

# pMT/BiP/V5-His A, B, and C

**Catalog no. V4130-20**

Version C  
100501  
28-0178



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## Important Information

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**Contents** 20 µg each of pMT/BiP/V5-His A, B, and C, lyophilized in TE Buffer, pH 8.0  
20 µg of pMT/BiP/V5-His/GFP, lyophilized in TE Buffer, pH 8.0

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**Shipping/Storage** Lyophilized vectors are shipped at room temperature and should be stored at –20°C.

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**Product Qualification** The pMT/BiP/V5-His A, B, and C and pMT/BiP/V5-His/GFP vectors 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 (see below).

Vector	Restriction Enzymes	Expected Results (bp)
pMT/BiP/V5-His A	<i>Apa</i> I	3642
	<i>Bam</i> H I	307, 3335
	<i>Eco</i> R V	3642
	<i>Kpn</i> I	3642
	<i>Msc</i> I	3642
	<i>Sac</i> II	No site
	<i>Xba</i> I	3642
pMT/BiP/V5-His B	<i>Apa</i> I	3646
	<i>Bam</i> H I	311, 3335
	<i>Eco</i> R V	3646
	<i>Kpn</i> I	3646
	<i>Msc</i> I	3646
	<i>Sac</i> II	3646
	<i>Xba</i> I	3646
pMT/BiP/V5-His C	<i>Apa</i> I	No site
	<i>Bam</i> H I	303, 3335
	<i>Bst</i> E II	3638
	<i>Eco</i> R V	3638
	<i>Kpn</i> I	3638
	<i>Msc</i> I	3638
	<i>Sac</i> II	No site
	<i>Xba</i> I	No site
pMT/BiP/V5-His/GFP	<i>Msc</i> I	4327
	<i>Nco</i> I	195, 4132
	<i>Pme</i> I	4327

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## Purchaser Notification

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### License Information

This product is licensed under patents assigned to SmithKline Beecham Corporation (SB) for research use only. Customers may not sell or transfer this product to any other person. Customers agree to make no commercial use of this product. For further details, please refer to the licensing agreement. A copy of the licensing agreement is available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or by calling Technical Service (see page 14).

**Please note that you need a license to practice this technology. If you do not have a signed agreement on file, please use the contact information below to obtain a license agreement:**

Licensing Coordinator  
Invitrogen Corporation  
1600 Faraday Avenue  
Carlsbad, CA 92008  
Tel: 760-603-7200  
Fax: 760-602-6500

**OR**

Licensing Coordinator  
Invitrogen Ltd  
3 Fountain Drive  
Inchinnan Business Park  
Paisley PA4 9RF, UK  
Tel: (0) 800 5345 5345  
Fax: +44 (0) 141 814 6287

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## Accessory Products

### Introduction

The products listed in this section are intended for use with the pMT/BiP/V5-His vector and the DES<sup>®</sup> Inducible/Secreted Kits. For more information, please refer to our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or call Technical Service (see page 14).

### Products Available Separately

The following products may be used with pMT/BiP/V5-His and the DES<sup>®</sup> Inducible/Secreted Kits and are available separately from Invitrogen.

Product	Amount	Catalog no.
MT Forward Primer	2 µg, lyophilized in TE	N620-02
BGH Reverse Primer	2 µg, lyophilized in TE	N575-02
Hygromycin B	1 g	R220-05
Blasticidin S HCl	50 mg	R210-01
Schneider (S2) Cells	1 ml vial, 1 x 10 <sup>7</sup> cells/ml	R690-07
Schneider's <i>Drosophila</i> Medium	500 ml	11720-034
Calcium Phosphate Transfection Kit	75 reactions	K2780-01

### Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for detection of C-terminal fusion proteins expressed using pMT/BiP/V5-His. Horseradish peroxidase (HRP) or alkaline phosphate (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

The amount of antibody supplied is sufficient for 25 westerns.

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern <i>et al.</i> , 1991) GKPIP NPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection (Lindner <i>et al.</i> , 1997) HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

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## Accessory Products, continued

### Purification of Recombinant Protein

The metal binding domain encoded by the polyhistidine tag allows simple, easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using Invitrogen's ProBond™ Resin (see below). To purify proteins expressed from pMT/BiP/V5-His, the ProBond™ Purification System or the ProBond™ resin in bulk are available separately. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Metal-Binding Resin	50 ml	R801-01
(precharged resin provided as a 50% slurry in 20% ethanol)	150 ml	R801-15
ProBond™ Purification System (includes six 2 ml precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification)	6 purifications	K850-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
Purification Columns (10 ml polypropylene columns)	50	R640-50

