pBlueBacHis2 A, B, and C

A Baculovirus Transfer Vector for Expression and Purification of Recombinant Proteins

Catalog no. V375-20

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Important Information

Kit Contents

20 µg each of pBlueBacHis2 A, B, and C, lyophilized in TE, pH 8.0
Control vector pBlueBacHis2/CAT, lyophilized in TE, pH 8.0

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Accessory Products

The table below lists additional products available from Invitrogen which you may use in conjunction with the pBlueBacHis2 vectors.

Product	Quantity	Catalog no.
Anti-Xpress™ Antibody	50 µl	R910-25
Anti-Xpress™-HRP Antibody	50 µl	R911-25
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Metal-Binding Resin (Precharged resin provided as a 50% slurry in 20% ethanol)	50 ml	R801-01
	150 ml	R801-15
EKMax™	250 U	E180-01
	1000 U	E180-02
Bac-N-Blue™ Kit	5 transfections	K855-01

Methods

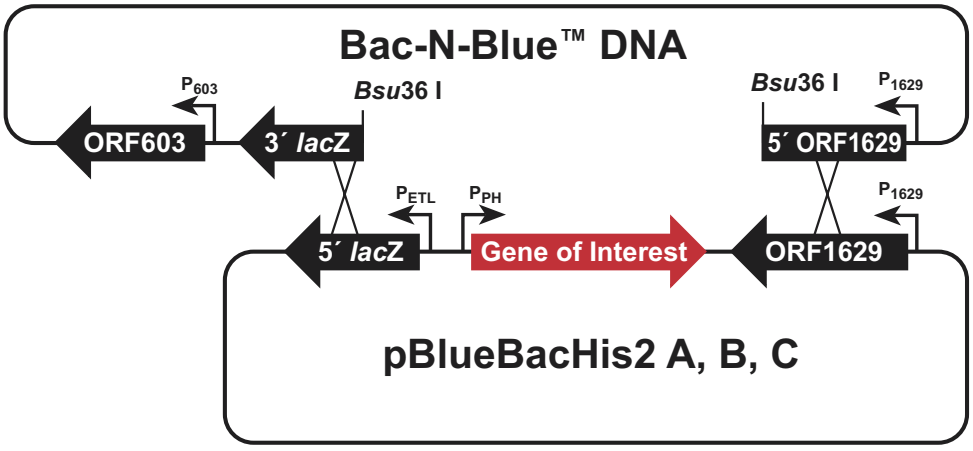
Overview

Introduction

The pBlueBacHis2 A, B, and C vectors are baculovirus transfer vectors designed for expression and purification of recombinant proteins in insect cells. Proteins expressed from pBlueBacHis2 are fused at the N-terminus to a tag of six tandem histidine residues and an enterokinase cleavage site (the Xpress™ tag). The histidine residues create a high-affinity metal binding site to allow purification of recombinant fusion proteins on nickel-chelating resin (i. e. ProBond™). The Xpress™ tag is easily cleaved away from your protein using enterokinase (i. e. EnterokinaseMax™). Expression and purification of the Xpress™ fusion protein is easily tracked using the Anti-Xpress™ Antibody which recognizes an epitope located in the Xpress™ tag. The pBlueBacHis2 vector is supplied in three different versions (A, B, and C) to allow correct in-frame fusion with the Xpress™ tag.

Recombination with Bac-N-Blue™ DNA

The pBlueBacHis2 transfer vectors contain *lacZ* and ORF1629 sequences homologous to those found in Bac-N-Blue™ AcMNPV DNA. Upon co-transfection and recombination, blue, occlusion body-negative (occ$^-$) recombinant plaques are formed. Bac-N-Blue™ DNA is linearized to remove sequence at the C-terminus of ORF1629 that are essential for efficient propagation of the virus. When recombination occurs between pBlueBacHis2 and Bac-N-Blue™ DNA, these essential sequences are restored, and viable, recombinant virus is produced. The diagram below shows where recombination occurs.



Important

pBlueBacHis2 A, B, and C can only be used with Bac-N-Blue™ DNA. The vectors **cannot** be used with Invitrogen's original linear AcMNPV DNA, BaculoGold™ (PharMingen) or BacPAK6™ (Clontech) linear AcMNPV DNA.

Cloning into pBlueBacHis2

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, see *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Maintenance of pBlueBacHis2 A, B, and C

In order to propagate and maintain pBlueBacHis2 A, B, and C, we recommend that you take each vector and resuspend the lyophilized material in 20 µl sterile water to make a 1 µg/µl stock solution. Store at -20°C.

