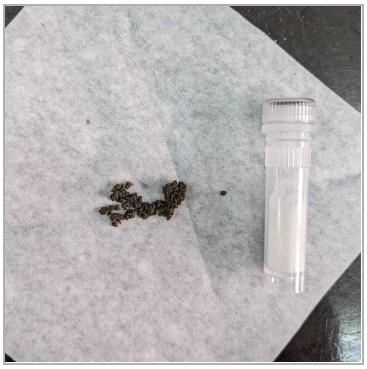

MAR 23, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.4r3l27864g1y/v1

Protocol Citation: Jie Hao Ou, Yin-Tse Huang 2023. SuperSoil - Soil DNA Extraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l27864g1y/v1

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

Created: Sep 26, 2022

Last Modified: Mar 23, 2023

PROTOCOL integer ID: 70491

Keywords: Soil DNA extraction

SuperSoil - Soil DNA Extraction

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ABSTRACT

SuperSoil - Soil DNA Extraction

GUIDELINES

Keep all solutions at room temperature

MATERIALS

1. Bead (ball mill)

	A	B
	Zirconia Balls	150 µm : 500 µm = 1 : 2

2. Solution SD1 (Chaotropic)

	A	B	C
		(w/w)	Price
	Sodium thiocyanate	5%	1400/250 g
	Na2HPO4	2.5%	1350/500 g
	pH	~8.4	

3. Solution SD2

	A	B	C
		(w/w)	Price
	AlCl3 · 6H2O	1.5%	900/500 g
	Ammonium acetate	25%	820/ 500 g
	pH (Adjust with acetic acid)	6.3	

4. Solution SD3 (Chaotropic)

	A	B	C
		(w/w)	Price
	Guanidinium thiocyanate	65%	1800/250 g
	pH	~7.0	

5. Solution SEA (Wash buffer 1)

	A	B	C
		(w/w)	Price
	Ethanol	50%	1000/4000 mL
	Isopropanol	10%	150/ 500 g
	Guanidine hydrochloride	30%	2900/1000 g

6.Solution S5 (Wash buffer 2)

	A	B	C
		(w/w)	Price
	Ethanol	70%	1000/4000 mL

7.Solution S6 (Elution buffer)

	A	B	C
		(w/w)	Price
	Tris Base	0.12%	3300/1000 g
	pH (Adjust with HCl)	8	

SAFETY WARNINGS

Some of the chemicals in this protocol may produce hazardous gases if mixed with bleach.

Lysis

1 Add **following materials** to 1.8 ml centrifuge tube

1. 250 mg soil

2. 1.5 g Bead
3. 800 µl SD1

2 **Vortex** for 00:10:00 at max speed 10m

Note

Vortex horizontally and ensure the cap is tightly sealed

3 14000 rpm, 00:01:00 1m

4 **Transfer the supernatant** to a clean 1.5 ml or 2 ml tube

Note

Expect 500–600 µl

Remove inhibitor 1m

5 Add **200 ul SD2**

Note

White precipitate forms.
If too much AlCl3 (SD2) is added, it will cause all the DNA to be precipitated along with the inhibitory substances.
See Mustafa (2017) for more info.

CITATION

Irfan Mustafa, Hadiatullah and Sustiyah (2017). Removal of humic acid from peat soils by using AlCl3 prior to DNA extraction. AIP Conference Proceedings.

LINK

doi.org/10.1063/1.4983434

6 **Vortex** for 00:00:05 at max speed 5s

7 14000 rpm, 00:01:00 1m

Note

Don't worry if the supernatant is yellow.

8 **Transfer the supernatant** to a clean 1.5 ml or 2 ml tube

Note

Expect 500–600 µl

Bind DNA to silica membrane 2m 5s

9 Add **600 µl SD3**

10 **Vortex** for 00:00:05 at max speed 5s

11 **Transfer 650 ul lysate** to spin column

12 14000 rpm, 00:01:00, discard flow-through 1m

13 **Transfer remaining lysate** to spin column

Wash silica membrane 1m

14 Add **500 ul SEA** to spin column

15 14000 rpm, 00:01:00, discard flow-through 1m

16 Add **500 ul S5** to spin column

17 14000 rpm, 00:01:00, discard flow-through 1m

18 14000 rpm, 00:01:00 , Centrifuge again to remove remaining ethanol (S5) , discard flow-through 1m

Elution

19 Add **50–100 ul S6**