DETERMINING INTRACELLULAR LOCATION OF RECOMBINANT PROTEINS

N.B. 500 ml starting culture ~ 2g cells.
Grow and induce cells under previously determined optimum conditions

Day 1

1. Inoculate 2 x 5ml LB + antibiotic with bacteria containing the expression vector. Grow overnight.

2. Prewarm 2 x 250 ml flasks of LB overnight.

Day 2

3. Add antibiotic to 250 ml LB flasks and then add 5 ml overnight culture. Grow cells until OD_{600} is greater than 0.6.

4. Induce with IPTG for optimum time and then harvest.

5. Spin 5K 10' in 2 bottles at 4°C.

6. Resuspend cell pellet in 5ml **lysis buffer** (2.5ml per bottle).
   Resuspend
   completely or unlysed cells will be spun down with inclusion bodies.

7. Combine resuspended pellets in 30 ml centrifuge tube.

8. Snap freeze in liquid nitrogen and thaw to break cell membrane.

9. Add 1.25 ml 10 $mgml^{-1}$ lysozyme. Incubate 30' on ice. The solution will become very viscous.

10. Add: 72.5 µl 1 M $MgCl_2$
    7.25 µl 1 M $MnCl_2$
    72.5 µl 1$mgml^{-1}$ DNAase 1 (made up from powder)

    Incubate 37°C 30' with gentle shaking.

11. Add 10 ml **detergent buffer** and spin 5,000g for 10'.

12. Take off the orange supernate. This contains all of the soluble proteins. KEEP THIS for analysis later.

13. Completely resuspend the pellet (insoluble inclusion bodies) in 2 ml **wash buffer** and spin 5,000g for 10'

14. Remove the milky supernate, and resuspend the pellet in 0.5 ml **wash buffer**. Transfer to an eppendorf and spin 13K for 5'.

15. Continue washing until a tight pellet has been obtained (~4 washes). Collect each supernate for analysis.

16. Run samples on an acrylamide gel:
    soluble proteins
    wash supernates
    final pellet – resuspend in 100μl 1 x **loading buffer**
    Load 20 μl of soluble proteins and 10 μl of rest.

Check which sample contains the recombinant protein. If the recombinant protein is soluble in the bacterial cell, it will be predominantly in the orange soluble protein fraction. The washes should contain the major bacterial inclusion body membrane proteins. If the recombinant protein is mainly in the final pellet it is localized in the bacterial inclusion bodies.

Lysis buffer (50 ml)

50 mM Tris pH8	1.25 ml 2 M
25% sucrose (w/v)	12.5 g
1 mM EDTA	100 μl 0.5 M

Loading buffer (10ml 5x)

0.5M	
3% β-mercaptoethanol	1.5ml
3% SDS	1.5g
0.3% bromophenol blue	150mg
10% glycerol	5ml

Detergent buffer (50 ml)

0.2 M NaCl	2 ml 5 M
1% deoxycholic acid (w/v)	0.5 g
1% Nonidet P-40 (v/v)	0.5 ml
20 mM Tris pH 7.5	500 μl 2 M
2 mM EDTA	200μl

Wash buffer (50 ml)

0.5% Triton X-100	250 μl
1mM EDTA	100 μl 0.5 M

Store all at room temperature. Do not autoclave.