PROTEIN PURIFICATION

Part 1: Isolation of GFP or RFP

1. Add 500ul of Tris, EDTA, Glucose (TEG) to labeled 1.5ml microreaction tube. (for each sample
2. Using a sterile inoculating loop scrape a large clump of cells (from pellet) and add to labeled TEG tube
3. Repeat with other samples
4. For protein in solution) Take 1ml of protein solution and add to TEG tube
5. Place in microcentrifuge and spin for 1 minute
6. Then place tubes in -20C freezer for 15 min or until frozen, lay tubes on side to ensure rapid freezing.
7. After GFP\RFP cells or protein extract are completely frozen, put in -37C water bath to thaw.
8. Repeat steps 6-7 at least 2 more times (this freezing and thawing, will help lyse open cells)
9. Place tubes in microcentrifuge for 5 minutes.
10. Transfer 200ul of the supernatant (should be glowing) to new tube and label appropriately.
11. STORE leftover supernatant in -20C freezer.

This is optional stopping point: If stopping all(freeze all samples in -20C)

Part II Purification of Protein Samples (tagged with GFP or RFP) using Column Chromatography

Preparing the Column (packing and equilibrating the column)

1. Vertically mount the column on ring stand
2. Slide the cap onto spout at bottom of the column. Fill about ⅓ of column with elution buffer (do NOT let column dry out
3. Mix the molecular sieve thoroughly by swirling or stirring (find on stirring plate)
4. Carefully pipet the mixed slurry (molecular sieve) into the column by letting it stream down the inside walls of the column. (about 6ml)- just use transfer plastic sterile pipet

If flow is stopped by air pocket, stop adding the slurry and firmly tap the column until air pocket is removed and slurry continues to flow down side of column

5. Place empty small beaker under column to collect wash buffer.
6. Remove the cap from the bottom of column and allow the sieve matrix to pack into Column
7. Wash the packed column with 5 ml of 1x elution buffer. Do not allow column to dry
8. Place cap back onto spout and make sure it does not drip.

Optional stopping point. (column can stay like this over weekend as long as it is full of elution buffer and has cap on.

Column and Elution buffer (kept at room temp) but the collection samples (beaker under the spout needs to be on ice and all collection samples should be on ice

Part III Collecting the Column Fractions of Protein

1. Label eight microreaction tube (1-8) for each sample testing. Make the outside of tube at the 0.5ml volume mark with a permanent pen (use clear tubes)
2. Slowly load the column with 200ul of the protein sample from Part 1,step 10. Allow the extract to completely enter column.
3. Elute (wash) the column with 1x Elution butter (add buffer slowly (several drops at a time) to avoid diluting protein sample
4. Using graduated marks on side of tubes (collect 0.5ml fraction) in tube 1, then move to tube 2 etc- until you have 8 samples. All samples immediately after individual collection should be stored on ice.
5. Monitor progress with uv light (may have to turn out class lights)

Note: tube fractions after tube 3 or 4 should not have noticeable protein in them.

6. Save the fractions with highest visual signal (tubes 1-3?) for SDS gel electrophoresis.