**Lab# 2 BCH 462**

**Competent Cells Formation and Transformation of Competent Cells with Plasmid DNA.**

**Experimental protocol:**

**A] Competent cells formation:**

1. Centrifuge cells 7 minutes at 3000 rpm at 4°C. allow centrifuge to decelerate without break.

2. Pour off supernatant and resuspend each pellet in 10 ml ice-cold CaCl2 solution.

3. Centrifuge cells 5 minutes at 2500 rpm at 4 °C discard supernatant and resuspend each pellet in 10 ml ice-cold CaCl2 solution. Keep resuspended cells on ice for 10 minutes.

4. Centrifuge cells 5 minutes at 2500 rpm at 4 °C discard supernatant and resuspend each pellet in 2 ml ice-cold CaCl2 solution.

**B] Transformation of competent cells with DNA :**

1. Aliquot 100 ng of DNA plasmid in a volume of 25 µl in to a eppendorf tube and place it on ice.

2. Rapidly add 100 µl of competent cells immediately in to eppendorf tubes containing the DNA plasmid. Gently swirl tubes to mix, then place on ice for 10 minutes. Competent cells should be used immediately after thawing. Remaining cells should be discarded rather than refroze it.

3. Heat shock cells by placing tubes in to a 42 °C water bath for 45 sec.

4.Add 1 ml LB medium (without antibiotic). Place each tube on a roller drum at 250 rpm for 30 minutes at 37°C.

5. Plate aliquot of transformation culture on LB/Ampicillin or other appropriate antibiotic containing plates. When plates are dry, incubate 24 to 48 hours at 37 °C.

6. A negative control should also be included that contains cells with no added DNA.

7. calculate the transformation efficiency.

 **C] Transformation efficiency calculation:**

Transformation efficiency=total number of colonies on LB/Amp plate CFU/µg

 amount of DNA plated [µg/ml]