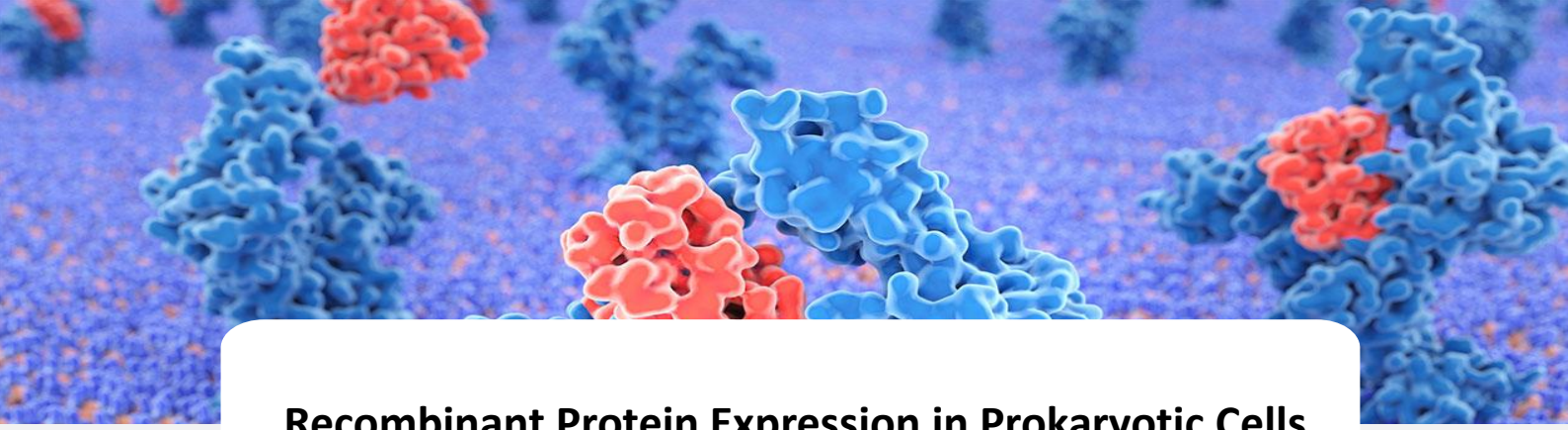

Recombinant Protein Expression in Prokaryotic Cells

Alpha Lifetech

1. Introduction

Prokaryotic cells (BL21(DE3)) require an inductor, IPTG, for protein expression after plasmid transformation. BL21(DE3), as a commonly used prokaryotic expression cell, which is selected by most researchers due to its advantages of short cycle and low cost. The basic flow of recombinant protein expression in prokaryotic cells is shown in Figure 1.

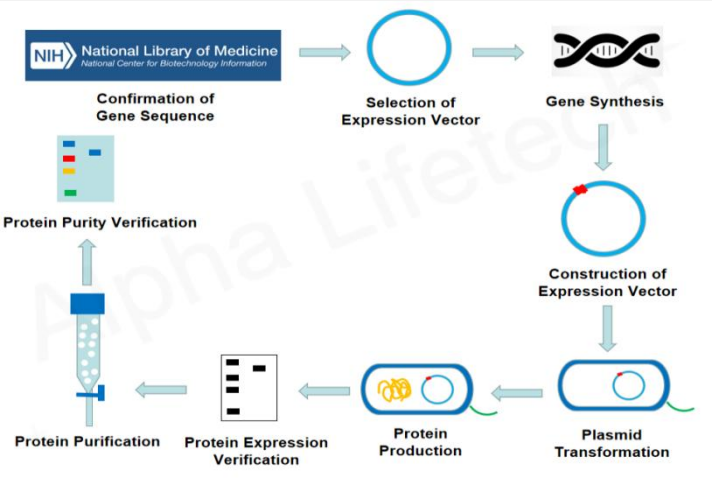

Fig 1 Recombinant Protein Expression in Prokaryotic

2. Q&As for Recombinant Protein Expression

Q1: What if the soluble expression cannot be expressed?

A1: Corresponding measures are as follows:

In order to promote soluble expression of protein, fusion tags can be added to the N or C terminus of the target protein, commonly including GST and SUMO tags. However, considering the downstream application of protein, label removal should be carried out in the later stage.

Q2: What are the causes of low protein expression?

A2: Possible causes:

(1) Before induction, the protein is toxic. Corresponding measures are as follows:
Control the background expression level:

A. Use tightly controlled plasmids to reduce copy number.
B. lac-based promoter expression with glucose added.
C. Glucose was selected as the carbon source medium.
D. based on T7-based promoter expression, plasmid containing T7 lysozyme was used.

(2) After induction, the protein becomes toxic. Corresponding measures are as follows:
Control induced expression level:

A. An adjustable promoter is used.
B. Controllable inducible strains were used.
C. Reduce copy number.
D. Suitable virulent protein expression strains were used.
E. Secretory expression strategy was adopted.

(3) Codon preference. Corresponding measures are as follows:

A. Optimization of plasmid DNA codons to fit the host codon system.
B. Codons were used to adjust the strain.
C. Add new substances: Try a new medium; Improve ventilation condition and avoid soaking.

Q3: What causes inclusion bodies to form?

A3: Possible causes:

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(1) Incorrect disulfide bond formation. Corresponding measures are as follows:

A. Secretory expression strategy was adopted.

B. E.coli, which has cytoplasmic oxidative function, is used.

(2) Incorrect folding. Corresponding measures are as follows:

A. Co-expressed molecular chaperones.

B. Supplemented with medium containing compound chaperones and cofactors.

C. Remove the inducer and add fresh medium: Low temperature induced expression; Adjust the inducer concentration.

(3) Low soluble protein is formed. Corresponding measures are as follows:

A. Change the fusion chaperone protein and promote the expression of soluble protein.

(4) Post-translational embellishment is crucial. Corresponding measures are as follows:

A. Changes in the expression host, such as prokaryotic changes in the eukaryotic expression system.

Q4: What's the reason for the lack of activity?

A4: Possible causes:

(1) Incorrect folding. Corresponding measures are as follows:

A. Low temperature induced expression.

B. Monitor disulfide bond formation and in vitro folding.

(2) cDNA mutation. Corresponding measures are as follows:

C. Sequencing before and after plasmid induction.

D. The recA− strain was used to ensure plasmid host stability.

E. Changes in the expression host, such as prokaryotic changes in the eukaryotic expression system.

3. Characteristics of prokaryotic expression systems

(1) Clear genetic background.

(2) Rapid propagation.

(3) Low cost.

(4) High expression.

(5) Easy purification of expression products.

(6) Good stability.

(7) Strong anti-pollution ability.

(8) Wide application range.