

INTRODUCTION

Baculoviridae is a family of viruses that infect insect hosts, such as lepidoptera. Many of the genes encoded by baculoviruses are nonessential for replication in cell culture and were replaced with recombinant DNA to create the Baculovirus Expression Vector System (BEVS). The technology is utilized world-wide to produce a range of protein classes, including enzymes, antigens, and virus-like particles. In the past 10 years, there have been significant investments in developing BEVS technology for manufacturing vaccines (Flublok) and gene therapy vectors (AAV) and for use as a gene delivery vehicle for mammalian cells.

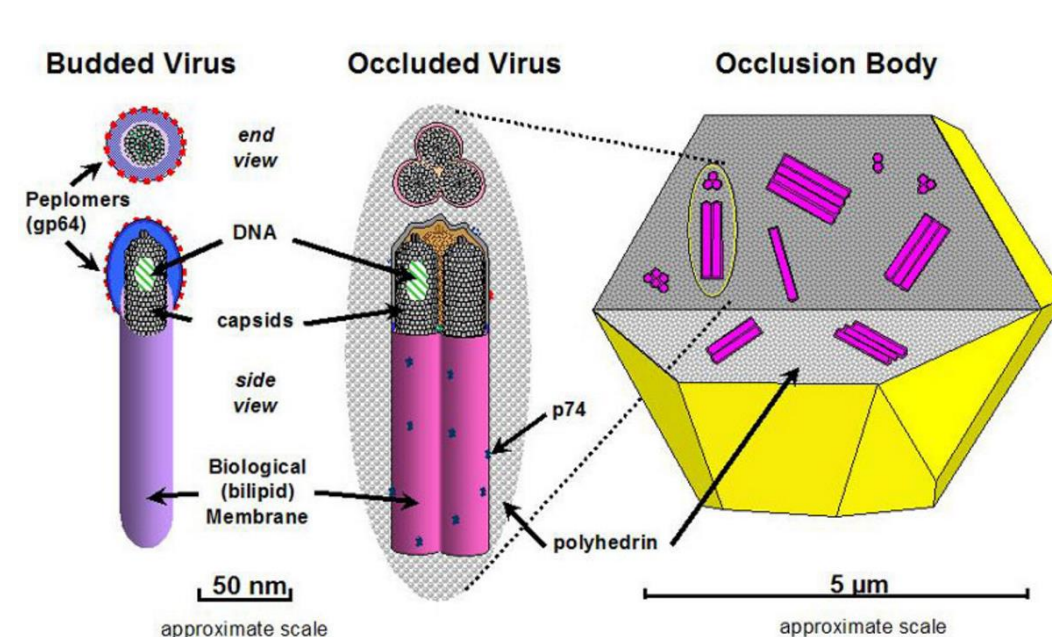
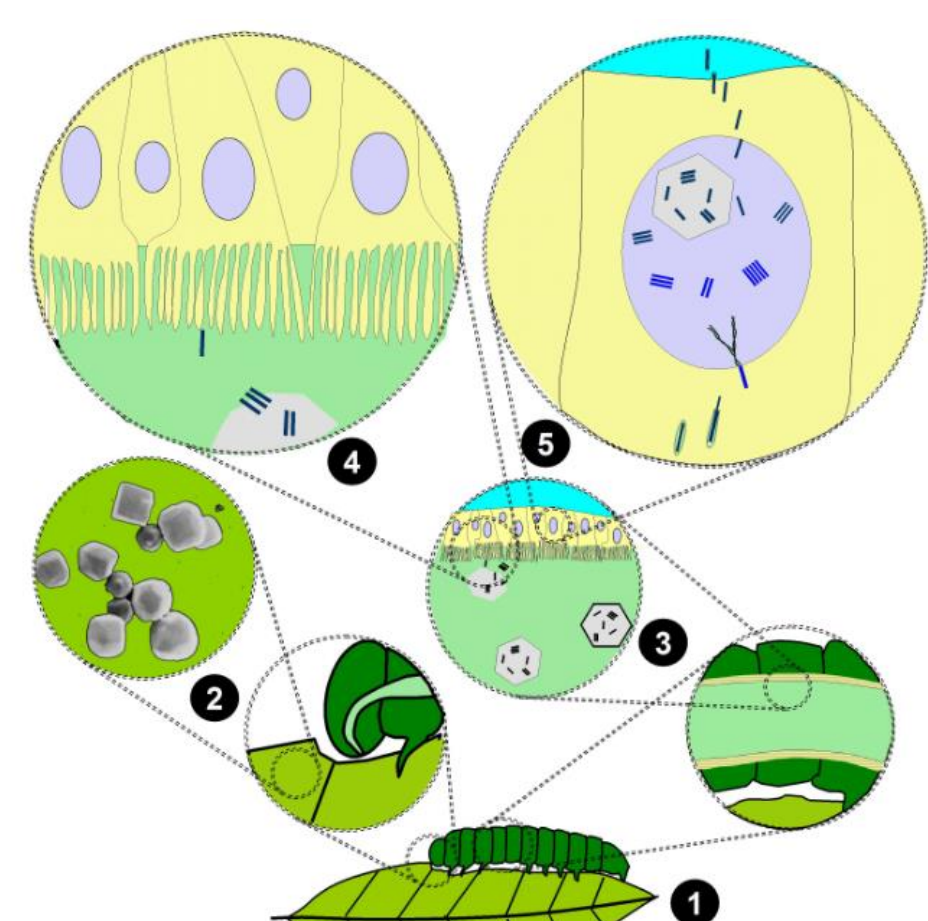
Key Highlights of LakePharma's BEVS Platform

