病毒载体

杆状病毒-昆虫细胞表达系统

真核表达系统

表达系统（Expression system）

- **Prokaryote** expression
  —*Escherichia coli*

- **Eukaryote** expression:
  —Yeast
  —**Insect-Baculovirus（昆虫细胞-杆状病毒）**
  —Mammalian

原核表达系统的缺点

- 真核基因在原核的系统中并不真正 “at home” 。
- 原核细胞会频繁识别并排异真核基因表达产物。
- 原核细胞不能对表达产物进行有效的翻译后加工修饰 *(less activity and stability)*。
- 原核细胞不能对表达产物进行正确的折叠 *(improperly folded)*。
- 易产生不溶的、没有活性的包涵体。

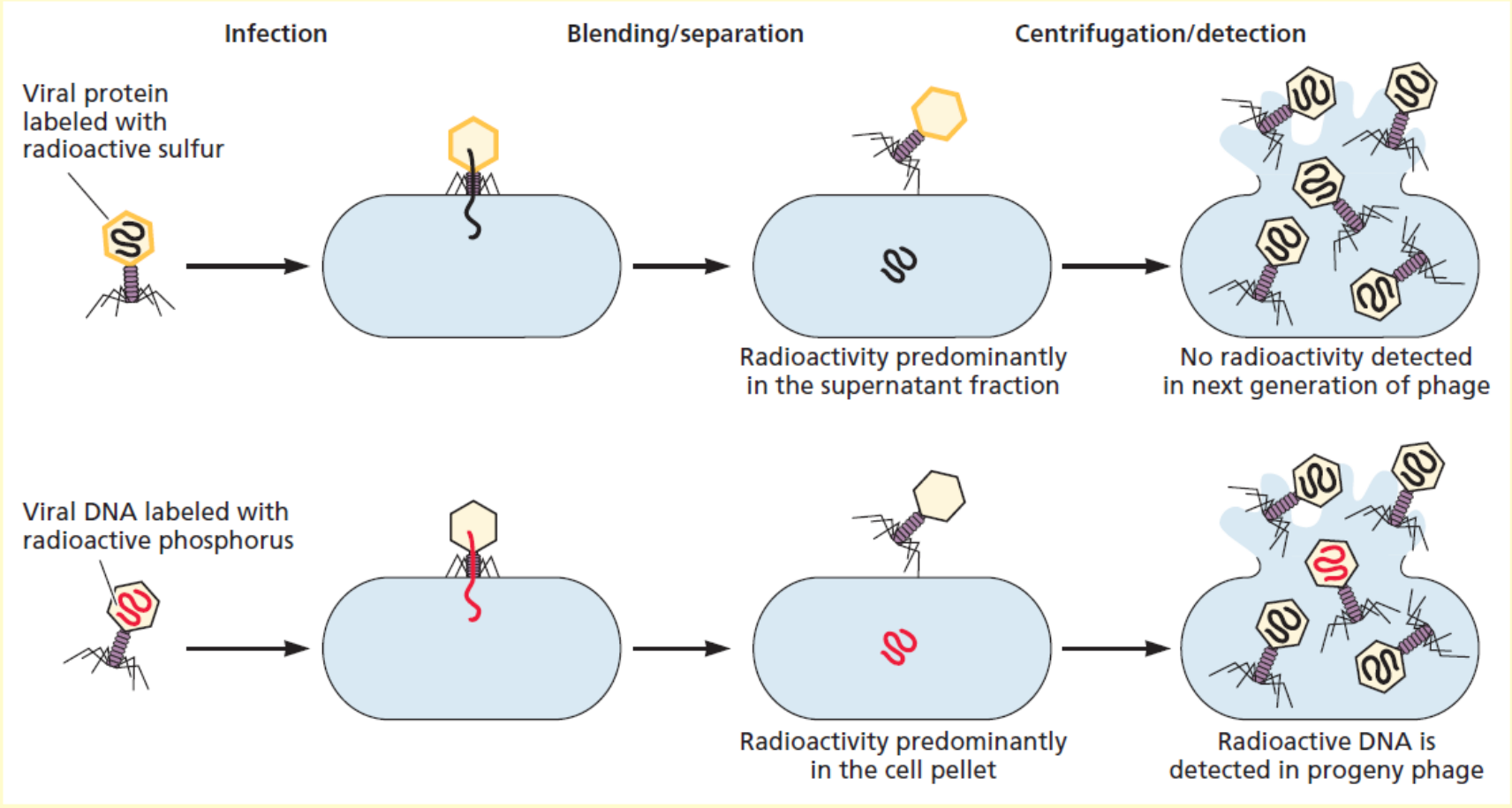
病毒做为载体的基础

- 病毒（真病毒）结构简单，只由**蛋白质**和**核酸**组成，没有细胞结构。

- In 1952, a definitive **experiment** established that viral **nucleic acid** carries **genetic information**.

The Hershey-Chase experiment

By differentially labeling the nucleic acid and protein components of virus particles with radioactive phosphorus (^{32}P) and radioactive sulfur (^{35}S), respectively, Alfred Hershey and Martha Chase showed that the protein coat of the infecting virus could be removed soon after infection by agitating the bacteria for a few minutes in a blender. In contrast, ^{32}P-labeled phage DNA entered and remained associated with the bacterial cells under these conditions. Because such blended cells produced a normal burst of new virus particles, it was clear that the DNA contained all of the information necessary to produce progeny phages.



病毒—载体

（一）植物病毒载体

（二）细菌病毒（噬菌体）载体

（三）动物病毒载体

--SV40

--痘苗病毒载体

--逆转录病毒载体

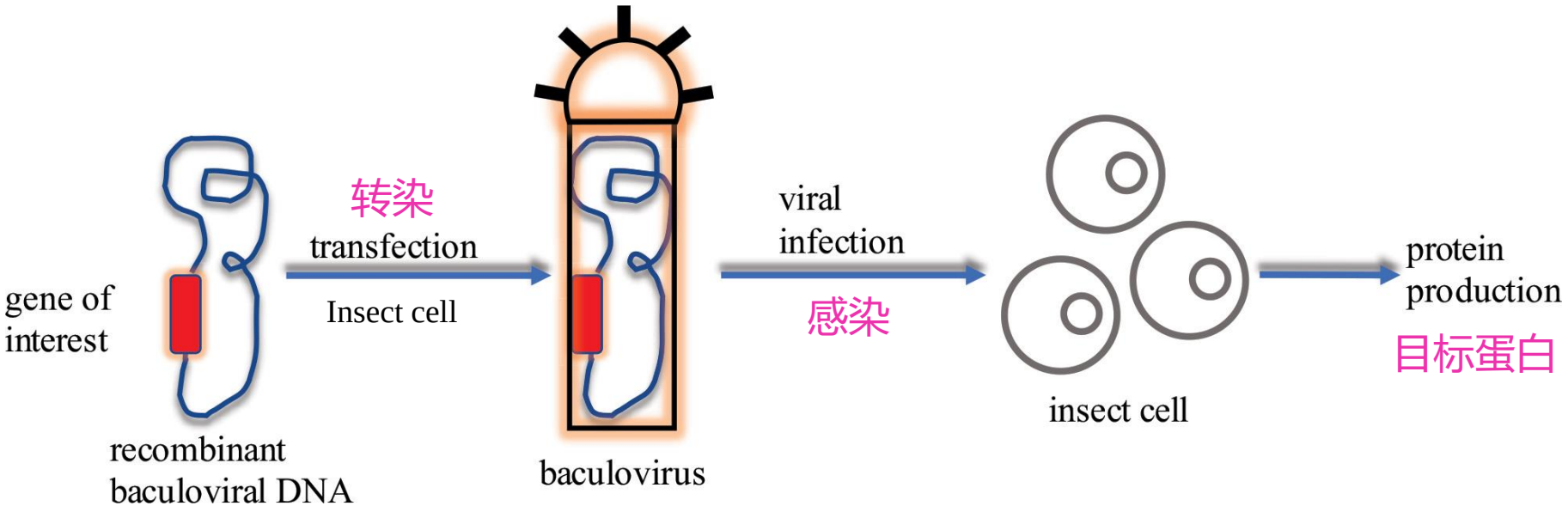
--慢病毒载体

--腺病毒载体

--腺相关病毒载体

--单纯疱疹病毒载体

--杆状病毒载体


gene of interest
recombinant baculoviral DNA
转染
transfection
Insect cell
baculovirus
viral infection
感染
insect cell
protein production
目标蛋白

Background—**Baculovirus**

- 杆状病毒是一类重要的无脊椎动物病毒。

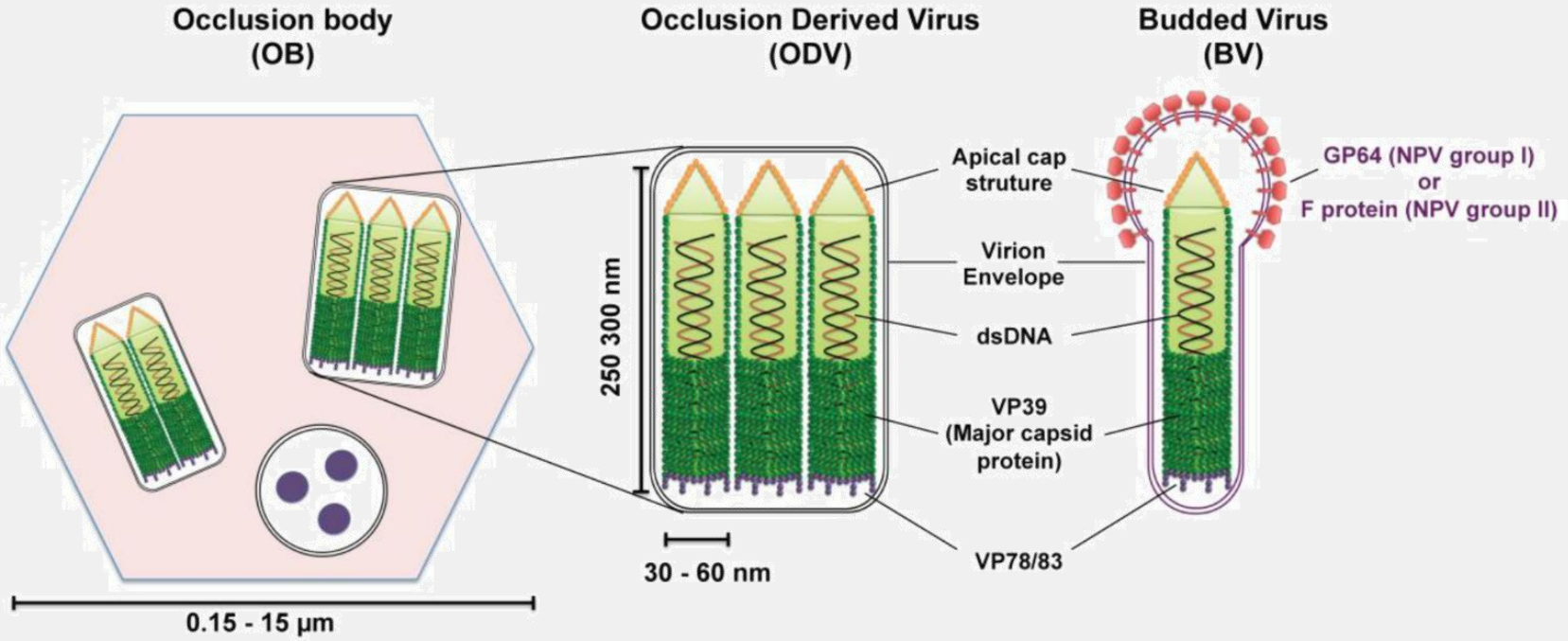
- 基因组为闭合环状的双链DNA，分子大小在 80-180kb, 编码 90-180 个基因。

- 研究比较清楚的杆状病毒有两个:

- **AcMNPV** (Autographa californica nucleopolyhedrovirus) (苜蓿丫纹夜蛾核多角体病毒)
- BmNPV (Bombyx mori nucleopolyhedrosis virus) (家蚕核多角体病毒)