Use this stock solution to transform a recombination deficient (*rec*A) and endonuclease A deficient (*end*A) *E. coli* strain like TOP10, TOP10F´, DH5α, or equivalent.

For your convenience, Invitrogen offers TOP10 and TOP10F´ cells in chemically competent and electrocompetent form. See below for purchasing information.

Item	Quantity	Catalog no.
One Shot™ TOP10 Chemically Competent *E. coli*	10 x 50 µl	C4040-10
One Shot™ TOP10 Electrocomp™ *E. coli*	20 x 50 µl	C4040-52
One Shot™ TOP10F´ Chemically Competent *E. coli*	21 x 50 µl	C3030-03

Important

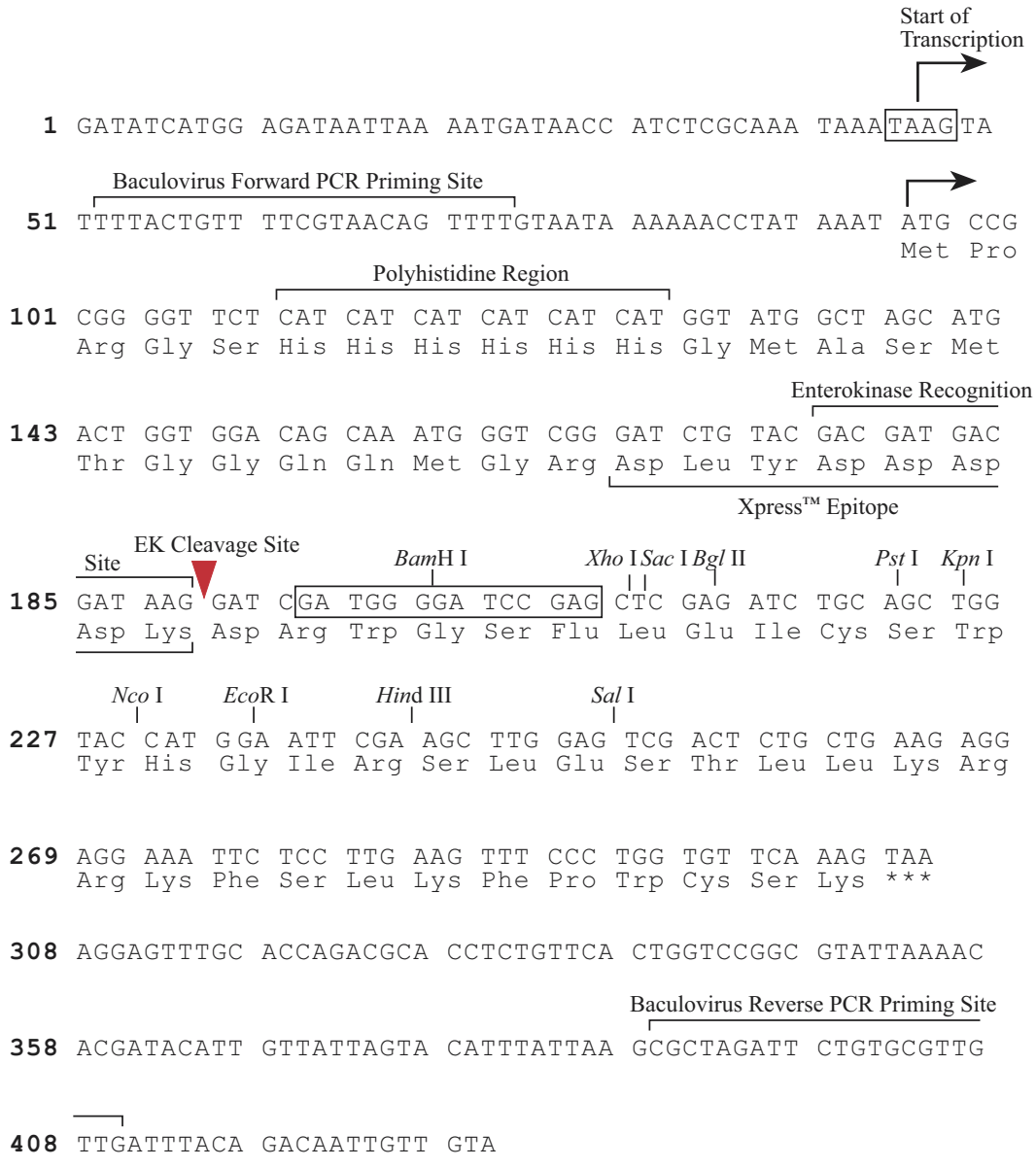
pBlueBacHis2 A, B, and C vectors are fusion vectors requiring that you clone your gene in-frame with the Xpress™ N-terminal peptide. We provide three versions of this vector to facilitate correct fusion of your gene to the signal sequence. Carefully inspect your gene and the multiple cloning site of each vector before cloning your gene of interest.

