Insect viruses as vectors for highly efficient expression of foreign genes

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Subject: Genetic engineering

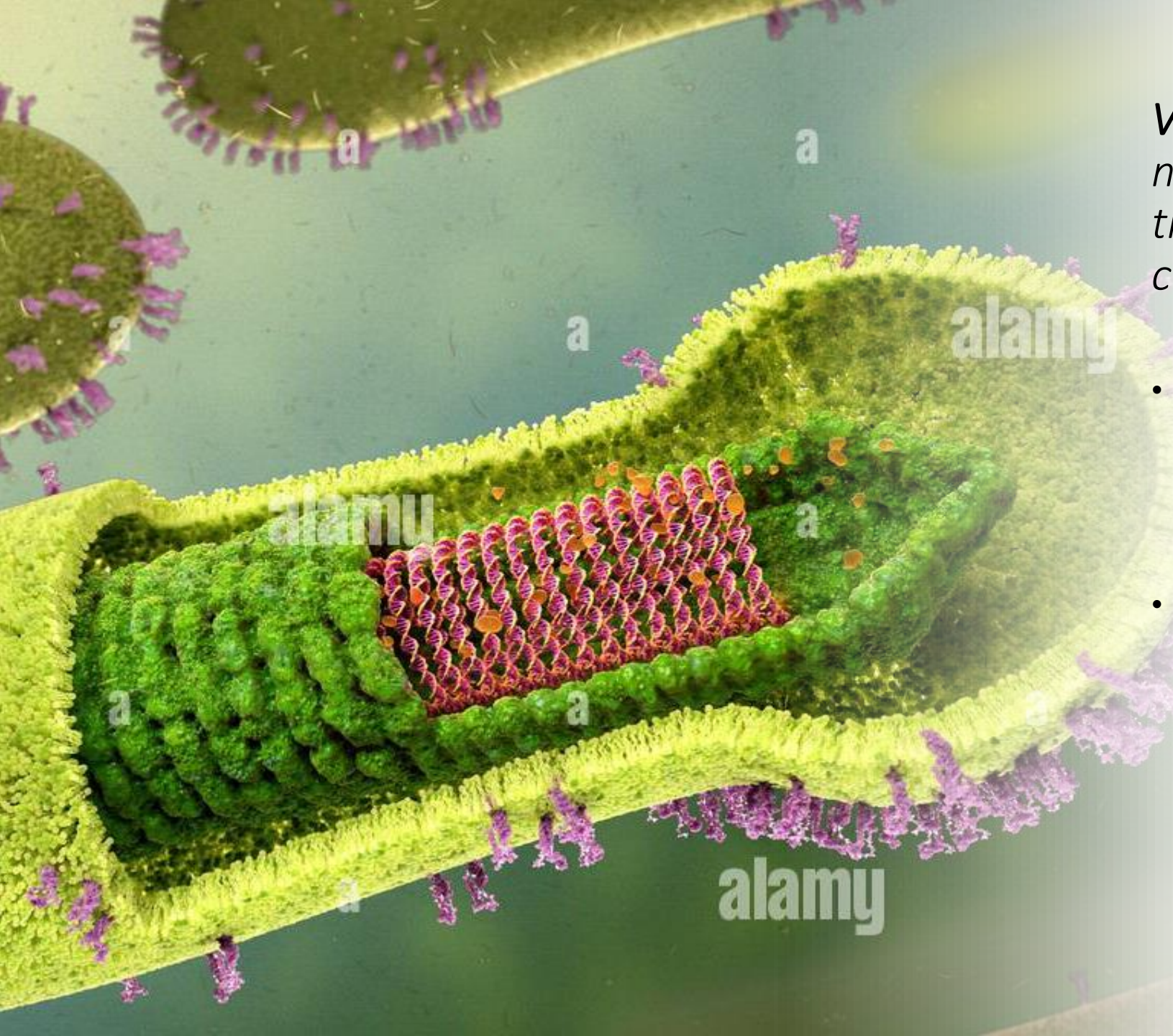
(Lecture 7)

Lecture Goal:
To explore the use of insect viruses as vectors for the efficient expression of foreign genes, focusing on the Baculovirus expression system and the Bac-to-Bac hybrid system.

Tasks:

1. Explain the advantages of insect viruses, particularly Baculovirus, as vectors for the expression of foreign genes in biotechnology.
2. Describe the Baculovirus Expression System, including its mechanism and applications for high-level protein expression.
3. Outline the Bac-to-Bac hybrid Baculovirus creation system, detailing its methodology and benefits for producing recombinant viruses.

Keywords: *Insect viruses, Baculovirus, Baculovirus Expression System, Bac-to-Bac system, recombinant protein expression, foreign gene expression, viral vectors, insect cell expression system*



__Viruses__ are often considered non-living things because they lack some of the basic characteristics of life.

- **Insects** are also like human attacked by a great diversity of viruses, and frequently their infection may cause the death of the infected individuals.

- Although there is a great diversity of insect viruses, only a few are frequently observed in insect populations, such as the ***ascovirus, iridovirus, polydnavirus, baculovirus, cypovirus, entomopoxvirus***; and among these, only few show potential to be used as control agents, mainly the *baculoviruses*.

For what?

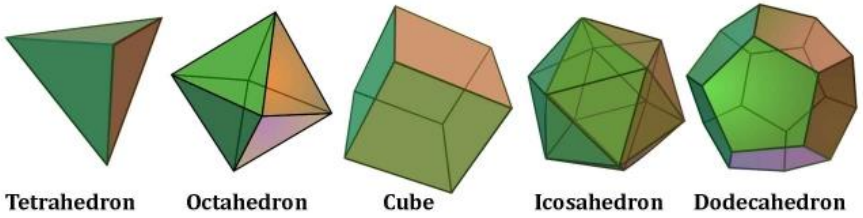
- Overexpression to obtain sufficient amounts;
- Easy cleaning.

Application:

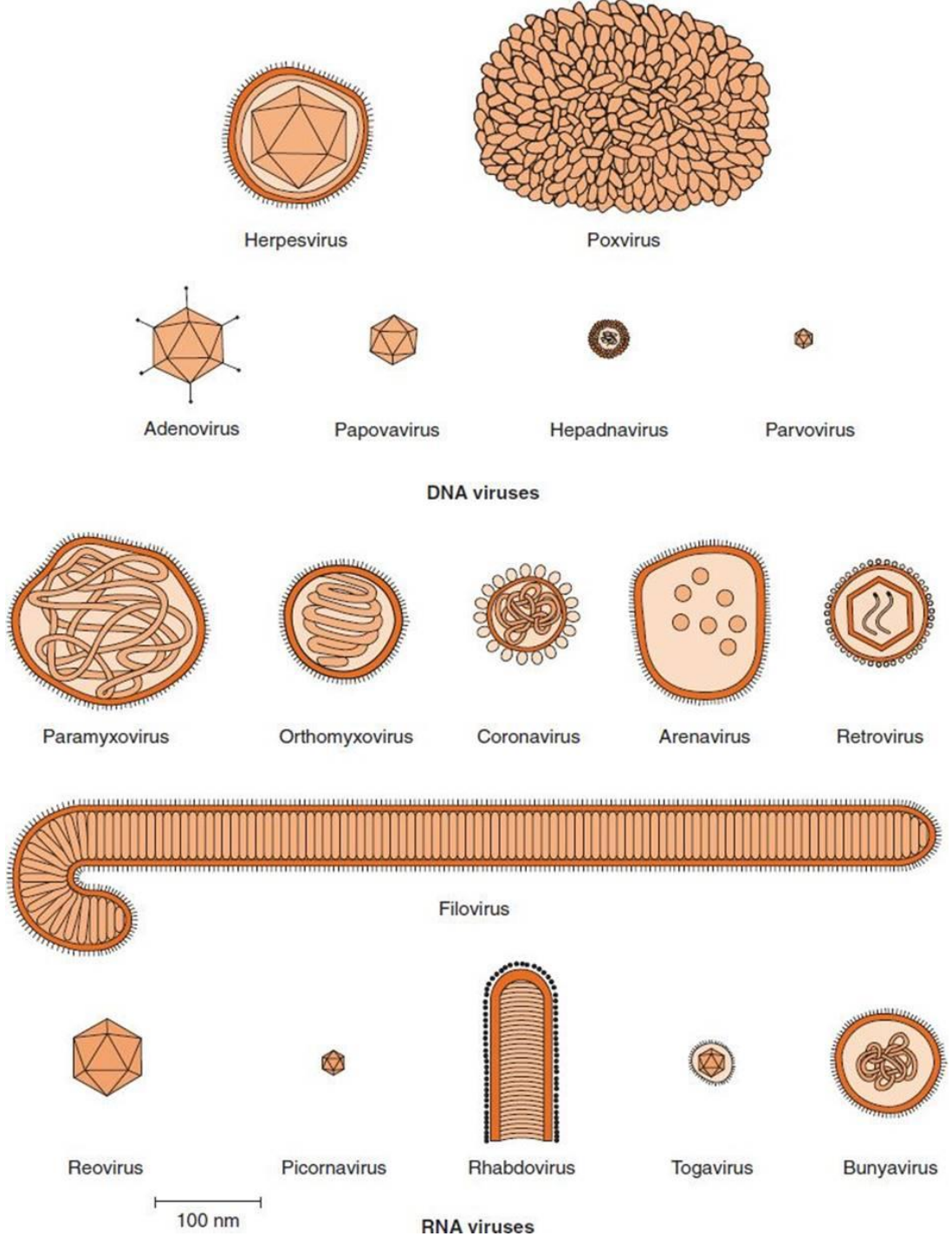
- Functional studies;
- Structural studies;
- Vaccine/antigen/antibodies;
- Therapeutic drugs;
- Industrial enzymes for the reaction.

