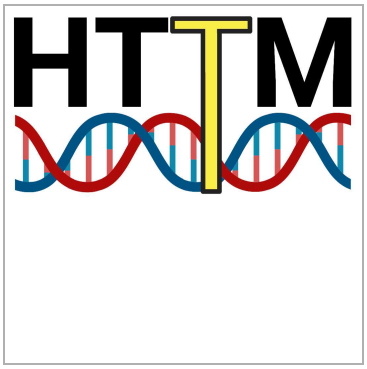

VERSION 1

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

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HTTM : DNA Extraction V.1

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ABSTRACT

Part two of the HTTM protocol. A low-cost and high-throughput Tn-seq protocol. This part cover the DNA extraction from cell pellets of transposon insertion mutants and subsequent silica columns regeneration.

MATERIALS

- **Homemade DNA lysis Buffer :**

	A	B
	Component	Amount for 1000 ml of solution
	CTAB 2%	20 g
	1.5 M Guanidine HCl	143.2 g
	10 mM Tris HCl	1.57 g

Mix well and adjust volume to 1 l with water and adjust pH to 8.0.

- **Homemade wash solution :**

	A	B
	Component	Amount for 1000 ml of solution
	Ethanol 100%	800 ml
	Tris HCl 1 M pH 8.0	10 ml
	NaCl 4 M	25 ml
	EDTA 0.5 M	2 ml

Mix well and adjust volume to 1 l with water 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 X-100 0.15% (v/v)**

	A	B
	Component	Amount for 1000 ml of solution
	Water	960 ml
	NaOH	40 g
	Triton X-100	1.5 ml

Mix well and store in a base resistant container.

- **HCl 1.5N + Triton X-100 0.15% (v/v)**

	A	B
	Component	Amount for 1000 ml of solution
	Water	873.5 ml
	HCl Stock (37%)	125 ml
	Triton X-100	1.5 ml

Mix well and store in an acid resistant container.

Silica columns array come from the following commercially available kit :
96-Well Plate Bacteria Genomic DNA Miniprep Kit from Biobasic. CAT#: SK1295

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 cover 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) 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, 00:05:00 . 5m

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, 00:05:00 . 5m

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, 00:05:00 . 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_2O 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.