

Protein Expression in Prokaryotic Rosetta (DE3) *E. coli*

- (1) Transformant **Rosetta (DE3)** cells with **pET30a** alone or recombinant **pET30a/IL3** were grown on LB/Kanamycin/Chloramphenicol agar plates overnight.
- (2) Next day, a single separate colony was isolated and inoculated in 5ml **LB/Kana/Chloramp** broth, placed in shaking incubator at 250rpm, 37C overnight. This is done for both transformant Rosetta with plasmid alone or recombinant plasmid.
- (3) On the third day, 3ml of O/N cultures were placed in 100ml fresh LB/Kana/Chloramph culture in 250ml flask (sterile flasks). Determine the start reading OD₆₀₀ using Blank LB/Kana/Chloramph and take 1ml of these cultures.

Growth and induction

- (4) Place the flasks in shaking incubator and measure their OD₆₀₀ every 30 minutes. When OD₆₀₀ reaches 0.6-1.0, split the 100ml culture into 2 fractions (2 x 50ml in new flasks). Add 500µl of 100mM IPTG for one flask (induced flask, final conc is **1mM IPTG**, since our vector is T7lac promoter). The other flask will receive nothing (i.e. uninduced).

Note: In vectors which are T7 (i.e. lack lac operator or lac I gene), the final concentration of IPTG should be 0.4mM.

- (5) After adding IPTG, incubate for 2hours at 37C in shaking incubator.
- (6) Near the end of induction period, pre-chill centrifuge tubes (50ml tubes)

*** Harvesting***

- (7) At the end of induction incubation, place the flask(s) on ice for 5minutes and pipette 40ml of cultured cells in the pre-chill tubes.
- (8) Centrifuge at 5000xg, 5min, 4°C. **Keep the pellet** and discard the supernatant.
- (9) Add 10ml of **ice- cold 50mM Tris-HCl, pH 8.0** (resuspension buffer) and resuspend the pellet.
- (10) Centrifuge at 5000xg, 5min, 4°C. **Keep the pellet** and discard the supernatant.
- (11) Place the pellet on ice and store it at -80C till lysis.

*** Protein extract and *E. coli* lysis***

- (1) Cells that were kept at -80C are taken out and 5ml of ice cold 1X start buffer (10mM imidazole, 0.5 NaCl, 20mM PBS, pH 7.4).
- (2) Add 50µl of protease inhibitor PMSF (PMSF in ethanol, it is very toxic, wear gloves).
- (3) Resuspend by pipetting up and down. Transfer to non disposable centrifugation tube.
- (4) Freeze thaw twice (in liquid nitrogen). Cell Suspension becomes viscous.
- (5) Sonicate briefly to complete the cell lysis. Keep the tube on ice and submerge the Sonicator probe in the liquid and turn it on for 10seconds, turn it off for 30 seconds. Repeat it twice.
- (6) Centrifuge at 12000 x g for 15 min at 4°C. **Keep the supernatant.**
- (7) Keep 20µl of this supernatant in a clean eppendorf tube (**S1-fraction** or Crude soluble protein extract). Label and freeze it at -20C.
- (8) Purify the rest with His-Tag column.