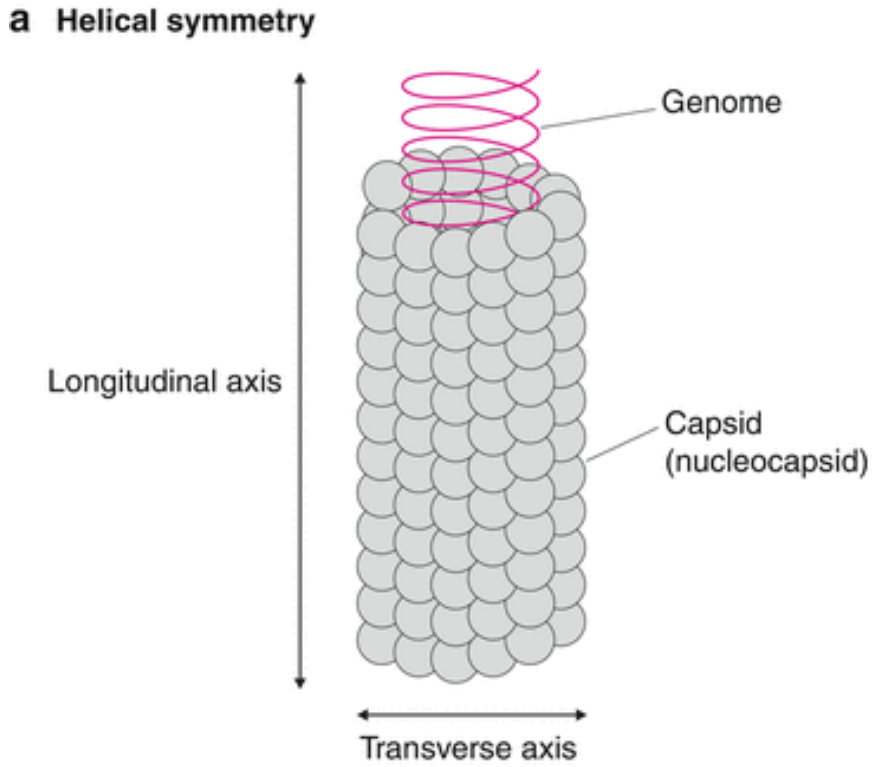
Insects constitute the most divers group of living things on the world.

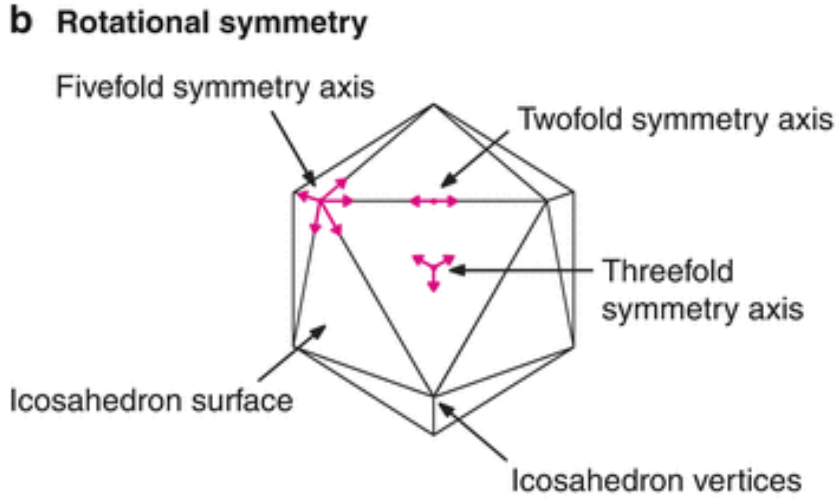
To date studies on the genetics of entomopathogenic viruses are focused on the study of complete genomes as a consequence of the development of genomics. So far, more than 29 complete sequenced genomes have been obtained.



FAMILY	NUCLEIC ACID	NUCLEOCAPSID SIMETRY	OCCLUSION BODY
Baculoviridae	dsDNA	Baciliform	+
Reoviridae	dsRNA	Isometric	+
Poxviridae	dsDNA	Ovoid	+
Iridoviridae	dsDNA	Icosahedral	-
Parvoviridae	ssDNA	Isometric	-
Picornaviridae	ssRNA	Spherical	-
Ascoviridae	dsDNA	Allantoid	-
Polydnaviridae	dsDNA	Ovoid	-
Rhabdoviridae	ssRNA	Baciliform	-
Nodaviridae	ssRNA	Icosahedral	-
Rhabdoviridae	ssRNA	Baciliform	-
NON-CLASSIFIED RNA VIRUSESs			
Divided genome	ssRNA	Isometric	-
β *Nodaurelia*	ssRNA	Isometric	-
Kelply group	ssRNA	Isometric	-
5-virus group	ssRNA	Isometric	-
Minivirus	ssRNA	Isometric	-
Ovoid virases	ssRNA	Ovoid	-


Herpesvirus
Poxvirus
Adenovirus
Papovavirus
Hepadnavirus
Parvovirus
DNA viruses
Paramyxovirus
Orthomyxovirus
Coronavirus
Arenavirus
Retrovirus
Filovirus
Reovirus
Picornavirus
Rhabdovirus
Togavirus
Bunyavirus
100 nm
RNA viruses


a Helical symmetry
Genome
Longitudinal axis
Capsid
(nucleocapsid)
Transverse axis


b Rotational symmetry
Fivefold symmetry axis
Twofold symmetry axis
Threefold
symmetry axis
Icosahedron surface
Icosahedron vertices

These **insect-specific viruses** have a strict tropism and are unable to replicate in vertebrate cells, these properties are interesting for many reasons.

- The first insect-specific virus (ISV) was discovered over 40 years ago by Stollar and Thomas [1]. It was isolated from an *Aedes aegypti* cell culture where a large number of syncytia were observed and the virus was named cell fusing agent virus (CFAV). Further, when inoculated on different vertebrate cell lines no cytopathic effect (CPE) could be observed and the virus could not be re-isolated, suggesting that the virus must be insect-specific [1].

Aedes aegypti

- Insect-specific viruses are believed to be restricted by the innate immune system in the vertebrate cell, but there is evidence pointing to that this is not the only mechanism causing the restriction of host-range [34]. In contrast to arboviruses, who can replicate at temperatures up to 42 °C insect-specific viruses replicate only at ambient temperatures.

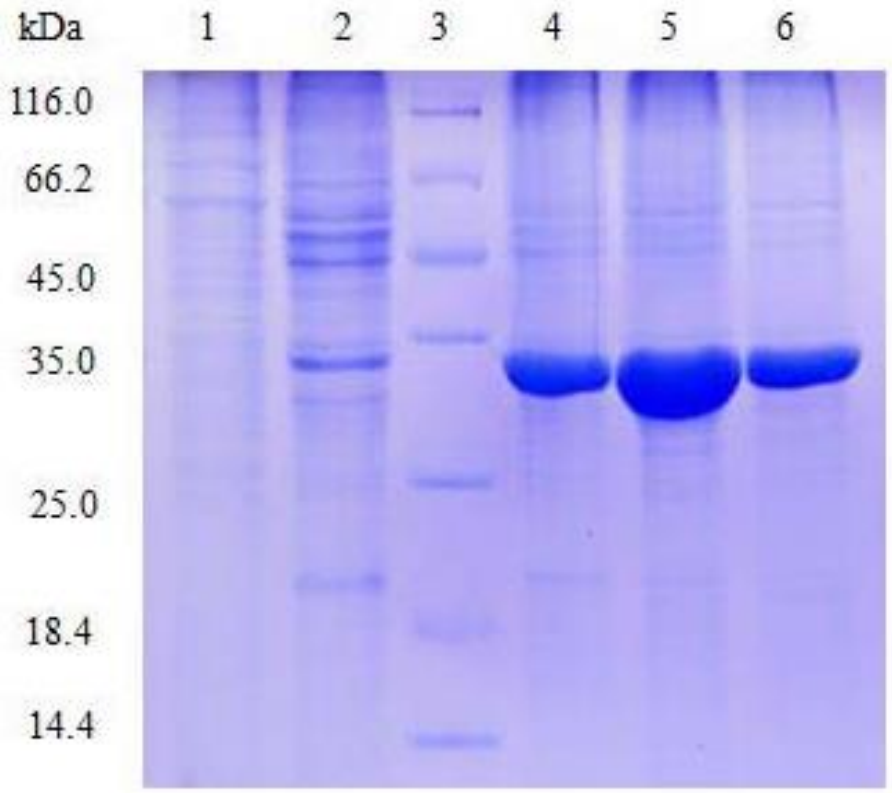
Insect Baculovirus Expression System

- **Insect baculovirus expression vector system (BEVS)** belongs to the *eukaryotic expression system*, and it's an expression system with high safety.
- It has a large genome, which enables the insertion of large exogenous genes, therefore has the great advantage of expressing proteins with large molecular weight.
- It also has the ability to achieve complete post-translational modification and efficiently express exogenous genes.
- The system consists of transfer vector, baculovirus vector and the host cell. The system uses one or more baculovirus super-strong promoters, and gets the recombinant virus after the exogenous target gene is inserted into the promoter.
- The highly efficient expression of the exogenous gene is achieved while the recombinant viruses replicate themselves in the insect cells.
- BEVS is widely used in virus vaccine development (such as the development of influenza virus vaccine and HPV vaccine), preparation of cell signaling proteins and cytokines, as well as kinase development, etc.

