BACTERIAL CELL TRANSFORMATION

This procedure is used to get DNA into bacteria. It requires fresh cells for optimal transformation – but it is quite reliable. Supercoiled DNA works much better than ligated DNA in transformations (why?). the important part of this protocol is to plan ahead, and once cells are in $CaCl_2$, to keep them ice-cold.

1. Grow a 5ml overnight culture of XL1-Blue in LB broth at 37 oC with vigorous shaking.

2. The next morning, add 50ul of this culture to a fresh tube of 5ml LB. Use the plastic 15ml snap top tubes. Again incubate at 37 o C with vigorous shaking, and grow to A_{600} = 0.6-0.7. This takes a couple of hours, and the suspension will begin to take on a silky appearance. Do not let this culture overgrow - the health of the cells declines with confluence. An alternative procedure is to start a culture of XL1-Blue cells from a recently streaked plate, (streaked on LB/tetracycline) and to grow for several hours.
you are starting here

3. Cool cells on ice for 10 min., then take the tops off the tubes and while using the green tube adapters, spin in Sorvall SS-34 rotor for 5 minutes, 4 o C, at 3,000 rpm.

4. Discard the supernatant and quickly put cells back on ice. [From here on, keep cells on ice as best you can, even when resuspending them in fresh buffers.].

5. Resuspend the cells in 2mls of ice cold (sterile) $CaCl_2$ (50mM) using your P-1000 Pipettman. Leave on ice for 15 minutes.

6. Centrifuge the cells as in #3 above. Discard the supernatant and resuspend cells in 500 ul $CaCl_2$. .

7. To transform these competent cells, put 100-200ul of the suspension in a **precooled** microfugetube. Add DNA ligation mixture to the cells, mixing gently with your pipette on ice. [For a positive control of transformation, use 1 ul of supercoiled plasmid pBS DNA from any miniprep.] . Close the tube and incubate for 20-30 minutes on ice. Prepare a beaker of water (tap water is fine) at 42o C. Heat shock the cells by placing the glass culture tube at 42o C for 2 minutes, and then back on ice. Add 0.5ml LB media and then incubate cells in the 37 oC water bath for 1 hour, making sure the tube is capped with Parafilm.

9. Spread the cells over one or two LB/Amp plates, invert in 37o C incubator for overnight. (spin out cells to concentrate by using a microfuge mid-speed for 30 seconds, and then resuspend in 50microliters ofLB and plate.

For 50 mM $CaCl_2$ use 0.73 gms of $CaCl_2$ - $2H_2O$ per 100 mls of water, and autoclave