Day 2: April 5, 2005- Bacterial transformation

Today you will transform your recombinant DNA construct into *E. coli* so that it can be amplified. We will use JM109 Competent cells (Promega, see product info on class website for more information). They have been made competent for transformation using the method of Hanahan 1983 (J. Mol Biol. 166:557-580).

1. Chill polypropylene culture tubes on ice.

2. Thaw frozen competent cells on ice (do not let cells warm up).

3. Gently mix the thawed competent cells by flicking the tube. Transfer 100 µl to each of the chilled tubes (pipette carefully as each group only has 3 tubes of competent cells, each with 200 µL of cells). Keep these tubes on ice.

4. For your ligations: Add 2 µL of each of your 4 ligations to an aliquot of competent cells.

5. Control 1- For the competent cell DNA control reaction, add 1 µl (0.1ng) of competent cell control DNA to an aliquot of the competent cells. Quickly flick the tubes a few times to mix and return to ice. You will use this reaction to calculate the transformation efficiency of the competent cells (see below).

6. Control 2- As an additional control, you will use 1 µl of the cDNA construct (plasmid miniprep of pGem7 and insert #2 or #4) that I supply to you. Each group should have 6 different transformation reactions.

7. Incubate the tubes (bacterial cells + DNA) on ice for 10 min.

8. Heat shock the cells for 45 sec in a water bath at 42 º C (Do not shake).

9. Immediately place the tubes on ice for 2 min.

10. Add 900 µl of cold (4º C) SOC or LB (no antibiotics) to each transformation reaction. Incubate for 60 min at 37 º C with shaking.

11. Label all of your LB plates on the bottom.

12. For each transformation reaction, dilute some of the cells 1:10. Plate 100 µl of undiluted and 100 µl of each diluted (1:10) transformation reaction on LB amp, X-gal, IPTG plates. Plate 100 µl of only the diluted (1:10) control #1 reaction.

(LB plates contain 50 µg/ ml ampicillin, 40 µg/ ml X-gal, 0.5 mM IPTG)

13. Incubate the plates overnight at 37 º C. Plates should be upside down.

14. The TA will take your plates out of the incubator and put them in the refrigerator on the 6th (AM).

Reminder: we will need to start the liquid cultures on Wed April 6th.