Baculovirus as a highly efficient expression vector in insect and mammalian cells

Case 1

The protein was highly expressed in our company's Insect baculovirus expression vector system. A clear band was observed from cell lysate by SDS-PAGE. The yield was up to 20 mg/L after purification.



Protein purification image
Lane 1: Flow through.
Lane 2: Cell lysate
Lane 3: Marker
Lane 4: 30 mM imidazole elution
Lane 5: 60 mM imidazole elution
Lane 6: 250 mM imidazole elution

Baculovirus has been widely used for the production of recombinant proteins in insect cells. Since the finding that baculovirus can efficiently transduce mammalian cells, the applications of baculovirus have been greatly expanded.

Baculoviruses make up a family of viruses and are grouped into nuclear polyhedrosis viruses (NPV) and granulosis viruses. More than 500 different types of baculoviruses have been discovered and the host range is restricted to invertebrates, mostly to insects (eg, moths and butterflies);

- Among the numerous baculoviruses, ***Autographa californica*** multiple NPV (**AcMNPV**) is the most well studied and most extensively used.
- **AcMNPV** has a circular double-stranded DNA genome of approximately 130 kb, which is condensed with a protamine-like protein into the core and packed into the nucleocapsids.
- Nucleocapsids are synthesized in the nucleus of infected cells (typically 40 nm–50 nm in diameter and 200 nm–400 nm in length). Membrane-enveloped nucleocapsids are referred to as virus particles or virions.

Alfalfa looper

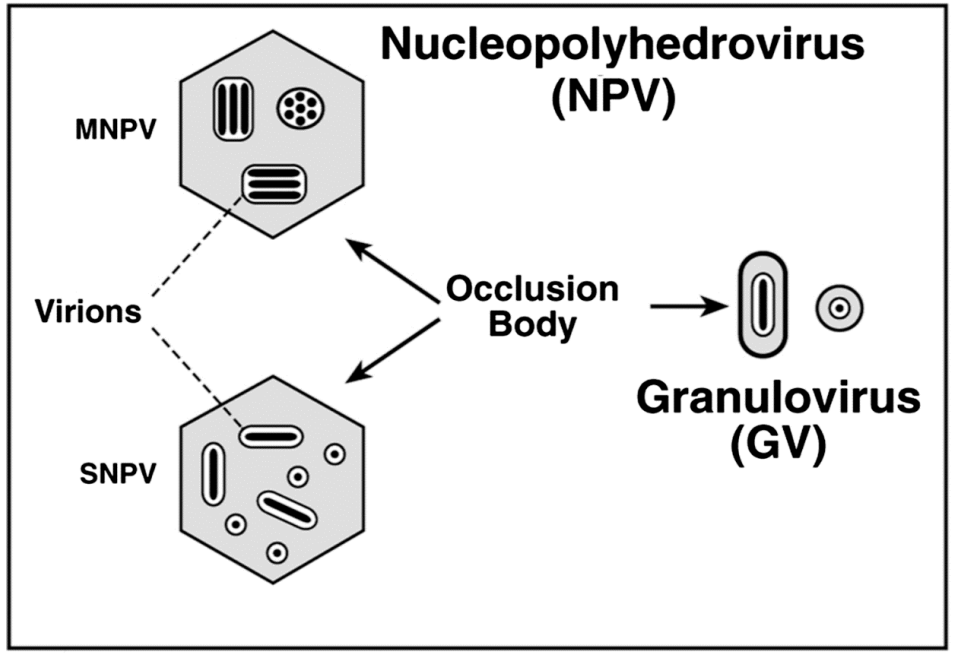
- *Autographa californica* multiple nuclear polyhedrosis virus (Baculovirus)
- The virus usually infects the cells of the *alfalfa* (small beetle) or cutworm insects (and their larvae).
- Uses an ultra-strong promoter from the polyhedron coat protein to enhance protein expression while the virus is inside the insect

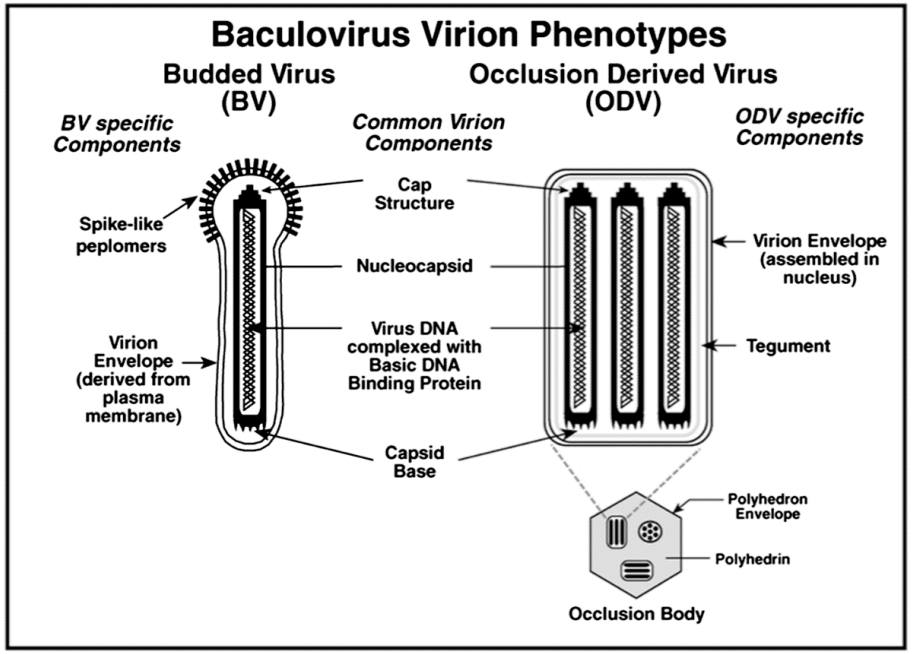
Autographica californica

- In nature, **AcMNPV** are occluded in a polyhedron (2 µm–15 µm in size) mainly consisting of polyhedrin protein. After ingestion by insects, the polyhedrin matrix is dissolved in the alkaline midgut, thus releasing the embedded virions, which subsequently infect the epithelial cells of the intestine. Early in the infection cycle, the DNA genome is replicated and transcribed in the nucleus and the nucleocapsids are assembled. The budding of nucleocapsids through plasma membrane results in the release of budded virus, which is responsible for systemic transmission within an infected insect. Late in the infection cycle, progeny nucleocapsids become membrane-bound within the nucleus and are embedded into the polyhedra. After cell death and lysis, the polyhedra are released in the wild to spread the infection.

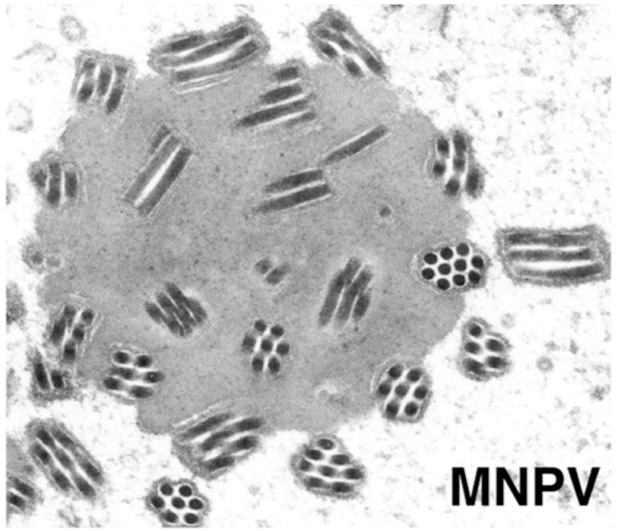
➢ **Budded virus is highly infectious to cultured insect cells and is the primary form used in the laboratory as an expression vector.**

