Testing for Recombinant Protein Expression and Solubility in *E. coli*

This protocol is for proteins expressed under the control of the *lac*, *tac*, or T7 promoters.

Transformation:

- Transform 10 μL competent BL21(DE3) cells (or derivatives) with 10 ng of plasmid DNA. Plate the cells on LB-agar containing the appropriate antibiotics. Grow colonies at 37 ºC overnight. See the protocol page for "Transformation of *E. coli*."

If using IPTG induction:

- Inoculate ~10 colonies into a 14-mL tube containing 5 mL of liquid LB and the appropriate antibiotics.
- Grow cells for a few hr at 37 ºC, shaking at 250-300 rpm. Make sure the **tubes are tilted**.
- Watch the turbidity. Once the culture reaches an OD at 600 nm (OD_{600}) of 0.4-0.6 (takes 2-4 hr, depending on the sample), take out 2 mL of the culture. Measure the actual OD_{600}. Aliquot the equivalent of 1 mL of cells at OD_{600} = 0.8 in a 1.5-mL microfuge tube, i.e., volume in mL = $0.8/OD_{600}$ of sample. Spin down at maximum speed for at least 1 min. Carefully remove all of the supernatant with a vacuum line. This is your uninduced sample. Store the cells at -20 ºC.
- Add 3 μL of 1 M IPTG to the remaining culture. Continue shaking at 300 rpm overnight but at a lower temperature of 23 ºC.
- The next day, measure the OD_{600}. Spin down two tubes containing the equivalent of 1 mL of cells at OD_{600} = 0.8 and remove the supernatant. These are your induced samples — one tube will be used to test for expression and the second for solubility. Store the cells at -20 ºC.

If using autoinduction:

- Inoculate ~10 colonies into a 14-mL tube containing 3 mL of ZY-5052 and the appropriate antibiotics.
- Grow cells for at least 4 hr at 37 ºC, shaking at 300 rpm. Make sure the **tubes are upright**.
- Take out 2 mL of the culture. Measure the actual OD_{600}. Aliquot the equivalent of 1 mL of cells at OD_{600} = 0.8 in a 1.5-mL microfuge tube, i.e., volume in mL = $0.8/OD_{600}$ of sample. Spin down at maximum speed for at least 1 min. Carefully remove all of the supernatant with a vacuum line. This is your uninduced sample. Store the cells at -20 ºC.
- Continue shaking the remaining culture at 300 rpm overnight but at a lower temperature of 23 ºC.
- The next day, dilute 200 μL of the culture 10-fold with 1x PBS and measure the OD_{600}. Spin down two tubes containing the equivalent of 1 mL of cells at OD_{600} = 0.8 and remove the supernatant. These are your induced samples — one tube will be used to test for expression and the second for solubility. Store the cells at -20 ºC.

Testing for expression:

- For each construct, take the tube of uninduced and 1 tube of induced cells and resuspend each in 100 μL of 1x SDS-PAGE sample buffer.
- Boil the samples for 10 min, then cool down to room temperature.
- Centrifuge for 5 mins at maximum speed at room temperature.
- Analyze 10 μL of each sample using SDS-PAGE, with western blotting if necessary.

Testing for solubility:

- Take the remaining tube of induced cells and resuspend in 50 μL of B-PER containing protease inhibitors (PMSF or Complete).
- Incubate at room temperature for 10 mins.
- Spin down in a microcentrifuge at maximum speed for 10 min at 4 ºC.
- Carefully transfer all of the supernatant into a new microfuge tube. Add 50 μL of 2x SDS-PAGE buffer. This is the soluble fraction.
- Resuspend the pellet in 100 μL of 1x SDS-PAGE buffer. This is the insoluble fraction.
- Boil the samples for 10 min, then cool down to room temperature.
- Centrifuge for 5 mins at maximum speed at room temperature.
- Analyze 15 μL of each sample using SDS-PAGE, with western blotting if necessary.

Things to look up / think about:

Learn about the *lac* operon. How do lactose and IPTG induce gene expression via the *lac* promoter? How is the *tac* promoter different from the native *lac* promoter?
Learn about the T7 system. What is a DE3 lysogen?
Learn about other promoters used for expression in *E. coli*. What are their advantages or disadvantages relative to the *lac*-based systems?
How does the autoinduction medium work?
How does B-PER work? What is the correlation between a protein's solubility in B-PER and other buffer systems?
What are the factors that affect a recombinant protein's solubility?