# Methods

## Overview

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### Introduction

pMT/BiP/V5-His is a 3.6 kb expression vector designed for use with the *Drosophila* Inducible/Secreted Expression System (DES<sup>®</sup>; Catalog nos. K4120-01 and K5120-01) available from Invitrogen. Upon transfection, the vector allows transient, inducible, secreted expression of your protein of interest in *Drosophila* cells. When cotransfected with the selection vector, pCoHygro or pCoBlast, included with the appropriate DES<sup>®</sup> Inducible/Secreted Kit, pMT/BiP/V5-His allows selection of stable cell lines exhibiting inducible, secreted expression of the protein of interest. The vector contains the following elements:

- The *Drosophila* metallothionein (MT) promoter for high-level, metal-inducible expression of the gene of interest in S2 cells (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992)
- *Drosophila* BiP secretion signal for secreted expression of the gene of interest (Kirkpatrick *et al.*, 1995)
- Multiple cloning site to facilitate cloning the gene of interest
- C-terminal peptide containing the V5 epitope and polyhistidine (6xHis) tag for detection and purification of your protein of interest (if desired)
- Three reading frames to facilitate in-frame cloning with the C-terminal peptide
- Ampicillin resistance gene for selection of transformants in *E. coli*

The control plasmid, pMT/BiP/V5-His/GFP, is included for use as a positive control for transfection and expression.

For more information about the DES<sup>®</sup> Inducible/Secreted Kits, pCoHygro, and pCoBlast, refer to the *Drosophila* Expression System manual. The manual is supplied with each DES<sup>®</sup> Inducible/Secreted Kit, but is also available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or by contacting Technical Service (see page 14).

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### Description of MT Promoter

The *Drosophila* MT promoter allows high-level, inducible expression of the gene of interest in *Drosophila* S2 cells. When used to express heterologous proteins, the promoter is extremely efficient and tightly regulated, even at high copy number (Johansen *et al.*, 1989). The MT promoter is well characterized (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992), with regulatory elements and the start of transcription well defined.

The MT promoter is inducible by addition of copper sulfate or cadmium chloride to the culture medium (Bunch *et al.*, 1988). Copper sulfate is generally the preferred inducer due to its reduced toxicity as compared to cadmium. While cadmium is an effective inducer, it also induces a heat-shock response in S2 cells.

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### BiP Secretion Signal

In addition to the MT promoter, the pMT/BiP/V5-His vector contains the *Drosophila* BiP secretion signal upstream of the multiple cloning site. The *Drosophila* BiP protein encodes an immunoglobulin binding chaperone protein (Kirkpatrick *et al.*, 1995). The secretion signal of the BiP protein was chosen because it efficiently targets high levels of BiP to the endoplasmic reticulum in the S2 cell line (Kirkpatrick and Shatzman, 1997). Its efficiency is comparable to that of the tPA secretion signal.

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## Overview, continued

### Experimental Outline

The table below describes the general steps needed to clone and express your gene of interest. For more details, please refer to the manual and pages indicated.

Step	Action	Source
1	Develop a cloning strategy to ligate your gene of interest into pMT/BiP/V5-His A, B, or C in frame with the C-terminal peptide encoding the V5 epitope and the polyhistidine tag (if desired).	Pages 4-7, this manual
2	Transform your ligation reactions into a <i>recA</i> , <i>endA</i> <i>E. coli</i> strain (e.g. TOP10). Select on LB agar plates containing 50-100 µg/ml ampicillin.	Page 8, this manual
3	Analyze your transformants for the presence of insert by restriction digest.	Page 8, this manual
4	Select a transformant with the correct restriction pattern and sequence it to confirm that your gene is cloned in frame with the C-terminal peptide.	Page 8, this manual
5	Transfect your pMT/BiP/V5-His construct into S2 cells and induce expression of the gene of interest with copper sulfate.	Page 9, this manual and DES <sup>®</sup> manual
6	Assay for transient expression of your recombinant protein.	Page 9, this manual and DES <sup>®</sup> manual
7	To generate stable cell lines, cotransfect your pMT/BiP/V5-His construct and pCoHygro or pCoBlast into S2 cells and select for hygromycin resistant clones.	DES <sup>®</sup> manual
8	Scale up expression for purification.	DES <sup>®</sup> manual
9	Purify your recombinant protein by chromatography on metal-chelating resin (i.e. ProBond <sup>™</sup> ).	DES <sup>®</sup> manual

# Cloning into pMT/BiP/V5-His A, B, and C

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## Introduction

Diagrams are provided on pages 5-7 to help you clone your gene of interest into pMT/BiP/V5-His. General considerations for cloning and transformation are discussed below.

