Per Widlund's XMAP215-GFP purification protocol v. 08.10.08
(adapted from Jeff and Gary's Xmap215 protocol)

Cells
Infect 500mL of SF+ cells at 1 x 10^6/mL with 200uL BIIC stock (1:2,500 dilution)
Harvest at peak expression (72 hours)

Harvesting Cells
Spin down SF+ cells for 15min at 1700rpm.
Resuspend in 40mL Lysis buffer with 1x Pi
Freeze in 2x~25mL in Falcon tubes.
Store at -80°C

Purification
Purification is suitable for 50mL of cell suspension. Scale up may require larger columns.

Lysis and clarification

1. Turn on Beckman Ultra Max and set chamber to 4 degrees.
2. Thaw suspension in RT water and transfer to ice
3. Adjust to 10mM CaCl2, 1x Pi's
4. Dounce for ten strokes with a pre-chilled dounce
5. Spin for 45' at 80,000 rpm in MLA80 rotor and collect supernatant (tubes fit about 6-7mL each – 8 tubes fit one rotor)
6. Collect supernatant.
7. Adjust NaCl concentration to 100mM by diluting 1:2 with 50mM Hepes pH7.5, 5% glycerol, 0.1% TritonX-100. This is necessary to allow robust binding to the cation exhange column.

Cation exchange column

8. Equilibrate a 5mL HiTrap SP HP column with cation exhange buffer
9. Load cleared lysate onto equilibrated SP HP column
10. Wash with 5CV of 75mM NaCl/1x Cation Buffer
11. Wash with 5CV of 150mM NaCl/1x Cation Buffer
12. Elute with 600mM NaCl/1x Cation Buffer
13. Wash column with a 5CV 1.5M NaCl/1x Cation Buffer
14. Run SDS-PAGE to determine peak fractions

Nickel column

15. Add imidazole to 9mM final
16. Load supernatant over pre-equilibrated 1mL His-Trap Nickel column (3% buffer B)
17. Wash with 5 CV of 3% buffer B
18. Wash with 5 CV of high salt buffer (to reduce anion exhange effects)
19. Wash with 5 CV of 10% buffer B
20. Elute with 100% buffer B
21. Run SDS-PAGE to determine peak fractions

Gel Filtration Column

22. Collect peak fractions and pool. Load onto equilibrated Superdex 200 16/60
23. Determine peak fractions by denaturing A280 on NanoDrop.
24. Determine concentration using extinction coefficient: 154900
25. Adjust to 10% Glycerol, 1mM DTT.

Column Set-up

Nickel Column

1. Wash out 20% ethanol with 10 CV water
2. Strip column if necessary
    -10 CV of 50mM EDTA, pH 8.0
    -8 CV water
    -1 CV of 100mM NiCl2
    -8 CV water
3. Equilibrate with 10 CV of 3% Buffer B

Cation exchange column

1. Wash out 20% ethanol with 10 CV water
2. Wash with 10 CV of 1.5M NaCl/20mM Cation Buffer
3. Equilibrate with 10 CV 75mM NaCl/20mM Cation Buffer

Gel filtration column

1. Wash out 20% ethanol with 2 CV water
2. Equilibrate with 2 CV Anion buffer, 100mM KCl

Buffers

Lysis Buffer

50mM Hepes pH 7.5
5% glycerol
0.1% Triton X-100
200mM NaCl

Cation buffers

100mM Cation Buffer
- 33.3 mM MES (6.5g/L)
- 33.3 mM HEPES (7.94g/L)
- 33.3 mM Acetate (4.5g/L of NaAc3H2O)

pH to 7.5 with NaOH

Ni column buffers

Buffer A:
- 25mM Tris-HCl pH 8.0 (3.03g for 1L)
- 300mM NaCl (17.53g for 1L)
- 20% glycerol

Buffer B:
- As above, but with 300mM imidazole (20.4g for 1L)

High Salt Wash
- 1.5mL Buffer B
- 48.5mL Buffer A
- 3.0g NaCl

Gel Filtration Buffers

100mM Anion Buffer
- 50mM Tris Base(6.1g/L)
- 50mM Bis-Tris (14.1g/L)

Adjust to pH 6.6 with HCl

XMAP215 gel filtration buffer
- 20mM anion buffer pH6.6
- 100mM KCl (14.9g/L)