**Preparation of electrocompetent cells** Entered by Cheryl Borgman
Janes Lab Protocols 8/19/18

Protocol adapted from *Molecular Cloning, A Laboratory Manual 4th edition* by Green and Sambrook from CSH Press. 2012

1. Inoculate a single colony of the specific *E. coli* strain from a fresh agar plate into a flask containing 50 mL LB medium. Incubate the culture overnight at 37°C with vigorous aeration (250 rpm).
2. Inoculate 500 mL of prewarmed LB medium in a 2 L flask with 25 mL of the overnight culture. Incubate the flasks at 37°C in a shaking incubator.
3. Measure the $OD_{600}$ of the growing culture at least every 20 min.
   - *The spectrophotometer is kept in the cabinet over bench #7.*
4. When $OD_{600}$ is approaching 0.4, rapidly transfer the flask to an ice-water bath for 15–30 min. Swirl the culture occasionally to ensure that cooling occurs evenly. In preparation for the next step, place the centrifuge bottles in the ice-water bath.
   - *It is critical that the temperature of the bacteria be maintained at 4°C.*
5. Transfer the culture to ice-cold centrifuge bottles.
   - *For best yield use conical centrifuge bottles. We use two 250 ml conical bottles.*
6. Centrifuge at 1000 rcf for 15 min at 4°C. Decant the supernatant and resuspend the cell pellet in equal volume (250 mL each) of pure ice-cold Milli-Q water by gently pipetting up and down.
7. Centrifuge at 1000 rcf for 20 min at 4°C. Decant the supernatant and resuspend the cell pellet in half volume (125 mL each) ice-cold 10% glycerol.
8. Centrifuge at 1000 rcf for 20 min at 4°C. Decant the supernatant and resuspend the cell pellet in 10 mL ice-cold 10% glycerol.
   - *Take care when decanting as bacterial pellets are not very adherent in 10% glycerol.*
9. Centrifuge at 1000 rcf for 20 min at 4°C. As soon as the centrifuge stops, carefully decant the supernatant and use a Pasteur pipette attached to a vacuum line to remove any remaining drops of buffer. Resuspend the pellet in 1 mL of ice-cold GYT medium.
   - *Resuspend by swirling rather than pipetting or vortexing.*
10. Measure the $OD_{600}$ of a 1:100 dilution of the cell suspension. Dilute the cell suspension to a concentration of $2 \times 10^{10}$ to $3 \times 10^{10}$ cells/mL (1.0 $OD_{600}$ = ~$2.5 \times 10^8$ cells/ml) with ice-cold GYT medium.
11. Dispense 50 μL aliquots of the cell suspension into sterile, ice-cold 1.5 mL microfuge tubes, drop them into a bath of $LN_2$ before transfer to a –80°C freezer.
12. Remove one aliquot and test the efficiency of the preparation using 10 pg and 50 pg of supercoiled plasmid DNA (20 uL cells/concentration). Expect the efficiency of transformation of the preparation to be ~$10^9$ colonies/mg of plasmid DNA and the number of transformants should be proportional to DNA concentration.

**Buffer recipes**

- GYT medium (for 100 mL total volume):
  10 ml of Glycerol (final conc: 10% v/v)
  0.125 g of Yeast Extract (final conc: 0.125% w/v)
  0.25 g of Tryptone (final conc: 0.25% w/v)
  Bring total volume to 100 mL with MilliQ water
  Sterilize the medium by passing through a pre-rinsed 0.22 µm filter. Store in 2.5 mL aliquots at 4°C.

- SOB medium (for 1 L final volume):
  Start with 950 ml Milli-Q water
  Add 20 g Tryptone
  5 g Yeast Extract
  0.5 g NaCl
  Dissolve completely, then add
  10 ml of a 250 mM solution of KCl (Dissolve 1.86 g of KCl in 100 mL Milli-Q water)
  Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL)
  Bring volume to 1 L with Milli-Q water
  Sterilize by autoclaving
  Just before use, add 5 mL of sterile 2 M $MgCl_2$ (final conc: 10 mM, see preparation below)

- 2 M $MgCl_2$ (for 100 mL final volume):
  90 mL Milli-Q water
  19 g $MgCl_2$ (40.66 g of $MgCl_2$-$8H_2O$)
  Bring total volume to 100 mL
  Sterilize by autoclaving

- SOC medium (for 1 L final volume):
  Start with 950 mL Milli-Q water
  Add 20 g Tryptone
  5 g Yeast Extract
  0.5 g NaCl
  Dissolve completely, then add
  10 ml of a 250 mM solution of KCl (Dissolve 1.86 g of KCl in 100 mL Milli-Q water)
  Adjust the pH to 7.0 with 5 N NaOH (~0.2 mL)
  Bring volume to 1 L with Milli-Q water
  Sterilize by autoclaving
  Once cooled, add
  20 mL 1 M Glucose (Dissolve 18 g Glucose in 90 mL Milli-Q water, bring total volume to 100 mL and sterilize the medium by passing through a pre-rinsed 0.22 µm filter)

- LB medium (for 1 L total volume):
  To 950 mL Milli-Q water, add
  5 g of Yeast Extract
  10 g of Tryptone
  10 g NaCl (Janes Lab conc – CSH protocol recommends 5 g NaCl)
  pH to 7.0
  Bring total volume to 1 L with Milli-Q water
  Sterilize by autoclaving