pcDNA™3.1/*myc*-His(-) A, B, and C

Catalog no. V855-20

Version G
October 28, 2010
28-0128

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Important Information

Contents

20 µg each pcDNA™3.1/*myc*-His(-) A, B, and C, lyophilized
20 µg pcDNA™3.1/*myc*-His(-)/*lacZ*, lyophilized

Shipping/Storage

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

Product Qualification

The pcDNA™3.1/*myc*-His(-) and pcDNA™3.1/*myc*-His(-)/*lacZ* 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)
pcDNA™3.1/*myc*-His(-) A	*Apa* I	92, 5430
	*Sna*B I	5522
	Xba I	5522
pcDNA™3.1/*myc*-His(-) B	*Apa* I	5520
	*Sna*B I	5520
	Xba I	86, 5434
pcDNA™3.1/*myc*-His(-) C	*Apa* I	5521
	*Sna*B I	412, 5109
	Xba I	5521
pcDNA™3.1/*myc*-His(-)/*lacZ*	*Nhe* I	8592
	Xba I	8592
	Xba I + *Eco*R V	1151, 7441

Overview

Introduction

pcDNA™3.1/*myc*-His(-) A, B, and C are 5.5 kb vectors designed for overproduction of recombinant proteins in mammalian cell lines. The vectors are supplied in three reading frames to facilitate in frame cloning, with a C-terminal peptide containing a polyhistidine metal-binding tag and the *myc* (*c-myc*) epitope. The human cytomegalovirus immediate-early (CMV) promoter provides high-level expression in a wide range of mammalian cells. In addition, the vector will replicate episomally in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS7). High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The control plasmid, pcDNA™3.1/*myc*-His(-)/*lac*Z, is the pcDNA™3.1/*myc*-His(-) A vector with a 3.2 kb fragment containing the β-galactosidase gene cloned in frame with the C-terminal peptide (see page 10). It is included for use as a positive control for transfection, expression, 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 5-7 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 to 100 µg/ml ampicillin.
- Analyze your transformants for the presence of insert by restriction digestion.
- Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in frame with the C-terminal peptide.
- Transfect your construct into the cell line of choice using your own method of transfection. Generate a stable cell line, if desired.
- Test for expression of your recombinant gene by western blot analysis or functional assay. If you do not have an antibody to your protein, you may use the Anti-*myc* Antibody (Catalog no. R950-25) or the Anti-Hi (C-term) Antibody (Catalog no. R930-25) to detect your recombinant protein.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see below for ordering information).

ProBond™ Resin

Ordering information for ProBond™ resin is provided below.

Item	Amount	Catalog no.
ProBond™ Purification System	6 x 2 ml precharged ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Purification System with Anti-*myc*-HRP Antibody	1 Kit	K852-01
ProBond™	50 ml	R801-01
	150 ml	R801-15

pcDNA™3.1/*myc*-His(-) A, B, and C Vectors

Features of pcDNA™3.1/*myc*-His(-)

pcDNA™3.1/*myc*-His(-) A (5522 bp), pcDNA™3.1/*myc*-His(-) B (5520 bp), and pcDNA™3.1/*myc*-His(-) C (5521 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 C-terminal polyhistidine tag
myc epitope (*c-myc*)	Allows detection of your recombinant protein with the Anti-*myc* Antibody (Catalog no. R950-25) or Anti-*myc*-HRP Antibody (Catalog no. R951-25) (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 (Catalog no. R930-25) and the Anti-His (C-term)-HRP Antibody (Catalog no. R931-25)
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 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*

