powersoil kits work effectively.

Reagent preparation

3 Prepare the following reagents:

Bead solution:

[M] 181 millimolar (mM) Sodium phosphate

[M] 121 millimolar (mM) Guanidinium isothiocyanate

pH 8.8 - 9.2

For 500 mL of 1M Na_3PO_4 stock:

- In a 500 mL volumetric flask, dissolve 81.97g Na_3PO_4 in molecular biology grade water

For 500 mL of 1M guanidinium isothiocyanate stock:

- In a 500 mL volumetric flask, dissolve 59.08g guanidinium isothiocyanate in molecular biology grade water

For 250 mL of bead solution:

- Add 45.25 mL of 1M Na_3PO_4 stock and 30.25 mL of 1M guanidinium isothiocyanate stock to a 250 mL pyrex media bottle. Bring volume to approximately 200 mL with molecular biology grade water. **Slowly** adjust pH to 8.8-9.2 using 5N hydrochloric acid. Decant solution into 250 mL volumetric flask and bring to 250 mL with molecular biology grade water.

Solution 1:

[M] 150 millimolar (mM) Sodium chloride

[M] 4 % (v/v) Sodium dodecyl sulfate

[M] 0.5 Molarity (M) Tris

For 10 mL:
0.0877 g NaCl
0.6057 g Tris

- Dissolve in 6 mL of molecular biology grade water. Add 4 mL of 10% SDS stock. Do not autoclave. Adjust pH to 10.75-11.25.

Solution 2:

[M] 133 millimolar (mM) ammonium acetate

For 25 mL:
0.2563 g ammonium acetate

- Dissolve in 25 mL of molecular biology grade water.

Solution 3A:

[M] 180 millimolar (mM) aluminum ammonium sulfate dodecahydrate

For 100 mL:
8.16 g aluminum ammonium sulfate dodecahydrate.

- Dissolve in 100 mL of molecular biology grade water and adjust pH to 3.1-3.3 using 5N HCl.

Solution 3B:

[M] 204 millimolar (mM) calcium chloride dihydrate

For 100 mL:
3 g calcium chloride dihydrate

- Dissolve in 100 mL of molecular biology grade water.

Solution 4:

[M] 5 Molarity (m) Guanidine hydrochloride
[M] 30 millimolar (mM) Tris
[M] 9 % (v/v) Isopropanol
pH 6.69 – 6.72

For 500 mL of 1M Tris stock (unadjusted pH):

- In a 500 mL volumetric flask, dissolve 60.568g Tris in molecular biology grade water

For 250 mL Solution 4:

- In a 250 mL pryex media bottle, dissolve 7.5 mL of 1M Tris stock and 119.4125g GuHCl in approximately 150 mL of molecular biology grade water. Add 22.5 mL isopropanol. **Slowly** adjust to pH 6.69-6.72 using 5M HCl.

Solution 5:

[M] 10 millimolar (mM) Tris (pH 7.5)

[M] 100 millimolar (mM) Sodium chloride
[M] 60 % (v/v) Ethanol

For 100 mL:

- In a 100 mL pyrex media bottle, add 1 mL 1 M Tris pH 7.5 stock, 2 mL 5 M NaCl stock, and 37 mL of molecular biology grade water. Autoclave for 20 minutes and add 60 mL 100% ethanol. Confirm solution pH.

Solution 6:

[M] 10 millimolar (mM) Tris (pH 8.5)

Preparation of bead tubes:

Add 0.25 mL each of 0.7 mm and 1.0 mm beads to 2mL screwcap bead tube.

Soil DNA extraction procedure

30s

4 This protocol is written for mineral soil DNA extraction. For fresh mineral soil samples 200-225 mg is optimal for most soil types. For freeze-dried and homogenized mineral soils, 100-150 mg is optimal. For freeze-dried and homogenized forest floor samples, 50 mg is optimal. Optimize the sample mass for your sample type before increasing throughput.

If **Solution 1** has precipitated, heat at 60 °C in a dry bath to dissolve.
Warm **Solution 6** in a 37 °C dry bath before beginning.

4.1 To each 2mL screwcap bead tube, add 750 µL of bead solution and 60 µL of **Solution 1**

4.2 Add 225 mg of fresh mineral soil to a prepared 2mL bead tube and secure lid tightly.

4.3 Secure bead tube to vortex genie homogenizer and vortex at maximum speed for 20 minutes. Adjust homogenization step to suit your homogenization setup.

4.4 Centrifuge tubes at 17000 × g for 2 minutes

4.5 Transfer the supernatant to a clean 1.5 mL tube. Expect 400-500 µL of supernatant.

4.6 Add 250 uL of **Solution 2** and vortex for 5 s. Incubate in freezer -20 °C for 5 minutes.

4.7 Centrifuge for 2 minutes at 17000 × g

4.8 Avoiding the pellet, transfer up to 600 µL of supernatant to a clean 1.5 mL tube

4.9 Make an equal parts master mix of **Solution 3A and 3B**, adding 200 µL of this mix to each tube. Incubate in freezer -20 °C for 5 minutes.