Continued on next page

Cloning into pBlueBacHis2, continued

Multiple Cloning Site A

Below is the multiple cloning site for pBlueBacHis2 A. Restriction sites are labeled to indicate the cleavage site. The variable region is the boxed region located after the enterokinase cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.



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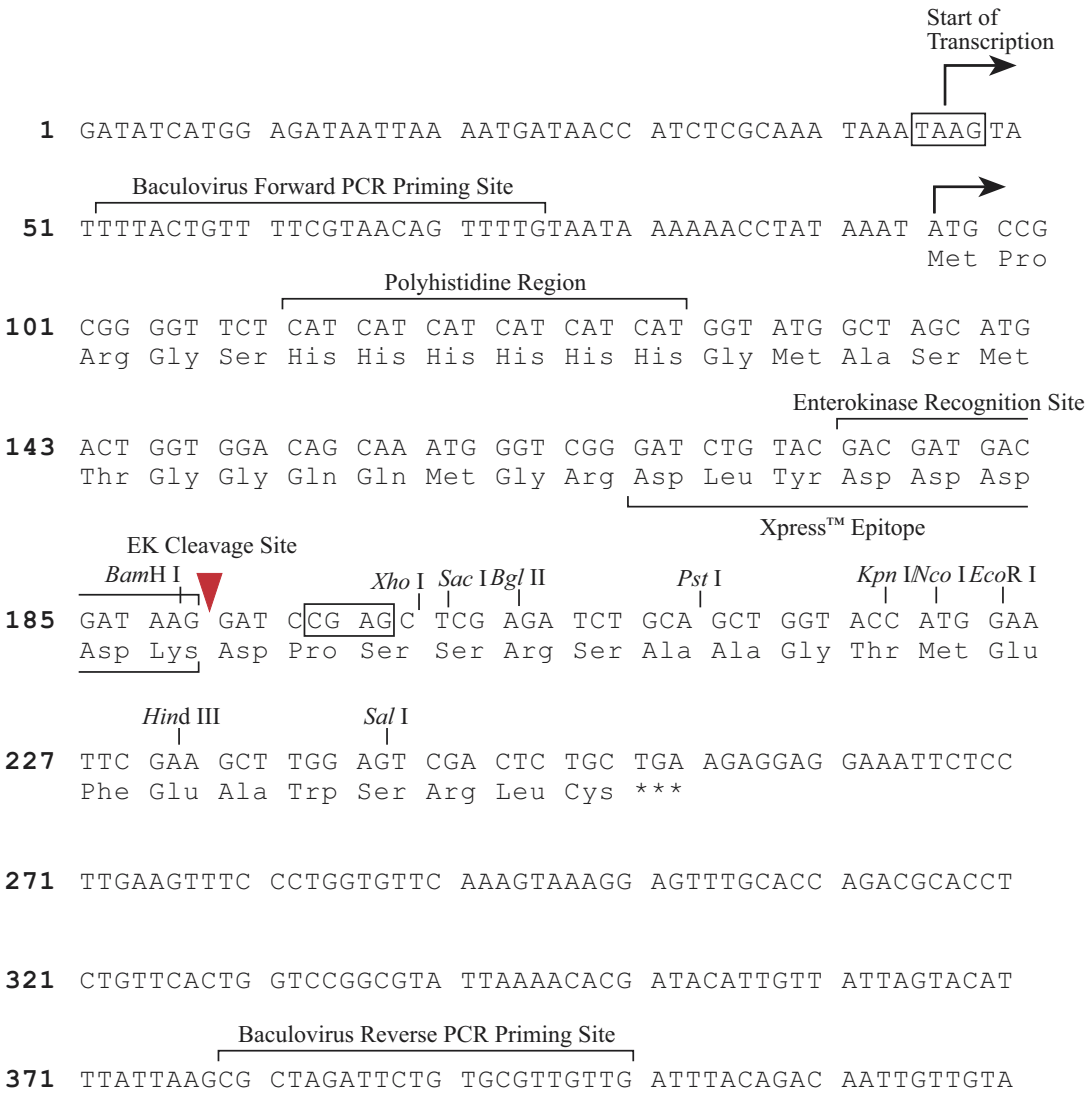
Cloning into pBlueBacHis2, continued

Multiple Cloning Site B

Below is the multiple cloning site for pBlueBacHis2 B. Restriction sites are labeled to indicate the cleavage site. The variable region is the boxed region located after the enterokinase cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.



