USER GUIDE

invitrogen™
by life technologies™

pcDNA™5/TO

Hygromycin-resistant expression vector
designed for use with the T-REx™ System

Catalog Number V1033-20

Revision Date 12 September 2012

Publication Part Number 25-0407

MAN0000221

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

life technologies™

Contents

Important Information

pcDNA™ Vectors

This manual is supplied with the following products.

Product	Catalog No.
pcDNA™5/TO Vector	V1020-20

Shipping/Storage

Plasmids are shipped on wet ice and should be stored at –20°C.

Contents

20 µg of pcDNA™5/TO vector at 0.5 µg/µL, in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Volume: 40 µL.

20 µg of pcDNA™5/TO/*lac*Z vector at 0.5 µg/µL, in TE buffer, pH 8.0. Volume: 40 µL

Product Use

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

Methods

Overview

Introduction

pcDNA™5/TO is a 5.7 kb expression vector designed for use with the T-REx™ System (Catalog Nos. K1020-01 and K1020-02) available from Life Technologies. The vector allows tetracycline-regulated expression of the gene of interest in mammalian host cells expressing the Tet repressor (TetR) from the pcDNA™6/TR vector (Catalog No. V1025-20). The pcDNA™5/TO vector contains the following elements:

- Hybrid promoter consisting of the human cytomegalovirus immediate-early (CMV) promoter and tetracycline operator 2 ($TetO_2$) sites for high-level tetracycline-regulated expression in a wide range of mammalian cells (see below)
- Hygromycin resistance gene for selection of stable cell lines

The control plasmid, pcDNA™5/TO/*lacZ*, is included for use as a positive control for transfection and tetracycline-regulated expression in the cell line of choice.

For more information about pcDNA™6/TR and the T-REx™ System, refer to the T-REx™ System manual, our website (**www.lifetechnologies.com**), or call Technical Support (see page 16). The T-REx™ System manual is supplied with the T-REx™ Core Kit or T-REx™ Complete Kit and is also available for downloading from our website or by contacting Technical Support (see page 16).

A Note About pcDNA™5/TO

The pcDNA™5/TO vector contains two tetracycline operator 2 ($TetO_2$) sites within the human cytomegalovirus immediate-early (CMV) promoter for tetracycline-regulated expression of your gene of interest (Yao *et al.*, 1998). The $TetO_2$ sequences serve as binding sites for 4 Tet repressor molecules (comprising two Tet repressor homodimers) and confer tetracycline-responsiveness to your gene of interest. The Tet repressor is expressed from the pcDNA™6/TR plasmid. For more information about the $TetO_2$ sequences, see the next page. For more information about the pcDNA™6/TR plasmid and the Tet repressor, refer to the T-REx™ System manual.

In the absence of tetracycline, expression of your gene of interest is repressed by the binding of Tet repressor homodimers to the $TetO_2$ sequences. Addition of tetracycline to the cells derepresses the hybrid CMV/$TetO_2$ promoter in pcDNA™5/TO and allows expression of your gene of interest.

Continued on next page

Tet Operator Sequences

The promoters of bacterial *tet* genes contain two types of operator sequences, O_1 and O_2, that serve as high affinity binding sites for the Tet repressor (Hillen and Berens, 1994; Hillen *et al.*, 1983). Each O_1 and O_2 site binds to one Tet repressor homodimer. While Tet repressor homodimers bind to both *tet* operators with high affinity, studies have shown that the affinity of the Tet repressor homodimer for O_2 is three- to five-fold higher than it is for O_1 (Hillen and Berens, 1994).

Tet operators have been incorporated into heterologous eukaryotic promoters to allow tetracycline-regulated gene expression in mammalian cells (Gossen and Bujard, 1992; Yao *et al.*, 1998). In the T-REx™ System, two copies of the O_2 operator sequence ($TetO_2$) were inserted into the strong CMV promoter of pcDNA™5/TO to allow regulated expression of your gene of interest by tetracycline. We use the $TetO_2$ operator sequence in pcDNA™5/TO to maximize repression of basal gene expression. For more detailed information about *tet* operators, refer to Hillen and Berens (1994).

Yao *et al.* (1998) have recently demonstrated that the location of *tet* operator sequences in relation to the TATA box of a heterologous promoter is critical to the function of the *tet* operator. Regulation by tetracycline is only conferred upon a heterologous promoter by proper spacing of the $TetO_2$ sequences from the TATA box (Yao *et al.*, 1998). For this reason, the first nucleotide of the $TetO_2$ operator sequence has been placed 10 nucleotides after the last nucleotide of the TATA element in the CMV promoter in pcDNA™5/TO. Refer to the diagram on page 5 for the sequence and placement of the $TetO_2$ sequences in relation to the TATA box.

In other tetracycline-regulated systems, the $TetO_2$ sequences are located upstream of the TATA element in the promoter of the inducible expression vector (Gossen and Bujard, 1992). These systems differ substantially from the T-REx™ System in that they use regulatory molecules composed of the Tet repressor fused to a viral transactivation domain. The presence of viral transactivation domains appears to overcome the requirement for specific positioning of the $TetO_2$ sequences in relation to the TATA box of the heterologous promoter. However, the presence of viral transactivation domains has been found to have deleterious effects in some mammalian cell lines.

