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HTTM : gDNA extraction V.3

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

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Abstract

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

Attachments

HDTM__Protocol-2.pd...

298KB

Image Attribution

Make with BioRender.com

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

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

	A	B
	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 µL of proteinase K to 66 mL of homemade lysis buffer and mix well.

2 Add 600 µL 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 3270 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, 00:05:00 to eliminate traces of wash solution. 5m

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 3270 x g, 00:05:00 . 5m

Silica array regeneration (Optional) 1h 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, 00:02:00 2m

19 Add 200 µL 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, 00:02:00 2m

22 Add 150 µL 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, 00:02:00 2m

24.1 Collect the flowthrough in a beaker. Neutralize pH if needed and dispose of the flow through.

25 Add 600 µL of ddH_2O to each well.

26 Centrifuge 3000 x g, 00:05:00 5m

27 Silica columns array are ready to be reused.