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Soil sample DNA extraction (SuperSoil)

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

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Abstract

A method for fungal and bacterial DNA extraction.

Materials

1. Bead (ball mill)

	A	Diameter (mm)	Density (g/ml)	Amount (ml)	Catalog name
	Zirconia beads	0.5	6	0.2	CAAR-ZB-0.5-1000BX
	Zirconia beads	1.0	6	0.2	CAAR-ZB-1-1000BX

2. Solution SD1 (Chaotropic)

	A	B
		w/w
	sodium thiocyanate	2.5%
	Na_2HPO_4	5%
	pH	~8.4

3. Solution SD2

	A	B
		w/w
	$AlCl_3 \cdot 6H_2O$	1.5%
	Ammonium acetate	25%
	pH (Adjust with acetic acid)	6.3

4. Solution SD3 (Chaotropic)

	A	B
		w/w
	Guanidinium thiocyanate	65%
	pH	~7.0

Solution SEA (Wash buffer 1)

	A	B
		w/w
	Ethanol	50%
	Isopropanol	10%
	Guanidine hydrochloride	30%

80% Ethanol (Wash buffer 2)
DEPC treated water (Elution buffer)

Safety warnings

1. Solution SEA and Ethanol are flammable
2. DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Sample preparation & Cell lysis

2m

1 Add 0.2 mL ⌀=1.0 mm zirconia beads, 0.2 mL ⌀=0.5 mm zirconia beads, 800 µL SD1 solution, 0.5 g Soil into a 1.5 ml microcentrifuge tube. Vortex briefly to mix.

Note

If the SD1 solution is precipitated, leave it at room temperature for 30 minutes, it will become transparent again.

Note

If the soil is too loose and fills up the whole microcentrifuge tube, you can separate them into 2 microcentrifuge tubes for the downstream workflow. And centrifuge 2 microcentrifuge tubes at the final step to merge them.

Note

If your sample is hard to lyse, you can try the following methods:
1. After adding solution SD1, incubate at 65 ℃ for 10 minutes
2. Add 3 additional stainless steel beads and use 2.0 ml screw vials for your samples

2 Homogenize samples using Vortex-Genie® 2 with SI-H524 Horizontal microtube holder. Vortex at maximum speed for 00:04:00

4m

Note

Be careful when removing the microcentrifuge tube from the holder. Press the lid and slowly loosen the tube with another hand.

3 Centrifuge the 1.5 ml microcentrifuge tube at 14000 rpm, 25°C, 00:02:00

2m

Inhibitor precipitation

2m

4 Add 200 µL SD2 solution to a clean 1.5 ml microcentrifuge tube.

5 Transfer 500 µL supernatant to the tube in the previous step. Vortex 00:00:05 for mixing.

5s

> Note
>
> White precipitate forms.
> If too much AlCl3 (CD2) is added, it will cause all the DNA to be precipitated along with the inhibitory substances.

> Note
>
> If there is any suspended substance, you can use P200 and turn it to 150 µl and suck the supernatant up 3 times.

6 Centrifuge at 14000 rpm, 25°C, 00:02:00

2m

DNA binding

2m

7 Add 600 µL SD3 solution to a clean 1.5 ml microcentrifuge tube. Avoiding the pellet, transfer up to 700 µL of supernatant from the previous step to that tube. Vortex 00:00:05 for mixing.

5s

> Note
>
> If Solution CD3 has precipitated, heat at 60°C until the precipitate dissolves.

8 Take 650 µL to spin column (with collection tube) and centrifuge at 14000 rpm, 25°C, 00:02:00 2m

9 Discard the flow-through and repeat step 8 to ensure all the lysates have passed through the spin column.

10 Carefully place the spin column into a clean 2 ml collection tube. Avoid splashing any flow-through onto the spin column.

Wash 7m

11 Add 500 µL of Solution SEA to the spin column. Centrifuge at 14000 rpm, 25°C, 00:02:00 2m

> Note
>
> Solution SEA may leaks or suck back causing contamination.

12 Discard the flow-through and return the spin column to the same 2 ml collection tube.

13 Add 500 µL 80% ethanol to the spin column. Centrifuge at 14000 rpm, 25°C, 00:02:00 2m

> Note
>
> Ethanol may leaks or suck back causing contamination.

14 Discard the flow-through and place the spin column into a new 2 ml collection tube.

15 Centrifuge at 14000 rpm, 25°C, 00:03:00 Carefully place the spin column into the new 1.5 ml microcentrifuge tube 3m

Note

Ethanol can interfere with downstream DNA applications, such as PCR, restriction digests, and gel electrophoresis. Make sure to centrifuge for 3 minutes.

Elute

2m

16 Add 50 µL RNase-free water (DEPC-treated water) to the center of the white filter membrane.

Note

Alternative for RNase-free water: TE(Tris-EDTA) buffer

17 Centrifuge at 14000 rpm, 25°C, 00:02:00 Discard the spin column. The DNA will be in the microcentrifuge tube. 2m

Quality and quantity assessment

18 Use BioTek Take3 & Take3 Trio Microvolume Plates to measure the quality and quantity of your specimens. And then upload them to Google Drive (1 MolecularWork (Direct edit) 分生實驗號碼 (直接編輯) w_ Primer list)

Protocol references

Protocol

NAME

SuperSoil - Soil DNA Extraction

CREATED BY

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Preview