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

Created: Sep 14, 2023

DNA extraction v9.0 (modified BOMB)

Forked from DNA extraction (BOMB)

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ABSTRACT

DNA extraction using yttria-stabilized zirconia beads lysing and automated magnetic bead-based extraction.

MATERIALS

1. Lysis master mix 870 ml (870 µl/sample)

	Chemical	Volume
	TE buffer	225 µl
	Lysis buffer	375 µl
	10M Ammonium acetate	270 µl

2. TE buffer stocks 225 ml

	Chemical	Volume	Notes
	1M Tris-HCl pH8.0	2.25 ml	10mM
	1M EDTA	0.225 ml	1mM
	ddH2O	adjust the volume with water to 225 ml	

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Note

Prepare 1M Tris-HCl and 1M EDTA first.

	Chemical	Mass	Total volume
	Tris-HCl	7.88 g	50 ml
	EDTA	14.61	50 ml

Note

adjust the pH of EDTA with solid NaOH or 1M NaOH to 8.0. The undissolved EDTA will be dissolved entirely when the pH reaches 8.0.

3. Lysis buffer stocks 375 ml

	Chemical	Volume/Mass	Notes
	Guanidine thiocyanate (GITC)	177.3 g	4M
	1M Tris HCl pH8.0	18.75 ml	50mM
	Sodium Lauryl Sulfate	3.75 g	0.5 g
	1M EDTA	7.5 ml	20mM
	ddH2O	adjust the pH with HCl to 7.6–8.0 and the volume with water to 375 ml	

4. 10M Ammonium acetate 270 ml

	Chemical	Volume/Mass
	Ammonium acetate	208.116 g
	ddH2O	adjust the volume with water to 270 ml

Note

Ammonium acetate is hygroscopic. Do not add more than half of the water needed to dissolve it.

