Baculovirus-Efficient Tool for Protein Expression

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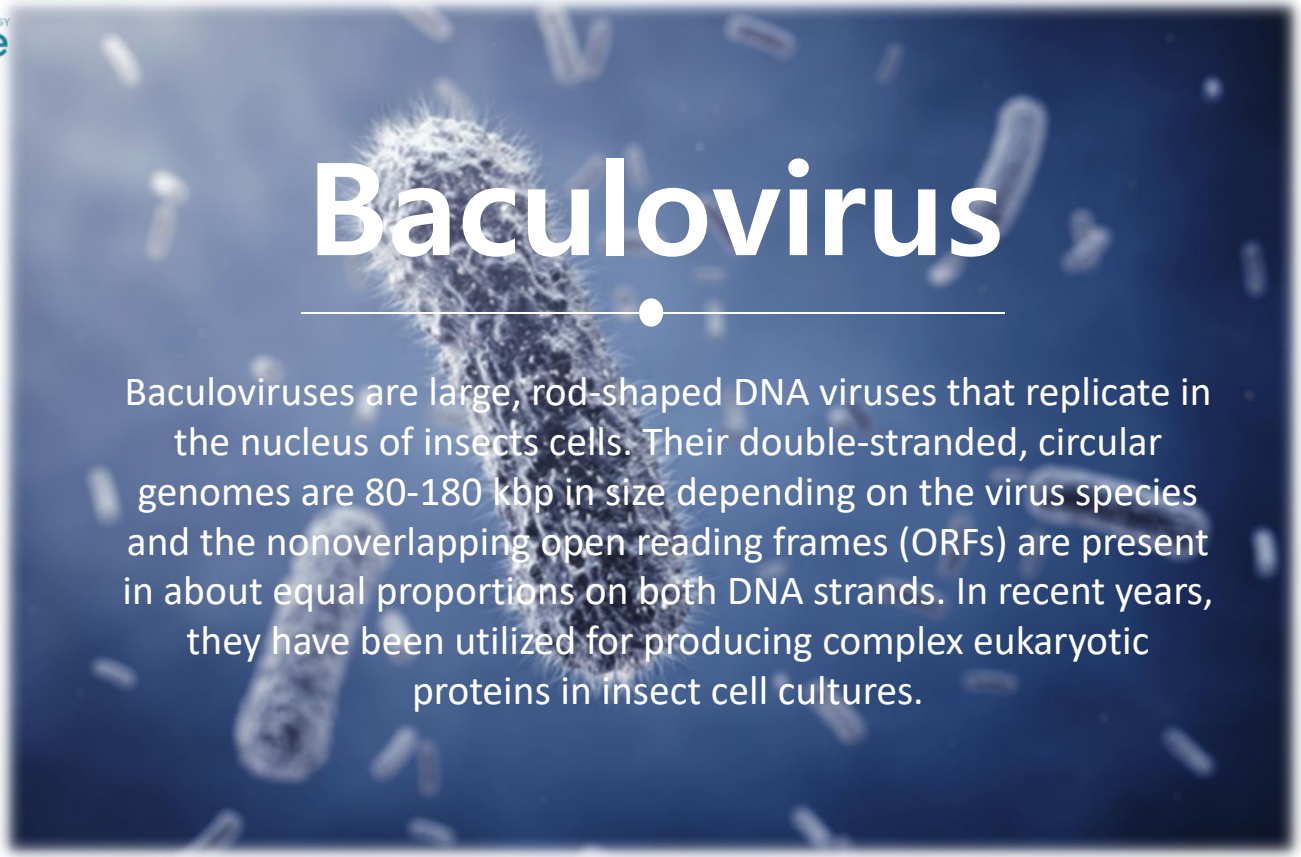
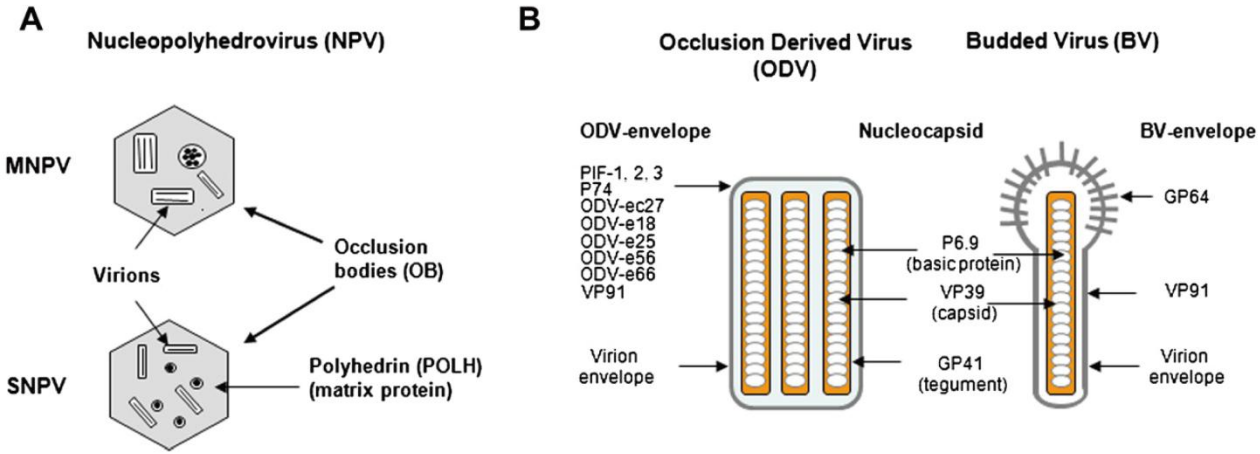


