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DNA Extraction and Purification from Soil

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Justin

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Affordable DNA extraction and purification from soil samples at scale.

This protocol outlines an affordable DNA extraction and purification technique to be used with soil samples processed at scale. In the materials section, I provide details on how to make up reagents for each step. I provide the steps of the protocol in detail. The protocol steps follow the physical and chemical lysis of cells present in soils, the flocculation of various inhibitors of PCR, and the purification of extracted DNA by repeated centrifuging steps that bind and then elute DNA using a silica filter.

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Soil, DNA Extraction, DNA Purification

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When eluted in 1 mM Tris, the purified DNA can survive several freeze-thaw cycles. Depending on the samples analysed the purified DNA may benefit from additional dilution prior to PCR, especially if the end material is discoloured to a soil-brown.

Buffer components

A	B	C	D	E	F	G
Component	**Contents**	**Required**	**Chemical molarity**	**per L**	**per 100 mL**	**pH**
Lysis solution 1	guanidine thiocyanate	147 mM	118.16	17.33 g	1.73 g	9.0
	trisodium phosphate	228 mM	380.13	86.67 g	8.67 g	-
	sodium chloride	26 mM	58.44	1.5 g	0.15 g	-
	1 M Tris HCl	67 mM	-	67 mL	6.7 mL	-
	0.5 M EDTA	27 mM	-	53 mL	5.3 mL	-
Lysis solution 2	aluminium ammonium sulphate*	90 mM	453.33	40.8 g	4.08 g	-
	SDS	1.25 %	-	12.5 g	1.25 g	-
Protein flocculant	ammonium acetate	5 M	77.0825	385.41 g	38.54 g	-
Inhibitor flocculant 1	aluminium ammonium sulphate*	180 mM	453.33	81.6 g	8.16 g	-
Inhibitor flocculant 2	calcium chloride dihydrate	204 mM	147.01	30 g	3 g	-
Binding solution	guanidine HCl	5.5 M	95.53	525.42 g	52.54 g	-
Wash solution	EtOH	80 %	-	-	-	-
Elution buffer	Pure H2O or 1mM Tris	-	-	-	-	8.0

*aluminium ammonium sulphate = aluminium ammonium sulphate dodecahydrate (CAS 7784-26-1), if using anhydrous powder, adjust the calculation above.

Adjust the pH of **Lysis solution 1** to 9.0 with 5M HCl and bring to volume with ddH2O. This will probably require much less than 20 ml of HCl and should be mixed in the fume hood. All other components apart from **Elution buffer** are used at the pH of the mixture without modification. Sterilise all solutions in a suitable manner (autoclave or filter).

Follow appropriate precautions for molecular laboratory work, including following the COSHH guidelines for the reagents in use.

The soil samples used in this work were collected using a soil auger from the upper 10cm

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of the soil core by a collaborator. Where possible, I removed soil from the centre of the core for analysis in order to minimise the potential for sample cross-contamination.

Sample Lysis 6m 30s

1 Add 2g of 1.0mm to 1.4mm diameter acid-washed garnet beads to a 5ml Eppendorf screw-cap tube

2 Add 2200µL of ***Lysis Solution 1*** and vortex briefly.

3 Add 0.25g of sample to the tube, and shake briefly by hand to mix the contents.

4 Parafilm the lids of the tubes to prevent leaks.

5 Place in Geno/Grinder 2010 with appropriate adapters and shake at 1750 RPM for 2 mins 2m

Geno/Grinder 2010
Pulverizer and Cell Lyser
SPEX CertiPrep™ **12605297**

6 Wait 30 seconds 30s

7 Grind again for an additional 2 mins at 1750 RPM 2m

8 Centrifuge at 1000xg for 30 seconds to remove liquid from the lids of tubes 30s

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1000 x g, 25°C, 00:00:30

9 Add 800µL of ***Lysis Solution 2***

10 Centrifuge at 4,000xg for 1 min at room temperature. 1m
4000 x g, 25°C, 00:01:00

11 Transfer the supernatant to a fresh 1.5ml tube - or Transfer 1ml and save 500µl.

12 Centrifuge at 10,000xg for 1 min at room temperature. Transfer 500µl of supernatant to fresh tube 1.5ml tube. 1m
10000 x g, 25°C, 00:01:00

DNA Purification 18m

13 Add 200 µl volume of ***Protein flocculant***, vortex briefly, and incubate on ice for a minimum of 10 mins. 10m

14 Centrifuge at 10,000xg for 1 min at room temperature. 1m
10000 rpm, 25°C, 00:01:00

15 Transfer supernatant to fresh tube 1.5ml tube.

16 Make an ***Inhibitor flocculant master mix*** composed of:

n x 110 µl of ***Inhibitor flocculant 1***
and
n x 110 µl of ***Inhibitor flocculant 2***

Where **n** is the number of samples purified

17 Add 200 µl of ***Inhibitor flocculant mastermix*** to each sample

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18 Centrifuge at 10,000xg for 1 min at room temperature. 1m

10000 rpm, 25°C, 00:01:00

19 Transfer supernatant to fresh 5ml tube.

20 Add 1568µl of ***Binding Solution*** and invert several times to mix.

21 Fill a silica spin column to capacity with the above mixture, centrifuge at 10,000xg for 1 min at room temperature, discard flow-through and repeat until all mixture has passed through the spin column. 1m

10000 rpm, 25°C, 00:01:00

EZ-10 Spin Column & Collection Tube
Consumables
Bio Basic **SD5005.SIZE.100**

21.1 As an alternative, 96 well silica plates may be used for processing at scale. 5m

When using these, fill the column with 600µl of the above mixture and seal the plate with a breathable film. Centrifuge at 4,000 xg for 5 minutes over a 2.2ml deep-well plate to collect the flow-though. Repeat until all mixture has passed through the spin column using a new breathable film each time the column if filled.

4000 x g, 25°C, 00:05:00

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96 well DNA plate with membrane (960ul each well)
Consumables

Bio Basic **SD5007.SIZE.12**

Breathable Film
Plate Seal

StarLab **E2796-3005**

Natural, opaque, porous self-adhesive seal. Allows effective gas exchange for cellular and bacterial cultivation, while preventing contamination.

22 Add 392 µl of ***Wash Solution***, centrifuge at 10,000xg for 1 min at room temperature, discard flow-through. 1m

10000 rpm, 25°C, 00:01:00

23 Centrifuge at 10,000xg for 1 min at room temperature, replace collection tube with a fresh 1.5ml tube. 1m

10000 rpm, 25°C, 00:01:00

24 Add 313µl of ***Elution buffer*** heated to 70°C directly to the silica filter membrane. Leave for 2 min at room temperature. 2m

25 Centrifuge at 10,000xg for 1min at room temperature. 1m

10000 rpm, 25°C, 00:01:00

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26 Purified DNA is now in solution in the collection tube.

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