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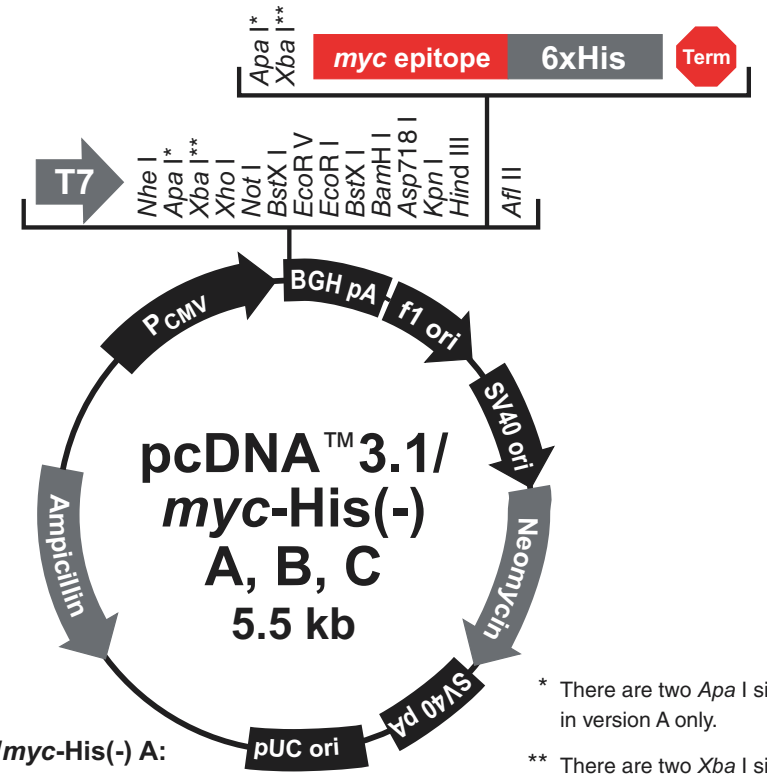
pcDNA™3.1/*myc*-His(-) A, B, and C Vectors, continued

Map of pcDNA™3.1/*myc*-His(-)

The figure below summarizes the features of the pcDNA™3.1/*myc*-His(-) vectors. **The sequences for pcDNA™3.1/*myc*-His(-) A, B, and C are available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 11).** Details of the multiple cloning sites for pcDNA™3.1/*myc*-His(-) A, B, and C are shown on pages 5-7.



* There are two *Apa* I sites in version A only.

** There are two *Xba* I sites in version B only.

Comments for pcDNA™3.1/*myc*-His(-) A: 5522 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1006
myc epitope: bases 1007-1036
Polyhistidine tag: bases 1052-1069
BGH reverse priming site: bases 1113-1130
BGH polyadenylation signal: bases 1116-1343
f1 origin: bases 1389-1817
SV40 promoter and origin: bases 1844-2152
Neomycin resistance gene: bases 2227-3021
SV40 polyadenylation signal: bases 3195-3325
pUC origin: bases 3708-4381
Ampicillin resistance gene: bases 4526-5386 (complementary strand)

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 growth of this vector including TOP10F´. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A-deficient (*end*A).

For your convenience, TOP10F´ is available as chemically competent or electrocompetent cells from Invitrogen.

Item	Quantity	Catalog no.
Electrocomp™ TOP10F´	5 x 80 µl	C665-55
One Shot® TOP10F´ (chemically competent cells)	21 x 50 µl	C3030-03

Maintenance of pcDNA™3.1/*myc*-His(-)

To propagate and maintain the pcDNA™3.1/*myc*-His(-) vectors, we recommend resuspending each vector in 20 µl sterile water to make a 1 µg/µl stock solution. Store the stock solution at -20°C.

Use this stock solution 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.

Cloning Considerations

Your insert should contain a Kozak translation initiation sequence for proper initiation of translation (Kozak, 1987; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Note that other sequences are possible, but the G or A at position -3 and the G at position +4 are the most critical nucleotides for function (shown in bold). The ATG initiation codon is shown underlined.

(G/A)NN<u>ATG</u>**G**

To express your gene as a recombinant fusion protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. See pages 5-7 to develop a cloning strategy.

If you wish to express your protein **without** the C-terminal peptide, be sure to include a stop codon.