Baculovirus

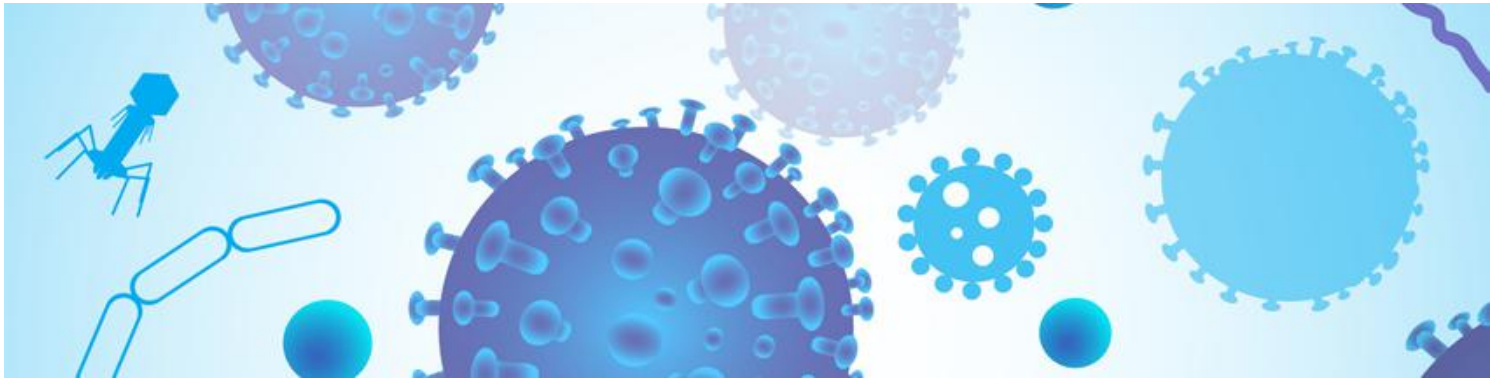
Baculoviruses are large, rod-shaped DNA viruses that replicate in the nucleus of insects cells. Their double-stranded, circular genomes are 80-180 kbp in size depending on the virus species and the nonoverlapping open reading frames (ORFs) are present in about equal proportions on both DNA strands. In recent years, they have been utilized for producing complex eukaryotic proteins in insect cell cultures.

Overview of Baculovirus

Baculoviruses are characterized by having two different virion types (Figure 1A and B): budded virions (BVs) that bud from the cell membrane and spread infection from cell to cell in an infected host insect and a second type, called occlusion-derived virus (ODV), which is assembled entirely in the nucleus of infected cells and is occluded in large proteinaceous occlusion bodies (OBs). Depending on the genus, baculoviruses occlude their nucleocapsids in granular OBs carrying only one virion (*granuloviruses*, genus *Betabaculovirus*) or in large polyhedral shaped OBs that may harbor over 100 virions (NPVs, genera *Alphabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus*).



