

Novel BaculoDirect™ and pFastBac™-TOPO® expression vectors

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Abstract

The baculovirus-insect cell expression system has proven to be an extremely valuable tool for recombinant protein production as it addresses the need for large scale and cost effective production of functionally active recombinant mammalian proteins. Here we report the addition of 3 novel vectors compatible with our Bac-to-Bac® and BaculoDirect™ expression systems: First, we generated BaculoDirect™ N-GST, a new BaculoDirect vector that produces N-terminal GST-fusion proteins. The vector has been engineered to include *attR* sites for quick and efficient recombination with Gateway® entry clones using a simple, one-hour LR reaction. We have also generated two new pFastBac™-TOPO® vectors with a hexa histidine tag at their N- or C-terminus. The tag can be easily removed from the final protein by acTEV protease cleavage. Performance of these vectors has been evaluated and implications are discussed.

Figure 1 – Maps of pFast Bac-TOPO vectors

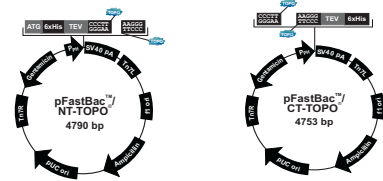


Figure 2 – Diagram of the Bac-to-Bac System

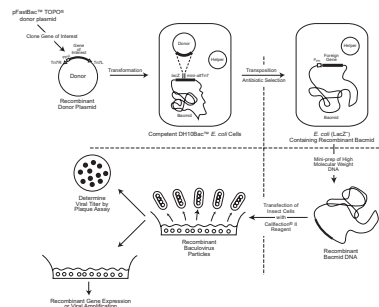
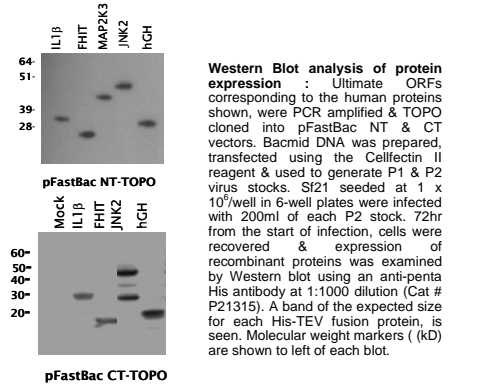


Figure 3 - Proteins expression using Bac-to Bac system

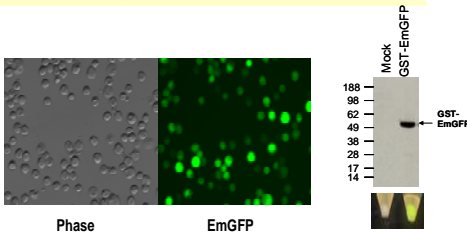


Western Blot analysis of protein expression - Ultimate ORFs corresponding to the human proteins shown, were PCR amplified & TOPO cloned into pFastBac NT & CT vectors. Bacmid DNA was prepared, transfected using the Cellfectin II reagent & used to generate P1 & P2 virus stocks. Sf21 seeded at 1×10^6 /well in 6-well plates were infected with 200ml of each P2 stock. 72hr from the start of infection, cells were recovered & expression of recombinant proteins was examined by Western blot using an anti-penta His antibody at 1:1000 dilution (Cat # P21315). A band of the expected size for each His-TEV fusion protein, is seen. Molecular weight markers (kD) are shown to left of each blot.

Figure 4 – Diagram of BaculoDirect GST tag genomic DNA



Figure 6 – Expression of GST-EmGFP fusion protein using BaculoDirect-GST



Expression of GST-EmGFP fusion protein: EmGFP gene was cloned via Gateway LR reactions into the BaculoDirect-GST genomic DNA. Sf21 cells were transfected using the Cellfectin II reagent to generate P1 & P2 virus stocks. Sf21 seeded in 24-well plates were infected with the P2 stock. Cells were observed under the microscopy 48 h post infection (Right panel). The expression of recombinant proteins was examined by Western blot using GST antibodies. A band of the expected size is indicated. Molecular weight markers are shown to left (kD).

