USER GUIDE

invitrogen™
by life technologies™

pcDNA™4/HisMax A, B, and C

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For Research Use Only. Not for diagnostic procedures.

life technologies™

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General Information

Contents	20 µg each pcDNA™4/HisMax A, B, and C, lyophilized in TE, pH 8.0 20 µg pcDNA™4/HisMax/*lacZ*, lyophilized in TE, pH 8.0
Shipping/Storage	Lyophilized plasmids are shipped at room temperature and stored at -20°C.
Product Use	**For research use only.** Not intended for any human or animal diagnostic or therapeutic uses.

Methods

Overview

Introduction

pcDNA™4/HisMax A, B, and C are 5.3 kb vectors derived from pcDNA™4/His and designed for overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow purification and detection of expressed proteins (see pages 16-17 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- QBI SP163 translational enhancer for increased levels of recombinant protein expression (Stein *et al.*, 1998) (see page 4 for more information)
- Three reading frames to facilitate in-frame cloning with an N-terminal peptide encoding the Xpress™ epitope and a polyhistidine metal-binding tag
- Zeocin™ resistance gene for selection of stable cell lines (Mulsant *et al.*, 1988) (see page 12 for more information)
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA™4/HisMax/*lacZ*, 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™4/HisMax.

- 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 N-terminal Xpress™ 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 or 25-50 µg/ml Zeocin™.
- 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 N-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. For antibody to the Xpress™ epitope, please see the next page.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see next page for ordering information).

Accessory Products

Introduction

The products listed below are designed to help you detect and purify your recombinant fusion protein expressed from pcDNA™4/HisMax. In addition, Life Technologies has a wide variety of mammalian expression vectors, many of which can be utilized with pcDNA™4/HisMax to express and detect multiple proteins in the same cell (see below).

Antibodies for Detection

If you do not have an antibody to your protein, Life Technologies offers the Anti-Xpress™ antibodies and Anti-HisG antibodies to detect your recombinant fusion protein. For more information, please refer to our website (www.lifetechnologies.com) or call Technical Support (see page 19).

ProBond™ Resin

Ordering information for ProBond™ resin is provided below.

Item	Amount	Catalog no.
ProBond™ Purification System	6 x 2 ml precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Purification System with Anti-Xpress™ Antibody	1 kit	K851-01
ProBond™ Resin	50 ml	R801-01
	150 ml	R801-15

Expression Vectors

We have a wide variety of mammalian expression vectors utilizing the CMV or EF-1α promoter. Vectors are available with the Xpress™ (N-terminal), *c-myc* (C-terminal), or V5 (C-terminal) epitope for detection and either the neomycin, blasticidin, or Zeocin™ resistance genes. All vectors utilize the polyhistidine tag for purification using ProBond™ resin. For more information on the mammalian expression vectors available, please see our website (www.lifetechnologies.com) or call Technical Support (see page 19).

Cloning into pcDNA™4/HisMax A, B, and C

Introduction

Diagrams are provided on pages 5-7 to help you ligate your gene of interest in frame with the N-terminal peptide. General considerations for cloning and transformation are listed below.

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).

E. coli Strain

Many *E. coli* strains are suitable for the propagation of this vector. 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 Life Technologies.

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

Transformation Method

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

Maintenance of pcDNA™4/HisMax

To propagate and maintain the pcDNA™4/HisMax vectors, we recommend resuspending each vector in 20 µl sterile water to prepare 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 TOP10F´, DH5α™, JM109, or equivalent. Select transformants on LB plates containing 50 to 100 µg/ml ampicillin or 25 to 50 µg/ml Zeocin™ in Low Salt LB. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 8 for protocol).

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Cloning into pcDNA™4/HisMax A, B, and C, continued

QBI SP163 Translational Enhancer

The QBI SP163 element is a 163 nucleotide splice variant derived from the 5´ untranslated region (UTR) of the mouse vascular endothelial growth factor (VEGF) gene (Stein *et al.*, 1998). The splice variant is composed of a 31 nucleotide fragment containing the 5´ cap sequence of the VEGF gene fused to a 132 nucleotide fragment of the 5´ UTR immediately preceding the translational start site of the VEGF gene. Please refer to the diagrams on pages 5-7 for the sequence of the QBI SP163 element.

The QBI SP163 element functions as a strong translational enhancer and acts to increase recombinant protein production when placed directly upstream of the ATG initiation codon of the gene of interest. The increase in protein expression is thought to occur through ribosome recruitment and a cap-independent translation mechanism. (Stein *et al.*, 1998).

In general, expression levels of recombinant protein from pcDNA™4/HisMax are 2-5 fold greater than the levels obtained with the pcDNA™4/His expression vector. The amount of recombinant protein expressed will vary depending on the nature of the gene of interest.

Note

The pcDNA™4/HisMax vectors are fusion vectors. To ensure proper expression of your recombinant protein, you must clone your gene in frame with the ATG at base pairs 1080-1082. This will create a fusion with the N-terminal polyhistidine tag, Xpress™ epitope, and the enterokinase cleavage site. The vector is supplied with the multiple cloning site in three reading frames relative to the N-terminal peptide to facilitate cloning. See pages 5-7 to develop a cloning strategy.

