Making Competent Cell Stock

1. **DAY 1**: Between 4 and 6 pm, streak an antibiotic free LB plate with competent cells. Use a sterile pipette tip and serially dilute the cells onto the plate. Place the plate at 30C overnight.
2. **DAY 2**: Between 10-12 pm the next day, remove the plate from the incubator and pick a single colony (2-3 mm diameter) and transfer to a sterile flask (250 ml) that contains 25 ml of SOB medium.
3. Place the flask in a shaking incubator set at 37C for 6-8 hours. Make sure the 18C shaking incubator is set up in the 4C room.
4. At 6 pm, remove the starter culture from the shaking incubator to prepare for the overnight cultures. Obtain 3 sterile 1 L flasks (if not available, choose the smaller flasks)
5. Each flask gets 250 ml of SOB (with $MgCl_2$).
6. Each flask will get a different volume of starter culture. Flask 1 gets 10 ml, flask 2 gets 4 ml, and flask 3 gets 2 ml.
7. Place all three flasks into a shaking incubator set to 18C in the 4C room to incubate overnight.
8. **DAY 3**: Take the OD_{600} of all three flasks, using base SOB medium to blank the spectrometer. Monitor the cultures every 45 minutes. When the target value is close, remove a 50 ml and 20 ml falcon tube of Inoue transformation buffer from -20C and allow to thaw on bench top. Once they are thawed, place on ice. Prepare an ice bath as well as turn on all centrifuges and allow them to reach temperature (4C). Prepare space in the 4C room.
9. Once a culture reaches 0.55, that culture is ready while the other two flasks can be discarded. Place the saved culture in an ice bath and incubate for 10 minutes.
10. Pour culture into a 500 ml centrifuge container and harvest cells by centrifugation at 2500g (rpf setting) for 10 minutes at 4C.
11. Pour off medium, store open container for 2 minutes on a stack of paper towels in 4C room. Aspirate excess medium from the container.
12. Add 50 ml of ice-cold Inoue Transformation Buffer to the dried pellet and gently resuspend through swishing while in the 4C room. A wide nosed pipette can be used to dislodge the pellet from the side of the container to promote re-suspension. Limit pipetting though to prevent cell shearing. This step could take up to 20 minutes, be patient.
13. Centrifuge cell suspension at 2500g (3900 rpm in a Sorvall GSA rotor) for 10 minutes at 4C.
14. Pour off the supernatant and allow the pellet to dry on a stack of paper towels for 2 minutes in 4C room.
15. Vacuum aspirate any excess liquid.
16. Resuspend cells in 20 ml of ice-cold Inoue Transformation Buffer. Follow the same method outlined in step 12. This step should also be conducted in 4C room.
17. Once re-suspended, add 1.5 ml of DMSO. Mix by swirling and incubate on ice for 10 minutes.
18. While incubating, make a liquid nitrogen bath. Also get another ice bath to store microtubes for aliquots. Find your aliquot buddy and prepare to work quickly to distribute cells.
19. Each microtube gets 50 µl of cell suspension. After adding cells, close the tube and drop into liquid nitrogen. About 430 aliquots can be made with this volume of cell suspension. Try to snap-freeze all of the cells as fast as possible.
20. Pour the tubes and liquid nitrogen into freezer boxes. Be careful when handling the boxes to dump out all liquid nitrogen. Place filled boxes into the -80C freezer for storage.

SOB Medium

1. Put 950 ml of d.d. H_2O into a 1 L sterile bottle.
2. Add 20 g of Tryptone
3. Add 5 g of Yeast Extract
4. Add 0.5 g of NaCl
5. Shake unitl the solutes have dissolved
6. Add 10 ml of a 250 mM KCl solution to the medium (see prep below)
7. Adjust the pH to 7.0 using 5 M NaOH.
8. Adjust the total volume to 1 L
9. Autoclave
10. Right before use, add 5 ml of 2 M $MgCl_2$ (sterile by autoclave, see prep below) to the 1 L bottle (or 0.05% of a smaller volume).

Note 1: 250 mM KCl solution: 1.86 g of KCl in total volume of 100 ml d.d. H_2O. Dissolve and use.

Note 2: 2 M $MgCl_2$ solution: 19 g $MgCl_2$ in total volume of 100 ml d.d. H_2O. Dissolve, autoclave, and use.

SOC Medium

1. Follow the same procedure above, including the addition of 2 M $MgCl_2$.
2. Make a 1 M glucose solution (See below) and sterilize through filtration.
3. Add 20 ul of this sterile solution to 1 ml of SOB medium (or 2%).

Note 3: Dissolve 6 g of glucose in 40 ml of d.d. H_2O. Adjust volume to 50 ml once glucose is dissolved. Sterilize through syringe powered filtration through a 0.22 μm filter.

Inoue Transformation Buffer

1. Prepare a 0.5 M PIPES solution. Dissolve 15.1 g PIPES in 80 ml d.d. H_2O. Adjust pH to 6.7 with 5 M KOH. Adjust volume to 100 ml. Sterilize through filtration through a 0.45 μm filter. Divide into 20 ml aliquots and store at -20C.
2. In a sterile bottle, collect 800 ml of d.d. H_2O.
3. Add 10.88 g of $MnCl_2*4H_2O$ (55mM concentration)
4. Add 2.2 g of $CaCl_2*2H_2O$ (15 mM concentration)
5. Add 18.65 g of KCl (250 mM concentration)
6. Add 20 ml of the PIPES solution from above.
7. Dissolve these solutes and adjust the total volume to 1 L with d.d. H_2O.

8. Sterilize by filtration through 0.45 μm filter. Divide into 50 ml and 20 ml aliquots and store at -20C.