**OBJECTIVE**: Preparation of competent cells from *E. coli* DH5α cells

**Chemicals required**: $MgCl_2$, $CaCl_2$, glycerol, luria agar (HiMedia), luria broth (HiMedia), ddH2O/MilliQ.

**Plastic ware/ glassware**: measuring cylinder, autoclaved micro centrifuge tubes, 1.0 ml tips, 0.02ml-0.2 ml tips, enzyme tips, 100ml/250 ml flasks, reagent bottles, Test tubes, OakRidge tubes, Uvettes.

**Equipment required**: refrigerated centrifuge, vortex, UV spectrophotometer,

**Recipe for solutions:**

**Luria broth**: 2.0 g in 100 ml (autoclaved)

**Solution I (100 ml)**

| S.No. | Chemical(s) | Concentration | Weight |
|---|---|---|---|
| 1. | $MgCl_2$ | 80 mM | 1.642 g |
| 2 | $CaCl_2$ | 20 mM | 0.294 g |

**Solution II (100 ml)**

| S.No. | Chemical(s) | Concentration | Weight |
|---|---|---|---|
| 1. | $CaCl_2$ | 0.1 M | 1.4702 g |

**Solution III (100 ml)**

| S.No. | Chemical(s) | Concentration | Weight |
|---|---|---|---|
| 1. | $CaCl_2$ | 0.1 M | 1.4702 g |
| 2 | Glycerol | 15% | |

Solutions were prepared and autoclaved

**PROTOCOL:**

Inoculate a single colony of *E. coli* DH5α in 5ml LB (primary inoculum) and incubate at 37°C, 180 rpm overnight.

Transfer 1 percent of primary inoculum in 100 ml LB and grow at 37°C, 180 rpm until $O.D_{600nm}$ reaches ~0.5-0.6.

After attaining appropriate absorbance keep the culture on ice for 20 min.

Transfer the culture in pre-chilled OakRidge tube and harvest cells at 4000 rpm for 10 min, 4°C.

Discard supernatant and resuspend the pellet in 30 ml of chilled Solution I

Recover cells by centrifugation at 4,000 rpm for 10 min at 4°C.

Discard supernatant and resuspend the pellet in 2-4ml of solution II.

Recover cells by centrifugation at 4,000 rpm, for 10 min at 4°C.

Discard supernatant without disturbing pellet, and resuspend in 0.8 ml-1.0 ml of solution III.

Prepare aliquots of 100µl and store at -80°C.