- 病毒粒子有两种形态：芽生型（budded virions, **BV**) 和包埋型（occluded virions, ODV）。

Baculovirus—AcMNPV



Cited from Shelly Au, 2013

有囊膜的闭合环状双链DNA病毒(130kb)，编码154个ORF
主要感染鳞翅目、膜翅目和双翅目昆虫

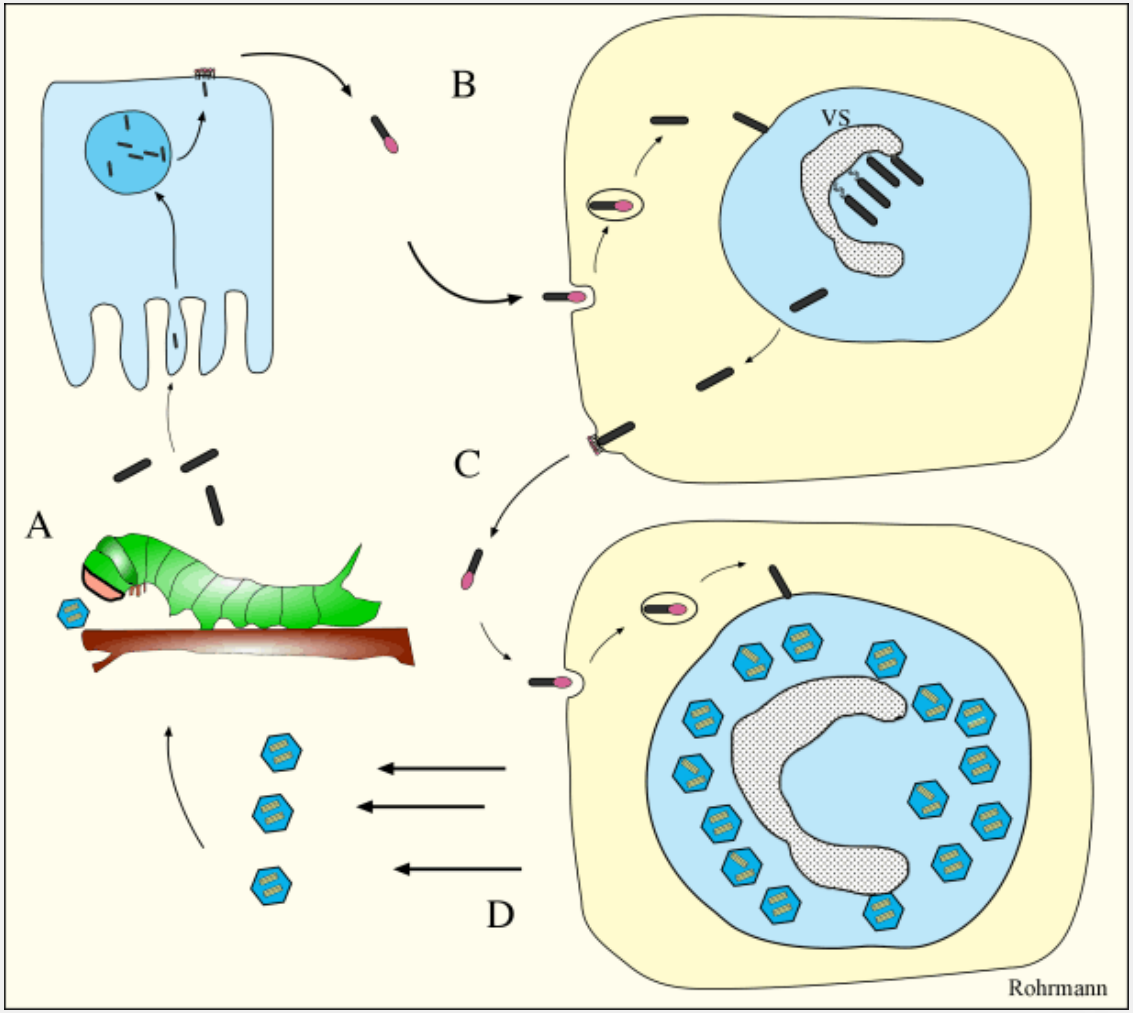
杆状病毒的感染（生活）周期

A: 环境中的包涵体(**OB**)被昆虫摄食后进入中肠，裂解，释放出包埋型病毒粒子（**ODV**）感染中肠上皮细胞

B: 细胞释放的芽生型病毒粒子（**BV**）引发系统感染

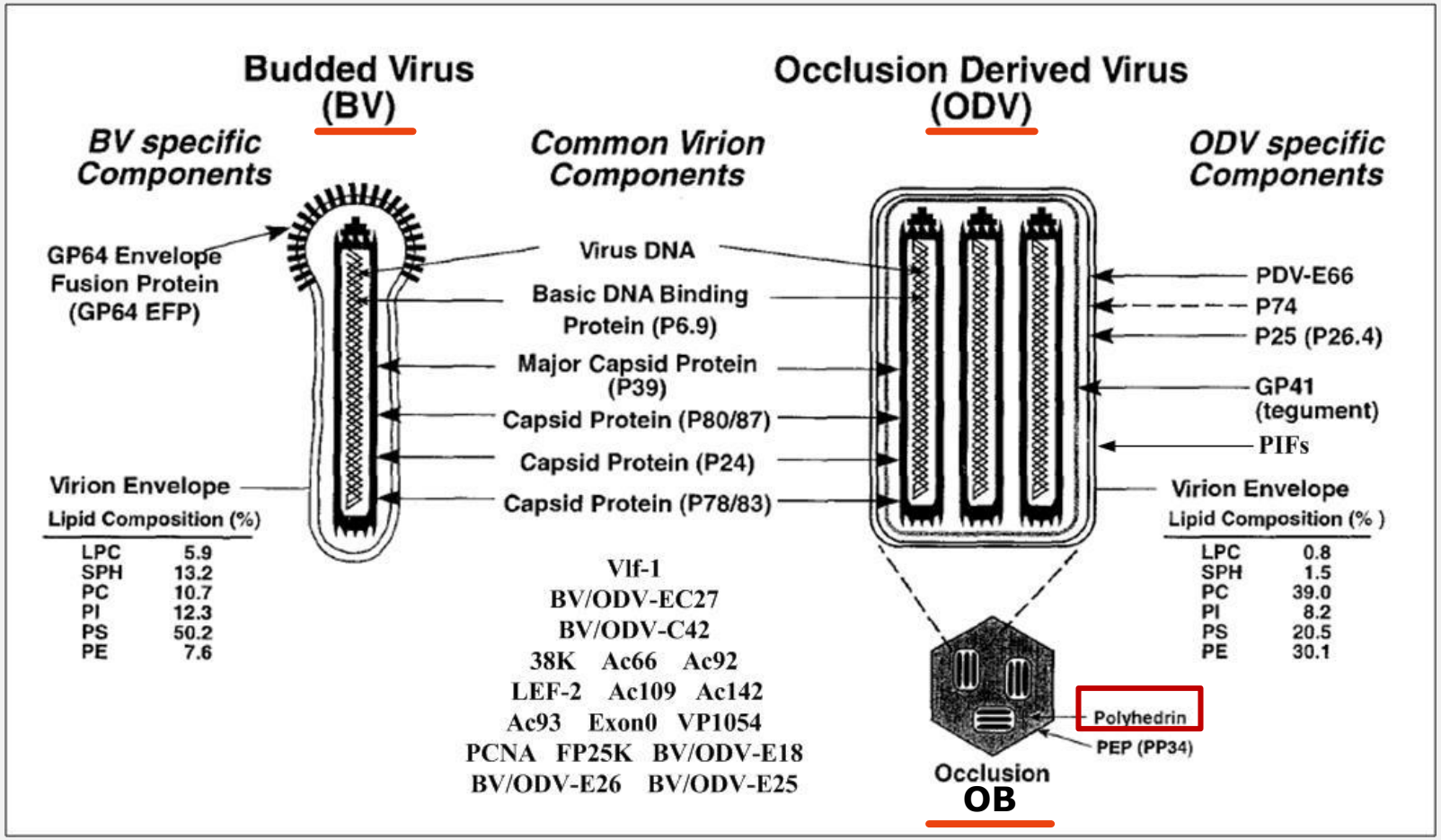
C: 感染的早期，主要产生**BV**，使感染在昆虫的细胞、组织间传播

D： 感染晚期形成大量**ODV**，细胞死亡，释放出**OB**到环境中



图（引自 *G. F. Rohrmann*, 2011）

BV（早期病毒粒子），ODV（晚期病毒粒子），OB（包涵体）



病毒感染晚期大量表达*Polyhedrin*基因和*P10*基因，前者作为基质蛋白主要用于包埋 ODV

大量表达的基因一般都有很强的启动子

杆状病毒表达系统的构建历程

Table 1. Milestones leading to the invention of the baculovirus expression system

Description	Impact for research and development	References
Isolation of *Ac*MNPV	Initial discovery	Vail *et al.* (1971)
Establishment of first insect cell lines	*In vitro* studies	Gaw *et al.* (1959), Grace (1962), Hink (1970)
Infectivity of haemolymph for cells in culture	BV/ODV difference	Vaughn & Faulkner (1963),
Characteristics of virus produced in cell culture	Infection in cell culture	Henderson *et al.* (1974)
*Ac*MNPV plaque assay	Purification of genetic variants	Brown & Faulkner (1977)
Infectivity of baculovirus DNA	Transfection	Burand *et al.* (1980)
Physical map of *Ac*MNPV	Cloning of individual fragments	Vlak & Smith (1982)
Mapping and sequencing of the polyhedrin gene	Vector construction	Adang & Miller (1982), Vlak *et al.* (1981)
Polyhedrin protein is not essential in cell culture	Use of polyhedrin promoter for foreign gene expression	Smith *et al.* (1983b)

Cited from Monique M. van Oers, JGV, 2015

Polyhedrin (polh) gene：多角体基因

病毒极晚期基因：多角体基因 和 *p10*基因

AcMNPV的基因表达分为四个阶段：立早期，早期，晚期和极晚期。在极晚期基因表达过程中有两个高表达的蛋白，分别是多角体蛋白和P10蛋白。

多角体蛋白是形成包涵体**OB**的主要成分，感染后期在细胞中的累积可高达30%～50%，是病毒复制的**非必需**成分，但对病毒粒子有保护作用，使之保持稳定和感染力。

P10蛋白也是病毒复制非必需成分，可在细胞中形成纤维状物质。

- 多角体基因和p10基因的**启动子**具有很强的启动能力，因此这两个基因的基因座，成为杆状病毒表达载体系统理想的外源基因插入位点。

昆虫细胞（insect cell）

草地贪夜蛾（*Spodoptera frugiperda*）昆虫细胞（Sf9, Sf21… ）

昆虫细胞培养条件:

- Temperature: **27—28℃**
- pH: **6.1 to 6.4**
- Grace’s Insect cell culture medium

杆状病毒表达系统

Baculovirus Expression Vector Systems

(BEVS)

（1）通过**同源重组（Homologous Recombination）**的方式产生**重组 Virus** --（早期的方法）

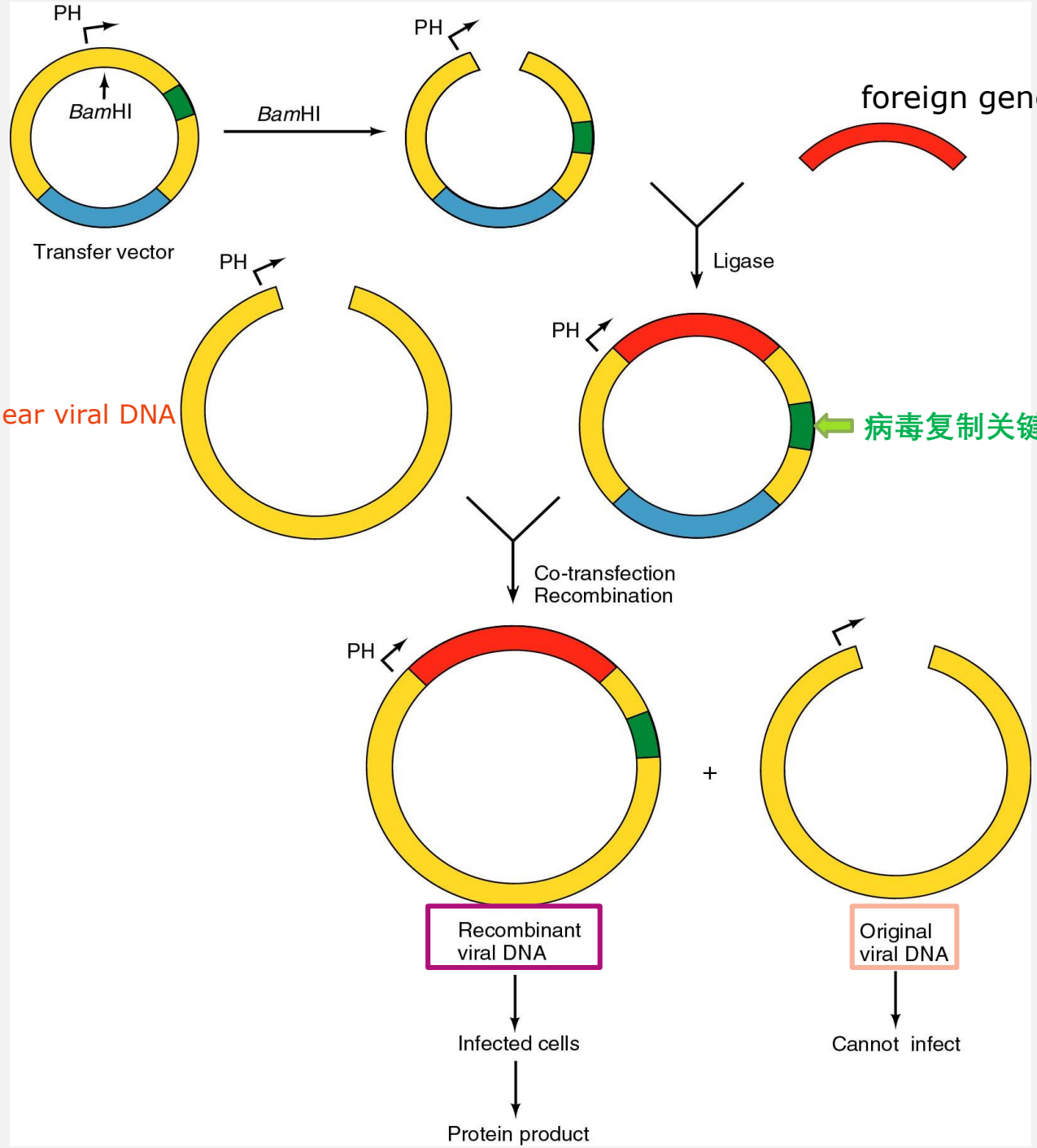
（2）通过**位点特异性转座（Site-Specific Transposition）**的方式产生**重组 Virus** -- **（Bac-to-Bac）**

