**Competent Cells Formation and Transformation of Competent Cells with 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 20 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 in a volume of 25 µl in to a sterile 15 ml round – bottom test tube ad place on ice.

2. Rapidly thaw competent cells by worming between hands and dispense 100 µl immediately in to test tubes containing DNA. 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) to each tube . Place each tube on a roller drum at 250 rpm for 45 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 number of transformed colonies per aliquot volume [µl] x 105 : this is equal to the number of transformants per microgram of DNA.

**C] Transformation efficiency calculation:**

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

amount of DNA plated [µg/ml]