Sample Collection

3m

1 Add 200 µL of **0.5 mm beads** to a 2mL screw tube. 30s

2 Add 200 µL of **1 mm beads** to a 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 lysis 4m

5 Put the 2mL screw tube on vortex for sample lysis, at 3200 rpm 00:04:00 4m

Note

Remember to balance if you have odd number of samples

Centrifugation

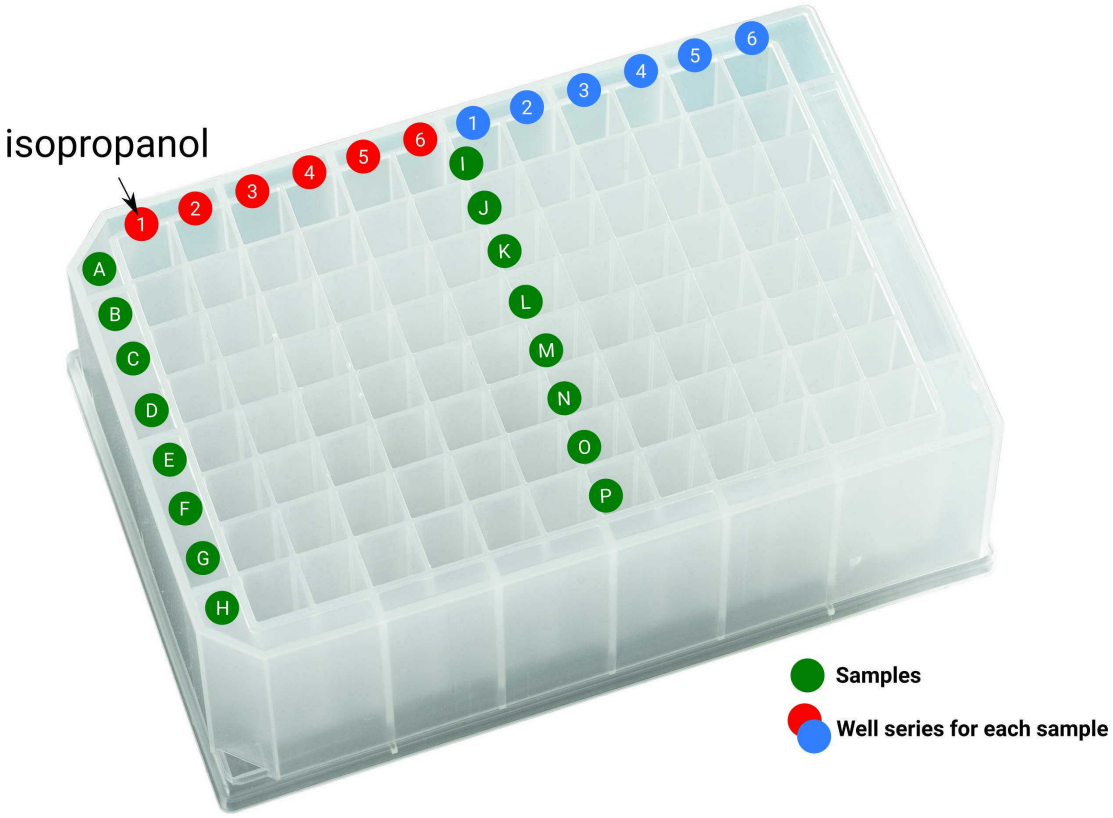
3m

6 Put 2mL screw tube in centrifuge for centrifugation, at this condition: 10 x g, 25°C, 00:03:00 3m

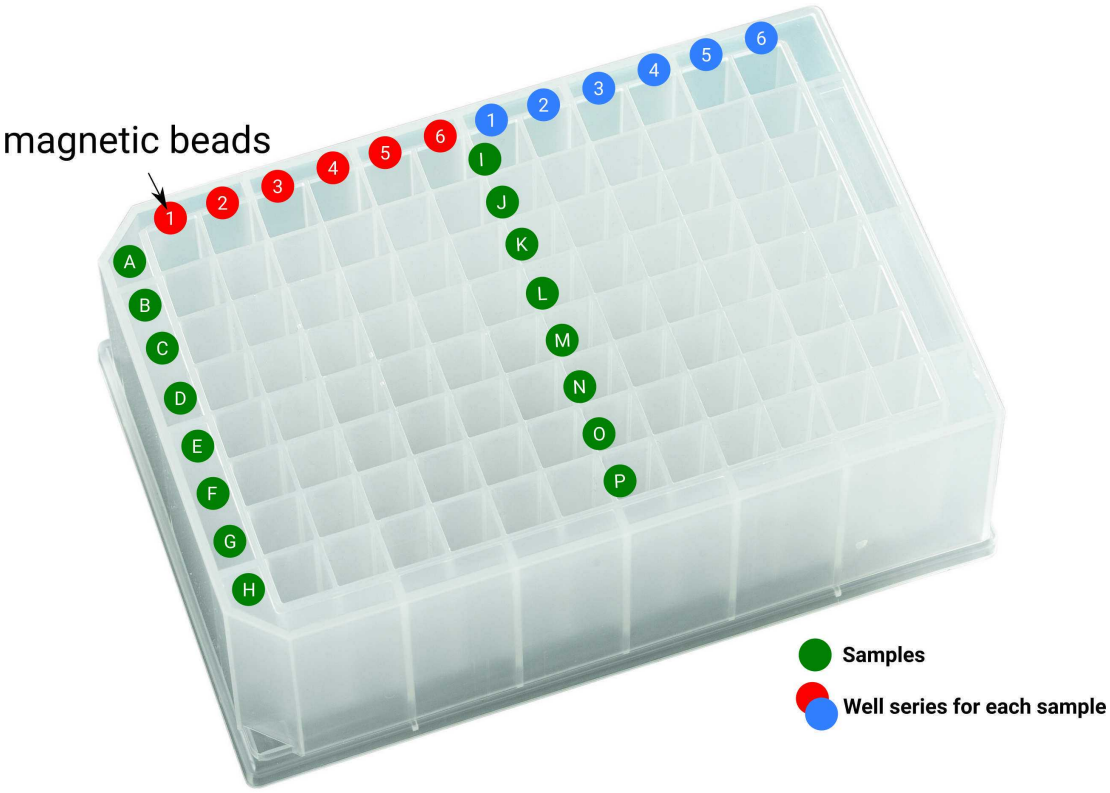
DNA extraction

37m 30s

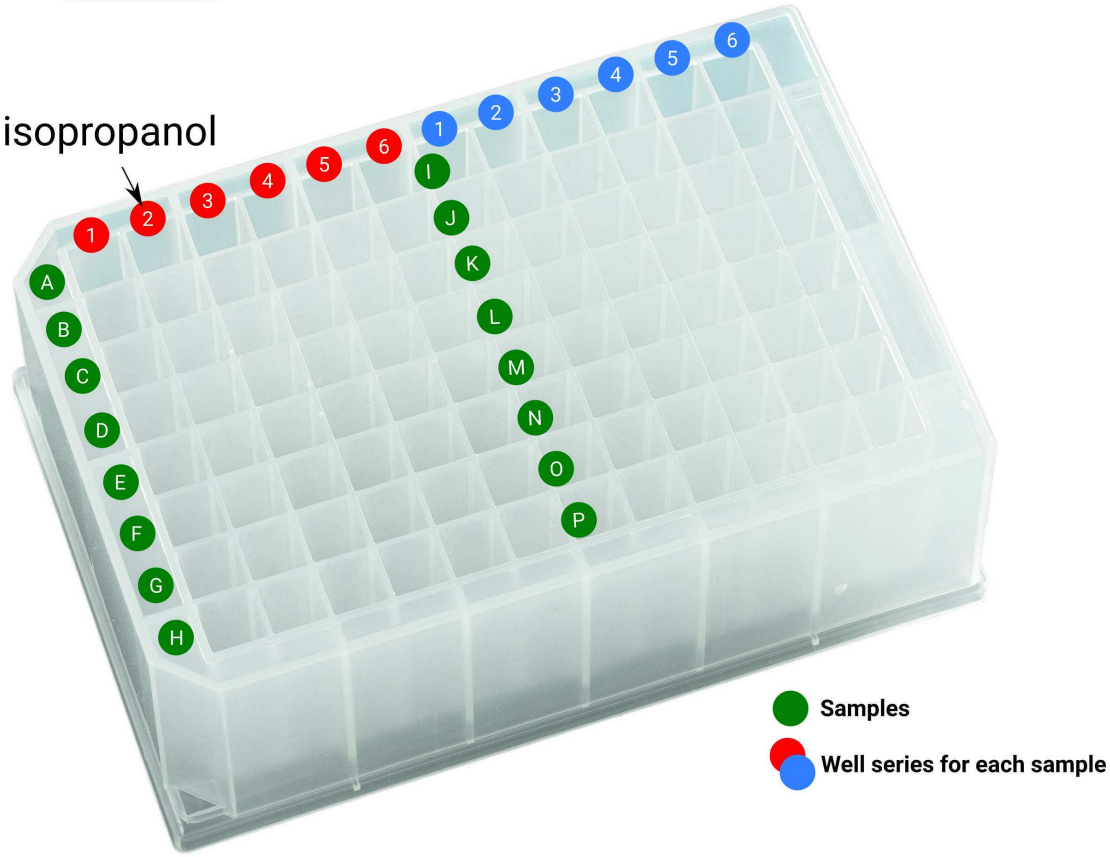
7 Add 350 µL of **isopropanol** to the 1st well of 96 well plate 30s



