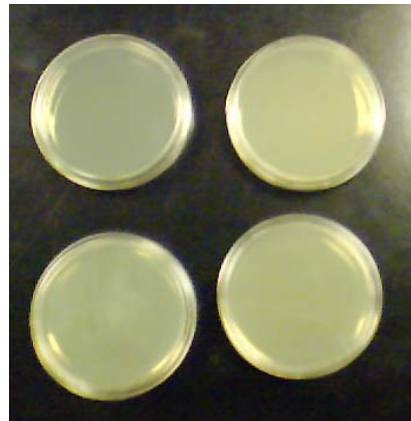


# Making Competent Cells

The following protocol will produce enough competent cell culture for a maximum of twenty transformations. As it was designed for execution during standard sixty-minute periods, some yield has been sacrificed in order to expedite the production of culture. Note that the “days” are suggestions only. The preparation of agar plates can be carried out any time from an hour before to many weeks preceding the transformation, as uncontaminated agar tends to remain intact. Because most schools lack freezing-apparatus capable of sustaining subzero temperatures, the production of competent cells is carried out the same day as the transformation. Normally, competent cells may be able to survive as long as a week in temperatures of  $-20^{\circ}\text{C}$  and much longer stored at  $-80^{\circ}\text{C}$ .

## Day 1

1. Prepare and pour of LB agar plates:



2. Using a sterile loop, streak with the *E. Coli* HB101 or K12 culture one of the LB plates. Incubate overnight at  $37^{\circ}\text{C}$ .



3. Add 50 mL of LB-Broth to a culture flask and autoclave.

## Day 2

4. Inoculate the LB-Broth with a colony of *E. Coli* from the LB control plate by lifting it with sterile loop.



5. Grow the liquid culture at 37 °C (if possible) shaking at 300 rpm overnight. (If the lab lacks a shaker, use a rocker.)



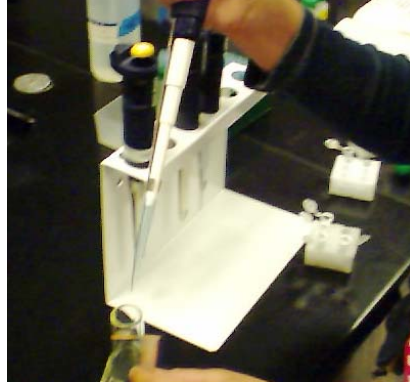
6. All containers must be chilled as close as possible to 4°C. So placing the rotor from a centrifuge into the refrigerator is important for the centrifuging steps. Also place the electroporation cuvettes, and 10% Glycerol and ddH<sub>2</sub>O into the refrigerator as well.

## Day 3

7. Place the liquid culture, ten 2 mL tubes, and one .2 cm electroporation cuvette on ice.



8. Transfer the culture to the ten 2 mL tubes.



9. After twenty minutes on the ice, transfer the cells to the centrifuge and spin them for five minutes at 6000 x g.



10. Carefully pour off (or aspirate if you have devise) all the supernatant. (It is better to sacrifice the yield by pouring off a few cells than to leave any supernatant.)



11. Gently resuspend the cell pellets in 2 mL of ice-cold ddH<sub>2</sub>O.



12. Centrifuge for five minutes at 6000 x g at 4 °C.

13. Carefully pour off all the supernatant.

14. Gently resuspend the cell pellets in 1 mL of ice-cold ddH<sub>2</sub>O

15. Centrifuge for five minutes at 6000 x g at 4 °C.

16. Carefully pour off all the supernatant.

17. Gently resuspend the cell pellets in approximately 800 µL of ice-cold 10% glycerol.

18. Aliquot into 80 ul into 1.5ml or 2ml cryogenic tubes.

19. Snap freeze using Dry Ice or Liquid Nitrogen

20. Freeze at -80 °C until transformation.

Yield: Ten 2 mL tubes filled with 80 µL each of competent cells; enough for two transformations each and twenty transformations total.



References:

Sambrook, et al *Molecular Cloning: A Laboratory Manual* 3<sup>rd</sup> Ed Cold Spring Harbor Press 2001

Leachman, Samuel Pictures of Procedure Added April 2004