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Bac-to-Bac®
Baculovirus Expression System

Blast off to expression

The Bac-to-Bac® Baculovirus Expression System offers:

- Tremendous time-savings over traditional baculovirus methods
- High-level recombinant protein yields
- Gateway™-compatible expression vectors for easy cloning

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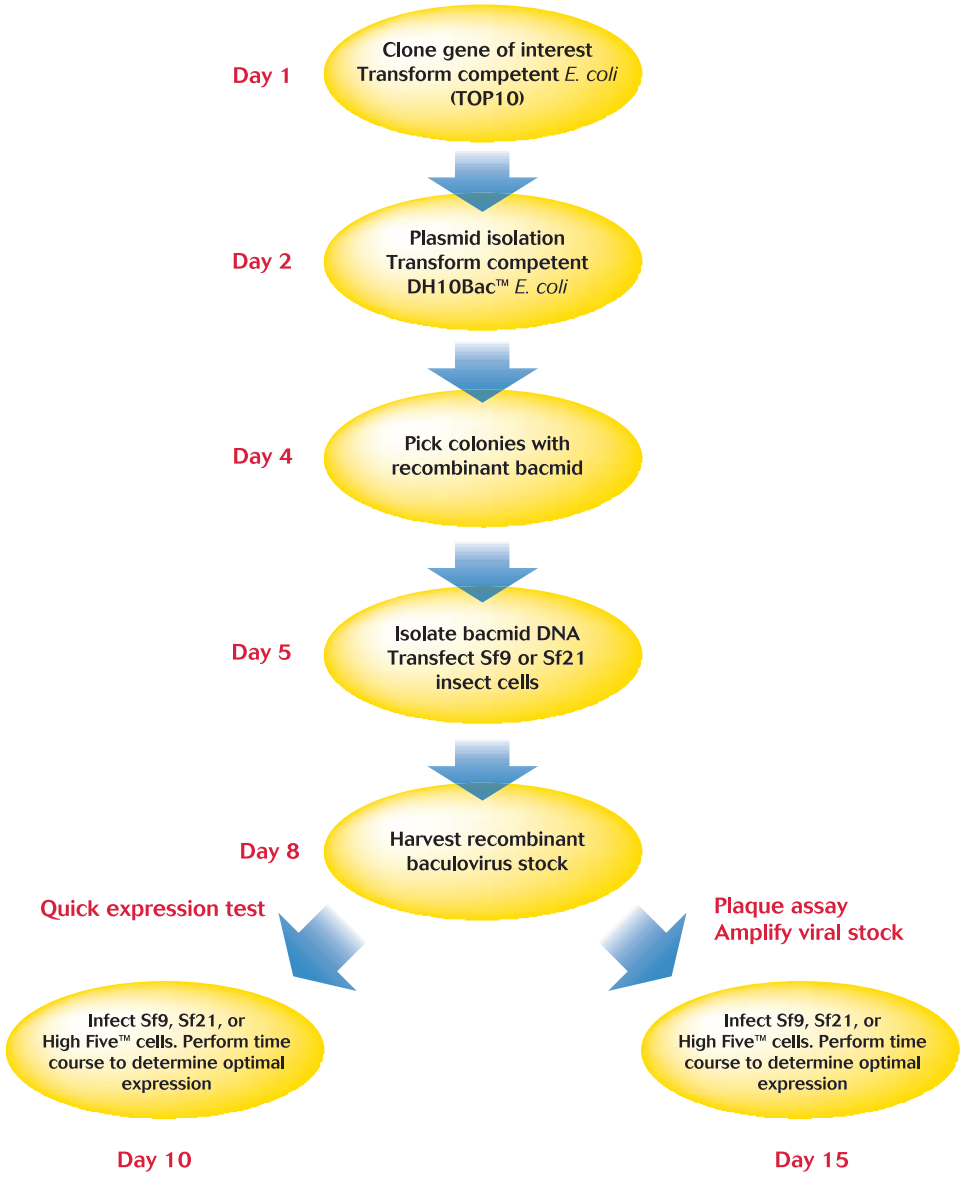
If high-level gene expression is where you want to be, you'll get there faster with the Bac-to-Bac® Baculovirus Expression System. Bac-to-Bac® is the fastest route available for producing recombinant baculovirus (Figure 1). With Bac-to-Bac® you can express your protein of interest in as little as two weeks—saving you weeks of time compared to traditional homologous recombination baculovirus systems.

