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Version 4

HTTPM : Transposon mutagenesis V.4

 [PLOS One](#)

✓ Peer-reviewed method

DOI

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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.

Attachments



[HDTM_Protocol.pdf](#)

444KB

Image Attribution

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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 with  180 rpm .
- 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 with  180 rpm .

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 100-150 µL (total volume) donor culture into each recipient well.
- 8 (3-D) Pellet the cells by centrifugation 3270 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 50-100 µL (total volume) 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 at 30 °C 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) Transfert 400 µL (total volume) 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 at 37 °C with 180 rpm 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 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: pellet cells of the culture after passage  3270 x g and remove supernatant, add  75 μL of 50 % glycerol solution and resuspend by agitating  900 rpm  00:10:00 . Store it at  -80 °C .

10m

Day 8

10m

- 17 (8-A)/(8-B) Pellet cells by centrifugation  3270 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