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Version 5

DNA extraction (BOMB) V.5

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Yin-Tse Huang¹, Tsu-Chun Hung¹

¹KMU



Yin-Tse Huang

Kaohsiung Medical University

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Protocol status: Working

We use this protocol and it's working

Created: June 08, 2023



Last Modified: September 12, 2023

Protocol Integer ID: 83099

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 μ L of **0.5 mm beads** to 2mL screw tube

30s



- 2 Add  200 μ L 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:

3m

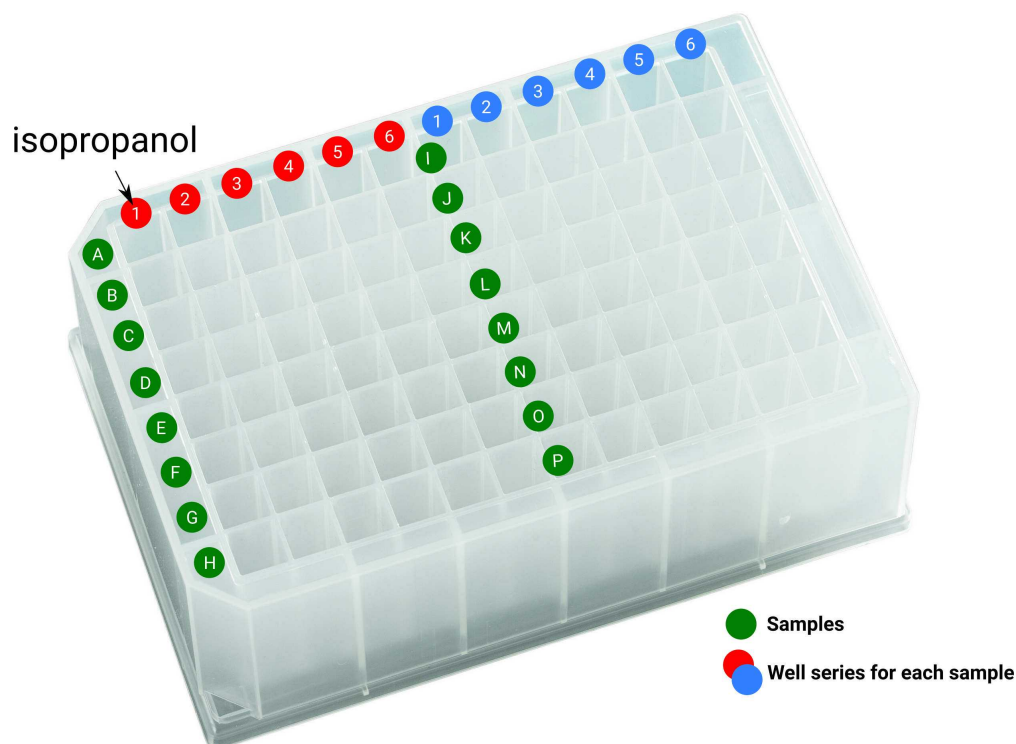
 10 x g, 25°C, 00:03:00

DNA purification

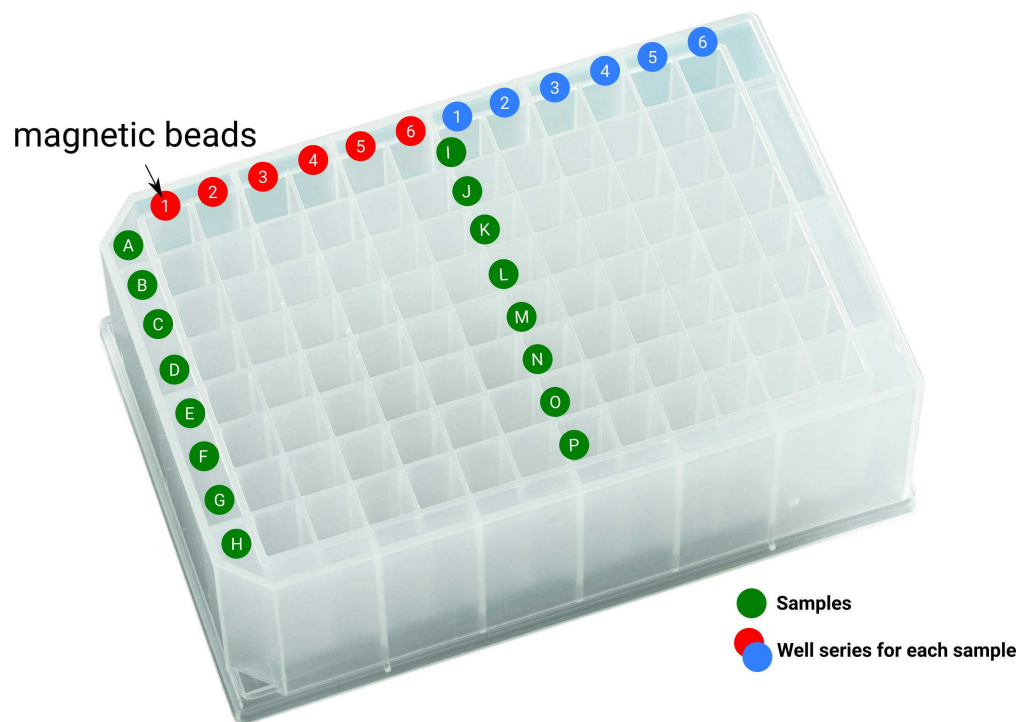
37m 30s

- 7 Add  350 μ L of **isopropanol** to the 1st well of 96 well plate

30s

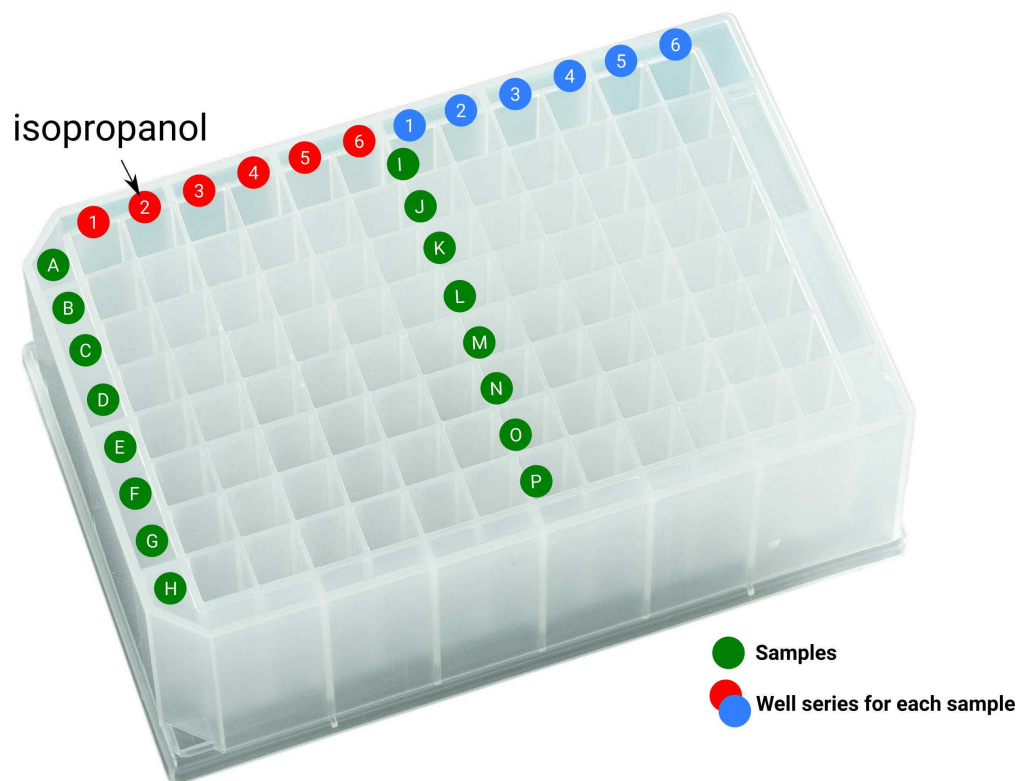


- 8 Add  100 μ L of **magnetic beads (10mg/ml)** to the 1st well of 96 deep well plate



