TRANSFORMATION OF *E. coli*

Before starting: pull LB pates with appropriate antibiotic out of the refrigerator to warm to room temperature and heat up water bath to 42ºC.

1. Thaw $DH_{5\alpha}$ competent cells on ice for about 10 minutes.

   (# of desired transformations + controls) x 100 µl = total µl of cells needed

2. In an ice bucket chill labeled 1.5 ml eppendorf tubes.

3. Aliquot 100 µl of thawed competent cells to chilled eppendorf tubes with wide tip pipette tips (blue tips)

4. Add 1 to 3 µl of plasmid DNA or ligation reaction to appropriate tube of cells and stir with tip gently. Keep tubes on ice for 30 minutes.

5. Heat shock the cells for 60 seconds in a water bath at exactly 42ºC.

6. Gently transfer cells to ice for at least 1.5 minutes

7. Add 400 µl to 900 µl liquid LB to each tubes. Shake the tubes for 1 hour at 200 rpm and at 37ºC. For Ampicillin resistant cells this 1 hour incubation step can be skipped.

8. Plate 50 µl and 200 µl of transformed bacteria by hockey-stick method. Plate negative controls first.

9. Incubate the plates in 37ºC incubator for 14-16 hours.

Making plasmid glycerol stocks: Prior to plasmid DNA extraction, add equal amounts of 80% glycerol and bacterial cells in a 1.5 ml eppendorf tube and store in -80ºC.