Figure 1 - Timeline of protein expression with the Bac-to-Bac® Baculovirus Expression System



Powerful vectors

The Bac-to-Bac® system includes six powerful expression vectors for recombinant protein expression (Figure 2). The pFastBac™1 vector offers the strong polyhedrin promoter for high-level, native protein expression and a large multiple cloning site for simplified cloning.

The pFastBac™ HT vector (Figure 3) offers the strong polyhedrin promoter as well as an N-terminal 6xHis tag for simple purification of recombinant proteins. The vector also contains a TEV protease cleavage site for removal of the histidine tag following protein purification.

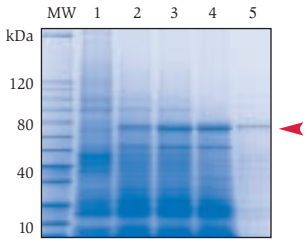
The pFastBac™ Dual (Figure 4) is a single vector featuring two strong promoters, the polyhedrin promoter and the p10 promoter in a single vector for simultaneous expression of two proteins in insect cells.

The pDEST™8, pDEST™10, and pDEST™20 vectors (Figure 5) are Gateway™-adapted* Bac-to-Bac® vectors. The vectors are designed for rapid cloning with a Gateway™ entry clone using site-specific recombination. All three vectors contain *att*R sites for efficient recombination with any *att*L-flanked Gateway™ vector.

- pDEST™8 is designed for native expression
- pDEST™10 contains an N-terminal 6xHis tag for rapid purification of recombinant proteins with a nickel column
- pDEST™20 has an N-terminal GST tag for easy column purification of recombinant proteins

Using the powerful vectors of the Bac-to-Bac® system will give you the high protein yield of baculovirus and streamlined Bac-to-Bac® protocol to save you time.

Figure 2 - Expression of β-glucoronidase using the Bac-to-Bac® system



SDS-PAGE analysis of β-glucoronidase expression. Sf9, Sf21, and High Five™ cells were infected at an MOI of 10 with β-glucoronidase-recombinant virus. Samples were analyzed by SDS-PAGE. 25 µg protein were loaded into each lane (48 h post-infection).

MW. 10 kDa Protein Ladder.
Lane 1. Uninfected Sf9.
Lane 2. Sf9 infected cells.
Lane 3. Sf21 infected cells.
Lane 4. High Five™ infected cells.
Lane 5. 2 µg purified β-glucoronidase.

Figure 3 - pFastBac™1 and pFastBac™ HT

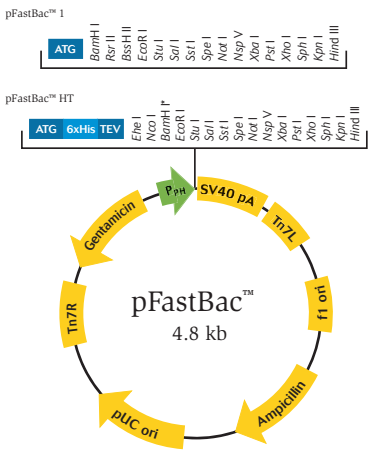


Figure 4 - pFastBac™ Dual

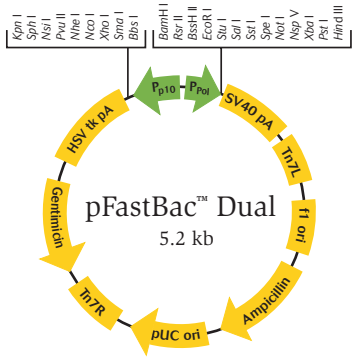
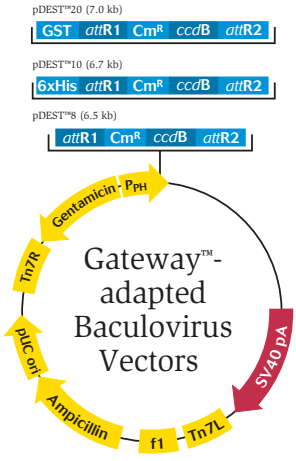


Figure 5 - Gateway™ destination vectors



* See our website at www.invitrogen.com for more information on Gateway™.