If you wish to clone your gene as close as possible to the enterokinase cleavage site, follow the guidelines below:

- Digest pcDNA™4/HisMax A, B, or C with *Kpn* I.
- Create blunt ends with T4 DNA polymerase and dNTPs.
- Clone your blunt-ended insert in frame with the lysine codon (AAG) of the enterokinase recognition site.

If you wish to separate your protein of interest from the N-terminal peptide tag, you may use any suitable enterokinase including EnterokinaseMax™ (EKMax™, Catalog no. E180-01) from Life Technologies. Following enterokinase cleavage, no vector-encoded amino acid residues will be present in your protein.

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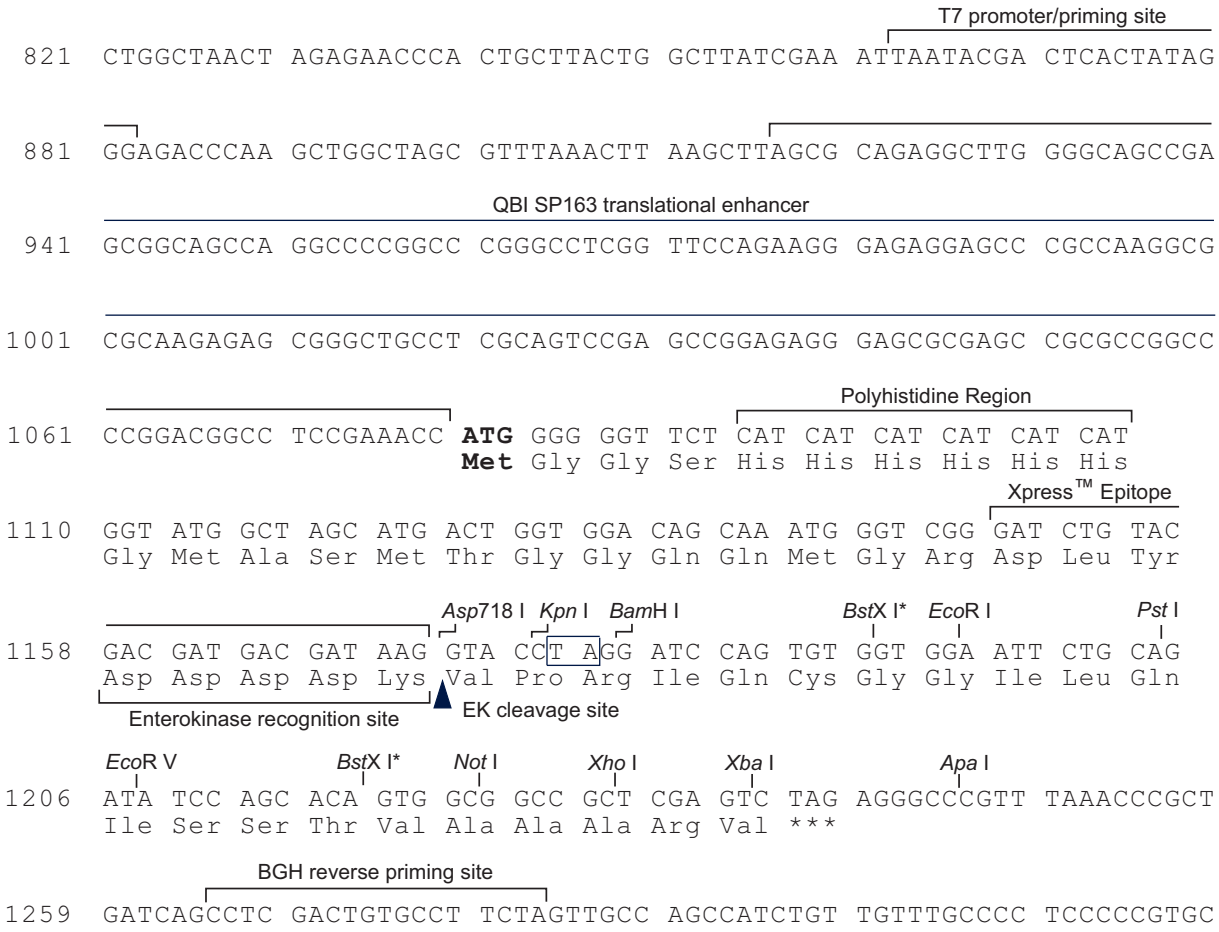
Cloning into pcDNA™4/HisMax A, B, and C, continued

Multiple Cloning Site of Version A

Below is the multiple cloning site for pcDNA™4/HisMax A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Please note that there is a stop codon within the *Xba* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™4/HisMax A is available for downloading from our website (www.lifetechnologies.com) or from Technical Support (see page 19).**