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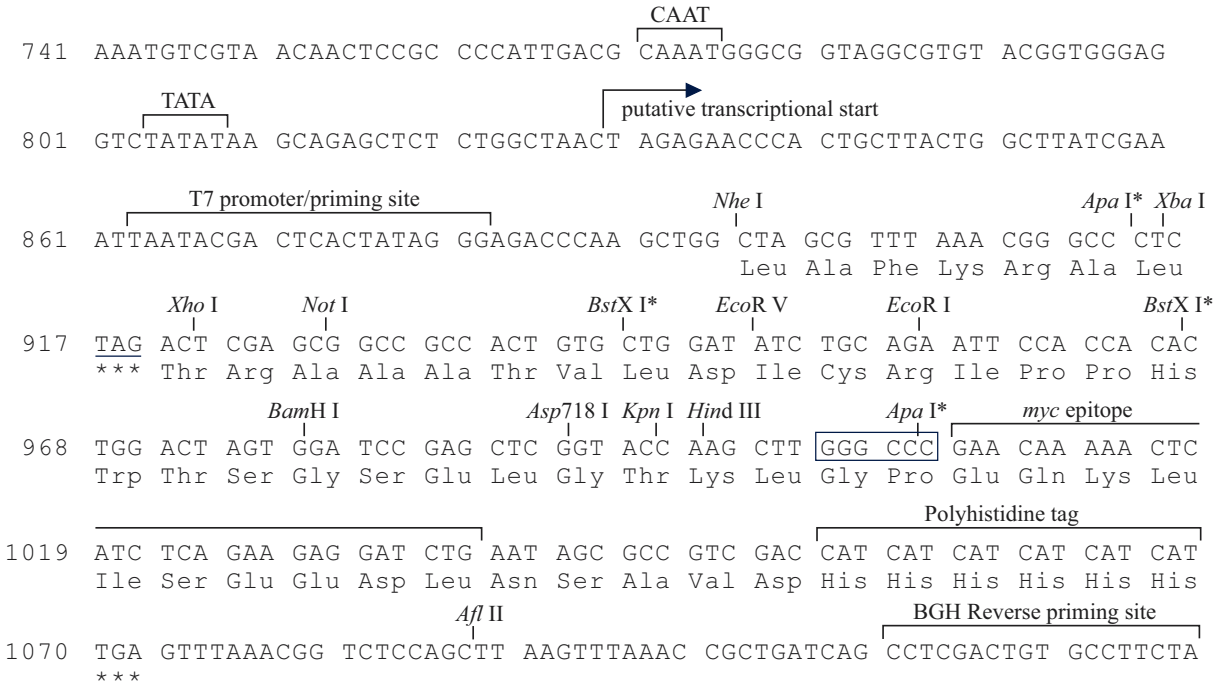
Cloning into pcDNA™3.1/*myc*-His(-) A, B, and C, continued

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. The boxed nucleotides indicate the variable region. **Note that there is a stop codon after the *Xba* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™3.1/*myc*-His(-) A is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 11).**



*Please note that there are two *Bst*X I sites and two *Apa* I sites in the polylinker.

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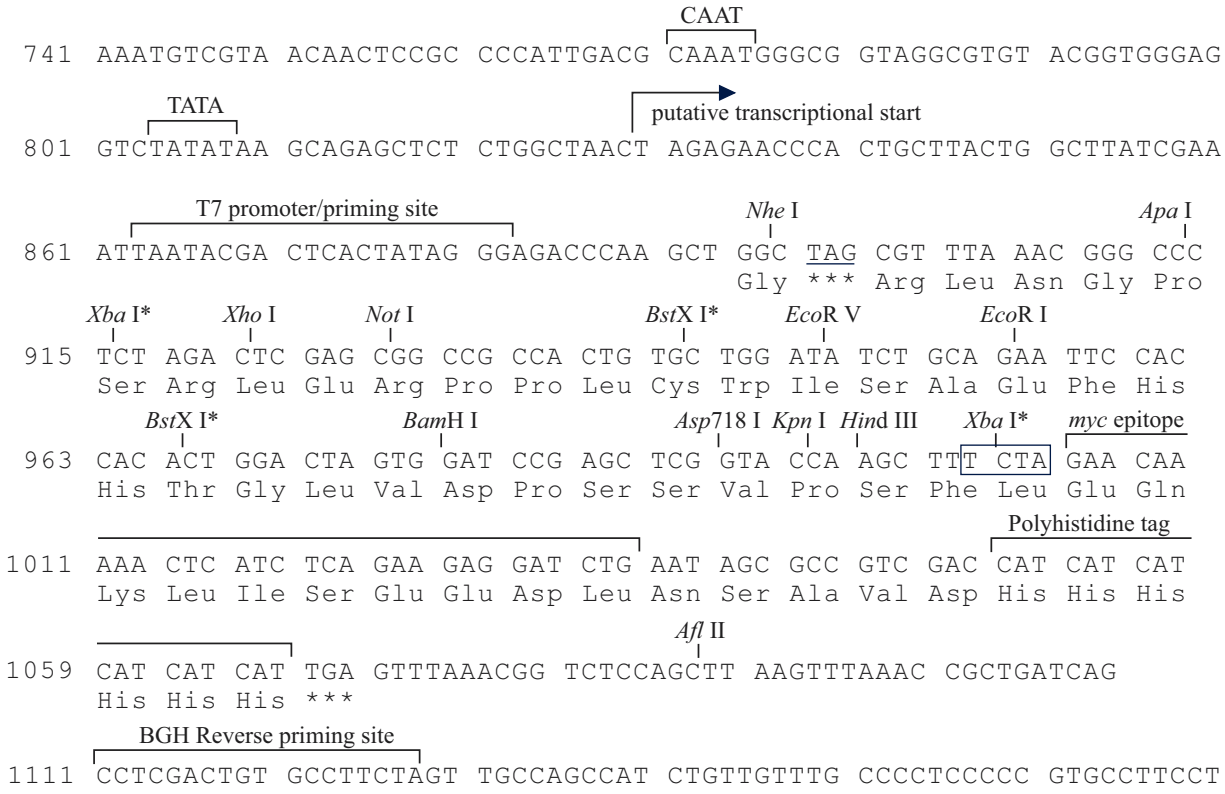
Cloning into pcDNA™3.1/*myc*-His(-) A, B, and C, continued

