Per Widlund's ZYG9-GFP purification protocol v. 29.09.2009
(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, and increase salt to 300mM if necessary
4. Dounce for ten strokes with a pre-chilled dounce
5. Spin for 45' at 50,000 rpm in MLA80 rotor and collect supernatant (tubes fit about 6-7mL each – 8 tubes fit one rotor)
6. Collect supernatant.

Nickel column

7. Add imidazole to 9mM final
8. Load supernatant over pre-equilibrated 1mL His-Trap Nickel column (3% buffer B)
9. Wash with 5 CV of 3% buffer B
10. Wash with 5 CV of high salt buffer (to reduce anion exchange effects)
11. Wash with 5 CV of 10% buffer B
12. Elute with 100% buffer B
13. Run SDS-PAGE to determine peak fractions

Gel Filtration Column

14. Collect peak fractions and pool. Load onto equilibrated Superdex 200 16/60
15. Determine peak fractions by denaturing A280 on NanoDrop.
16. Determine concentration using extinction coefficient: 154900
17. 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

Gel filtration column
1. Wash out 20% ethanol with 2 CV water
2. Equilibrate with 2 CV Gel filtration buffer

Buffers

Lysis Buffer
50mM Hepes pH 7.5
5% glycerol
0.1% Triton X-100
300mM NaCl

Ni column buffers

Buffer A:
    25mM Tris pH 8.0
    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
20mM Tris

300mM KCl

Adjust to pH 7.5 with HCl

Protease Inhibitors

1 Complete tablet/50mL
50ul 10mg/mL Pepstatin
1mL 100mM (17mg/mL) PMSF