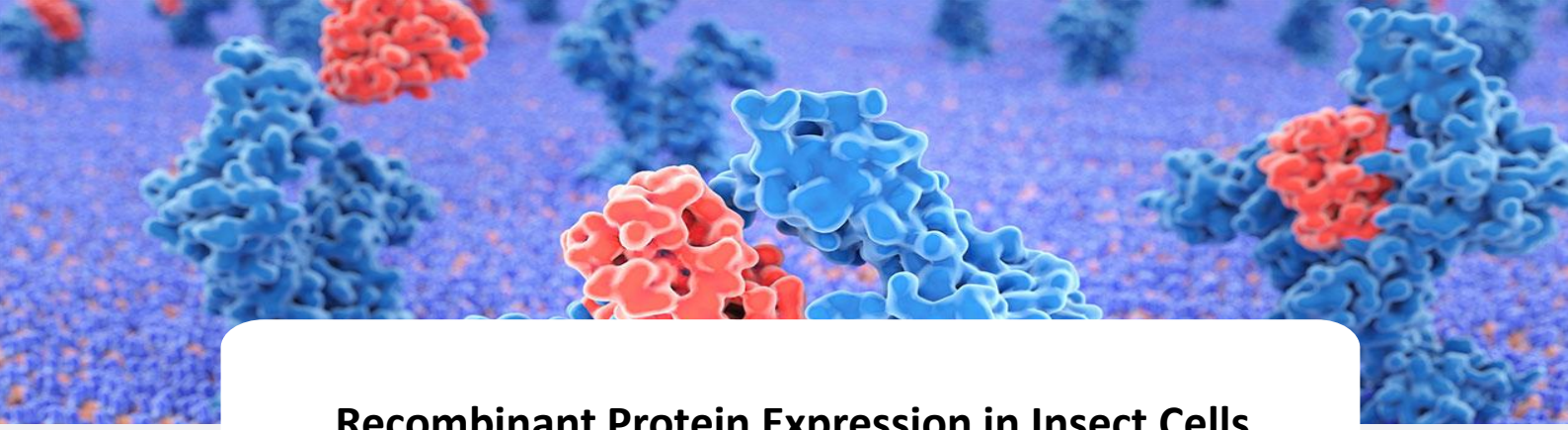

Recombinant Protein Expression in Insect Cells

Alpha Lifetech

1. Introduction

Insect cells require an intermediate, baculovirus, for protein expression. Baculoviruses are a diverse group of DNA viruses that are capable to infect various (>600) insect cells. They serve as a shuttle for the introduction of the target gene into a given host cell. A flow chart of the process is summarized in Figure 1. Briefly, a gene encoding the protein-of-interest is inserted into a primary vector, which is subsequently cloned into a secondary vector called “Bacmid”. The Bacmid is transferred into a bacteria strain (commonly E.coli) for preliminary virus production and assembly to acquire generation 1 baculovirus (P1). The P1 virus is amplified in an insect cell (e.g. sf9) to reach the suitable titer (P2) and the P2 virus is then used to infect the same or a different insect cell line (e.g. High-five) for protein expression.

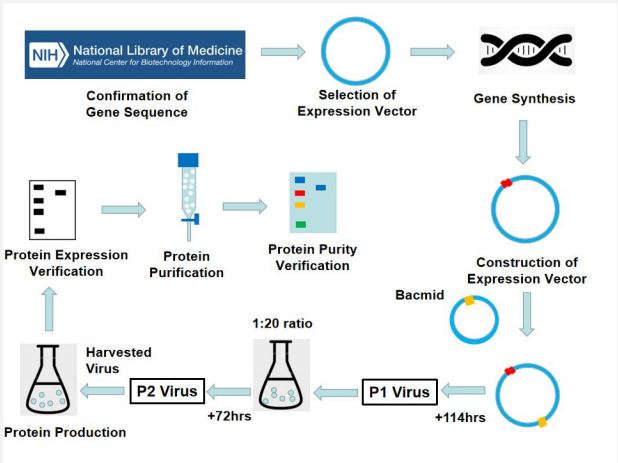

Fig 1 Recombinant Protein Expression in Prokaryotic

2. Q&As for Recombinant Protein Expression

Q1: When is baculovirus (insect) expression system used?

A1: Baculovirus (insect) expression is ideal for the production of mature, folded and processed recombinant proteins. In contrast to E. coli, insect cells are able to integrate post-translational modifications, and they are also particularly suited to expressing secreted proteins.

Q2: Why the target protein "disappears" during purification

A2: Possible causes:

(1) The protein may have been degraded by the protease. Corresponding measures are as follows:

A. Protease inhibitors were added to the sample and buffer to prevent proteolytic digestion.

(2) Protein adsorption by filtration during sample preparation. Corresponding measures are as follows:

A. Use different membrane types of filters. Regenerated cellulose, PVDF, and PES generally exhibit low protein binding and are a good starting point to determine the most appropriate membrane type for your application.

(3) Samples of precipitation. It may be caused by salt removal or inappropriate buffering conditions.

(4) Hydrophobic protein is still attached to the ligand. Corresponding measures are as follows:

A. Use a release agent, polarity reducer, or cleaner.

Q3: Will affinity tags be used to purify proteins? What if you need to delete the label?

A3: We will use an affinity tag (such as His or GST) to purify your protein. For proteins that are more difficult to express, we can also try other tags (including SUMO or thioredoxin) to increase solubility and expression. If you have a need, we can cut the label and provide the label-free protein for delivery as the final product.

+1 609-736-0910
www.alpha-lifetech.com
802 N 3th St, Ocean City, NJ 08226, USA

Q4: Suggestions on the use of experimental materials for insect expression systems

A4: Corresponding suggestions are as follows:

(1) The recommended insect cell is Sf9.

(2) Serum-free medium was used.

(3) For transfection, cells in logarithmic growth phase should be taken, and the viability requirement should be greater than 95%.

Q5: How many steps are involved in Bac-to-Bac insect baculovirus expression?

A5: The steps are as follows:

(1) The target gene was cloned into pFastBac vector.

(2) The above plasmids were transformed into DH10Bac strain to prepare recombinant Bacmid.

(3) Recombinant Bacmid was transformed into insect cells to prepare recombinant baculovirus.

(4) Baculovirus was amplified to infect insect cells and express recombinant protein.

Q6: What are the characteristics of the pFastBac 1 vector?

A6: Characteristics of the pFastBac 1 vector are as follows:

(1) It contains a strong AcMNPV polyhedrosomal protein promoter (pPH), which promotes high protein expression.

(2) Multiple MCS for easy cloning.

3. Characteristics of insect expression systems

(1) Histones have complete biological functions, such as proper protein folding and matching of disulfide bonds.

(2) Post-translational processing and modification of protein.

(3) The expression level was high, up to 50% of the total protein.

(4) An insert that can accommodate a macromolecule.

(5) Can express multiple genes at the same time.