Protein Expression:

freshly transform pInt DNA into BL21 strain
pick an isolated colony and inocculate 100mL of LB + Amp100ug/ml
incubate overnight at 37°C in orbital shaker
subculture 1:100 into 2 x 500 mL of LB + Amp100
grow at 37°C in orbital shaker until OD600 of around 0.6
induce with IPTG to a final concentration of 0.5mM
grow an additional 4 hours at RT in orbital shaker
spin down at 10,000 x g for 30 minutes
pour off supernatant
store pellet at -20°C

Protein Extraction: ***all steps on ice**

hit bottle on bench to break off pellet while still frozen, this usually shatters the pellet into about 20 pieces which facilitates easy transfer
transfer to a 30mL homogenizer (glass mortar and teflon pestle type)
combine both 500 mL's worth of pellets into same homogenizer
add 5mL of extraction buffer (50mM Tris-HCl pH 8.0, 10% sucrose, 1mM EDTA, 0.4M KCl)
let thaw on ice, mix thoroughly by vortexing
add protease inhibitors (per g of cell weight):

soybean trypsin inhibitor	final 2ug/ml
leupeptin	final 2ug/ml
pepstatin A	final 1ug/ml
PMSF	final 100ug/ml

add BME to 10mM
add 1/20th volume lysozyme (10mg/ml in 0.25M Tris pH8)
incubate on ice for 1 hour, homogenizing every few minutes
transfer to 1.5mL microcentrifuge tubes
spin at 45,000 rpm for 45 minutes at 4°C
transfer supernatant to new tube
quick freeze in dry ice/EtOH bath and store at -80°C

Protein Purification

A. Prep Whatman Phosphocellulose P-11 Resin
   -Hydrate resin as per manufacturers directions
   -Wash resin 4 times with 5 volumes wash buffer + 10 mM BME (same as extraction buffer above except substituting 10% glycerol for sucrose)
B. Protein Binding
   -Thaw protein extracts on ice and add to washed resin (200 ul of resin per 800 ul of cell extract)
   -Rotate at 4C for 1 hour
C. Wash Nonspecific Proteins
   -Gently pellet resin and remove supernatant
   -Add 3 volumes of wash buffer (with 0.4M KCl and 10mM BME), resuspend
   -Rotate at 4C for 30 min

-Repeat wash steps if desired

D. Elute Protein
-Gently pellet resin and remove supernatant
-Resuspend in 2 volumes elution buffer (50 mM Tris-HCl pH 8.0, 10% glycerol, 1mM EDTA, 10 mM BME) + 0.6M KCl
-Rotate at 4C for 30 min
-Gently pellet resin
-Repeat elution steps with 0.8, 1.0, and 1.2 M KCl
-Perform activity assay to determine in which fraction protein resides in.