Chemocompetent *Escherichia coli* cells

This protocol is from the iGEM team Aix-Marseille 2014: http://2014.igem.org/Team:Aix-Marseille/Protocol:chemocompetent_ecoli_cells

Chemoconpetent *Escherichia coli* cells

1. Streak *E.coli* cells on an LB plate with or without antibiotics.
2. Allow cells to grow at 37°C overnight.
3. Place three or four colonies in 5 mL LB media (+ antibiotic selection if necessary), grow overnight at 37°C.
4. Dilute the preculture to OD600 = 0,05 in 100 mL LB.
5. Allow cells to grow at 37°C (250 rpm), until OD600= 0.4 (about 2-3 hours).
6. Transfer cells to 2 centrifuge bottles (50 mL), and place cells on ice for 20 minutes.
7. Centrifuge cells in Sorval GSA rotor at 4°C for 10 minutes at 5 000 rpm.
8. Subsequent resuspensions may be done in the same bottle. Cells must remain cold for the rest of the procedure: Transport tubes on ice and resuspend on ice in the cold room. Discard the supernatant and resuspend cells in 1/2 Vol of cold 50 mM $CaCl_2$. Incubate on ice for 20 minutes.
9. Centrifuge cells using Sorval RT6000B rotor at 4°C for 10 minutes at 5 000 rpm.
10. Discard the supernatant and resuspend cells (by pipetting) in 1/20 Vol cold 50 mM $CaCl_2$ containing 15% glycerol. Transfer 500 µL of cells into (1.5 mL) Ependorff tubes placed on ice. Cells stored at -80°C can be used for transformation for up to ~6 months.

Note: Through the process, cells should be treated with care. No vortexing or excess pipetting should be performed, especially when the cells have been resuspended in $CaCl_2$ because lysis will result, decreasing the amount of competent cells. Also, depending on the density of the cells, higher or lower volumes $CaCl_2$ can be used to increase the concentration of cells per tube.

Transformation

11. Add 2 µL of 0,5, 10 and 50 ng/L of Transformation Efficiency Kit DNA to 100 µl of competent cells.
12. Incubate the mixture on ice for 35 minutes.
13. Transfer the reaction to a 42°C water bath for exactly 2 minutes.
14. Incubate on ice for 5 minutes.

15. Add 900 µL of LB medium to each tube and incubate at 37°C for 1 hour to allow cells to recover and express the antibiotic resistance marker.
16. Spread the appropriate quantity of cells (50 to 200 µl) on selective media. Store the remaining cells at 4°C.
17. Incubate all plates overnight at 37°C (agar side up).