Preparation of Electrocompetent cells

1. Inoculate single colony of DH5α into 50 ml No Salt LB medium and culture at 37˚C O/N.
2. Add 25 ml culture medium into 1 L of pre-incubated No Salt LB medium to 37˚C.
3. Grow the cells at 37˚C at approximately 200 rpm.
4. Grow cells to an A_{600} of 0.6~7.5.

1. Preparation for cell stock

1. Keep EtOH (>500 ml) at –20˚C
2. Chill four 250 ml tubes, microcentrifuge tubes, tips and vortex mixer at 4˚C
3. Prepare two ice boxes A(ice/water/NaCl) and B(dry ice/EtOH)
4. Set up the centrifugation machine at 8,000 rpm at 0˚C
5. Transfer culture to box A and chill completely for 10 min

2. Cell Stock

1. Transfer of chilled culture into two 250 ml tubes
2. Centrifuge at 8,000 rpm for 10 min at 0˚C and remove the supernatant carefully and vortex pellets mildly.
3. Resuspend all four pellets in a total volume of 200 ml cold 10% glycerol-dH_2O. Combine all resuspended cells in one 250 ml bottle.
4. Centrifuge at 8,000 rpm for 10 min at 0˚C and remove the supernatant carefully and vortex pellets mildly.
5. Resuspend pellets in 150 ml cold 10% glycerol-dH_2O by vortex mildly.
6. Centrifuge at 8,000 rpm for 10 min at 0˚C and remove the supernatant carefully and vortex pellets mildly.
7. Resuspend pellets in 100 ml cold 10% glycerol-dH_2O by vortex mildly.
8. Centrifuge at 8,000 rpm for 10 min at 0˚C and remove the supernatant carefully and vortex pellets mildly.
9. Resuspend pellets in 2 ml cold 10% glycerol-dH_2O with 1 ml pipetteman.
10. Transfer 100 μl aliquot into 1.5 ml microcentrifuge tubes
11. Freeze immediately in box B for 5 min
12. Transfer tubes to deep freezer and freeze overnight at –80˚C

Electro-Transformation

(using Bio-Rad Electroporator)

-Note: These are high efficiency cells. 1 pg of pUC19 yielded>1000 colonies when 10% was plated. Be warned and use good judgment when determining how much DNA to

transform with.

1. Thaw one tube of cells on ice. Keep cells cold until electroporation.
2. Dispense 50 µl homogenous cell prep to a chilled microcentrifuge tube on ice.
3. Add 1 µl DNA solution and mix gently.
4. Set Bio-Rad electroporator to 1.8 kV, 200 Ω resistance, and 25 µF capacitance.
5. Transfer the cell and DNA mixture to a chilled 0.2 cm electroporation cuvette sterilized with 70% EtOH..
6. Have 1 ml SOC at r.t. ready in a pipette before electroporation of the sample.
7. Apply one pulse by pressing and holding both buttons simultaneously until the readout flashes ‘Ch9’ several times and a beep is heard.
8. Add I ml SOC immediately and mix by pipetting several times.
9. Transfer the electroporated sample to a microcentrifuge tube.
10. Incubate for 1 h at 37˚C with agitation at about 250 rpm. Pre-incubate LB agar plate with appropriate antibiotics at 37˚C.
11. Add variable volumes of your transformation mix to LB agar plates and spread the plates.
12. Incubate plates 12~16 h at 37˚C