The Liu Laboratory protocol — OCP isolation
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1. Protease inhibitor cocktail and DNase were added to the cells (usually 10 L cells pellet resuspended in Tris-HCl pH 7.5 from culture in 15 L carboy)

2. The suspension was broken by passing French Press cylinder, three cycles (incubate the cell on ice (several mins) if the cell solution gets warm).

3. Spin cell lysate, SS34 30 min (16,500 rpm), keep supernatant.

3. Apply supernatant to ultra, Ti 45 (rotor), spin 90 min (45,000 rpm) and clarify the supernatant by using sterile filter (can be omitted). Keep supernatant.

4. Add imidazole, NaCl, Glycerol to supernatant to final concentration of 20 mM, 0.2 M, 20% (v/v) respectively using stock solution.

5. Load sample (flow rate of 5 ml/min) on to Ni-NTA column (1 ml) pre-equilibrated with 20 mM Tris-HCl pH 7.5, 20% glycerol, 200 mM NaCl, 20 mM imidazole. (1ml column is good for 40L OCP Gm and even for 30 L OCP Km). Overnight loading is OK with large volume.

6. The column was washed using 10 volumes of the equilibrating buffer.

7. Wash column using Tris-HCl 7.5, 0.04% DDM (5 column volume)

8. Wash column using Tris-HCl 7.5, 20 mM NaCl, 5 column volume.

9. The OCP was eluted using 160 mM imidazole in buffer (50 mM Tris-HCl pH7.5, NaCl 20 mM)

10. Dilute sample using 20 mM Tris-HCl pH 7.5 to 15 ml and apply to 30 kDa centricon filter, discard the flowthrough.

11. Repeat step 10 four times (removing imidazole and salts).

12. The OCP was loaded onto Q-HP column (sample can be dilute to 2-5 ml) pre-equilibrated with Tris-HCl 7.5. The column was washed with 10 volumes of equilibration buffer. The OCP was eluted using 160 mM NaCl in 20 mM Tris-HCl pH 7.5 buffer. Pool the OCP fraction and concentrate using centricon (MWCO, 30 kDa). Resuspend the concentrated sample using pain brush if necessary (pay attention to buffer use).

13. OCP can be clarified by centrifugation to eliminate some pellet if present.

14. NiNTA column regeneration (Buffer 1

OCP isolation

Buffer 1: Tris 8.0 25% glycerol 0.5 M NaCl+10 imidazole

Washing : Buffer 1+0.4% DDM :3 volume of column.

Washing: Buffer 1+20 mM imidazole : 10 volume of column.

Elution: Tris+200 imidazol+50 mM NaCl

Polish: QHP 1ml good enough for 50 mg OCP (or at least 50L ocpkm cells)

Tris-HCl 8.0+80 mM NaCl elution

To clean (regenerate) QHP use 0.5 M NaCl and H2O and followed by 25% Ethanol (for reuse).

Pass another nickel column if necessary.

UV-Vis and SDS-PAGE quality control as follows