Preparing competent cells using CaCl2

Buffer:
60 mM CaCl2, 10 mM PIPES, 15% v/v glycerol, pH 7.0
For 500 mL: 4.4 g CaCl2, 1.73 g PIPES, 75 mL glycerol
(PIPES won't dissolve until you adjust the pH- put pH meter in undissolved solution and add NaOH to indicated pH, let stir more, then adjust pH again)

1 plate of LB, no antibiotics: 0.45 g Bacto Agar in 30 mL LB (autoclave)
200 mL LB (autoclave)

Autoclave buffer, place in ice water bath and let cool before use

For cells containing pLysS, add chloramphenicol to all media- otherwise, use no antibiotics, use good sterile technique

Day 1:
1. Streak cells onto plain LB plate (no antibiotics) and incubate at 37°C overnight

Day 2:
2. Evening- pick a single colony to inoculate 30 mL plain LB (no antibiotics) and incubate at 37°C overnight with shaking

Day 3:
3. Use 10 mL of overnight culture to inoculate 200 mL of plain LB (no antibiotics), grow at 37°C with shaking until OD at 600 nm reaches ~0.6 (check OD at 1 hour)
4. Pre-chill four 50 mL tubes in ice water bath
5. Once OD is reached, pour cells into pre-chilled tubes and incubate at 0°C for 15 minutes
6. Spin in large tabletop centrifuge at 4°C, 4000 rpm, 10 minutes
7. Resuspend each cell pellet with 15 mL sterile ice cold CaCl2 buffer by gentle pipetting (do not vortex)
8. Incubate cells in ice water bath 15 minutes
9. Spin cells again at 4°C, 4000 rpm, 10 minutes
10. Resuspend each cell pellet in 4 mL of sterile ice cold CaCl2 buffer (do not vortex)
11. Aliquot cells into 100 µL aliquots in 1.5 mL tubes
12. Let cells incubate on ice for 1 hour
13. Place tubes in racks and freeze in -80°C