DNA extraction (BOMB) V.5

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

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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 **0.5 mm beads** to 2mL screw tube

30s

2 Add 200 µL of **1 mm beads** to 2mL screw tube

30s

3 Add 870 µL Lysis master mix to 2mL screw tube. The final look:

30s

Note

In 11F, 4°C fridge
Lysis master mix: **225 µL** of TE buffer + **375 µL** of lysis buffer + **270 µL** of 10M ammonium acetate

4 Collect 20-50 mg of **sample** to 2mL screw tube 1m

Note

You can collect up to 100 mg of sample if you can until you bump into the low DNA quality or PCR success rate; by then it means too many inhibitors in the sample and you have to lower the input.

Sample crush 4m

5 Put the 2mL screw tube in mixmill for sample crush, at 3200 rpm 00:04:00 4m

Note

Remember to balance if you have odd number of samples

Centrifugation

3m

6 Put 2mL screw tube in centrifuge for centrifugation, at this condition: 10 x g, 25°C, 00:03:00

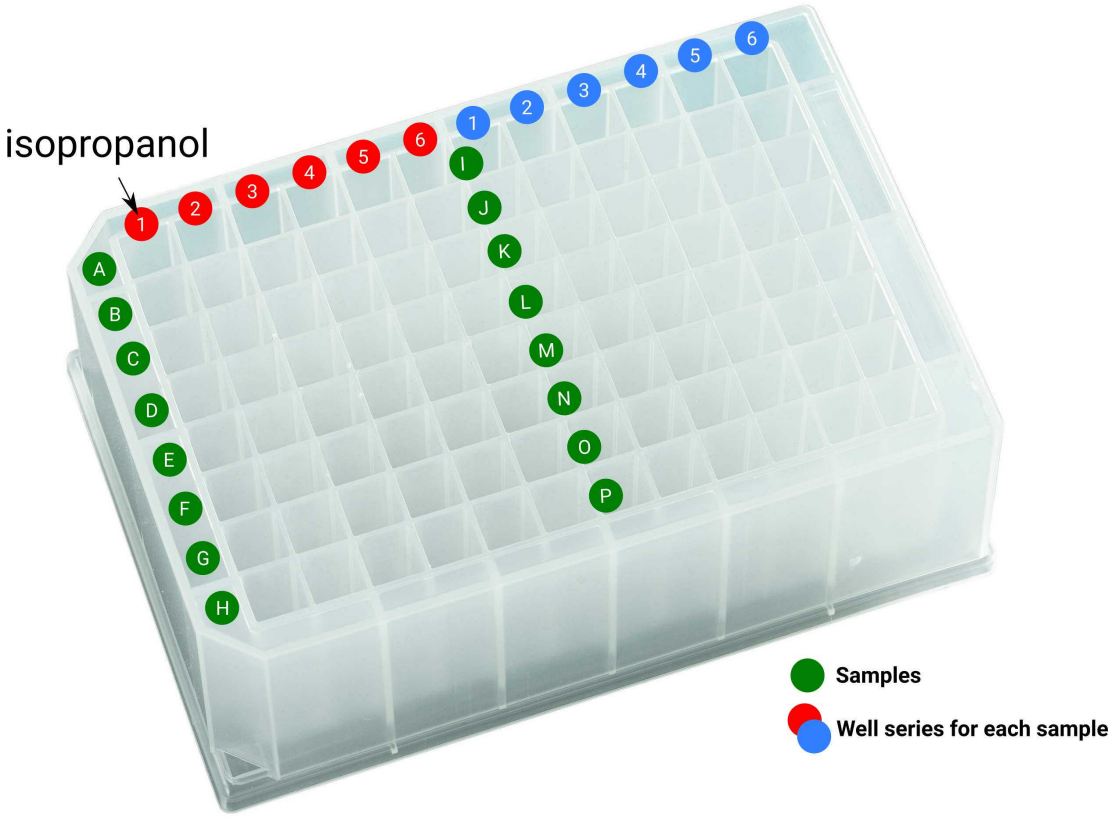
3m

DNA purification

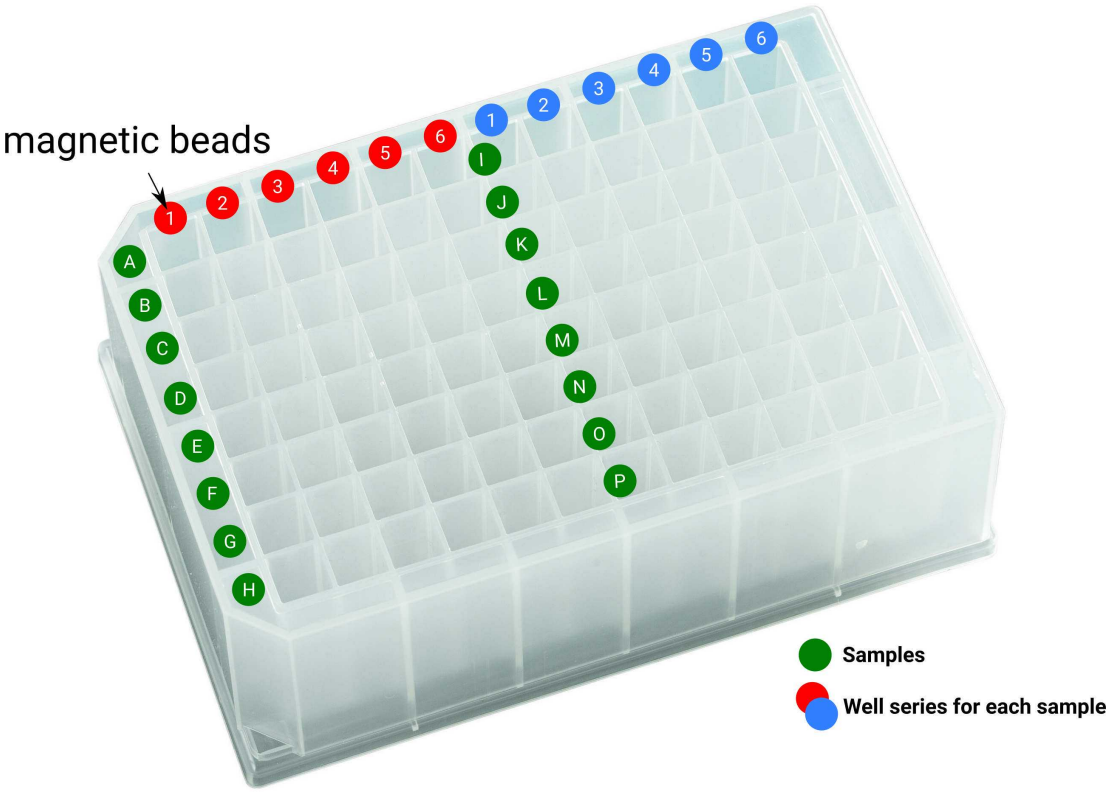
