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Version 3

HTTPM : 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 HTTP protocol dedicated to the extraction of gDNA from transposon mutated cell pellets.

Attachments



[HDTM_Protocol-2.pd...](#)

298KB

Image Attribution

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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
	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 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ 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 ddH₂O to each well.
- 26 Centrifuge  3000 x g, 00:05:00 5m
- 27 Silica columns array are ready to be reused.