invitrogen™

pcDNA™3.1/*myc*-His A, B, and C

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User Manual

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Kit Contents and Storage

Shipping and Storage

pcDNA™3.1/*myc*-His vectors are shipped on wet ice. Upon receipt, store vectors at –20°C.

Kit Contents

All vectors are supplied in aliquot detailed below. **Store the vectors at –20°C.**

Vector	Composition	Amount
pcDNA™3.1/*myc*-His A, B, and C	40 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg
pcDNA™3.1/*myc*-His/*lacZ*	40 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg

Introduction

Overview

Description of the System

pcDNA™3.1/*myc*-His A, B, and C are 5.5-kb vectors derived from pcDNA™3.1(+) and designed for high-level expression, purification, and detection of recombinant proteins in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Three reading frames to facilitate in frame cloning with a C-terminal tag encoding the *myc* (*c-myc*) epitope and a polyhistidine metal-binding peptide
- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (*e.g.*, COS7)

The control plasmid, pcDNA™3.1/*myc*-His/*lac*Z, is the pcDNA™3.1/*myc*-His C vector with a 3.2-kb fragment containing the β-galactosidase gene cloned in frame with the C-terminal peptide (see page 9). It is included for use as a positive control for transfection, expression, purification, and detection in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™3.1/*myc*-His:

- Consult the multiple cloning sites described on pages 3-4 to determine which vector (A, B, or C) should be used to clone your gene in frame with the C-terminal *myc* epitope and the polyhistidine tag.
- Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50–100 µg/ml ampicillin.
- Analyze your transformants for the presence of insert by restriction digestion.
- Select a transformant with the correct restriction pattern and confirm that your gene is in frame with the C-terminal peptide by sequencing.
- Transfect your construct into the cell line of choice using your own method of transfection.
- Test for expression of your recombinant gene by western blot analysis or functional assay. If you do not have an antibody to your protein, Invitrogen offers the Anti-*myc* antibody or the Anti-His(C-term) antibody to detect your recombinant protein (see page 10).
- To purify your recombinant protein, you may use a metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 10).

Methods

Cloning into pcDNA™3.1/*myc*-His A, B, and C

General Molecular Biology Techniques

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

E. coli Strain

Many *E. coli* strains are suitable for the propagation of pcDNA™3.1/*myc*-His vectors, including TOP10, TOP10F′, DH5™-T1^R. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombinant deficient (*rec*A) and endonuclease A-deficient (*end*A).

For your convenience, TOP10F′ is available as chemically competent or electrocompetent cells from Invitrogen (see page 10).

Maintenance of pcDNA™3.1/*myc*-His

To propagate and maintain the pcDNA™3.1/*myc*-His vectors, use the supplied 0.5 µg/µl stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10, TOP10F′, DH5α, JM109, or equivalent. Select transformants on LB plates containing 50–100 µg/ml ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage.

