Preparation of Utral-Competent Cell

The following procedure can be used to obtain competent cells with a transformation frequency of 10^7 - 10^8 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 RbCl2
10 mM RbCl2
50 mM MnCl2
15% Glycerol (v/v)
pH = 5.8

Transformation buffer II (TfbII) :

10 mM MOPS (or PIPES)
75 mM CaCl2
10 mM RbCl2
15% Glycerol (v/v)
pH = 6.5

How to MAKE TRANSFORMATION BUFFERS

For 100 ml TfbI

Reagents	Volume(ml)	Final Concentration
0.3MKOAc	10	30mM
1MRbCl2	10	100mM
1MCaCl2	1	10mM
1MMnCl2	5	50mM
Glycerol	15	15%

pH to 5.8 with HOAc, make up to 100 ml with ddH2O, Nalgene filter sterilize.

For 20ml TfbII

Reagents	Volume(ml)	Final Concentration
1MMOPS (PIPES)	0.2	10mM
1MRbCl2	0.2	10mM
1MCaCl2	1.5	75mM
Glycerol	3	15%

Add H2O to 15 ml, readjust pH to 6.5 with KOH, and make up to 20 ml with H2O. Filter sterilize through disc.

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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.

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