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## General Molecular Biology Techniques

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

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## *E. coli* Strain

Many *E. coli* strains are suitable for the propagation and maintenance of the pMT/BiP/V5-His vectors including TOP10 (Catalog no. C610-00), DH5 $\alpha$ <sup>TM</sup>-T1<sup>R</sup>, and JM109. We recommend that you propagate the vectors in *E. coli* strains that are recombination deficient (*recA*) and endonuclease A deficient (*endA*).

For your convenience, TOP10 and DH5 $\alpha$ <sup>TM</sup>-T1<sup>R</sup> *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot<sup>®</sup> format from Invitrogen.

Item	Quantity	Catalog no.
One Shot <sup>®</sup> TOP10 (chemically competent cells)	21 x 50 $\mu$ l	C4040-03
One Shot <sup>®</sup> TOP10 Electrocomp <sup>TM</sup> (electrocompetent cells)	21 x 50 $\mu$ l	C4040-52
One Shot <sup>®</sup> DH5 $\alpha$ <sup>TM</sup> -T1 <sup>R</sup> Max Efficiency (chemically competent cells)	10 x 50 $\mu$ l	12297-016

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## Transformation Method

You may use any method of your choice for transformation. Chemical transformation is the most convenient method for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

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## Maintenance of Plasmids

To propagate and maintain the pMT/BiP/V5-His and pMT/BiP/V5-His/GFP vectors, we recommend resuspending each vector in 20  $\mu$ l sterile water to prepare a 1  $\mu$ g/ $\mu$ l stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *recA*, *endA* *E. coli* strain like TOP10, DH5 $\alpha$ <sup>TM</sup>-T1<sup>R</sup>, or equivalent. Select transformants on LB agar plates containing 50 to 100  $\mu$ g/ml ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 8).

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## Cloning into pMT/BiP/V5-His A, B, and C, continued

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### Cloning Considerations

Please consider the following points when designing a strategy to clone your gene of interest into pMT/BiP/V5-His.

- pMT/BiP/V5-His is a terminal fusion vector. To express your gene as a recombinant fusion protein, you **must** clone your gene in frame with the N-terminal BiP secretion signal. If you use the *Bgl* II site, only two amino acids (arginine and serine) will be fused to your protein. Be sure to remove the native signal sequence, if present, before fusing your gene to the BiP signal sequence.
- If you wish to use the V5 epitope and the polyhistidine (6xHis) tag for detection and purification of your recombinant protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. Please refer to the diagrams on pages 5-7 to develop a cloning strategy. **Be sure that your gene does not contain a stop codon upstream of the C-terminal peptide.**
- If you do not wish to include the C-terminal peptide, include the native stop codon for your gene of interest.

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# Cloning into pMT/BiP/V5-His A, B, and C, continued

## Multiple Cloning Site of pMT/BiP/V5-His A

Below is the multiple cloning site for pMT/BiP/V5-His A. The metal regulatory regions are marked as per Maroni, *et al.*, 1986. The start of transcription is at nucleotide 778. The BiP signal cleavage site is also indicated (Rubin *et al.*, 1993). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. **The complete sequence of pMT/BiP/V5-His A is available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or from Technical Service (see page 14).** For a map and a description of the features of pMT/BiP/V5-His A, refer to pages 11-12.

```

      ┌── 5' end of metallothionein promoter
411  CGTTGCAGGA CAGGATGTGG TGCCCGATGT GACTAGCTCT TTGCTGCAGG CCGTCCATC
      Metal regulatory region
471  CTCTGGTTCC GATAAGAGAC CCAGAACTCC GGCCCCCACC CGCCCACCGC CACCCCCATA
      Metal regulatory
531  CATATGTGGT ACGCAAGTAA GAGTGCTGCT GCATGCCCCA TGTGCCCCAC CAAGAGTTTT
      region
591  GCATCCCATC CAAGTCCCCA AAGTGAGAGAA CCGAACCAAT TCTTCGCGGG CAGAACAAAA
      Metal regulatory regions
651  GCTTCTGCAC ACGTCTCCAC TCGAATTTGG AGCCGGCCGG CGTGTGCAAA AGAGGTGAAT
      Metal regulatory region
711  CGAACGAAAG ACCCGTGTGT AAAGCCGCGT TTCCAAAATG TATAAACCG AGAGCATCTG
      Metal regulatory regions
771  GCCAATGTGC ATCAGTTGTG GTCAGCAGCA AAATCAAGTG AATCATCTCA GTGCAACTAA
      Start of transcription
      MT Forward priming site
831  AGGGGGGATC CGATCTCAAT ATG AAG TTA TGC ATA TTA CTG GCC GTC GTG GCC TTT
      BiP signal sequence
      Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe
      Bgl II Nco I Sma I Kpn I Spe I BstX I
887  GTT GGC CTC TCG CTC GGG AGATCTCCAT GGCCCGGGGT ACCTACTAGT CCAGTGTGGT
      Val Gly Leu Ser Leu Gly Signal cleavage site
      EcoR I EcoR V BstX I Not I Xho I Xba I Apa I
945  GGAATTCTGC AGATATCCAG CACAGTGGCG GCCGCTCGAG TCTAGAGGGC CTTTCGAA GGT AAG
      V5 epitope Age I Polyhistidine
      Region Pme I BGH Reverse priming site
1009 CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC
      Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His
1063 CAT CAC CAT TGA GTTTAAACCC GCTGATCAGC CTCGACTGTG CCTTCTAAGG CCTGAGCTCG
      His His His ***
1125 CTGATCAGCC TCGATCGAGG ATCCAGACAT GATAAGATAC ATTGATGAGT TTGACAAAC
1185 CACAACCTAGA ATGCAGTGAA AAAAAATGCTT TATTTGTGAA ATTTGTGATG CTATTGCTTT
      SV40 late polyadenylation signal
1245 ATTTGTAACC ATTATAAGCT GCAATAAACA AGT
  
