HTTM : Transposon mutagenesis V.3

PLOS One ✓ Peer-reviewed method

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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 INSTRUCTIONS

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

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

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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 10m

- 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 the 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 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 °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 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 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. 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.

Spotlight video

18

h