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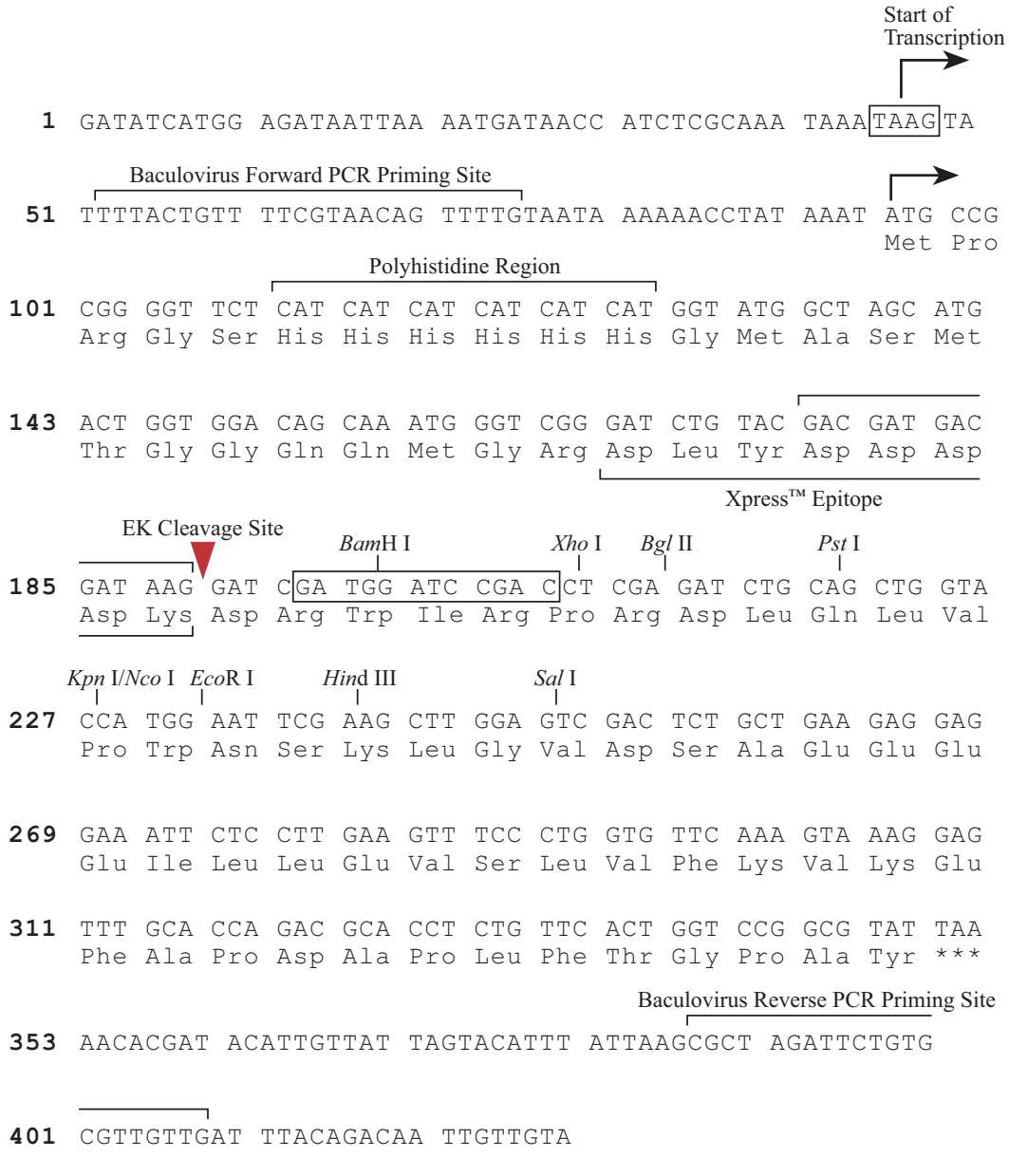
Cloning into pBlueBacHis2, continued

Multiple Cloning Site C

Below is the multiple cloning site for pBlueBacHis2 C. Restriction sites are labeled to indicate the cleavage site. The variable region is the boxed region located after the enterokinase cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.

Note that there is no *Sac* I site in this version of pBlueBacHis2.



