# Experiment 7: Protein expression and SDS-PAGE (I)

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**Purpose**
• To express green fluorescence protein (GFP)
• To make SDS-PAGE gel for the next experiment

**Materials**
• LB media (LPS solution, LB-05)
• 1M isopropyl-β-D-thiogalactopyranoside (IPTG) (GOLDBIO, I2481C100)
• 40 % Acrylamide:Bis solution (BioRad, 1610144)
• 10 % ammonium persulfate (APS) (Sigma Aldrich, A7460)
• 10 % sodium dodecyl sulfate (SDS) (BIOMAX, BS001)
• tetramethylethylenediamine (TEMED) (Sigma-Aldrich, T9281)
• 1.5 M Tris-HCl (pH8.0)
• 1.0 M Tris-HCl (pH6.8)
• Ethanol

**Machines to use**
• SDS-PAGE gel electrophoresis kit (BioRad)
• UV spectrometer

**Principles**
**1. Cell growth curve & doubling time**

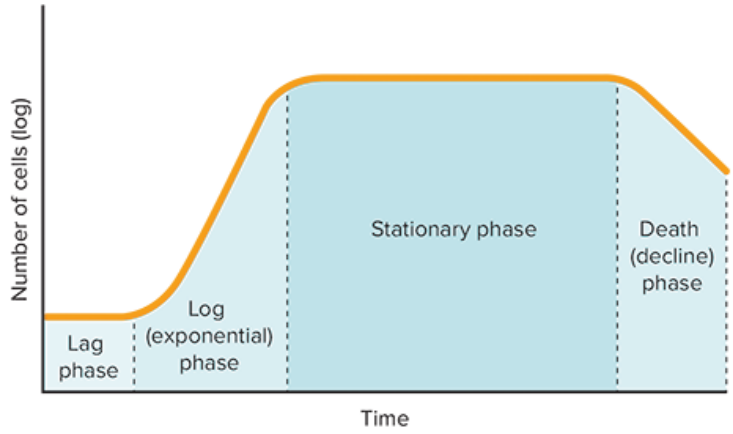


Figure 1 Cell growth curve

The cell growth curve shows the number of cells as time passes**.** The curve is divided in four phases: lag phase, log (exponential) phase, stationary phase, and death phase.

At log phase, cell grows exponentially, showing cells are healthy. Doubling time is the time taken to the number of cells increases in twice, and it can be measured in the log phase.

**2. OD600**

OD600 indicates the optical density of a sample measure at a wavelength of 600 nm. OD600 is measured to estimate the density of growing bacteria cell. 600-nm wavelength light does not affect the cell growth.