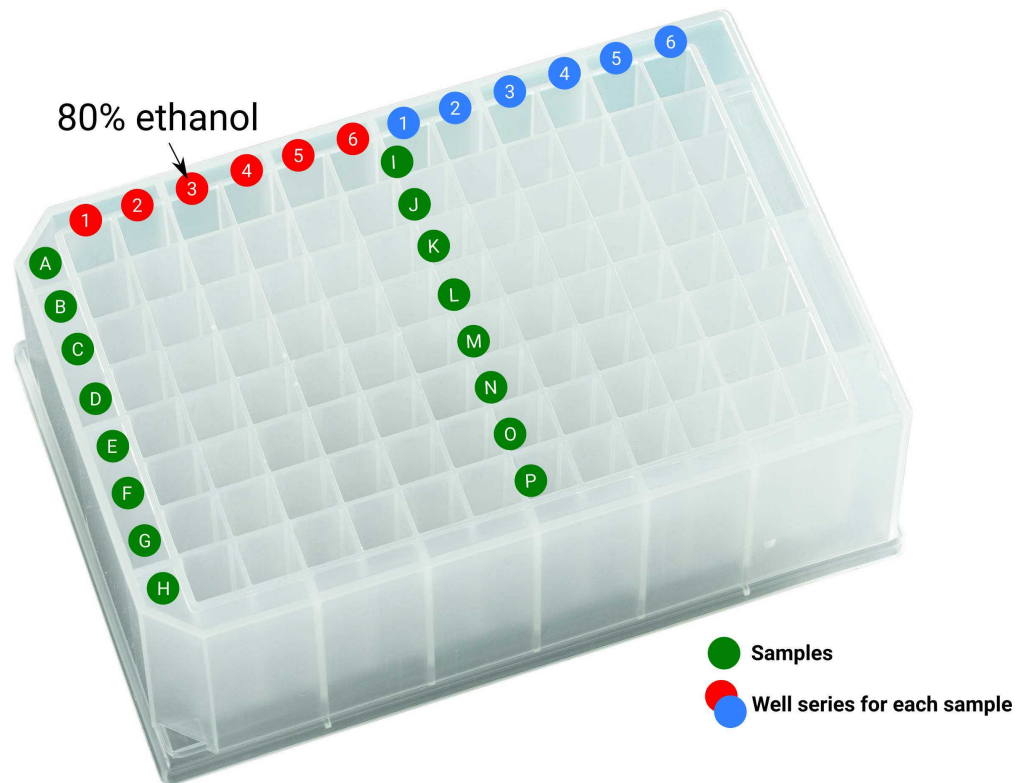
9 Add  400 μL of **isopropanol** to the 2nd well of 96 deep well plate

