Transformation and Production of Chemically Competent Cells

Buffers needed for the Procedure

WASH BUFFER:	STORAGE BUFFER:	10 X SOC	LB MEDIUM
100 mM RbCl	10 mM RbCl	0.2 % (w/v) KCL	0.5 % (m/v) Yeast Extract
50 mM $MnCl_2$	75 mM $CaCl_2$	2.0 % (w/v) $MgCl_2$	1 % (m/v) Tryptone
30 mM KOAc	10 mM MOPS	2.0 % (w/v) $MgSO_4$	1 % (m/v) NaCl
10 mM $CaCl_2$	15 % Glycerol	4.0 % (w/v) Glucose	*(1.5 % (m/v) Agar for plates only)*
15 % Glycerol		In *A. dest* (sterile filtered)	In *A. dest* (autoclave)

Production of Chemically Competent Cells (D. Hanahan, J. Mol. Biol.. 1983, 166, 557-580.)

- Create an Overnight Culture (LB medium, 37°C, 200 rpm) of your desired *E. coli* cell (i.e. DH5α)
- Inoculate 100 ml main culture 1 : 100 with the overnight culture (1 ml overnight culture in 100 ml LB)
- Cultivate the cells at 37°C and 200 rpm until the OD_{600} = 0.3 – 0.4 is reached
- Chill the culture on ice for 10 min
- Centrifuge the cold cells at 4000 x g at 4°C and discard the supernatant
- Resuspend the pellet in 20 ml Wash Buffer and incubate the cells on ice for 15 min
- Centrifuge the cold cells at 4000 x g at 4°C and discard the supernatant
- Resuspend the cells in 4 ml Storage Buffer and incubate the cells on ice for 15 min
- Aliquot the cells in your desired volume (i. e. 50 µl each aliquot)
- Shock freeze the aliquots immediately with liquid nitrogen or a “Dry Ice – Water – NaCl” solution
- Store the cells at -80°C until use

Chemical Transformation

- Thaw an aliquot of the chemical competent cells on ice for 5 min
- Add 1 µl plasmid (10 – 100 ng) to the cells and stir the tube with the pipet
- Incubate the cells on ice for 30 min
- Heat shock the cells exactly 30 seconds at 42°C (use a water bath)
- Chill the cells on ice for 2 min
- Add 450 µl 1 x SOC (dilute the 10x SOC 1:10 in LB)
- Incubate the cells at 37°C and 200 rpm for 1 h
- Plate the cells on an agar plate with the desired antibiotic (you may use different dilutions of your transformation approach to gain single colonies)