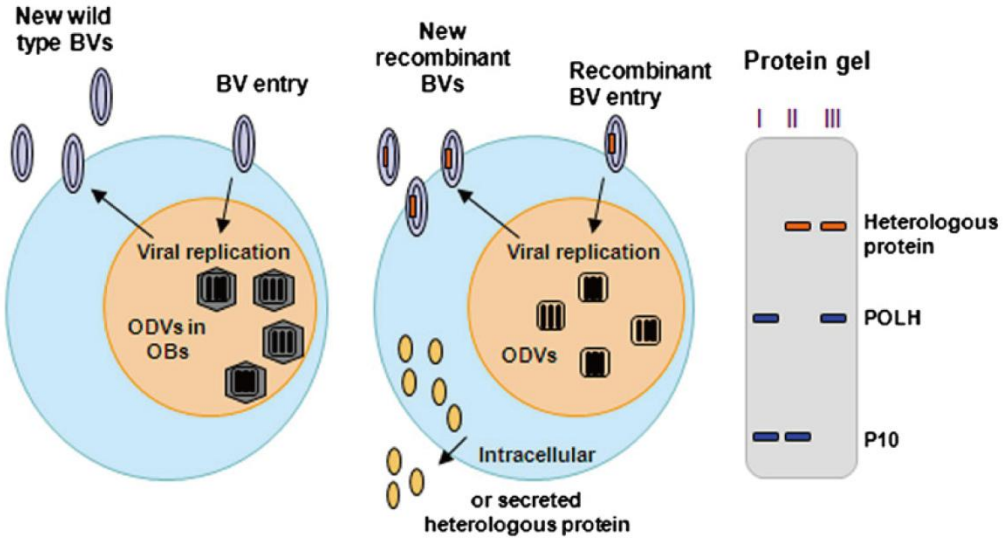
Overview of Baculovirus



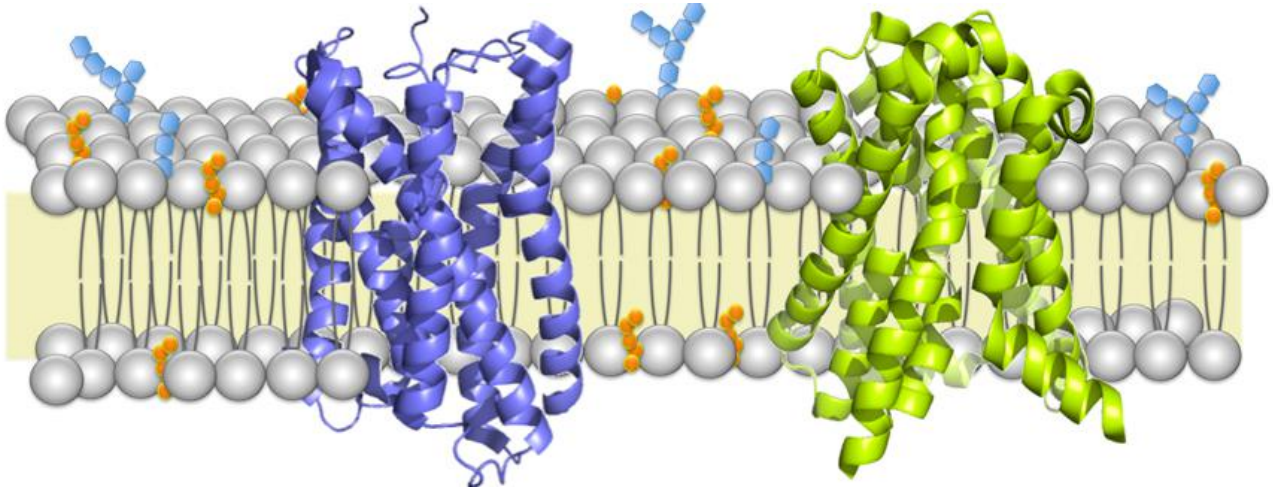
In contrast to most other DNA viruses, baculovirus gene expression occurs in four phases: immediate-early, delayed-early, late and very late. In the very early phase, host-encoded RNA polymerase II transcribes immediate early-genes that activate delayed-early and late genes. In the early phase, genes are transcribed, which encode proteins required for DNA replication and late gene expression. Proteins that prevent host defense mechanisms, such as inhibitors of apoptosis are also made in the early phase. In the late phase, the DNA is being replicated and proteins needed for virion assembly and virus budding, are produced including nucleocapsid and viral envelope proteins. In the very late phase, the virions are occluded and two proteins, polyhedrin (ca. 33 kDa) and a 10 kDa protein (P10), are produced in very high amounts.

The principle of the BES

The P10 and polyhedrin proteins are needed to complete the infection cycle in a larval population via horizontal transmission, but are not required to produce BVs, the form of the virus that is responsible for the systemic infection of the larva and for infection of insect cells in culture. As a consequence, the polh and p10 promoters can be used to drive the expression of foreign genes in cultured cells. This forms the basis of the baculovirus insect cell expression system. These very late promoters, containing a canonical TAAG transcription initiation site, can also be exploited in the presence of the polh and p10 genes, by adding single or multiple promoter constructs with foreign genes into the baculovirus genome, which facilitates protein production in insect larvae.