```

\*Note that there are two *BstX I* sites in the polylinker.

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# Cloning into pMT/BiP/V5-His A, B, and C, continued

## Multiple Cloning Site of pMT/BiP/V5-His B

Below is the multiple cloning site for pMT/BiP/V5-His B. The metal regulatory regions are marked as per Maroni, *et al.*, 1986. The start of transcription is at nucleotide 778. The BiP signal cleavage site is also indicated (Rubin *et al.*, 1993). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. **The complete sequence of pMT/BiP/V5-His B is available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or from Technical Service (see page 14).** For a map and a description of the features of pMT/BiP/V5-His B, refer to pages 11-12.

```

    ┌── 5' end of metallothionein promoter
411  CGTTGCAGGA CAGGATGTGG TGCCCGATGT GACTAGCTCT TTGCTGCAGG CCGTCCTATC

    Metal regulatory region
471  CTCTGGTCC GATAAGAGAC CCAGAACTCC GGCCCCCAC CGCCACC GCACCCATA

    Metal regulatory
531  CATATGTGGT ACGCAAGTAA GAGTGCCTGC GCATGCCCA TGTGCCAC CAAGAGTTT
    region

591  GCATCCATA CAAGTCCCA AAGTGGAGAA CCGAACCAAT TCTTCGCGG CAGAACAAA

    Metal regulatory regions
651  GCTTCTGCAC ACGTCTCCAC TCGAATTGG AGCCGGCCGG CGTGTGCAA AGAGGTGAAT
    Metal regulatory region

711  CGAACGAAAG ACCCGTGTGT AAAGCCCGT TTCCAAAATG TATAAAACCG AGAGCATCTG
    TATA box
    Metal regulatory regions

771  GCCAATGTGC ATCAGTTGTG GTCAGCAGCA AAATCAAGTG AATCATCTCA GTGCAACTAA
    └─ Start of transcription
    MT Forward priming site

831  AGGGGGGATC CGATCTCAAT ATG AAG TTA TGC ATA TTA CTG GCC GTC GTG GCC TTT
    Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe
    BiP signal sequence

887  GTT GGC CTC TCG CTC GGG AGATCTCCAT GGCCCGGGT ACCTACTAGT CCAGTGTGGT
    Val Gly Leu Ser Leu Gly Signal cleavage site
    Bgl II Nco I Sma I Kpn I Spe I BstX I
    EcoR I EcoR V BstX I Not I Xho I Xba I Apa I Sac II

945  GGAATTCTGC AGATATCCAG CACAGTGGCG GCCGCTCGAG TCTAGAGGGC CCGCGGTTTCG AA GGT
    Gly

1010 AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT
    Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His
    V5 epitope Age I Polyhistidine

1064 CAC CAT CAC CAT TGA GTTTAAACCC GCTGATCAGC CTCGACTGTG CCTTCTAAGG
    His His His His ***
    Region Pme I BGH Reverse priming site

1119 CCTGAGCTCG CTGATCAGCC TCGATCGAGG ATCCAGACAT GATAAGATAC ATTGATGAGT

1179 TTGGACAAAC CAACTAGA ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG

    SV40 late polyadenylation signal
1239 CTATTGCTTT ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAAACAAC AACAAATTGCA
  
```

\*Note that there are two *BstX* I sites in the polylinker.

