Protocol: Enzyme expression in *E. coli* BL21(DE3) from pET-11a

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Reviewed by: Giulia, Rytis

Note: The steps described are starting after successful transformation of *E. coli* BL21 with overnight formation of colonies. For details on transformation in *E. coli* BL21 competent cells with vector, check *E. coli* transformation protocol:
E. coli Transformation - Heat Shock Method.docx

Note: For the activities described in this protocol, **work under sterile conditions**: wipe the lab bench with 70% ethanol, work with bunsen burner to maintain sterility.

Materials

- Host strain: *E. coli* BL21(DE3), transformed with vector containing gene of interest.
- Media: LB ; TB
- Antibiotic: Ampicillin (final concentration 100 µg/mL)
- IPTG stock: 100 mM (filter sterilized)
- Equipment:
    - Shaking incubators*2 (37 °C and 25°C(?), 150–170 rpm)
    - Spectrophotometer (OD_{600})
    - Cuvettes + sterile H_2O
    - Shake flasks
- Consumables: Sterile pipettes, tubes, SDS-PAGE sampling supplies.

Instructions

Day 1 – Starter Culture

1. In the afternoon add **5 mL sterile LB medium + 5 µL of 100 mg/mL ampicillin stock (to obtain a concentration of 100 µg/mL)** in a culture tube (when working with the LB medium, always close the tube lids to avoid contamination)
2. Pick a colony from a plate and inoculate the culture tube. ***Make sure all cell clumps are fully suspended*** !
3. Incubate overnight at **37 °C and 150 rpm** in a shaking incubator (no longer than 16 hours to avoid antibiotic amount depletion).
4. If a large main culture is to be prepared, inoculate several starter tubes.
   *Example: a **500 mL main culture** usually needs **~5–10 mL starter culture**.*
5. **Prepare a control culture as well.**

Day 2 – Main Culture

4. In the morning, place overnight starter cultures **on ice.**
    - If multiple tubes contain the same construct, **pool them** using a pipette.
    - Measure **OD_{600}** (dilute 1:10 in water in the cuvette).

5. **Calculate inoculation volume** needed (this will normalize the inoculation volume based on OD. Use the following standard condition: to inoculate 100 ml media (main culture) inoculate with 0.5 ml started culture with OD600 = 3. For example, to inoculate 500 ml media 6.25 ml starter culture is needed if the OD is 1.2.
    - 0.5 mL starter culture (OD_{600} = 3) → inoculates 100 mL main culture.
    - Example: 6.25 mL starter (OD = 1.2) → inoculates 500 mL main culture.

6. Prepare media in shake flasks:
    - Mix components A and B for TB-media.
    - Add ampicillin to obtain an **appropriate concentration**.

      $$(V_{amp} = C_{stock} \times V_{culture} / C_{final})$$

      C_{final} = final ampicillin concentration (µg/mL); $V_{culture}$ = culture volume (mL); C_{stock} = stock ampicillin concentration (x µg/mL)

      C_{final} should be **100 µg/mL**.

- **Take 1 mL sample before inoculation for blank OD measurement**

7. Inoculate the main culture shake flasks with the calculated starter volume. **Take 1 mL sample for OD measurement at time zero**
8. Incubate at **37°C, 170 rpm** in a shaking incubator.
9. Turn on another shaker incubator and keep it at the induction temperature of the insert. (37°C for us?)
10. Monitor cell growth via **OD_{600}** (10× dilution with water in the cuvette). Take OD measurements every 30 minutes until induction (doubling time in growth phase around 30 minutes). Post induction, measure OD every 30 minutes. Ensure appropriate dilution such that the OD never goes beyond 0.3. (i.e. if OD at t=n is 0.25, for t=n+1, take dilution of 1:10)

    Prepare cuvettes with H_2O to facilitate sampling, follow the separate SOP (see "Taking samples from E. coli cultivations for SDS-PAGE" for SDS-PAGE sampling).

11. **Induction point:**
    - **TB-medium** induce at **OD_{600} = 1.5**.
    - **LB medium**: induce at **OD_{600} ≈ 0.7**
    - Consider that measuring OD takes some time and that the cells grow rapidly in the meantime. You may need to induce the cells before you actually can read OD 1.5 in step 9. Try to predict a suitable timepoint based on previous measurements.

Regarding induction time, for future reference: doubling time of E. coli is ~30 mins, and the OD600 should be 'foreseen' by previous measurements and growth curve, so in the future if doing it, it would be good to take continuous measurements and construct this growth curve

You can use this file to log the curve values (OD/time of cultivation) protein expression data logging.xlsx and the table in Appendix at the end of this document.

Induction

11. At induction:
    I. Take a sample from each flask for OD measurement (transfer to cuvette with H_2O; **measure later**).
    II. Collect an **uninduced sample** for SDS-PAGE from the control culture. **DO NOT INDUCE THE CONTROL.**
    III. Add **100 mM IPTG stock** to reach a final concentration of 0.25 mM. (**V_{IPTG} = 0.0025 × $V_{culture}$** i.e. 250 µL of IPTG for 100mL culture). Return flasks to **170 rpm** incubation at the **desired temperature of induction.**

Expression Monitoring

12. Collect samples for SDS-PAGE:
    - Take samples **every hour** after induction **until a minimum of 3-4 h after induction**, and **after overnight cultivation;**
    - Follow the separate SOP for SDS-PAGE sampling.

Log all values in the provided table to facilitate following calculations.

Post-Culture

13. After overnight growth, cool cultures (ideally to **4°C**) and follow the **acid heat treatment SOP**.

Harvesting Cells for Enzyme Expression

Materials:

- Cultured cells (100 mL total per sample)
- 50 mL Falcon tubes (×2 per sample)
- Centrifuge capable of 5,000 × *g*
- Freezer (–20 °C)

Procedure:

1. Transfer 50 mL of the culture into a 50 mL Falcon tube.
2. Centrifuge at 5,000 × *g* for 5 minutes at room temperature.
3. Discard the supernatant carefully without disturbing the pellet.
4. Transfer the remaining 50 mL of the culture into another 50 mL Falcon tube.
5. Centrifuge again at 5,000 × *g* for 5 minutes.
6. Combine the pellets or keep them separate as needed.
7. Store the pellets at –20 °C until further use.
    - *Note: Proceed with enzyme purification only if SDS-PAGE results are satisfactory.*
    - *Penning protocol: washes the pellet, THEN stores at -80C (resuspend each pellet in 50 mL of sonication buffer, then pool slurries into two bottles and centrifuge at 6500 rpm for 15 min at 4 °C)*

Induction condition

Gene	Study (year)	OD_{600}	IPTG	Induction Temp	Time	Outcome
ci2350	ci2350 pap… (2025)		0.4 mM	25 °C	Overnight (~16h)	
ci2350 with solubility tags	N/A		0.4 mM	25 °C	~16h	
AKR1D1	Steroid 5β-… Penning (2023)		1 mM	37 °C	14 h	
AKR1D1 with solubility tags	N/A		1 mM	37 °C	14 h	
atcABC	⑤Four Mol… (2018)	0.7	1 mM	°C	h	
			mM	°C	h	

Appendix

OD measurements logging

Host organism: E.coli BL21(DE3)
Plasmid: pET11a
Enzyme:
Solubility tag:
Inoculum volume mL:
Medium volume mL:
Inoculum OD:
Medium OD:
Shaking speed:

Time	Cult time	OD	DF	Actual OD	Volume	lnOD