Larger scale protein expression (500 ml Batch)

The day before:

1. Inoculate 3-10 single colonies (of the expression strain cells transformed with plasmids carrying the gene of interest) in 25mL of LB medium with the appropriate antibiotic.
2. Incubate at +30°C with shaking overnight.

The next day:

3. Prewarm 500mL of LB medium to +37°C in a 2L Erlenmeyer flask. Add antibiotics.
4. Dilute 3-5mL of the overnight grown preculture in 500mL prewarmed LB.
5. Incubate at +37°C with shaking until OD_{600} value reaches 0.6.
6. Take a 500µL sample of the culture before induction of protein expression, and process the sample as in small-scale protein expression. Induce protein expression with a final concentration of 0.5mM IPTG.
7. Incubate the culture at +37°C for 4 more hours (or what was decided based on the small-scale expression test). Take samples for SDS-PAGE (optionally also 2h after induction).
8. Transfer the culture in a 1L sorvall centrifuge bottle (balance carefully!) and harvest cells by centrifuging at 5,000 x g for 10 minutes at +16°C.
9. Discard supernatant and resuspend pellet in Buffer A (50mM NaPi, 300mM NaCl, pH 8.0) to obtain a final volume of ~35mL.
10. If cells are stored at this point, transfer into a 50mL Falcon tube, freeze in liquid nitrogen at store at -20°C or -80°C.

Cell lysis with AVESTIN EmulsiFlex-C3:

1. Thaw the frozen cells in a hand-warm water bath.
2. Turn on air valve for Emulsiflex.
3. Flush Emulsiflex with 20% ethanol. Close the lid of the inlet cylinder when flushing.
4. Refill with Milli-Q and flush twice.
5. Refill with Milli-Q and wash with pressure. Release the stop button and press start. Wait until no air bubbles in the pipe. Turn on the air regulation button to pressurize the system to 10,000-15,000 psi. Let the washing run for a while. Stop and flush.
6. Add sample buffer (Buffer A) and flush.
7. Add sample into the machine with a pipette. Make sure that the sample is homogenous and there are no chunks.
8. Pressurize the system to 10,000-15,000 psi. Repeat 2 or 3 times or alternatively let the the running continue for 10 minutes keeping the outlet pipe in the inlet cylinder.
9. Release the pressure by pressing the stop button and let the circulation continue. Collect the sample into a 50mL Falcon tube.
10. Flush with 10% deconex (detergent) in Milli-Q.
11. Refill with Milli-Q and wash with pressure twice. Flush.
12. Wash with 20% ethanol and flush.
13. Fill the cylinder with some more ethanol and flush some of it so that the system still contains ethanol for the appropriate storage of the machine.
14. Centrifuge the sample at 18,000 x g for 30 minutes at +4°C.
15. Take samples for SDS-PAGE from supernatant and pellet.
16. Filter supernatant with 0.45µm filter.
17. Purify supernatant using ÄKTA.

Protein purification with ÄKTA:

1. Wash away ethanol from the machine.
2. Change lanes A1 and B1 into filtered (degassed) Milli-Q. Wash the pumps and system with MQ. Then wash the injection loop with 10 x column volume of MQ. Then wash the column to be used with 10 x column volume of MQ.
3. Change lanes A1 and B1 into filtered Wash Buffer (50mM NaPi, 300mM NaCl, 30mM imidazole, pH 8.0) and Buffer B (50 mM NaPi, 300 mM NaCl, 250 mM imidazole, pH 8,0). Repeat the same washes as in step 2. Then change lane A1 into Buffer A.
4. Make a suitable program for the purification in the software.
5. Inject sample into the sample loop with a syringe. (When purifying sample for the first time, it is recommended to inject only half of the sample in the first run.)
6. Start the run. Fractions will be collected in tubes/plates.
7. After the run wash the machine with filtered MQ, as at the beginning but in reverse order.
8. Wash the machine with 20% ethanol, in reverse order. Store the machine in ethanol.
9. Select elution fractions with the desired protein from the graph, and run flow-through and elution fractions on an SDS-PAGE. Pool fractions that contain the desired protein, freeze in liquid nitrogen and store at -20°C or -80°C.

Buffer exchange and concentration of protein samples

1. Wash filter columns (we used, Sartorius Vivaspin 20 ultrafiltration centrifugal tubes (5,000 MWCO)) with Milli-Q (~20mL). Centrifuge at 3,000 x g for 45 minutes - 1 hour at +4°C. NOTE: If the columns are reused after storing in ethanol, centrifuge and discard ethanol first.
2. Fill filter columns with 20mL of the protein sample. Centrifuge at 3,000 x g for 30-45 minutes at +4°C (approximately half of the sample will go through the filter). Mix the remaining sample by pipetting up and down. Repeat the centrifugation. Collect flow-through fractions and run samples on SDS-PAGE to verify that the filter is not leaking.
3. Load the remaining samples in the columns. Centrifuge at 3,000 x g for 30-45 minutes at +4°C, collect flow-through fractions. Repeat centrifugation if needed.
4. When ~5mL of the old buffer is left above the filter, fill the column up to 20mL with the new buffer. Centrifuge at 3,000 x g for ~1 hour at +4°C. Collect flow-through fractions. Note down the dilution of the old buffer to know e.g. the amount of salts left from the old buffer at the end.
5. Repeat the step above (filling up the column with the new buffer) twice (or such that the amount of old buffer left in the sample is sufficiently low). In the end the desired sample volume in the column is ~5mL.
6. Freeze the protein samples in liquid nitrogen and store at -20°C or -80°C. Flow-through fractions may be stored in cold room on ice until the next day before running samples on an SDS-PAGE.
7. Wash the empty columns twice with MQ by centrifuging. Fill the columns with 20% ethanol and store at +4°C.