37m 30s

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

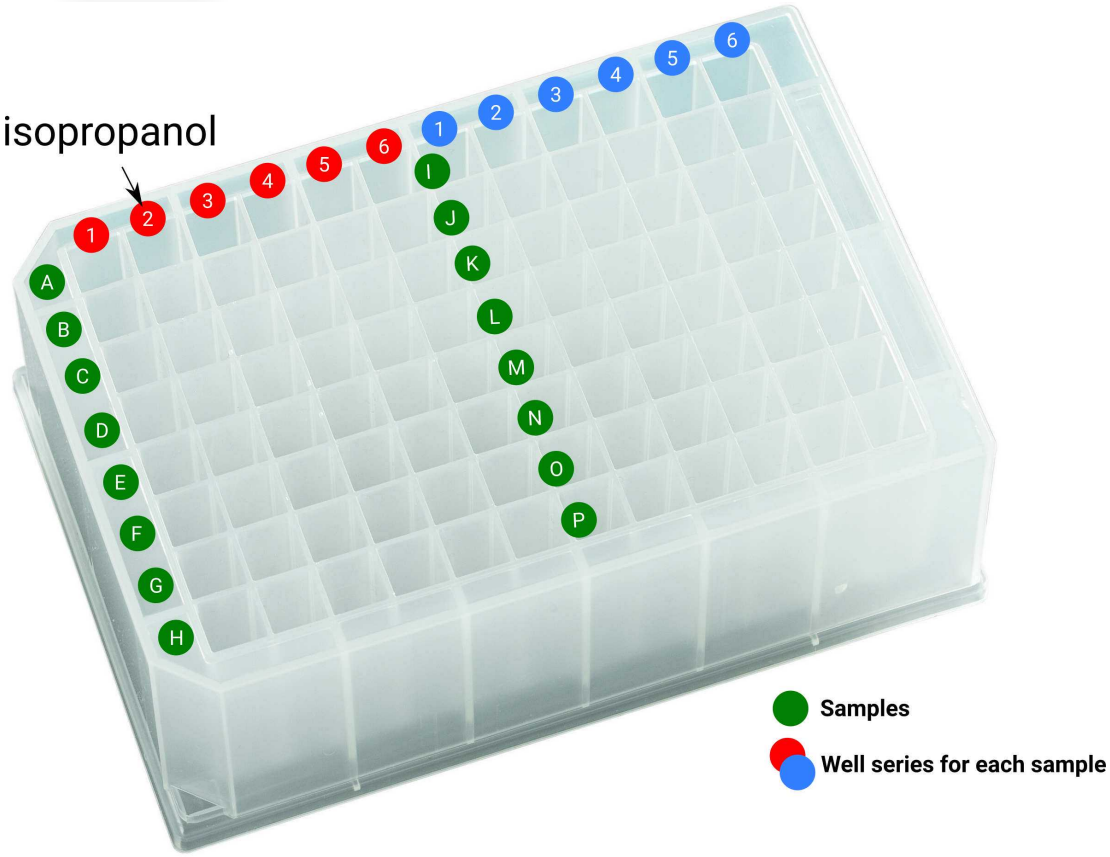
30s



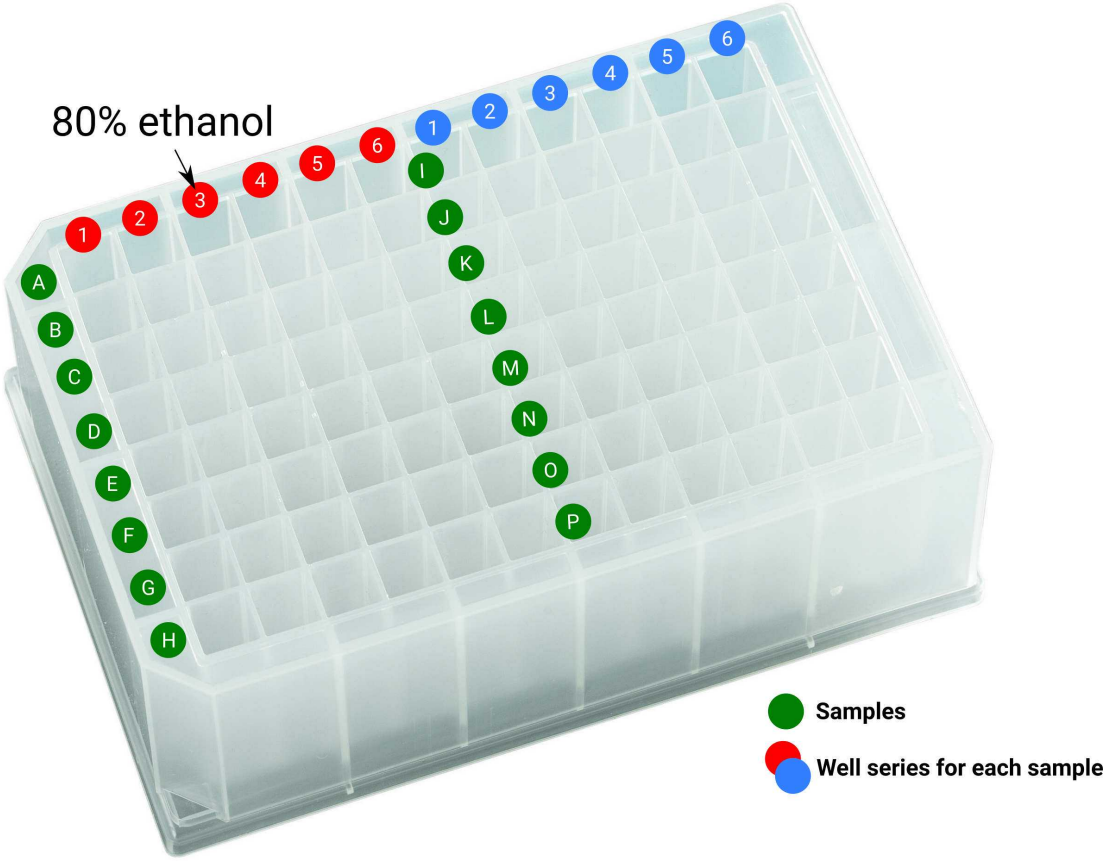
8 Add 100 µL of **magnetic beads (10mg/ml)** to the 1st well of 96 deep well plate



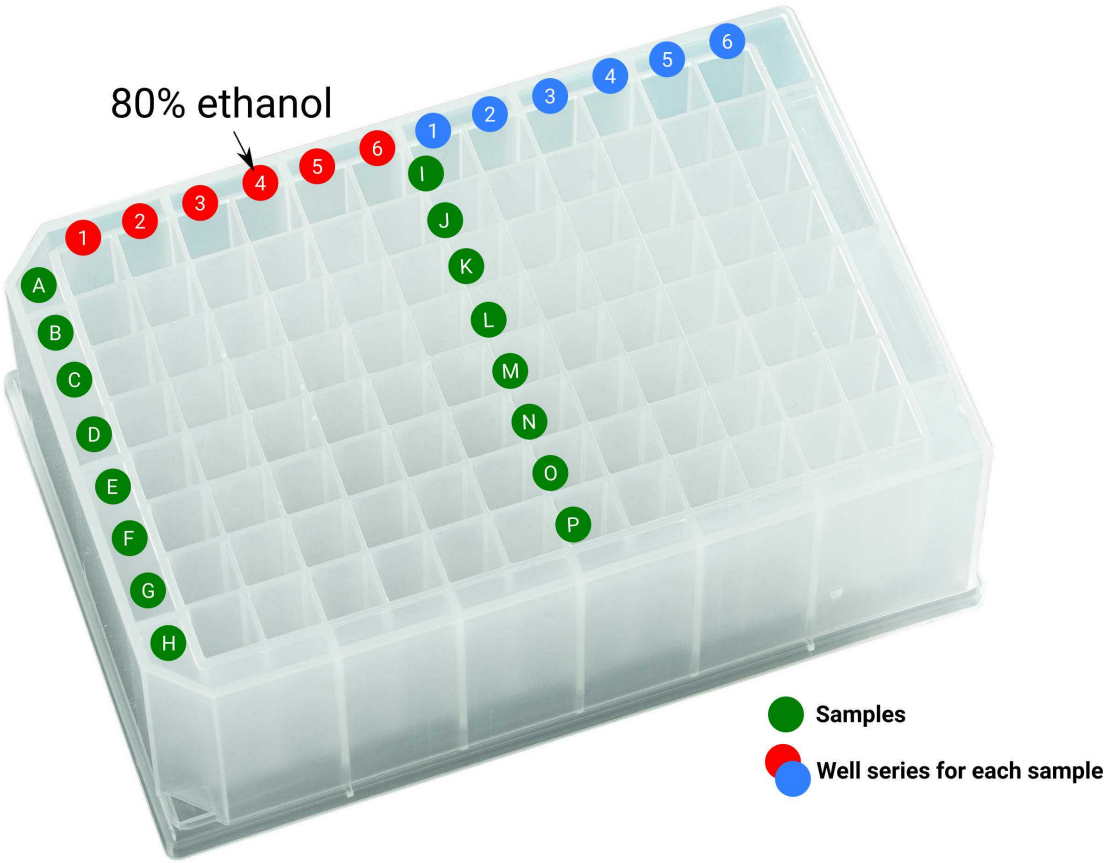
9 Add 400 µL of **isopropanol** to the 2nd well of 96 deep well plate 30s



