Protein production IPTG INDUCTION

Aim: To increase the production of our protein which is cloned downstream of a promoter from T7 phage regulated by lacI from the Lac operon.

Materials:

- Microbiology equipment: 37 °C incutator (static, and shaking), Bunsen burner, sterile rake, petri dishes with appropriate antibiotic on agar, SDS-PAGE gel, electrophoresis tank and power supply
- Antibiotics stock (carbenicillin 50 mg/ml, chloramphenicol 34 mg/ml)
- BL21(DE3) competent cells
- Isopropyl β-D galacto pyranoside (IPTG) 0.5 M stock
- Gel Code blue Coomassie blue protein stain (Pierce, or EZ-Stain Sigma)

Protocol:

1. Transform expression plasmid into BL21(De3) *Escherichia coli* cells. Plate on antibiotic selection plates and incubate overnight at 37°C.

2. Resuspend a single colony in 10 ml liquid LB with antibiotic.

3. Incubate at 37°C until OD_{600nm} reaches 0.4–0.8.

4. Induce with 10 or 20 µl of a 0.5 M stock of IPTG
(final concentration of IPTG varies from 0.5 mM or 1.0 mM) and induce for 3 to 5 hours at 37°C.

5. Check for expression by Sodium dodecyl sulfate – poly acrylamide gel electrophoresis (SDS-PAGE) protein gel electrophoresis, Western blot or activity assay. Check expression in both the total cell extract (soluble + insoluble) and the soluble fraction only. *If a fraction of the target protein is insoluble, repeat expression at a lower temperature.

6. For large scale, inoculate 1 liter of LB medium (with antibiotic) with 10 ml of freshly grown culture. Incubate at 37°C until OD_{600nm} reaches 0.4–0.8. Add 0.5 mM or 1 mM IPTG and express protein using optimal time/temperature determined in a small scale trial.