Experiment (2)

Competent Cells Formation and Transformation of Competent Cells with recombinant plasmid DNA

Materials:

Chemical

CaCl2, LB medium, NaCl, Tryptone, Yeast extract, Ampicillin.

Preparation of solutions

1- LB medium

To 950 ml of deionize H_2O add 10g Tryptone, 5g yeast extract, and 10g NaCl. Shake until the solution dissolve. Adjust pH to 7.0 with 5N NaCl. Adjust the volume to 1L with deionize H_2O. Sterilize by autoclave for 20 minutes at 15 psi on liquid cycle.

2- 50 M CaCl2 solution

Dissolve 7.4g of CaCl2.2H2O in d.H2O, make up the volume up to 1L by d.H2O. Autoclave the solution then store it at 4°C

3- Ampicillin

Dissolve ampicillin sodium salt in AnalaR water to a concentration of 100 mg/ml. Filter the solution through a 0.2µm filter. It should be added to a final concentration of 100 µg/ml. Store at 20 °C**.**

Equipment and Glassware

Microfuge centrifuge, electronic balance, water bath, roller, plate, incubator, microcentrifuge tube, centrifuge tube, Pasteur pipette, micropipette, tips.

Protocol:

A) Competent cells formation:

1. Centrifuge 10-15 ml of bacterial sample for 7 minutes at 3000 rpm at 4°C. allow centrifuge to decelerate without break.
2. Discard the supernatant and resuspend each pellet gently in 10 ml ice cold $CaCl_2$ solution.
3. Centrifuge the cells for 5 minutes at 2500 rpm at 4 °C.
4. Discard the supernatant and resuspend each pellet in 10 ml ice cold $CaCl_2$ solution.
5. Keep resuspended cells on ice for 10 minutes.
6. Centrifuge the cells 5 minutes at 2500 rpm at 4 °C.
7. Discard the supernatant and resuspend each pellet in 2 ml ice cold $CaCl_2$ solution.

B) Transformation of competent cells with DNA:

1. Transfer 100 ng of plasmid DNA in a volume of 25 µl into clean microcentrifuge tube and place it on ice.
2. Rapidly add 100 µl of competent cells immediately into microcentrifuge tubes containing the plasmid DNA. Gently swirl tubes to mix, then place them on ice for 10 minutes.
3. Store the remaining competent cells at -80 °C.
   Note: Competent cells should be used immediately after thawing. Remaining cells should be discarded rather than refroze it.
4. Using the thermomixer, heat shock the cells by placing the tubes into a 42 °C for 45 sec.
5. Add 1 ml LB medium (without antibiotic) → **Tube (A)**
6. Transfer (10-50µl) of **tube (A)** into clean microcentrifuge and complete the volume to 1000µl with 1 ml LB medium →**Tube (B).**
7. Place **tube (B)** on the thermomixer at 250 rpm for 20-60 minutes at 37°C.
8. Plate aliquot of transformation culture on LB/Ampicillin or other appropriate antibiotic containing plates.
9. When plates are dry, incubate 24 to 48 hours at 37 °C.
10. A negative control should also be included that contains cells with no added plasmid DNA.
11. Calculate the transformation efficiency.

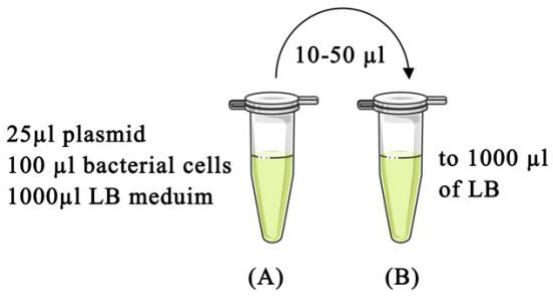

Figure (1) Schematic representation of sample dilution

Results:

Transformation efficiency calculation:

Count white colonies as recombinant transformants and test for insert. Calculate the transformation efficiency in terms of the number of colony-forming units (CFU) per microgram of transforming DNA as follows:

Transformation efficiency $= \frac{\text{Total number of colonies}}{\text{Amount of DNA plated (µg)}}$ X Dilution Factor

= _____ CFU/ µg