**3. BL21(DE3) and IPTG induction**

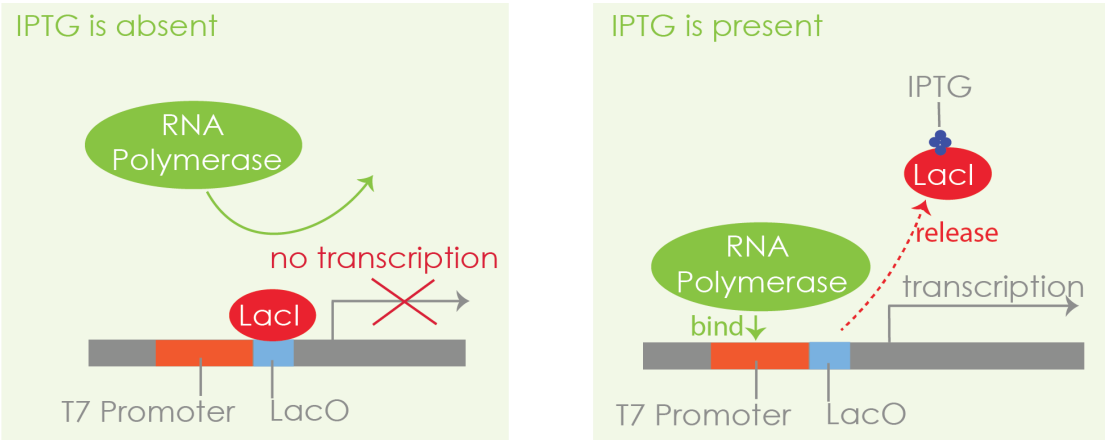


Figure 2 Principles of protein overexpression by IPTG induction

IPTG is a compound mimicking allolactose and bind to LacI protein like allolactose. IPTG induction is a method of regulating protein synthesis by triggering transcription of the lac operon. BL21(DE3) strain contains LacI and T7 RNA polymerase genes, and the T7 RNA polymerase gene is at the downstream of *LacO* sequence. When IPTG is absent, LacI binds to the *LacO*, but the LacI is released from *LacO* upon IPTG binding. Then, T7 RNA polymerase can be transcribed.

Our target protein sequence (in this experiment, GFP) is cloned in pET vector (Cloning GFP gene in the pET vector is what you did in experiment 6 and 7 through PCR, RE digestion, and ligation). pET vector genes can be transcribed by T7 RNA polymerase. (Adding IPTG → T7 RNA polymerase made → GFP gene in the pET vector transcribed)

**4. SDS-PAGE (polyacrylamide gel electrophoresis)**

SDS (sodium dodecyl sulfate) is a detergent that denatures proteins. It binds to the amino acid backbone and provides negative charges to the denatured (unfolded) protein proportional to the length of amino acid chain. The negatively charged peptide chain moves from the top to the bottom of the polyacrylamide gel according to the

electric field applied to the gel. The moving distance of the proteins is inversely proportional to the length (the molecular weight) of protein (Figure 3).

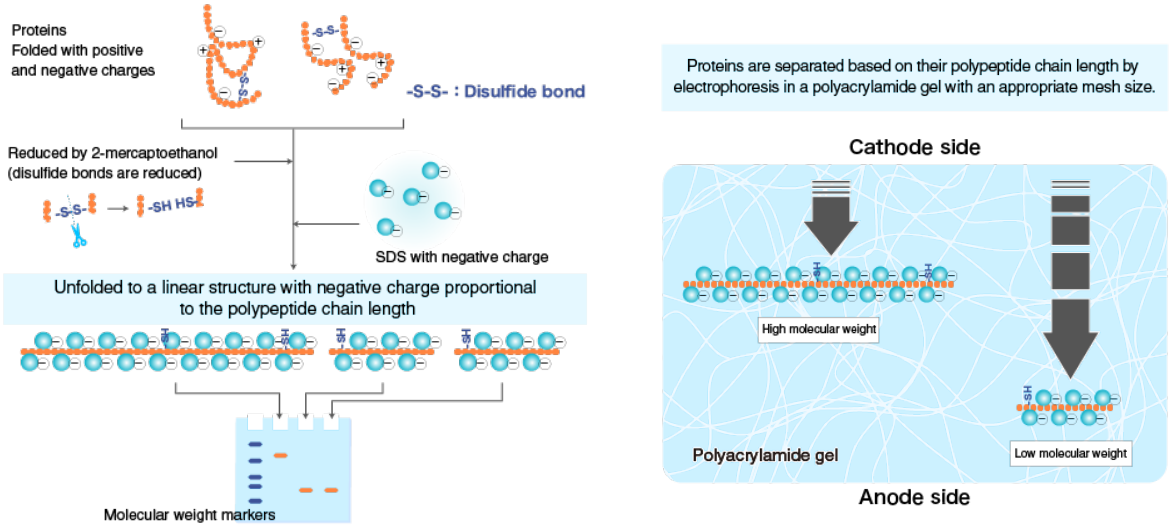


Figure 3 Principles of SDS-PAGE

In making polyacrylamide gel, APS and TEMED catalyze the polymerization of acrylamide. Stacking gel has larger pore size compare to resolving gel, so protein can move faster. It aligns the sample and makes proteins to enter to the resolving gel area at the same line. Resolving gel is for protein separation by protein size.