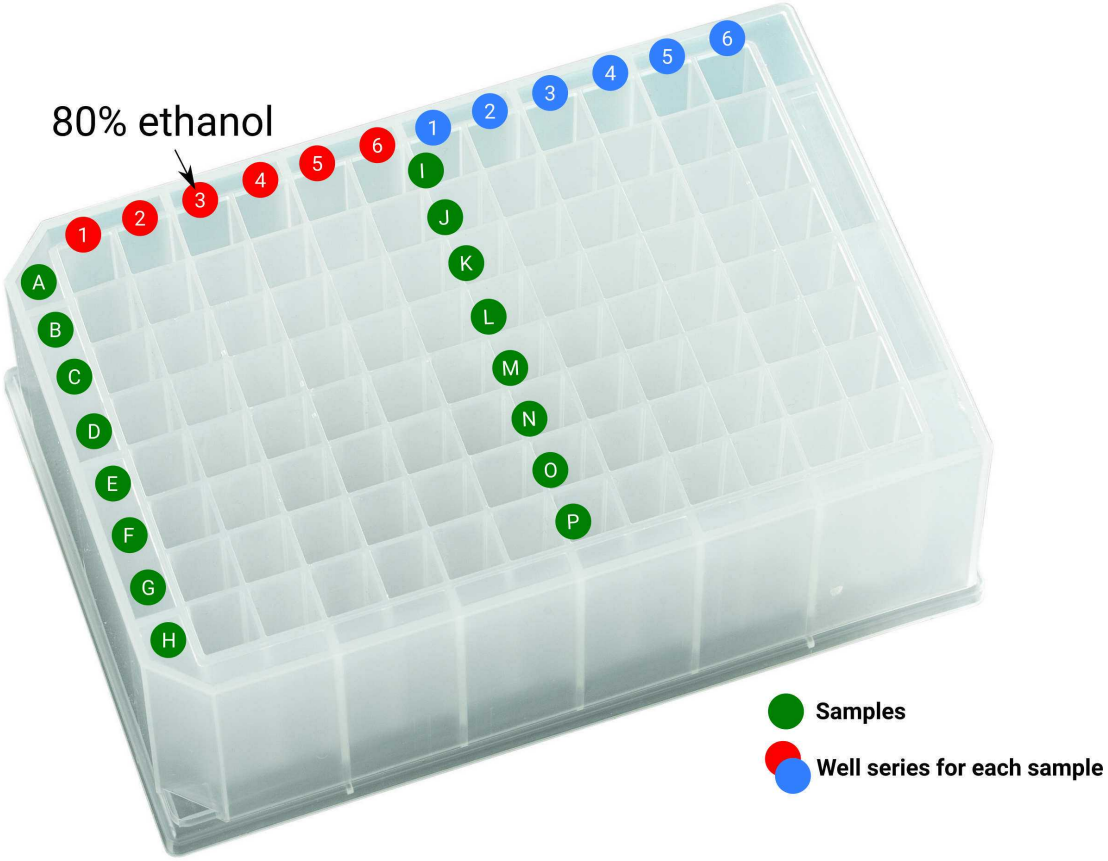
8 Add 50 µL of **magnetic beads (10mg/ml)** to the 1st well of 96 deep well plate



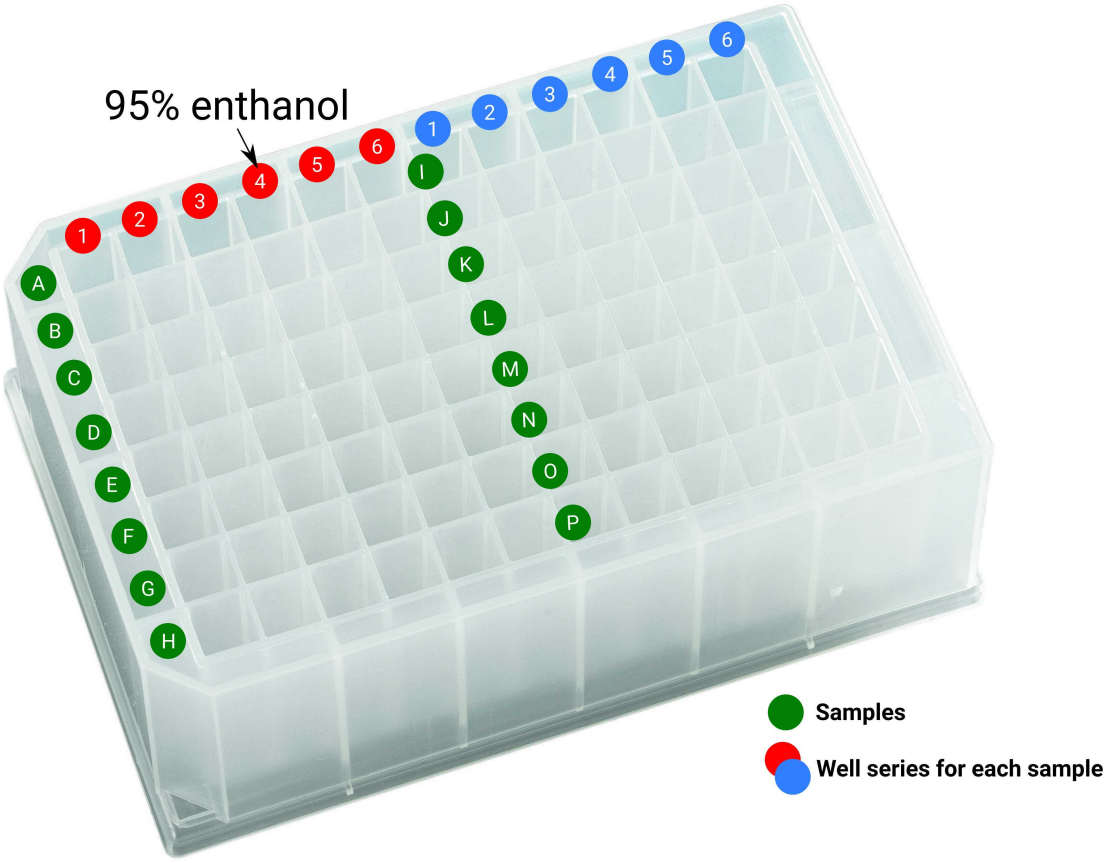
9 Add 400 µL of **isopropanol** to the 2nd well of 96 deep well plate 30s



