Molecular cloning and genetic engineering – Competent Cell Production

Aim

Competent cell: physical and chemical methods induce the cells to be in the optimum physiological state of uptake and accommodation of foreign DNA.

Materials

0.1mol/L $CaCl_2$

LB plate

ddH_2O

Procedure

1. Ethanol and ultraviolet treat all working areas for sterility.

2. Streak DH5α cells on an LB plate and grow for single colonies at 37°C;

3. Fill an ice bucket halfway with ice. Use the ice to pre-chill centrifuge tubes and 0.1mol/L $CaCl_2$;

4. Pick a single colony in 30 ml LB medium, shake it at 37℃, 160 r/min, until OD_{600} = 0.3, then take it out and put it on ice for 15minutes

5. Pipette 1ml of bacterial solution and sterilized in 1.5 ml centrifugal tube.

Centrifugation at 4℃ for 5min at 5000r/min;

6. Discarded the supernatant, suck up the residual culture medium and gather cells.

7. Add 500 ul pre-chilled 0.1mol/L $CaCl_2$ to resuspend the cells, ice bath foe 20 min.

8. Centrifugation at 4℃ for 5 min at 5000r/min;

9. Discarded the supernatant, suck up the residual water. Add 100 ul pre-chilled 0.1mol/L $CaCl_2$, place at 4℃ for confirmation.