Important

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

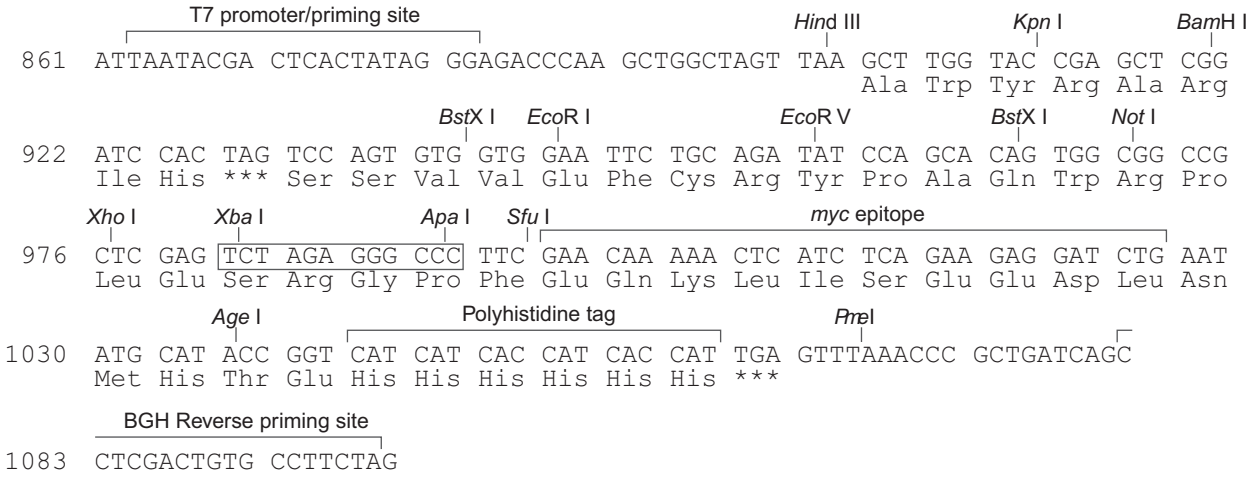
(G/A)NNATG**G**

Continued on next page

Cloning into pcDNA™3.1/*myc*-His A, B, and C, Continued

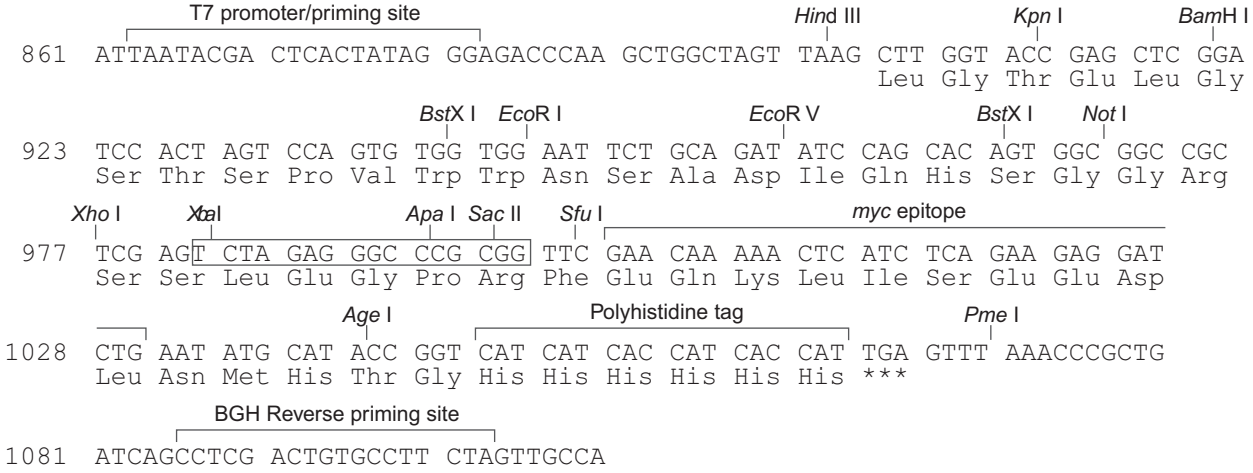
Multiple Cloning Site of Version A

Below is the multiple cloning site for pcDNA™3.1/*myc*-His A. Restriction sites are labeled to indicate the cleavage site. **Note that there is a stop codon between the *Bam*H I site and the *Bst*X I site.** The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™3.1/*myc*-His A is available for downloading from our website at www.invitrogen.com or from **Technical Support** (see page 11).



Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA™3.1/*myc*-His B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™3.1/*myc*-His B is available for downloading from our website at www.invitrogen.com or from **Technical Support** (see page 11).



