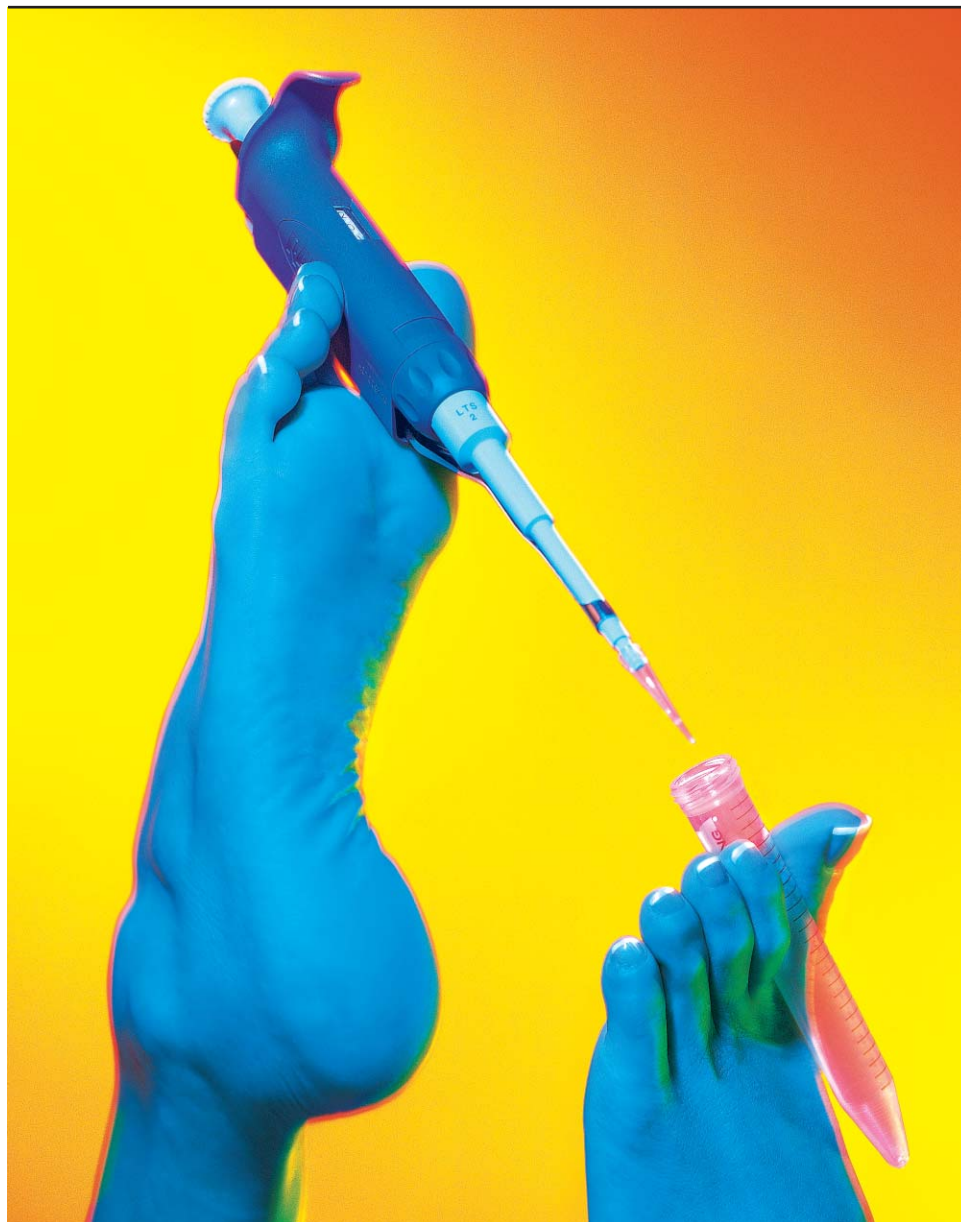




Free your hands with the BaculoDirect™ Baculovirus Expression System



The BaculoDirect™ Baculovirus Expression System gives you:

- Unique speed and simplicity
- High-throughput capabilities
- Efficient recombinational cloning

Fast and easy baculovirus expression

The BaculoDirect™ Baculovirus Expression System is the fastest and easiest method for generating recombinant baculovirus. Typically, baculovirus expression systems utilize tedious site-specific transposition in *E. coli* or lengthy homologous recombination methods in insect cells to generate recombinant baculovirus. The BaculoDirect™ Baculovirus Expression System eliminates these processes, saving you time and getting your baculovirus expression results quicker than ever.

