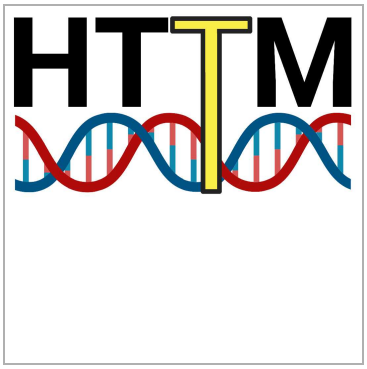

VERSION 1

MAR 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.36wgq72n3vk5/v1

Protocol Citation: Antoine Champie, Amélie De Grandmaison 2023. HTTM : Transposon mutagenesis . **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq72n3vk5/v1 Version created by Antoine Champie

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

Created: Oct 27, 2022

Last Modified: Mar 30, 2023

PROTOCOL integer ID: 71935

HTTM : Transposon mutagenesis V.1

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ABSTRACT

Part one of the HTTM protocol. A low-cost and high-throughput Tn-seq protocol. This part cover the transposon mutagenesis and selection steps.

MATERIALS

Main materials used in this protocol. Exhaustive list available in the twinned publication.

	A	B	C
	Aluminum cover	Ultident	PS-FOILP-100
	Deep well	Greinerbio-one/Fisher	780271-FD
	p200 tips	Sarstedt	70,303
	p300 tips	Sarstedt	70,304
	200µl 96 well plate	Ultident	87-C96-NS
	Dilution plate	fisher	12-556-008
	Petri dish	SARSTEDT	82,1473,001
	LB Broth	Biobasic	SD7002

Antibiotics and additives concentrations used in this protocol :

	A	B
	Ampicilin	100ng/µl
	Spectinomycin	100ng/µl
	2,6-Diaminopimelic acid	55ng/µl

BEFORE START INSTRUCTIONS

Per plate refers to the number of 96 well plates of target cells that need to be processed.

Keywords: HDTM, TnSeq, HTTM

Day 1

3m

1 (1-A) Make a 15 mL LB (Diaminopimelic acid [Dap], Ampicillin [Amp], Spectinomycin [Spec]) pre-culture (2 mL per plate minimum) of the donor strain eAC494 and incubate with agitation at 37 °C overnight.

2 (1-B) Prepare the 96 deep-well plates for conjugation :

10m

2.1
- Preheat the deep-well plates at 60 °C in a sterile incubator for 00:10:00
- Prepare 50 mL of LB-Agar for each plate and keep it above 70 °C

2.2 Using a multichannel pipette transfer 300 µL of molten LB-Agar in each well of the deep-well plates, paying attention not to create bubbles by keeping the tips on the side of the wells and not dispensing all the liquid.

2.3 Let dry in a biological hood for 3 days or until well dried but not cracked. (Optional : can be placed on a heating mat set at 30 °C to shorten the drying time to 2 days).

Day 2

3 (2-A) Prepare a 500 mL LB (Dap, Amp, Spec) culture of the donor strain per plate by making a 1/250 dilution of the pre-culture and incubate overnight at 37 °C.

4 (2-B) Fill the deep-well plates with chosen medium (1.5 mL per well) and inoculate each well with the recipient strains. Incubate overnight at 37 °C.

Day 3

20m

5 (3-A) Pellet the donor strain by centrifugation 6000 x g, 00:10:00 and discard the liquid. 10m

6 (3-B) Resuspend the pellet in 10 mL LB per plate.

7 (3-C) Dispense 100 µL of concentrated donor culture into each recipient well.

8 (3-D) Pellet cells by centrifugation 4000 x g, 00:10:00 and remove the supernatant with the Aspir-8 + 50 µL guide. 10m

8.1 If not using the Aspir-8 + 50 µL guide, remove all supernatant and add 50 µL of LB to each well.

9 (3-E) Resuspend by agitating on a shaker 900 rpm, 00:10:00 and do a quick spin to recover all the cells at the bottom of the plate.

10 (3-F) Take 50 µL from the resupended cells and deposit them on the dried agar at the bottom of the prepared deep-well plate. Let dry 01:00:00 in a biological hood and cover with a gas permeable plate seal. 1h

11 (3-G) Incubate the deep-well plates 02:00:00 at 37 °C for conjugation. 2h

12 (3-H) Add 400 µL of selection media to each well and resuspend by agitating on a shaker at 900 rpm, 00:10:00 and do a quick spin to recover all the cells at the bottom of the plate.

13 (3-I) Transfer 250 µL of the resuspended cells to a new deep-well filled with 1500 µL of selection media (with antibiotics to select for newly obtained mutants). Cover with a gas permeable plate seal and incubate with agitation at 37 °C overnight.

14 (3-J)/(3-K) (Optional) Using 20 µL of the conjugation mix make serial dilutions and spot on selective plates to estimate the number of mutants obtained per well.
Selection markers :
- Donor strain : Dap, Amp, Spec
- Recipient : Target-dependant
- Transposon mutants : Target-dependant + Spec

Days 4 to 7

15 Make a passage from the previous plate to a new deep-well plate filled with selective medium.
The volume of the passage (optimized to pass 3 million mutants in *E.coli*) varies from day to day :
- 200 µL of day 4 (4-A)
- 100 µL on day 5 (5-A), 6 (6-A) and 7 (7-A)

16 (7-B) (Optional) In order to have a backup in case of an issue during DNA extraction, make a glycerol stock using 150 µL of the culture after the passage, and store it at -80 °C .

Day 8

10m

17 (8-A)/(8-B) Pellet cells by centrifugation 4000 x g, 00:10:00 and remove the supernatant. 10m
Aspir-8 can be used to accelerate this step. Cells are ready for DNA extraction and can be stored at -80 °C until ready to process.