Molecular cloning and genetic engineering - Induction of Recombinat Protein

Aim

Like allolactose, IPTG binds to the lac repressor and releases the tetrameric repressor from the lac operator in an allosteric manner, thereby allowing the transcription of genes in the lac operon, such as the gene coding for beta-galactosidase, a hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides. But unlike allolactose, the sulfur (S) atom creates a chemical bond which is non-hydrolyzable by the cell, preventing the cell from metabolizing or degrading the inducer. It is a highly active inducer of β-galactosidase activity, which is not metabolized by bacteria and is very stable. Therefore, IPTG has been widely used in laboratories.

Material

LB media

kanamycin

IPTG

phosphate buffer

Procedure

1. Vector construction: pET-28a (+) expression vector is used in this study.

The cloned receptor bacteria are E. coli DH5α and the expressed host is E. coli BL21 (DE3).

2. A single colony of transformed recombinant plasmid is inoculated in LB liquid medium (containing 100 ug/ml kanamycin) and cultured overnight at 37 ℃.

3. 1 ml overnight culture is transferred to 100 ml liquid culture medium containing 100 ug/ml kanamycin and cultured at 37℃ for 1.5-2 hours to logarithmic growth period.

4. IPTG is added to the culture until the final concentration was 0.2 mM, and the expression is induced according to the pre-established optimal expression conditions.

5. 1200 rpm, centrifuge for 2 minutes, and collect bacteria.

6. The supernatant was discarded and 500 ml Starting buffer (20 mM phosphate buffer (Na_2HPO_4 and NaH_2PO_4), 200 mM NaCl, 10% glycerol and pH 7.0) is added to the sediment to suspend and wash the bacteria.

7. 12000 rpm, centrifuge for 2 minutes, and collect bacteria.

8. 15 ml of starting buffer is added to the sediment to re-suspend the bacteria.

9. Under ice-bath condition, the bacterial liquid is cooled by ultrasonic

treatment for 3 minutes at intervals of 1 minute per 30 sec. The output strength is 5-6 and the frequency is 60-70%. No bubbles are observed during the treatment, overheat is not allowed, and the bacterial liquid become clear as the termination criterion.

10. 12000 rpm, centrifuge at 4℃ for 20 min, the supernatant is moved to a 50 ml sterilized centrifugal tube. SDS-PAGE is used to analyze the protein expression. If the target protein is distributed in the supernatant, the following purification operation is continued.