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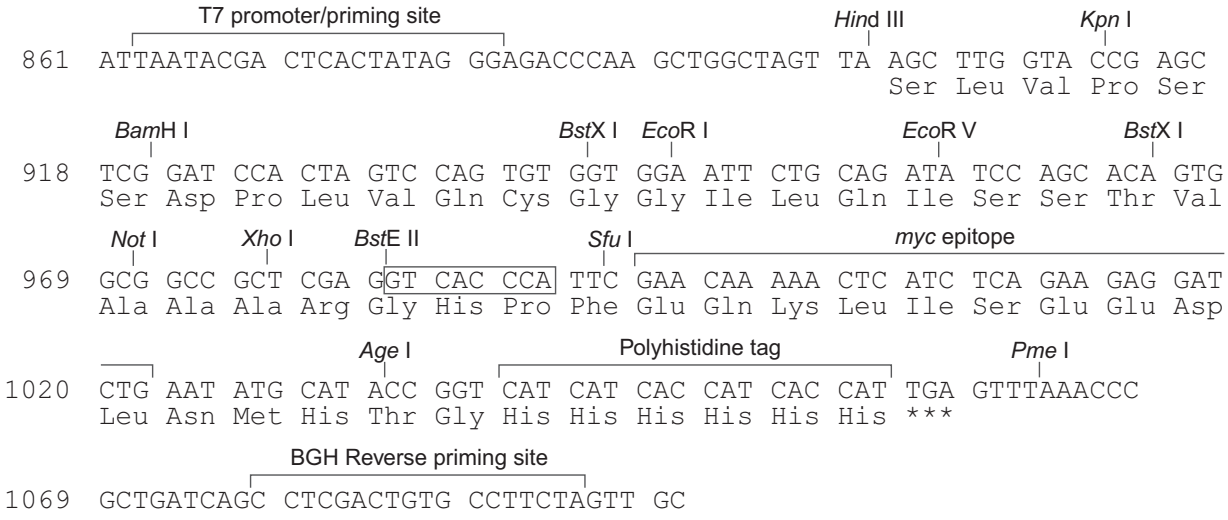
Cloning into pcDNA™3.1/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA™3.1/*myc*-His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pcDNA™3.1/*myc*-His C is available for downloading from our website at www.invitrogen.com or from **Technical Support** (see page 11).



Transformation and Transfection

Introduction

If you need more details about the techniques discussed, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al*., 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al*., 1994).

Method of Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (*e.g.* TOP10, TOP10F´, 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. For your convenience, TOP10F′ is available as chemically competent or electrocompetent cells from Invitrogen (see page 10).

We recommend that you sequence your construct to confirm that your gene is fused in frame with the *myc* epitope and the C-terminal polyhistidine tag. We suggest using the T7 Promoter and BGH Reverse primer sequences. Refer to the diagrams on pages 3–4 for the sequence and location of the primer binding sites.

For your convenience, Invitrogen offers a custom primer synthesis service. For more information, visit www.invitrogen.com or contact **Technical Support** (page 11).

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HiPure Miniprep Kit or the PureLink™ HiPure Midiprep Kit (see page 10 for ordering information).

Methods of Transfection

For established cell lines (*e.g.*, HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology*.

Methods of transfection include calcium phosphate (Chen & Okayama, 1987; Wigler *et al*., 1977), lipid-mediated (Felgner *et al*., 1989; Felgner & Ringold, 1989) and electroporation (Chu *et al*., 1987; Shigekawa & Dower, 1988). For high efficiency transfection in a broad range of mammalian cells, we recommend using Lipofectamine™ 2000 Reagent available from Invitrogen. For more information on Lipofectamine™ 2000 and other transfection reagents available, visit our website at www.invitrogen.com or contact **Technical Support** (page 11).

Positive Control

pcDNA™3.1/*myc*-His/*lac*Z is provided as a positive control vector for mammalian transfection and expression (see page 9). It may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the CMV promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see below).

Detection of Fusion Proteins

A number of antibodies are available from Invitrogen (see page 10) that can be used to detect expression of your fusion protein from pcDNA™3.1/*myc*-His.

Continued on next page

Assay for β-galactosidase Activity

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (*e.g.*, TOP10, TOP10F´, 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.

Geneticin® Selection Guidelines

Geneticin® is available separately from Invitrogen. Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982). Use as follows:

- Prepare Geneticin® in a buffered solution (*e.g.*, 100 mM HEPES, pH 7.3).
- Use 100–1000 µg/ml of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check lot label).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take from 3 to 6 weeks of growth in selective medium.

Preparing Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purification of your protein on ProBond™. You will need 5×10^6 to 1×10^7 cells for purification of your protein on a 2-ml ProBond™ column (see the ProBond™ Purification manual).

1. Seed cells in five T-75 flasks or two to three T-175 flasks.
2. Grow the cells in selective medium until they are 80–90% confluent.
3. Harvest the cells by treating with trypsin-EDTA for 2–5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 1,500 rpm for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at –70°C until needed.

