PURIFICATION OF GST-TAGGED PROTEINS (pGEX)

THINGS YOU MUST KNOW FIRST:

- optimum protein induction conditions
- rough idea of protein yield – estimate by comparing to known amounts of BSA on a Coomassie stained gel and calculate mg protein per ml culture
- that protein is soluble in bacteria

1. Amersham GSTrap FF columns have a capacity of 10mg recombinant GST per 1ml resin. Want to load resin at 80% capacity so that as many sites as possible are specifically bound. This will avoid non-specific binding of contaminants. Thus you should aim to load 8mg protein onto a prepacked 1ml column.

2. Assuming protein expression conditions have been optimized, pellet an appropriate amount of *E. coli* culture containing GST-tagged protein (i.e. amount that will yield 8mg protein). Store at –80°C.

3. Resuspend pellet in 5ml ice cold **binding buffer** per 100ml original culture.

4. **Either:** disrupt cells by sonicating in short bursts on ice. Take care to avoid frothing and sonicate until partial clearing is observed. **Or:** add 250µl 10mgml^{-1} lysozyme per 100ml original culture and incubate on ice for 30 minutes.

5. Add a 20ml syringe to the top of the column and fill with 5ml **binding buffer**. Remove twist off end of column and use syringe to force buffer through. Recap temporarily if necessary while preparing sample.

6. Spin lysed bacteria to pellet debris. Filter lysate through 45µm filter. Save 10µl for gel analysis.

7. Add lysate to syringe on top of column. Use syringe to regulate flow rate of less than 1ml/min (less than 30 drops a minute). Save 10µl for gel analysis.

8. Reapply flow through a second time. Save 10µl for gel analysis.

9. Wash column with 10ml **binding buffer** at a flow rate of 1-2ml/min. Save 10µl for gel analysis.

10. Elute with 10ml **elution buffer** (see below for choice) at a flow rate of 1-2ml/min. Collect 1ml fractions. Remove 10µl of each for gel analysis.

Binding Buffer (100 ml)

140mM NaCl	2.8ml 5M
2.7mM KCl	2.7ml 100mM
10mM Na_2HPO_4	1ml 1M
1.8mM KH_2PO_4	1.8ml 100mM
pH7.3	

Elution Buffer (10 ml)

50mM Tris-HCl pH8.0	250µl 2M
10mM reduced glutathione	1ml 100mM (stored in aliquots at –20°C)

Note: if you want to immobilize protein on CNBr activated supports, DO NOT ELUTE IN TRIS. Instead, use:

Phosphate Elution buffer (10 ml)

200mM sodium phosphate	2 ml 1M
10mM glutathione	1 ml 100mM