Efficient transfection

Cellfectin® Reagent is a powerful cationic lipid designed for the optimal transfection of DNA into insect cells. Transfection with Cellfectin® leads to consistent and highly efficient transfection of Sf9, Sf21, and High Five™ cells. Cellfectin® is the recommended transfection agent for use with Bac-to-Bac®. It can be used to transfect adherent or suspension cells in serum-free or serum-containing media.

Superior growth

A number of different GIBCO™ products are available for your insect cell culture needs. Sf900 II SFM is a serum-free media for growth and protein expression in Sf9 and Sf21 cells. It is supplied in a ready-to-use format and is optimized for maximum cell growth as well as recombinant baculovirus production and protein expression. Express Five® SFM is optimized for growth of High Five™ cells in monolayer or suspension cultures. The media is glutamine-free for maximum shelf life. Grace's Insect Medium is also available for growth and maintenance of Sf9 and Sf21 cells in serum. It is supplied supplemented with glutamine, lactalbumin hydrolysate, and yeastolate.

Expression host options

Sf9 and Sf21 (*Spodoptera frugiperda*) insect cells are available for recombinant baculovirus generation and protein expression. Both Sf9 and Sf21 cells are available in serum-free and serum-containing media. High Five™ (BTI-NT-5BI-4) insect cells are also available as an alternative protein expression host. This cell line has been shown to produce higher levels of secreted recombinant proteins than Sf9 or Sf21.

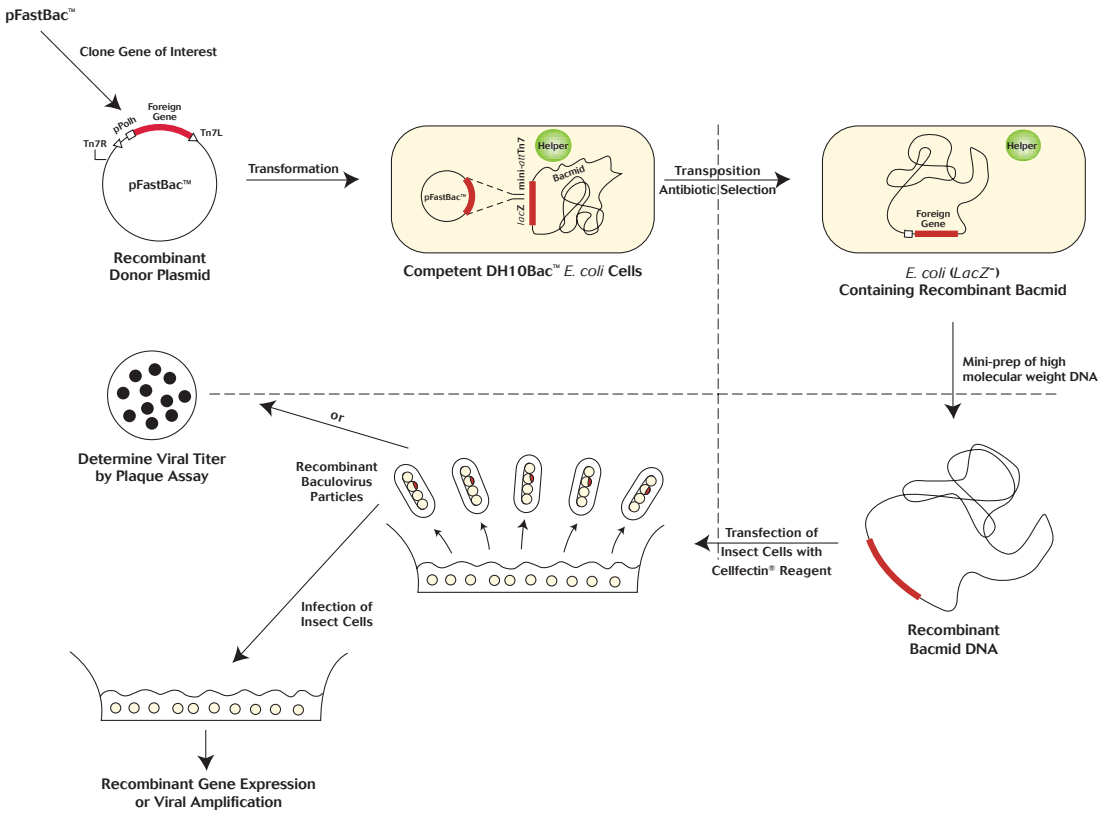
Table 1 - Insect cell lines used for baculovirus expression

