

BISHOP LAB (Modified from Red Book Protocol)

PRODUCTION AND TRANSFORMATION OF ELECTROCOMPETENT E. COLI

See “*Helpful Hints*” at the bottom of the protocol

PREPARING E. COLI

1. Inoculate a single colony of *E. coli* into 5mL LB (set up 2-4 overnight cultures)
2. Grow overnight, shaking at 37°C
3. Inoculate 5mL overnight culture into 1L LB in a 2L flask (set up **AT LEAST** 2L culture)
4. Grow at 37°C, shaking at 250-300rpm, until density reaches OD₆₀₀ of 0.5-0.6

GO TO THE COLD ROOM – ALL STEPS NOW ARE AT 4°C

5. In the cold room, chill cells (in flask) in an ice-water bath for 10-15 minutes
6. Transfer cultures into pre-chilled 250mL centrifuge bottles (4 bottles per 1L culture)
7. Spin cells in the Sorvall for 5 minutes at 5000rpm at 4°C
8. Pour off supernatant and resuspend the pellet in 250mL sterile ice-cold H₂O
9. Spin as in step 7
10. Repeat wash and spin (steps 8 and 9)
11. Pour off supernatant and resuspend the pellet by swirling in remaining liquid (at this step, pellets should be combined so that there is 1 pellet per 500mL culture)
12. Add 40mL ice-cold 10% glycerol to the pellet and mix well
13. Spin cells for 10 minutes at 5000rpm at 4°C
14. Pour off supernatant
15. Estimate the pellet volume and add an equal volume of ice-cold 10% glycerol to resuspend the cells (Note: there is usually enough liquid left in the bottle to resuspend the pellet to determine the volume)
16. In the cold room, aliquot 50μL cells per pre-chilled microcentrifuge tubes on ice and freeze tubes on dry ice (not liquid nitrogen)
17. Store aliquots at –80°C
18. Test cells for transformation efficiency and contamination immediately

HELPFUL HINTS:

1. It takes almost the same amount of work to make 2-4L worth of competent cells as it does to make 1L. Be a good lab citizen and make a large stock of competent cells.
2. To maximize competency, all reagents and equipment must be pre-chilled to 4°C or lower before use. Place several bottles of autoclaved H₂O, 10% glycerol (filter sterilized), and centrifuge bottles in the –20°C freezer at the start of the day when making competent cells. The water may freeze, but can be thawed right before use.
3. This protocol works best with 250mL centrifuge bottles, because the pellets are most stable in these particular bottles. If the cell pellet is very loose in step 8, do the following wash step with 1mM HEPES in ice-cold water.
4. Set up a workstation in the cold room to aliquot cells prior to the last spin, so that the competent cells can be frozen as quickly as possible, including an ice bucket with dry ice/ethanol to freeze the cells and a bucket with wet ice to aliquot the cells.
5. **IMPORTANT NOTE:** If you are making competent cells from a stock carrying a plasmid (i.e. BLR pLysS, BL21 pBirA, etc.) you **MUST** grow your cells on plates and in all liquid cultures to select for the plasmid. Otherwise, the plasmid will be lost and your competent cells will be useless.

ELECTROPORATION

1. Settings for Bishop Lab electroporator (BioRad Gene Pulser) for *E. coli* transformation:
 - a. Resistance: 200 ohms
 - b. Capacitance: 25 μ FD (do not attach capacitance extender)
 - c. Volts for brown-capped cuvettes: 1.8kV (use 0.1cm electrode)
 - d. Volts for green-capped cuvettes: 2.5kV (use 0.2cm electrode)
2. Add 5pg to 0.5 μ g plasmid DNA in 1 μ L to tubes containing fresh or thawed cells (on ice)
Note: Adding too much DNA (up to 1/10 the cell volume) will decrease transformation efficiency 2- to 3-fold. Also, resistance should be high, so addition of DNA should not increase the total salt concentration in cuvette >1mM
3. Mix by tapping tube or swirling the cells with pipet tip
4. Transfer the DNA and cells into chilled cuvette (5 minutes on ice)
5. Shake slightly to settle cells to the bottom and wipe ice and water from the cuvette
6. Place cuvette into sample chamber and apply the pulse
7. Remove the cuvette and immediately add 1M SOC medium
8. Transfer to a sterile culture tube with a Pasteur pipet
9. Incubate 30 to 60 minutes with moderate shaking at 37°C
10. Plate aliquots of the transformation culture on LB plates containing appropriate antibiotics

LB Media

10g Bacto tryptone
5 g Yeast extract
5g NaCl

Dissolve ingredients in 1L dH₂O
Adjust pH to 7-7.5 with NaOH
Autoclave

SOC

10g Bacto tryptone
2.5g Yeast extract
1.8g Glucose
0.3g NaCl
0.625mL 2M KCl
1.017g MgCl₂
1.232g MgSO₄

Add dH₂O to 500mL
Mix well until all ingredients are dissolved
Aliquot into individual bottles
Autoclave