Guidelines for Isolating Recombinant Virus

Introduction

The following guidelines and recommendations are provided for your convenience. If you need more details about the techniques discussed, refer to *Current Protocols in Molecular Biology*, Unit 16.9-16.11 (Ausubel *et al.*, 1994), *The Baculovirus Expression System: A Laboratory Guide* (King and Possee, 1992) , or *Baculovirus Expression Vectors: A Laboratory Manual* (O'Reilly *et al.*, 1992).

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e. g. TOP10, DH5α) and select on LB plates containing 50-100 µg/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct to confirm that your gene is correctly fused to the Xpress™ tag. For your convenience, Invitrogen offers a Custom Primer service and can construct primers according to your specifications. For more information on this service, visit our Web site (www.invitrogen.com).

Transfection into Insect Cells

In addition to your construct in pBlueBacHis2, we recommend that you include the control vector pBlueBacHis2/CAT as a positive control for expression. Plasmid DNA for transfection into insect cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and, in addition, salt will interfere with the Insectin™ liposomes, decreasing transfection efficiency.

For methods to transfect insect cells, screen for recombinant plaques, PCR confirmation of recombinant plaques, and expression, see the Bac-N-Blue™ Transfection and Expression Guide or the references listed above.

Important

We recommend isolating recombinant virus containing the CAT control protein as a positive control for expression.

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Guidelines to Isolating Recombinant Virus, Continued

Designing PCR Primers

To perform PCR analysis, you will need to design appropriate PCR primers to allow verification of recombinant viruses containing inserts. We recommend designing forward and reverse primers with the following sequence:

Baculovirus Forward primer: 5′-TTTACTGTTTTCGTAACAGTTTTG-3′

Baculovirus Reverse primer: 5′-CAACAACGCACAGAATCTAGC-3′

These primers flank the polyhedrin region and are compatible with all polyhedrin promoter based baculovirus transfer vectors. Refer to the diagrams on pages 3-5 to locate the primer binding site for each pBlueBacHis2 vector. Note that these are suggested primer sequences, and are not available from Invitrogen. If you would like information on placing custom primer orders, visit our Web site (www.invitrogen.com) or contact Technical Service (see page 14).

PCR Analysis

If you use PCR to analyze putative recombinant plaques in order to isolate a pure clone, you will need to know the expected size of your PCR fragment. Using the Baculovirus Forward and Reverse Primers discussed above, the following PCR products are generated:

Vector	PCR Product (bp)
pBlueBacHis2 A	358
pBlueBacHis2 B	348
pBlueBacHis2 C	356
pBlueBacHis2/CAT	1135

Add the size of the PCR product from the pBlueBacHis2 vector alone to the size of your insert to calculate the size of the expected PCR product. For example, if you have a 1000 bp fragment cloned into the *Pst* I site of pBlueBacHis2 C, the expected size of your PCR fragment will be 1356 bp.

Expression of Recombinant Fusion Protein

Introduction

To evaluate expression of your recombinant fusion protein, it is very helpful to have recombinant virus containing the CAT control protein cloned in-frame with the Xpress™ N-terminal peptide. Expression of the CAT control protein will rule out any problem with the cells or the multiplicity of infection (MOI). Every protein has its own requirements for optimal expression and you need to adjust conditions to optimize expression of your particular protein.

For optimal expression of your construct, we recommend that you:

- Use cells adapted to suspension culture for large-scale expression of recombinant proteins
- Perform a time course of expression to determine the maximum point of expression
- Have a detection method for your protein

Important

If you do not have a method for detection of your protein, you may wish to use the Anti-Xpress™ Antibody (see page iv for purchasing information) from Invitrogen to detect your Xpress™ fusion protein. The Antibody recognizes the Xpress™ tag, allowing you to track your Xpress™ fusion protein through expression and purification.

Assay for CAT Protein

You may assay for CAT expression by ELISA assay, Western blot analysis, fluorometric assay, or radioactive assay.

Before Starting

Be sure to generate a high-titer stock of your recombinant virus and the CAT recombinant virus. Refer to the Bac-N-Blue™ Transfection and Expression Guide.

You will need to have on hand or prepare the following:

- Log-phase suspension cultures of Sf9, Sf21, or High Five™ cells at 95-98% viability
- 20 mM sodium phosphate buffer, pH 7
- SDS-PAGE apparatus and solutions

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Expression of Recombinant Fusion Protein, Continued

Time Course of Expression

The following procedure describes how to carry out a time course to determine the point of maximum expression.