Cell	Use	Advantage
Sf9 (*Spodoptera frugiperda*)	Recombinant baculovirus production, protein expression	Well-characterized host cell, good for plaquing
Sf21 (*Spodoptera frugiperda*)	Recombinant baculovirus production, protein expression	High yield of intracellular proteins
High Five™ (*Trichoplusia ni*)	Protein expression	High yield of secreted proteins

How it works

Bac-to-Bac® relies on the generation of recombinant baculovirus by site-specific transposition in *E. coli* rather than homologous recombination in insect cells (Figure 6). Clone your gene of interest into a pFastBac™ vector and transform into DH10Bac™ competent *E. coli*. DH10Bac™ contains a parent bacmid with a *lacZ*-mini-*att*Tn7 fusion. Transposition occurs between the elements of the pFastBac™ vector and the parent bacmid in the presence of the transposition proteins provided by a helper plasmid. When the transposition is successful, the expression cassette disrupts the *lacZ* gene. The result: you can easily visualize the new expression bacmid as white bacterial colonies. Isolate high molecular weight DNA and transfect Sf9 or Sf21 cells using Cellfectin® transfection reagent. After two days, you can isolate pure, high-titer recombinant baculovirus for amplification and expression. Cells are infected using the viral stock and protein expression can be detected within 24-48 hours. The whole process takes as little as ten days.

Figure 6 - Generation of recombinant baculovirus and gene expression with the Bac-to-Bac® Expression System



Let us do the work

Save your time and resources and let the Invitrogen experts do the work. Our Custom Services group is trained to perform all the steps for expression of your protein using the Bac-to-Bac® Expression System. Call our Custom Services Representative today at 800 955 6288, ext. 67265.

Get started

Blast off to expression. The Bac-to-Bac® System includes the components you need to get started (Table 2). For the fastest production of recombinant baculovirus, choose Bac-to-Bac®. Call Invitrogen and order today.

Table 2 – Bac-to-Bac® complete kit contents

Components	
• Your choice of FastBac™ vector (pFastBac™1 or pFastBac™ HT) • A positive expression control • MAX Efficiency® DH10Bac™ Chemically Competent *E. coli*	• Cellfectin® Reagent • Ni-NTA resin and column (pFastBac™ HT only)

Order Information

Description	Quantity	Cat. no.
Bac-to-Bac™ Baculovirus Expression System *with pFastBac™1*	1 kit	10359-016
Bac-to-Bac™ HT Baculovirus Expression System *with pFastBac™ HT*	1 kit	10608-016
pFastBac™1	10 µg	10360-014
pFastBac™ Dual	10 µg	10712-024
pDEST™8	6 µg	11804-010
pDEST™10	6 µg	11806-015
pDEST™20	6 µg	11807-013
MAX Efficiency® DH10Bac™ Competent Cells	0.5 ml	10361-012

References

Jarvis, D.L. *et al.* (1990) *Bio/Technology* **8:** 50-55.
Wickham, T.J. *et al.* (1992) *Bio/Technology Prog.* **8:** 391-396.
Davis, T.R. *et al.* (1992) *Bio/Technology* **10:** 1148-1150.
Davis, T.R. *et al.* (1993) *In Vitro Cell Dev Biol* **29A:** 388-390.

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