Expression Construct: _____ Cell type_____

Day 1, late PM (date = _____)

1. Grow overnight culture at 37°C, with shaking. Inoculate at _____PM

Culture volume/container size:

Broth:

Additives:

Day 2 (date = _____)

1. Harvest overnight culture at _____AM, pellet(1400 x *g*, 4°C, 10 min) and resuspend in _____ml fresh LB broth.
2. Inoculate new culture(s) with _____ml of resuspended cells (dilution = _____), grow at 37°C. Time inoculated: _____AM

_____(sample 1) _____(sample 2) _____(sample 3) _____(sample 4)

Culture volume/container size:

Broth:

Additives:

3. Make 100 mM IPTG stock, and keep on ice. (_____mg in _____μl PBS).
4. Remove 1 ml pre-induction sample for SDS-PAGE analysis. Pellet cells (11,000 x *g*, 5 min, RT), remove SN, resuspend in 100 μl PBS, add 100 μl sample buffer – freeze.
5. Induce with IPTG. _____(sample 1) _____(sample 2) _____(sample 3) _____(sample 4)

OD_{600} at induction:

Time induced:

IPTG volume added: _____μl of 100 mM IPTG to each container (giving a final concentration of _____mM IPTG).

6. Remove 1 ml post-induction sample for SDS-PAGE analysis. Pellet cells (11,000 x *g*, 5 min, RT), remove SN, resuspend in 100 μl PBS, add 100 μl sample buffer – freeze.

OD_{600} at end of induction: _____(sample 1) _____(sample 2) _____(sample 3) _____(sample 4)

(1:2 with LB)

Time harvested: _____PM (Total length of induction _____hrs). Average OD_{600} at harvest:_____

7. Store cultures at 4°C, O/N.

Day 3 (date = _____)

1. Pool and pellet cultures (6,000 x *g*, 10 min, 4°C). Culture volume harvested: _____. (÷ _____ = _____ml/bottle).
2. Remove, neutralize (10% bleach-final concentration, let sit 1 hour under hood), then discard supernatant.
3. Resuspend cells in ice-cold 20 mM Tris, pH 8.0, 20% sucrose, to an OD_{600} of 5 U/ml, by SWIRLING (no pipeting!). This takes ~20 min.

Volume:_____ _____ml/pellet

4. Once cells are completely resuspended, incubate on ice (with gentle shaking), 10 min.
5. Pellet cells (15,000 x *g*, 30 sec, 4°C).

6. Remove, neutralize and discard supernatant.

7. GENTLY resuspend cells by SWIRLING (no pipeting!) in ice-cold 15 mM Tris, pH 8.0, to an OD_{600} of 5 U/ml. This takes ~20 min.

Total volume:_____ (_____ml/pellet)

8. Once cells are completely resuspended, incubate for 45 min. on shaker in cold room, setting 4.

9. Pellet cells (15,000 x *g*, 10 min, 4°C).

10. Remove and **save the periplasmic fraction** (supernatant).

11. Remove 30 µl of periplasmic fraction for SDS-PAGE analysis. Add 30 µl sample buffer – freeze.

12. Resuspend the pellet in PBS (1/10 volume used during osmotic shock). Volume:_____.

13. Remove 10 µl of the resuspended pellet for SDS-PAGE analysis. Add 10 µl sample buffer – freeze.

14. Wash (5 min on rotator) 4 ml Ni beads with 40 ml pre-elution 10 buffer (300 mM NaCl, 50 mM Tris-Cl, pH 8.0, 10 mM imidazole, 10% glycerol), then pellet (10 min, 1,400 *g*, RT) and discard S/N wash.

15. Incubate in 250 ml conical bottles, the periplasmic fraction with the pre-washed Ni beads (2 hours, 4°C, on rotator). Parafilm the lids.

16. Pellet the Ni beads (10 min, 2,500 rpm, RT), remove and save the Unbound fraction. Combine 20 µl of Unbound with 20 µl sample buffer – freeze.

17. Add the Ni beads to a column and allow the beads to settle for 30 min.

18. Wash the beads with pre-elution buffer 10, until baseline is flat. Collect and analyze this wash by SDS-PAGE.

19. Elute the bound protein with elution buffer (300 mM NaCl, 50 mM Tris-Cl, pH 8.0, 250 mM imidazole, 10% glycerol). Collect the eluted protein in smallest volume possible – watch chart recording to ascertain when the majority of the protein has eluted from the column.

20. Take A_{280} measurements of the eluted protein. A_{280}: _____(= _____ mg/ml) blank = _____

Eluate volume: _____, Yield from Ni column: _____mg.

21. Remove 10 µl of eluted protein for SDS-PAGE analysis. Add 10 µl sample buffer – freeze.

22. Remove and save the Ni beads, recharge as described below.

23. Concentrate the eluate using the AMICON spin cell concentrator and Millipore membrane #13612. Starting volume _____, ending volume _____.

24. A_{280} post concentration_____ (1:100 with water). (= _____ mg/ml)

25. Filter through pre-rinsed (with 50 mM Tris, pH 8.0, 150 mM NaCl) 0.2 µm filter.

26. A_{280} post filtration _____ (1:100 with water). (= _____ mg/ml)

27. Load onto pre-equilibrated Superdex 75. Buffer used to wash and elute the Superdex 75 column:_____.

28. Analyze FPLC fractions by SDS-PAGE and pool the fractions of interest.

29. Concentrate the pooled fractions to desired concentration using either an AMICON spin cell concentrator/Millipore membrane or Amicon Ultra-4 centrifugal filter device (10,000 MWCOs).

Recharging Ni beads:
1. Wash Ni beads in 5 column volumes of nanopure water.
2. Wash Ni beads in 3 column volumes of strip buffer (100 mM EDTA, 500 mM NaCl, 20 mM Tris, pH 8.0). Beads should turn white.
3. Wash Ni beads in 10 column volumes of nanopure water.
4. Wash Ni beads in 5 column volumes of charge buffer (100 mM $NiCl_2$).
5. Wash Ni beads in 10 column volumes of nanopure water.
6. Wash Ni beads in 5 column volumes of pre-elution buffer 10. Store beads in pre-elution buffer 10 plus either 10% EtOH or NaN_3.