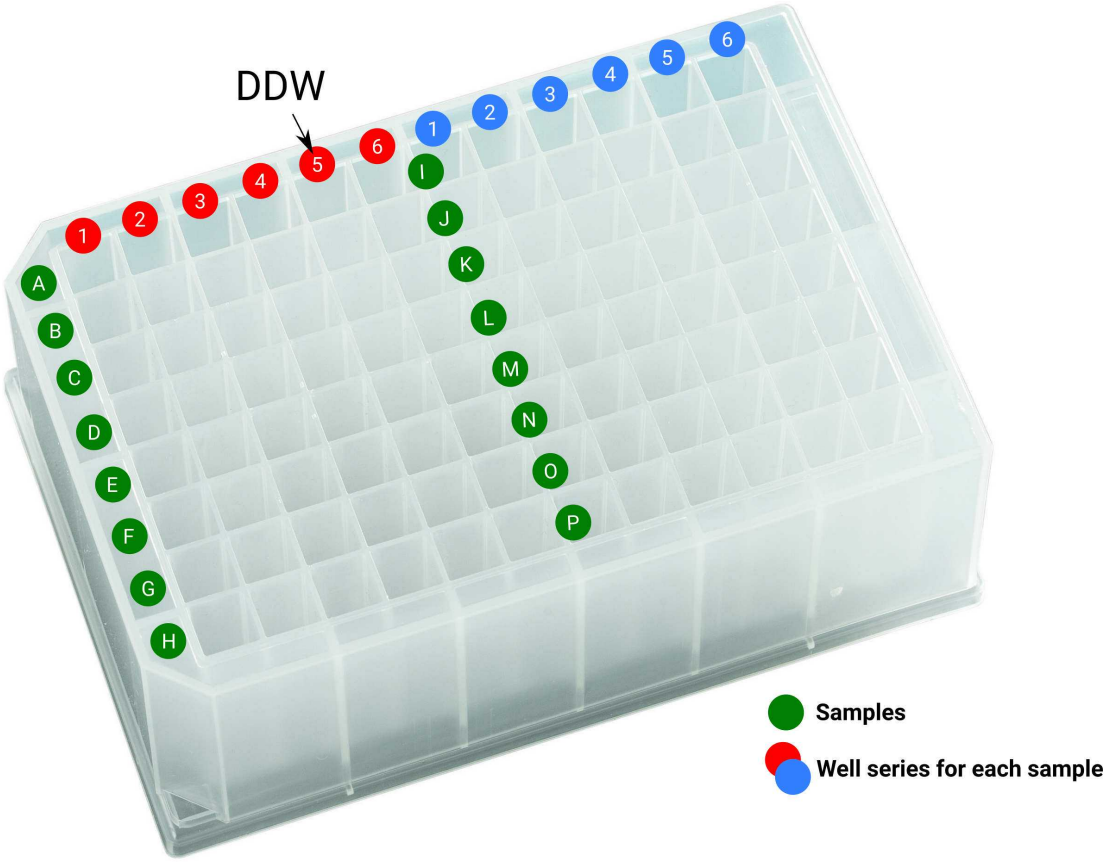
10 Add 300 µL of **80% ethanol** to the 3rd well of 96 deep well plate 30s