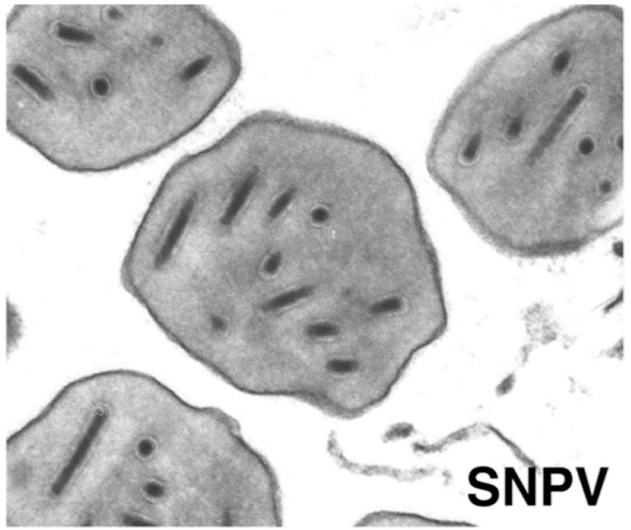
Baculovirus

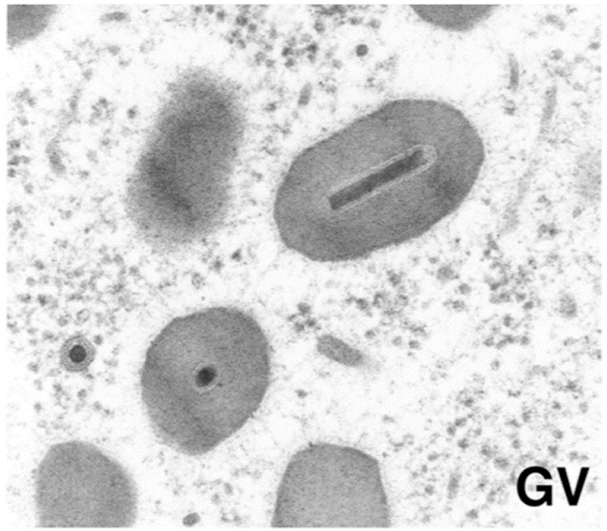


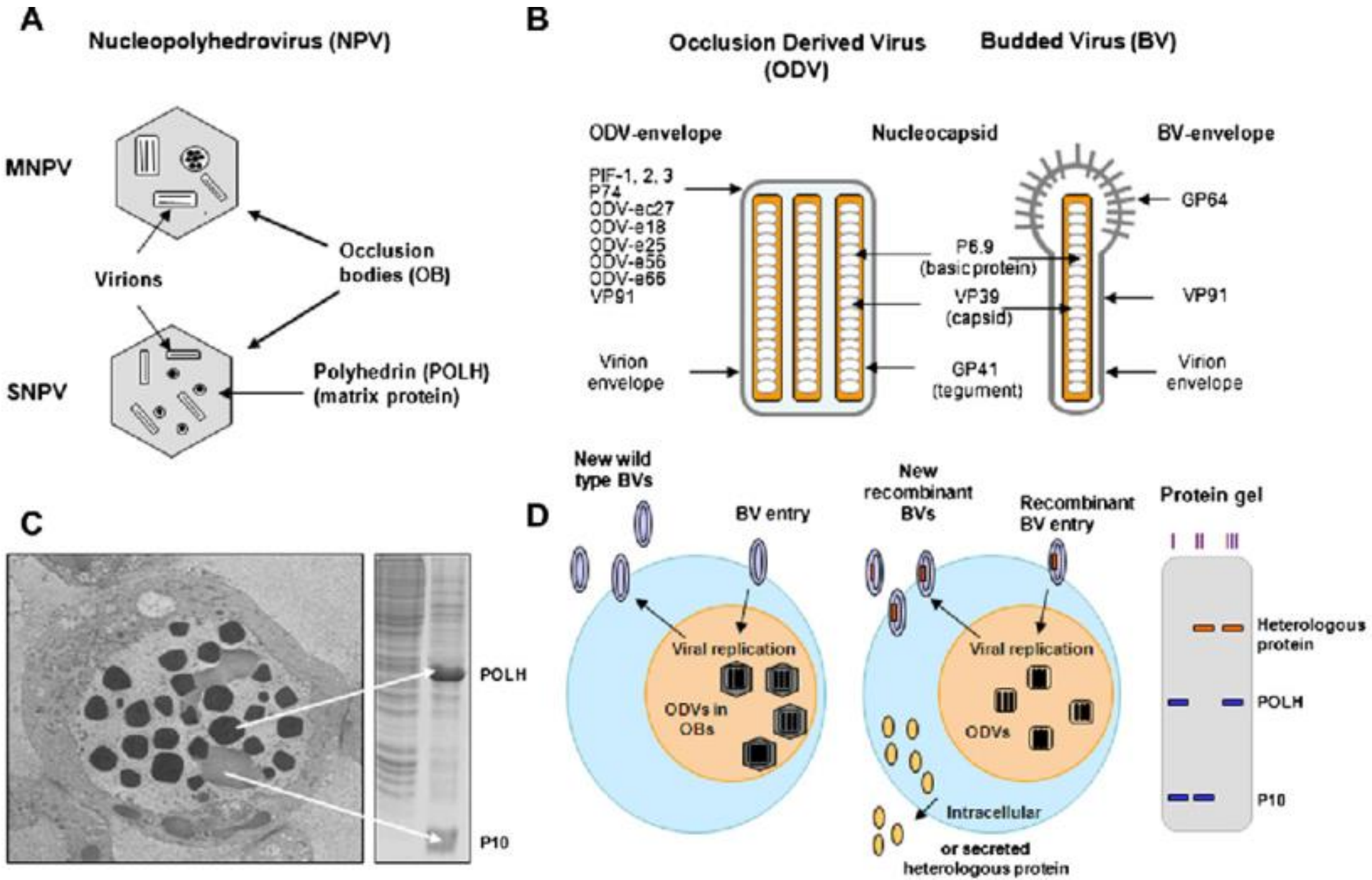


Occlusion Bodies



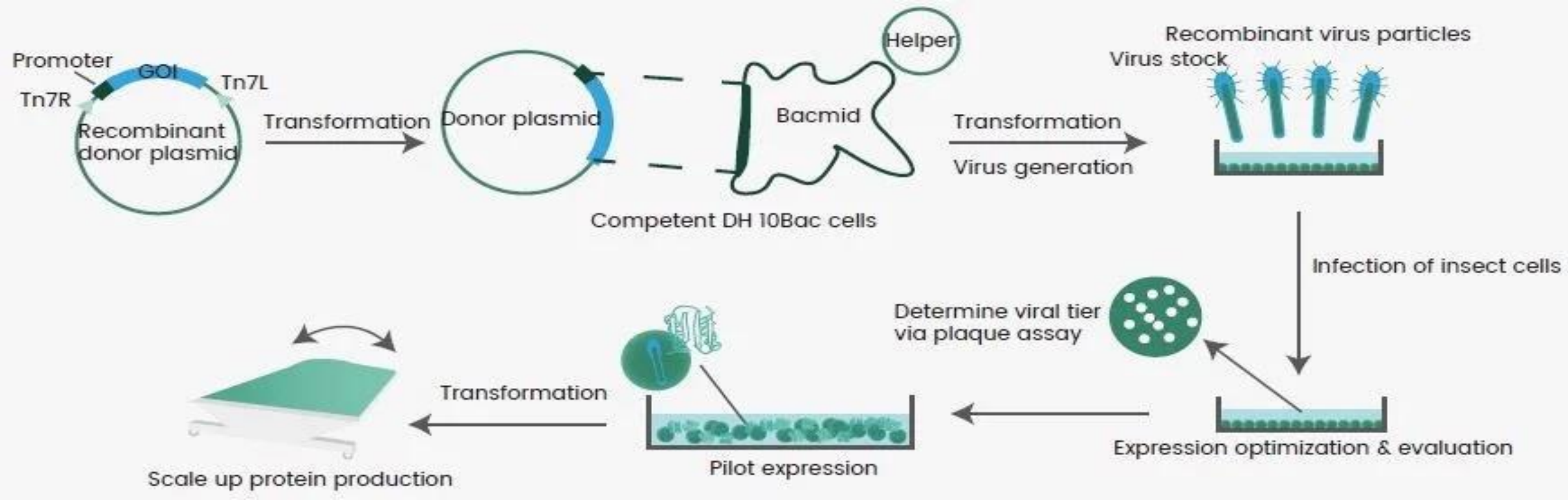


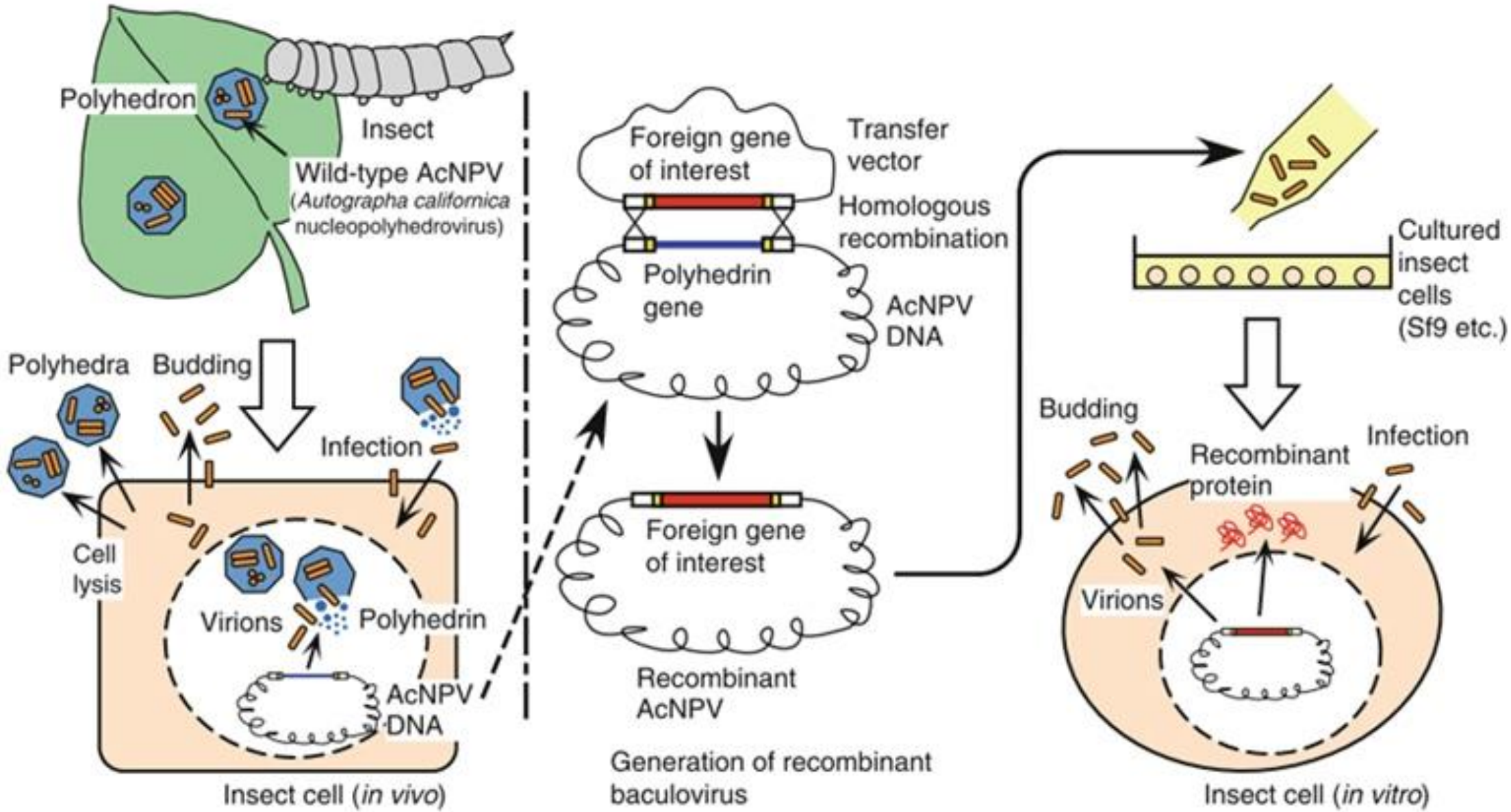



A
Nucleopolyhedrovirus (NPV)
MNPV
Virions
Occlusion bodies (OB)
SNPV
Polyhedrin (POLH) (matrix protein)
B
Occlusion Derived Virus (ODV)
Budded Virus (BV)
ODV-envelope
Nucleocapsid
BV-envelope
PIF-1, 2, 3
P74
ODV-ec27
ODV-e18
ODV-e25
ODV-e56
ODV-e66
VP91
Virion envelope
P6.9 (basic protein)
VP39 (capsid)
GP41 (tegument)
GP64
VP91
Virion envelope
C
POLH
P10
D
New wild type BVs
BV entry
Viral replication
ODVs in OBs
New recombinant BVs