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

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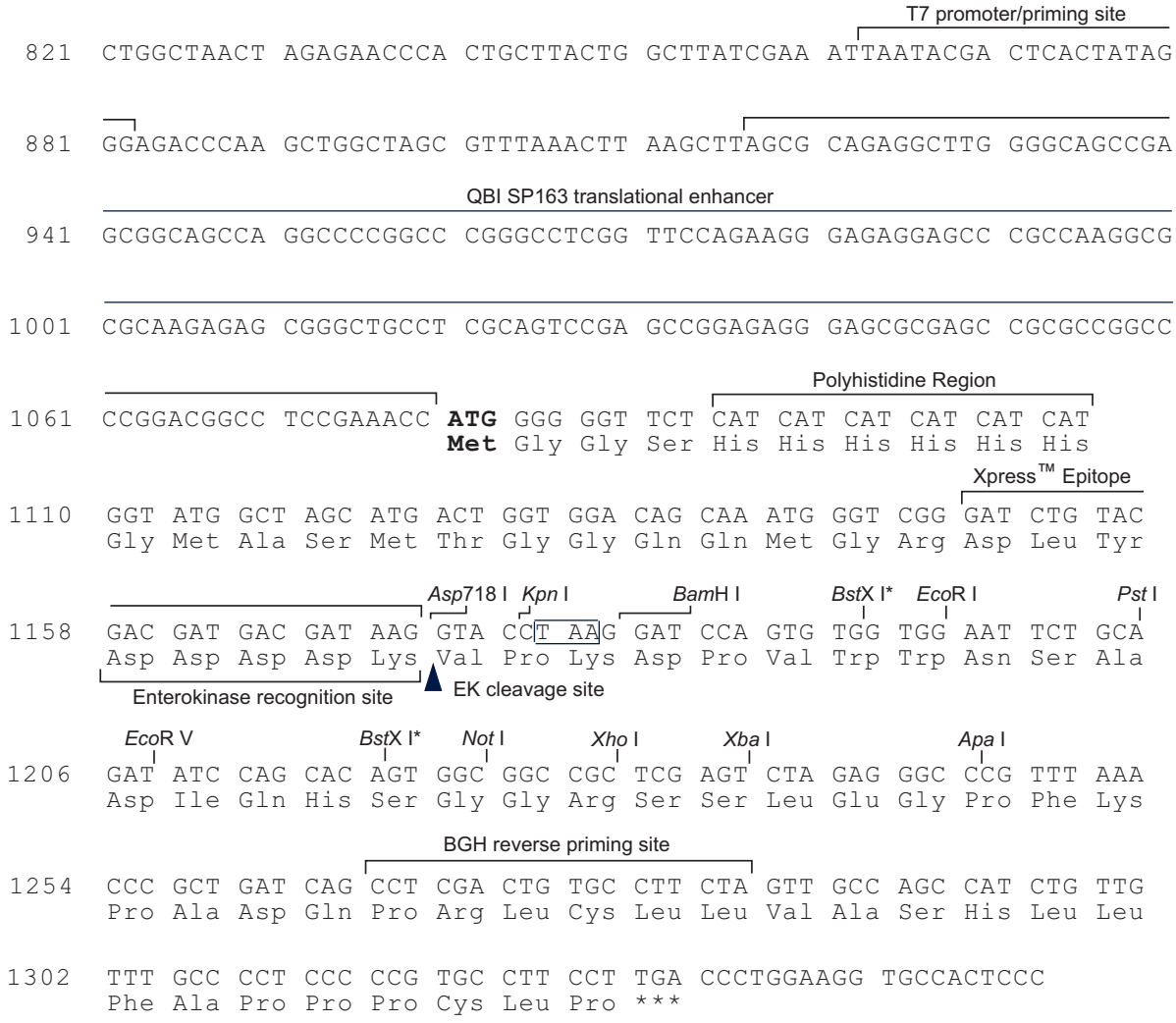
Cloning into pcDNA™4/HisMax A, B, and C, continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pcDNA™4/HisMax 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 complete sequence of pcDNA™4/HisMax B is available for downloading from our website (www.lifetechnologies.com) or from Technical Support (see page 19).**