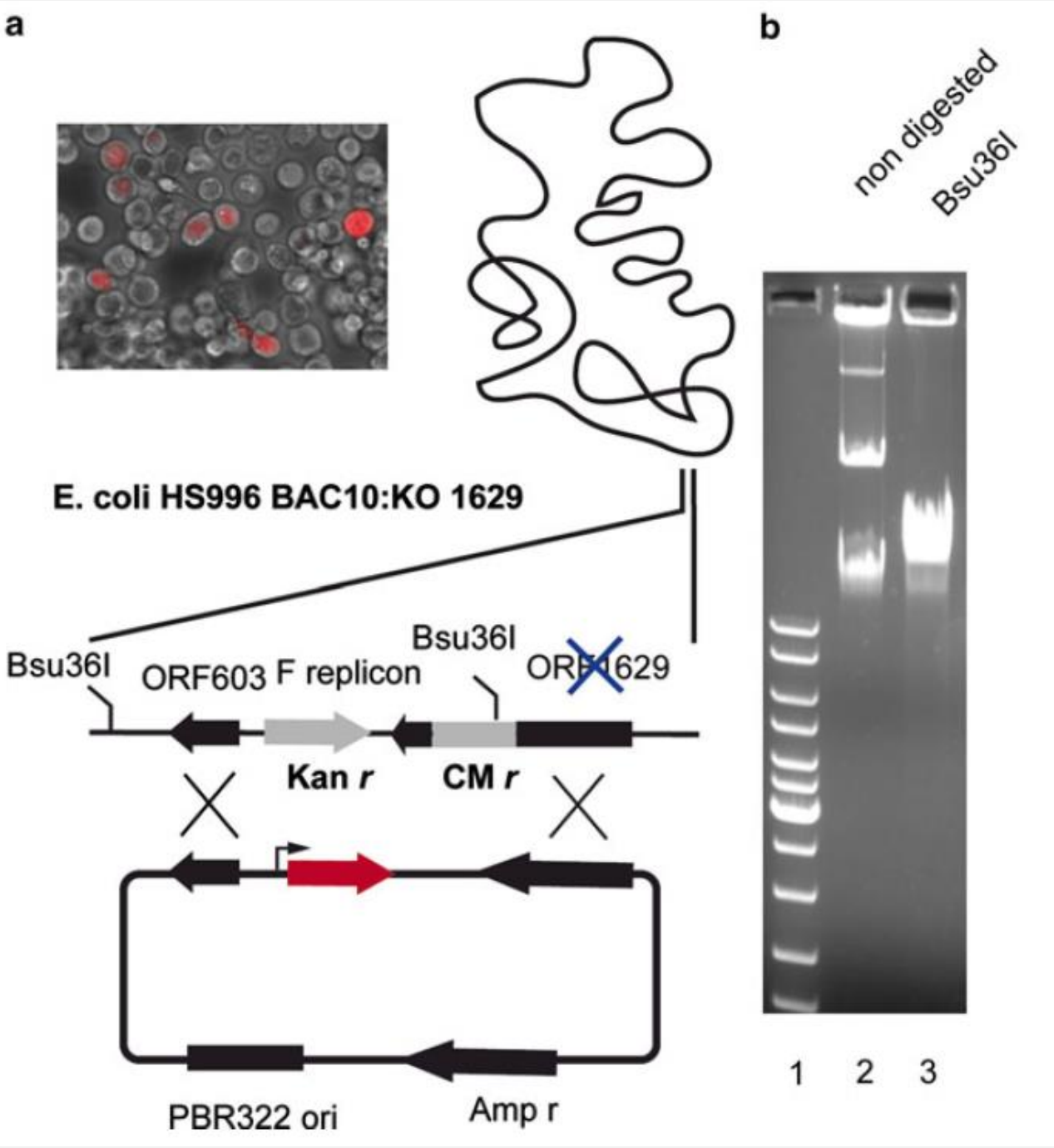
（1）通过**同源重组**（Homologous Recombination）的方式产生**重组病毒**

- 首先，将 foreign gene 克隆到 baculovirus transfer vector 中，该载体含有多角体基因启动子(P_{PH})，其侧翼序列包含了一个病毒复制的必需基因。
- Transfer vector 中缺失了多角体基因的编码序列
- 紧邻P_{PH}的下游有一个 *Bam*H I 的酶切位点，可将其切开插入 **foreign gene**。

- 然后，将重组 transfer vector 和 linear viral DNA（缺失了病毒复制的关键基因）一起共转染（**co-transfection**）昆虫细胞；
- 进入细胞后，载体和线性病毒DNA发生双交叉（double-crossover）**重组**，将外源基因与病毒复制关键基因一起插入 viral DNA，从而产生**重组病毒** DNA，其上携带具有P_{PH}的 **外源基因；**
- 用**重组病毒**感染昆虫细胞；
- 最后，用**重组病毒**感染昆虫细胞，收集外源基因表达的**蛋白产物**。

同源重组


PH
BamHI
Transfer vector
BamHI
PH
foreign gene
Ligase
PH
linear viral DNA
PH
病毒复制关键基因
Co-transfection
Recombination
PH
+
Recombinant
viral DNA
Original
viral DNA
Infected cells
Cannot infect
Protein product



Construction of recombinant baculovirus by homologous recombination.
(**a**) Linearized viral DNA (purified BAC10:KO1629) is co-transfected with a transfer vector containing the cDNA (DsRed) to be inserted into the viral genome.
Homologous recombination replaces the F replicon by the expression cassette for the gene of interest (DsRed) and restores the essential ORF1629, allowing viral replication and expression of the desired protein. 2–3 days after transfection, fluorescence of the DsRed protein should be visible. In this case only a few cells are infected.
(**b**) Analysis of purified BAC10:KO1629 on a 0.8 % agarose gel before (*lane 2*) and after digestion with Bsu36I (*lane 3*).
(Cited from "Insect Cells–Baculovirus System for the Production of Difficult to Express Proteins")

Notice:

- 开始所用的 viral DNA 是线性的，并且缺失了病毒复制的关键基因，所以它本身无法感染细胞。这种自身缺乏感染性反而方便筛选最终的重组**病毒**，因为只有成为重组病毒才具有感染性。

- 线性化 viral DNA 大约是转移载体的 15 倍 大。

（2）通过转座（Site-Specific Transposition）的方式产生重组 Virus

↓

Bac-to-Bac

1993年，Luckow等采用与BAC载体原理类似的F-复制子首次成功构建了可在大肠杆菌中复制的杆状病毒，该重组杆状病毒称为AcMNPV Bacmid，即Bac-to-Bac（Bacteria-to-Baculovirus）系统 。

Bac-to-Bac系统，是将一个表达框位点特异性地转座到杆状病毒穿梭载体（Bacmid）中，并在大肠杆菌中进行增殖，以获得重组Bacmid 。

Bac-to-Bac系统，是一种快速省时的表达系统，菌落筛选简单，能快速对重组蛋白进行纯化，适合外源蛋白的高效表达。

位点特异性转座

- 利用 **Tn7** 转座序列将 **foreign gene** 转座插入到在 *E.coli* 中增殖的bacmid中。
- 将 **foreign gene** 克隆进 pFastBac 载体，然后转化 DH10Bac 大肠杆菌细胞，该细胞含有带mini-attTn7靶序列的 bacmid 和一个辅助质粒（helper plasmid）。
- pFastBac 载体上的左右 mini-Tn7 可将插入其中的外源基因一起转座到 bacmid 中的 mini-attTn7 靶接受位点，转座酶由 helper plasmid 提供。
- 通过抗生素和蓝白斑筛选鉴定含 **recombinant bacmid** 的阳性克隆。
- 用获得的 **recombinant bacmid DNA** 转染昆虫细胞。

Bac-to-Bac 表达系统

- 供体质粒（**pFastBac**）：可将外源基因插入到该质粒的左右 mini-Tn7 中间，构成表达框。外源基因的表达受控于杆状病毒特异性启动子 PpH or Pp10。

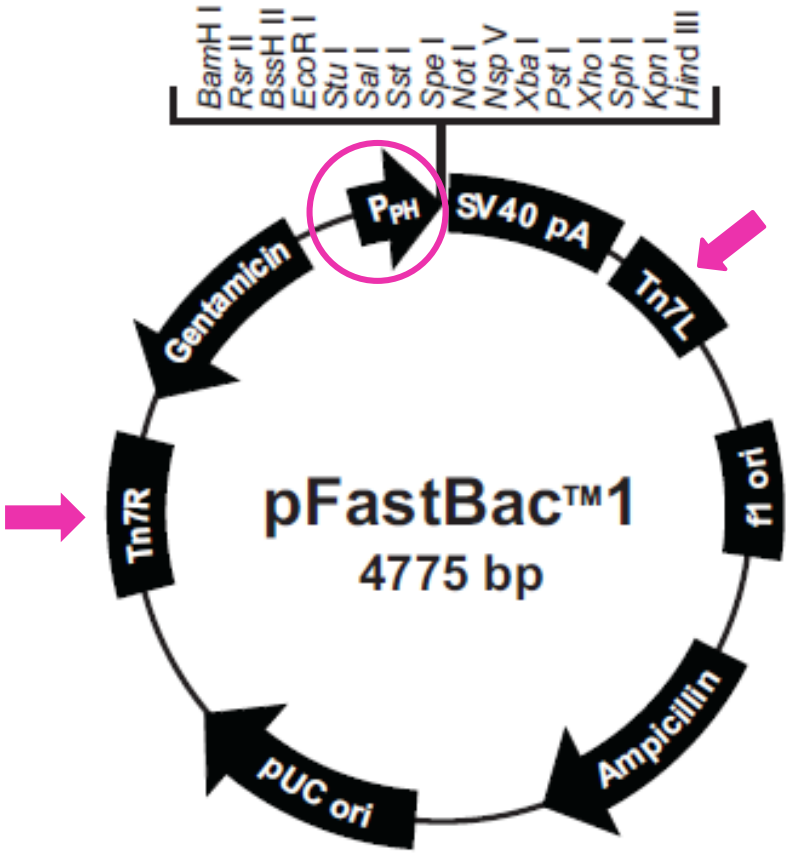
- *E. coli* 菌株, **DH10Bac**：包含了一个杆状病毒穿梭载体 (**bacmid**) 和一个辅助质粒。可通过转座 pFastBac 上的表达框而产生**重组 bacmid**。

供体质粒--pFastBac

- 强多角体启动子可驱动外源基因蛋白的高水平表达。
- 有大的 MCS 并提供 **3** 种读码框，方便克隆外源基因。
  - pFastBacHT： N-端有 **6 X His** tag 方便纯化重组蛋白
  - pFastBac Dual：既有$\mathbf{P_{PH}}$ 又有$\mathbf{P_{p10}}$，可同时表达两个蛋白

pFastBac™1 Map

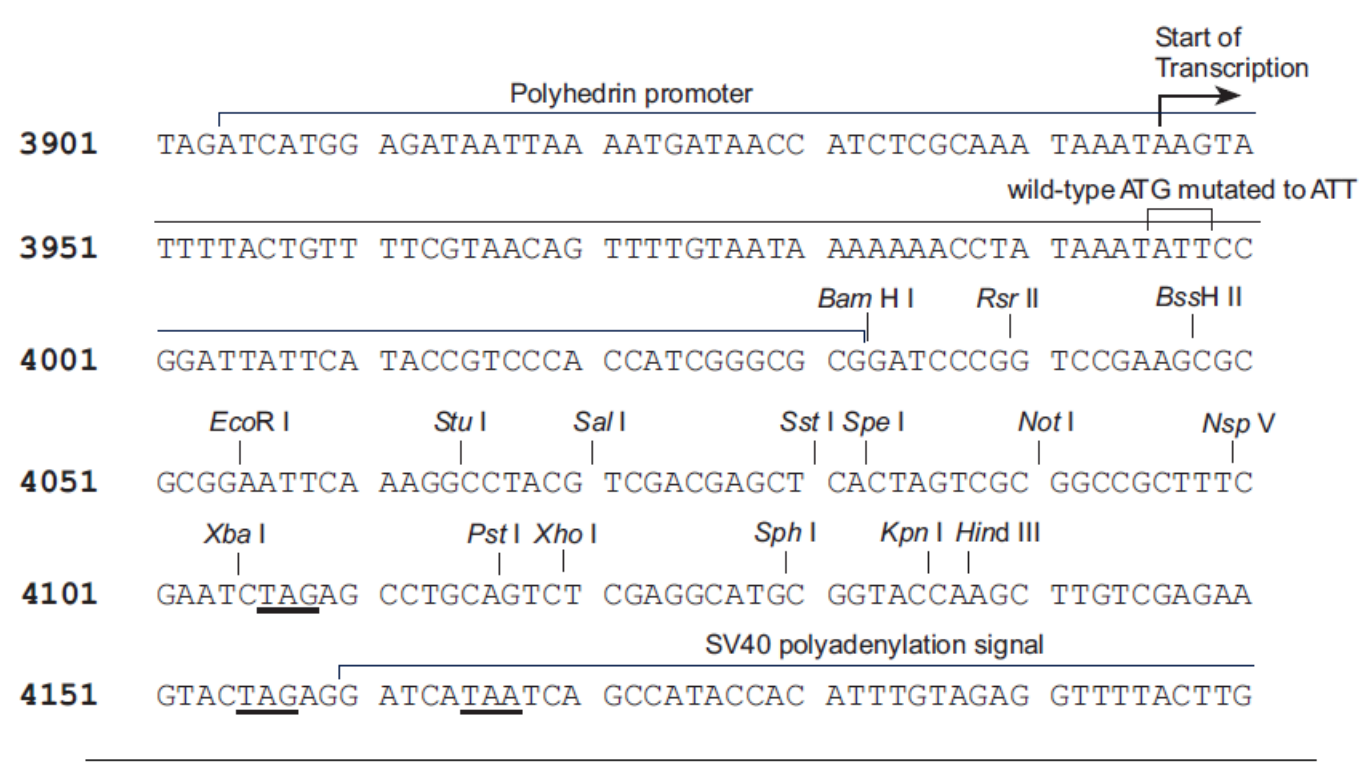
The map below shows the elements of pFastBac™1. **The vector sequence of pFastBac™1 is available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**



