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Ef_electocomp_cells_OG1RF

Elizabeth Fozo¹¹In-house protocol

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Works for me

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PROTOCOL CITATION

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MATERIALS TEXT

Electroporation Buffer (EB) 50 mL

0.5 M sucrose 8.56 g
 10% glycerol 5 mL 100% stock bottle
 Bring volume to 50 mL with dH₂O.
 Autoclave or filter sterilize.

Lysozyme Solution (LS) 50 mL

10 mM Tris pH 8.0 0.5 mL 1 M
 20% sucrose 10 g or 40 mL 25%
 10 mM EDTA 1 mL 0.5 M pH 8.0
 50 mM NaCl 0.5 mL 5 M
 Bring volume to 50 mL with dH₂O.
 Autoclave or filter sterilize.

*25 µg/ml lysozyme (add to desired volume of LS just before use)

Make 10 mg/ml stock lysozyme soln in LS → Dilute lysozyme stock 1:10 (50 µl 10 mg/ml stock +450 µl LS, concentration 1 mg/ml)

Use 1 mg/ml lysozyme solution to make 25 µg/ml lysozyme stock → Add 30 µl 1 mg/ml stock to 1.170 ml LS

STHB 100mL

0.5M Sucrose 17.115g
 THB powder 3g
 Bring up to 100 mL with dH₂O.
 Autoclave

DISCLAIMER:

DISCLAIMER: THIS WORK IS IN PROGRESS. IT IS FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

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BEFORE STARTING

Go to Materials for recipes for:

- Electroporation Buffer (EB)
- Lysozyme solution (LS)
- 25 µg/ml lysozyme for step 5
- STHB

E. faecalis Electrocompetent Cells (with Lysozyme)

- 1 Inoculate 5-10 mL BHI or THB (I add fusidic acid at 25 µg/mL but not rifampicin) and incubate o/n at 37°C.
- 2 Dilute o/n culture 1:50 or 1:100 with in 100 mL THB (with FA25) and incubate until culture OD at 600 nm reaches 0.5-1.0 (original protocol dilutes 1:10)
- 3 Chill cells on ice for 15-20 min. Use floor centrifuge to pellet cells (in Oakridge tubes or 50 mL Falcon tubes) at 6,000 rpm for 10 minutes (this step doesn't need to be at 4 °C).
- 4 Resuspend pellet in 2 mL 10% glycerol or sterile H₂O and split into two 1.5 mL tubes. Pellet 13,000 rpm for 1 min. in a tabletop centrifuge (this step doesn't need to be at 4 °C).
- 5 Resuspend each pellet in 500 µL lysozyme solution (LS) containing **25 µg/ml lysozyme*** (add just before use- see recipe in Materials). Incubate at 37°C for 20 min (water bath or incubator).
- 6 Pellet as in step 4.
- 7 Wash 3 times with 1 ml ice-cold electroporation buffer (EB – see recipe below). **Keep pellets and buffers cold from this point forward!**
- 8 Resuspend each tube in 300 µL EB/tube (total from both tubes pooled will be approx. 500-600 µL, including volume of cells) and split into 100 µL aliquots. Store at -80°C. Efficiency will decrease after freezing

Electroporation

- 9 Thaw cells on ice. I use ~50 µL per electroporation.
- 10 Chill DNA and tubes for recovery (1 tube per electroporation). Transfer an appropriate volume of STHB to a test tube (do NOT put on ice). Chill electrocuvettes (1 per electroporation and a few extras, we use 0.1 cm cuvettes).

- 11 Open program on Gene Pulser (Dawn has a standard *E. faecalis* one saved, settings are .6 kV, 200 Ω , 25 μ F).
- 12 Transfer 50 μ L cells to the first microfuge tube. Add ice-cold DNA (~100 ng, 1-3 μ L of most minipreps will be fine) and pipette a couple of times to mix. Immediately transfer to pre-chilled electrocuvette and electroporate. Immediately add 1 mL STHB to the electrocuvette after the pulse is completed. The time constant should be ~5.0 (higher for pure cells, lower with more or low-quality DNA). Continue even if it's lower or it arcs. Pipette up and down a few times to wash cells out of cuvette. Transfer back to the microfuge tube you mixed your cells and DNA in during step 2. **Keep at room temp/recovery temperature from here on out!**
- 13 Recover cells with static incubation at 37°C (or whatever temperature you need) for 2-4 hours
- 14 Plate 50 μ L undiluted cells. Pellet the remaining volume and resuspend in 100 μ L STHB. Plate 50 μ L of the resuspended cells. Save the rest on your benchtop overnight. Incubate plates at 37 °C until colonies appear (1-2 days).