B. Purifying affinity-tag adapter protein, MS2-MBP

The affinity-tag adapter protein is a recombinant MS2-MBP fusion expressed in *E. coli* (construct a gift from Josep Vilardell). This fusion places MBP N-terminal to MS2, and the MS2 portion carries a double mutation (V75Q and A81G) that prevents oligomerization (LeCuyer et al., 1995). Single-step purification of MS2-MBP over an amylose column yields a single band on a Coomassie-stained gel, but the A_{280}/A_{260} ratio (<1) reveals that a significant amount of bound nucleic acid remains as a contaminant. Heparin chromatography as a second purification step eliminates this contaminant.

Solutions:

1. *AB1*: 20 mM HEPES, pH 7.9, 200 mM KCl, 1 mM EDTA. Make 500 ml by combining 10 ml of 1 M HEPES, pH 7.9 stock solution, 50 ml of 2 M KCl stock solution, 1 ml of 0.5 M EDTA stock solution, and 439 ml of H_2O.

2. *AB2*: 20 mM HEPES, pH 7.9, 20 mM KCl, 1 mM EDTA. Make 500 ml by combining 10 ml of 1M HEPES, pH 7.9 stock solution, 5 ml of 2 M KCl stock solution, 1 ml of 0.5 M EDTA stock solution, and 484 ml of H_2O.

3. *ABE*: 20 mM HEPES, pH 7.9, 20 mM KCl, 1 mM EDTA, 10 mM maltose. Make 100 ml by combining 2 ml of 1 M HEPES, pH 7.9 stock solution, 1 ml of 2 M KCl stock solution, 200 µl of 0.5 M EDTA stock solution, 2 ml of 0.5 M maltose and 474 ml of H_2O.

4. *PMSF*: 100 mM. To make 5 ml, dissolve 87.1 mg PMSF in 5 ml of ethanol. Store at 4°C.

5. *IPTG*: 1 M. To make 10 ml, dissolve 1.19 g of IPTG in 5 ml of H_2O. Store in 1 ml aliquots at –20°C.

6. *HB1*: 20 mM HEPES, pH 7.9, 1 mM EDTA. Make 500 ml by combining 10 ml of 1 M HEPES, pH 7.9 stock solution, 1 ml of 0.5 M EDTA stock solution, and 489 ml of H_2O.

7. *HB2*: 20 mM Hepes, pH 7.9, 1 M KCl, 1 mM EDTA. Make 500 ml by combining 10 ml of 1 M HEPES, pH 7.9 stock solution, 250 ml of 2 M KCl stock solution, 1 ml of 0.5 M EDTA stock solution, and 239 ml of H_2O.

Steps:

1. Inoculate a 5 ml culture of Luria broth (LB) with single bacterial colony of DH5α cells transformed with a plasmid expressing MS2-MBP and grow overnight to saturation at 37°C with shaking. The next morning inoculate 1 L of LB plus 2% glucose with the 5 ml culture. Grow the cells at 37°C with shaking to an OD_{600} of ~0.5 and then induce expression of the protein by adding 1 ml of 1M IPTG. Continue to grow the cells for 2-3 hours and harvest by centrifugation at 6000 rpm for 10 minutes. Pour off the supernatant and freeze the cell pellet and store at –20°C.

2. Thaw and resuspend ~1 g cells in 10 ml cold AB1 plus 200 µl PMSF. Break open the cells by sonication on ice. Centrifuge 30 minutes at 15,000 rpm at 4°C.

3. All the following steps of the purification are performed at 4°C. Load the supernatant on a ~5 ml amylose column equilibrated with AB1, running the column at 0.3 ml/min. Wash the column with 40 ml of AB1, followed by 10 ml of AB2 to lower the salt concentration in preparation for heparin chromatography.

4. Elute the protein with 20 ml of ABE, taking 1 ml fractions. Check the OD_{280} of the fractions and pool the peak fractions. (The column can be cleaned with 5 ml of 0.1% SDS and re-equilibrated with AB1 for future use).

5. Concentrate the pooled peak fraction to about 1 ml in a Centricon-30.

6. On an FPLC, equilibrate a 1 ml heparin column with a mixture of HB1 and HB2 to 20 mM KCl. Load the concentrate on the column and wash with 5 ml at 20 mM KCl.

7. Run a gradient from 20 to 400 mM KCl over 10 column volumes. The MS2-MBP protein elutes at ~60 mM KCl. Pool peak fractions and concentrate to ~500 µl in a Centricon-30. Add glycerol to 10% and freeze at –20°C in 100 µl aliquots.

8. Determine the protein concentration for MS2-MBP. An OD_{280} of 1 corresponds to 16.5 µM or 0.89 mg/ml.