Murphy Lab, Emory University, Spring 2000

Preparation of frozen competent *E. coli Top 10 F'* cells

IMPORTANT! All chemicals, glassware, bottles etc must be sterile. The bottles and glassware you use to culture and spin down bacteria may have residual plasmid material. Rinse these with 0.25 M HCl/1.5 M NaCl, and then with denaturation solution (0.5 M NaOH, 1.5 M NaCl) and then copious amounts of RO water before autoclaving. Only this rinsing will destroy plasmid DNA, autoclaving will not. It is best to use only spin bottles reserved for competent cell preps. Follow aseptic technique. Compulsive attention to detail is essential to make a good batch of cells.

1. Inoculate 20 ml of TMY broth (supplemented with 50 µl tetracycline, 5 mg/ml in 100% ethanol) using 100 ul of E.coli TOP 10 F' (from frozen stock (e.g. frozen competent cells)) and grow in a 250 ml flask at 37C at 300 rpm shaking.
2. Grow to midlog phase (OD_{600} ~ 0.2 - 0.8).
3. Transfer culture to 2L flask containing 100 ml of TMY (supplemented with 250 µl of tetracycline).
4. Grow with vigorous shaking to OD_{600} of 0.5 - 0.9.
5. Dilute culture again by adding to the flask 400 ml of TMY, supplemented with 1 ml tetracycline, and grow to OD_{600} = 0.6.
6. Place flask in ice-water bath and shake gently to ensure rapid cooling.
7. Spin at 4200 rpm at 4C for 10 min using the "competent cell only" centrifuge bottles.
8. Decant supernatant and resuspend pellet in 100 ml of COLD **TFBI** by gentle pipetting on ice.
9. Spin at 4200 rpm at 4C for 8 min.
10. Decant supernatant and resuspend pellet in 20 ml of COLD **TFBII** by gentle pipetting on ice.
11. Dispense 600 ul aliquots in pre-chilled microfuge tubes and snap-freeze immediatel in a dry ice/ETOH bath (ETOH is cheaper than MeOH and works just as well).
12. Store at –70C.
13. Run a positive control by transforming frozen cell with 5 ng of plasmid, and plate 1/5th on LB amp. Should back calculate to get >10^6 colonies per µg of plasmid.

TMY Broth (1 L)	***TFBI (200 ml)***	***TFBII (200 ml)***
20 g bacto-tryptone 5 g yeast extract 5.84 g NaCl 1.2 g MgSO4 Dissolve in QH2O and autoclave	0.59 KOAc 1.98 g MnCl2 1.50 g KCl 0.29 g CaCl2 30 ml glycerol Dissolve all components in QH2O and filter sterilize	0.419 g MOPS 2.2 g CaCl2 0.15 g KCl 30 ml glycerol Dissolve MOPS in QH2O and adjust pH to 7.0 with I N NaOH. Add remaining components and filter sterilize.