Applied Biology and Bioengineering Laboratory (BT520)

EXPERIMENT 8

PROTEIN EXPRESSION ANALYSIS by SDS-PAGE

Purpose Recombinant protein expression and analysis by SDS-PAGE.

Principle

The expression vectors contains IPTG inducible promoter. The gene encoding a protein when cloned to an expression vector can be expressed by the induction using IPTG. The *E. coli* BL-21 cells containing recombinant expression plasmid are grown to mid-exponential phase and then IPTG is added to the growing cells to induce the protein expression. The cell samples before and after the IPTG induction can then be analyzed for protein expression by SDS-PAGE analysis.

Materials requirement

E. coli BL-21 cells harbouring the expression plasmid containing the target protein

LB medium ingredients, IPTG, Ampicillin, SDS, Bromophenol Blue, Coommassie Brilliant Blue, Acrylamide, Bis, Tris-HCl

Buffers and reagents for SDS-PAGE (Provided)

Blue and yellow sterile tips, sterile 1.5 ml eppendorf tubes, floating racks, sterile 15 ml test tubes, conical flasks, micropipettes.

Equipment

Incubators shaker 37°C, Protein electrophoresis kit and power supply, boiling water bath, micro-centrifuge,

PROCEDURE

Day 0

1. Inoculate 50 µl of *E. Coli* BL-21 cells harboring the recombinant plasmid to 5 ml LB medium supplemented with Ampicillin (100 µg/ml) at 5 pm and incubate in shaker at 37ºC, 200 rpm for 12h.

Day 1

I. Culture

1. Inoculate 1 ml of 12h grown *E. coli* culture to 100 ml LB medium supplemented with ampicillin (100 µg/ml) contained in conical flask at 12 Noon and incubate in shaker at 37ºC and 200 rpm for 4-6 h.
2. Periodically (every hour) check the OD at 600 nm till it reaches to mid exponential phase A600 = 0.4 - 0.6. (It takes around 4-6 h)
3. At this point take a broth sample (1 ml) for analysis and keep in refrigerator
4. Add IPTG to the remaining broth in flask to a final concentration of 1 mM and incubate the flask culture at 5-6 P.M. for further 10-12 h for induction at 37ºC and 130 rpm.

II. Polymerize 10% SDS-Gel

Wrap the gel casted plates in cling film and keep in refrigerator till use.

Day 2

Sample preparation and SDS-PAGE

1. Centrifuge 200 µl of the un-induced and induced cell samples, discard the supernatant and re-suspend the cells in 40 µl buffer and 80 µl buffer and add 10 and 20 µl of 5X sample buffer, respectively. The samples are boiled for 3 min and run on 10 % SDS PAGE
2. The gels are stained with Coomassie Brilliant Blue R-250.
3. The expressed protein is compared in un-induced and induced cells samples in the lanes of the gel.
4. The size of the expressed protein can be analyzed by comparing with protein marker.

Observation

Result