- Utilizes Bac-to-Bac baculovirus system (Thermo),
- Involves suspension culture-based virus generation
- Utilizes Baculovirus-Infected Insect Cell (BIIC) Research Cell Banks for long-term storage of virus (>10 years) without loss in potency
- Utilizes Infection Kinetics Monitoring (IKM), a "titerless" system that obviates the need for plaque assays.

Advantages of LakePharma's BEVS Platform

- Accelerates protein manufacturing by up to several weeks
- Improves Lot-to-Lot reproducibility in protein yield and quality
- Scales easily from flasks to Wavebag production.

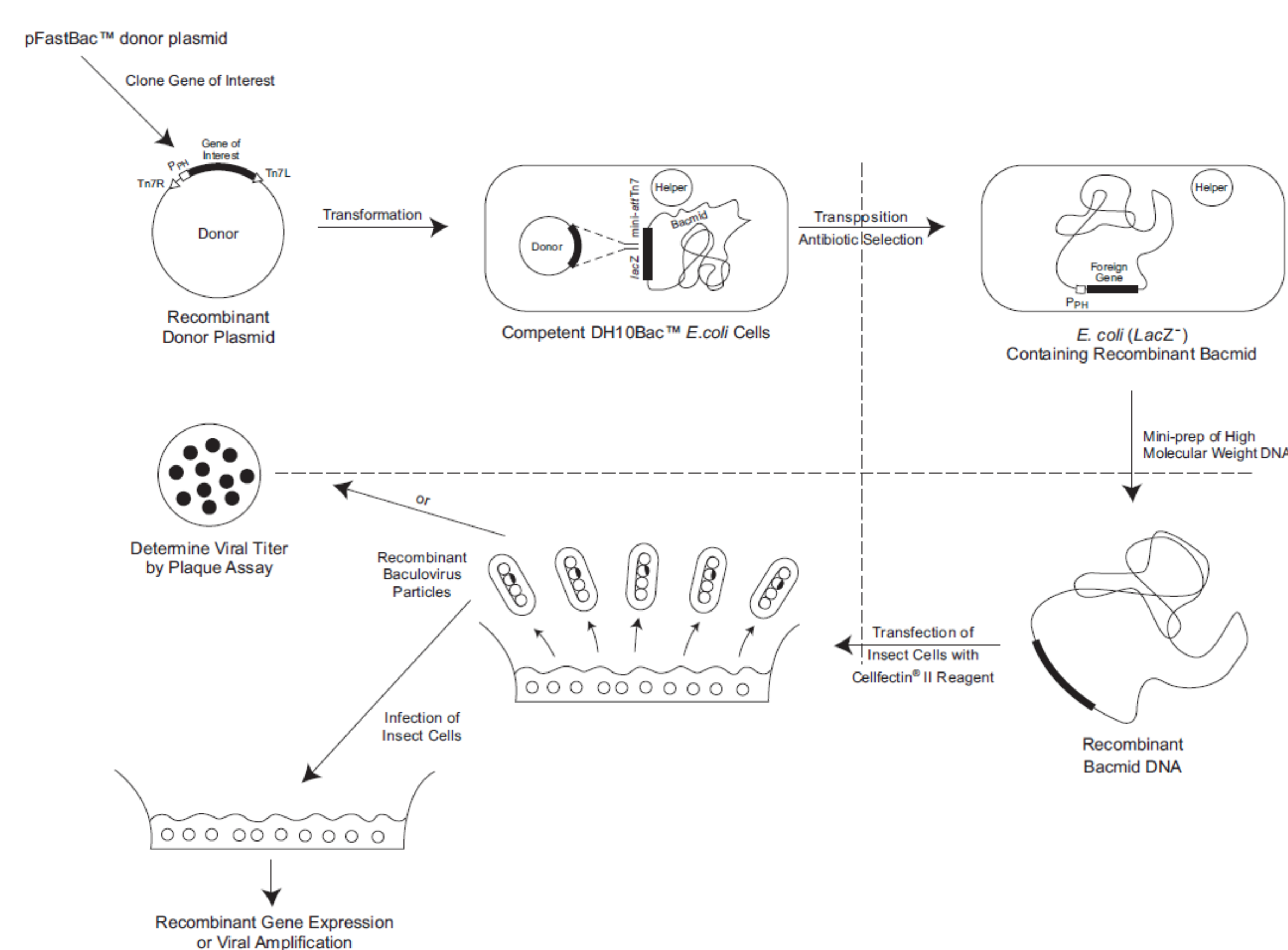
Baculovirus life cycle Multicapsid nucleopolyherovirus



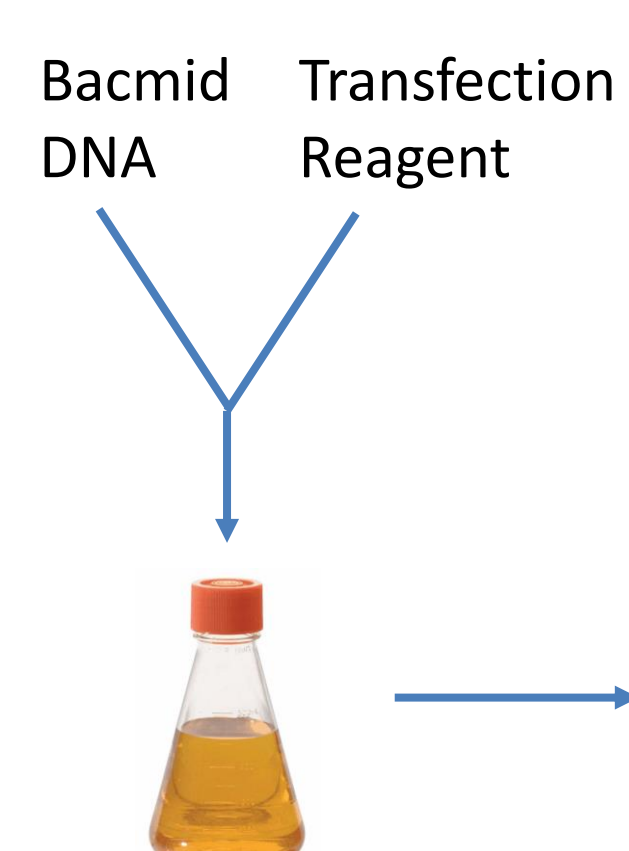
- 1 Insect feeding on virus-contaminated foliage
- 2 Close up of occlusion bodies (OBs)
- 3 Lumen of digestive tract (alkaline conditions)
- 4 Virus particles being released from OBs and attaching to brush border of gut cells
- 5 Replication of virus in insect cell

