Small-scale protein expression

1. Inoculate 3-5 colonies (of the expression strain cells transformed with plasmids carrying the gene of interest) in 7mL of LB medium with the appropriate antibiotic.
2. Grow cells at +37 °C with shaking until OD_{600} reaches ~0.6 (e.g. 1:2 dilution of the culture can be used for measurements).
3. Before inducing the protein expression take a 500µL sample from the culture (=non-induced). **Sample processing**: Centrifuge at 12,000 x g for 1 minute, remove supernatant and resuspend pellet in 1X SDS Loading Dye according to the OD_{600} value of the culture in the end. Add 10µL of 1X SDS Loading Dye for each OD_{600}=0.1, so if the value is 0.5, resuspend in 50µL, 0.6 in 60µL etc.
4. Induce protein expression by adding a final concentration of 0.5mM of IPTG in the culture. Continue growing the cells at +37°C.
5. Take 500µL samples 2 and 4 hours after induction. Process the samples as the non-induced one.
6. Pellet cells by centrifuging at 4,500 x g for 10 minutes.
7. Resuspend pellet in 100µL of ThermoFisher Scientific B-PER Bacterial Protein Extraction Reagent (https://www.thermofisher.com/order/catalog/product/78248).
8. Incubate at RT for 10 minutes with shaking.
9. Centrifuge at 15,000 x g for 5 minutes.
10. Take a 5µL sample of supernatant (=lysate) and add 20µL of 1X SDS Loading Dye. Alternatively, take e.g. 10µL sample of supernatant and add 10µL of 2X SDS Loading Dye (if rather small amount of protein expected).
11. Take a sample for SDS-PAGE from pellet (due to the viscosity of the pellet, a small amount of H_2O may be added into it prior to pipetting the sample), and continue purifying proteins from supernatant.

Protein purification with Qiagen Ni-NTA spin columns

1. Recharge the columns (soaked in EDTA for storage). Add 400µL of 0.1M EDTA and centrifuge at 700 x g for 1 min.
2. Add 400µL of Milli-Q and centrifuge at 700 x g for 1 min. Repeat this step.
3. Add 100µL of 0.1M $NiSO_4$ and incubate for 30 minutes.
4. Centrifuge at 700 x g for 1 min.
5. Add 400µL of Milli-Q and centrifuge at 700 x g for 1 min.
6. Add 400µL of NPI-10 buffer (=Buffer A) (50mM NaPi, 300mM NaCl, pH 8.0) and centrifuge at 700 x g for 1 min.
7. Equilibrate the spin columns with 600µL of NPI-10 buffer. Centrifuge twice at 2,900 rpm for 2 minutes.
8. After equilibration start the protein purification: load the remaining supernatant onto the spin columns and centrifuge at 1,600 rpm for 5 minutes. Prepared a sample for SDS-PAGE from the flow-through: 4µL of the flow-through + 20µL of 1X SDS Loading Dye.
9. Wash the column with 600µL of NPI-20 buffer (=Wash Buffer) (50mM NaPi, 300mM NaCl, 30mM imidazole, pH 8.0). Centrifuge at 2,900 rpm for 2 minutes. Repeat the wash step. Take samples for SDS-PAGE from both washing steps.
10. Elute the proteins in 300µL of NPI-500 buffer (=Buffer B) (50mM NaPi, 300mM NaCl, 250mM imidazole, pH 8.0). Centrifuge at 2,900 rpm for 2 minutes. Take a sample for SDS-PAGE. Repeat the elution step and take a sample for SDS-PAGE.
11. Store the spin columns soaked in 0.1M EDTA solution, at +4 °C.