Protein Expression in BL21(DE3) Protocol

1. Resuspend a single colony from the transformation plate in 5 mL of LB media containing 50 µg/mL ampicillin (AMP) in a Falcon tube.

2. Incubate at 37 °C with shaking at 260 rpm overnight.

3. Inoculate 200 mL of fresh LB media containing 50 µg/mL AMP with 2 mL of the overnight culture in a 500 mL Erlenmeyer flask.

4. Incubate at 37 °C with shaking at 260 rpm until the OD600 reaches 0.5–0.6 (about 2 hours).

5. Induce protein expression by adding 200 µL of 100 µM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and incubating for an additional 3 hours at 37 °C with shaking.

6. Take 100 µL of the sample for SDS-PAGE.

7. Harvest the cells by centrifugation at 4,000 x g for 10 minutes at 4 °C.

8. For SDS-PAGE:
   - Pellet the 100 µL sample by centrifugation (1–2 min at 13,000 rpm).
   - Resuspend the pellet in 10 µL 2× loading buffer + 10 µL MQ water.
   - Incubate at 95°C for 5 min.
   - Briefly spin down and load 8 µL onto the gel.