Electroporation Protocol
Vaillancourt Laboratory
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Preparation of Electrocompetent Cells

1. Streak E. coli DH5a from the frozen stock of ElectroMAX DH5 alpha-E cells (Invitrogen catalog # 11319019) onto a fresh LB plate. Incubate plate upside-down at 37° overnight.

2. Use a single colony to inoculate 10 mls of LB broth. Place on shaker at 250rpm, 37°, overnight

3. Inoculate 1 liter of fresh LB broth with 10 ml of the fresh overnight LB culture.

4. Grow cells at 37° with vigorous shaking to an A600 of 0.5-1.0 (for DH5a, this is about 3-4 hours).

5. To harvest, chill the flask on ice for 15-30 minutes, then centrifuge in a cold rotor at 4000 X gmax for 15 minutes.

6. Remove as much of the supernatent as possible. Resuspend pellets in a total of 1 liter of sterile cold milli-Q water. Centrifuge as in step 5.

7. Resuspend in 0.5 liter of sterile cold water. Centrifuge as in step 5.

8. Resuspend in 20 mls of cold sterile 10% glycerol. Centrifuge as in step 5.

9. Resuspend to a final volume of 2 or 3 mls in cold, sterile 10% glycerol. The cell concentration will be about 1-3 X 10^{10} cells/ml.

10. Divide the cell suspension into aliquots of 120 microliters/tube in screw-cap eppendorf tubes. Dip the tubes into liquid nitrogen to freeze, store at -80 until used. Cells will be good for 6 months to a year.

Electroporation Procedure

1. Remove 1 tube of frozen competent cells from the freezer for every 3 transformations that will be done. Thaw the cells slowly on ice. Mix gently to resuspend, and return to the ice.

2. Pre-chill required number of electroporation vials on ice. Label tops.

3. Mix DNA and 40 microliters of chilled cells in cold microfuge tubes. Transfer to chilled electroporation vials, knock sharply on the desk to remove any bubbles. Return vials to ice.

4. Set up the gene pulser apparatus by removing the electroporation chamber from the refrigerator and attaching the leads to the apparatus. Turn on the apparatus and check the settings:

    a. Voltage, 2.5kV
    b. Capacitor, 25uF
    c. Pulse controller setting: 200 ohms

5. Set the indicator to read the time constant. If all goes well, this should be around 4.5-4.7 for each sample.

6. Place the electroporation vial containing the DNA and bacterial cells into the chamber (dry off the bottom with a kimwipe, first), slide it into place so that contact is made with the electrodes, then push both buttons on the apparatus at the same time, until the buzzer sounds.

7. AS QUICKLY AS POSSIBLE: slide the electroporation cell out of the chamber, take off the lid, and add 1 ml of chilled SOC medium. Mix briefly, then return the cells to the ice until you have finished electroporating all your samples.

8. Transfer samples to microfuge tubes, incubate the tubes for 20 minutes on the shaker at 37°.

9. Spread 50-150 microliters of the cells onto LBamp plates, incubate upside-down at 37° overnight.

Recipes

LB (Luria-Bertani) medium

Commercially available: or use the following recipe:

To 950 mls of ultrapure water, add

10g bacto-tryptone

5g bacto-yeast extract

10g NaCl.

15g bacto-agar, to solidify.

Mix, autoclave.

LBamp: add 1 ml of the 10mg/ml stock ampicillin to each liter of cooled broth or cooled (not solidified) agar. Swirl to mix.

SOC medium

To 950 mls of ultrapure water, add

20g bacto-tryptone

5g bacto-yeast extract

0.5g NaCl

Mix until dissolved. Also, make up a 1M solution of glucose (18 grams of glucose in 90 mls of ultrapure water). Autoclave both solutions separately. Allow the medium to cool to 60 degrees or less, then add 20 ml of the sterile glucose solution.