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# Cloning into pMT/BiP/V5-His A, B, and C, continued

## Multiple Cloning Site of pMT/BiP/V5-His C

Below is the multiple cloning site for pMT/BiP/V5-His C. The metal regulatory regions are marked as per Maroni, *et al.*, 1986. The start of transcription is at nucleotide 778. The BiP signal cleavage site is also indicated (Rubin *et al.*, 1993). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. **The complete sequence of pMT/BiP/V5-His C is available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or from Technical Service (see page 14).** For a map and a description of the features of pMT/BiP/V5-His C, refer to pages 11-12.

```

      ┌── 5' end of metallothionein promoter
411  CGTTGCAGGA CAGGATGTGG TGCCCGATGT GACTAGCTCT TTGCTGCAGG CCGTCCTATC
      Metal regulatory region
471  CTCTGGTTCC GATAAGAGAC CCAGAACTCC GGCCCCCCAC CGCCACCCGC CACCCCCATA
      Metal regulatory
531  CATATGTGGT ACGCAAGTAA GAGTGCCTGC GCATGCCCCA TGTGCCCCAC CAAGAGTTTT
      region
591  GCATCCATA CAAGTCCCA AAGTGGAGAA CCGAACCAAT TCTTCGCGGG CAGAACAAAA
      Metal regulatory regions
651  GCTTCTGCAC ACGTCTCCAC TCGAATTGG AGCCGGCCGG CGTGTGCAA AGAGGTGAAT
      Metal regulatory region
711  CGAACGAAAG ACCCGTGTGT AAAGCCGCGT TTCCAAAATG TATAAAACCG AGAGCATCTG
      Metal regulatory regions
      Start of transcription
771  GCCAATGTGC ATCAGTTGTG GTCAGCAGCA AAATCAAGTG AATCATCTCA GTGCAACTAA
      MT Forward priming site
      BiP signal sequence
831  AGGGGGGATC CGATCTCAAT ATG AAG TTA TGC ATA TTA CTG GCC GTC GTG GCC TTT
      Met Lys Leu Cys Ile Leu Leu Ala Val Val Ala Phe
      Bgl II Nco I Sma I Kpn I Spe I BstX I
887  GTT GGC CTC TCG CTC GGG AGATCTCCAT GGCCCGGGGT ACCTACTAGT CCAGTGTGGT
      Val Gly Leu Ser Leu Gly Signal cleavage site
      EcoR I EcoR V BstX I Not I Xho I BstE II
945  GGAATTCTGC AGATATCCAG CACAGTGGCG GCCGCTCGAG GTCACCCA TT CGAA GGT AAG CCT
      Gly Lys Pro
      V5 epitope Age I Polyhistidine Region
1008 ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT
      Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His His
      Pme I BGH Reverse priming site
1062 CAC CAT TGA GTTTAAACCC GCTGATCAGC CTCGACTGTG CCTTCTAAGG CCTGAGCTCG
      His His ***
1121 CTGATCAGCC TCGATCGAGG ATCCAGACAT GATAAGATAC ATTGATGAGT TTGGACAAAC
1181 CACAAC TAGA ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG CTATTGCTTT
      SV40 late polyadenylation signal
1241 ATTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC AACAATTGCA TTCATTTTAT
  
```

\*Note that there are two *BstX* I sites in the polylinker.

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## Cloning into pMT/BiP/V5-His A, B, and C, continued

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### ***E. coli*** **Transformation**

Transform your ligation mixtures into a competent *recA*, *endA* *E. coli* strain (e.g. TOP10, DH5 $\alpha$ <sup>™</sup>-T1<sup>R</sup>) and select on LB agar plates containing 50 to 100  $\mu$ g/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

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We recommend that you sequence your construct with the MT Forward and BGH Reverse primers (Catalog nos. N620-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and is cloned in frame with the BiP secretion signal and the C-terminal peptide. The MT Forward and BGH Reverse primers are included in each DES<sup>®</sup> Inducible/Secreted Kit. Refer to the diagram on pages 5-7 for the sequences and location of the priming sites.

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### **Preparing a** **Glycerol Stock**

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at -20°C.