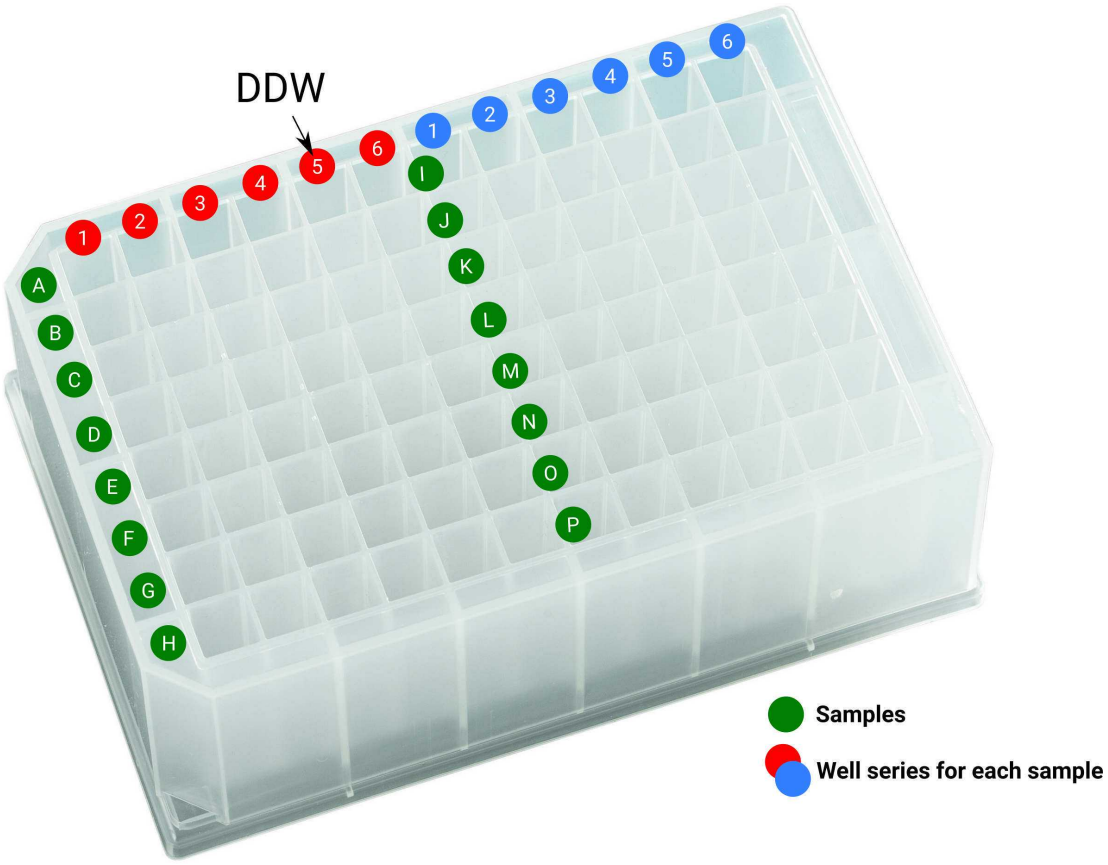
10 Add 300 µL of **80% ethanol** to the 3rd well of 96 deep well plate 30s



