# Protein Expression

1. **Transforming the fusion plasmid into the competent T7 Express:**

   Prepare competent T7 Express cell and stock in -80°C refrigerator first. Take 33µl competent T7 Express from -80°C refrigerator and mix with 1µl fusion plasmid, then put on the ice for 20 minutes followed by heat-shock in 42°C water for 35 seconds. Incubate the mixed cell in 37°C for 30 minutes. Spread the cells evenly on the labelled plates, incubating in 37°C for 16 hours.

2. **Inoculating colonies:**

   Prepare PCR mixture solution and dispense 10µl into each PCR tube. Dispense LB broth 4ml with Ampicillin 100 µg/ml. Pick colony on the plate with toothpick into the PCR solution first, then into LB media. Incubate the culture in incubator at 37°C for 12-16hours.

3. **Preparation of *E. coli* culture glycerol stocks:**

   Label an eppendorf and Extract 750µl culture mixed with 250µl 80% glycerol and then stock in -80°C refrigerator.

4. **Inducing protein expression with IPTG:**

   Pour the remaining culture into a flask with 100ml LB broth with Ampicillin 100 µg/ml. Incubate the culture at 37°C until the OD level reach 0.6-0.7, then induce with 40mM IPTG, and remove the flask into 6°C incubator for 12-16 hours.

5. **Cell harvest:**

   Centrifuge cell culture at 4,000 rpm for 10 minutes at 4°C. Discard supernatant. Resuspend cell pellet in column buffer (20mM Tris-HCl, 0.5M NaCl, pH8.5). Then break cells by sonication on ice, centrifuge the mixture at 4,000 rpm for 10 minutes at 4°C and collect the supernatant. Stock the protein solution in labelled eppendorf at -80°C.

# Protein Refolding

If target protein is insoluble, protein refolding is necessary for our target protein to show the function.

**1. Preparation of renaturation buffer**

prepare the followings:

Renaturation Buffer A: 20 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 8 M urea,

Renaturation Buffer B: 20 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 6 M urea,

Renaturation Buffer C: 20 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 4 M urea,

Renaturation Buffer D: 20 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 2 M urea,

Renaturation Buffer E: 20 mM Tris-HCl, pH 8.5, 0.5 M NaCl, 0 M urea

and stock in 4°C.

**2. Use urea to resuspend the protein**

Resuspend the cell harvest pellet in Column Buffer and break cells with sonication. Then spin down the debris containing the inclusion bodies at 4000rpm,4°C for 10 minutes and resuspend it in Renaturation Buffer A. Centrifuge it at 4000rpm,4°C for 10 minutes and collect the supernatant.

**3. Preparation of dialysis bag**

Cut the dialysis bag to appropriate length, put in $DDH_2O$, and boil it. After boiling in $DDH_2O$, soak the dialysis bag in column buffer (20 mM Tris-HCl, pH 8.5, 0.5 M NaCl) for 15 minutes.

**4. Dialysis**

Load the supernatant (protein resuspended in urea) into the dialysis bag and seal it. Dialyze it against Renaturation Buffer B, C, D, E. Each dialysis step takes at least 2 hours at 4°C. During dialysis the buffer should be continuously stirred.

# Protein Purification & Condensation

**The following** is required for protein purification

| | |
|---|---|
| Column buffer | 20 mM Tris-HCl, pH 8.5, 0.5 M NaCl |
| Chitin resin | 3ml |
| DTT | 0.5ml |
| Column | volume=15ml |
| PBS | NaCl 0.137 M\KCl 0.0027 M<br>$Na_2HPO_4$ 0.01 M\$KH_2PO_4$ 0.0018 M\pH7.4 |

**1. Preparation of purification column:**

Sterilize the 15ml column and centrifuge tube in advance. Pour chitin resin 3ml and wash with column buffer 50ml to equilibrate the chitin.

**2. Loading protein solution:**

Load the refolded protein solution/lysate onto the chitin column. Seal the column with parafilm and stir the column with rotator mixer at 4°C for 1 hour to connect our protein chitin binding domain to chitin resin.

**3. Washing column to remove the impurities:**

After stirring the tube for 1 hour, load 100ml column buffer to remove those incorrect proteins. Do not let the chitin resin dry out during the whole purification process.

**4. Cleavage of target protein and chitin binding domain:**

Add cleavage buffer 10ml (Column buffer containing 50 mM DTT) to quickly wash the column to ensure the whole chitin resin soaking in the cleavage buffer. Stop the flow to keep the same volume of cleavage buffer staying in the column to cut off the connection between chitin binding domain and the target protein at 4°C overnight.

**5. Elution of target protein:**

Elute the target protein with Column Buffer 10ml by continuing the column flow.

**6. Dialysis of target protein:**

Collect the elution flow and dialyze the target protein into PBS; this will remove the excess thiol reagent used in the Cleavage Buffer.