

flashBAC™ One-step baculovirus protein expression

flashBAC™

The **flashBAC™** system is a **new platform technology** for the production of recombinant baculoviruses. Most importantly, **flashBAC™** has been specifically designed to remove the need to separate recombinant virus from parental virus by plaque-purification or any other means. The production of recombinant virus has been reduced to a **one-step procedure** in insect cells and is thus **fully amenable to high throughput and automated production systems**.

The **flashBAC™** technology has been developed by the same team that produced the triple-cut, linear DNA (BacPAK6) system that has been the stalwart of the baculovirus expression system for the past 10 years. At the heart of **flashBAC™** technology is an baculovirus genome that lacks part of an essential gene (ORF 1629) and contains a bacterial artificial chromosome (BAC) at the polyhedrin gene locus, replacing the polyhedrin coding region. The essential gene deletion prevents virus replication within insect cells but the BAC allows the viral DNA to be maintained and propagated, as a circular genome, within bacterial cells. Circular viral DNA is then isolated from the bacterial cells and purified. This is the **flashBAC™** DNA provided in our kit.

A recombinant baculovirus is produced by simply transfecting insect cells with **flashBAC™** DNA and a transfer vector containing 'the gene under investigation'. Homologous recombination within the insect cells (1) restores the function of the

essential gene allowing the virus DNA to replicate and produce virus particles and (2) simultaneously inserts 'the gene under investigation' under the control of the polyhedrin gene promoter and **removes the BAC sequence**. The recombinant virus genome, with the restored essential gene, replicates to produce BV that can be harvested from the culture medium of the transfected insect cells (and forms a seed stock of recombinant virus). As it is not possible for non-recombinant virus to replicate there is no need for any selection system.

This one-step procedure greatly facilitates the high throughput production of baculovirus expression vectors via automated systems. However, it is also of benefit to the small research group just requiring one or a few recombinant baculoviruses prepared in individual dishes of cells.

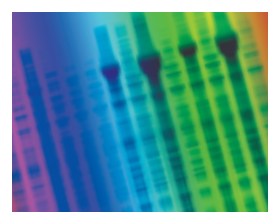
The **flashBAC™** system is **back compatible** with all baculovirus transfer vectors based on homologous recombination in insect cells at the polyhedrin gene locus. This includes vectors using the polyhedrin promoter, dual, triple and quadruple expression vectors and those that use other gene promoters such as *p10*, *ie1* etc. Examples include pBacPAK8/9, pAcUW31 and pBacPAK-His1/2/3 (BD Biosciences Clontech) but not vectors such as pFastBac™, which are designed for site-specific transposition in *E. coli* using the Bac-to-Bac® system (GibCo-BRL)¹⁴.

the science of baculovirus expression™

PRODUCTS ARE FOR RESEARCH PURPOSES ONLY, NOT FOR DIAGNOSTIC OR THERAPEUTIC USE



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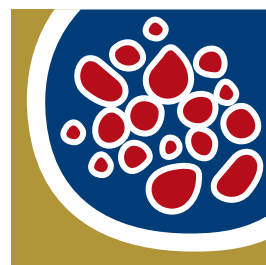
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The **flashBAC™** system maximises protein secretion and membrane protein targeting. Baculovirus genomes contain several auxillary genes, which are non-essential for replication in insect cell culture. One of these is chitinase (*chiA*), which encodes an enzyme with exo- and endochitinase activity. In an infected insect, chitinase (together with cathepsin) facilitates host cuticle breakdown and tissue liquefaction at the very late stages of infection, so releasing the virus to infect more hosts. Confocal and electron microscopy observations of insect cells infected with

AcMNPV have shown that chitinase is targeted to the endoplasmic reticulum where it is densely packed in a para-crystalline array, severely compromising the function and efficacy of the secretory pathway. Deletion of *chiA* from **flashBAC™** has improved the efficacy of the secretory pathway and resulted in a greatly enhanced (up to 60-fold in some instances) yield of recombinant proteins that are secreted or membrane targeted (in comparison with recombinant viruses that synthesise chitinase).

Advantages of the **flashBAC™** system:

- Simple to use
- One step production of recombinant virus in insect cells
- No steps needed to purify recombinant virus
- Amenable to high throughput and automated systems
- Back-compatible with a huge range of transfer vectors



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Oxford Expression Technologies Ltd., developers of world-leading baculovirus protein expression kits and an exciting new range of products and services for the medical, research, biotechnology and pharmaceutical markets.