Comments for pFastBac™1
4775 nucleotides

f1 origin: bases 2-457
Ampicillin resistance gene: bases 589-1449
pUC origin: bases 1594-2267
Tn7R: bases 2511-2735
Gentamicin resistance gene: bases 2802-3335 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 3904-4032
Multiple cloning site: bases 4037-4142
SV40 polyadenylation signal: bases 4160-4400
Tn7L: bases 4429-4594

pFastBac 1



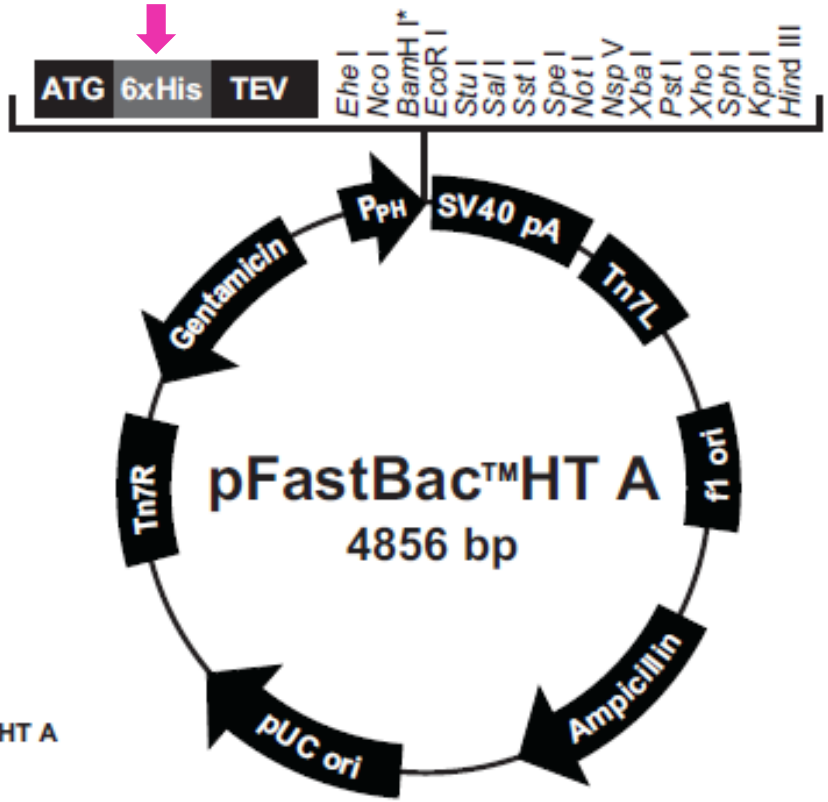
Non-fusion vector. To ensure proper expression of recombinant protein, the **insert** must contain:

- An **ATG** start codon for initiation of translation
- A stop codon for termination

➢ *Stop codons are included in the MCS in all three reading frames*

pFastBac™HT A Map

The map below shows the elements of pFastBac™HT A. **The vector sequences of the pFastBac™HT A, B, and C vectors are available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**

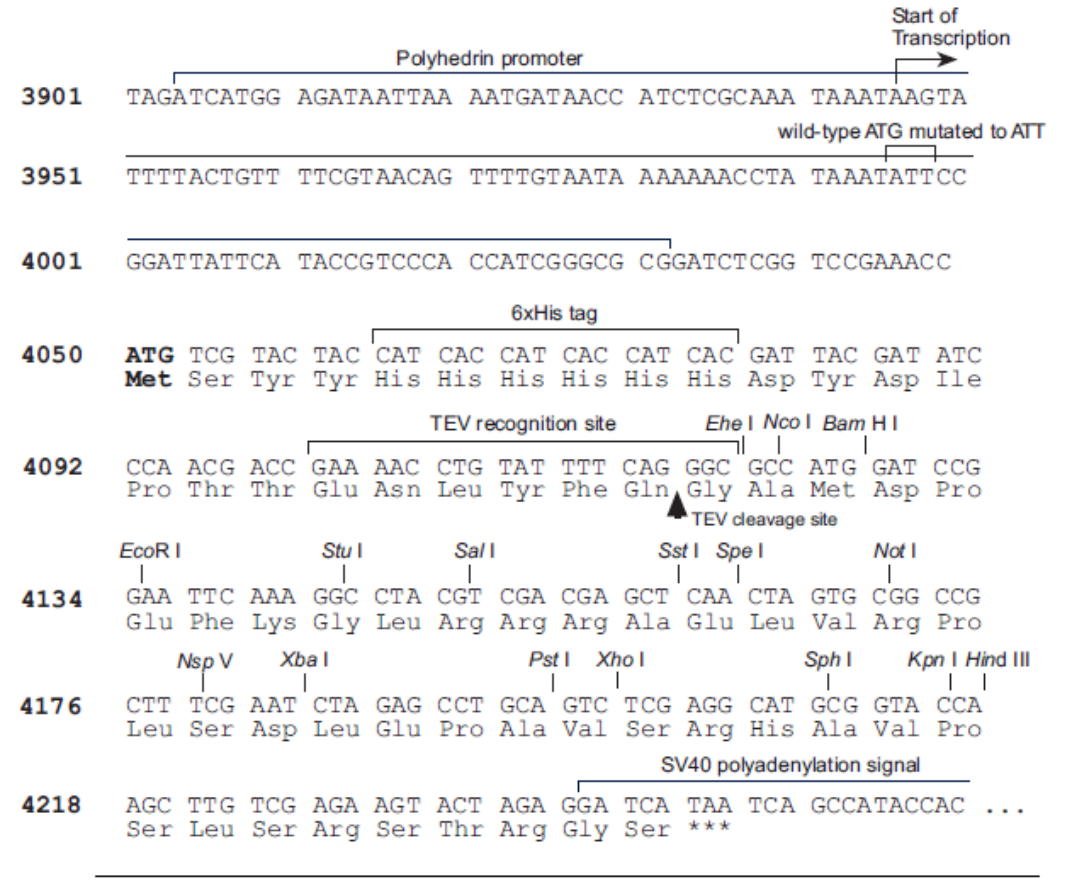


Comments for pFastBac™HT A
4856 nucleotides

f1 origin: bases 2-457
Ampicillin resistance gene: bases 589-1449
pUC origin: bases 1594-2267
Tn7R: bases 2511-2735
Gentamicin resistance gene: bases 2802-3335 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 3904-4032
Initiation ATG: bases 4050-4052
6xHis tag: bases 4062-4079
TEV recognition site: bases 4101-4121
Multiple cloning site: bases 4119-4222
SV40 polyadenylation signal: bases 4240-4480
Tn7L: bases 4509-4674

*Frameshift occurs at the *Bam*H I site in each vector

pFastBacHT A, B, and C



Fusion vectors. To ensure proper expression of recombinant protein:

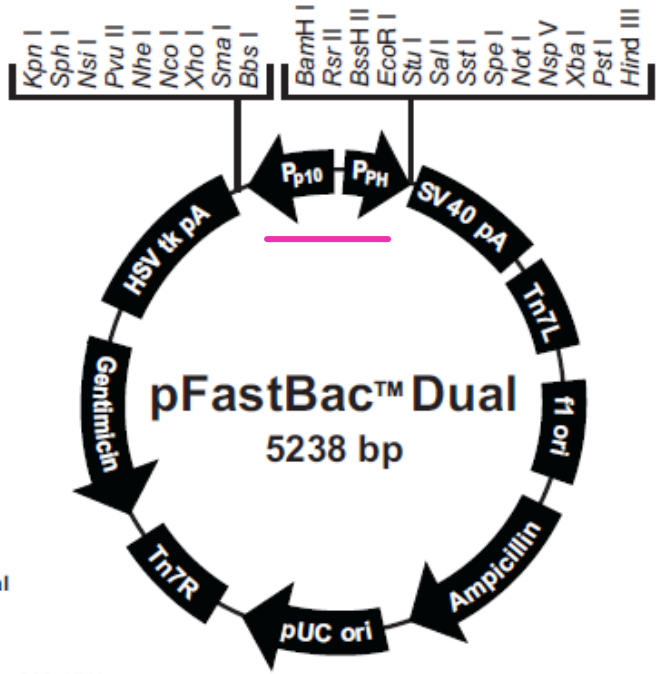
- Clone gene **in frame** with the initiation ATG. Fusing with a N-terminal **6xHis** tag and a cleavage site for the AcTEV Protease.
- Include a stop codon with insert.

pFastBac Dual

Contains **two** MCS to allow expression of **two** heterologous genes; one controlled by P_{PH} and one by P_{P10}

pFastBac™ Dual Map

The map below shows the elements of pFastBac™ Dual. **The vector sequence of pFastBac™ Dual is available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**



Comments for pFastBac™ Dual
5238 nucleotides

f1 origin: bases 102-557
Ampicillin resistance gene: bases 689-1549
pUC origin: bases 1694-2367
Tn7R: bases 2611-2835
Gentamicin resistance gene: bases 2902-3435 (complementary strand)
HSV tk polyadenylation signal: bases 3992-4274 (complementary strand)
Multiple cloning site: bases 4274-4337 (complementary strand)
p10 promoter (P_{p10}): bases 4338-4459 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 4478-4606
Multiple cloning site: bases 4606-4704
SV40 polyadenylation signal: bases 4722-4962
Tn7L: bases 4991-5156

将pFastBac 重组质粒转化进 *E. coli* 细胞

将外源基因插入pFastBac供体质粒后，转化*E. coli* 细胞，筛选 Amp^r 的转化子。
(May use *rec*A, *end*A *E. coli* strain including TOP10 or DH5α for transformation)

- 筛选和提取含外源基因的重组pFastBac质粒，下一步将其转化进入DH10Bac 细胞。

转化 DH10Bac *E. coli* 细胞

获得了重组pFastBac后，可将其转入 DH10Bac *E. coli* 细胞，以将外源基因转座至 bacmid。

可通过抗生素和蓝白斑筛选、鉴定**重组 bacmid**。

DH10Bac *E. coli* strain

- DH10Bac 细胞含有一个杆状病毒穿梭载体（bacmid) 和一个辅助质粒。Bacmid 上带有 mini-*att*Tn7 靶接受位点。
- 将pFastBac 转化进入 DH10Bac 细胞后，pFastBac上mini-Tn7所携带的外源基因表达框，在辅助质粒提供的转座酶的作用下转座至 bacmid上的 mini-*att*Tn7 靶接受位点，从而产生**重组 bacmid**。
- DH10Bac 含有的 helper plasmid, pMON7124 (13.2 kb), 编码转座酶，并且携带四环素（tetracycline）抗性。

杆状病毒穿梭载体：**Bacmid**

Bacmid，bMON14272 (136 kb), 包含：

- mini-F replicon
- Kan^r marker
- LacZα 肽段编码序列，其中插入了mini-*att*Tn7序列（不影响 LacZα肽段)

在 DH10Bac 细胞中增殖的 bacmid 携带有卡那霉素（ kanamycin）抗性，可以进行蓝白斑筛选。

分离 Recombinant Bacmid DNA

分离用作转染昆虫细胞的重组bacmid DNA

*Inoculate a single **white colony** into 2 ml LB medium with 50 μg/ml kanamycin, 7 μg/ml gentamicin, and 10 μg/ml tetracycline.*

Incubate the culture at 37℃ in a shaking at 250 rpm overnight.

