CHARACTERIZATION

A. Materials

10X TE Buffer
1M Tris HCl Buffer
Protein lysis buffer (PLB): 50 mM Tris (pH 7.5), 50 mM NaCl, dtt. sterilized by autoclaving
Start buffer: 20 mM Tris-HCl, pH 8.0 (At least 1 pH unit above the pI of substance)
Elution Buffer (20mM Tris-HCl, pH 8.0, 1 M NaCl).
Binding buffer: 4 M ammonium sulfate 132.14 g/mol in TE (Tris- EDTA)
Wash buffer: 1.3 M ammonium sulfate in TE
Equilibrating buffer: 2 M ammonium sulfate in TE

B. Expression

Day 1

1. Pick single colony of C41 cells to 5ml LB solution with 1x antibiotics to grow starter. Prepare frozen stock (culture with 25% glycerol) afterward.

Day 2

1. 1% Inoculation in two 1L conical flask, each with 250 ml 2XYT solution 1x antibiotics overnight.

Day 3:

1. Spin down 100ml cells in 50 ml falcon.
2. Discard the supernanatent.
3. Wash cell pellet with 40 ml cool TE buffer.
4. Re-suspend cells with cold 15 ml Protein Lysis Buffer (PLB).
5. Sonicate on ice.
6. Spin at 4°C at 13000 speed for 5 min
7. Transfer supernatants to new set of tubes.
8. Dialysis overnight.

C. Purification: HiTrap Q HP Ion exchange column (Ge Health Care)

1. Fill the syringe or pump tubing with start buffer (low ionic strength). Remove the stopper and connect the column to the syringe (with the provided luer connector), “drop to drop” to avoid introducing air into the column.
2. Remove the snap-off end at the column outlet.
3. Wash out the preservatives with 5 column volumes of start buffer, at 1 ml/min for the 1 ml column and 5 ml/min for the 5 ml column.
4. Wash with 5 column volumes of elution buffer (start buffer with 1 M NaCl).
5. Finally equilibrate with 5 to 10 column volumes of start buffer.
6. Apply the sample at 1 or 5 ml/min for the 1 ml and 5 ml columns respectively, using a syringe fitted to the luer connector or by pumping it onto the column.

7. Wash with at least 5 column volumes of start buffer or until no material appears in the effluent.
8. Elute with 5 to 10 column volumes of elution buffer.
10. After the completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 column volumes of start buffer. The column is now ready for a new sample.

D. Purification: HIC column (Biorad)

1. Re-suspend HIC matrix vigorously.
2. Aliquot 3 ml HIC suspension per column.
3. Settle HIC matrix at room temperature.
4. Remove supernatant.
9. Re-suspend HIC matrix with shaking in 1.5 ml Equilibration buffer.
10. Securely clamp a column perpendicularly on the stand such that the tip of the column is on top of the collection tube which sits in the tube rack.
11. Aliquot 3 ml water to the column with collection tube in place.
12. Let water drip through.
13. Empty waste collection tube.
14. Aliquot 1 ml Equilibration buffer.
15. Slowly add 3 ml washed and resuspended HIC matrix to the column.
16. Let Equilibration buffer drain off the column and fasten the female luer at the tip of column. Note: never dry the resin at any time.
17. Empty waste collection tube.
18. Slowly add 3 ml Equilibration buffer against the inner wall of the column.
19. Close the column with the female luer when the Equilibration buffer has successfully drained and meniscus has reached the top of the bed.
20. Mix 1 volume of protein sample to 1 volume of Binding Buffer (~200 µl).
21. Transfer all 400 µl of protein/Binding Buffer mix to the column carefully without disturbing the top of the bed containing the settled HIC matrix.
22. Remove the female luer for buffer running through the column to the waste collection tube.
23. Trace the chromoprotein.
24. Wash the resin with 1 ml Wash buffer when the meniscus reaches the top of the bed and the run-through is collected in the waste collection tube.
25. Trace the chromoprotein.
26. Add 1 ml TE buffer to the resin and the run-through is collected in the waste collection tube.
27. Trace chromoprotein.
28. Add 1 ml TE buffer to resin again.
29. Collect run-through with fluorescent protein in a new collection tube and the run-through without fluorescent protein into waste collection tube.

30. Cap the tip of the column with female luer when meniscus reaches top of the bed.
31. Wash resin with 6 ml Wash Buffer.
32. Aliquot 6 ml water to column once the Wash Buffer has run through the column.
33. Cap the column top and attach the female luer to the tip of the column when meniscus reaches the top of the column.
34. Run SDS-PAGE to determine purity.
35. Determine protein concentration by refractometer.

E. pH- stability test

1. Prepare the buffers as follow:

pH	Composition
2	0.2M KCl/HCl
4	3M acetate buffer
6	0.5M MES buffer
7	1M PIPES buffer
8	1M HEPES buffer
10	0.2M NaHCO3/NaOH
12	0.2M KCl/NaOH

2. Diluted into buffers ranging in pH from 3.75–8.50 in 96-well plates.
3. Determine absorbance/ fluorescence by Plate reader.