CHEMICALLY COMPETENT CELLS

Judelson method, adapted from D. Alexander in Methods in Enzymology vol. 154.
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Preparing solutions and supplies

Glassware and plasticware are rinsed extensively with distilled water to remove all traces of detergents. This includes vessels used to make the various solutions. The recipe can be scaled down.

Growth of cells

E. coli cells (DH5-alpha) are innoculated into 25 ml of 2xL broth and grown overnight at 30°C (NOT 37°C!!). 2xL is 2% bacto-tryptone, 1% yeast extract, 0.1% NaC1, 0.2% glucose, pH 7.0.

Each of four 2-liter flasks, containing 500 ml 2xL broth prewarmed to 30°, are inoculated with 5 ml of the overnight growth. The ratio of liquid to flask size is important for good aeration.

The flasks are shaken at 150-200 rpm, 30°, until the A600 reaches 0.45-0.55. NO HIGHER–or the competency level goes way down!

The cells are chilled in ice water for 2 hr and then centrifuged in polypropylene bottles at 2500 g (maximum) for 15-20 min, 4°. KEEP EVERYTHING AT 4° FROM THIS STEP ONWARDS.

Cells in each bottle are triturated (resuspended) with a pipet in a small volume (10-20 ml) of ice-cold 100 mM CaCl2, 70 mM MnCl2, 40 mM NaOAc, pH 5.5 (made fresh from the three salts and filter sterilized, not autoclaved!!).

Cells are then diluted to 500 ml with the same solution, and incubated 45 min in an ice water bath.

The cells are centrifuged at 1800 g (maximum) for 10 min and then very gently suspended in a total of 100 ml (relative to the 2000 ml starting culture) of the same ice cold solution ($CaCl_2$, $MnCl_2$, NaOAc). Pool the cells into a single plastic bottle.

80% glycerol is added dropwise with gently swirling to give a final concentration of 15% (v/v).

Cells are aliquoted into 1.5 ml microfuge tubes on ice (or in a cold room), most into aliquots of 1 ml, some into aliquots of 0.2 ml, and placed directly into -80° for storage.

Transformation

Typically about 100 µl of bacterial cells are used per transformation, but depending on the experiment this can be scaled up or down.

After making cells, you need to determine how competent they are. To do this, test the cells using a 1 ng or 10 ng of supercoiled DNA (diluted in TE or water):

Transformation procedure: Thaw an aliquot of the cells on ice, and use immediately for transformation. In a pre-chilled tube, combine the DNA with the cells by gently tapping the tube with your finger. For optimal results, the volume of DNA should be 1/2 of the cell volume (for example, 100 µl of DNA with 200 µl of cells). After mixing the cells, and DNA, wait 30 min (on ice). Mix by gentle inversion, and place in a 37C water bath for 5 min. Add a ten-fold excess of media (SOC or 2x L or LB), i.e. 1 ml of media per 100 µl of cells. Incubate at 37C for 45-60 min (shaking is optimal, but not essential: placing in a stationary heat block works fins). Plate aliquots on selection media; larger volumes may be briefly centrifuged and resuspended before plating.

This should give a transformation efficiency of about 10^8 transformants per microgram DNA.