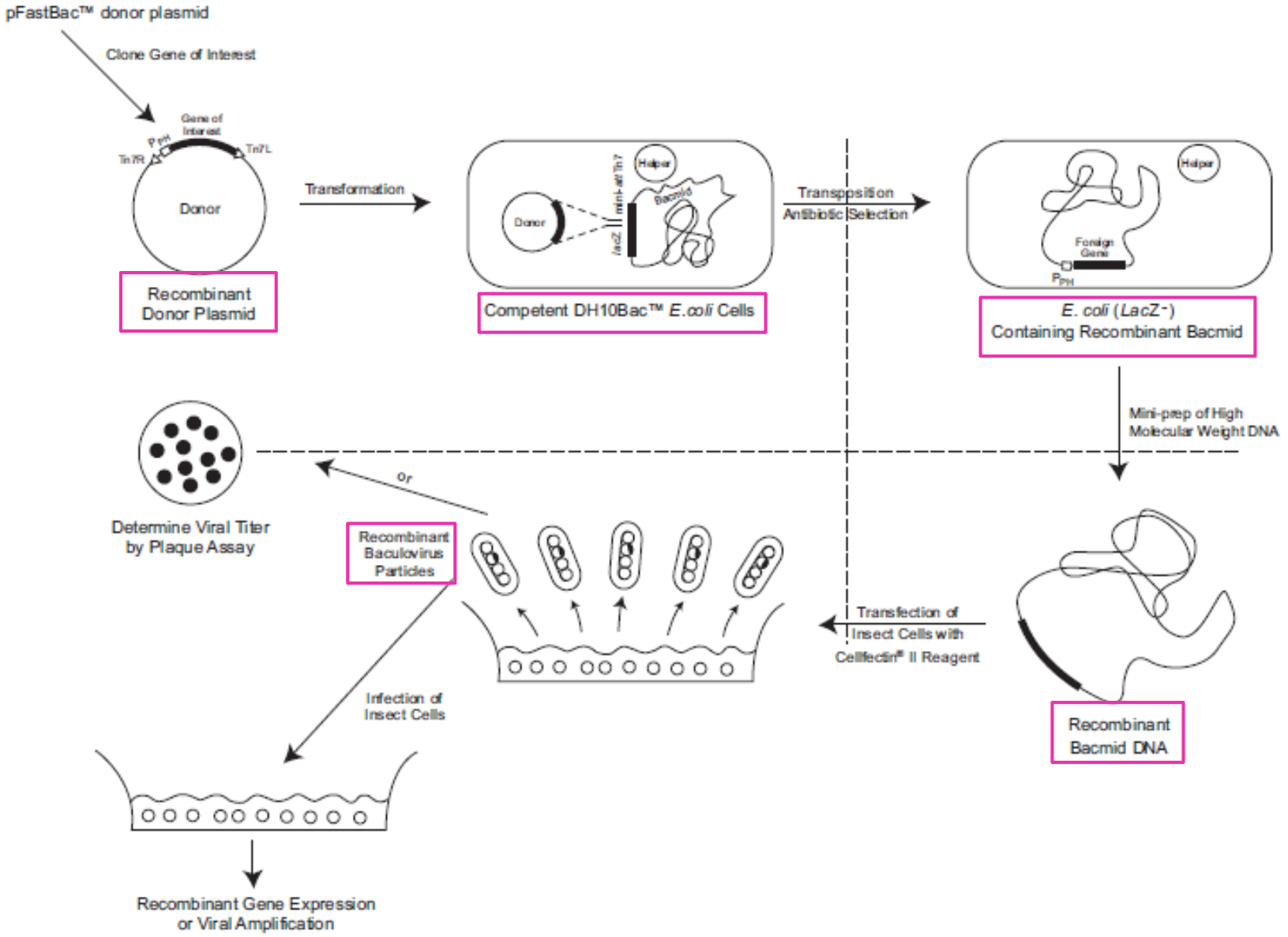
多重抗性 + 蓝白斑筛选

用分离到的重组 bacmid DNA 转染昆虫细胞，以产生重组 baculovirus，产生的重组 baculovirus 可用于外源蛋白的表达。

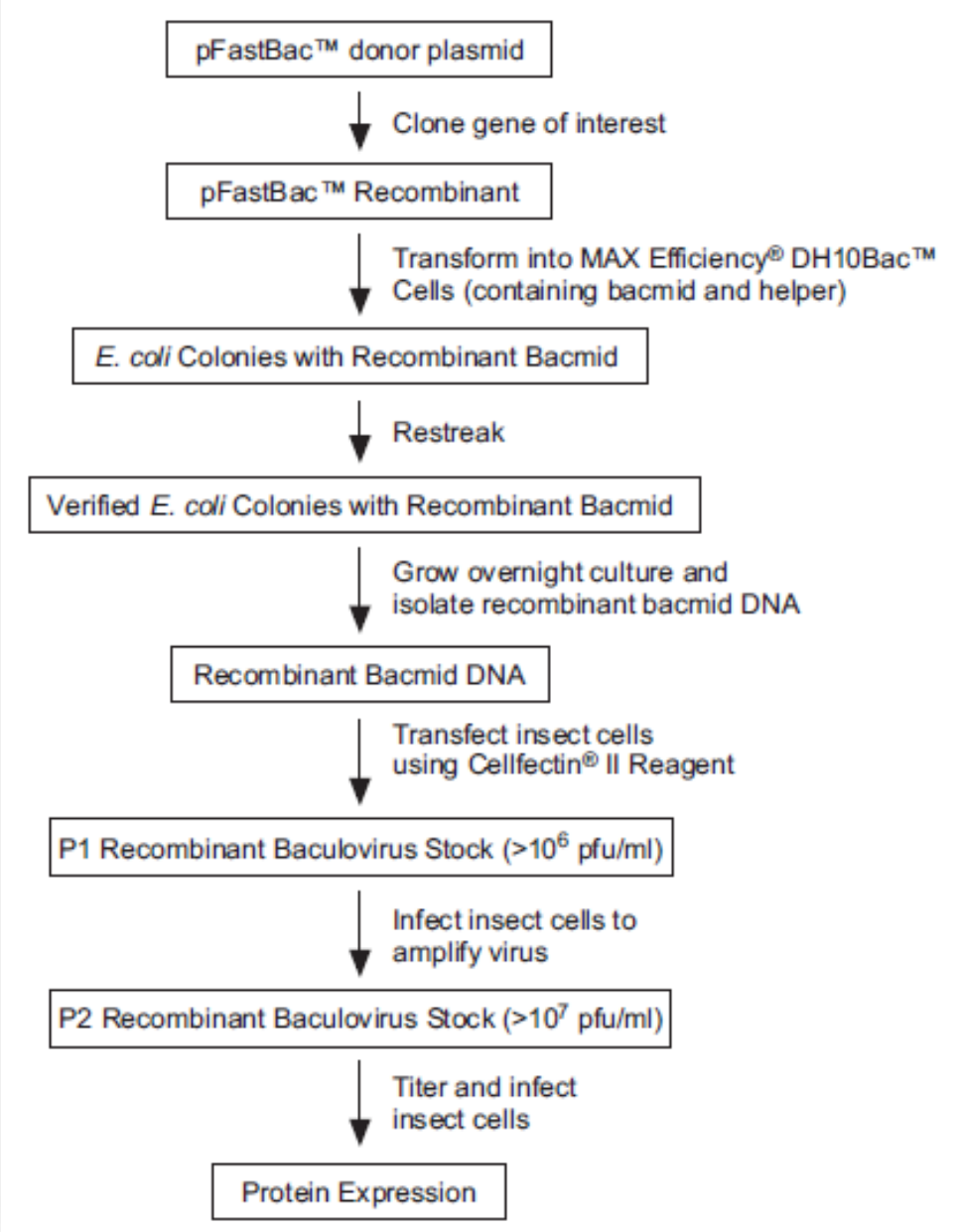
将重组 baculoviral stock 扩增并测滴度（titered），高滴度的病毒株可用来感染昆虫细胞，以获得大量表达的**重组蛋白**。

Diagram of the Bac-to-Bac® System

The figure below depicts the generation of recombinant baculovirus and the expression of your gene of interest using the Bac-to-Bac® Baculovirus Expression System.



General steps required to express the gene of interest using the Bac-to-Bac Expression System

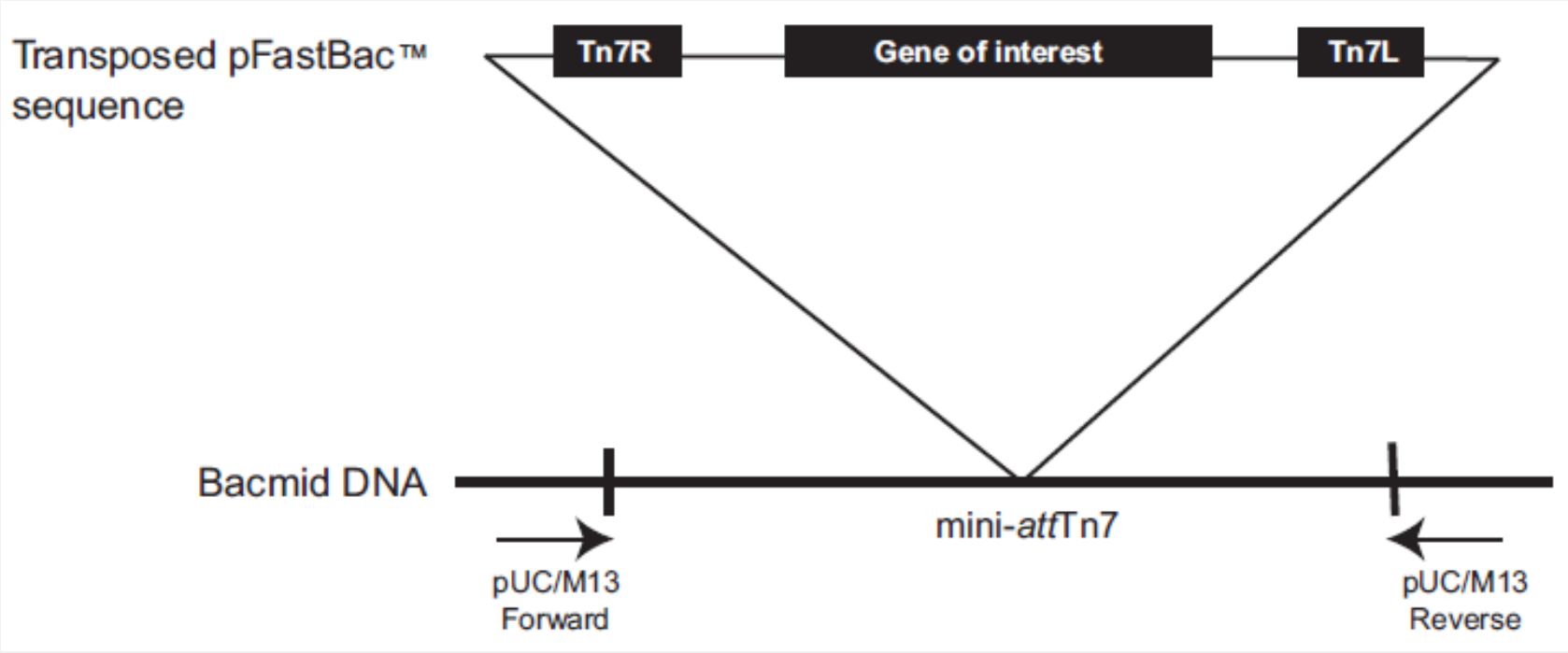


分析重组Bacmid DNA

重组 bacmid DNA很大，≥135 kb.

可采用 PCR 验证重组 bacmid上的外源基因

(by pUC/M13 Forward and Reverse primers)



外源基因表达框的插入破坏了**LacZα肽**段的表达, 所以含重组 bacmid 的克隆是白色的

True positive colonies tend to be **large**

转染昆虫细胞

- 用含外源基因的重组 bacmid**转染昆虫细胞**以产生**重组 baculovirus**

- Using Sf9 or Sf21 cells
- Add DNA transfection mixture
- Incubate cells at 27°C for 3–5 hours
- Remove the transfection mixture and replace with complete medium
- Incubate cells at 27°C for 72 hours or until see **signs of viral infection**（细胞感染的迹象—细胞病理效应）

分离 P1 代病毒（Viral Stock）

- 转染后早中期，BV 会释放进培养基；
- 随着细胞出现明显的病毒感染迹象，至晚期或极晚期时，收集细胞培养上清中的病毒，此为P1代 viral stock.

Signs of Infection	Phenotype	Description
Early (first 24 hours)	Increased cell diameter	A 25–50% increase in cell diameter may be seen.
	Increased size of cell nuclei	Nuclei may appear to "fill" the cells.
Late (24–72 hours)	Cessation of cell growth	Cells appear to stop growing when compared to a cell-only control.
	Granular appearance	Signs of viral budding; vesicular appearance to cells.
	Detachment	Cells release from the plate or flask.
Very Late (>72 hours)	Cell lysis	Cells appear lysed, and show signs of clearing in the monolayer.

一些要点

- 使用**低 MOI**（0.05–0.1）的病毒液去扩增重组病毒。
- 不要无限扩增病毒, 因为会累积突变。**P3** 代病毒是可使用的最高扩增代数病毒。
- 所使用的昆虫细胞应处于良好的生长状态, 低传代数(5–20), 对数生长, 以及>95% viability.
- If the titer of P1 viral stock have not determined, it can be assumed that the titer ranges from $1x10^6$ to $1X10^7$ pfu/ml (plaque forming units/ml).
- **MOI** (multiplicity of infection，感染复数)：给予每个细胞的病毒粒子数量。

扩增 Baculoviral Stock

P1 代viral stock 通常量比较少且低滴度。需要用它去感染细胞，获得高滴度的 **P2 代** stock.

扩增 viral stock 时，通常采用**低 MOI**（0.05–0.1）感染细胞。

$$\text{Inoculum required (ml)} = \left(\frac{\text{MOI (pfu/cell)} \times \text{number of cells}}{\text{titer of viral stock (pfu/ml)}}\right)$$

Example：infect 10 ml culture at $2X10^6$ cells/ml using an MOI = 0.1. Assume that the titer of P1 viral stock is $5X10^6$ pfu/ml.

$$\text{Inoculum required (ml)} = \left(\frac{0.1\,\text{pfu/cell} \;\; 2\times10^7\;\text{cells}}{5\times10^6\;\text{pfu/ml}}\right)$$

Inoculum required (ml) = 0.4 ml

Case: Amplify viral stock

(1) Prepare insect cells at 2X10^6 cells/well. Incubate at 27℃ (1 hour) to allow attachment.
(2) After attachment, Add appropriate amount of **P1** viral stock to each well.
(3) Incubate cells at 27℃ for 48 or 72 hours.
(4) Collect the medium containing virus.
(5) Centrifuge the collection to obtain clarified viral stock. This is the **P2 viral stock**.
(6) Determine the titer.

Next: scale-up the amplification procedure to produce the high-titer **P3 stock**

关于病毒储存

- 首先：分装成小份

- 日常使用：4℃保存，避光
- 长期保存：–80℃保存

- 日常使用的病毒液不要低于4℃放置。 储存在 –80℃的病毒，每冻融一次滴度下降 10% 左右。

避免反复冻融

表达重组蛋白

获得了合适滴度 (e.g.,1X10^8 pfu/ml）的重组 baculoviral stock 后，可用其感染昆虫细胞进行 **重组蛋白**的**表达**检测。

As a starting point, infect cells using an **MOI** of **1 to 5** perform a time course to determine the expression kinetics, as many proteins may be degraded by cellular proteases released in cell culture.

Note: Maximum expression of secreted proteins is generally observed between 30 and 72 hpi and non-secreted proteins between 48 and 96 hpi.

Use the MOI that provides the optimal level of expression. Infect cells at varying MOIs (*e.g.,* 1, 2, 5, 10, 20) and assay for protein expression.

Time course（表达时相）：Infect cells at a constant MOI and assay for recombinant protein expression at different times post-infection (*e.g.,* 24, 48, 72, 96 hpi.)