- Virus
- Occlusion body
- Nucleus
- Cytoplasm
- Hemocel
- Gut lumen
- Plant

Bac-to-Bac System (Thermo)



Scalable Virus Generation in Suspension Culture

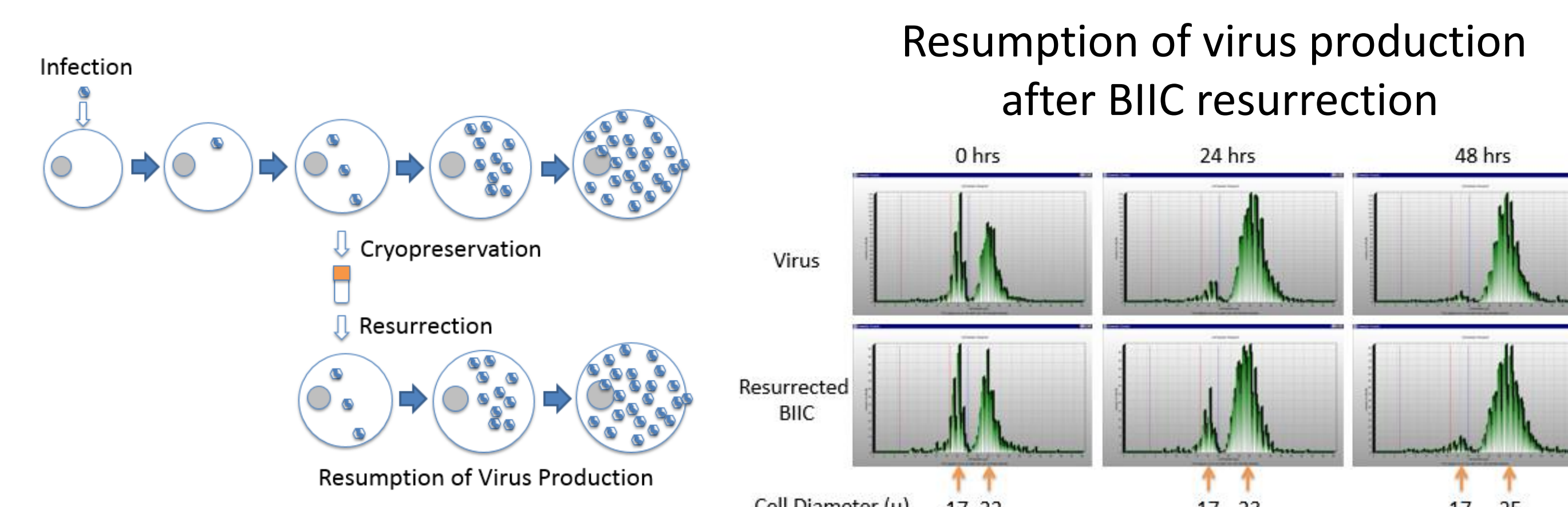


- A scalable transfection process utilizing suspension cell lines was developed
- Accelerates timeline for high-titer virus generation
- Titers: 1-2 X 10e8 pfu/ml
- ~40 ml of P0 Virus equivalent to a P1.
- Harvest supernatant a few days post-transfection.