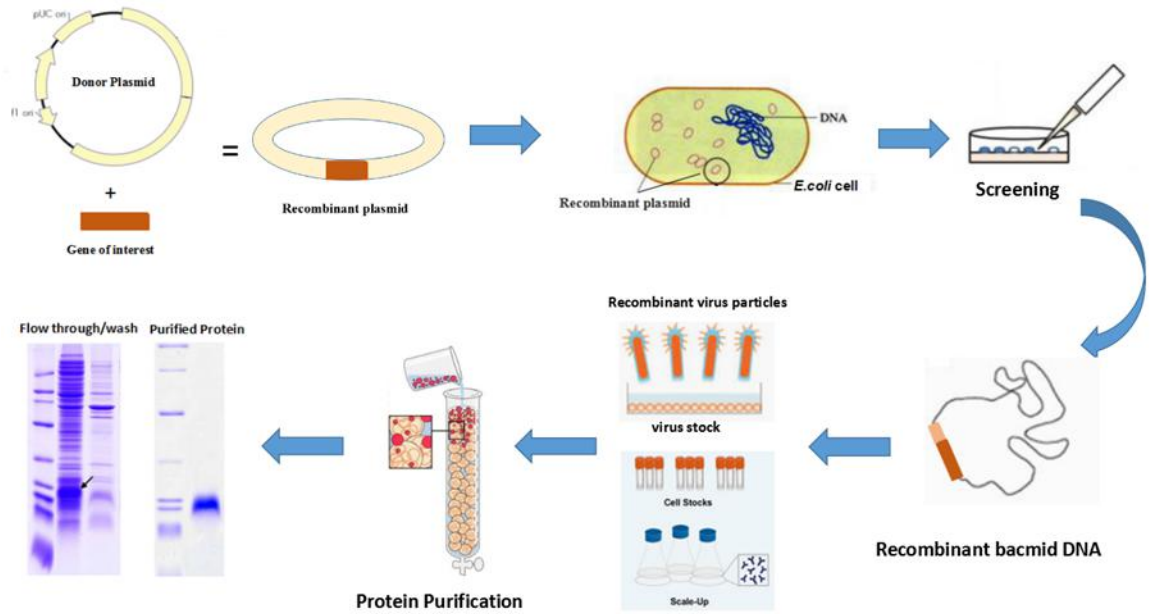


BES as a platform for protein expression



The baculovirus expression system (BES) has been one of the versatile platforms for the production of recombinant proteins requiring multiple post-translational modifications, such as folding, oligomerization, phosphorylation, glycosylation, acylation, disulfide bond formation and proteolytic cleavage. Advances in recombinant DNA technology have facilitated application of the BES, and made it possible to express multiple proteins simultaneously in a single infection and to produce multimeric proteins sharing functional similarity with their natural analogs.

Experiment processes



① Subcloning/PCR cloning ② Recombinant virus production ③ Optimization of expression

④ Protein characterization ⑤ Protein production and purification

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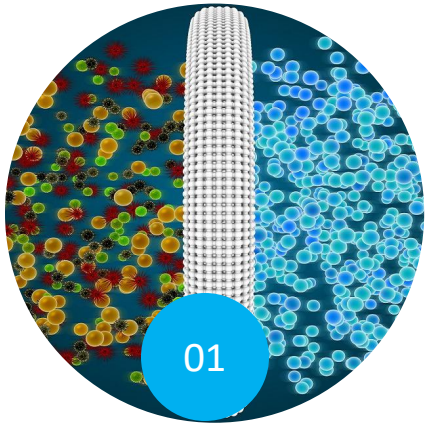
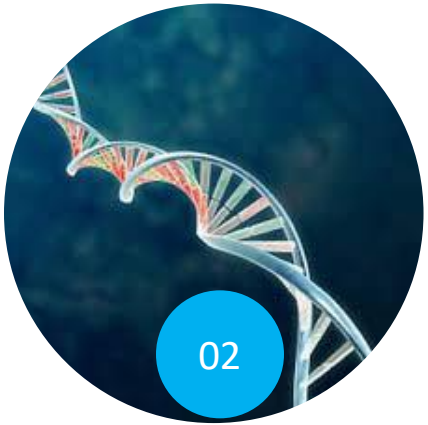
Applications



Eukaryotic protein processing



The production of recombinant proteins

The development of subunit vaccines

The construction of virus-like particles (VLPs)

Challenges for protein quantity and quality

- ✓Insert properties
- ✓Preventing proteolytic cleavage by viral enzymes
- ✓Routing and surface display
- ✓Humanizing protein glycosylation
- ✓Expression at the optimal time
- ✓Co-expression of foldases

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Creative Biogene is a biotechnology company specializing in custom baculovirus production service.

Creative Biogene's sophisticated equipments, advanced technologies and highly experienced staffs are available to assist in all aspects of baculovirus transfer vector construction, recombinant bacmid DNA preparation, as well as the generation of the baculovirus in high titer.

Baculovirus service at Creative Biogene

- Gene synthesis or subcloning of customer’s DNA into baculovirus transfer vector
- Generation of recombinant bacmid DNA
- Transfection of insect cells with bacmid DNA
- Generation of P1 stock (low titer)
- Amplification of high titer recombinant baculovirus stock (P2)

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THANKYOU

THANKS FOR WATCHING THIS PRESENTATION