Preparation of Competent Cells

**Microbiology**

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* [Protocol](https://protocols.scienceexchange.com/protocols/preparation-of-competent-cells)
* [Discussion](https://protocols.scienceexchange.com/protocols/preparation-of-competent-cells/discussion)

**Time required:**

* Day 1: Overnight
* Day 2: Overnight
* Day 3: 4 hours to grow culture
* 2 hours to prepare the competent cells

Procedure:

**Day 1**

1. Streak out the E.coli strain on an LBM plate (no ampicillin!) to isolate colonies and incubate at 37 degrees C overnight (16-20 hours).

**Day 2** 1. Use a sterile inoculating loop to collect cells from a single colony and inoculate 50 ml sterile 1X LBM Grow at 37 degrees C overnight (16-20 hours) in a shaker incubator. Also place 2 flasks of 250 ml 1X LBM in the incubator to equilibrate the temperature of the medium.

**Day 3**

1. Add 25 ml of the overnight culture to each 250 ml LBM flask. Place another flask of 150 ml 1X LBM in the incubator to equilibrate the temperature of the medium. Grow the cultures to OD650 = O.2. (not dense approximately 3 hours). Add 75 ml of equilibrated 1X LBM to each flask and continue incubating for 30 minutes.
2. Pellet the cells in chilled autoclaved large centrifuge bottles using the Beckman J-6 centrifuge and JA 10 rotor (must be cold!) at 5000 rpm for 10 minutes. Subsequent resuspensions may be done in the same bottle. Cells must remain cold for the rest of the procedure: Transport tubes on ice and resuspend on ice in the cold room.
3. Decant supernatant and resuspend the cells in 1/4 original volume (87.5 ml) ice cold 100 mM MgCl2. Hold on ice for 5 minutes. Transfer the cells to pre-chilled sterile large centrifuge bottles. Spin in the Beckman J- 6 centrifuge for 10 minutes using the JA-20 rotor 4000 rpm at 4 degrees C.
4. Decant the supernatant and resuspend the cells in 1/20 original volume (17.5 ml) of ice cold 100 mM CaCl2. Hold on ice for 20 minutes. Pellet as above 4000 rpm for 10 minutes.
5. Decant the supernatant and resuspend the cell pellet in 1/100 original volume (3.5 ml) of a solution that is 85% v/v 100 mM CaCl2 and 15% v/v glycerol (100%). For each culture processed chill approximately 15 labeled eppendorf tubes in a dry ice-EtOH bath. Pipet 300 ul cells into each tube and place immediately into the dry ice-EtOH bath. Transfer the frozen competent cell aliquots to -80 degrees C.
6. After the competent cells have been stored for 24 hours check the efficiency of transformation: Use 1 ng 10 ng and 100 ng of any ampicillin resistant plasmid on LBM + Amp plates as per transformation protocol for intact plasmids. Check the background level by plating 50 ul of cells alone on an LBM + Amp plate. Expect yields to be approximately 5x10e7 colonies per ug of supercoiled DNA.

Solutions:

1. 100 mM MgCl2:
   * 1:10 dilution of lab stock; use sterile ingredients or filter
   * sterilize
2. 100 mM CaCl2:
   * 1:10 dilution of lab stock; use sterile ingredients or filter
   * sterilize
3. 85% 100 mM CaCl2, 15% glycerol:
   * 42.5 ml 100 mM CaCl2
   * 7.5 ml 100% glycerol
   * 50.0 ml total volume; mix well and use sterile ingredients or filter sterilize

Precautions:

Plasmid/cosmid DNA should be considered biohazards and wastes should be disposed of appropriately.