Lysis of Cells

If you are using ProBond™ resin, refer to the ProBond™ Purification manual for details about sample preparation for chromatography. If you are using another resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Note

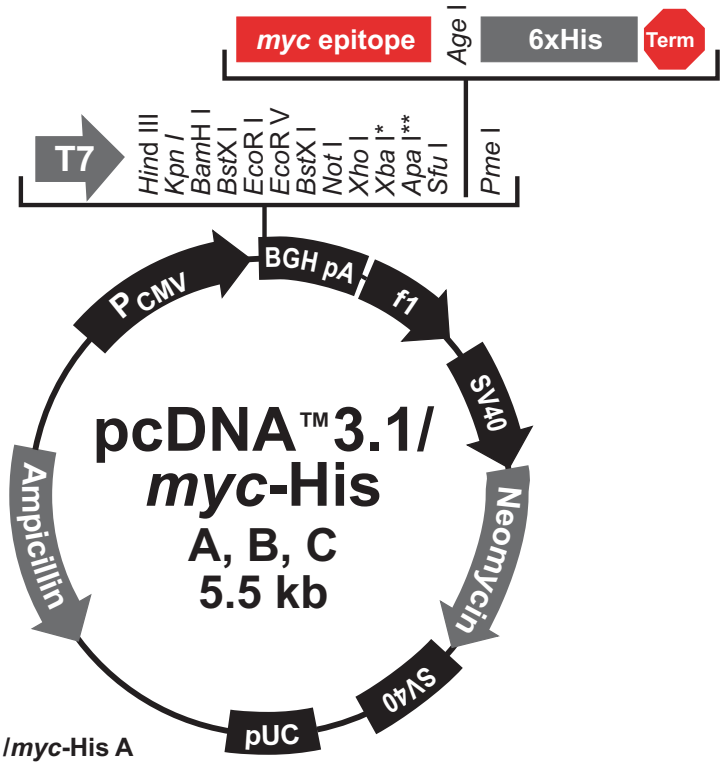
The C-terminal peptide containing the *myc* epitope and polyhistidine tag will add approximately 3 kDa to the size of your protein. The size of the lacZ/*myc*-His fusion protein is approximately 121 kDa.

Appendix

pcDNA™3.1/*myc*-His A, B, and C

Map of pcDNA™3.1/*myc*-His

The figure below summarizes the features of the pcDNA™3.1/*myc*-His vectors. The nucleotide sequence for pcDNA™3.1/*myc*-His A is available for downloading from www.invitrogen.com or from **Technical Support** (page 11). Details of the multiple cloning sites for pcDNA™3.1/*myc*-His A, B, and C are shown on pages 3–4.



Comments for pcDNA™3.1/*myc*-His A
5493 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 902-999
myc epitope: bases 997-1026
Polyhistidine tag: bases 1042-1059
BGH reverse priming site: bases 1082-1099
BGH polyadenylation signal: bases 1081-1295
f1 origin of replication: bases 1358-1771
SV40 promoter and origin: bases 1836-2160
Neomycin resistance gene: bases 2196-2990
SV40 polyadenylation signal: bases 3166-3296
pUC origin: bases 3679-4352
Ampicillin resistance gene: bases 4497-5357 (complementary strand)

* There is a unique *Bst*E II site, but no *Xba* I or *Apa* I sites in version C.

** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.

Features of pcDNA™3.1/*myc*-His

pcDNA™3.1/*myc*-His A (5,493 bp), pcDNA™3.1/*myc*-His B (5,497 bp), and pcDNA™3.1/*myc*-His C (5,489 bp) contain the following elements. All features have been functionally tested.