1. Seed two 100 ml spinner flasks with 50 ml of Sf9, Sf21, or High Five™ cells at a density of 2 x 10^6 cells/ml.
2. Infect one flask with the high-titer stock of your recombinant virus and the other with the CAT recombinant virus. Use an MOI of 5 to synchronously infect the cells. This will insure that all the cells will be expressing recombinant protein at the same time.
3. Remove a 1 ml aliquot at time zero and transfer to a microcentrifuge tube.
4. Centrifuge the cells at 1000 x g for 10-15 minutes at room temperature and decant the supernatant to a fresh microcentrifuge tube.
5. Store the cells and the supernatant at -70°C.
6. Collect 1 ml samples every 12-24 hours for a period of 2-3 days as described in Steps 3-5. The time course may be carried out for a longer period of time; however, cell lysis will occur around 72 hours postinfection. This will release protein to the supernatant.
7. When all the time points have been collected, proceed to **Analysis of Time Course,** below.

Analysis of Time Course

At this point you should have both cells and supernatant for each time point. The procedure below describes how to prepare your time points for analysis.

1. Prepare an SDS-polyacrylamide gel for analysis of your time points. For each time point, there will be two samples: a pellet fraction and a supernatant fraction. Remember to include molecular weight standards.
2. Resuspend each cell pellet in 100 µl of 20 mM sodium phosphate buffer, pH 7.0.
3. Freeze solid in liquid N_2 or an ethanol/dry ice bath.
4. Quickly thaw the frozen cells at 42°C.
5. Repeat Steps 3 and 4 two more times to make a cell lysate.
6. Centrifuge at maximum speed in a microcentrifuge for 10 minutes at +4°C to pellet cell debris and insoluble protein.
7. Transfer the supernatant to a fresh tube and add an equal volume (100 µl) of SDS-PAGE sample buffer.
8. Resuspend the cell pellet in 100 µl SDS-PAGE sample buffer.
9. Analyze 10 µl of each supernatant sample and 20 µl of each pellet sample on the SDS-polyacrylamide gel.

Results

Calculate the expected size of your recombinant fusion protein. Examine a Coomassie- or silver-stained SDS-polyacrylamide gel and determine the time point at which maximal expression occurs. Most of the recombinant fusion protein should be in the supernatant; there should be very little protein in the pellet fraction.

If you do not see expression of your fusion protein, perform a Western blot. Use an antibody to your protein or the Anti-Xpress™ Antibody (Catalog no. R910-25) to detect your recombinant fusion protein. This antibody recognizes the Xpress™ N-terminal peptide.

Continued on next page

Expression of Recombinant Fusion Protein, Continued

Large-Scale Expression

Once you have determined the point of maximal expression, you may scale up your protein expression using the following guidelines:

- Use 1 liter spinner flasks seeded with cells at a density of 2 x 10^6 cells/ml in a total volume of 500 ml
- Use a high-titer stock of your recombinant virus and infect with an MOI of 5
- Harvest the cells in 50 ml aliquots at the time point of maximal expression
- Pellet the cells by centrifugation and store at -70°C until needed

Purification of Recombinant Fusion Protein

To prepare cell lysates from insect cells and to purify your Xpress™ fusion protein using ProBond™ resin (see page iv for purchasing information), refer to the ProBond™ Protein Purification System manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 14).

If you are using other Ni^{2+}-chelating resins, refer to the manufacturer's instructions for purification of your fusion protein.

Digestion with Enterokinase

After obtaining purified recombinant fusion protein, you may wish to cleave the Xpress™ tag away from your protein. Invitrogen has a recombinant preparation of the catalytic subunit of enterokinase (EKMax™, see page iv for purchasing information). This enzyme has high specific activity, leading to more efficient cleavage with less enzyme. For more information, contact Technical Service (page 14).

Appendix

pBlueBacHis2 Vector

Features of pBlueBacHis2

The important elements of pBlueBacHis2 are described in the following table. All features have been functionally tested, except where noted.

Feature	Benefit
Polyhedrin promoter	Allows efficient, high-level expression of your recombinant fusion protein
ATG Initiation Codon	Permits initiation of translation of your Xpress™ fusion protein in insect cells
Polyhistidine Region	For high affinity binding to Ni-chelating resin (i. e., ProBond™) and easy purification (see page iv for ordering information)
Xpress™ Epitope	Allows detection of your Xpress™ fusion protein with the Anti-Xpress™ Antibody (Catalog no. R910-25)
Enterokinase Cleavage Site	For the removal of the Xpress™ tag from your protein
Multiple Cloning Sites in three reading frames (A, B, and C)	Allows insertion of your gene in-frame with the Xpress™ tag for purification of your fusion protein
ORF1629 recombination sequences	Permits integration of your gene into the Bac-N-Blue™ DNA and restores the essential ORF1629 for production of viable, recombinant virus
*lac*Z recombination sequences	Permits integration of your gene into the Bac-N-Blue™ DNA and production of blue, recombinant plaques for easy selection
Baculovirus early-to-late (ETL) promoter	Allows expression of the intact *lac*Z gene to produce blue, recombinant plaques in insect cells
Small size (4.9 kb, half the size of most baculovirus transfer vectors)	Permits efficient and easy cloning of inserts
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*
pUC origin	High-copy number replication and growth in *E. coli*

