**Preparation of Competent Cells**

1. Inoculate 2ml of LB with a single DH5α colony. Incubate culture overnight at 37°C while shaking at 250 RPM.
2. The following morning, inoculate 500ml of LB with 1ml of saturated overnight culture. Incubate culture at 37°C while shaking at 250 RPM until OD600 = 0.5 (3-5 hours).
3. Transfer culture to 2 pre-chilled sterile 250ml centrifuge tubes. Pellet bacteria cells with a 5000 RPM spin for 10 minutes at 4°C. Discard supernatant. Place pellets on ice.
4. Resuspend cells in 10ml cold CaCl2 solution. Pool cells together into one pre-chilled 50ml Oakridge tube.
5. Pellet cells with a 2500 RPM spin for 5 minutes at 4°C. Discard supernatant and resuspend cells in 10ml cold CaCl2 solution. Set on ice 30 minutes.
6. Pellet cells with a 2500 RPM spin for 5 minutes at 4°C. Discard supernatant and resuspend cells in 2ml cold CaCl2 solution.
   *At this point you can leave cells on ice overnight at 4°C – this increases competency in some cases*
7. Dispense cells into 50ul aliquot in pre-chilled sterile polypropylene tubes. Store cells at −80°C.

**Test for Competency**

Transformation:

8. Remove competent DH5a cells from the −80°C and immediately place on ice. Once thawed, add >10ng of plasmid DNA to a 50ul aliquot of competent cells. Place cells/DNA on ice for 3 minutes.
9. Heat shock cells at 42°C for 3 minutes.
10. Place cells back on ice for 3 minutes.
11. Add 1ml LB to cells/DNA. Tape tube onto shaking incubator platform and incubate cells/DNA for 1 hour at 37°C while shaking at 250 RPM.
13. Plate 100ul and 200ul of transformation onto 2 LB+Amp plate. Place plates inverted at 37°C overnight.