



VERSION 2

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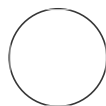
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## HTTM : gDNA extraction V.2

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### ABSTRACT

Part of the HTTM protocol dedicated to the extraction of gDNA from transposon mutated cell pellets.

### MATERIALS

#### ■ Homemade DNA lysis Buffer :

A	B
Component	Amount for 1000ml of solution
CTAB 2%	20g
1,5M Guanidine HCl	143,2g
10mM Tris HCl	1,57g

Mix well and adjust pH to 8.0.

#### ■ Homemade wash solution :

A	B
Component	Amount for 1000ml of solution
Ethanol 100%	800ml
Tris HCl 1M pH 8,0	10ml
NaCl 4M	25ml
EDTA 0,5M	2ml

Mix well and adjust pH to 8.0.

#### ■ Elution Buffer (Low TE Buffer): 10 mM Tris-HCl (pH 8.0) + 0.1 mM EDTA

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

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**PROTOCOL integer ID:**  
69131

### Solutions for plate regeneration, from this protocol :

(1)<https://doi.org/10.1016/j.ab.2008.10.021>.

#### ■ NaOH 1N + Triton X100 0,15% (v/v)

A	B
Component	Amount for 1000ml of solution
Water	960ml
NaOH	40g
Triton X-100	1,5ml

Mix well and store in a base resistant container.




#### ■ HCl 1.5N + Triton X100 0,15% (v/v)



A	B
Component	Amount for 1000ml of solution
Water	873,5ml
HCl Stock (37%)	125ml
Triton X-100	1,5ml

Mix well and store in an acid resistant container.

## DNA extraction

2h 5m

- 1 Prepare the lysis solution by adding  165  $\mu\text{L}$  of proteinase K to  66 mL of homemade lysis buffer and mix well.
- 2 Add  600  $\mu\text{L}$  of lysis solution to each well of the deep-well plate and resuspend the pellet.

3 Cover with an adhesive aluminum foil and incubate at  55 °C for  01:00:00 .


1h

4 While still warm, add  260 µL of ethanol 100%, without overmixing.


#### Note

Overmixing will result in DNA agglomeration and difficulty with the extraction.

5 Transfer immediately to a deep-well plate fitted with an array of silica columns.

6 Centrifuge twice at  4000 x g, 00:10:00 .

10m






7 Discard flowthrough and add  500 µL of wash solution.

8 Centrifuge at  3000 x g, 00:10:00

10m


8.1 Repeat steps 7 and 8.

9 Discard flowthrough.

- 10 Centrifuge at  3000 x g, 00:10:00 to eliminate traces of wash solution. 10m
- 11 Discard flowthrough.
- 12 Add a collector plate between the silica column array and the deep-well plate.
- 13 Add  50 µL of low TE to the silica matrix in each well.
- 14 Cover with an adhesive aluminum foil and incubate at  55 °C for  00:15:00 . 15m
- 15 Centrifuge at  3000 x g, 00:10:00 . 10m

### Silica array regeneration (Optional)

1 h 5m

- 16 Put the contaminated silica array on an empty deep-well plate.  
Add  150 µL of 1N NaOH + 0.15%(v/v) Triton X-100 to each well.

- 17 Incubate at  Room temperature for  00:05:00 5m
- 18 Centrifuge  3000 x g, 00:05:00 5m
- 19 Add  200  $\mu$ L of 1,5N HCl+ 0,15% (v/v) Triton X-100 to each well.
- 20 Incubate at  Room temperature for  00:30:00 30m
- 21 Centrifuge  3000 x g, 00:05:00 5m
- 22 Add  150  $\mu$ L of 1N NaOH + 0,15%(v/v) Triton X-100 to each well.
- 23 Incubate at  Room temperature for  00:05:00 5m
- 24 Centrifuge  3000 x g, 00:05:00 5m
- 24.1 Collect the flowthrough in a beaker. Neutralize pH if needed and dispose of the flow through.

**25** Add  200 µL of ddH<sub>2</sub>O to each well.

**26** Centrifuge  3000 x g, 00:05:00

5m

**26.1** Repeat steps 25 and 26.

**27** Silica columns array are ready to be reused.