Genetic Engineering Laboratory Protocol

Preparation of Competent Cells

Aim: Preparation of Competent Cells using *E.coli* DH5α cells

Principle: The ability of taking the DNA by a bacterial cell is called Competence. *E.coli* cells can be made competent chemically by adding $CaCl_2$ (or) $MgCl_2$. These cells are able to take foreign DNA (recombinant plasmids/ amplicons). The bacterial cell surface is negatively charged and the DNA molecule is also negatively charged as a result, the bacteria cannot take the foreign DNA in to it. The addition of $CaCl_2$ the net charge (or) the charge of the cell wall and the foreign DNA is neutralized, and promotes the opening of Ca^{2+} ion channels present on the surface of bacterial cells. Thus promotes the easy entry of foreign DNA inside the bacterial cell.

Requirements: *E.coli* DH5α cells, Luria Bertani medium, $CaCl_2$ (0.1M, filter sterile).

L.B medium composition:

1. a. Bactotryptone: 10.0 g /L
   b. Yeast Extract: 5.0 g/l
   c. Sodium Chloride: 10.0 g/l.
   pH: 7.0 ± 0.2 (Make up the pH by NaOH (or) HCl.

2. One can use readymade L.B medium for preparing L.B Broth (for preparing the media follow the instruction of manufacturer)

Procedure:

1. **Day 1:** Inoculate single colony of *E.coli* DH5α cells to 3.0 ml of autoclaved L.B broth in test tube and incubate at 37°C, 180 rpm for 12-16 hours.
2. **Day 2:** 500 µL of overnight culture inoculate to 25 ml of Autoclaved L.B broth in conical flask and incubate at 37°C, 180 rpm for 2-3 hours (or) till log phase is attained (or) the cell O.D reaches to 0.4-0.6.

3. After O.D reaches to 0.4-0.6 , take out the flask from incubator and keep in ICE for 10 min
4. Transfer the above pre cooled E.coli DH5α cells in to 50 ml centrifuge tubes and do centrifugation at 3,500 rpm for 15 min at 4°C.
5. Decant the supernatant without disturbing the pellet. Now resuspend the above pellet in 3.0 ml of $CaCl_2$ (0.1M).
6. Keep the above mixture on ice for 45 min. Centrifuge the above suspension at 3,500 rpm, for 10 min at 4°C.
7. Decant the supernatant and resuspend in 1 ml of 20% Glycerol solution prepared in 0.1M $CaCl_2$ .Aliquot 200 µL of competent cells into five sterile centrifuge tubes (sterile) and store the above competent cells at -80°C, for transformation.