

## Preparation of Supercompetent Cells (DH5 $\alpha$ )

### Reagents

#### SOB medium

	<u>quantities for 1 L</u>
2.0% tryptone	20 g
0.5% yeast extract	5 g
10 mM NaCl	2 mL of 5 M stock (146.1 g NaCl/500 mL)
2.5 mM KCl	1.25 mL of 2 M stock (74.56 g KCl/500 mL)
	H <sub>2</sub> O for final volume of 1L

Autoclave and add 5 mL of sterile Mg solution per 500 mL (Mg solution is 1 M MgCl<sub>2</sub> and 1 mL MgSO<sub>4</sub>; 20.3 g MgCl<sub>2</sub>•6H<sub>2</sub>O, 24.7 g MgSO<sub>4</sub>•7H<sub>2</sub>O/100 mL)

#### CMG buffer

	<u>quantities for 1 L</u>
50 mM CaCl <sub>2</sub>	7.35 g CaCl <sub>2</sub> •2H <sub>2</sub> O
50 mM MgCl <sub>2</sub>	6.02 g MgCl <sub>2</sub> (anhydrous)
	H <sub>2</sub> O for final volume of 1L

### Procedure

- 1) Inoculate an LB culture with DH5 $\alpha$  cells (directly from the frozen stock without thawing) and grow overnight at 37 °C.
- 2) Add 5 mL of this overnight culture to 500 mL of SOB medium in a 2 L flask.
- 3) Grow cells to an OD<sub>600</sub> between 0.4 or 0.6. **Do not exceed 0.6.**
- 4) Chill culture on ice for 10 minutes.
- 5) Transfer culture to a sterile 500 mL centrifuge bottle and pellet the cells at 4000 g for 5 minutes at 4 °C. Decant off the SOB medium.
- 6) Gently resuspend the pellet in 150 mL of cold CMG buffer. A large fraction of the cells will remain as clumps.
- 7) Incubate the cell suspension on ice for 15 minutes.
- 8) Pellet the cells at 4000 g for 5 minutes at 4 °C. Decant off the CMB buffer.
- 9) Gently resuspend the cells in 36 mL of cold CMB buffer and transfer the suspension to a 50 mL disposable centrifuge tube. The cells should be thoroughly dispersed.
- 10) Incubate the cell suspension on ice for 5 minutes.
- 11) Add 1.26 mL of high quality DMSO, mix well, and incubate on ice for 5 minutes.
- 12) Add another 1.26 mL of DMSO, mix well, and incubate on ice for 5 minutes.
- 13) Dispense the suspension into sterile microcentrifuge tubes (250  $\mu$ L aliquots is generally appropriate) and flash freeze in liquid nitrogen.
- 14) Store competent cells at -80 °C.

### Notes

- 1) Have centrifuge bottles and microfuge tubes autoclaved and prechilled prior to use. You will need approximately 150 microfuge tubes per 500 mL of starting culture.

- 2) Aliquoting of the competent cell suspension is tedious and time consuming. An extra pair of hands at this stage would help speed up the process.