Ef_electocomp_cells_OG1RF

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[1]In-house protocol

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

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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

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety

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 ug/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 oC).

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 oC).

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 uL EB/tube (total from both tubes pooled will be approx. 500-600 uL, including volume of cells) and split into 100 uL aliquots. Store at -80°C. Efficiency will decrease after freezing

Electroporation

9 Thaw cells on ice. I use ~50 uL 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 uF).

12 Transfer 50 uL cells to the first microfuge tube. Add ice-cold DNA (~100 ng, 1-3 uL 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 uL undiluted cells. Pellet the remaining volume and resuspend in 100 uL STHB. Plate 50 uL of the resuspended cells. Save the rest on your benchtop overnight. Incubate plates at 37 oC until colonies appear (1-2 days).