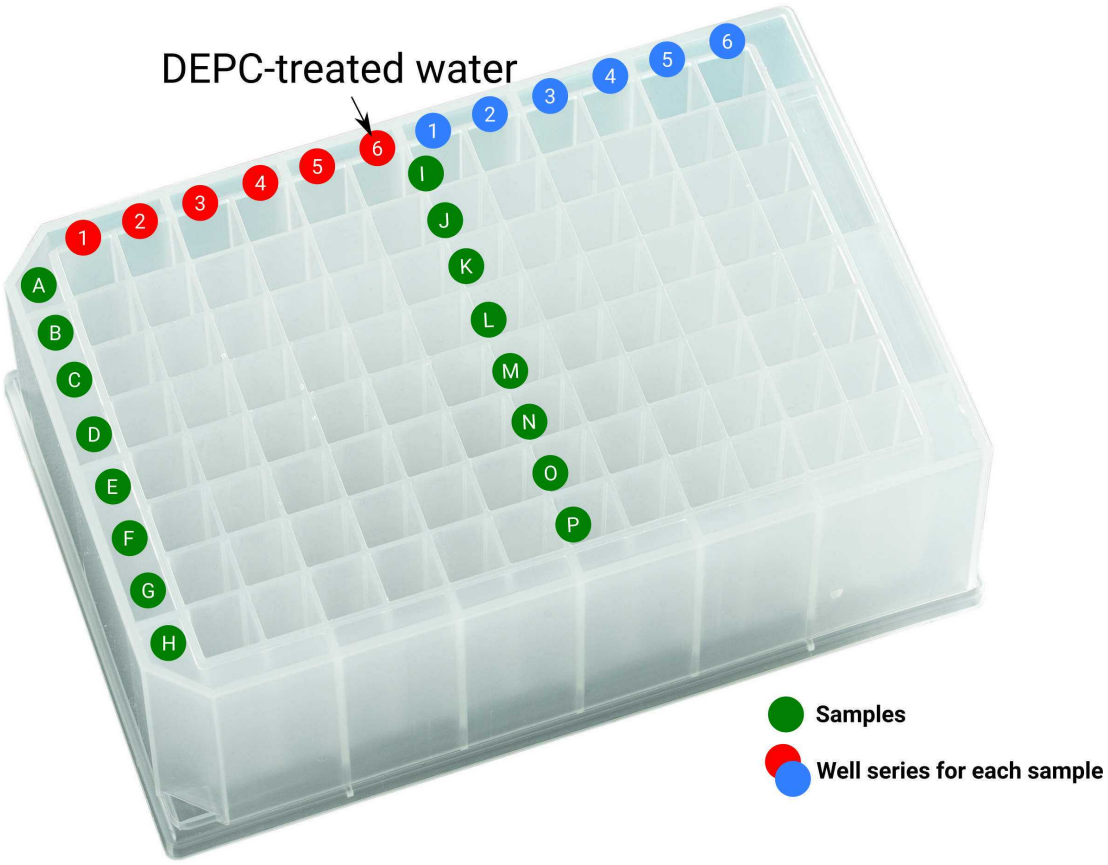
11 Add 300 µL of **95% ethanol** to the 4th well of 96 deep well plate 30s



