Making Competent Cells

A. The Day Before

Rinse centrifuge bottles and pyrex bottles at least six times with MilliQ dH_20. If possible set aside designated bottles for making electrocompetent cells that will remain free of detergent and bleach.

Sterilize

- 500 ml of **low salt** media (5 g Tryptone, 2.5 g Yeast Extract, **2 g NaCl**) in a 2 L flask.
- 2 × 500 ml MilliQ dH_20 in rinsed bottles
- 400 ml 10% glycerol (360 ml dH_20 + 50 g glycerol)
- 500 ml Centrifuge bottle
- 0.5 ml microcentrifuge tubes

Refrigerate the bottles of dH_20 and 10% glycerol at 4°. Chill centrifuge bottles and about 20 labeled microcentrifuge tubes at –20°.

Start a 10 ml LB supplemented with 0.2% w/v glucose overnight culture from a colony of your favorite strain.

B. The Next Morning

- Add glucose to the 500 ml of media (e.g. 5 ml of 20% stock solution), and inoculate with the 5 ml of overnight culture. Grow at 37° to OD_{600}=0.7 – 0.8 (~3 to 3.5 hrs for a normal strain).
- Incubate the flask on ice for 15 min. From this point on all steps are performed on watery ice (i.e. have ice chunks floating in the water, but not a lot).
- Transfer the cells to the cold centrifuge bottle and spin for 20 min at 3,000 ×g (4°C).
- Pour off the supernatant; add *5-10* ml of cold water, and resuspend the pellet by swirling in an ice bath. When the pellet is completely dissolved, add cold water up to the top, invert gently several times to mix and centrifuge as before.
- Repeat.
- Pour off the supernatant; add 5-10 ml of cold 10% glycerol, and resuspend as before. Add remaining cold 10% glycerol, and centrifuge for 10min at 3,000 ×g (4°).
- Pour off the supernatant immediately, and resuspend the pellet by swirling in the remaining supernatant (only about 1 ml liquid + 0.5 ml pellet for "supercompetent" or 2 ml for subcloning efficiency).

- Aliquot 110 uL of the cell suspension to cold, sterile 1.5 ml tubes, and freeze immediately at –80°C. You should get 15-20 aliquots of 70-100 µl each. Store at –80°C.

C. Testing the Competency

Chill a 0.1 cm electroporation cuvette on ice. Add 1 µl of 0.01 ng/µl pUC18 (i.e. 10 pg) to a sterile microcentrifuge tube on ice. Thaw competent cells on ice for a few minutes. Add the cells to the tube containing pUC18, mix by pipetting once, and transfer to the cuvette. Pulse at 1.8 kV, 100 Ω, 50 µF. Wash twice with 0.75 ml SOC. Grow at 37° for 60 min (90 min for ElectroTen Blue).

Make dilutions of 1:10, 1:100 and 1:1000, and plate 100 µl to Amp plates. Grow overnight at 37°. Count the colonies and figure out the CFU/µg pUC18 (e.g. for the 1:100 plate, multiply the number of colonies by 1500 to get the number of transformants per 10 pg. Then multiply by 10^5 to get the CFU/µg. 100 colonies on the 1:1000 plate corresponds to 1.5×10^{11} CFU/µg).