1. Streak the original colony out on an LB plate containing 50  $\mu$ g/ml ampicillin. Incubate the plate at 37°C overnight.
  2. Isolate a single colony and inoculate into 1-2 ml of LB containing 50  $\mu$ g/ml ampicillin.
  3. Grow the culture to mid-log phase (OD<sub>600</sub> = 0.5-0.7).
  4. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
  5. Store at -80°C.
-

# Transfection and Analysis

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## Introduction

Once you have cloned your gene of interest into pMT/BiP/V5-His and have prepared purified plasmid DNA, you are ready to transfect your construct into S2 cells. If you are assaying for transient, inducible expression of your gene of interest, you may transfect your pMT/BiP/V5-His construct alone into S2 cells. If you wish to generate stable cell lines, you **must** cotransfect your pMT/BiP/V5-His construct and pCoHygro or pCoBlast into S2 cells. Note that the pMT/BiP/V5-His vector does not contain a resistance marker for selection in *Drosophila* cells. We recommend that you include the pMT/BiP/V5-His/GFP positive control vector and a mock transfection (negative control) in your experiments to evaluate your results. Specific guidelines and protocols for transient transfection and generation of stable cell lines can be found in the DES<sup>®</sup> manual.

**Note:** Either pCoHygro or pCoBlast is supplied with the appropriate DES<sup>®</sup> Inducible/Secreted Kit. For detailed information about each vector, refer to the DES<sup>®</sup> manual.

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## Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be clean and free from phenol and sodium chloride. Contaminants will kill the cells, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.<sup>™</sup> MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.<sup>™</sup> MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

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## Positive Control

pMT/BiP/V5-His/GFP is provided as a positive control vector for *Drosophila* cell transfection and expression (see page 13 for a map) and may be used to optimize transfection conditions for S2 cells. Transfection of pMT/BiP/V5-His/GFP results in induction of GFP expression upon addition of copper sulfate. A successful transfection will result in GFP expression that can be easily assayed (see the next page). **Note:** The GFP gene in pMT/BiP/V5-His/GFP is a mutated form of GFP and is known as cycle 3-GFP (Cramer *et al.*, 1996) (see below).

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## GFP Gene Used in pMT/BiP/V5-His/GFP

The GFP gene used in pMT/BiP/V5-His is described in Cramer *et al.*, 1996. The codon usage was optimized for expression in *E. coli* and three cycles of DNA shuffling were used to generate a collection of mutants. The GFP mutant that exhibited the greatest fluorescence in mammalian cells is used in pMT/BiP/V5-His/GFP and is known as cycle 3-GFP. Cycle 3-GFP has the following characteristics:

- Excitation and emission maxima that are the same as wild-type GFP (395 nm and 478 nm for primary and secondary excitation, respectively, and 507 nm for emission)
  - High solubility in *E. coli* for visual detection of transformed cells (if expressed from a promoter recognized by *E. coli*)
  - >40-fold increase in fluorescent yield over wild-type GFP
- 

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## Transfection and Analysis, continued

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### Assay for Cycle 3-GFP

You may assay for GFP expression in the following ways:

- Use fluorescence microscopy to visualize GFP-expressing cells  
To detect fluorescent cells, it is important to pick the best filter set to optimize detection. The primary excitation peak of cycle 3-GFP is at 395 nm. There is a secondary excitation peak at 478 nm. Excitation at these wavelengths yield a fluorescent emission peak with a maximum at 507 nm.
- Use fluorescence spectroscopy to assay the medium  
You can detect cycle 3-GFP fluorescence in the medium using fluorescence spectroscopy. Be sure to run a mock sample (medium alone) as the Schneider's *Drosophila* Medium has some autofluorescence (Zylka and Schnapp, 1996) and will interfere with detection of cycle 3-GFP fluorescence.
- Use western blot analysis to assay for GFP protein  
GFP Antiserum is available from Invitrogen (Catalog no. R970-01).

After transfection, allow the cells to recover for 24 to 48 hours before inducing expression of cycle 3-GFP with copper sulfate. Induce for ~20 hours before assaying for fluorescence.

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### Induction of Recombinant Protein Expression

Once you have transfected your pMT/BiP/V5-His construct into S2 cells, you will induce expression of recombinant protein using copper sulfate. In general, we recommend that you add copper sulfate directly to the culture medium to a final concentration of 500  $\mu$ M and incubate the cells for 24 hours to obtain maximal induction of your protein of interest. Refer to the DES<sup>®</sup> manual for more details. Copper sulfate is provided in each DES<sup>®</sup> Inducible/Secreted Kit.

