Gene Expression in the bacteria *Escherichia coli*

1. **Induction of maltose-binding protein (MBP) in bacteria**
2. **Isolation of total protein from bacteria**
3. **Separation of proteins by SDS-PAGE**

Theory:

Escherichia coli is widely employed for expression and purification of foreign proteins. In this exercise you will induce expression of maltose-binding protein (MBP) and monitor its induction in *E. coli* by separating proteins in SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis).
You will then, in the Next Class further confirm the induction by looking at the Expressed Proteins in cells by lysing the cells and then separating proteins in SDS-PAGE (sodium-dodecyl sulfate polyacrylamide gel electrophoresis).

You will be provided with logarithmically growing culture of *E.coli* BL21 strain transformed with a plasmid encoding MBP. *E. coli* BL21 strain lacks proteases, thus it allows accumulation of foreign proteins in large amount. The plasmid harbors ampicillin resistance marker for its selection in bacteria. The coding sequence of MBP is under the indirect regulation of the Lac Promoter - a promoter induced by lactose, which works on the principle of *lac operon*. However, more robust induction of proteins can be obtained by IPTG, a non-hydrolysable analog of lactose.

Following expression, cells are lysed and total proteins are isolated. The induction of proteins will be monitored by separating proteins by SDS-PAGE. This is a technique for separating proteins based on their molecular weight. It is achieved first by denaturing protein with the detergent SDS to remove secondary and tertiary structures of proteins. SDS, in addition, coats proteins with uniform negative charge. Under electric field, proteins can then be separated/resolved into the pores of acrylamide based on their sizes.

MATERIALS:

- *E. coli* strain BL21 transformed with a plasmid expressing MBP protein. Logarithmically growing culture in LB media containing ampicillin of above bacteria.
- 0.05M IPTG (Isopropyl β-D-1-thiogalactopyranoside) for induction of MBP. IPTG is a non-hydrolysable lactose analog.
- Incubator, micropipettes, microfuge tubes, vortex machine
- Cell lysis buffer (Na-Phosphate buffer pH 7.5, non-ionic detergents). Bacteria contain DNA and RNA, which should be digested for isolation of proteins of good quality.
- Protein sample buffer (50mM Tris-HCl pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue).
- Coomassie brilliant blue staining solution (0.1% Coomassie Brilliant Blue R250, 50% Methanol, 10% Acetic acid, 40% water)
- Distaining solution (Water:Methanol:Acetic Acid = 50:40:10)

PROCEDURE:

1. In a group of four students, prepare total four samples for the induction and expression of MBP as per the following table. One student should prepare at least one sample. Add required amounts of IPTG and mix by tapping the tubes. Incubate the samples at 37°C for the given time duration.
2. Aliquot the bacterial culture into 2 ml Microcentrifuge tubes (NOT 1.5 ml). This ensures better aeration.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Volume of culture (in mL) (0.8 – 1.0 OD)	0.5	0.5	0.5	0.5
Final IPTG concentration (mM)	0	0.1	0.5	1
Volume of 0.05M IPTG to be added (µ l)	0	1	5	10
Time of incubation at 37°C (in hrs)	1	1	1	1

3. After induction harvest the cells by centrifugation at 6000 rpm for 2 min.
4. Carefully discard the supernatant containing LB media. Centrifuge once more. Remove all traces of LB using a pipette.
5. Add 100µl of cell lysis buffer. Mix the cells by vortexing or pipetting. Incubate at 37°C for 15 minutes.
6. Add 100µl protein sample buffer. Heat at 95°C for 10 minutes.
7. Store at -20°C.

In the next class:

Run SDS- PAGE to quantitate the level of expression of MBP after IPTG induction.

1. Vortex the samples. Heat at 95°C for 1 minute. Centrifuge at 6000 rpm for 2 min.
2. Load 10µl of each sample into the gel lanes of SDS-PAGE (SDS-PAGE gels will be provided by the teaching assistants). Load protein ladder as reference into another lane.
3. Run the gel by applying a potential of 80 Volts for the initial 20min followed by 100 Volts until bromophenol blue reaches the bottom of the gel.
4. Remove the gel carefully from the plate assembly.
5. Stain the gel with Commassie Brilliant Blue (CBB) staining solution.
6. Destain the gels with destaining solution. Observe the induction of MBP protein. Note down the approximate size of the Induced MBP protein.