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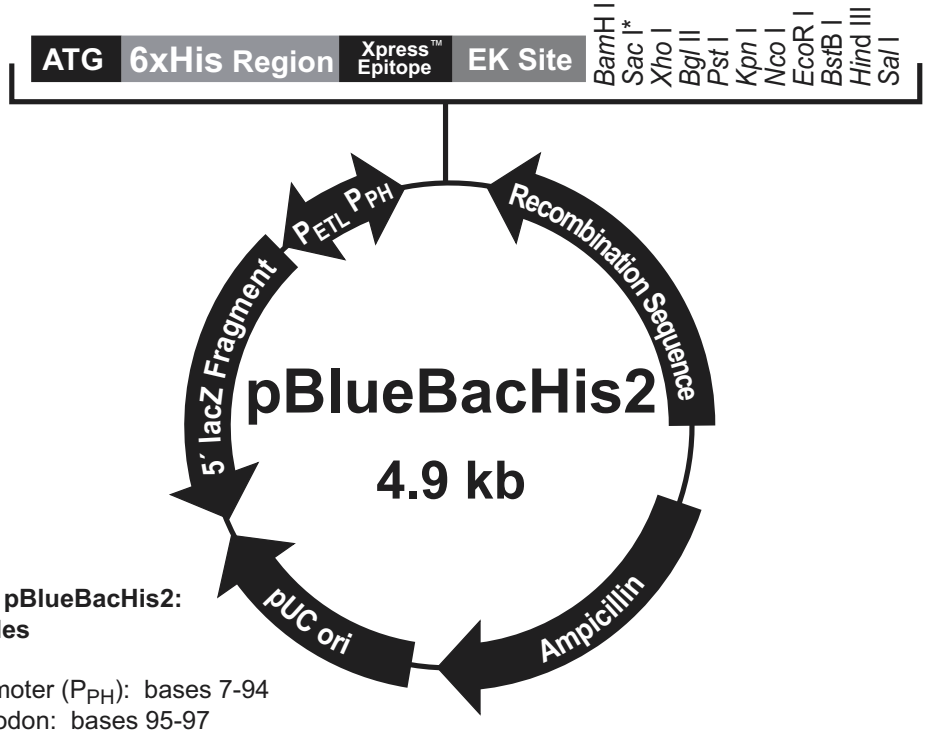
pBlueBacHis2 Vector, continued

Description

pBlueBacHis2 A (4863 bp), pBlueBacHis2 B (4853 bp), and pBlueBacHis2 C (4861 bp) are baculovirus transfer vectors that contain the Xpress™ N-terminal tag for expression and purification of recombinant fusion proteins. The pBlueBacHis2 vector is provided in three different versions for simplified cloning of your gene in-frame with the Xpress™ tag. The vectors contain sequences homologous to the *lac*Z gene and ORF1629 sequences in Bac-N-Blue™ linear DNA, allowing production of blue, recombinant plaques. Expression of your recombinant fusion protein is driven by the polyhedrin promoter.

Map of pBlueBacHis2

The figure below summarizes the features of the pBlueBacHis2 vector. The complete nucleotide sequence for pBlueBacHis2 B is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 14). Details of the multiple cloning site, including the variable region that determines the reading frame, are shown on pages 3-5.



Comments for pBlueBacHis2:
4853 nucleotides

Polyhedrin promoter (P_{PH}): bases 7-94
ATG initiation codon: bases 95-97
Polyhistidine region: bases 110-127
Xpress™ epitope: bases 167-190
Enterokinase recognition site: bases 176-190
Multiple cloning site: bases 190-245
Recombination sequences (ORF1629): bases 1256-456 (C)
Ampicillin resistance gene: bases 1643-2500
pUC origin: bases 2648-3321
5´ *lac*Z fragment: bases 4551-3439 (C)
*lac*Z sequence homologous to *lac*Z sequence in Bac-N-Blue™ DNA: bases 4327-3439
Early-to-late promoter (P_{ETL}): bases 4853-4552 (C)
(C) = complementary strand

