Transformation of Escherichia coli DH5 and Top 10

Protocol for Chemically Competent E. coli DH5a Cells Transformation

Materials:

- · DNA
- · Chemically Competent E. coli DH5a Cells or Top 10 Cells
- · SOC media (preheated at 37°C)
- · LB plates with the respective antibiotic

Transformation:

- 1. Place competent cells in ice and add 1μ L of DNA (if it is a ligation add 3μ L).
- 2. Incubate at 4°C for 30 minutes and preheat the heat block at 42°C.
- 3. Place cells in the heat block at 42°C for 90 seconds.
- 4. Incubate in ice for 10 minutes.
- 5. Add $200\mu L$ of preheated SOC and incubate at $37^{\circ}C$ and 180rpm for 2 hours. LB can be used if SOC media is not available.
- 6. Centrifuge cells at 6000 rpms for 2 minutes.
- 7. Discard 100µL of supernatant and resuspend pellet.
- 8. Add 100μ L of cells to the plate with the respective antibiotic and spread with the Drigalski spatula.
- 9. Incubate overnight at 37°C.