Unique in speed and simplicity

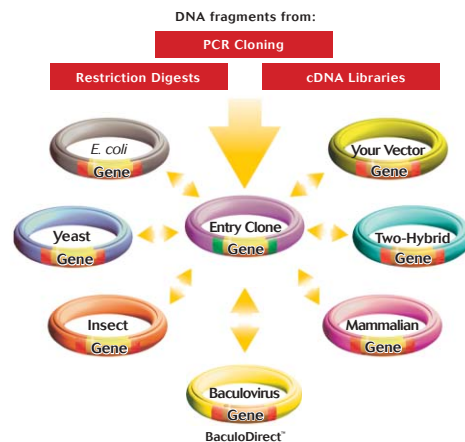
The BaculoDirect™ Baculovirus Expression System makes baculovirus expression more convenient, requiring less than half the hands-on time necessary for traditional systems. Baculovirus expression systems typically require bacterial transformation and isolation of a large bacmid or co-transfection of a transfer vector and linear baculovirus DNA into insect cells. The BaculoDirect™ System accelerates your

research by eliminating these time-consuming steps. Hands-on time necessary for expression is dramatically reduced. From initial transfection to isolation of your baculovirus stock takes approximately 8 hours of hands-on time. The BaculoDirect™ System gives you rapid results, with less effort, and fewer tedious steps.

The power of Gateway® Technology

By incorporating Gateway® Technology, the BaculoDirect™ Baculovirus Expression System streamlines your research and accelerates your results. Gateway® Technology offers a rapid and efficient route to protein expression and functional analysis. Based on lambda-phage recombination, Gateway® Technology enables you to easily transfer your gene of interest into a variety of expression systems without the usual subcloning steps. Once your gene of interest is cloned into a Gateway® entry vector, you have nearly limitless access to a variety of expression options including the BaculoDirect™ System. When analysis of your protein is required in more than one system, Gateway® Technology is the ideal choice (Figure 1).

Figure 1 - Gateway® Technology overview



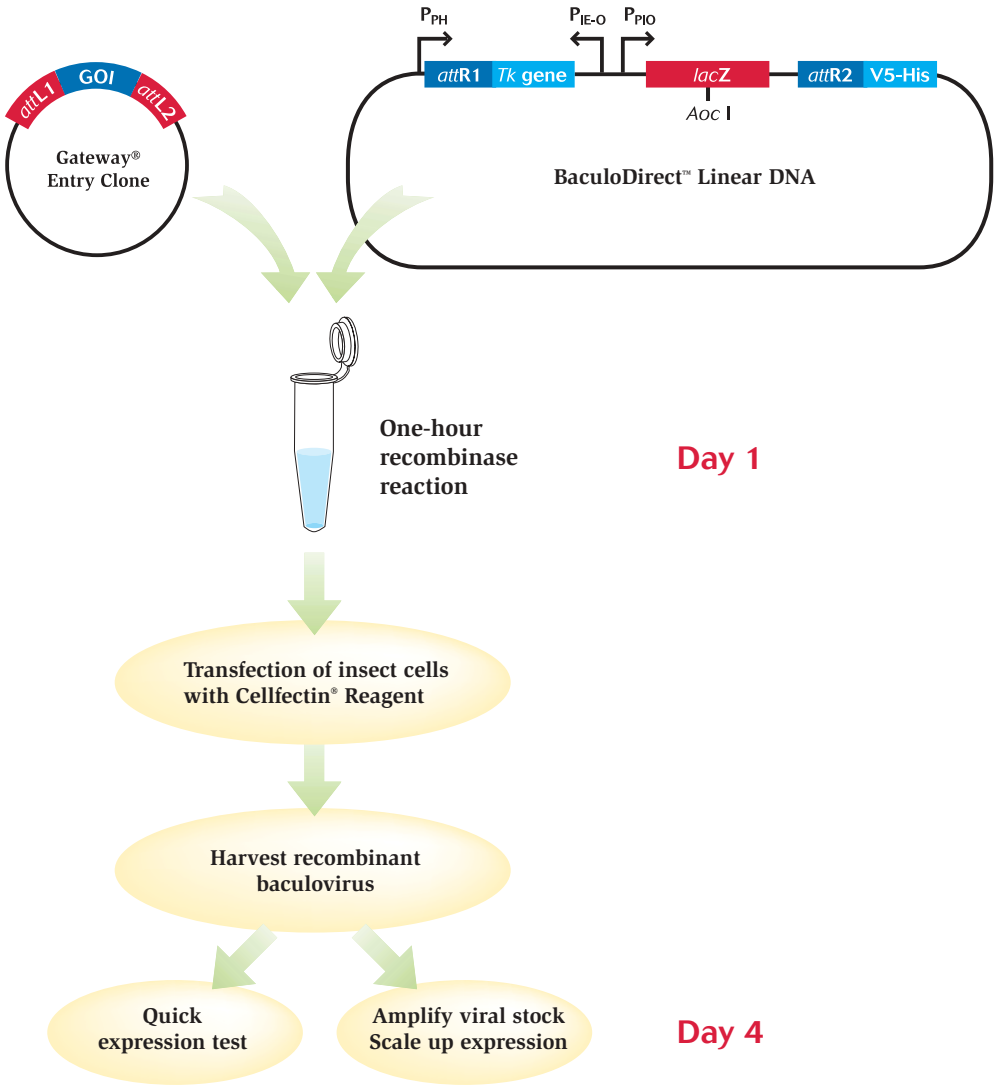
1. Create a Gateway® entry clone by PCR or traditional cloning.
2. Mix an entry clone with the BaculoDirect™ Linear DNA.
3. Use for baculovirus expression. Once a gene is cloned into an entry vector, you can move the DNA fragment into one or more destination vectors simultaneously.

