Making Competent Cells

Day 1:

1. Grow 5 mL LB culture of cells overnight at 37 ºC
   For XL1-Blue add 12.5 µg/mL Tetracycline

Day 2:

1. Dilute overnight culture into LB
   For XL1-Blue – 1 mL of culture into 500 mL LB + 12.5 µg/mL Tet + 15 mM $MgCl_2$
   For DH5α – 2 mL of culture into 120 mL LB
2. Grow cells at 37 ºC until OD_{600} = 0.4
3. Pellet cells at 3000 rpm for 15 min at 4 ºC
4. Resuspend pellet in 150 mL cold MES A
5. Incubate on ice 20 min
6. Spin down at 3000 rpm for 15 min at 4 ºC
7. Resuspend pellet in 30 mL of cold MES A + 15 % Glycerol
8. Aliquot (200-300 µL) and snap freeze in liquid nitrogen or dry ice ethanol bath
9. Transform cells – 100 µL for ligation or 50 µL for plasmid:
   a. Add DNA – 10 µL of 20 µL ligation or 0.5 – 1.0 µL plasmid
   b. Incubate on ice for 2 min
   c. Heat shock 2 min at 42 ºC
   d. Incubate on ice for 2 min
   e. Add prewarmed LB - 100 µL for ligation or 950 µL for plasmid
   f. Plate - All 200 µL for ligation or 50 µL for plasmid

MES A (500 mL)

100 mL - 50 mM MES Salts pH 6.3 with KOH
2.77 g – $CaCl_2$-$2H_2O$
5 mL – 1M $MnCl_2$-$4H_2O$
dH_2O to 500 mL

Make fresh, filter sterilize, wrap in foil to protect from light, and store at 4 ºC.