Apr 19, 2023 Version 4

DNA extraction (BOMB) V.4

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvj6mdnlk5/v4

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DOI: https://dx.doi.org/10.17504/protocols.io.n2bvj6mdnlk5/v4

Protocol Citation: Tsu-Chun Hung, Yin-Tse Huang 2023. DNA extraction (BOMB). **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj6mdnlk5/v4Version created by Yin-Tse Huang

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

Created: April 06, 2023

Last Modified: September 13, 2023

Protocol Integer ID: 80088

Keywords: dna extraction, bomb, dna, extraction

Abstract

DNA extraction (BOMB)

Materials

1. Lysis master mix (870 uL/sample)

	A	B
	TE buffer	225 uL
	Lysis buffer	375 uL
	Ammonium acetate	270 uL

2. TE buffer

	A	B
	Tris HCl pH8.0	10mM
	EDTA	1mM

3. Lysis buffer

	A	B
	GITC	4M
	Tris HCl pH8.0	50mM
	SDS	0.5g
	EDTA	20mM

Sample Collection

3m

1 Add 200 µL of **1mm beads** to 1.5mL eppendorf tube

30s

2 Add 200 µL of **0.5mm beads** to 1.5mL eppendorf tube

30s

3 Add 870 µL Lysis master mix to 1.5mL eppendorf tube

30s

Note

In 4°C fridge
Lysis master mix: **225 µL** of TE buffer + **375 µL** of lysis buffer + **270 µL** of 10M ammonium acetate

4 Collect 10-20 mg of **sample** to 1.5mL eppendorf tube

1m

Sample crush

4m

5 Put 1.5mL eppendorf tube in mixmill for sample crush, at this condition: 30 rpm/s, for 4mins 00:04:00

4m

Centrifugation

3m

6 Put 1.5mL eppendorf tube in centrifuge for centrifugation, at this condition: 17.0 x g, 25°C, 00:03:00

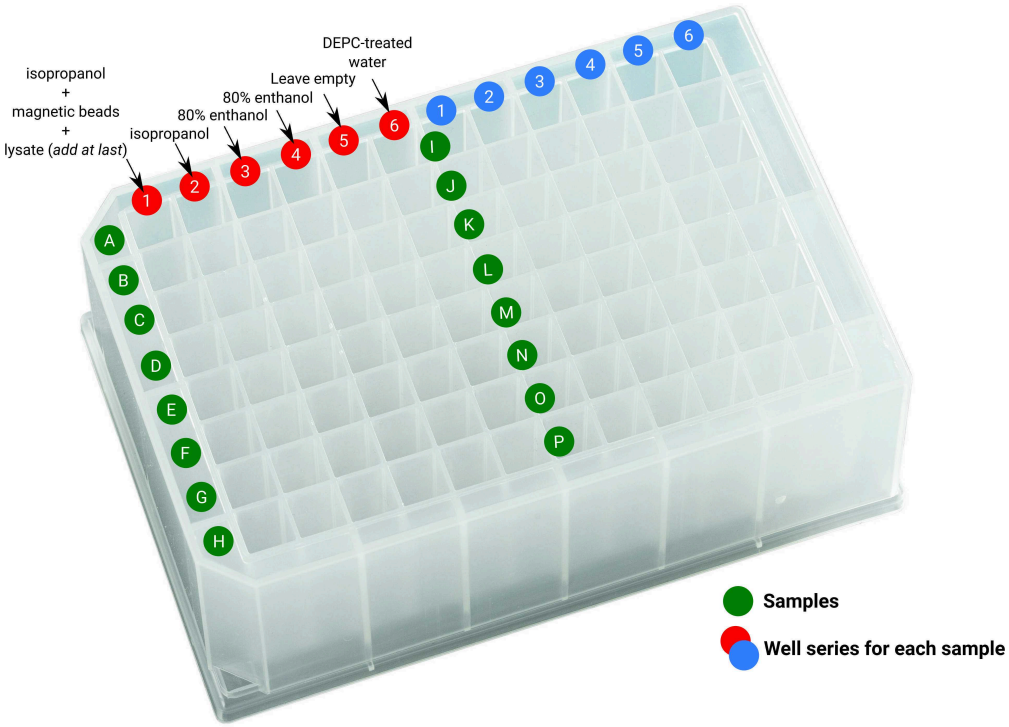
3m

DNA purification

37m 30s

7 Add 350 µL of **isopropanol** to the 1st well of 96 well plate

30s



7.1 Add 100 µL of **magnetic beads** (10 mg/ml) to the 1st well of 96 deep well plate 30s

Note

Vortex the bottle and pipetting before using magnetic beads; re-do vortex after adding to 3 samples to prevent set down of magnetic beads

7.2 Add 200-300 µL of the **sample (lysate)** from the 1.5mL centrifuged tube to the 1st well of 96 deep well plate 30s

Note

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

ADD at LAST

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

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

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

11 Add 300 µL of **DDW** to the 5th well of 96 deep well plate

12 Add 100 µL of **DEPC-treated water** to the 6th well of 96 deep well plate 30s

13 Put the prepared 96 deep well plate in the automated DNA extraction machine and select the BOMB protocol 34m

14 After the extraction is done, collect 50-100 µL of the **eluted sample** as the DNA template for downstream experiments