Recombinant BV entry
Viral replication
ODVs
Intracellular
or secreted heterologous protein
Protein gel
I II III
Heterologous protein
POLH
P10

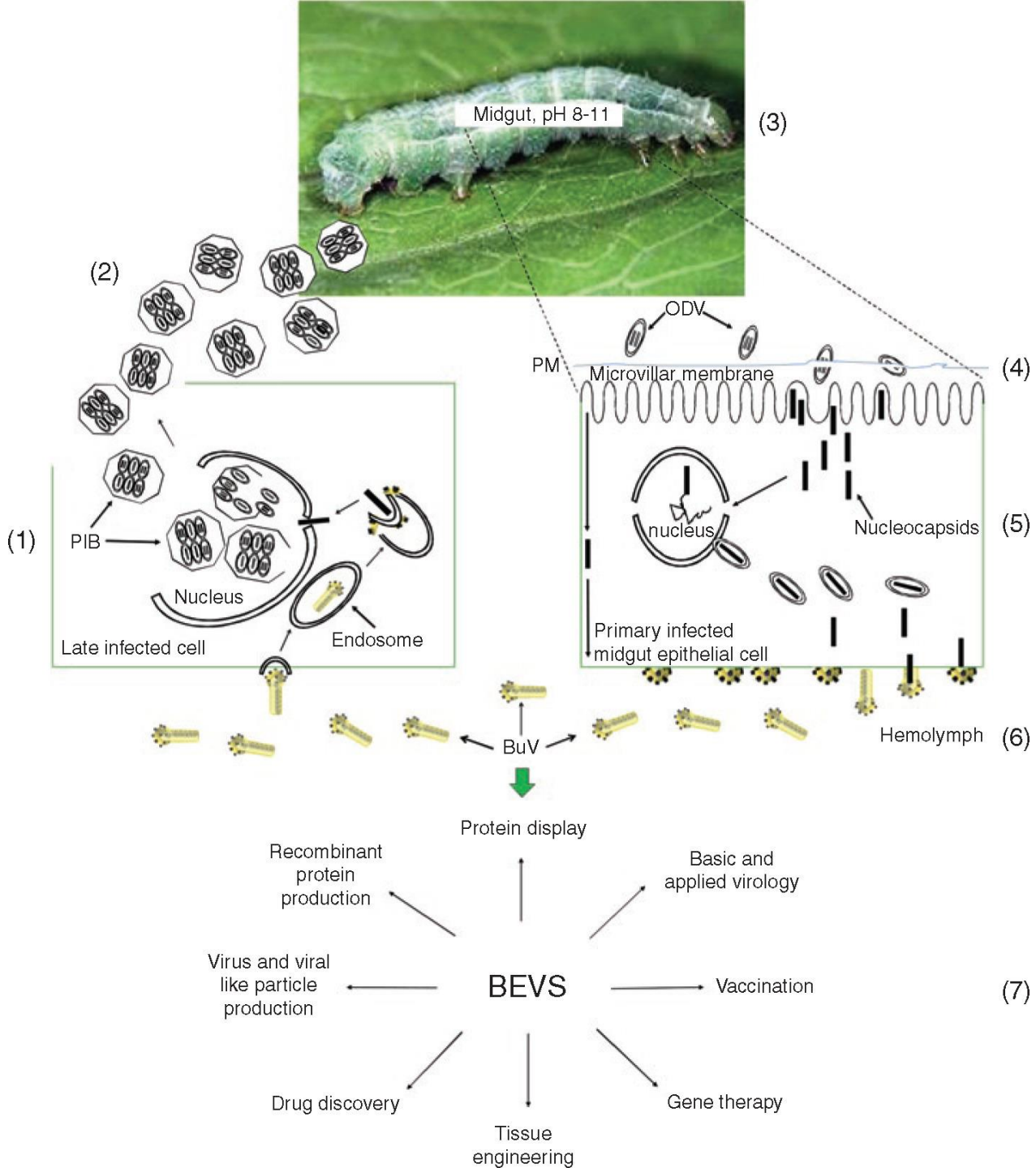
The baculovirus has been widely used for the production of numerous recombinant proteins in insect cells because it has the following advantages:

(i) proper post-translational modification, because insect cells are higher eukaryotes;
(ii) a high capacity for multiple genes or a large insert, because of the huge and flexible viral genome (130 kb);
(iii) biosafety, because baculovirus naturally does not infect humans;
(iv) a very high yield driven by the strong promoters polyhedrin or p10.

