Gene expression in and protein extraction from *E. coli*

The T7 expression system, e.g., pET (Novagen), is commonly used to generate heterologous (recombinant) proteins. This system is based on the T7 promoter and its cognate T7 RNA polymerase. Gene expression is turned on in the presence of the inducer isopropyl-β-thiogalactoside or IPTG. IPTG induces the transcription of the T7 RNA polymerase gene from the host's chromosome, e.g., BL21 (DE3) *E. coli*. As T7 RNA polymerase becomes more prevalent, transcription from the T7 promoter of the recombinant vector increases. Consequently, the concentration of heterologous protein rises.

Keep cells on ice at all times!

1. Grow overnight seed culture. Inoculate 10 mL Luria-Bertani (LB) media + antibiotic (if any) with single colony. Grow overnight at 37 ºC with shaking (150—200 rpm).

2. Inoculate fresh LB + antibiotic (if any) with 0.5% (v/v) seed culture.

3. Grow at 37 ºC with shaking until OD_{600} = 0.3—0.6.

4. Induce expression with an IPTG final concentration of 0.1—1.0 mM.

5. Incubate at 30 ºC with shaking.

6. For a time-profile of expression, take 1 mL samples at t = 0, 1, 2, 4, 6, 8, 10, and 24 h after induction. For each sample, pellet the cells by centrifuging at 16,000 x g for 2 min, decant the supernatant, and store the spun-down cells at -20 ºC.
   For large-scale protein production, grow until expression is maximum (as determined by time-profile expression), pellet cells, e.g., centrifuge at 5000 x g for 20 min at 4 ºC, and proceed to step 7.

7. Resuspend the cells in lysis buffer. Use 0.2 mL of lysis buffer per 1 mL of culture.

Lysis buffer = 100 mM sodium phosphate, 300 mM NaCl, 10% (v/v) glycerol, 1 mg/mL lysozyme, 1 µg/mL pepstatin A, 1—2 µg/mL leupeptin, pH 8.0

8. Lyse the cells by sonication on ice. Sonicate samples for 30 sec. Small suspensions, e.g., 0.2 mL, require only 1 sonication for 30 sec while larger volumes, e.g., 10 mL, require 3 sonications of 30 sec each.

9. Pellet cell debris from lysis reaction by centrifuging at max speed for 30—60 min.

10. Transfer the cleared lysate (supernatants) to clean tube(s).

11. Proceed onto nickel purification for his-tagged proteins (see protocol "Ni column chromatography for 6X his-tagged proteins") or analyze lysate with SDS-PAGE.