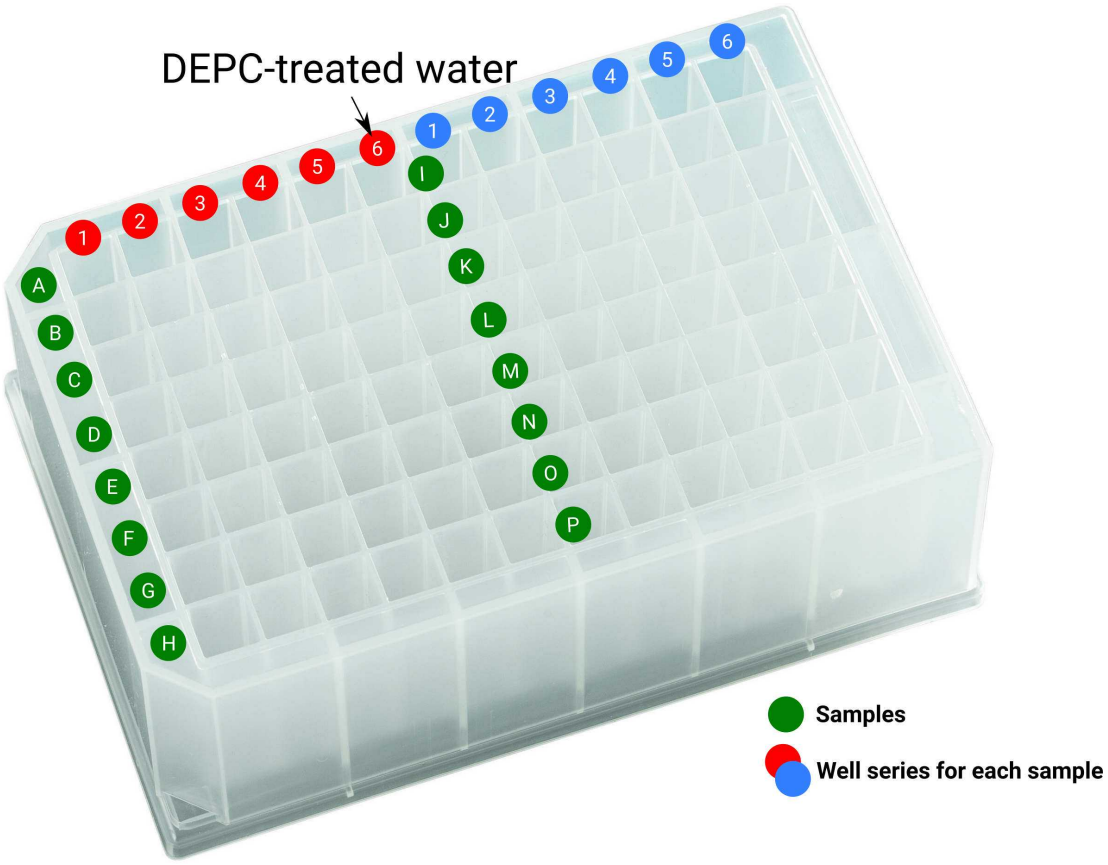
11 Add 300 µL of **80% ethanol** to the 4th well of 96 deep well plate 30s



