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Version 1

# HTTPM : Transposon mutagenesis V.1

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Antoine Champie<sup>1</sup>, Amélie De Grandmaison<sup>1</sup>

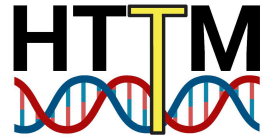
<sup>1</sup>Université de Sherbrooke

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**Antoine Champie**

Université de Sherbrooke



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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

Part one of the HTTPM 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

## Troubleshooting












### Before start

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







## 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 :
- 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. 







- 6 (3-B) Resuspend the pellet in  10 mL LB per plate.
- 7 (3-C) Dispense  100  $\mu$ L 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  $\mu$ L guide. 10m
- 8.1 If not using the Aspir-8 + 50  $\mu$ L guide, remove all supernatant and add 50  $\mu$ L 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  $\mu$ L from the resuspended 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  $^{\circ}$ C for conjugation. 2h
- 12 (3-H) Add  400  $\mu$ L 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  $\mu$ L of the resuspended cells to a new deep-well filled with  1500  $\mu$ L of selection media (with antibiotics to select for newly obtained mutants). Cover with a gas permeable plate seal and incubate with agitation at  37  $^{\circ}$ C overnight.
- 14 (3-J)/(3-K) (Optional) Using  20  $\mu$ L 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  $\mu\text{L}$  of day 4 (4-A)
  -  100  $\mu\text{L}$  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  $\mu\text{L}$  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. 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.

10m