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Version 3

HTTPM : Transposon mutagenesis V.3

 [PLOS One](#)

✓ Peer-reviewed method

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Spotlight series



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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 transposon mutagenesis of targets cells.

The **last step** in this version contains a supplemental video with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

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 μ L of concentrated donor culture into each recipient well.
- 8 (3-D) Pellet the cells by centrifugation  4000 x g, 00:10:00 and remove the supernatant with the Aspir-8 + 50 μ L guide. 10m
- 8.1 If not using the Aspir-8 + 50 μ L guide, remove all supernatant and add 50 μ 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 μ 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 μ 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) Transfert  250 μ L of the resuspended cells to a new deep-well filled with  1500 μ 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 μ 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 millions 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. 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

Spotlight video

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https://www.youtube.com/embed/KvjKKpLHSKE?si=eQurl-NSlvCrzQ_Z