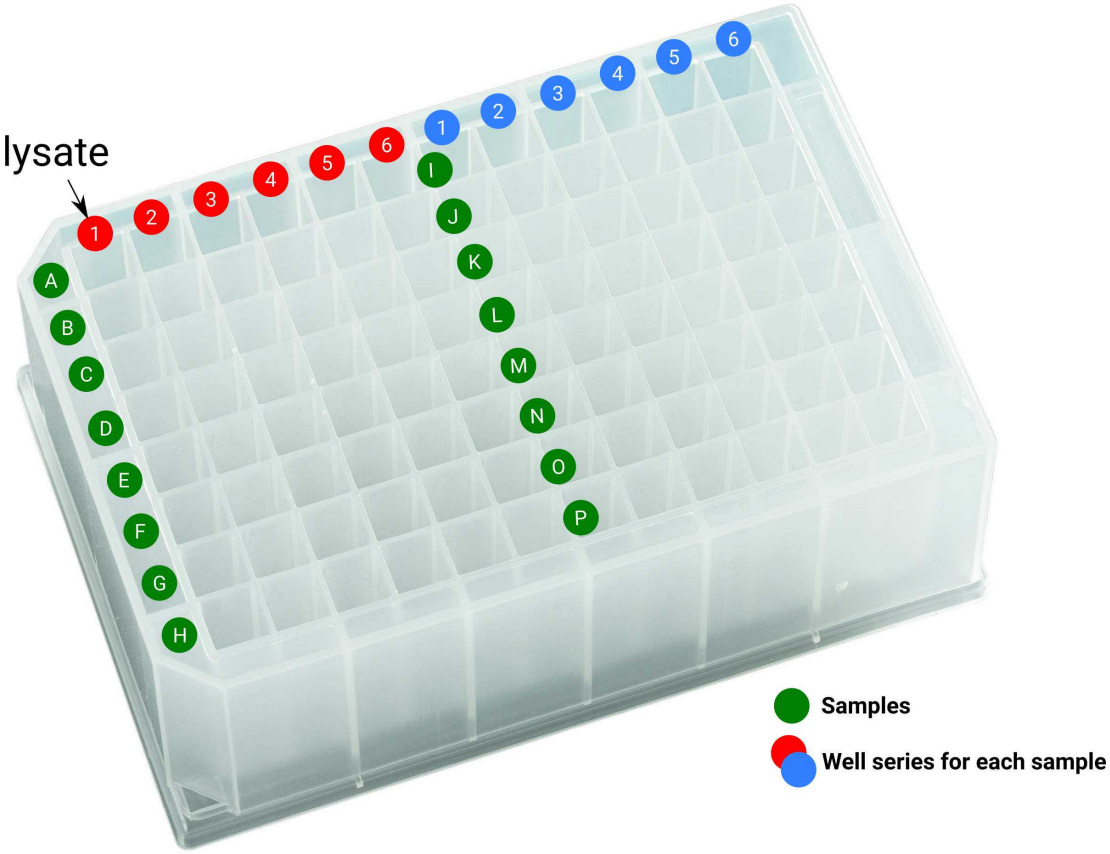
12 Add 300 µL of **DDW** to the 5th well of 96 deep well plate