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### Detection and Purification of Recombinant Fusion Proteins

If you have cloned your gene of interest in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use the Anti-V5 antibodies or Anti-His(C-term) antibodies available from Invitrogen to detect secreted expression of your recombinant fusion protein by western blot analysis. The 6xHis tag also allows purification of recombinant protein using metal-chelating resins including ProBond<sup>™</sup>. Refer to the DES<sup>®</sup> manual for more detailed guidelines and instructions to detect and purify your recombinant fusion protein.

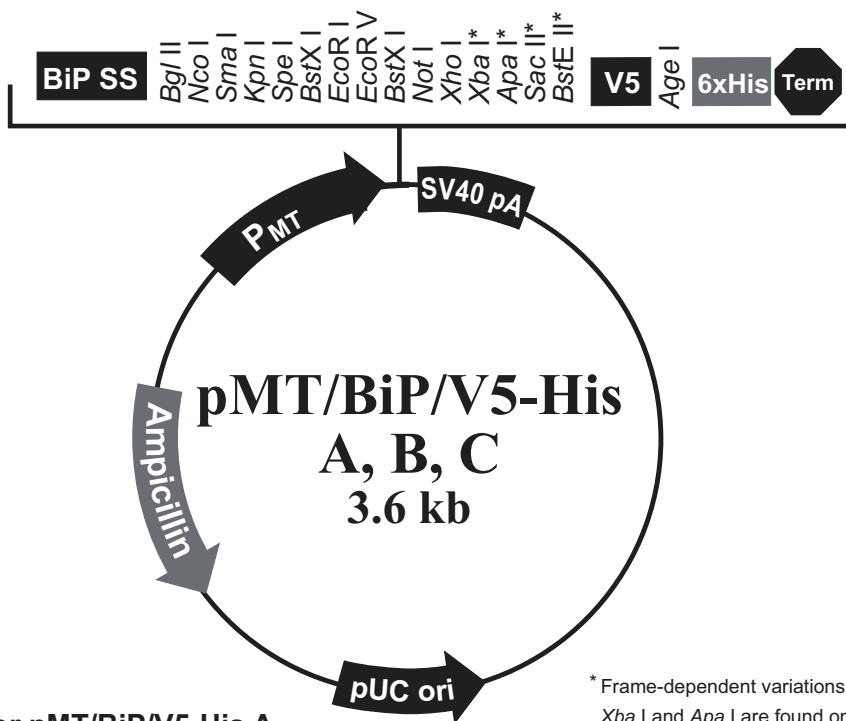
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## Appendix

### pMT/BiP/V5-His Vector

#### Map of pMT/BiP/V5-His

The figure below summarizes the features of the pMT/BiP/V5-His A, B, and C vectors. For a more detailed description of each feature, please see the next page. The complete sequences of pMT/BiP/V5-His A, B, and C are available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or from Technical Service (see page 14).



#### Comments for pMT/BiP/V5-His A 3642 nucleotides

Metallothionein promoter: bases 412-778  
 Start of transcription: base 778  
 MT Forward priming site: bases 814-831  
 BiP signal sequence: bases 851-904  
 Multiple cloning site: bases 906-999  
 V5 epitope tag: bases 1003-1044  
 Polyhistidine region: bases 1054-1074  
 BGH Reverse priming site: bases 1094-1111  
 SV40 late polyadenylation signal: bases 1267-1272  
 pUC origin: bases 1765-2438 (complementary strand)  
*bla* promoter: bases 3444-3542 (complementary strand)  
 Ampicillin (*bla*) resistance gene ORF: bases 2583-3443 (complementary strand)

\* Frame-dependent variations

*Xba* I and *Apa* I are found only in versions A and B.

*Sac* II is found only in version B.

*BstE* II is found only in version C.

*continued on next page*

## pMT/BiP/V5-His Vector, continued

### Features of pMT/BiP/V5-His

The features of pMT/BiP/V5-His A (3642 bp), pMT/BiP/V5-His B (3646 bp), and pMT/BiP/V5-His C (3638 bp) are described below. All features have been functionally tested. The multiple cloning site has been tested by restriction enzyme analysis.

