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DNA extraction (BOMB) V.2

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ABSTRACT

DNA extraction (BOMB)

PROTOCOL CITATION

Tsu-Chun Hung, Yin-Tse Huang 2022. DNA extraction (BOMB). **protocols.io** https://protocols.io/view/dna-extraction-bomb-cdk7s4zn Version created by Yin-Tse Huang

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Sample Collection 3m

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

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

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

TE buffer is in 4°C fridge

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

Lysis buffer is in 4°C fridge

5 Add **267 µL** of **10M ammonium acetate** to 2ml enppendorf tube

10M ammonium acetate is in 4°C fridge

6 Collect **10-20 mg** of **sample** to 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

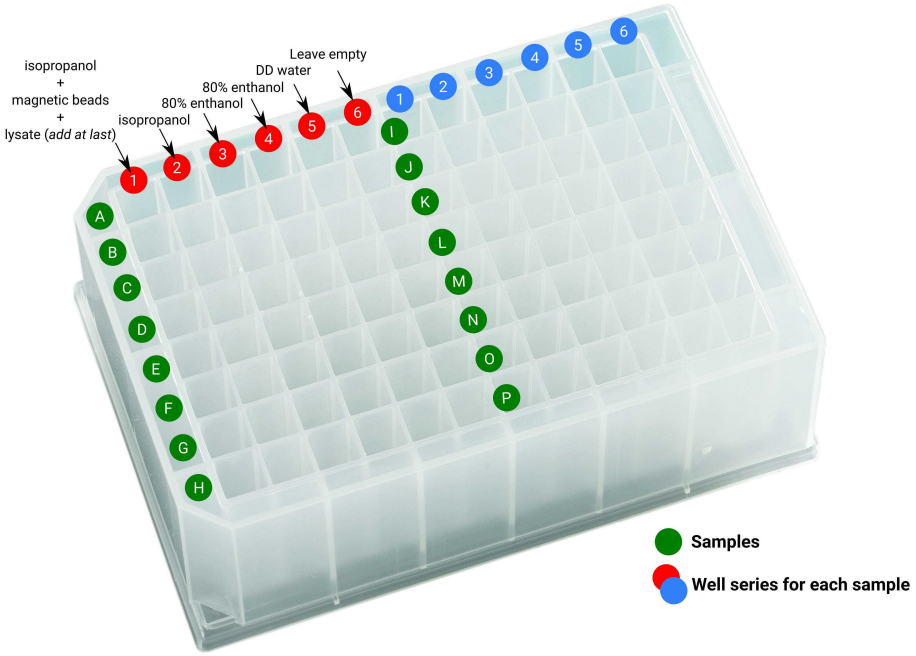
Centrifugation 3m

8 Put 2ml eppendorf tube in centrifuge for centrifugation, at this condition: 3m

17.0 x g, 25°C, 00:03:00

DNA purification 37m 30s

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



9.1 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

9.2 Add **200-300 µL** of the **sample (lysate)** from the 2ml centrifuged tube to the 1st well of 96 deep well plate 30s

Pipetting **as much lysate as you can**, as long as it's free of any cell debris (no solids in your tip)

USUALLY ADD at LAST

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

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

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

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

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

15 After the extraction is done, collect **50 µL** of the **eluted sample** as the DNA template for downstream experiments