INTEIN MEDIATED PURIFICATION OF PROTEINS (IMPACT)

THINGS YOU MUST KNOW FIRST:

optimum protein induction conditions
rough idea of protein yield
the capacity of the chitin beads is 2mg eluted protein per 1ml beads
generally 10 - 15 ml chitin beads are used for a 1 liter culture.

1. Assuming protein expression conditions have been optimized, pellet a 1L *E. coli* culture containing tagged protein. Store at –80°C.

2. Resuspend pellet in 25 ml ice cold **column buffer** per 1L culture.

3. Freeze in liquid nitrogen and thaw to break cell membranes.

4. Sonicate in water bath containing ice for 20 min.

5. Spin slurry at 10,000 g for 30' at 4°C and remove supernate. If supernate is too viscous, add 10$\mu g ml^{-1}$ DNAase and $MgCl_2$ to 5mM. Leave on ice for 30' and then pass through a 0.45μm filter (or cheesecloth if still too viscous).

6. Take 10μl aliquot for gel analysis. First time through, check on gel before proceeding to make sure that the fusion protein is in the soluble extract. Take 5ml aliquot for purification and freeze the remaining 20ml at –80°C for further purifications.

7. Equilibrate 3ml chitin beads at 4°C by passing the beads and 10 volumes (30ml) of **column buffer** over a sintered glass filter.

8. Add 5 ml protein extract to the beads and shake gently at 4°C for 2 hours.

9. Pour beads and extract into a column and collect flow through. Take 10μl aliquot for gel analysis.

10. Wash column with 20 volumes (60ml) of **column buffer** at a flow rate of 1 ml per minute.

11. To cleave the protein: remove resin from column and place in a 15ml falcon tube. Add three column volumes (9ml) of **cleavage buffer** containing 30 mM DTT and leave at 4°C overnight.

12. Pour resin back into a column and collect flow through. Take 10μl aliquot for gel analysis.

13. Elute the protein using **cleavage buffer minus DTT**. Collect 9 x 1 ml fractions. Take 10μl aliquot of each fraction for gel analysis and measure OD_{278} of each. Store protein-containing fractions at –80°C until further use.

14. Recycle the resin by passing the beads and 10 volumes (30ml) of **stripping buffer** over a sintered glass filter at room temperature.

Column Buffer (100 ml)

20 mM Na-HEPES pH 8	2 ml 1M
500 mM NaCl	10 ml 5 M
0.1% Triton X-100	0.1 ml
1 mM EDTA	200 μl 0.5 M

Cleavage Buffer (100 ml)

20 mM Na-HEPES pH 8	2 ml 1M
50 mM NaCl	1 ml 5 M
0.1 mM EDTA	20 μl 0.5 M
30 mM DTT	3 ml 1 M

Stripping Buffer (100 ml)

20 mM Na-HEPES pH 8	2 ml 1 M
500 mM NaCl	10 ml 5 M
1% SDS	10 ml 10%