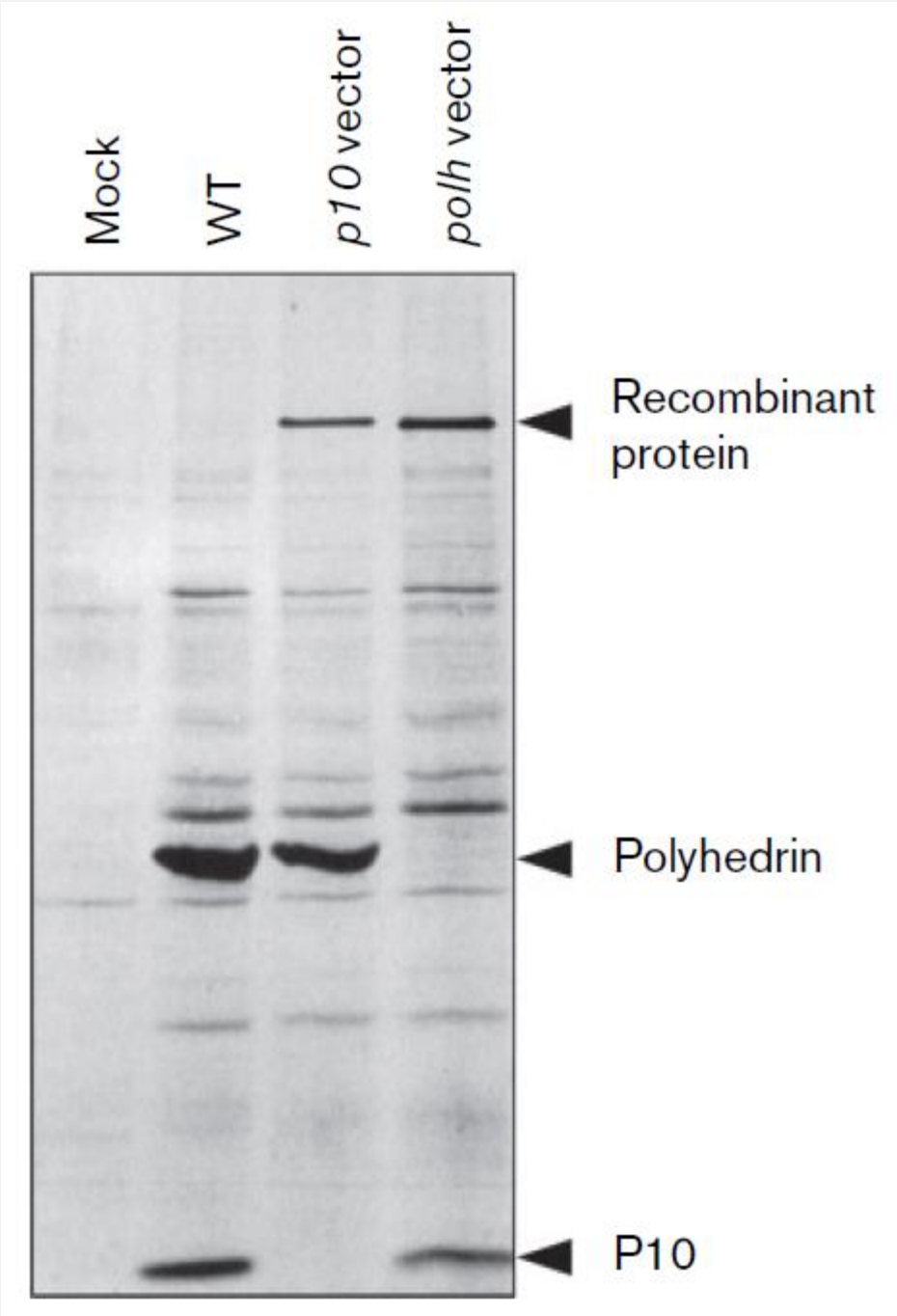
- 设置合适的**对照**(**controls**):

— mock-infected (uninfected) cells;
— pFastBac positive control baculovirus;
— previously characterized recombinant baculoviruses

- 在合适的时间点收集、裂解细胞

- 分析所表达的蛋白by SDS-PAGE or Western blot

- 纯化重组蛋白



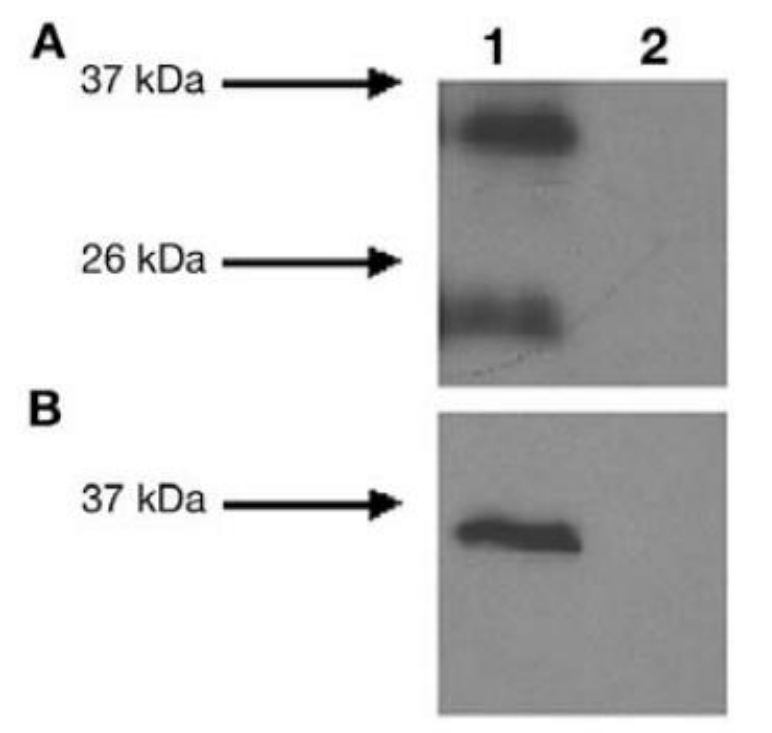
Case 1: 重组蛋白表达

Protein **gel electrophoresis** result with AcMNPV expression vectors.

The protein content is shown for mock- and WT-infected insect cells in comparison with cells infected with expression vectors based on the p10 or polh promoter.
The polyhedrin and P10 proteins are indicated as well as a recombinant protein product (ß-galactosidase in this case).

Cited from Monique M. van Oers, JGV, 2015

Case 2 重组蛋白表达



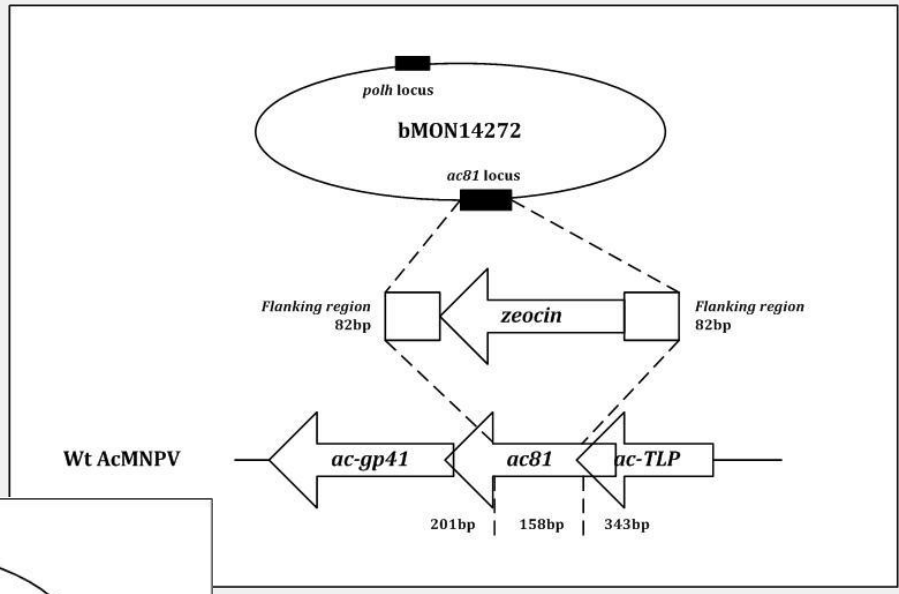
Expression of SARS-CoV 3a protein in insect cells.
Sf9 cells were infected with a recombinant myc-3a baculovirus at a MOI of 1 (lane 1). Cells were harvested at 72 h p.i., lysed, and subjected to Western blot analysis using (A) anti-3a antibody and (B) anti-myc antibody.
Two forms of myc-3a were detected by anti-3a antibody as previously reported.
Mock infected Sf9 cells were used as a negative control (lane 2).

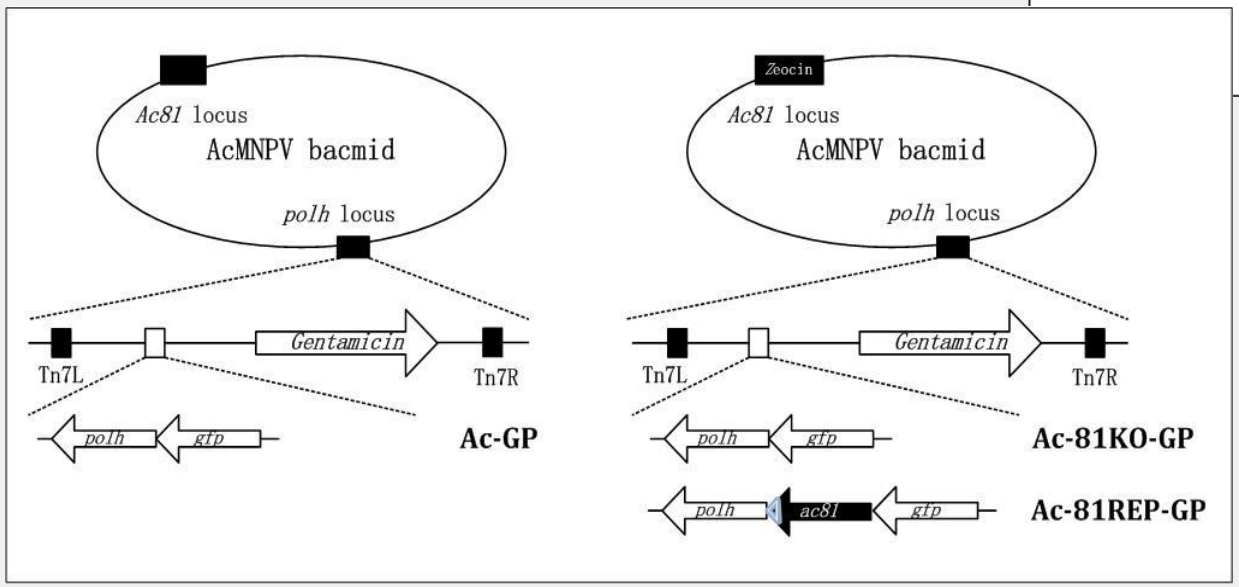
(cited from "Expression of the severe acute respiratory syndrome coronavirus 3a protein and the assembly of coronavirus-like particles in the baculovirus expression system")

Case 3: *ac81*基因功能研究

—采用Bac-to-Bac及ET重组技术

*ac81*缺失型重组bAc81KO 的构建流程图

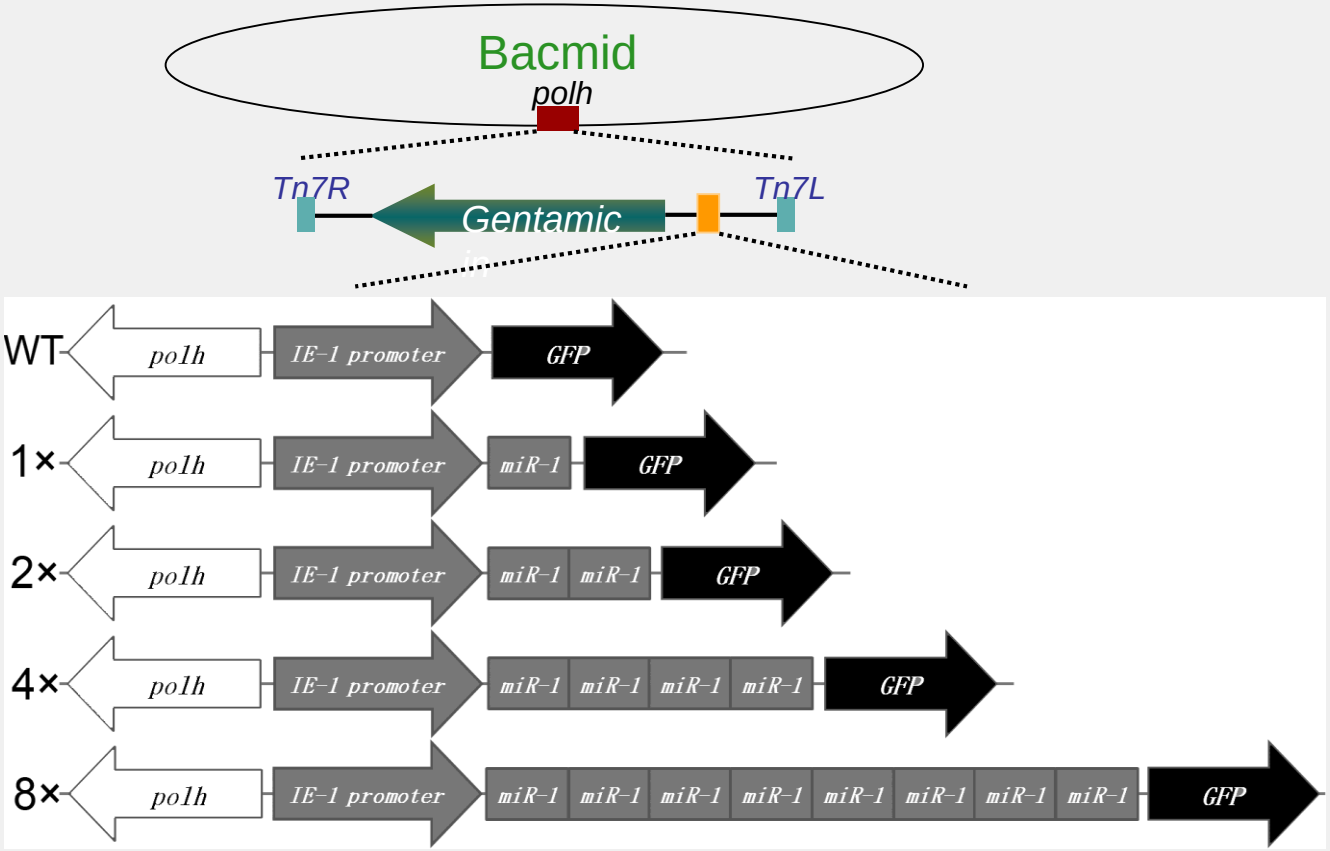




利用Bac-to-Bac杆状病毒表达载体系统构建带荧光和多角体标记的重组病毒

Case 4: 内源性过表达AcMNPV-miR-1的构建

—采用Bac-to-Bac及ET重组技术



小 结

杆状病毒基因组比较大，不易通过常规酶切、连接载入外源基因，所以通常：

（1）将外源基因克隆到转移载体或供体质粒上，载体上含有病毒的同源臂或转座位点，以及一个或多个杆状病毒的启动子。

（2）将外源基因插入到启动子下游，与病毒重组（或转座），获得重组病毒。

（3）将重组病毒纯化，感染昆虫细胞或虫体，则外源基因随着病毒的复制而获得表达。

优势：Bac-to-Bac VS homologous recombination

- 所需时间短：less than 2 weeks VS 4–6 weeks
- 不需要进行多轮空斑纯化
- 可以同时制备多个重组病毒，和表达不同的蛋白

Table 2. Comparison of various commonly used expression systems for mammalian proteins

