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HTTM : Transposon mutagenesis V.4

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Spotlight series

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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

Make with BioRender.com

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

10m

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 resupended 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

18

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