



## Introduction

The pAcPIE1 transfer plasmids are designed for the production of recombinant baculoviruses containing foreign genes to be expressed under *ie1* control (1). The transfer plasmids are analogous to classical polyhedrin-based transfer plasmids, except they are designed to express foreign genes much earlier in infection (usually beginning as early as four hours postinfection). These plasmids are well-suited for several different applications: production of recombinant glycoproteins in insect cells, engineering of insect cell metabolic pathways, production of recombinant proteins in insect larvae, and production of recombinant viruses for insect pest control (1).

Each of these plasmids has the *Autographa californica* nuclear polyhedrosis virus (AcNPV) *hr5* enhancer element (483 bp; 2, 3) positioned upstream of the *ie1* promoter. The pAcPIE1 transfer plasmids are used to construct recombinant baculoviruses by cotransfection with BacVector™ Triple Cut Virus DNA, as described in the BacVector™ Transfection Kits protocols (TB216).

## pAcPIE1 Descriptions

The pAcPIE1 transfer plasmid series includes pAcP(+)IE1-1, pAcP(+)IE1-2, pAcP(+)IE1-3, pAcP(+)IE1-4, pAcP(-)IE1-5, and pAcP(-)IE1-6.

### General characteristics

- These vectors are designed for the production of occlusion-negative (-) or occlusion-positive (+) recombinant baculoviruses, when used with appropriate baculovirus DNA. Occlusions produced by the occlusion-positive recombinant baculoviruses are environmentally stable, and are orally infectious to larvae of AcNPV host insects (*Trichoplusia ni*, *Spodoptera frugiperda*, etc.).
- Foreign protein expression driven by the *ie1* promoter may be detectable as early as 4 hours postinfection, and peak steady-state levels of accumulated protein are typically reached within 24 hours postinfection (1).
- The *ie1* promoter in each plasmid is followed by the multiple cloning sites indicated by the number following the name of the plasmid (refer to Technical Bulletins 169–174 for the circle maps of each).
- All pAcPIE1 vectors include the *hr5* transcription enhancer (2, 3) upstream of the *ie1* promoter, which provides optimal levels of *ie1*-mediated expression.
- Transfer plasmids with the *ie1* translational start site (mcs 1, 2, and 5) or without the *ie1* translational start site (mcs 3, 4, and 6) can be used to express fusion or native proteins, respectively.
- The *hr5* element, *ie1* promoter, and mcs sequences in the immediate early transfer plasmids are targeted for insertion into the polyhedrin region of the parental viral genome by long flanking sequences derived from that region. Therefore, all pAcPIE1 transfer plasmids are compatible with Triple Cut Virus DNA, such as BacVector-3000. Co-transfection of insect cells with BacVector-3000 Triple Cut Virus DNA and a pAcP(-)IE1 vector produces polyhedrin-negative, *lacZ*-negative recombinants, which can be identified in plaque assays by their lack of blue staining in the presence of X-gal.
- Conversely, the pAcP(+)IE1 plasmids, which include an intact polyhedrin gene, produce occlusion-positive, *lacZ*-negative recombinants when co-transfected into insect cells with genomic DNA from an occlusion-negative parental virus (e.g., BacVector-1000). *If occlusion-positive recombinants are desired, the pAcP(+)IE1 plasmids should not be used with viral DNAs containing additional deletions (e.g., BacVector-2000 or -3000) because additional genomic deletions may compromise the ability of the recombinant virus to form occlusions.*
- Availability of immediate early baculovirus transfer plasmids that can be used to isolate either occlusion-negative or occlusion-positive recombinant baculoviruses provides flexibility in choosing screening techniques. These plasmids make it possible to produce environmentally labile (occlusion-negative) recombinants, or environmentally stable (occlusion-positive)



# Using pAcPIE1 Transfer Plasmids

recombinants for use as biocontrol agents or to produce foreign proteins in insect larvae following oral infection.

## pAcP(+)IE1-1, pAcP(+)IE1-2

These transfer plasmids are designed for the isolation of recombinant baculoviruses capable of expressing fused proteins under *ie1* control and capable of forming occlusions. They are compatible with linearized polyhedrin-negative viral DNA, such as BacVector-1000 Triple Cut Virus DNA.

## pAcP(+)IE1-3, pAcP(+)IE1-4

These transfer plasmids are designed for the isolation of recombinant baculoviruses capable of expressing non-fused proteins under *ie1* control and capable of forming occlusions. They are compatible with linearized polyhedrin-negative viral DNA, such as BacVector-1000 Triple Cut Virus DNA.

## pAcP(-)IE1-5, pAcP(-)IE1-6

These transfer plasmids are designed for the isolation of recombinant baculoviruses capable of expressing fused [pAcP(-)IE1-5] or non-fused [pAcP(-)IE1-6] proteins under *ie1* control and not capable of forming occlusions. They are compatible with linearized polyhedrin-negative viral DNA such as BacVector-1000, -2000 or -3000 Triple Cut Virus DNA, or uncut polyhedrin-positive viral DNA, such as wild type AcNPV.

## Summary of Characteristics

Transfer plasmid	Polyhedrin* (occlusions)	Translation initiation <sup>†</sup>	Virus DNA Compatibility		
			BacVector-1000	BacVector-2000, BacVector-3000	Uncut wild type AcNPV
pAcP(+)IE1-1	√	ATG	√	NR	NR
pAcP(+)IE1-2	√	ATG	√	NR	NR
pAcP(+)IE1-3	√	-	√	NR	NR
pAcP(+)IE1-4	√	-	√	NR	NR
pAcP(-)IE1-5	-	ATG	√	√	√
pAcP(-)IE1-6	-	-	√	√	√

\* When used with BacVector-1000 Triple Cut Virus DNA.

<sup>†</sup> ATG = initiation codon provided by the vector; inserts must be in-frame with this codon to use it  
NR = not recommended if occlusion-positive phenotype or oral infection of insect larvae are to be used

# Using pAcPIE1 Transfer Plasmids



## Related Products

Product	Size	Cat. No.
BacVector-1000 Transfection Kit	12 transfections	70059-3
BacVector-2000 Transfection Kit	12 transfections	70030-3
BacVector-3000 Transfection Kit	12 transfections	70077-3
BacVector-1000 DNA Kit	12 transfections	70057-3
BacVector-2000 DNA Kit	12 transfections	70058-3
BacVector-3000 DNA Kit	12 transfections	70078-3
AcNPV Wild Type Virus DNA	2 µg	70061-1
Ready-Plaque™ Sf9 Cells	6 vials	70033-3
Ea4 Insect Cells	3 vials	70086-3
BacPlaque™ Agarose	30 g	70034-3
pAcP(+)IE1-1 transfer plasmid	10 µg	69092-3
pAcP(+)IE1-2 transfer plasmid	10 µg	69093-3
pAcP(+)IE1-3 transfer plasmid	10 µg	69094-3
pAcP(+)IE1-4 transfer plasmid	10 µg	69095-3
pAcP(-)IE1-5 transfer plasmid	10 µg	70170-3
pAcP(-)IE1-6 transfer plasmid	10 µg	69097-3

## References

1. Jarvis, D.L., Weinkauff, C. and Guarino, L.A. (1996) *Protein Expr. Purif.* **8**, 191–203.
2. Guarino, L.A., Gonzalez, M.A. and Summers, M.D. (1986) *J. Virol.* **60**, 224–229.
3. Guarino, L.A., and Summers, M.D. (1986) *J. Virol.* **60**, 215–223.

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