Property	Transgenic insect cells	Baculovirus vectors in insect cells	Mammalian cells (transient)	Vaccinia vectors	Lentivirus vectors
Post-translational modifications*	++	++	+++	+++	+++
Homogeneity of *N*-glycans	++	++	+	+	+
Biological activity	++	++	+++	+++	+++
Immunogenicity	+++	+++	+++	+++	+++
Production levels†	+	++	+	++	++
Safety concerns	++	++	+	+	-
Downstream processing efforts‡	++	+	++	+	+

*Post-translational modifications resembling those in mammalian cells.
†Expression levels per culture volume vary per product produced.
‡Removal of viral vectors requires extra processing.

Cited from Monique M. van Oers, JGV, 2015

杆状病毒表达系统的优点

（1）在病毒晚期基因强启动子的驱动下，外源基因可获高效表达。Polyhedrin和 P10是病毒复制非必需基因，启动子均很强，既可为外源片段提供插入位点，又可高水平表达外源片段；

（2）载体安全性高。因为杆状病毒对脊椎动物无致病性；

（3）双链环状的DNA基因组易于重组操作；

（4）具有克隆大片段外源基因的能力。病毒基因组大，容量大，可接纳大片段外源DNA，而不影响正常复制；

（5）昆虫细胞对表达产物的加工修饰接近于哺乳动物。如糖苷化、脂肪酸酰基化、羟基末端酰胺化、磷酸化等；

（6）杆状病毒重组载体分子不仅能在细胞培养物中表达，还能在昆虫活体中稳定、高效表达。

杆状病毒表达系统的优点-Furthermore

（1）利用晚期、极晚期基因启动子，即使外源基因产物对细胞有毒性，也不影响表达水平。因为在外源基因大量表达之前，病毒与宿主的大部分基因表达已关闭，病毒已完成复制过程并释放出大量成熟的子代病毒。

（2）外源基因插入多角体基因座引致多角体基因失活或缺失。因此，重组病毒不产生包涵体，病毒不能在环境中长期存在，所以更为安全。

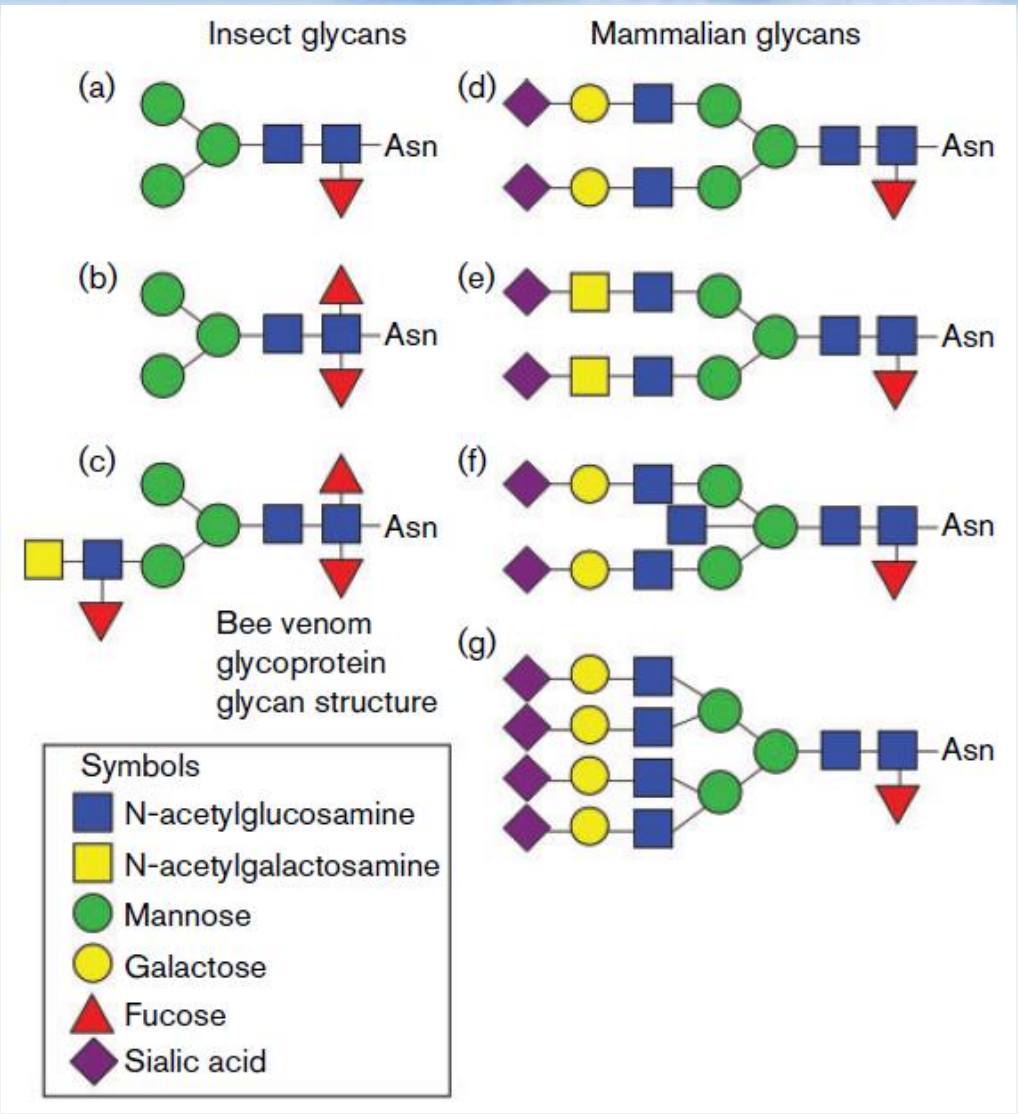
（3）允许多个外源片段的表达。

（4）可以获得大量抗原性、免疫原性较好的，与天然蛋白功能相似的可溶性重组蛋白。这一特点优于细菌、酵母表达体系。

尽管杆状病毒表达系统有着一系列的优点，但也存在一些不足…

杆状病毒表达系统的不足

- Bacmid DNA的拷贝数低，*lacZ*的互补能力不如高拷贝数质粒强，菌落显色较慢。
- 表达产物翻译后加工修饰较哺乳动物细胞相对简单。如：外源糖蛋白糖链短，种类单一，有时还会额外加入岩藻糖。另外，N糖苷链末端未唾液酸化。改进：将哺乳动物半乳糖苷转移酶及唾液酸合成酶导入昆虫细胞。
- 多角体启动子驱动表达强，能在短时间内产生大量外源蛋白，导致有些蛋白来不及正确折叠而形成不溶性颗粒。改进：共表达分子伴侣帮助蛋白折叠
- 晚期启动子启动表达落后于几丁质酶、胱氨酸蛋白酶等基因的表达，而上述两种酶能促进昆虫的死亡和降解。改进：删除病毒几丁质酶与组织蛋白酶基因。



昆虫细胞和哺乳动物细胞糖蛋白N-糖原的结构差异. (a, b) Two major products of insect N-glycan-processing pathway, classified as pauci-mannose structures. (c) The most complex insect glycan found in the bee venom（蜂毒）. (d–g) Various complex mammalian N-glycan structures. Data retrieved from Harrison & Jarvis (2006).

杆状病毒表达系统的应用

Table 3. Overview of approved vaccines and therapies based on baculovirus expression technology

Product name	Company	Expressed product	Purpose	Use	Year of release	Reference
Porcilis Pesti	MSD Animal Health	E2 glycoprotein	Subunit/marker vaccine against classical swine fever	Pigs	1998	–
Bayovac CSF E2*	Bayer Biologicals/Pfizer Animal Health	E2 glycoprotein	Subunit/marker vaccine against classical swine fever	Pigs	2001	Hulst *et al.* (1993)
Circumvent PCV†	MSD Animal Health	Porcine circovirus ORF2	VLP vaccine against porcine circovirus type 2	Pigs	2005	–
Cervarix	GlaxoSmithKline	Human papillomavirus L1 protein (serotypes 16 and 18)	VLP-based vaccine against cervical cancer	Girls	2007	Harper (2008)
CircoFLEX	Ingelvac	Porcine circovirus ORF2	VLP vaccine against porcine circovirus type 2	Pigs	2008	Desrosiers *et al.* (2009)
Porcilis PCV†	MSD Animal Health	Porcine circovirus ORF2	VLP vaccine against porcine circovirus type 2	Pigs	2009	–
Provenge (sipuleucel-T)	Dendreon	PAP-GM-CSF‡	Immunotherapy against prostate cancer	Men	2010	Kantoff *et al.* (2010)
Glybera	UniQure	AAV vector with lipoprotein lipase transgene	Gene therapy against familial lipoprotein lipase deficiency	Humans	2012	Haddley (2013)
Flublok	Protein Sciences	Influenza HA	Annual trivalent flu vaccine	Humans	2013	Cox (2009), Cox & Hashimoto (2011), Treanor *et al.* (2011)

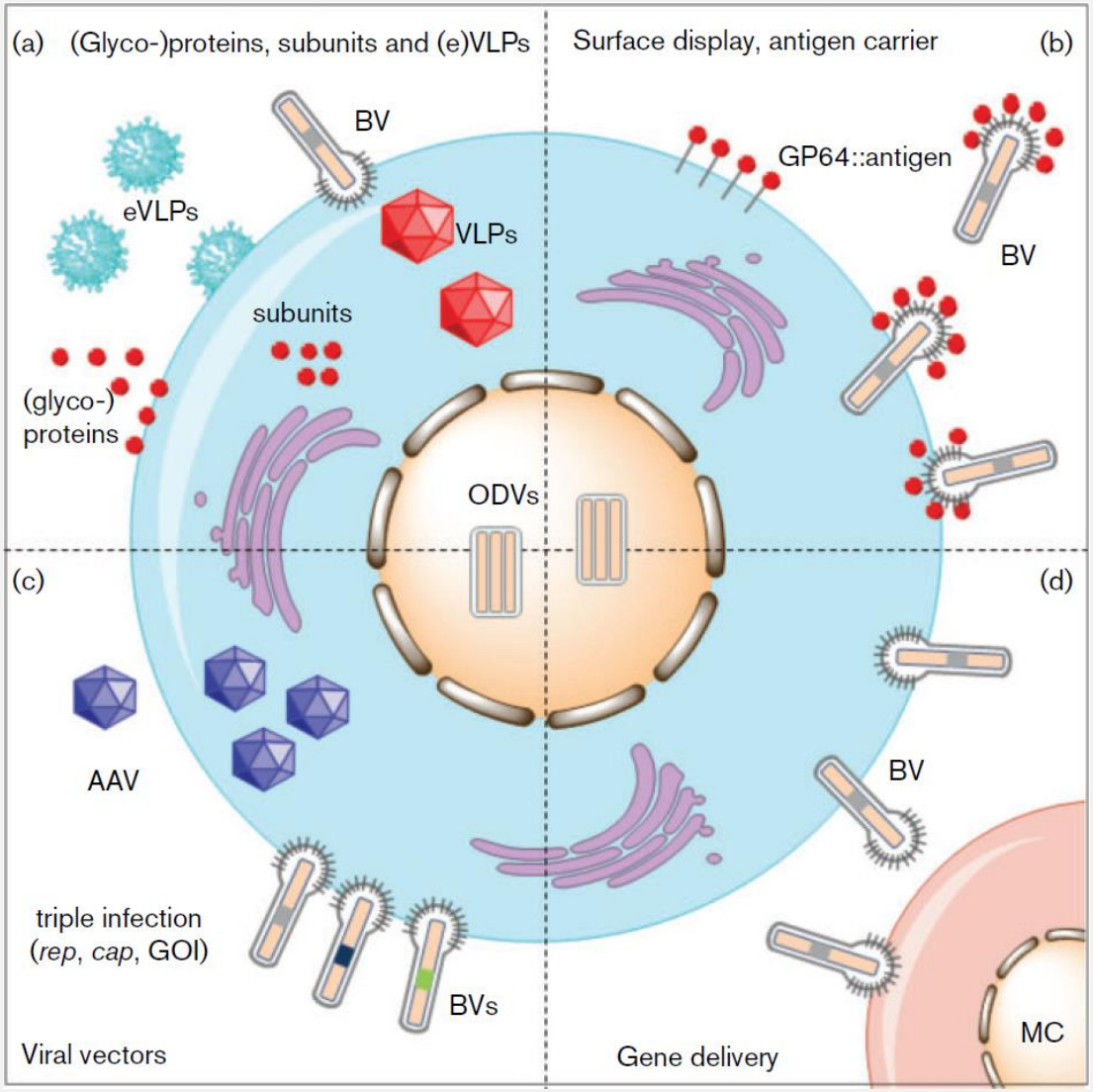
*Bayovac CSF E2 vaccine has been discontinued.
†The two porcine circovirus vaccines produced by MSD Animal Health are licensed in different geographical areas.
‡Prostatic acid phosphatase coupled to granulocyte–macrophage colony-stimulating factor.