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. **Note that there is a stop codon after the *Nhe* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™3.1/*myc*-His(-) B is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 11).**



*Please note that there are two *Bst*X I sites and two *Xba* I sites in the polylinker.

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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. **Note that there are two stop codons. One is located between the *Nhe* I site and the *Apa* I site, and the other is located before the *Bam*H I site.** This means the 3´ cloning site must be either *Bam*H I, *Kpn* I (*Asp*718 I), or *Hin*d III if you wish to clone your gene into pcDNA™3.1/*myc*-His(-) C so that it is expressed without interruption. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™3.1/*myc*-His(-) C is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 11).**

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                                                CAAT
741  AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG

          TATA                                  putative transcriptional start
801  GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG GCTTATCGAA

          T7 promoter/priming site                        Nhe I                          Apa I  Xba I
861  ATTAATACGA CTCACTATAG GGAGACCCAA GCTG GCT AGC GTT TAA ACG GGC CCT
                                        Ala Ser Val *** Thr Gly Pro
               Xho I      Not I                BstX I*    EcoR V       EcoR I
916  CTA GAC TCG AGC GGC CGC CAC TGT GCT GGA TAT CTG CAG AAT TCC ACC
     Leu Asp Ser Ser Gly Arg His Cys Ala Gly Tyr Leu Gln Asn Ser Thr
       BstX I*          BamH I              Asp718 I  Kpn I Hind III
964  ACA CTG GAC TAG TGG ATC CGA GCT CGG TAC CAA GCT TAC GTA GAA CAA
     Thr Leu Asp *** Trp Ile Arg Ala Arg Tyr Gln Ala Tyr Val Glu Gln
          myc epitope                                          Polyhistidine tag
1012 AAA CTC ATC TCA GAA GAG GAT CTG AAT AGC GCC GTC GAC CAT CAT CAT
     Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp His His His
                                          Afl II
1060 CAT CAT CAT TGA GTTTAAACG GTCTCCAGCT TAAGTTTAAA CCGCTGATCA
     His His His ***
          BGH Reverse priming site
1111 GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC
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*Please note that there are two BstX I sites in the polylinker.

General Guidelines and Special Information

Introduction

The following guidelines and recommendations are provided for your convenience. If you need more details about the techniques discussed, refer to the general molecular biology references in the **Reference** section.

E. coli 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.

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 5-7 for the sequence and location of the primer binding sites.

For your convenience, Invitrogen offers a custom primer synthesis service. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (see 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 DNA using the S.N.A.P.™ Miniprep Kit (10-15 µg, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg, Catalog no. K1910-01), or CsCl gradient centrifugation.

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 for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). Invitrogen offers the Calcium Phosphate Transfection Kit and a large selection of reagents for transfection. For more information on the reagents available, visit our Web site (www.invitrogen.com) or call Technical Service (see 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 10) and 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 next page).

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General Guidelines and Special Information, continued

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit (Catalog no. K1455-01) and the β-Gal Staining Kit (Catalog no. K1465-01) for fast, easy detection of β-galactosidase expression.

Geneticin® Selective Antibiotic

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).

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (Catalog no. 10131-035). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100 to 1000 μg/ml of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the 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.

Preparation of Cells for Purification

Use the procedure below to purify recombinant protein from a stable cell line. You will need 5 x 10^6 to 1 x 10^7 cells for purification on a 2 ml ProBond™ column (see ProBond™ Purification System manual).

1. Seed cells in either five T-75 flasks or 2 to 3 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 to 5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting in fresh medium (if necessary), and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 1500 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 System manual for details about sample preparation for chromatography.

