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 2 l 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

2% bactotryptone	20 g
0.5% yeast extract	5 g
0.1M NaCl	5.84 g
10mM MgSO4	2.46 g
Water to 1L	

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.