30s



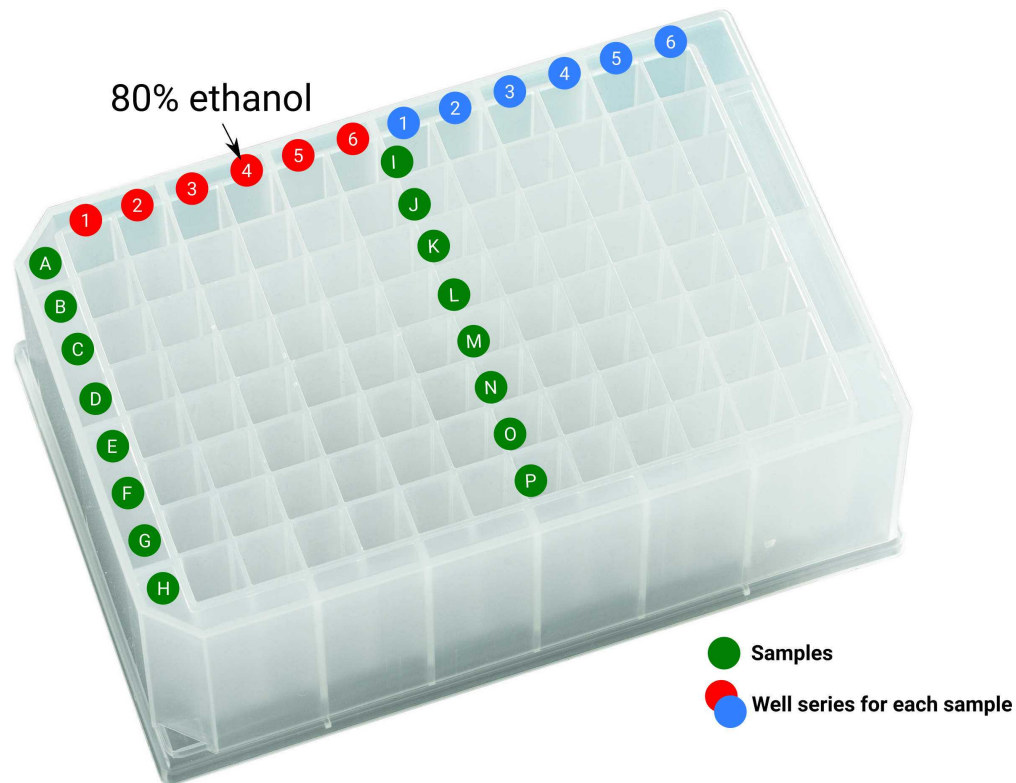
10 Add  300 μ L of **80% ethanol** to the 3rd well of 96 deep well plate