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

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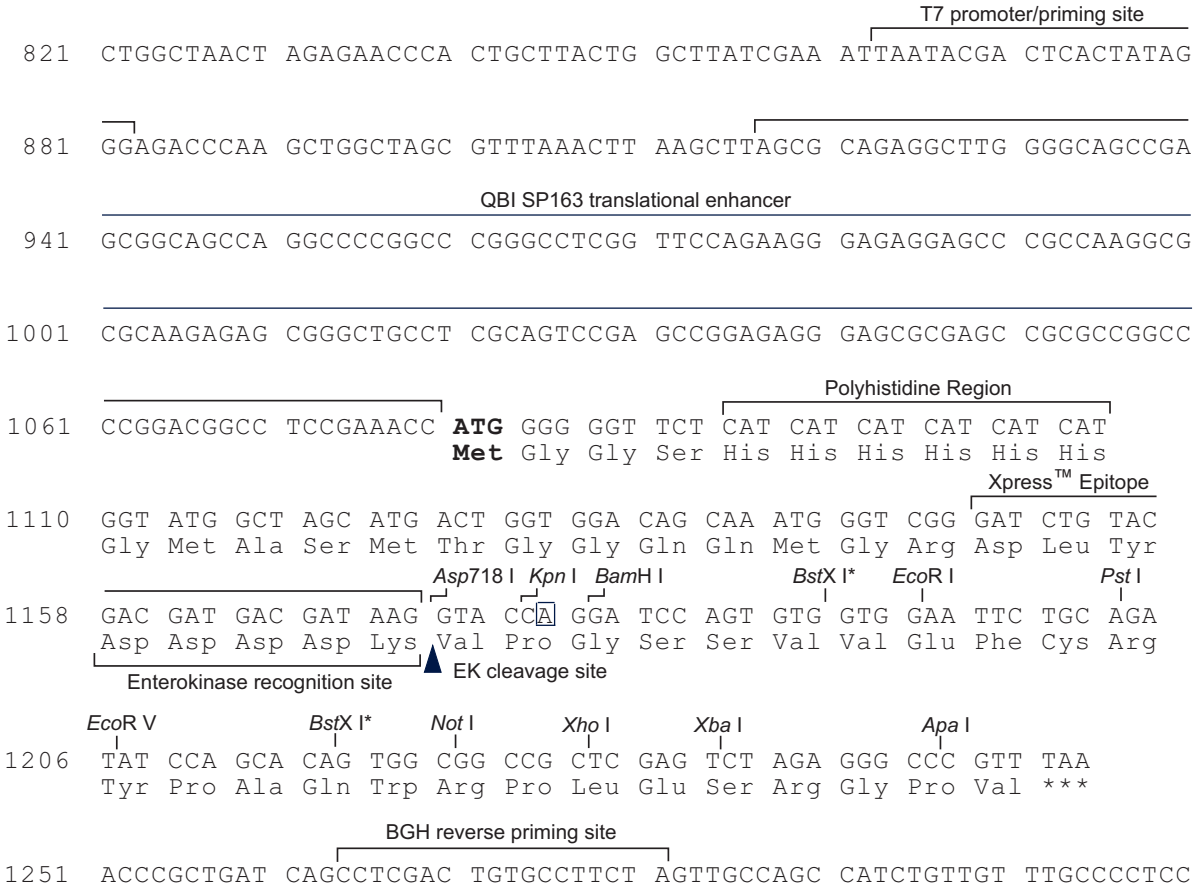
Cloning into pcDNA™4/HisMax A, B, and C, continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pcDNA™4/HisMax C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotide indicates the variable region**.** The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™4/HisMax C is available for downloading from our website (www.lifetechnologies.com) or from Technical Support (see page 19).**



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

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Cloning into pcDNA™4/HisMax A, B, and C, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F´, DH5α™) and select on LB plates containing 50-100 µg/ml ampicillin or 25-50 µg/ml Zeocin™ in Low Salt LB medium (see the following section). Select 10-20 clones and analyze for the presence and orientation of your insert.

Low Salt LB Medium with Zeocin™

For Zeocin™ to be active, the salt concentration of the medium must be low (< 90 mM) and the pH must be 7.5. For selection in *E. coli*, it is **imperative** that you prepare LB broth and plates using the following recipe. Please note the lower salt content of this medium. Failure to use low salt LB medium will result in non-selection due to inactivation of the drug.

Low Salt LB Medium:
10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 ml. Adjust pH to 7.5 with 5 M NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 lbs/sq. in. and 121°C for 20 minutes.
3. Thaw Zeocin™ on ice and vortex before removing an aliquot.
4. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 µg/ml final concentration.
5. Store plates at 4°C in the dark. Plates containing Zeocin™ are stable for 1-2 weeks.

Important

Any *E. coli* strain that contains the complete Tn*5* transposable element (i.e. DH5αF´IQ™, SURE, SURE2) encodes the *ble* gene (bleomycin resistance gene). These strains will confer resistance to Zeocin™. For the most efficient selection, we recommend an *E. coli* strain that does not contain the Tn*5* gene (i.e. TOP10, DH5α™, DH10, etc.).

We recommend that you sequence your construct with the T7 Forward and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is fused in frame with the N-terminal polyhistidine tag and the Xpress™ epitope. Please note that if you use the T7 Forward primer to sequence your insert, approximately 300 bp of sequence encoding the QBI SP163 element and the N-terminal tag will precede the sequence of your insert.

Preparing a Glycerol Stock

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage. It is also a good idea to keep a DNA stock of your plasmid at -20°C in case you lose the glycerol stock.

- Streak the original colony out on an LB plate containing 50 µg/ml ampicillin or 25 µg/ml Zeocin™ in Low Salt LB. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin or 25 µg/ml Zeocin™.
- Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
- Store at -80°C.

Transfection and Analysis

Introduction

Once you have confirmed that your construct is in the correct orientation and fused in frame with the N-terminal peptide, you are ready to transfect your cell line of choice. We recommend that you include the positive control vector and a mock transfection (negative control) to evaluate your results.

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 lipids, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. HeLa), please consult original references or the supplier of your cell line for the optimal method of transfection. We recommend 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* (Ausubel *et al.*, 1994).