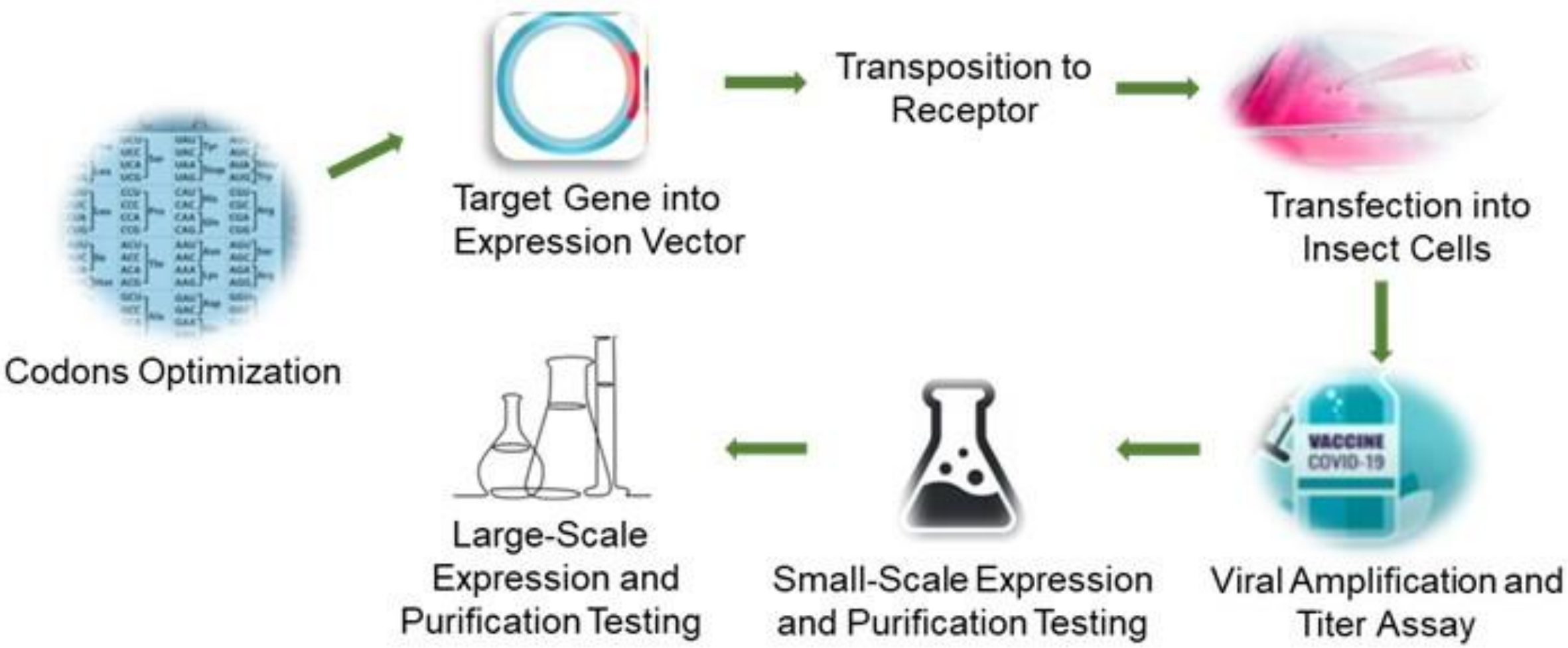




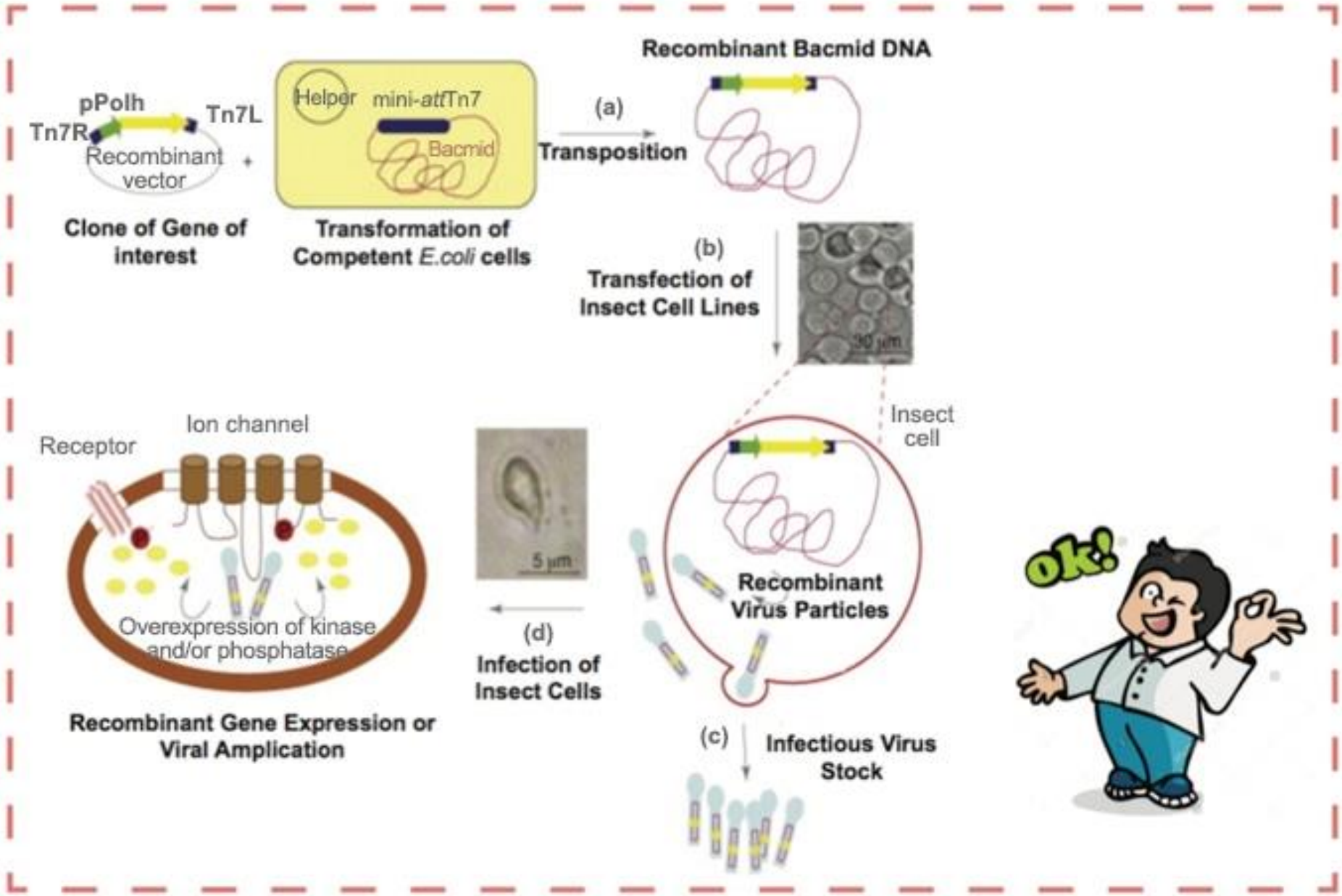
Recombinant protein production in the baculovirus-insect system
(Antibody Expression and Production, 2010)


Midgut, pH 8-11
(3)
(2)
ODV
PM
Microvillar membrane
(4)
(1)
PIB
Nucleus
Late infected cell
Endosome
nucleus
Nucleocapsids
(5)
Primary infected
midgut epithelial cell
Hemolymph
(6)
BuV
Protein display
Recombinant
protein
production
Basic and
applied virology
Virus and viral
like particle
production
BEVS
Vaccination
(7)
Drug discovery
Tissue
engineering
Gene therapy

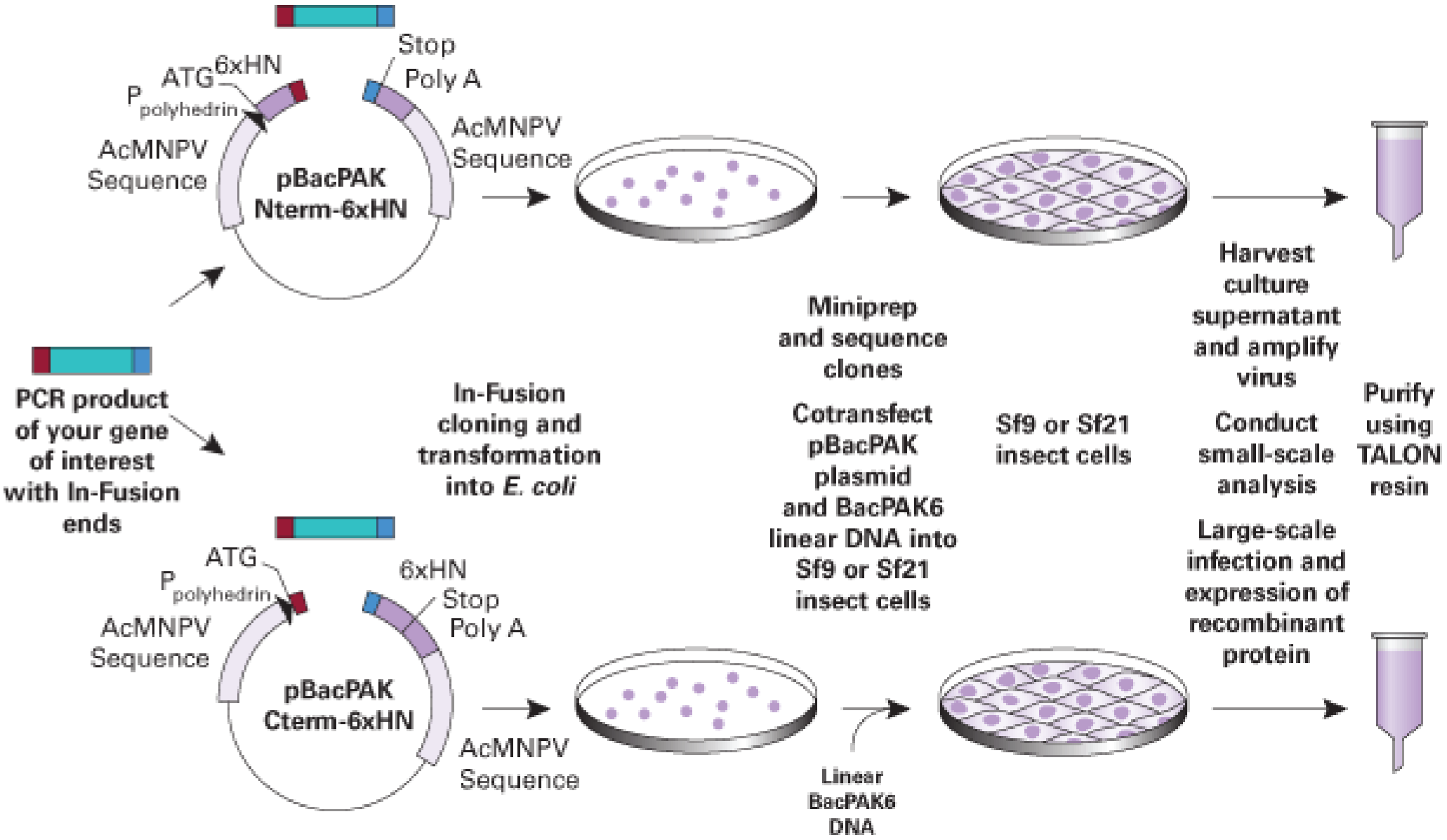
Bac-to-Bac Baculovirus Expression System - Lifeasible



