University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	Mini-purification of His-tagged proteins		
	Version:	2		
	Description:	How to purify His-tagged proteins using batch purification.		
	Trigger:			
Last updated	Name:	Sean Lowe	Date:	29.07.14
You will need	Time:			
	PPE:	Gloves Lab coat		
	Equipment:	Liquid nitrogen Dewar Plastic double walled well Ultracentrifuge pH Meter Perostaltic Pump Pipette and tips		
	Materials:	Frozen cell pellets Ni-NTA beads Lysis Buffer 100mL pH 8 o 20mM Tris o 2mM Benzamidine o 0.1mg/mL Soybean Trypsin Inhibitor o 10% Glycerol o 1% Triton x100 (if required) o 300mM NaCl o 5mM Imidazole Lysozyme Iodoacetamide DNase Equilibration buffer Bradford reagent Base Buffer 500mL pH 8 o 20mM Tris o 2mM Benzamidine o 10% Glycerol		

A) Prepare buffers	
Step 1	Prepare lysis buffer according to recipe listed above.
Step 2	Add lysozyme to the lysis buffer to a final concentration of 0.1 mg/mL. Add iodoacetamide now, if required.
B) On The Day: Lysis using freeze-thaw in Eppendorf tubes	
Step 1	Scrape off a bit of the frozen cell pellet into the lysis Eppendorf tube
Step 2	Add the prepared lysis buffer to the cell pellet in a ratio of 1ml:1g wet cell pellet.
Step 3	Freeze-thaw by dunking the lysis Eppendorf tube into liquid nitrogen, then into a 37 degree water bath. Repeat another 2 times.

Step 4	Add DNAse to the lysis Eppendorf tube at a concentration of 10 ug/mL. Add 5 mM MgCl2 (3.75 uL of a 2 M stock for 1.5 mL of lysis buffer)
Step 5	Incubate with the DNase for 15 min on the rotary suspension mixer at room temperature
Step 6	Centrifuge spin at 4 C at 12,000 rpm for 30 minutes
Step 7	Aliquot out 40µL of sample for running on a gel
C) While tubes are spinning in step B6: Preparation the Ni-NTA beads:	
Step 1	Vortex the slurry that the beads come in
Step 2	Add an appropriate amount of Ni-NTA resin to an eppendorf tube. (minimum 100µL)
Step 3	Centrifuge tube for 2 minutes at 700 × g and carefully remove and discard the supernatant.
Step 4	Add two resin-bed volumes of Equilibration Buffer (250 uL) and mix until the resin is fully suspended.
Step 5	Centrifuge tube for 2 minutes at 700 × *g* and carefully remove and discard Equilibration buffer.
D) Sample addition and incubation	
Step 1	Use a pipette to carefully take up the supernatant from the lysis Eppendorf tube. Save the insoluble pellet remaining for SDS-PAGE analysis later.
Step 2	Add the prepared protein extract to the tube from C5 and mix on an end-over-end rotator for at least 30 minutes in the cold room
E) Wash to remove nonspecific binding	
Step 1	Centrifuge the tube for 2 minutes at 700 × *g*. Save supernatant for downstream analysis, labelling "Unbound"
Step 2	Wash the resin with two resin-bed volumes (2x300µL) of Wash Buffer. Centrifuge the tube for 2 minutes at 700 × g. Save supernatant for downstream analysis.
Step 5	Repeat step E2 using a new collection tube until there is no more protein in the washes as detected using the Bradford reagent
F) Elution	
Step 1	Elute bound His-tagged proteins using one resin-bed volume of Elution Buffer (300 uL). Centrifuge tubes for 2 minutes at 700 × g. Carefully remove and save the supernatant. Repeat this step at least twice, saving each supernatant fraction in a separate tube.
Step 2	Monitor protein elution by measuring the absorbance of the fractions at 280nm or by Coomassie Plus (Bradford) Assay Reagent
Step 3	The eluted protein can be directly analyzed by SDS-PAGE.
Version history	*Describe the changes made in each new version of the protocol here.*