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). Life Technologies offers the Calcium Phosphate Transfection Kit (Catalog no. K2780-01) and a large selection of reagents for transfection. Please refer to our website (www.lifetechnologies.com) or call Technical Support (see page 19) for more information.

Positive Control

pcDNA™4/HisMax/*lacZ* is provided as a positive control vector for mammalian cell transfection and expression (see page 18) and may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the control of the CMV promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see next page).

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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. Life Technologies offers the β-Gal Assay Kit (Catalog no. K1455-01) and the β-Gal Staining Kit (Catalog no. K1465-01) for fast and easy detection of β-galactosidase expression.

Detection of Fusion Proteins

The Anti-Xpress™ antibodies and the Anti-HisG antibodies are available from Life Technologies to detect expression of your fusion protein from pcDNA™4/HisMax (see page 2).

To detect the fusion protein by western blot, you will need to prepare a cell lysate from transfected cells. We recommend that you perform a time course to optimize expression of the fusion protein (*e.g.* 24, 48, 72 hours, etc. after transfection). To lyse cells:

1. Wash cell monolayers (~10^6 cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 ml PBS and pellet the cells at 1,500 x g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (see recipe below). Other cell lysis buffers are suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
5. Centrifuge the cell lysate at 10,000 x g for 10 minutes to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration. **Note:** Do not use protein assays utilizing Coomassie Blue or other dyes. NP-40 interferes with the binding of the dye with the protein.
6. Add SDS-PAGE sample buffer to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

Cell Lysis Buffer

50 mM Tris-HCl, pH 7.8

150 mM NaCl

1% Nonidet P-40

1. This solution can be prepared from the following common stock solutions. For 100 ml, combine:

1 M Tris base	5 ml
5 M NaCl	3 ml
Nonidet P-40	1 ml

2. Bring the volume up to 90 ml with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 ml. Store at room temperature.

Note: Protease inhibitors may be added at the following concentrations:

1 mM PMSF

1 µg/ml pepstatin

1 µg/ml leupeptin

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Note

The N-terminal peptide containing the Xpress™ epitope and the polyhistidine tag will add approximately 3.4 kDa to the size of your protein. Please note that the QBI SP163 element is **not** translated.

Purification

You will need 5 x 10^6 to 1 x 10^7 **transfected** cells for purification of your protein on a 2 ml ProBond™ column (or other metal-chelating column). Please refer to the manufacturer's instructions before attempting to purify your fusion protein. To prepare cells for lysis, please refer to the protocol on page 15.

Creation of Stable Cell Lines

Introduction

The pcDNA™4/HisMax vectors contain the Zeocin™ resistance gene for selection of stable cell lines using Zeocin™. We recommend that you test the sensitivity of your mammalian host cell to Zeocin™ as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

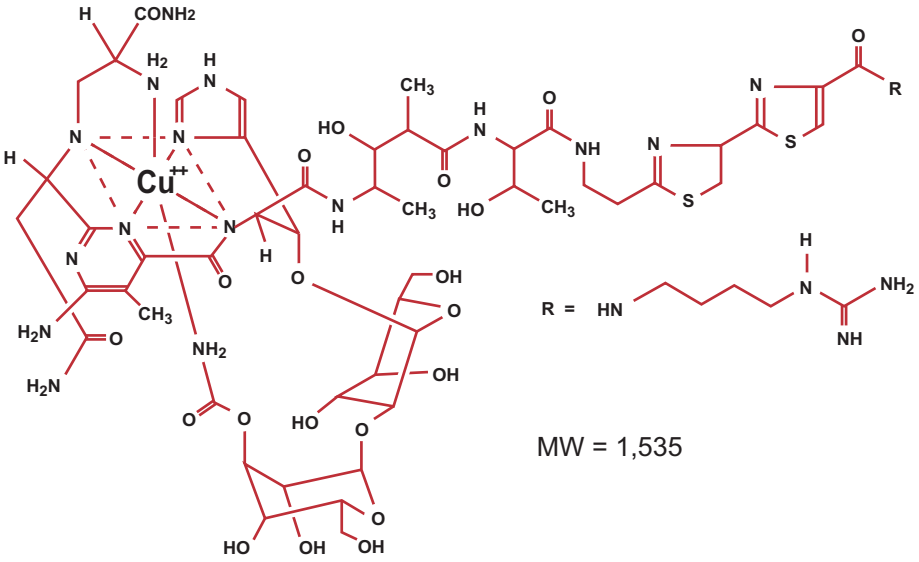
Zeocin™

Zeocin™ is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong anti-bacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells (Baron *et al.*, 1992; Drocourt *et al.*, 1990; Mulsant *et al.*, 1988; Perez *et al.*, 1989).

The Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13.7 kDa protein that binds Zeocin™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

Molecular Weight, Formula, and Structure

The formula for Zeocin™ is $C_{60}H_{89}N_{21}O_{21}S_3$ and the molecular weight is 1,535. The diagram below shows the structure of Zeocin™.