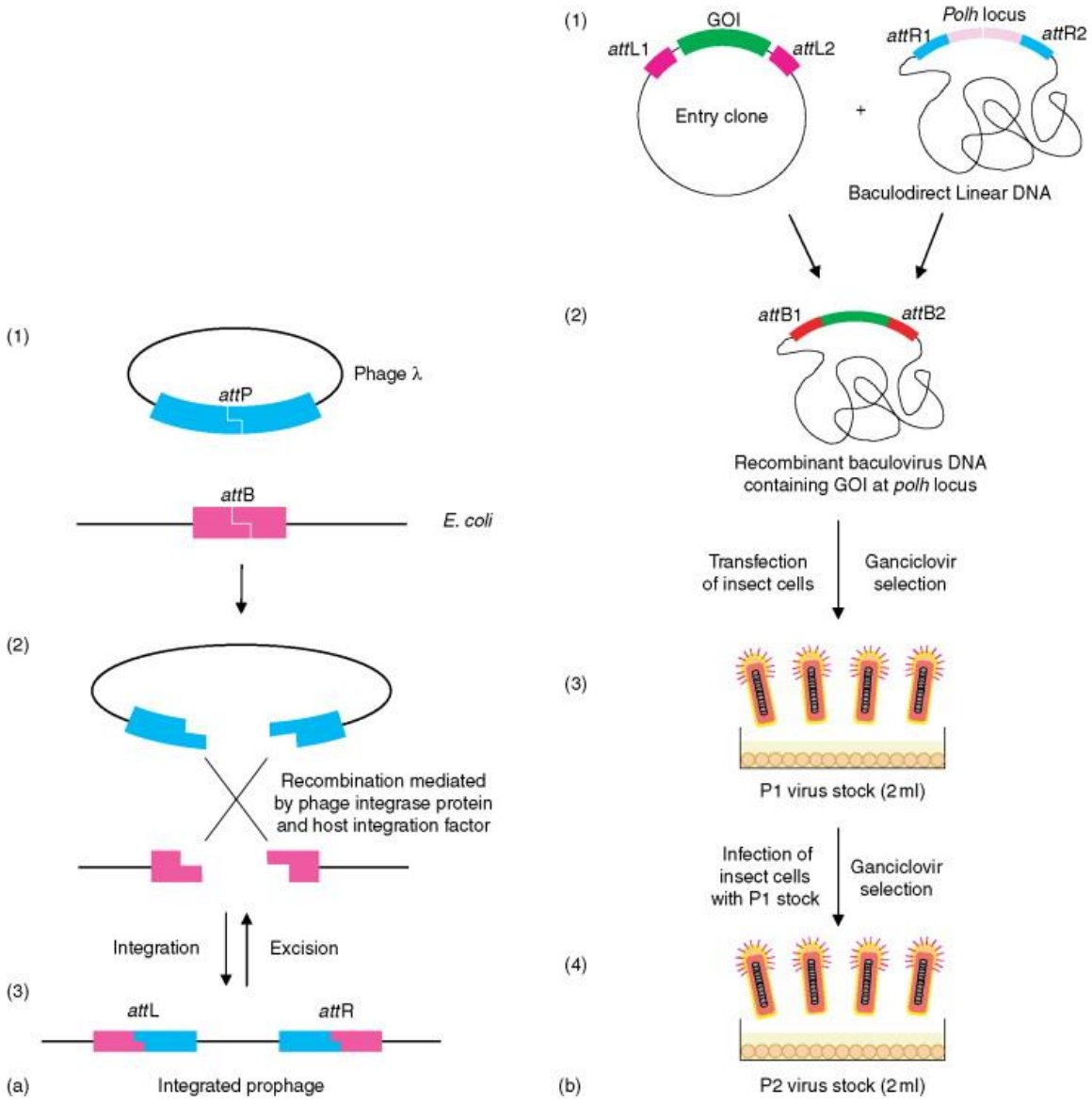
Generation of Recombinant Baculovirus and Gene Expression



