

SIMPLE ELECTROCOMPETENT CELLS -FROZEN

Materials

YETP: 0.75% Bacto yeast extract, 1.00% Bacto tryptone

10% Glycerol (v/v) filter sterilized

sterile water

Day 0 or 1

Streak out ccdB survival cells (should work if you start in the AM early)

Day 1

Pick colony and grow overnight, in LB.

Day 2

Pull O.N. out in morning and let sit on bench,

Place 2 500 ml cultures in 20°C shaker at 250 rpm to cool to 20°C .

Innoculate with 0.5 ml overnight at noon. Note that the dilution required to get mid log cells the next morning may differ if not using ccdB survival cells.

Day 3

Next morning collect cells at 9 am should be ~ OD₆₀₀=0.6 to 0.8

Chill cells on ice for 15 minutes

Spin down cells 10 minutes @ 5500 rpm in SLA1500 at 4°C.

Resuspend cells in 20 ml, dilute to 200 ml cold water

Spin, 10 min at 5,000 rpm in SLA1500

Resuspend cells in 20 ml, dilute to 200 ml cold water

Spin, 10 min at 5,000 rpm in SLA1500

Resuspend washed cells in total of 50 ml of cold 10% glycerol

Combine cells into single centrifuge tube

Spin down cells and resuspend in 8ml 10% glycerol

Dispense cells into 100 μ l aliquots and freeze in liquid nitrogen. Store at -80°C.

Day 4

titer cells using 0.1 ng of a standard plasmid.

Electroporation Protocol

1. Place cuvettes(s) and ependorf tubes on ice.
2. Thaw electrocompetent cells on ice. Dive 100 μ l aliquot into two tubes if needed.
3. Add ligation, gateway reaction, or plasmid DNA to cells (1-3 μ l) and gently mix by pipetting. Note that salt will be a major problem. Only use a few μ l of ligation mix. Incubate on ice for a few minutes and transfer cells to the cuvette.
4. Zap cells in the Salkoff lab Biorad Electroporator. Set apparatus to 2.5 kV, 25 μ F, 200 ohms. Cuvette holder is in the -20°C freezer.
5. Immediately add 1 ml LB to cuvette.
6. Transfer cells to an ependorf tube and incubate at 37°C for 30 to 60 minutes.
7. Plate 100 ul (and/or rest after spinning down cells).

NOTES:

Good high efficiency cells depends on keeping everything cold. I just do all the work in the cold room so I know nothing will warm up.