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Creation of Stable Cell Lines, continued

Applications of Zeocin™

Zeocin™ is used for selection in mammalian cells (Mulsant *et al.*, 1988); plants (Perez *et al.*, 1989); yeast (Baron *et al.*, 1992); and prokaryotes (Drocourt *et al.*, 1990). Suggested concentrations of Zeocin™ for selection in mammalian cell lines and *E. coli* are listed below:

Organism	Zeocin™ Concentration and Selective Medium
E. coli	25-50 μg/ml in **low salt LB** medium* (see page 8 for recipe)
Mammalian Cells	50-1000 μg/ml (varies with cell line)

*Efficient selection requires that the concentration of NaCl be no more than 5 g/liter (< 90 mM).

Handling Zeocin™

- High salt and acidity or basicity inactivate Zeocin™. Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active (see page 8).
- Store Zeocin™ at -20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store drug, plates, and medium containing drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling solutions containing Zeocin™.
- Zeocin™ is toxic. Do not ingest or inhale solutions containing the drug.

Ordering Information

Zeocin™ can be purchased from Life Technologies. For your convenience, the drug is prepared in autoclaved, deionized water and available in 1.25 ml aliquots at a concentration of 100 mg/ml. The stability of Zeocin™ is guaranteed for six months, if stored at -20°C.

Amount	Catalog no.
1 gram	R250-01
5 grams	R250-05

Effect of Zeocin™ on Sensitive and Resistant Cells

Zeocin™'s method of killing is quite different from neomycin and hygromycin. **Cells do not round up and detach from the plate.** Sensitive cells may exhibit the following morphological changes upon exposure to Zeocin™:

- Vast increase in size (similar to the effects of cytomegalovirus infecting permissive cells)
- Abnormal cell shape
- Presence of large empty vesicles in the cytoplasm (breakdown of the endoplasmic reticulum and golgi apparatus, or other scaffolding proteins)
- Breakdown of plasma and nuclear membrane (appearance of many holes in these membranes)

Eventually, these "cells" will completely break down and only cellular debris will remain.

Zeocin™-resistant cells should continue to divide at regular intervals to form distinct colonies. There should not be any distinct morphological changes in Zeocin™-resistant cells when compared to cells not under selection with Zeocin™.

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Creation of Stable Cell Lines, continued

Selection in Mammalian Cell Lines

To generate a stable cell line expressing your protein, you need to determine the minimum concentration of Zeocin™ required to kill your untransfected host cell line. In general, concentrations ranging from 50 to 1000 µg/ml Zeocin™ are sufficient to kill the untransfected host cell line, with the average being 250 to 400 µg/ml. Test a range of concentrations to ensure that you determine the minimum concentration necessary for your cell line.

- Seed cells (20-25% confluent) for each time point and allow cells to adhere overnight.
- The next day, substitute culture medium with medium containing varying concentrations of Zeocin™ (e.g. 0, 50, 100, 200, 400, 600, 800, and 1000 µg/ml).
- Replenish the selective medium every 3-4 days, and observe the percentage of surviving cells.
- Count the number of viable cells at regular intervals to determine the appropriate concentration of Zeocin™ that prevents growth. Select the concentration that kills the majority of the cells in the desired number of days (4-10 days).

Possible Sites for Linearization

To obtain stable transfectants, you may choose to linearize your vector before transfection. While linearizing your vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts the gene of interest. The table below lists unique sites that may be used to linearize your construct prior to transfection. **Other restriction sites are possible. Please note that for the enzymes listed below, the cleavage site is indicated for versions A, B, and C of pcDNA™4/HisMax.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Restriction Site (bp) (A,B,C)	Location	Supplier
Bgl II	12	Upstream of CMV promoter	Many
Mfe I	161	Upstream of CMV promoter	New England Biolabs
Nru I	208	Upstream of CMV promoter	Many
*Bst*1107 I	3063 (A), 3064 (B), 3062 (C)	End of SV40 poly A	AGS*, Fermentas, Takara, Boehringer-Mannhiem
*Eam*1105 I	4335 (A), 4336 (B), 4334 (C)	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	4557 (A), 4558 (B), 4556 (C)	Ampicillin gene	Many
Pvu I	4705 (A), 4706 (B), 4704 (C)	Ampicillin gene	Many
Sca I	4815 (A), 4816 (B), 4814 (C)	Ampicillin gene	Many
Ssp I	5139 (A), 5140 (B), 5138 (C)	Backbone	Many

*Angewandte Gentechnologie Systeme

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Creation of Stable Cell Lines, continued

Selection Tip

Some cells may be more resistant to Zeocin™ than others. If cells are dividing rapidly, Zeocin™ may not be effective at low concentrations. To overcome this resistance, we recommend that you place the cells at +4°C for 2 hours after plating (be sure to buffer the medium with HEPES). Then return the cells to 37°C. This will stop the cell division process for a short time and allow Zeocin™ to act.

Selection of Stable Integrants

Once the appropriate Zeocin™ concentration is determined, you can generate a stable cell line with your construct.