4.10 Centrifuge for 2 minutes at 17000 × g.

4.11 Avoiding the pellet, transfer up to 750 µL of supernatant to a new 2 mL collection tube.

4.12 Shake to mix solution C4 and add 1200 µL to the supernatant from step 4.11. Mix with pipette and let stand for 3 minutes.

4.13 Load 675 µL onto a spin column and centrifuge at 10000 ×g for 1 minute. Discard flow through. To reduce plastic waste, carefully eject pipette tip back into the collection tube for re-use in proceeding steps.

4.14 Repeat step 4.13 twice until all the sample has been processed.

4.15 Add 500 µL of **Solution 5**. Allow the solution to sit on the silica membrane for 1 minute. Centrifuge for 30 s at 10000 × g and discard the flow through.

4.16 Add 500 µL of [M] 80 % (v/v) Ethanol. Allow the solution to sit on the silica membrane for 1 minute. Centrifuge for 30 s at 10000 × g and discard the flow through.

4.17 Centrifuge again for 1 minute at 10000 × g to dry the column.

4.18 Add 50 µL of warm 37 °C **Solution 6** to the centre of the membrane and wait 5 minutes while the solution absorbs onto the membrane.

4.19 Centrifuge for 30s at 10 000 × g.

4.20 Check DNA concentration and repeat steps 4.17-4.18 only if yield is acceptable.

Magnetic bead-based clean up

5 Soil DNA from this protocol is often of poor quality (low 260/230 values) due to GuHCl carryover. This is common even in Qiagen's powersoil kits, which use similar chemistry. Often DNA cleanup is necessary to improve quality. DNA can be cleaned up using commercial bead kits (e.g. ProNex, AMP-pure), or with a non-commercial (DIY) solid-phase reversible immobilization (SPRI) bead solution. This following is a cleanup procedure that has been tested with a DIY SPRI bead solution. This solution can be used in size-selection protocols, but the protocol below is designed to return DNA fragments of all sizes. Expect a

30% reduction in DNA yield. For difficult samples, I have successfully used 2 or even 3 clean ups on the same sample, reducing elution volume to prevent overly diluting samples.

The recipe for the SPRI bead solution can be found here: https://openwetware.org/wiki/SPRI_bead_mix.

The SPRI solution recipe has been tested exactly as listed in the previous link, except Sera-Mag Carboxylate-Modified (E7) magnetic beads (Cytiva cat. no. 24152105050250) were used instead of the more expensive speed beads referenced in the recipe. Although Cytiva does not recommend its beads for purification of genomic DNA, testing revealed good recovery of high molecular weight DNA fragments from a DNA ladder.

Before beginning, the bead solution should be brought to room temperature and should be well mixed prior to use. Also, prepare an [M] 85 % (v/v) ethanol solution, being sure to measure water and ethanol separately before combining.

5.1 To each sample to be cleaned up, add 2.5x vol of the SPRI bead solution.

5.2 Vortex samples for 15 seconds and incubate at room temperature for 5 minutes. If solution is sticking to the sides of the tubes, briefly spin down.

5.3 Place tubes on magnetic stand for 5 minutes or until the beads have separated.

5.4 Aspirate the cleared solution using a pipette. The beads are easily drawn out with the supernatant, so carefully aspirate, leaving behind 5 uL of supernatant if necessary.

5.5 Without removing tubes from magnetic stand, add 200 µL of [M] 85 % (v/v) ethanol to each tube. Increase the volume of ethanol for larger samples, ensuring that the beads are covered with the ethanol solution.

5.6 Vortex for tubes 5 seconds and return tubes to magnetic stand.

5.7 Aspirate ethanol solution once cleared (usually 30 seconds).

5.8 Wash again with 200 µL of 85% ethanol. To speed up drying, aspirate as much ethanol as possible with a small volume pipette (without aspirating the beads).

5.9 Allow samples to air dry for 10 minutes. Drying time can be reduced to 3-5 minutes by placing tubes in 37 °C dry bath. Do not over dry – the beads should look slightly glossy but should not be cracked. The beads are too wet if when lightly flicking the tube with a finger the beads streak across the tube.

5.10 Remove tubes from magnetic plate and resuspend in 40 µL of **Solution 6** (10 mM tris pH 8.5), gently pipetting 10 times to homogenize. The amount of elution buffer can be increased but should not be decreased below 30 µL.

5.11 Incubate samples for 5 minutes.

5.12 Place tubes back on magnetic stand and allow beads to separate (1 minute).

5.13 Transfer eluate to a new tube, being careful not to draw up beads. In my experience, bead carryover does not interfere with PCR.