Jul 18, 2022

DNA extraction (BOMB_Soil)

Forked from DNA extraction (BOMB)

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dx.doi.org/10.17504/protocols.io.q26g74e8kgwz/v1

Hsin-Mao Wu

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ABSTRACT

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PROTOCOL CITATION

Tsu-Chun Hung, Yin-Tse Huang, Hsin-Mao Wu 2022. DNA extraction (BOMB_Soil). **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g74e8kgwz/v1

FORK NOTE

FORK FROM

Forked from DNA extraction (BOMB), Yin-Tse Huang

CREATED

Jul 18, 2022

LAST MODIFIED

Jul 18, 2022

PROTOCOL INTEGER ID

66907

MATERIALS TEXT

1. P1000 pipette
2. 96-deep well
3. MagBeads
4. TE buffer
5. Lysis buffer
6. Isopropanal
7. 80 % Ethanol
8. DEPC treated water
9. ZiXpress 32 System
10. 0.5mm zirconia beads
11. 1.0mm zirconia beads
12. 0.133M Ammonium acetate
13. 0.06M Aluminium sulfate

Sample Collection 3m

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1 Measuring **250 mg** for a soil sample

2 Add **200 µL** of **1mm beads** to 2ml enppendorf tube 30s

3 Add **200 µL** of **0.5mm beads** to 2ml enppendorf tube 30s

4 Add **225 µL** of **TE buffer** to 2ml enppendorf tube 30s

TE buffer is in 4°C fridge

5 Add **375 µL** of **lysis buffer** to 2ml enppendorf tube 30s

Lysis buffer is in 4°C fridge

6 Take 2ml enppendorf tube out of the laminar flow and transfer soil samples to the 2ml enppendorf tube 1m

Sample crush 4m

7 Put 2ml eppendorf tube in mixmill for sample crush, at this condition: 30 rpm/s, for 4mins 4m
00:04:00

Centrifugation 3m

8 Put 2ml eppendorf tube in centrifuge for centrifugation, at this condition: 3m
17.0 x g, 25°C, 00:03:00

Remove proteins and humic acid

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9 Add **250 µL** 0.133M Ammonium acetate in a new 2ml enppendorf tube

10 Transfer supernatant **300 µL** of step 8 into 2ml enppendorf tube in step 9

11 Incubate on ice for **00:10:00** 10m

12 Put 2ml eppendorf tube in centrifuge for centrifugation, at this condition: 3m
17.0 x g, 25°C, 00:03:00

13 Add **200 µL** 0.06M Aluminium sulfate in another 2ml enppendorf tube

14 Transfer step 12 supernatant **500 µL** in step 13's 2ml enppendorf tube

15 Incubate on ice for **00:10:00** 10m

16 Put 2ml eppendorf tube in centrifuge for centrifugation, at this condition: 10m
17.0 x g, 25°C, 00:10:00

DNA purification 37m 30s

17 Add **350 µL** of **isopropanol** to the 1st well of 96 well plate 30s

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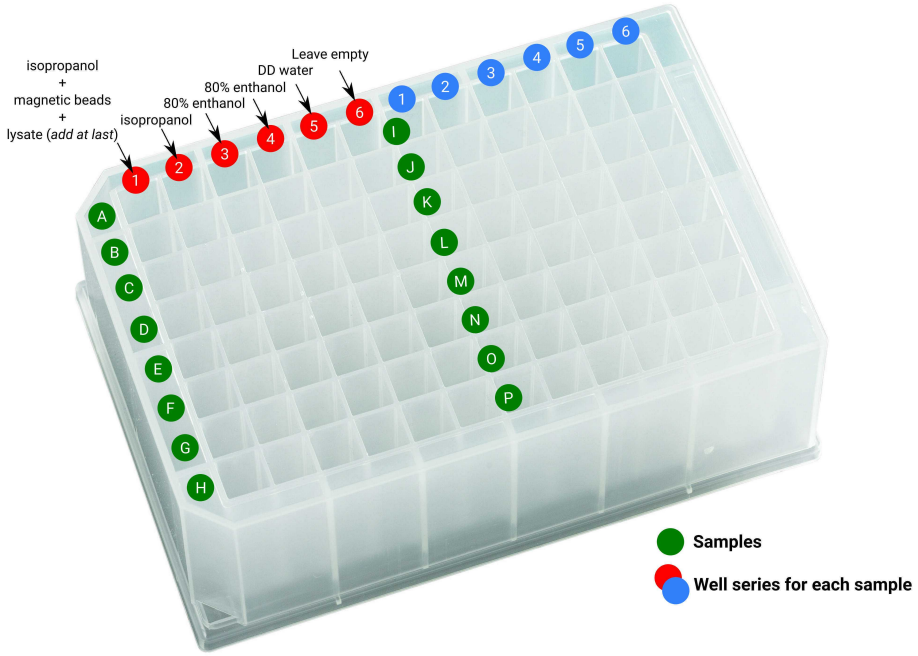

17.1 Add **125 µL** of **magnetic beads** (10 mg/ml) to the 1st well of 96 deep well plate 30s

Shake the bottle and pipetting before using magnetic beads

17.2 Add **200 µL** of the **sample (lysate)** from the 2ml centrifuged tube to the 1st well of 96 deep well plate 30s

USUALLY ADD at LAST

18 Add **400 µL** of **isopropanol** to the 2nd well of 96 deep well plate 30s

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19 Add **300 µL** of **80% enthanol** to the 3rd well of 96 deep well plate 30s

20 Add **300 µL** of **80% enthanol** to the 4th well of 96 deep well plate 30s

21 Add **100 µL** of **DD water** to the 5th well of 96 deep well plate 30s

22 Put the prepared 96 deep well plate in the automated DNA extraction machine 34m

23 After the extraction is done, collect **100 µL** of the **eluted sample** as the DNA template for downstream experiments

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