How it works

The BaculoDirect™ Baculovirus Expression System uses a straightforward and efficient Gateway® recombination reaction to speed your work. The specially engineered BaculoDirect™ Linear DNA (the baculovirus genome) contains *attR* sites for recombination of your gene of interest from a Gateway® entry clone*. Just mix the entry clone containing the gene of interest with the BaculoDirect™ Linear DNA and Gateway® LR Clonase™

enzyme mix for one-hour at room temperature (Figure 2). The resulting reaction mix contains the recombinant baculovirus carrying the gene of interest in the correct reading frame and orientation. Use this reaction mix to directly transfect insect cells (Sf9 or Sf21). Following seventy-two hours of growth, you'll have your recombinant baculovirus for amplification and expression. Baculovirus expression has never been easier.

Figure 2 - Streamlined protein expression using the BaculoDirect™ Baculovirus Expression System



* To create a Gateway® entry clone, clone your gene of interest into a Gateway® entry vector using PCR cloning or traditional restriction-enzyme digest and ligation. For more information, visit www.invitrogen.com/gateway.

Engineered for efficiency

The BaculoDirect™ Linear DNA is designed for simple, generation of recombinant baculovirus and expression in insect cells. In addition to *attR* sites for fast Gateway® cloning, the BaculoDirect™ Linear DNA includes:

- Strong polyhedrin promoter for high-level expression
- Thymidine kinase (TK) gene for negative selection of non-recombinant virus using ganciclovir

- *lacZ* gene for quick determination of recombinant virus purity
- C-terminal V5 epitope tag and polyhistidine (6xHis) sequence for easy detection and purification of recombinant proteins

The BaculoDirect™ Linear DNA makes cloning, expression, detection, and purification fast and easy.

Express multiple proteins simultaneously

The speed to gene expression and the reduced hands-on time makes the BaculoDirect™ System ideal for high-throughput expression. Gateway® entry clones and the BaculoDirect™ Linear DNA can be arrayed in multiple wells and the LR

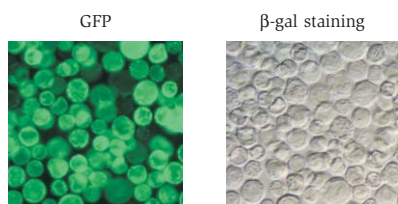
recombination reaction, transfection, and infection of insect cells can all be performed in multi-well plates. This allows quick analysis of a large number of proteins at once.

Easy selection, low background

The BaculoDirect™ Linear DNA includes two built-in time-saving checks—the *lacZ* gene and the thymidine kinase gene—to ensure the isolation of a pure baculovirus stock. During the LR recombination reaction with your Gateway® entry clone, the *lacZ* and the thymidine kinase (TK) genes are recombined out as by-products. Cells are then placed under selection with ganciclovir, a drug which selects against

the TK gene. Ganciclovir efficiently eliminates any remaining parental, non-recombinant virus. The purity of the viral stock can be quickly determined by staining cells with β -galactosidase to reveal any remaining *lacZ*-containing, parental, non-recombinant virus (Figure 3). You no longer have to go through multiple rounds of plaque purification to eliminate parental virus so you get to gene expression faster.

Figure 3 - Optimized expression and viral stock purity using the BaculoDirect™ Expression System



A one-hour Gateway® LR reaction was performed using 300 ng of BaculoDirect™ Linear DNA and 100 ng of a Gateway® entry clone containing GFP. Ten microliters of the LR reaction was used to transfect 2×10^6 Sf21 cells with Cellfectin® Reagent. Cells were grown 72 hours in Grace's Medium supplemented with 10% FBS and 100 μ M ganciclovir. Ten microliters of the resulting supernatant was used to infect 2×10^6 Sf21 cells. Cells were again grown in Grace's Medium supplemented with 10% FBS and 100 μ M ganciclovir. After 72 hours, cells were examined for expression by fluorescence. β -gal staining was performed on the cells and no background of non-recombinant virus was observed.