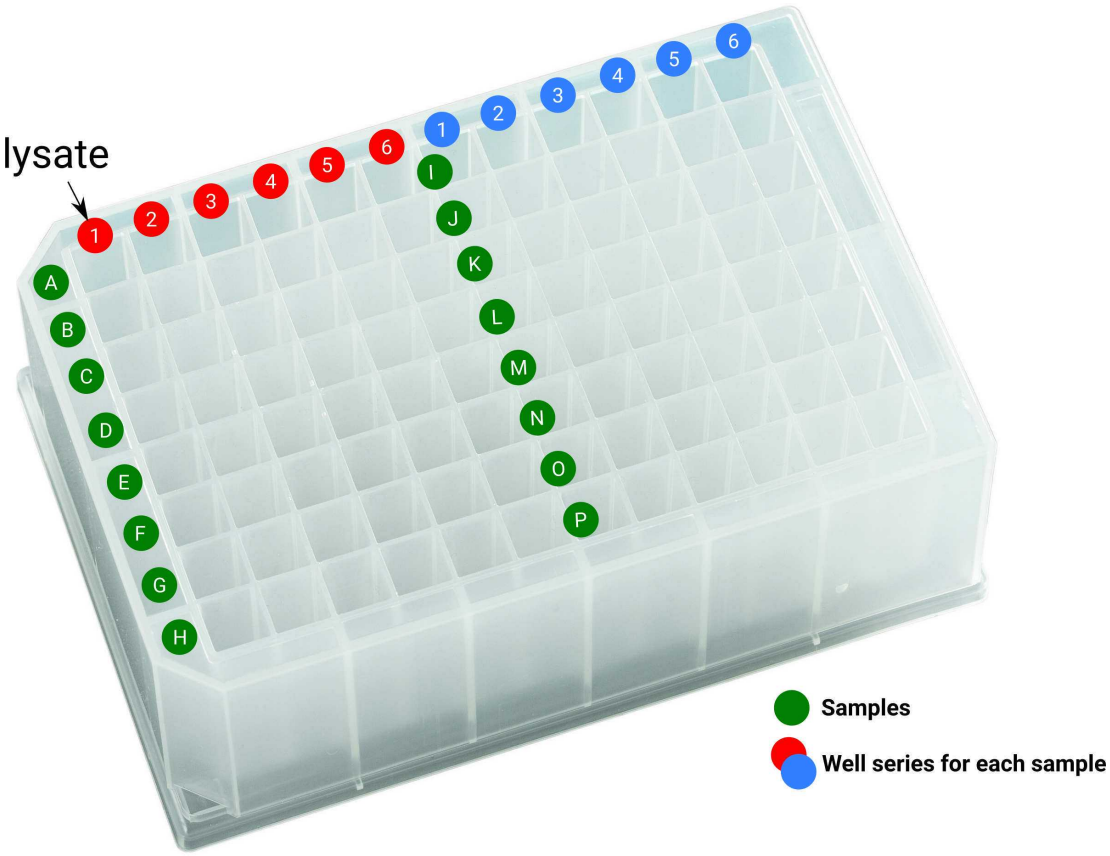
12 Add 300 µL of **DDW** to the 5th well of 96 deep well plate



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 (ZiXpress 32) and set up the settings as below. 34m

