Preparation of Competent Cells

1. Inoculate 2ml of LB with a single DH5α colony. Incubate culture overnight at 37oC while shaking at 250 RPM.
2. The following morning, inoculate 500ml of LB with 1ml of saturated overnight culture. Incubate culture at 37oC while shaking at 250RPM 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 4oC. 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 4oC. 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 4oC. Discard supernatant and resuspend cells in 2ml cold CaCl2 solution.

   At this point you can leave cells on ice overnight at 4oC – this increases competency in some cases
7. Dispense cells into 50ul aliquot in pre-chilled sterile polypropylene tubes. Store cells at –80oC.

Test for Competency

Transformation:

8. Remove competent DH5a cells from the –80oC 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 42oC 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 37oC while shaking at 250 RPM.
12. Pellet cells with a quick spin. Remove 800ul of supernatant. Resuspend cells in the remaining supernatant.
13. Plate 100ul and 200ul of transformation onto 2 LB+Amp plate. Place plates inverted at 37oC overnight.