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E_faecalis_conjugation_HR

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[1]In-house protocol

1 *Works for me* This protocol is published without a DOI.

Eadewunm

PROTOCOL CITATION

Elizabeth Fozo 2020. E_faecalis_conjugation_HR. **protocols.io** https://protocols.io/view/e-faecalis-conjugation-hr-bpz8mp9w

LICENSE

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MATERIALS TEXT

Donor (CK111/pCF101-10) Selection plates

Water – 500 mL
BHI – 18.5 g
Agar – 7.5 g

Autoclave 30 minutes
Cool to 65°C
Add spectinomycin – 1 mg/mL
Add erythromycin – 10 µg/mL
Mix and pour

Recipient (transconjugant) selection plates

Water – 500 mL
BHI – 18.5 g
Agar – 7.5 g

Autoclave 30 minutes
Cool to 65°C
Add erythromycin – 10 µg/mL
Add X-gal – 250 µg/mL
Add rifampicin – 250 µg/mL
Addfusidicacid – 25 µg/mL
Mix and pour

Counterselection plates

Water – 440 mL
Agar – 7.5 g
10 m9 salts – 50 mL
Yeast extract – 1.25 g
P-Cl-Phe– 998 mg

Autoclave 30 minutes
Mix thoroughly to dissolve the p-Cl-Phe
Cool to 65°C
Add 5 mL sterile 50% glucose
Add 250 µg/mL X-gal
Mix and pour

10x M9 salts (per liter)

Na_2HPO_4– 60 g
KH_2PO_4– 30 g
NaCl – 5 g
NH_4Cl – 10 g

DISCLAIMER:

BEFORE STARTING

Go to Materials for recipes for:

- Donor selection plates
- Recipient selection plates
- Counterselection plates
- 10x M9 salts

+*Enterococcus faecalis* conjugation and homologous recombination

1 Inoculate 5 to 10 mL BHI (add antibiotics if necessary) with donor and recipient strains and incubate overnight at 37°C

2 In the morning, wash cultures 2x with BHI, then resuspend cells in the original volume of BHI.
Dilute both cultures 1:10 in BHI and incubate at 37°C for 1 hour

3 Dilute both cultures 1:10 in BHI and incubate at 37°C for 1 hour

4 Mix donor and recipient (1 part donor to 9 parts recipient) to initiate conjugation

5 Put 800µL of the conjugation mix into a 1.5 mL Eppendorf tube and centrifuge for 2 min at 13000 RPM at 4°C

6 Resuspend in 150 µL BHI and spread on a BHI agar plate.

7 Mate for 5 hours.

8 Collect the cells from the surface by adding 2 mL of 1x PBS + 2 mM EDTA (20 mL 1x PBS + 80 µL 0.5M EDTA) and scraping the cells using a disposable hockey stick. Put the resuspended cells into a 1.5 mL Eppendorf tube.

9 Prepare 10-fold serial dilutions using 1x PBS as diluent and plate the suspensions on selective media for donors and transconjugants. Typical dilutions for donors (-4 to –6) and transconjugants (-3 to –5). Incubate at 37°C. Blue colonies appear after 24-48 hours.

10 Restreakblue colonies onto the same selective media 2x for single colony purification

11 Inoculate 10 mL BHI with a colony in the absence of selection until the culture reaches stationary phase (grow as overnight)

12 Prepare serial dilutions of the overnight: dilute the culture 1:100 three times, then 1:10 one time. Grow all as overnights.

- Rachel suggests: Do dilutions in 1.5 mL BHI; for final dilution, place 1 mL of diluent into 9 mL of BHI, leave 0.5 mL of higher concentrations in Eppendorf tubes; grow these in the incubator as well.
- Rachel suggests: There is no need to calculate CFUs of the original overnights before this step; just do the above dilutions – you will get growth!

13 Prepare 10-fold serial dilutions using 1x PBS and plate on counterselection plates. Incubate at 37°C until colonies appear and are large enough to pick.

- Rachel suggests: Incubating at 30°C may allow blue colonies to become more apparently blue, though growth will take longer.
- Rachel suggests: Placing plates with all apparently white colonies at 4°C may also help for blue color to develop
- Rachel suggests: hurting your eyes and squinting really hard at these plates will not necessarily help. Pick the whitest colonies of varying sizes if they all look white and screen these. Sometimes smaller slower growing colonies have had higher rates of successful recombination.

14 Restreak white colonies on BHI. Screen with check primers for the wild-type or mutant allele using the *E. faecalis* colony PCR protocol.