Competent Cell Prep: This procedure is used to create competent cells to use for transformation.

Materials/Reagents: *Everything needs to be chilled overnight at 4C

- LB Broth
- 900 mL 100mM $ClCl_2$
- 900 mL 100mM $MgCl_2$
- 100 mL 85mM $CaCl_2$ with15% glycerol
- Autoclaved H2O
- Microfuge tubes
- Centrifuge tubes
- Autoclaved Flasks

Protocol:

Day 1

1. Prepare & Autoclave:
    a. LB media
    b. 900 mL 100mM CaCl2
    c. 900 mL 100mM MgCl2
    d. 100 mL 85mM CaCl2 with 15% glycerol v/v
    e. microfuge tubes
2. Chill the following overnight at 4C
    a. the autoclaved 100mM CaCl2,
    b. 100mM MgCl2
    c. 85mM CaCl2 with 15% glycerol
    d. Chill the conical tubes for centrifugation x20
    e. Eppendorf Tubes for dilutions and storing
3. Create a starter culture of the desired *E. coli* cells for competent cell assay
    a. Select a single colony of *E. coli* from a fresh LB plate, inoculate in 15 mL LB, and grow overnight in 37C shaker 125 mL flask
        i. Strains Used: BL21

Day 2

1. Inoculate 1 L of LB media with 10 mL starter culture (made on Thursday) and grow in 37°C shaker.
2. Using a spectrophotometer measure the OD600 every hour, then every 15-20 minutes when the OD gets above 0.2. Check OD600 0.35-0.4 (~3 hours)
3. Split the media into 20 parts (50mL in each of the conical tubes that were chilled the previous day) and place on ice to chill for 20-30min to stop excess growth. Make sure to occasionally swirl to ensure even cooling.
4. ***Spin #I:*** Harvest the cells by centrifugation at 3000g (~4000 rpm) for 15 minutes at 4°C.

5. Decant the supernatant and resuspend the pellet in 5mL of cold $MgCl_2$
6. ***Spin #2:*** Harvest the cells by centrifugation at 2000g (~3000 rpm) for 15 minutes at 4°C.
7. Decant the supernatant and resuspend the pellet in 10 mL ice cold $CaCl_2$. Keep on ice for at least 20 min. Place the 1.5 mL microfuge tubes on ice as well.
8. **Spin #3:** Harvest the cells by centrifugation at 2000g (~3000 rpm) for 15 minutes at 4°C.
9. Decant the supernatant and resuspend the pellet in ~2.5 mL of ice cold 85 mM CaCl2, 15% glycerol. Transfer the suspensions into one 50 mL conical tube.
10. **Spin #4:** Harvest the cells by centrifugation at 1000g (~2100 rpm) for 15 minutes at 4°C
11. Decant the supernatant and resuspend the pellet in 1.8 mL of ice cold 85 mM CaCl2, 15% glycerol.
    a. The final OD600 of the suspended cells should be ~ 200-250.
12. Aliquot 50 µL into sterile 1.5 mL microfuge tubes and snap freeze with dry ice. Store frozen cells in the -80°C freezer.

Buffers/Solutions:

- 900 mL 100mM CaCl2
  - Add 13.23 g of CaCl2 *2H2O to ~700 mL of DI water, mix, fill to 900 mL, autoclave
- 900 mL 100mM MgCl2
  - Add 18.27 g of MgCl2*6H20 to ~700 mL of DI water, mix, fill to 900 mL, autoclave
- 100 mL 85mM CaCl2 with 15% glycerol v/v
  - Mix 8.5 mL of 1M CaCl2, 30 mL of 50% Glycerol and 61.5 mL of dH20

References:

http://mcb.berkeley.edu/labs/krantz/protocols/calcium_comp_cells.pdf