Protocol 10.14.15

* *E.coli* BL21 cells possessing recombinant plasmid were inoculated in 5ml culture 37oC overnight.
* The aforementioned culture will be transferred and grow in 200 ml of LB media containing Amp (50 mg/L) at 37OC until absorbance at 600 nm reached 0.6.
* Then induced with 1 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) and grow at 250 rpm 30oC.
* The cells were harvested overnight and weighed.
* The induced and uninduced cultures were analysed by SDS- PAGE to ensure the recombinant protein expression.
* Cell pellet were suspended in lysis buffer, sonication, and centrifugation.
* Inclusion bodies obtained after sonication of the 0.3 grams of harvested cells in 1.2 ml were re-suspended in Buffer A
* Centrifuged and the supernatant was loaded on 1 ml of Ni-NTA resin (Qiagen) pre equilibrated with Buffer A.
* The column was successively washed with Buffer B, Buffer C, Buffer D, and Buffer E.
* Finally, the recombinant protein was eluted with Buffer F in a refolded state.
  + Buffer A: 100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0 and 8 M urea
  + Buffer B: 100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 8 M urea and 20 mM imidazole
  + Buffer C: 100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0 and 20 mM imidazole
  + Buffer D: 100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole and 10% glycerol
  + Buffer E: 100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole, 10% Glycerol and 300 mM NaCl
  + Buffer F: 100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 300 mM imidazole and 10% glycerol and 300 mM NaCl
  + The Urea concentration will be used: 1M, 4M, and 6 M.
* Then SDS page gel will be run to decide which portion(s) of the elution will go to dialysis step.
* Dialysis overnight
* The purified protein concentration will be measured
* Activity assay will be performed for specific activity.