If you are using other resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Note

The C-terminal peptide containing the *myc* epitope and the 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.

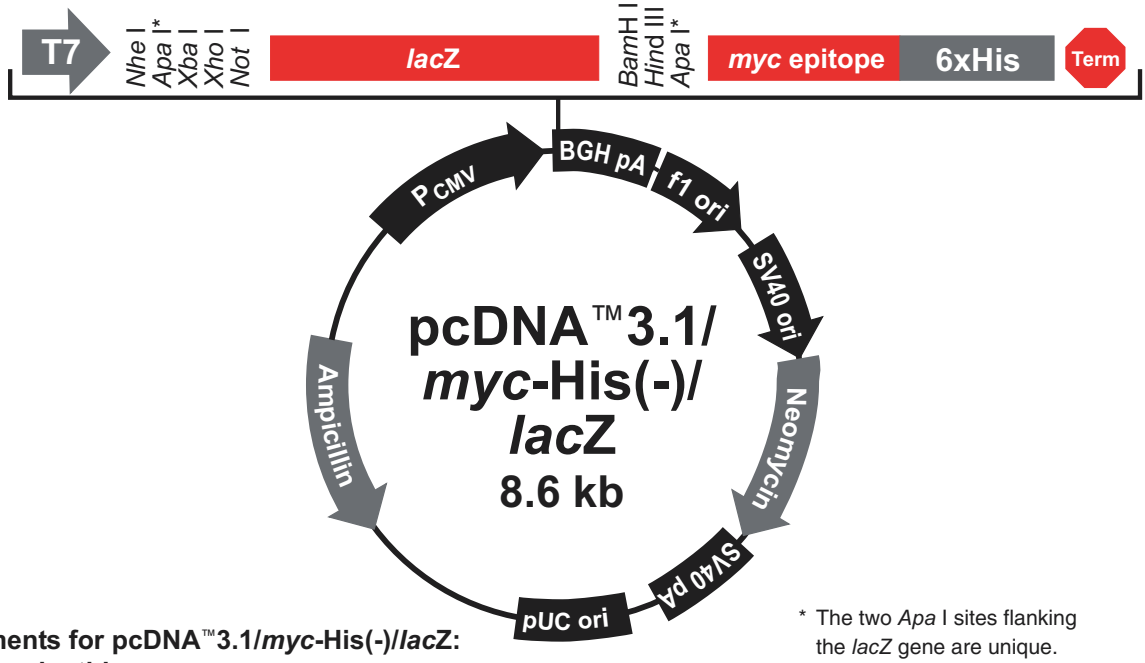
pcDNA™3.1/*myc*-His(-)/*lacZ*

Description

pcDNA™3.1/*myc*-His(-)/*lacZ* is a 8592 bp control vector containing the gene for β-galactosidase. pcDNA™3.1/*myc*-His(-) A was digested with *Eco*R V. A 3.2 kb blunt *Sfu* I-*Not* I fragment containing the β-galactosidase gene was then ligated into pcDNA™3.1/*myc*-His(-) A in frame with the C-terminal peptide.

Map of Control Vector

The figure below summarizes the features of the pcDNA™3.1/*myc*-His(-)/*lacZ* vector. **The complete nucleotide sequence for pcDNA™3.1/*myc*-His(-)/*lacZ* is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see next page).**



* The two *Apa* I sites flanking the *lacZ* gene are unique.

Comments for pcDNA™3.1/*myc*-His(-)/*lacZ*: 8592 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
LacZ ORF: bases 954-4010
myc epitope: bases 4077-4106
Polyhistidine tag: bases 4122-4139
BGH reverse priming site: bases 4183-4200
BGH polyadenylation signal: bases 4186-4413
f1 origin: bases 4459-4887
SV40 promoter and origin: bases 4914-5222
Neomycin resistance gene: bases 5297-6091
SV40 polyadenylation signal: bases 6265-6395
pUC origin: bases 6778-7451
Ampicillin resistance gene: bases 75967-8456 (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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MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

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Technical Service, continued

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Purchaser Notification

Introduction

Use of the pcDNA™3.1/*myc*-His(-) vectors is covered under a number of different licenses including those detailed below.

Limited Use Label License No. 22: Vectors and Clones Encoding Histidine Hexamer

This product is licensed under U.S. Patent Nos. 5,284,933 and 5,310,663 and foreign equivalents from Hoffmann-LaRoche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

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