30s

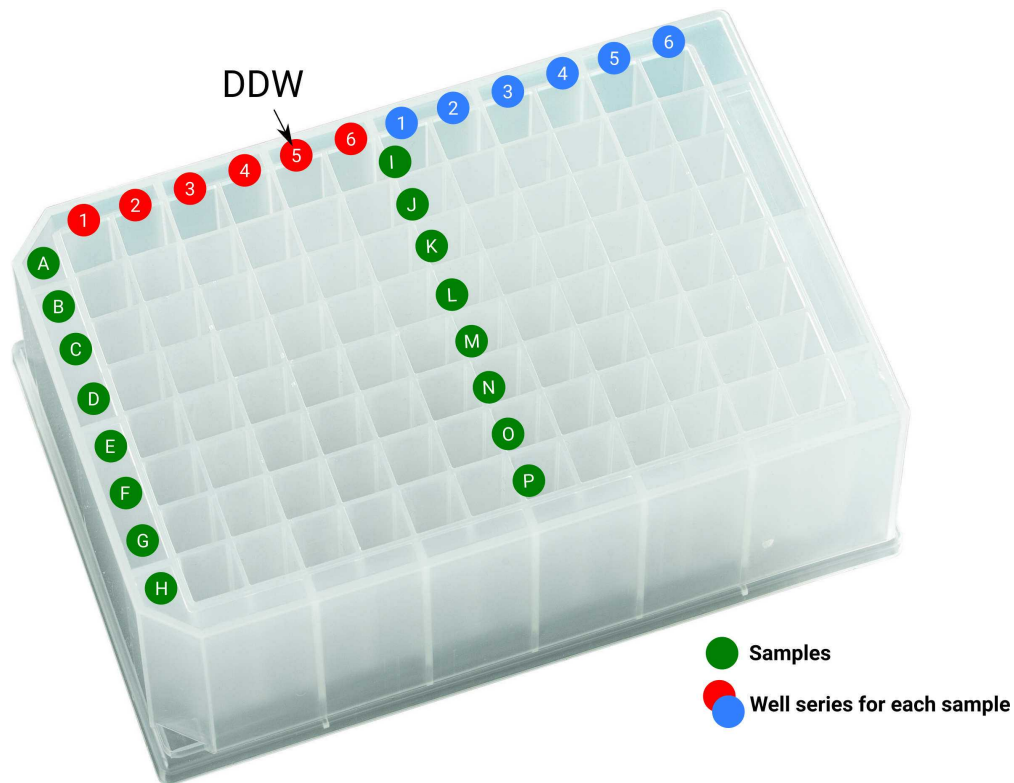


11 Add  300 μL of **80% ethanol** to the 4th well of 96 deep well plate

30s

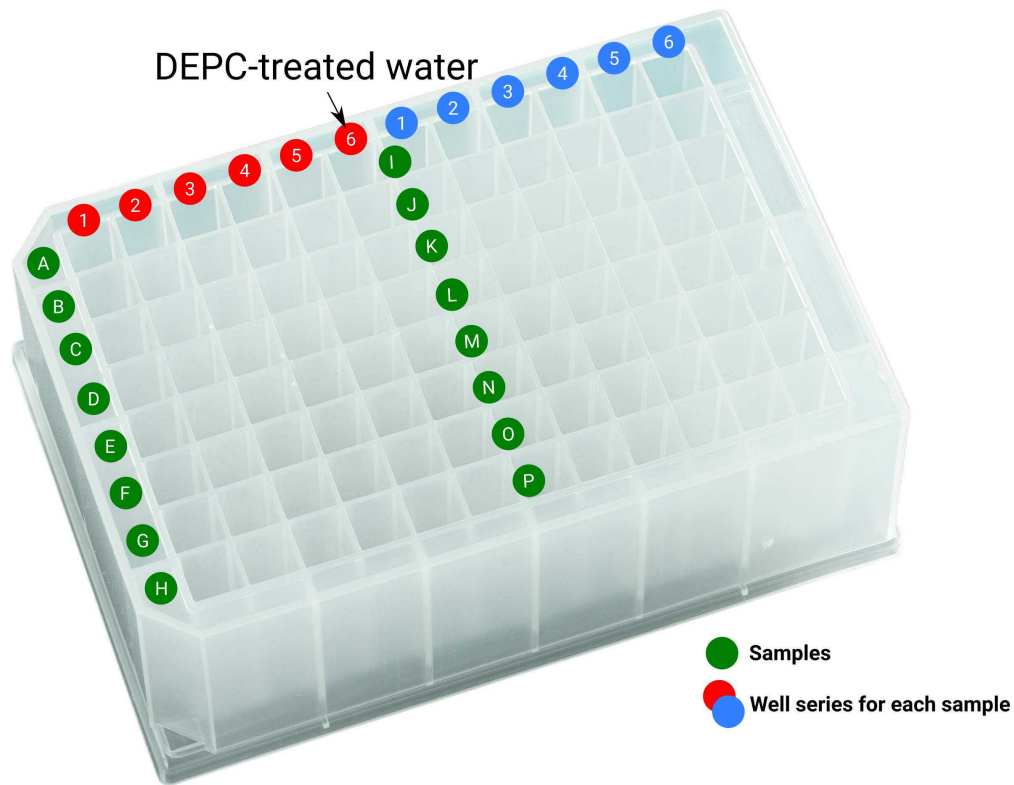


12 Add  300 μL of **DDW** to the 5th well of 96 deep well plate



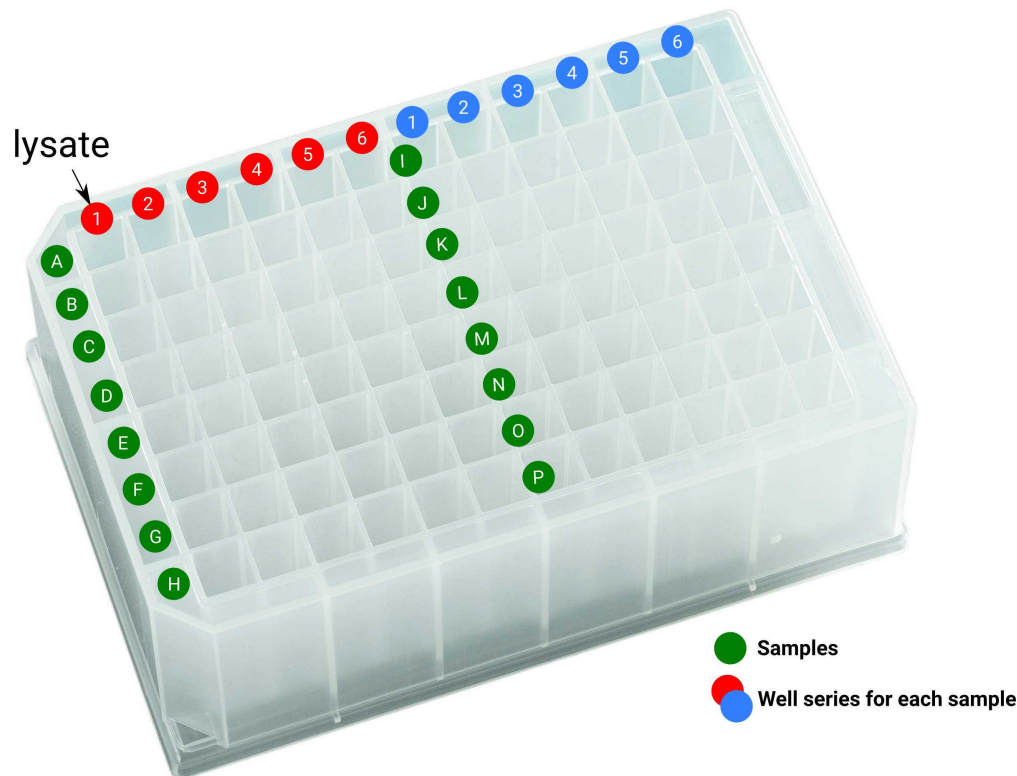
