UgtP PROTEIN PURIFICATION

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DAY 1:

- Streak out JC6 strain (*pBAD-ugtP, slyD*$^-$) onto LB Amp plate.
- Prepare 4L autoclaved LB for induction (45min liquid cycle for every 2L)

4L filter-sterilized ice-cold UgtP buffer with low salt concentration (10% glycerol, 50mM Tris pH8.0, 100mM NaCl) for dialysis

~100ml filter-sterilized ice-cold UgtP buffer with high salt concentration (10% glycerol, 50mM Tris pH8.0, 500mM NaCl)

4ml 100□g/ml Amp

40ml 20% filter-sterilized L-Arabinose

DAY 2:

- Grow JC6 from single colonies in 2X25 ml LB Amp O/N.

DAY 3:

- Add 1ml Amp to each 1L autoclaved LB prior to induction.
- Inoculate 10ml O/N culture into each 1L LB Amp. At OD_{600}~0.4 (~2-3hrs), induce each 1L culture with 10ml 20% L-Arabinose. Let grow for 4hrs at 37□C.
- Pellet the culture using the big rotor in the Kunkel centrifuge (always fill the rotor, balance, and never fill the bottle above the "line"): rotor code 3, 4000rpm, 10min.
- Resuspend each pellet in 10ml ice-cold UgtP buffer (high salt concentration: 500mM NaCl). Transfer to 50ml conical tubes and centrifuge again: 6000rpm, 15min, 4□C (fast cool before use). Pour off the supernatant and store pellets at -80□C or proceed to the next step.

DAY4:

- Resuspend 2 pellets in 30ml UgtP buffer with high salt concentration (10% glycerol, 50mM Tris pH 8.0, 500mM NaCl) with 300□l AEBSF.
- French Press 2-3 times.
- Transfer to 70Ti tubes. Balance to within 0.5g. Centrifuge in Ultra: 40Krpm, 45min, 4□C (70Ti rotor is kept in cold room. Cooling down Ultra takes ~1hr).
- Pre-run AKTA Prime and load the supernatant.

AKTA Prime Pre-run: 1) Run 20ml ddH_2O from A2 to Waste at 20ml/min

2) Fill superloop with ddH_2O
3) Inject 10ml ddH_2O from superloop at 5ml/min
4) Run 20ml ddH_2O to Load at 5ml/min
5) Run 20ml ddH_2O from A1 to Waste at 20ml/min
6) Put UgtP buffer (high salt) in A1
7) Run 20ml ddH_2O from A3 to Waste at 20ml/min
8) Put $NiSO_4$ in A3
9) Run 20ml ddH_2O from A4 to Waste at 20ml/min
10) Put EDTA in A4
11) Run 20ml ddH_2O from B to Waste at 20ml/min
12) Put UgtP buffer (high salt) with imidazole in B
13) Run 20ml of UgtP buffer from A1 to Waste at 20ml/min

14) Use a glass pipette to push superloop to 40ml
15) Fill superloop with UgtP buffer (high salt)
16) Inject 10ml UgtP buffer from superloop at 5ml/min
17) Load 30ml sample. Put in collecting tubes.
18) Turn on the lamp. Turn on the recorder.
19) Run stored method 38.
20) Fractions 6, 7, and 8 should contain the most protein
Note: Pressure should always be kept at 0.3

- Dialyze fractions 6, 7, and 8 into UgtP buffer with low salt concentration (100mM NaCl) twice (once O/N, and once for another 2hrs) in the cold room using dialysis tubing, clamps (Qingwei's drawer), 1L beakers, stir bars and stir plates.

DAY 5:

- Concentrate dialyzed UgtP fractions using Centricon YM-30 until ~0.5ml is left in the Centricon: 4500rcf , 30min or more, 4□C. Flip the Centricon upside down to elute the protein by centrifuging again: 1000rcf, 10min, 4□C.
- Aliquot 25□l UgtP into small eppi tubes and dump into liquid nitrogen right away. Store the aliquots at -80□C.
- Clean-up AKTA Prime.

AKTA Prime Clean-up: 1) Open superloop and rinse it well
2) Use a glass pipette to push superloop to 30ml
3) Fill the bottom of superloop with UgtP buffer (high salt)
4) Fill the top of superloop with ddH_2O
5) Inject 5ml UgtP buffer from superloop at 5ml/min
6) Replace UgtP buffer with ddH_2O in superloop
7) Inject 5ml ddH_2O from superloop at 5ml/min
8) Run 20ml EDTA from A4 to Waste at 20ml/min
9) Run 40ml EDTA from A4 to Load at 5ml/min
10) Run 40ml ddH_2O from A2 to Load at 5ml/min
11) Run 20ml ddH_2O (flask) from A1, B, A4 and A3 to Waste at 20ml/min
12) Put filter lines into EtOH flask (large EtOH flask for A4)
13) Run 20ml EtOH from A4 to Waste at 20ml/min
14) Empty superloop and fill with 20% EtOH
15) Inject 5ml EtOH from superloop at 3ml/min
16) Run 20ml EtOH from A4 to Load at 3ml/min
17) Replace large EtOH flask in A4 with a small one
18) Run 20ml EtOH from B, A1, A2 and A3 to Waste at 20ml/min
19) Turn off the machine. Refill ddH_2O flask with fresh.