Program settings

37m 30s

16 The automated extraction program settings for ZiXpress 32.

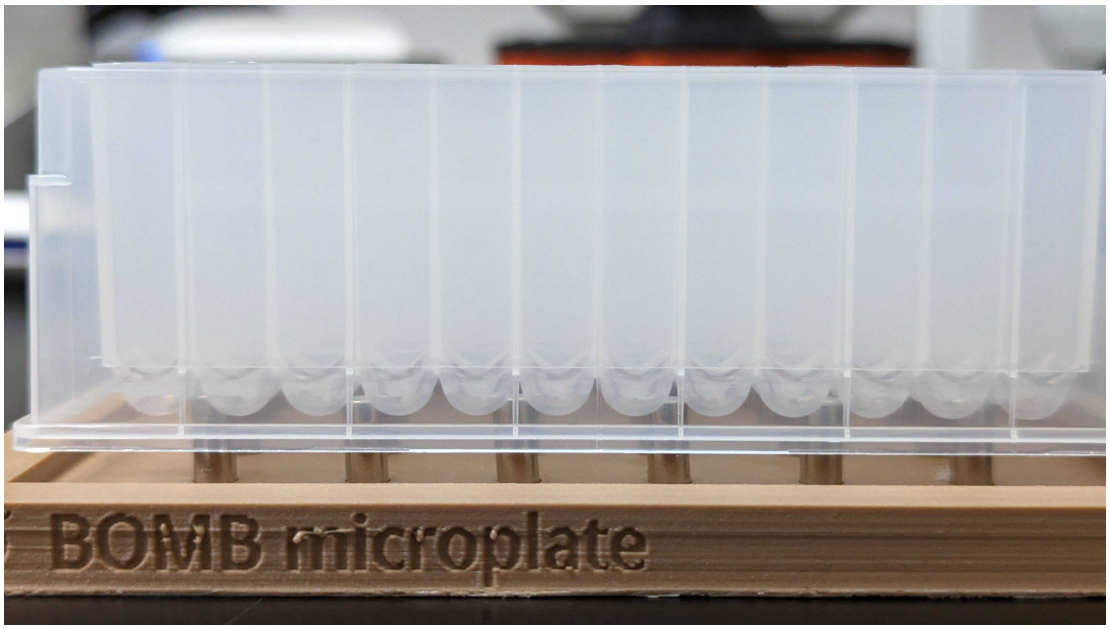
	Well no.	Standby(mins)	Mix(mins)	Volume(µl)	Mix speed	Mag(s)	Temp.(ºC)
	1	0	10	1000	3	120	0

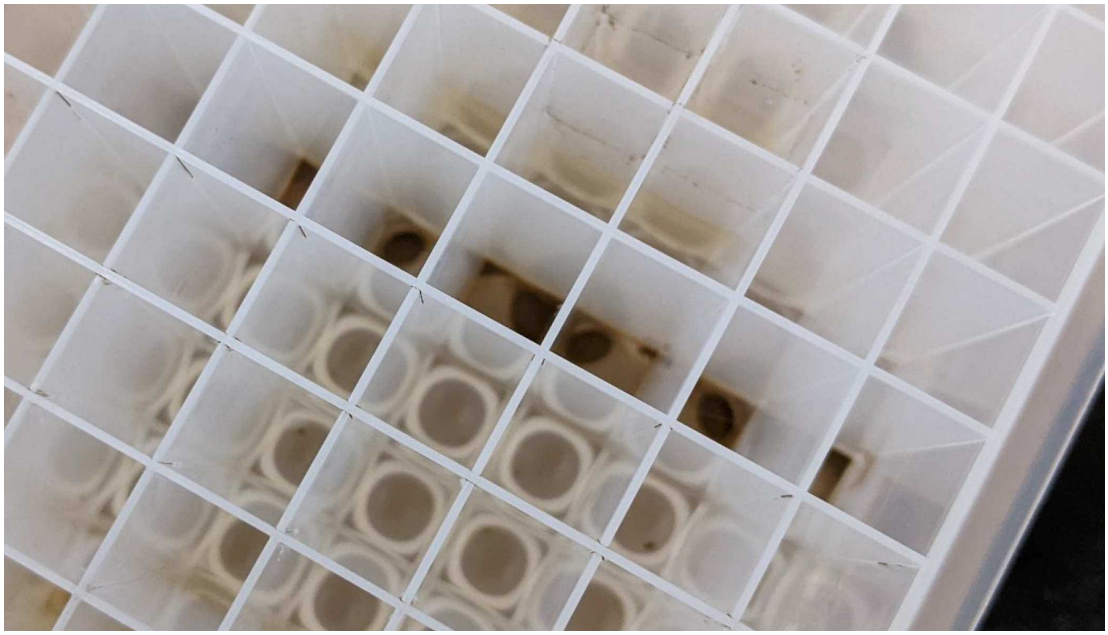
	Well no.	Standby(mins)	Mix(mins)	Volume(µl)	Mix speed	Mag(s)	Temp.(ºC)
	2	0	2	400	3	60	0
	3	0	2	300	3	60	0
	4	15	0	0	0	0	0
	4	0	2	300	3	60	0
	5	0	1	300	3	0	0
	6	0	5	100	3	120	55

gDNA collection

37m 30s

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





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