Figure 5 – Diagram of BaculoDirect-GST expression system

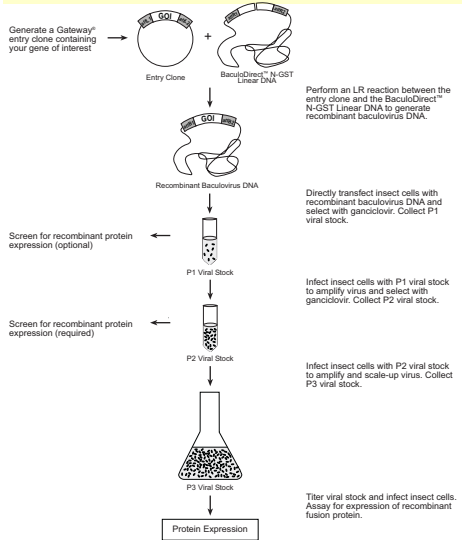
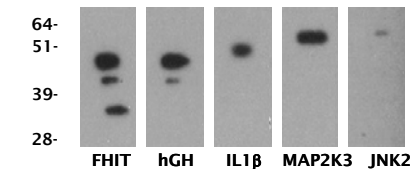
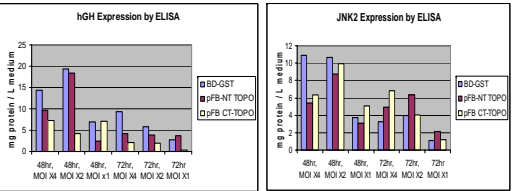


Figure 7 – Expression of GST-fusion proteins using BaculoDirect-GST



Western Blot analysis of GST fusion proteins: Ultimate ORFs corresponding to the human proteins shown, were cloned via Gateway LR reactions into the BaculoDirect-GST genomic DNA. Sf21 cells were transfected using the Cellfectin II reagent then P1 & P2 virus stocks were generated. Sf21 seeded in 24-well plates were infected with 50ml of each P2 stock. 72hr from the start of infection, soluble protein extracts were prepared from cells & expression of recombinant proteins was examined by Western blot using antibodies specific for each GST fusion protein. A band of the expected size for each fusion protein, is seen. Molecular weight markers are shown to left (kD).

Figure 8 – Expression soluble Proteins using BaculoDirect-GST and Bac-to Bac systems



Quantitation of hGH and JNK2 fusion protein production by ELISA: P2 virus stocks containing the human hGH & JNK2 genes were cloned into the BaculoDirect-GST genomic DNA & each pFastBac-TOPO vector. Sf21 seeded in 24-well plates were infected with P2 stock at MOIs of X4, X2 or X1. 48hr or 72hr from the start of infection, soluble protein extracts were prepared from cells. Expression of recombinant proteins at each time point & each relative MOI was quantitated by ELISA (Cat # KH00121, for JNK1/2 & KAQ1081 for hGH). Each bar is average of two infected wells.

Conclusions

- 1) We have generated two pFastBac TOPO cloning vectors including pFastBac-N-His TOPO and pFastBac-C-His TOPO vectors for Bac-to-Bac system. The TOPO vectors allow easy cloning the gene of the interest to the vectors. The vectors also provide a N-terminal or a C-terminal His tag to facilitate the protein expression, purification and detection. In addition, A TEV cleavage site that has been inserted between His tag and the gene of the interest provides the opportunity to generate tagless protein.
- 2) We have generated a BaculoDirect-GST genomic DNA with a GST tagged at the N-terminal. The GST tagged at the N-terminal could increase protein solubility, facilitate protein purification and be useful for proarray application.

Product Information

Bac-to-Bac® N-His TOPO® Cloning Kit	A11099
Bac-to-Bac® C-His TOPO® Cloning Kit	A11098
Bac-to-Bac® N-His TOPO® Expression System	A11101
Bac-to-Bac® C-His TOPO® Expression System	A11100
BaculoDirect™ GST Gateway® Transfection Kit	A10640
BaculoDirect™ GST Gateway® Expression Kit	A10641

