PREPARATION OF COMPETENT E. coli

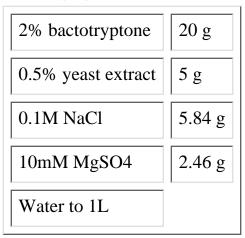
This protocol is a larger scale variant of one due to Mike Scott, Department of Neurology, UCSF.

- 1. Streak out the desired strain on an LB plate. Pick colony and grow in 3mL LB over night. (If sure cells, then use LB- Tetracycline.)
- 2. The next day, inoculate 100mL into 20 ml TYM broth (recipes next page) in a 250 ml flask. Preheat all broth solutions to 37C if you want to be done before midnight.
- 3. Grow the cells to midlog phase (OD600 ÷0.2 0.8), pour into 21 flask containing 100 ml TYM, continue vigorous agitation until cells grow to 0.5 0.9 OD, then dilute again to 500 ml in the same vessel.
- 4. When cells grow to OD600 0.6 put flask in ice-water, and shake gently to assure rapid cooling. When culture is cool, spin 4.2 k rpm 15 minutes (JA-10).
- 5. Pour off supernatant, resuspend pellet in ÷ 100 ml cold TfB I (next page) by gentle shaking on ice. Respin in same bottle 4.2 k rpm, 8 minutes (JA-10).
- 6. Pour off supernatant, resuspend pellet in 20 ml cold TfB II by gentle shaking on ice.
- 7. Aliquot 250 æl aliquots in prechilled microfuge tubes, freeze in liquid nitrogen, and store at -70ø. For transformation, see separate method.

Media and Buffers: (see reverse)

PROTOCOL FOR COMPETENT CELLS - see previous page

MEDIA + BUFFERS



(1L) TYM Broth

Separate into 20 ml, 100 ml, 500 ml and autoclave.

(250mL) TfBI

30mM KoAc	0.736g
50mM MnCl2	2.47g

100mM KCl	1.86g
10mM CaCl2 dihydrate	0.367g
15% (v/v) glycerol	37.5mL

(100mL) TfBII

10mM Na-MOPS pH 7.0 (stock: 50mM pH 7.0)	20 mL
75mM CaCl2 dihydride	1.1 g
10mM KCl	0.0745 g
15% glycerol	15 mL

TfBI and II : DO NOT AUTOCLAVE . FILTER THROUGH 0.2 u FILTER AND , STORE 4C.