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



14 Add 300-500 µL of the **sample (lysate)** from the 1.5mL centrifuged tube to the 1st well of 96 deep well plate 30s

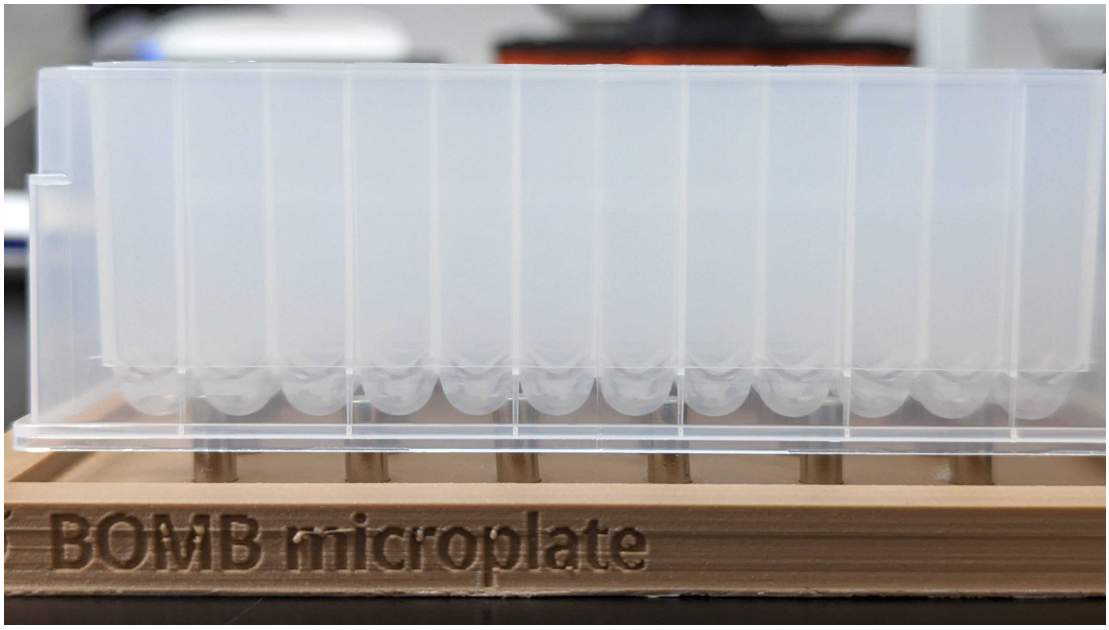


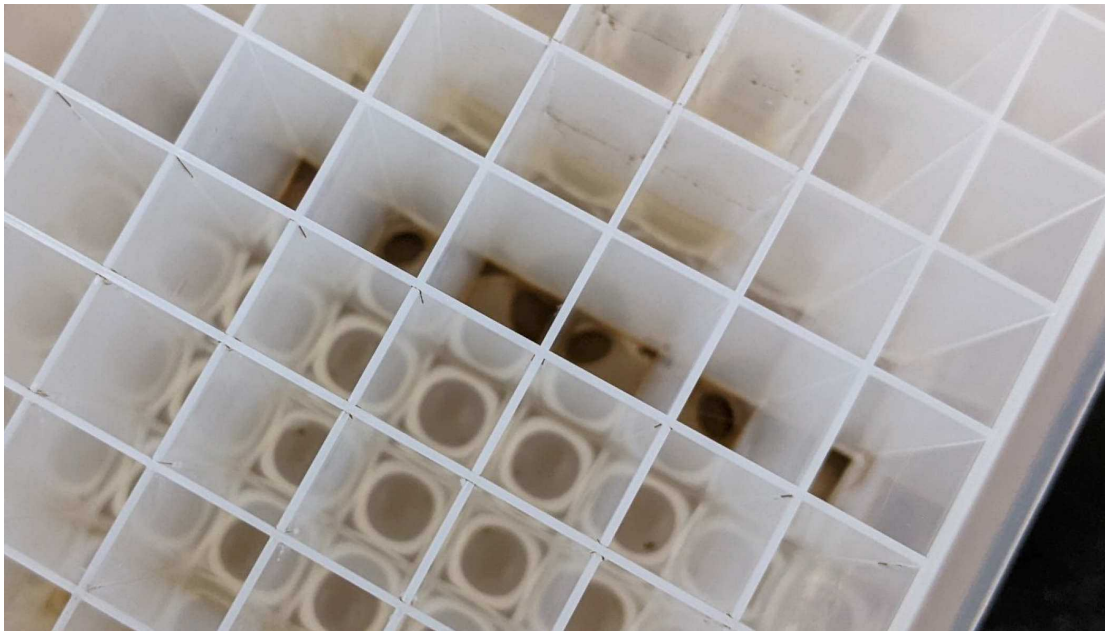
Note

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

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

16 After the extraction is done, put on the 96 magnetic plate to pellet the magnetic bead residues.





17 Collect 100 µL of the **eluted sample** (avoid getting magnetic bead) as the DNA template for downstream experiments


A
B
C
D
E
F
G
H