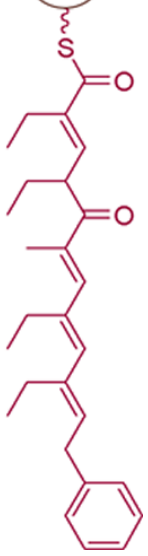

Procedure for Preparing Chemically Competent Cells

Adapted from http://www.molbio.wisc.edu/carroll/methods/Bacterial/CompetentCells.html

NOTE: Chill all centrifuge tubes and solutions before use! Try to keep cells cold at all times!

1. Prepare seed culture. Inoculate 5-10 mL LB broth and incubate overnight at 37C with shaking (200 rpm).
2. Inoculate fresh media (e.g. 400 mL Lauria-miller in a 2 L flask) with 0.05% (e.g. 2 mL) of seed culture.
3. Grow at 37C with shaking (200 rpm) until OD600 = 0.2—0.4.
4. Chill culture on ice for 10 minutes.
5. Pellet cells by centrifugation at 5,000 X g for 5 minutes at 4 oC.
6. Resuspend cells in 1:4 culture volume with 100 mM MgCl2, and ice for 5 minutes.
7. Pellet cells again by centrifugation at 4,000 X g for 5 minutes at 4 oC.
8. Resuspend cells in 1:4 culture volume with 100 mM CaCl2, and ice for 20
9. minutes.
10. Centrifuge at 4,000 X g for 5 minutes at 4 oC to pellet cells.
11. Resuspend cells in 1:40 culture volume of 100 mM CaCl2, 15% glycerol.
12. Aliquot, 50—200 µl, into prechilled 1.5 mL eppendorf tubes.
13. Immerse tubes in liquid nitrogen and store at -80 oC