**Procedure**
**<Large culture>**

(Day1)
1. Pick a colony from the saved agar plate (BL21(DE3), +Amp) and inoculate it to 10 mL LB + 100 ug/mL Ampicillin (starter culture). Grow the culture at 37'C overnight (O/N) (14~18 hr)

(Day2)
2. Add the 10 mL starter culture to 250 mL LB + 100 ug/mL Ampicillin, and grow at 37 °C, 200 rpm shaking incubator until OD600 reaches 0.6~0.7
   ❖ Need to measure OD600 frequently to check the cell growth.

3. Add 1 mM IPTG (250 uL of 1 M IPTG) to the culture, and incubate at 37 °C for 4 hr

4. Harvest the cells by centrifugation at 4000 rpm, for 30 min, 4 °C

5. Transfer the cell pellet into 50 mL tube and store the pellet at – 80 'C.

**<Making SDS-PAGE gel> <u>(Day2, during step4, takes 1~2 hr)</u>**

1. Build up the SDS-PAGE gel making cassette.

2. Mix the gel component as follows (Resolving and stacking gel solution):

| Resolving gel (13 %) | | | Stacking gel |
|---|---|---|---|
| 2.7 mL | $H_2O$ | | 2.7 mL |
| 2.1 mL | 40 % Acrylamide-Bis solution | | 0.7 mL |
| 1.7 mL | 1.5 M Tris (pH8.8) | 1.0 M Tris (pH6.8) | 0.5 mL |
| 66 uL | 10 % SDS | | 40 uL |
| 66 uL | 10 % APS | | 40 uL |
| 6.6 uL | TEMED | | 4 uL |
| 6.6 mL | Total volume | | 4 mL |

3. Pour the resolving gel solution using 1000p pipette and add 500 uL of 100 % EtOH on the top of the resolving gel. Wait until the gel solidified. (20 min)

- ❖ Make sure the cassette does not leak by pouring water between the gel plates before you pour the gel solution.
- ❖ Resolving gel takes ~70 % of the whole gel volume
- ❖ Adding EtOH makes the gel surface flat

4. Remove the EtOH by tilting the gel cast onto a paper towel and pour the stacking gel solution using 1000p pipette.

5. Insert a comb to the gel cast and wait until the gel solidified. (20 min)

6. After solidifying, remove the comb and store the gel at 4 ‘C for next experiment

**Result**

1. Describe your experiment experience by adding details to the procedure above and your observations.

2. How does the color of the cell culture changes as the cells grow? How does the OD600 increase?

3. Add the picture of harvested pellet and the SDS-PAGE gel you made.

**Analysis**

1. Change of OD600: Can you draw the growth graph? How long does it take to grown in twice? (doubling time)

## Discussion

1. Is there any other method for protein expression induction except IPTG induction?

2. Why do we add IPTG when OD600 = 0.7 (at log phase)? (Related to cell growing curve.)

3. Why do we need to grow cell at shaking incubator? What is the characteristic of cells that need ‘shaking’ when they grow up? Is there any other cell growing method without shaking?

4. For protein analysis, we used polyacrylamide gel. For nucleic acid analysis, we used agarose gel. How do they differ? Why do we use polyacrylamide and agarose gels for proteins and nucleic acids, respectively?

## Reference

https://www.moleculardevices.com/en/assets/app-note/br/measure-long-term-cell-growth-using-a-discontinuous-kinetic-reading#gref (Cell growth curve)
https://en.wikipedia.org/wiki/OD600 (OD600)
https://www.goldbio.com/articles/article/how-does-iptg-induction-work (IPTG induction)
https://ruo.mbl.co.jp/bio/e/support/method/sds-page.html (SDS-PAGE gel)