$CaCl_2$ Transformation of *E. coli*

1. Remove an aliquot of *E. coli* cells from the –80^{o}C and thaw on ice.

2. Mix ~ 5µl of DNA with 50µl of cells.

3. Incubate on ice 2 – 10 min (or more).

4. Transfer to water bath at 42^{o}C for 90 sec.

5. Remove to ice.

6. Add 1 ml SOC or LB.

7. Incubate at 37^{o}C for 1h with gentle shaking/agitation.

8. Spin cells down in microfuge.

9. Resuspend in 200µl SOC or LB.

10. Plate serial dilutions of the transformed cells; i.e., 10^{o}, 10^{-1}, 10^{-2} etc. onto antibiotic selection medium. Store half of the transformation mix (100µl) at 4^{o}C until you're confident all is well.

11. Incubate plates at 37^{o}C for ~ 18h.

Notes:

Discard unused *E. coli* cells (don't refreeze them).

Generally ~ 5 X 10^{6} to 2 X 10^{7} colony-forming units (cfu) can be expected per µg of supercoiled plasmid transforming DNA. (This number is based on transforming 50ng of DNA (in a volume of 10µl or less) into 200µl of cells. Transformation does not increase by adding more DNA. Thus if we transformed ~12.5ng DNA into 50µl of cells we should expect ~ 6 X 10^{4} to 3 X 10^{5} cfu. These volumes and numbers are not absolutes and results will vary. Closed circular DNA (i.e., an *in vitro* mutagenesis product) is approximately one order of magnitude less. Ligated products are yet another order of magnitude lower.