Feature	Benefit
<i>Drosophila</i> metallothionein (MT) promoter	Permits high-level, inducible expression of heterologous proteins (Bunch <i>et al.</i> , 1988; Maroni <i>et al.</i> , 1986)
MT Forward priming site	Allows sequencing in the sense orientation
<i>Drosophila</i> BiP secretion signal	Permits secreted expression of the gene of interest (Kirkpatrick <i>et al.</i> , 1995)
Multiple cloning site	Allows insertion of your gene of interest
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of your recombinant protein with the Anti-V5 Antibodies available from Invitrogen (Southern <i>et al.</i> , 1991)
Polyhistidine (6xHis) tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™.  In addition, the C-terminal 6xHis tag is the epitope for the Anti-His(C-term) Antibodies available from Invitrogen (Lindner <i>et al.</i> , 1997)
BGH Reverse priming site	Permits sequencing of the non-coding strand
SV40 late polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Angelichio <i>et al.</i> , 1991)
pUC origin	Allows high-copy number replication and growth in <i>E. coli</i>
<i>bla</i> promoter	Allows expression of the ampicillin ( <i>bla</i> ) resistance gene
Ampicillin ( <i>bla</i> ) resistance gene (β-lactamase)	Permits selection of transformants in <i>E. coli</i>

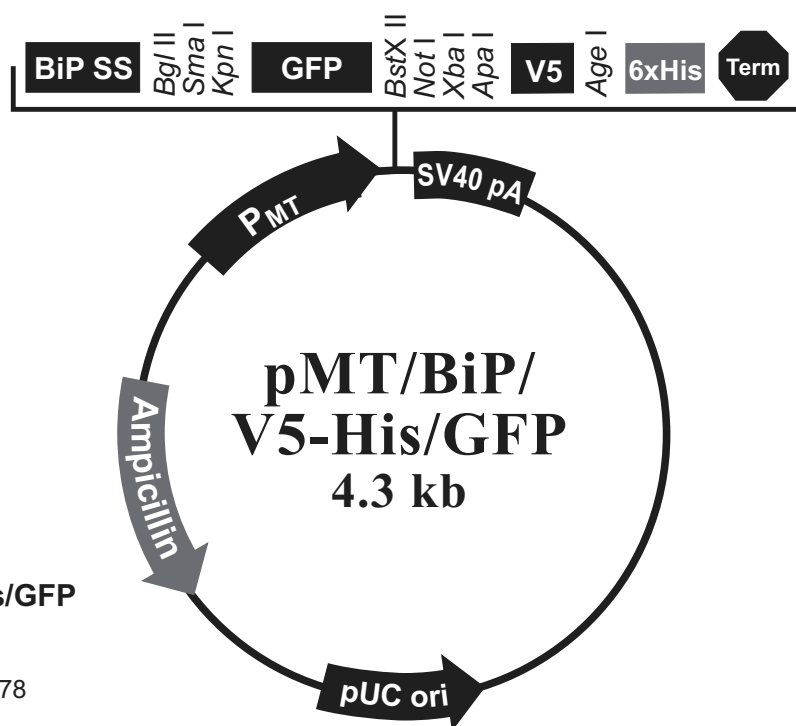
## pMT/BiP/V5-His/GFP Vector

### Description

pMT/BiP/V5-His/GFP is a 4327 bp control vector expressing Green Fluorescent Protein (GFP). The plasmid was constructed by digesting pMT/BiP/V5-His A with *Kpn* I and *EcoR* V and ligating a 721 bp *Kpn* I-*Sac* I (Klenow) fragment containing the cycle 3-GFP gene in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine tag.

### Map of pMT/BiP/V5-His/GFP

The figure below summarizes the features of the pMT/BiP/V5-His/GFP vector. **The complete nucleotide sequence for pMT/BiP/V5-His/GFP is available for downloading from our World Wide Web site ([www.invitrogen.com](http://www.invitrogen.com)) or by contacting Technical Service (see page 14).**



### Comments for pMT/BiP/V5-His/GFP

4327 nucleotides

Metallothionein promoter: bases 412-778

Start of transcription: base 778

MT Forward priming site: bases 814-831

BiP signal sequence: bases 851-904

GFP portion of fusion: bases 938-1687

V5 epitope tag: bases 1688-1729

Polyhistidine region: bases 1739-1759

BGH Reverse priming site: bases 1779-1796

SV40 late polyadenylation signal: bases 1952-1957

pUC origin: bases 2450-3123 (complementary strand)

*b/a* promoter: bases 4129-4227 (complementary strand)

Ampicillin (*b/a*) resistance gene ORF: bases 3268-4128 (complementary strand)

# Technical Service

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## 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):

**<http://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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## Contact us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page ([www.invitrogen.com](http://www.invitrogen.com)).

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## MSDS Requests

To request an MSDS, please visit our web site ([www.invitrogen.com](http://www.invitrogen.com)) and follow the instructions below.

1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
  2. Follow instructions on the page and fill out all the required fields.
  3. To request additional MSDSs, click the 'Add Another' button.
  4. All requests will be faxed unless another method is selected.
  5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.
- 

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## Technical Service, continued

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### Emergency Information

In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company  
Voice: 1-760-602-8700

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