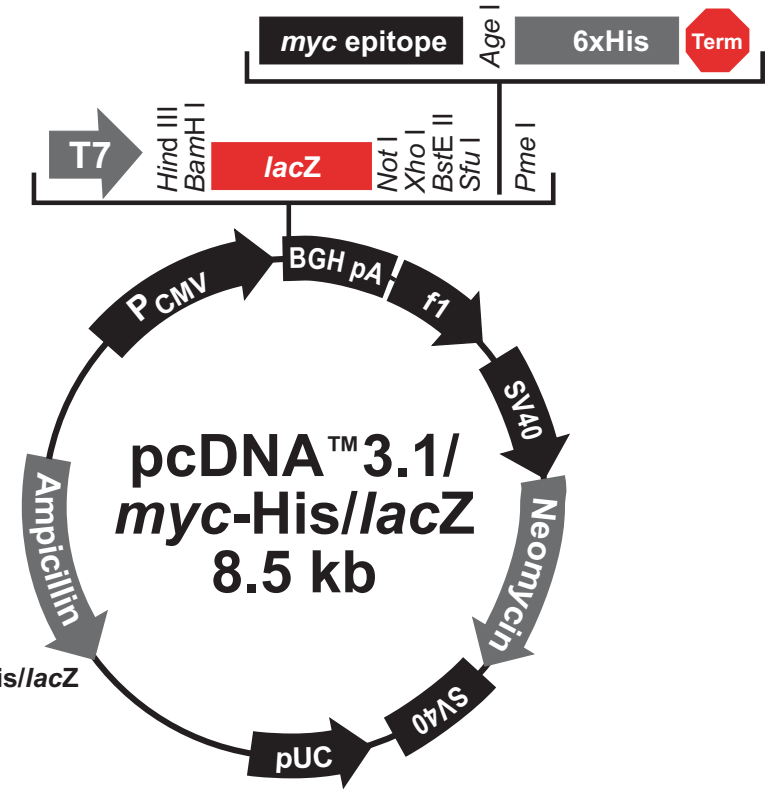
Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Allows efficient, high-level expression of your recombinant protein (Andersson *et al*., 1989; Boshart *et al*., 1985; Nelson *et al*., 1987).
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the *myc* epitope and polyhistidine C-terminal tag.
myc epitope (c-*myc*) (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of your recombinant protein with the Anti-*myc* Antibody or Anti-*myc*-HRP Antibody (Evan *et al*., 1985).
C-terminal polyhistidine tag	Allows purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His (C-term) Antibody and the Anti-His (C-term)-HRP Antibody.
BGH reverse priming site	Allows sequencing through the insert.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992).
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing the SV40 large T antigen.
Neomycin (Geneticin®) resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982).
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
pUC origin	High-copy number replication and growth in *E. coli.*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli.*

pcDNA™3.1/*myc*-His/lacZ

Map of Control Vector

pcDNA™3.1/*myc*-His/*lac*Z is a 8,540-bp control vector containing the gene for β-galactosidase. pcDNA™3.1/*myc*-His C was digested with *Eco*R V and *Not* I. A 3.2-kb blunt-*Not* I fragment containing the β-galactosidase gene was then ligated into pcDNA™3.1/*myc*-His C in frame with the C-terminal peptide.

The figure below summarizes the features of the pcDNA™3.1/*myc*-His/*lac*Z vector. The nucleotide sequence for pcDNA™3.1/*myc*-His/*lac*Z is available by downloading it from our website (www.invitrogen.com) or by contacting **Technical Support** (page 11).



Comments for pcDNA™3.1/*myc*-His/*lac*Z
8540 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
*lac*Z with C-terminal tag: 963-4106
  *lac*Z ORF: bases 963-4019
  myc epitope: bases 4044-4073
  Polyhistidine tag: bases 4089-4106
BGH reverse priming site: bases 4129-4146
BGH polyadenylation signal: bases 4128-4342
f1 origin of replication: bases 4405-4818
SV40 promoter and origin: bases 4883-5207
Neomycin resistance gene: bases 5243-6037
SV40 polyadenylation signal: bases 6213-63473
pUC origin: bases 67267-7399
Ampicillin resistance gene: bases 7544-8404 (complementary strand)

Accessory Products

Introduction

The following additional products may be used with the pcDNA™3.1/*myc*-His vectors. For more information, www.invitrogen.com or contact **Technical Support** (see page 11).

Product	Amount	Catalog no.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Resin	50 ml	R801-01
	150 ml	R801-15
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Electrocomp™ TOP10F′	2 × 20 rxns 6 × 20 rxns	C665-11 C665-24
One Shot™ TOP10F′ (chemically competent cells)	21 × 50 µl	C3030-03

Detection of Fusion Proteins

A number of antibodies are available from Invitrogen that can be used to detect expression of your fusion protein from pcDNA™3.1/*myc*-His. The table below describes the antibodies available and ordering information. The amount supplied is sufficient for 25 Westerns.

Product	Purpose	Catalog no.
Anti-*myc*	Detects 10 amino acid epitope derived from c-*myc*	R950-25
Anti-*myc*-HRP	See above. Provided as an HRP conjugate for time-saving detection.	R951-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection)	R930-25
Anti-His(C-term)-HRP	See above. Provided as an HRP conjugate for time-saving detection.	R931-25

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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