Baculovirus expression system: a complete system



Cloning and expression of foreign genes within the genome of baculoviruses



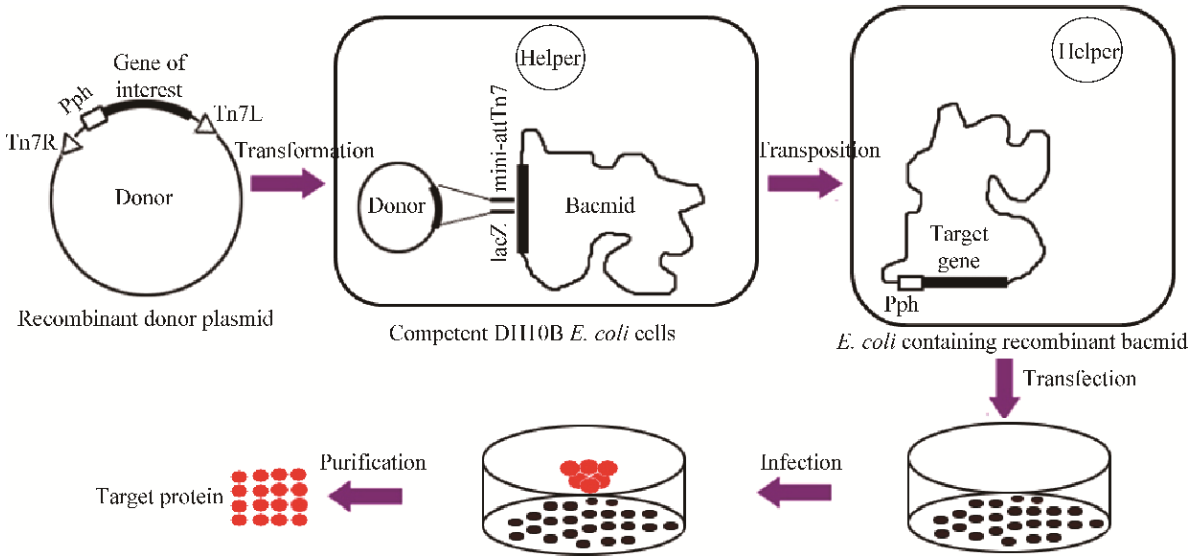


Figure - Schematic diagram of baculovirus expressing foreign gene

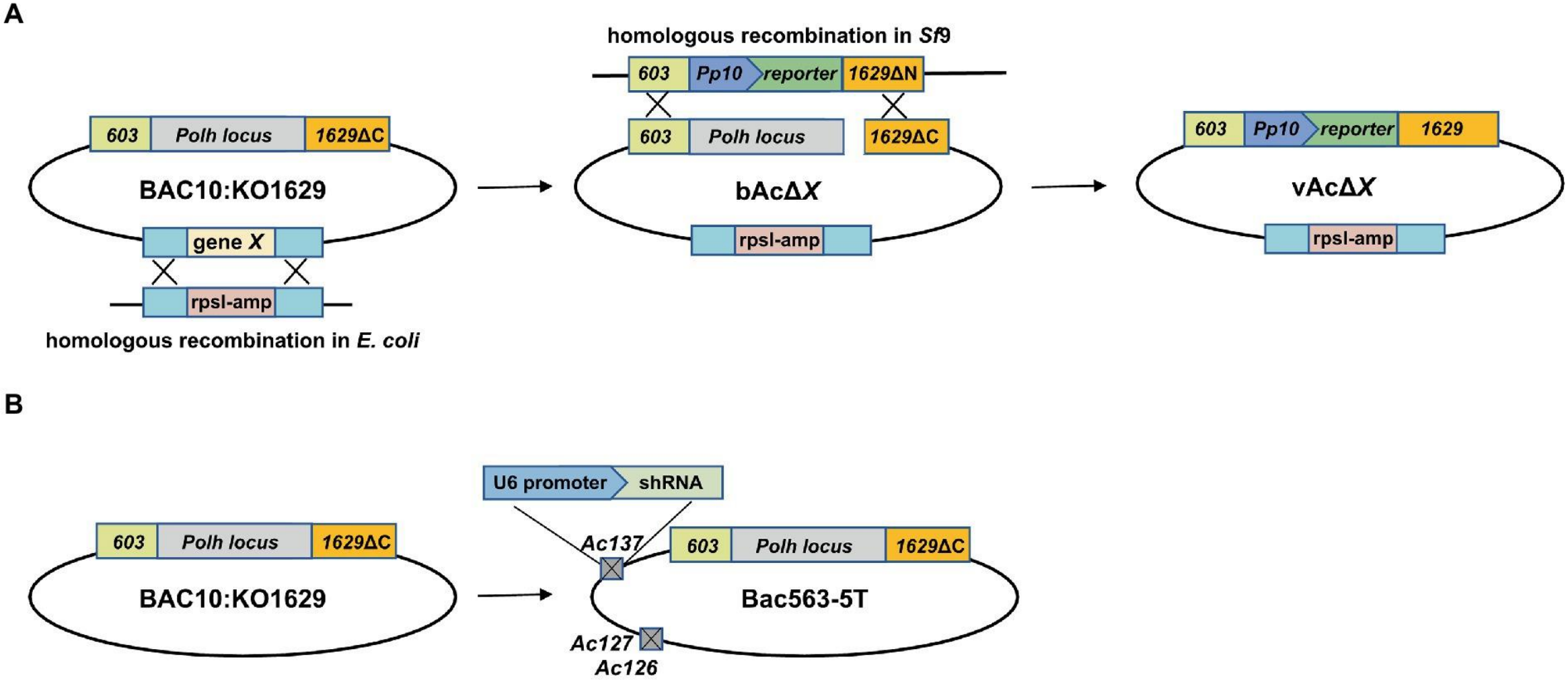
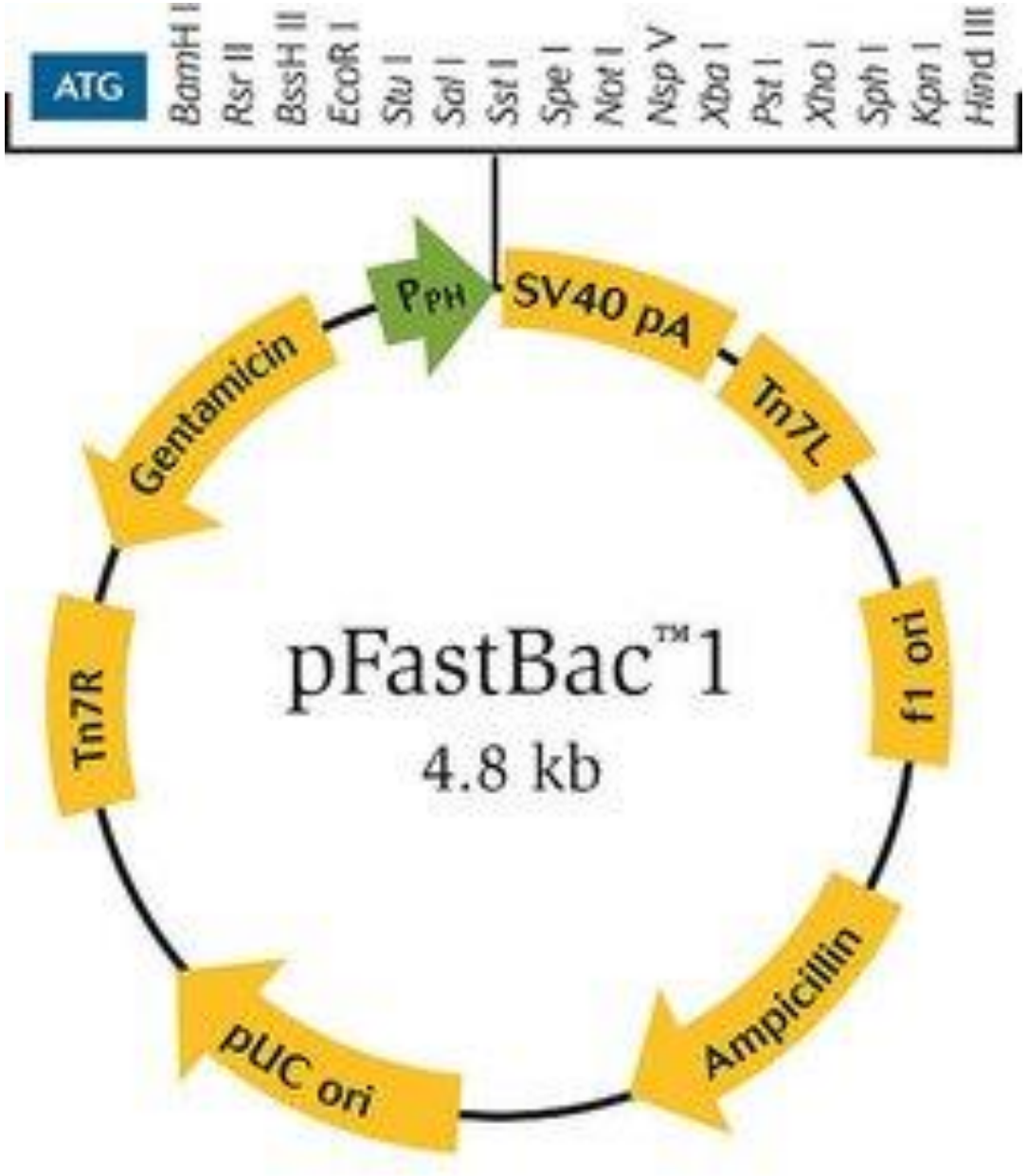


Figure 1. Schematic diagram of the construction of bacmids and generation of AcMNPV expressing a reporter gene. **(A)** Generation of fragment-knockout (KO) bacmid and AcMNPV expressing a reporter gene. DNA fragment containing predicted nonessential genes (gene X) was deleted from the AcMNPV bacmid BAC10:KO1629 by replacing the target fragment with a rpsl-Amp cassette *via* homologous recombination in *E. coli* strain HS996, and the KO bacmid was named as bAcΔX. The recombinant virus carrying the reporter gene expression cassette at the *polyhedrin* locus, vAcΔX, was generated in *Sf*9 cells by co-transfection with the linearized bAcΔX and pTriEx plasmid. The parental virus vAc generated from BAC10:KO1629 was used as a control in this study. **(B)** Schematic diagram of Bac563-5 T used in this study. Bac563-5 T was modified from BAC10:KO1629 by knocking out *ChiA/v-cath* (*Ac126-127*) and *p10* (*Ac137*) and inserting an shRNA expression cassette at the *Ac137* locus to inhibit the virus infection-induced apoptosis (https://doi.org/10.3389/fmicb.2023.1171500).

BAC-TO-BAC HYBRID BACULOVIRUS CREATION SYSTEM

The **Bac-to-Bac™ Vector Kit** contains a **pFastBac™ 1** vector, as well an expression control vector, intended for use as part of the Bac-to-Bac™ Baculovirus Expression System (Cat. No. 10359-016), which enables the efficent production of recombinant baculovirus for expression testing in insect cells. The Bac-to-Bac™ System relies on generation of recombinant baculovirus by site-specific transposition in *E. coli*rather than homologous recombination in insect cells (Figure 1). This system features:

• **Time-saving expression bacmid.** With Bac-to-Bac™, the expression cassette of the pFastBac™ vector recombines with the parent bacmid in DH10Bac™ *E. coli* Competent Cells (not included with this vector kit) to form an expression bacmid. The bacmid is then transfected into insect cells for production of recombinant baculovirus particles.
• **Easy colony screening.** The parent bacmid in DH10Bac™ *E. coli*contains a segment of the *lac*Zα gene. The *lac*Zα gene is disrupted upon transposition of the expression cassette into the bacmid allowing for blue/white selection of recombinants. This makes identification of recombinant colonies easy.
The Bac-to-Bac™ Baculovirus Expression System is designed for fast, small-scale production of recombinant baculovirus. The pFastBac™ 1 vector provided by this kit offers the strong polyhedrin promoter for protein expression and a large multiple cloning site for simplified cloning.



Baculovirus systems

Disadvantages:

- Grows very slowly (10-12 days to prepare);
- Cell culture is only viable for 4-5 days;
- Preparing takes a lot of time, not as easy as yeast.

Advantages:

- Can express large proteins (>50 kDa).
- Proper glycosylation and removal of signal peptide.
- It has chaperonins that help fold “strong” proteins.
- Very high yields, cheap