- Transfect your cells using the appropriate protocol for your cell line. Include a sample of untransfected cells as a negative control.
- After transfection, wash the cells once with 1X PBS and add fresh medium to the cells.
- 48 hours after transfection, split the cells into fresh medium containing Zeocin™ at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent.
- Replenish selective medium every 3-4 days until Zeocin™-resistant colonies are detected.
- Pick and expand colonies in 96- or 48-well plates. Grow cells to near confluence before expanding to larger wells or plates.

Preparation of Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purification of your protein on ProBond™. You will need 5 x 10^6 to 1 x 10^7 cells for purification of your protein 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 with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 1500 rpm for 5 minutes. Resuspend the cell pellet in PBS.
6. Centrifuge the cells at 1500 rpm for 5 minutes. Remove PBS. 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, please refer to the ProBond™ Purification System manual for details about sample preparation for chromatography.

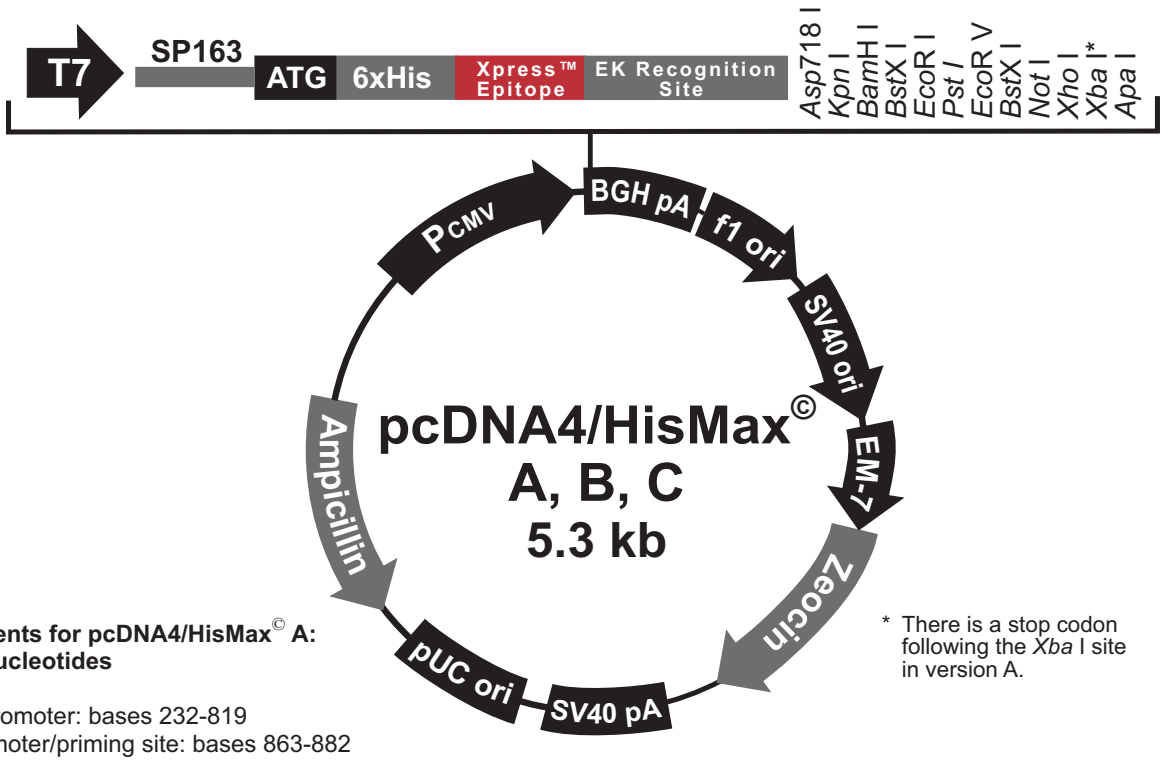
If you are using other metal-chelating resin, please refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

pcDNA™4/HisMax Vector

Map of pcDNA™4/HisMax

The figure below summarizes the features of the pcDNA™4/HisMax vectors. **The sequences for pcDNA™4/HisMax A, B, and C are available for downloading from our website (www.lifetechnologies.com) or from Technical Support (see page 19).**



* There is a stop codon following the *Xba* I site in version A.

Comments for pcDNA4/HisMax© A: 5258 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
QBI SP163 translational enhancer: bases 917-1079
ATG initiation codon: bases 1080-1082
Polyhistidine tag: bases 1092-1109
Xpress™ epitope: bases 1149-1172
Enterokinase recognition site: bases 1158-1172
Multiple cloning site: bases 1172-1245
BGH reverse priming site: bases 1265-1282
BGH polyadenylation sequence: bases 1268-1495
f1 origin: bases 1541-1969
SV40 promoter and origin: bases 1996-2305
EM-7 promoter: bases 2353-2408
Zeocin™ resistance gene: bases 2427-2801
SV40 polyadenylation sequence: bases 2931-3061
pUC origin: bases 3444-4117
Ampicillin resistance gene: bases 4262-5122

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pcDNA™4/HisMax Vector, continued