*There is no *Sac* I site in the 'C'

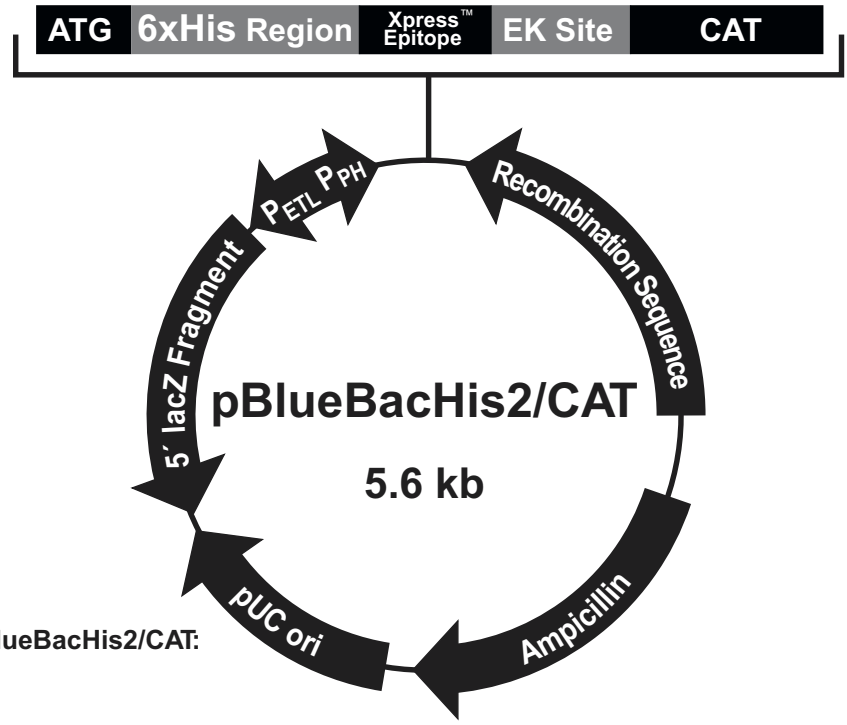
pBlueBacHis2/CAT

Description

pBlueBacHis2/CAT is a 5640 bp control vector containing the gene for chloramphenicol acetyl transferase (CAT). It was constructed by digesting pBlueBacHis2 B with *Hin*d III and dephosphorylating with calf intestinal alkaline phosphatase (CIAP). A *Hin*d III fragment containing the gene for chloramphenicol acetyl transferase was then ligated into pBlueBacHis2 B.

Map of Control Vector

The figure below summarizes the features of the pBlueBacHis2/CAT vector. The complete nucleotide sequence for pBlueBacHis2/CAT is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 14).



Comments for pBlueBacHis2/CAT:
5640 nucleotides

Polyhedrin promoter (P_{PH}): bases 7-94
ATG initiation codon: bases 95-97
Polyhistidine region: bases 110-127
Xpress™ Epitope: bases 167-190
Enterokinase recognition site: bases 176-190
CAT ORF: bases 269-925
Recombination sequences (ORF1629): bases 2043-1243 (C)
Ampicillin resistance gene: bases 2430-3287
pUC origin: bases 3435-4108
5´ *lac*Z fragment: bases 5338-4226 (C)
*lac*Z sequence homologous to *lac*Z sequence
in Bac-N-Blue™ DNA: bases 5114-4226
Early-to-late promoter (P_{ETL}): bases 5640-5339 (C)
(C) = complementary strand

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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Technical Service, continued

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Product Qualification

Vector Qualification

The pBlueBacHis2 vectors and the pBlueBacHis2/CAT control plasmid are qualified by restriction enzyme digestion with specific restriction enzymes as listed below. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel.

Vector	Restriction Enzyme	Expected Results (bp)
pBlueBacHis2 A	*Bam*H I	4861
	*Hin*d III	4861
	Pvu II	116, 500, 4245
	Sac I	4861
	*Bam*H I + *Nhe* I	66, 4795
pBlueBacHis2 B	*Bam*H I	4851
	*Hin*d III	4851
	Pvu II	116, 490, 4245
	Sac I	4851
	*Bam*H I + *Nhe* I	56, 4795
pBlueBacHis2 C	*Bam*H I	4859
	*Hin*d III	4859
	Pvu II	116, 498, 4245
	Sac I	No site
	*Bam*H I + *Nhe* I	64, 4795
pBlueBacHis2/CAT	*Bam*H I	5638
	*Hin*d III	787, 4851
	Pvu II	116, 170, 490, 4862

Purchaser Notification

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