
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<sub>600</sub> of the growing culture at least every 20 min.
   - The spectrophotometer is kept in the cabinet over bench #7.

4. When OD<sub>600</sub> 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<sub>600</sub> of a 1:100 dilution of the cell suspension. Dilute the cell suspension to a concentration of 2 x 10<sup>10</sup> to 3 x 10<sup>10</sup> cells/mL (1.0 OD<sub>600</sub> = ~2.5 x 10<sup>8</sup> 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<sub>2</sub> 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 µL cells/concentration). Expect the efficiency of transformation of the preparation to be ~10<sup>9</sup> 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 MgCl₂ (final conc: 10 mM, see preparation below)

- **2 M MgCl₂** (for 100 mL final volume):
  
  90 mL Milli-Q water
  19 g MgCl₂ (40.66 g of MgCl₂·8H₂O)

  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