13 Add  100 μL of **DEPC-treated water** to the 6th well of 96 deep well plate

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

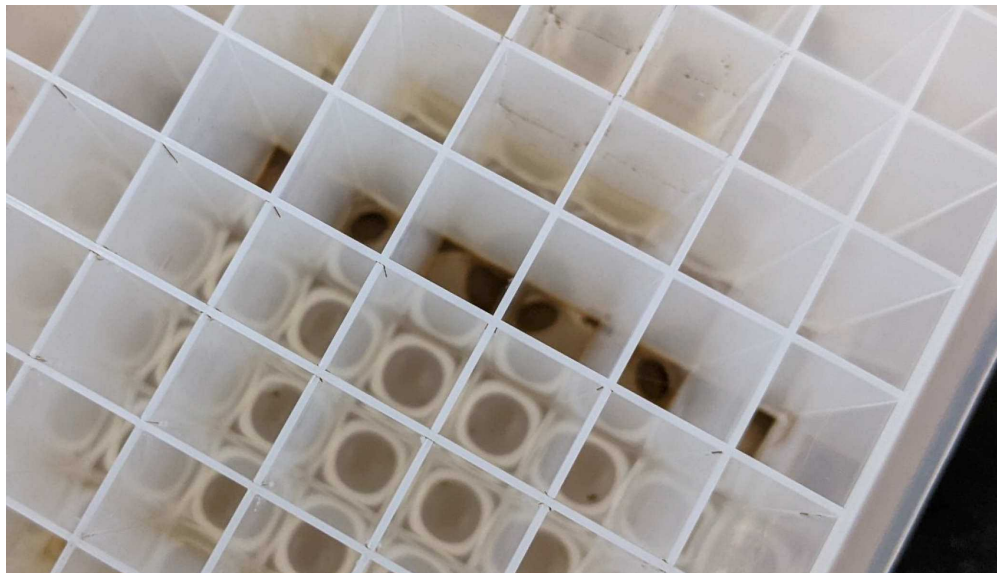


Note

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

34m



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