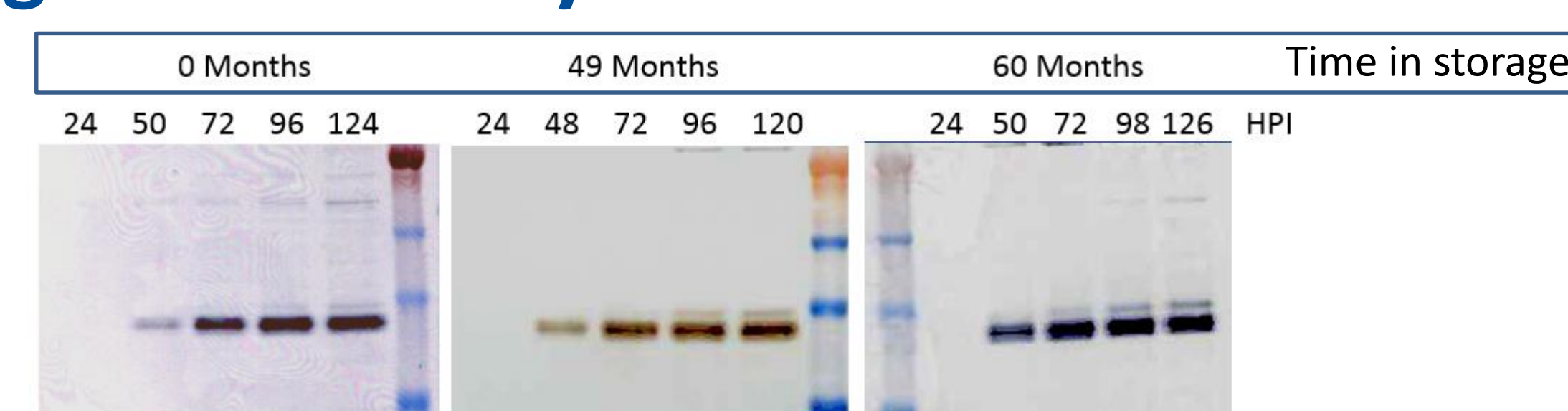
BIIC Virus Storage System

- Cells are infected, cryopreserved at a specific time in the viral life cycle, and stored at -140°C.
- Long term storage: >10 years without loss of potency
- BIICs can be used to directly infect insect cell cultures.
- Used in combination with Infection Kinetics Monitoring (IKM™) technology (below).

BIIC Research Cell Bank Generation & Resurrection



Long-term Stability of BIIC Research Cell Bank



Wasilko & Lee (2007)

Infection Kinetics Monitoring (IKM)

- Titerless system: no plaque assay required
- Monitors population dynamics in an infected insect cell culture using an automated cell analyzer (Cedex Bioanalyzer).
- Multiple cell lines are infected with dilutions of virus and harvested at different time points.
- Several culture parameters (cell density, viability, diameter) in infected cultures are measured daily using the Cedex Bioanalyzer (IKM data)
- IKM data is correlated with protein expression information to determine the optimal time of harvest in scale-up and resupply production.
- Improves scalability and reproducibility in protein yield and quality



IKM Enables Reproducible Protein Yield & Quality from Small-scale to Scale-up Production

50 ml Culture IKM Data

Time (Hrs)	Viable Cell Density (Cells/ml)	Cell Viability (%)	Cell Diameter (microns)
0	16.99x10E6	96.8	16.99
24	17.60x10E6	97.4	21.42
48	2.26x10E6	91.1	21.49
72	2.66x10E6	81.9	20.72

2L Culture IKM Data

Time (Hrs)	Viable Cell Density (Cells/ml)	Cell Viability (%)	Cell Diameter (microns)
0	2.07x10E6	95.6	16.89
24	2.67x10E6	95.8	20.18
48	2.65x10E6	82.5	20.88