Cited from Monique M. van Oers, JGV, 2015

杆状病毒表达系统的应用前景
—Gene delivery

杆状病毒在哺乳动物细胞中不能复制，也不整合到细胞基因组上，所以作为基因治疗的载体安全性高。但由于不能复制，其在哺乳动物细胞中的表达持续时间较短，疗效有限。

杆状病毒载体能有效地将基因导入哺乳动物细胞，但是目的基因必需在哺乳动物细胞可识别的启动子的启动下表达。杆状病毒对不同的哺乳动物细胞具有不同的转导效率。



杆状病毒表达系统的多种应用

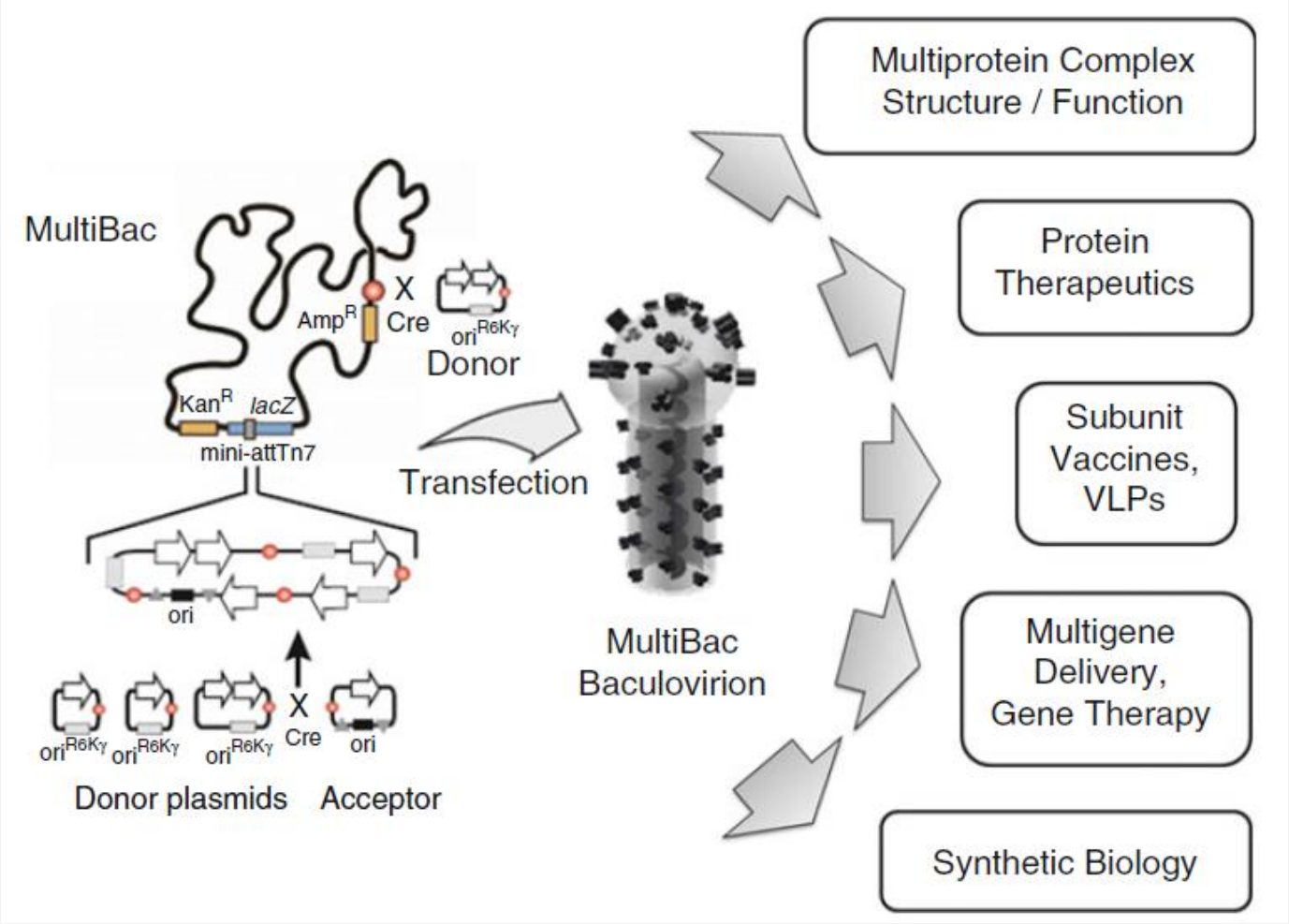
(a) BVs are used to produce foreign (glyco-)proteins in insect cells. The recombinant proteins may be synthesized as subunits, which may remain intracellular or in the case of glycoproteins will be transported to the cell surface or even be secreted. Alternatively, the recombinant proteins may form VLPs or enveloped, secreted VLPs (eVLPs). Protein subunits as well as (e)VLPs may be used as vaccines.

(b)When proteins are fused to the BV surface protein GP64 as a carrier, they are transported to the cell surface and become incorporated in the BV particles upon budding. This is called baculovirus surface display.

(c) Can be used to produce viral vectors such as AAV for gene therapy. Two baculovirus vectors induce the expression of AAV rep and cap genes, respectively, needed to amplify and package the transgene DNA encoded by the third baculovirus. GOI (gene of interest).

(d) BVs carrying a gene of interest under a promoter active in mammalian cells (MC) are produced in insect cells and used as gene delivery vectors.

The nucleus of the insect cell contains the ODV, which is not occluded in the absence of the polyhedrin protein.



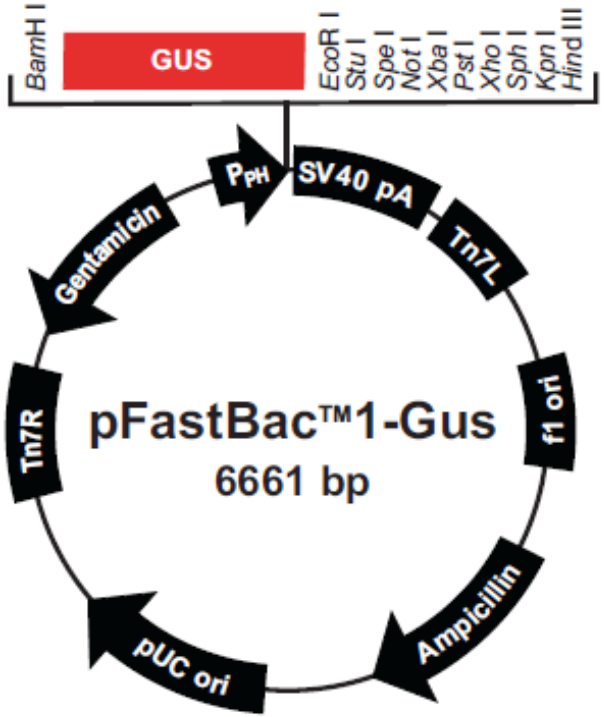
The MultiBac baculovirus/insect cell expression system.
MultiBac contains two integration sites for foreign genes, by Tn7 transposition or by site-specific recombination mediated by the Cre enzyme. It consists of an array of plasmids called Acceptors and Donors that facilitate multigene assembly.
(D. Sari. et al. The MultiBac Baculovirus/Insect Cell Expression Vector System for Producing Complex Protein Biologics. 2016)

对照表达质粒（Control expression plasmid）

Control plasmid-Gus (β-glucuronidase, 葡萄糖醛酸酶)

pFastBac™-Gus Map

The figure below summarizes the features of the pFastBac™-Gus vector. **The vector sequence of pFastBac™-Gus is available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**



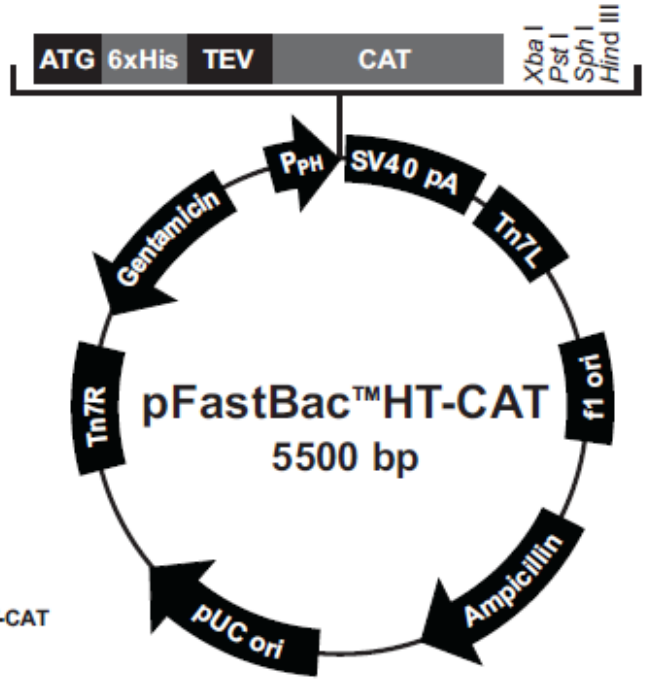
Comments for pFastBac™1-Gus
6661 nucleotides

f1 origin: bases 2-457
Ampicillin resistance gene: bases 589-1449
pUC origin: bases 1594-2267
Tn7R: bases 2511-2735
Gentamicin resistance gene: bases 2802-3335 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 3904-4032
GUS ORF: bases 4081-5892
SV40 polyadenylation signal: bases 6047-6287
Tn7L: bases 6315-6480

Control plasmid-CAT（chloramphenicol acetyltransferase）

pFastBac™HT-CAT Map

The figure below summarizes the features of the pFastBac™HT-CAT vector. **The vector sequence of pFastBac™HT-CAT is available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**



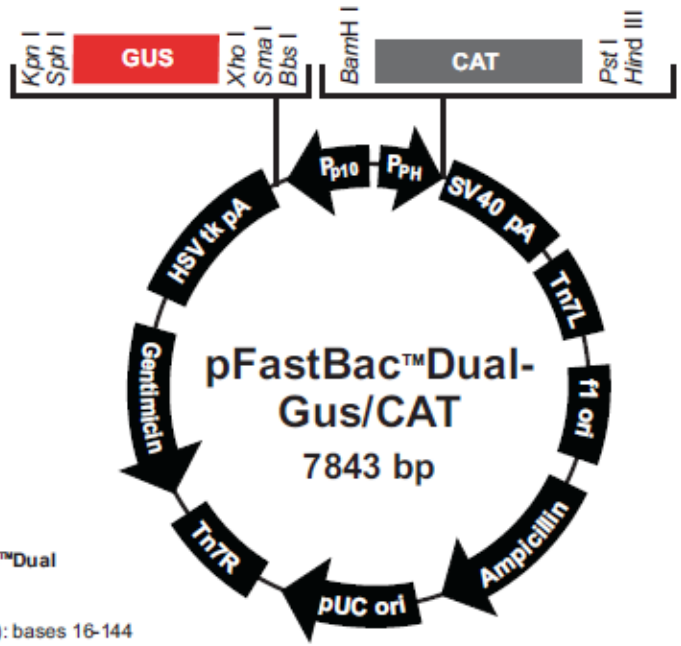
Comments for pFastBac™HT-CAT
5500 nucleotides

f1 origin: bases 2-457
Ampicillin resistance gene: bases 589-1449
pUC origin: bases 1594-2267
Tn7R: bases 2511-2735
Gentamicin resistance gene: bases 2802-3335 (complementary strand)
Polyhedrin promoter (P_{PH}): bases 3904-4032
Initiation ATG: bases 4050-4052
6xHis tag: bases 4062-4079
TEV recognition site: bases 4101-4121
CAT ORF: bases 4131-4790
SV40 polyadenylation signal: bases 4884-5124
Tn7L: bases 5153-5318

Control plasmid-Dual

pFastBac™ Dual-Gus/CAT Map

The figure below summarizes the features of the pFastBac™ Dual-Gus/CAT vector. **The vector sequence of pFastBac™ Dual-Gus/CAT is available from our website (www.invitrogen.com) or by contacting Technical Support (see page 66).**



Comments for pFastBac™Dual
5238 nucleotides

Polyhedrin promoter (P_{PH}): bases 16-144
CAT ORF: bases 181-840
SV40 polyadenylation signal: bases 964-1204
Tn7L: bases 4991-5156
f1 origin: bases 1582-2037
Ampicillin resistance gene: bases 2169-3029
pUC origin: bases 3174-3847
Tn7R: bases 4091-4315
Gentamicin resistance gene: bases 4382-4915 (complementary strand)
HSV tk polyadenylation signal: bases 5472-5754 (complementary strand)
GUS ORF: bases 5878-7689 (complementary strand)
p10 promoter (P_{p10}): bases 7719-7840 (complementary strand)