Superior transfection

Every BaculoDirect™ Expression Kit includes Cellfectin® Reagent, a powerful cationic lipid designed for the transfection of DNA into insect cells. Useful for cells in adher-

ent or suspension cultures, Cellfectin® Reagent mediates consistent and efficient transfection of Sf9, Sf21, High Five®, and Mimic™ Sf9 insect cells.

Flexible host and growth options

A variety of GIBCO™ insect cells and media are available for your insect cell culture needs. Sf9 (included in the BaculoDirect™ Expression Kit) or Sf21 cells are useful for generation of recombinant baculovirus and protein expression. High Five™ (BTI-NT-5BI-4) and Mimic™ Sf9 insect cells are available as alternative hosts for protein expression. For higher levels of secreted proteins, High

Five™ cells are a proven option. The Mimic™ Sf9 insect cells are engineered to express mammalian glycosyltransferases (glycosylation enzymes). As a result, the cells produce complex proteins, which are more similar to proteins expressed in mammalian cells. To determine which insect cell lines and media are right for you, check Table 1.

Table 1 - Insect cell lines and media for baculovirus expression

Cell	Advantage	Recommended Media
Sf9 (<i>Spodoptera frugiperda</i>)	Well-characterized host cell, good for plaque assay	Sf-900 II SFM Grace's Insect Cell Culture Medium, Supplemented or Unsupplemented (for transfection)
Sf21 (<i>Spodoptera frugiperda</i>)	High yield of intracellular proteins	Sf-900 II SFM Grace's Insect Cell Culture Medium, Supplemented or Unsupplemented (for transfection)
High Five™ (<i>Trichoplusia ni</i>)	High yield of secreted proteins	Express Five® SFM Grace's Insect Cell Culture Medium, Unsupplemented (for transfection)
Mimic™ Sf9 (<i>Spodoptera frugiperda</i>)	Engineered for high level expression of complex glycoproteins	Grace's Insect Cell Culture Medium, Supplemented or Unsupplemented (for transfection)

Custom services for you

Let the professional specialists at our Custom Services group assist your research efforts. From cloning to expression, our experts use the unique BaculoDirect™

System to give you rapid, reliable results. Call our Custom Services Representatives today at 800 955 6288, ext. 66024.

Everything you need

Get started with your baculovirus expression. The BaculoDirect™ Expression Kit and Transfection Kit contain

the reagents you need to begin your expression work (Table 2). Call Invitrogen and order today.

Table 2 - Kit components

BaculoDirect™ Expression Kit	BaculoDirect™ Transfection Kit
<ul style="list-style-type: none"> • BaculoDirect™ Linear DNA • Recombination and expression control • Cellfectin® Reagent • LR Clonase™ Enzyme Mix • Frozen Sf9 Cells in Grace's Medium • Grace's Insect Cell Culture Medium, Unsupplemented • Ganciclovir 	<ul style="list-style-type: none"> • BaculoDirect™ Linear DNA • Recombination and expression control • Cellfectin® Reagent

Ordering Information

Kits	Quantity	Cat. no.
BaculoDirect™ Expression Kit	5 reactions	12562-013
BaculoDirect™ Transfection Kit	5 reactions	12562-039
Cells		
Sf9 Frozen Cells (SFM adapted)	1.5 ml, 5 x 10 ⁶ cells/ml	11496-015
Sf9 Frozen Cells (Grace's Medium)	1 ml, 10 ⁷ cells	B825-01
Sf21 Frozen Cells (SFM adapted)	1.5 ml, 5 x 10 ⁶ cells/ml	11497-013
Sf21 Frozen Cells (Grace's Medium)	1 ml, 10 ⁷ cells	B821-01
High Five™ Frozen Cells	1 ml, 3 x 10 ⁶ cells	B855-02
Mimic™ Sf9 Cells	1 ml, 1 x 10 ⁷ cells	12552-014
Cell Culture Medium		
Sf-900 II SFM (1X), liquid	500 ml	10902-096
	1,000 ml	10902-088
Express Five® SFM	1,000 ml	10486-025
Grace's Insect Medium, Supplemented	500 ml	11605-094
Grace's Insect Medium, Unsupplemented	500 ml	11595-030
Accessories		
LR Clonase™ Enzyme Mix	20 reactions	11791-019
Cellfectin® Reagent	1 ml	10362-010



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