*PREPARATION OF COMPETENT CELLS*

*$RbCl_2$ Method*

The following procedure can be used to obtain competent cells with a transformation frequency of $10^6$ - $10^7$ colonies per microgram of DNA. Conveniently, these cells can be stored for months with relatively no loss in efficiency.

**Materials:**

*Buffers*

Transformation buffer I (TfbI):

30 mM KOAc
100 mM $RbCl_2$
10 mM $RbCl_2$
50 mM $MnCl_2$
15% Glycerol (v/v)
pH = 5.8

Transformation buffer II (TfbII) :

10 mM MOPS (or PIPES)
75 mM $CaCl_2$
10 mM $RbCl_2$
15% Glycerol (v/v)
pH = 6.5

*MAKING TRANSFORMATION BUFFERS*

| For 100 ml TfbI | Concentration Separately Autoclaved Stocks | Amt Added (ml) |
|---|---|---|
| 30 mM KOAc | 0.3 M | 10 |
| 100 mM $RbCl_2$ | 1 M* | 10 |
| 10 mM $CaCl_2$ | 1 M | 1 |
| 50 mM $MnCl_2$ | 1 M | 5 |
| 15% Glycerol | - | 15 |

pH to 5.8 with HOAc, make up to 100 mls with $H_2O$, Nalgene filter sterilize.

| For 20ml TfbII | Separately Autoclaved Stocks | Added(ml) |
|---|---|---|
| 10mM MOPS (PIPES) | 1 M (pH6.5) | 0.2 |

| | | |
|---|---|---|
| 75mM $CaCl_2$ | 1 M | 0.15 |
| 10mM $RbCl_2$ | 1 M* | 0.2 |
| 15% Glycerol | - | 3 |

Add $H_2O$ to 15 ml, readjust pH to 6.5 with KOH, and make up to 20 ml with $H_2O$.
Filter sterilize through disc.

**Procedure:**

1. Streak from DH-1 stock onto LB plate
   Incubate overnight at 37°C.
2. Pick a single colony and inoculate a 5 ml culture of LB. Incubate overnight at 37°C.
3. Add overnight culture to 100mls LB. Grow until OD = 0.5. This usually takes at least 2 hours. (optimal OD = 0.48).
4. Divide into 4 30ml Corex tubes.
   Chill 5' on ice.
   Spin 6000 rpm, 5', 4°C. Note shape of pellet.
5. Resuspend cells in 2/5 volume TfbI.
   (2/5V = 40ml, therefore 10 ml per tube.) (Use a 'greenie meanie')
6. Ice cells 5'.
   Spin 6000 rpm, 5' 4°C.
   Pellet should look more donut-like than in 4.
7. Resuspend cells in 1/25 original volume TfbII.
   (1/25v = 4 ml, therefore 1 ml per tube).
8. Pool. This helps to keep the number of cells per Eppendorf constant.
9. Ice 15'.
10. Aliquot 100 g into pre-chilled Eppendorfs with cold pipettes.
11. Snap freeze in powdered dry ice
    Store at -70°C.

**Comments:**

1. Keep everything cold. Work in cold room. Put pipettes and Eppendorfs in cold room at step 3.
2. Optimal OD is flexible. Cultures can be used with ODs as high as .9. Basically, a culture in mid to late log phase is all that is needed.
3. Times during which the cells are chilled are flexible. When handling many tubes don't worry about going over the suggested incubation times.
4. When many tubes are needed, freeze as 50g or 100g aliquots.