Features of pcDNA™4/HisMax

pcDNA™4/HisMax A (5258 bp), pcDNA™4/HisMax B (5259 bp), and pcDNA™4/HisMax C (5257 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
QBI SP163 translational enhancer	Increases expression of your recombinant protein via a cap-independent translation mechanism (Stein *et al.*, 1998)
N-terminal polyhistidine tag	Allows purification of your recombinant protein on metal-chelating resin such as ProBond™
Xpress™ epitope tag	Allow detection of your recombinant protein with the Anti-Xpress™ Antibody (Catalog no. R910-25)
Enterokinase cleavage site	Allows removal of the N-terminal tag from your recombinant protein using an enterokinase such as EnterokinaseMax™ (Catalog no. E180-01)
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the Xpress™ epitope and N-terminal polyhistidine tag
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 Zeocin™ resistance gene in mammalian cells and episomal replication in cells expressing the SV40 large T antigen
EM-7 promoter	Synthetic promoter based on the bacteriophage T7 promoter for expression of the Zeocin™ resistance gene in *E. coli*
Zeocin™ resistance gene	Selection of transformants in *E. coli* and stable transfectants in mammalian cells (Drocourt *et al.*, 1990; Mulsant *et al.*, 1988)
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 transformants in *E. coli*

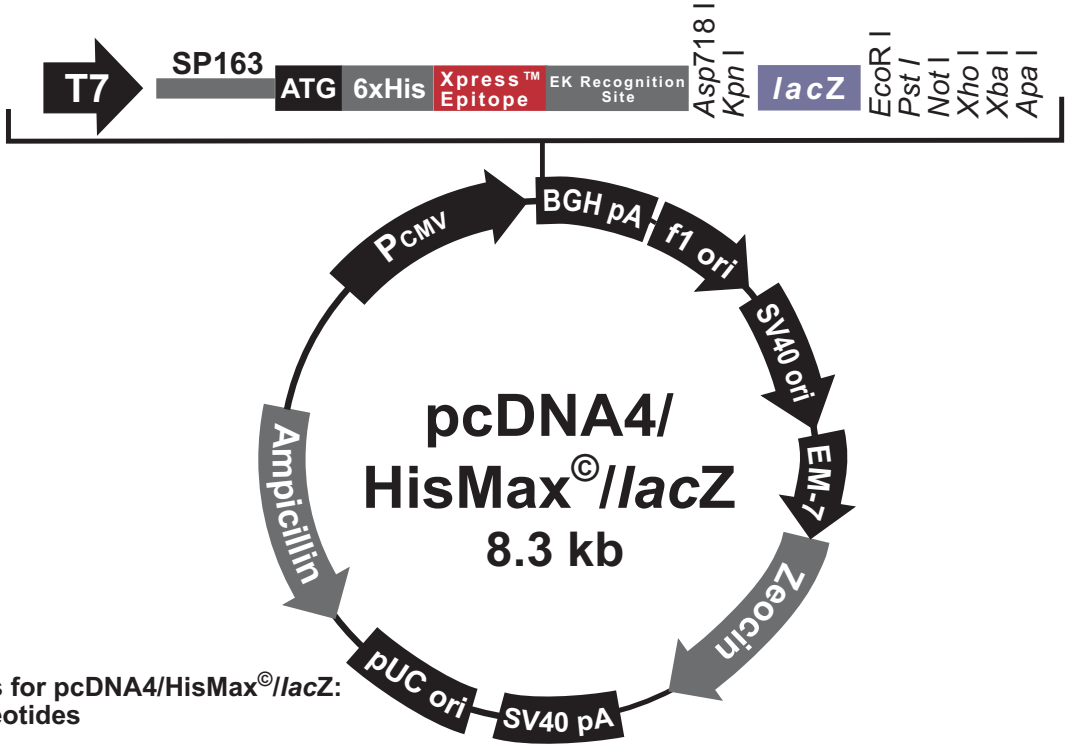
pcDNA™4/HisMax/*lacZ*

Description

pcDNA™4/HisMax/*lacZ* is a 8321 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3.1 kb *Kpn* I-*Eco*R I fragment containing the *lacZ* gene into the *Kpn* I-*Eco*R I site of pcDNA™4/HisMax.

Map of Control Vector

The figure below summarizes the features of the pcDNA™4/HisMax/*lacZ* vector. **The complete nucleotide sequence for pcDNA™4/HisMax/*lacZ* is available for downloading from our website (www.lifetechnologies.com) or from Technical Support (see page 19).**



Comments for pcDNA4/HisMax©/*lacZ*: 8321 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
QBI SP163 translational enhancer: bases 917-1079
ATG initiation codon: bases 1080-1082
Polyhistidine tag: bases 1092-1109
Xpress™ epitope: bases 1149-1172
Enterokinase recognition site: bases 1158-1172
LacZ ORF: bases 1197-4247
BGH reverse priming site: bases 4328-4345
BGH polyadenylation sequence: bases 4331-4558
f1 origin: bases 4604-5032
SV40 promoter and origin: bases 5059-5368
EM-7 promoter: bases 5416-5471
Zeocin™ resistance gene: bases 5490-5864
SV40 polyadenylation sequence: bases 5994-6124
pUC origin: bases 6507-7180
Ampicillin resistance gene: bases 7325-8185

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