Continued on next page

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™5/TO.

Step	Action
1	Consult the multiple cloning site diagrammed on page 5 to design your cloning strategy.
2	Ligate your insert into pcDNA™5/TO vector and transform into *E. coli*. Select transformants on 50–100 µg/mL ampicillin.
3	Analyze your transformants for the presence of insert by restriction digestion.
4	Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the correct orientation.
5	Cotransfect your pcDNA™5/TO construct and pcDNA™6/TR vector into the cell line of choice using your own method of choice, and induce expression of your gene of interest with tetracycline. Generate a double stable cell line, if desired. For more information about pcDNA™6/TR vector, refer to the T-REx™ System manual.

Cloning into pcDNA™5/TO

Introduction

A diagram is provided on the next page to help you clone your gene of interest into pcDNA™5/TO vector. 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, 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 and maintenance of this vector including TOP10 and DH5 ™-T1^R. We recommend that you propagate vectors containing inserts in *rec*A, *end*A *E. coli* strains.

For your convenience, TOP10 and DH5 ™-T1^R *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot® format from Life Technologies.

Item	Quantity	Catalog No.
One Shot® TOP10 (chemically competent cells)	21 × 50 µL	C4040-03
One Shot® TOP10 Electrocomp (electrocompetent cells)	21 × 50 µL	C4040-52
One Shot® MAX Efficiency® DH5 ™-T1^R (chemically competent cells)	21 × 50 µL	12297-016

Transformation Method

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

Maintenance of pcDNA™5/TO

To propagate and maintain the pcDNA™5/TO and pcDNA™5/TO/*lacZ* vectors, use 10 ng of the vector to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5 ™-T1^R, JM109, or equivalent. Select transformants on LB agar plates containing 50–100 µg/mL ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 6).

Cloning Considerations

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; 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™5/TO, continued

Multiple Cloning Site of pcDNA™5/TO

Below is the multiple cloning site for pcDNA™5/TO. Restriction sites are labeled to indicate the cleavage site. Potential stop codons are shown underlined. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™5/TO is available for downloading from our website (www.lifetechnologies.com) or from Technical Support (see page 16).** For a map and a description of the features of pcDNA™5/TO, refer to the **Appendix**, pages 12–13.

CMV Forward priming site

```
 721  AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG
```

TATA box; Tetracycline operator (TetO2)

```
 781  GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CCCTATCAGT GATAGAGATC
```

Tetracycline operator (TetO2)

```
 841  TCCCTATCAG TGATAGAGAT CGTCGACGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA

 901  GACGCCATCC ACGCTGTTTT GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA
```

Pme I* *Afl* II *Hin*d III *Asp*718 I *Kpn* I *Bam*H I *Bst*X I*

```
 961  CTCTAGCGTT TAAACTTAAG CTTGGTACCG AGCTCGGATC CACTAGTCCA GTGTGGTGGA
```

*Eco*R V *Bst*X I* *Not* I *Xho* I *Xba* I *Eco*0109 I *Apa* I *Pme* I*

```
1021  ATTCTGCAGA TATCCAGCAC AGTGGCGGCC GCTCGAGTCT AGAGGGCCCG TTTAAACCCG
```

BGH Reverse priming site

```
1081  CTGATCAGCC TCGACTGTGC CTTCTAGTTG CCAGCCATCT
```

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

Continued on next page

Cloning into pcDNA™5/TO, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, DH5 ™-T1^R) and select on LB agar 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 in the correct orientation for expression and contains an initiation ATG and a stop codon. Refer to the diagram on the previous page for the sequence and location of recommended primer binding sites.

For your convenience, Life Technologies a custom primer synthesis service. For more information, refer to our website (**www.lifetechnologies.com**) or contact Technical Support (see page 16).

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.

- Streak the original colony out on an LB plate containing 50 µg/mL ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL ampicillin.
- 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

Introduction

Once you have cloned your gene of interest into pcDNA™5/TO and have prepared clean plasmid preparations of your pcDNA™5/TO construct and pcDNA™6/TR, you are ready to cotransfect the plasmids into the mammalian cell line of choice. We recommend that you include the positive control vector and a mock transfection (negative control) to evaluate your results. Refer to the T-REx™ System manual for information on pcDNA™6/TR, transfection, and induction of expression using tetracycline.

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 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.

Positive Control

pcDNA™5/TO/*lacZ* is provided as a positive control vector for mammalian cell transfection and expression (see page 14) and may be used to optimize transfection conditions for your cell line. Cotransfection of the positive control vector and pcDNA™6/TR results in the induction of β-galactosidase expression upon addition of tetracycline. A successful cotransfection will result in β-galactosidase expression that can be easily assayed by staining with X-gal (following page).

Continued on next page

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.

Note

The *lacZ* gene in pcDNA™5/TO/*lacZ* is fused to an N-terminal peptide containing an ATG initiation codon, a 6xHis tag and the Xpress™ epitope. The Xpress™ epitope allows detection of the β-galactosidase fusion protein on a western blot using the Anti-Xpress™ Antibody. The N-terminal peptide adds approximately 4.3 kDa to the size of the β-galactosidase fusion protein (total size of the fusion protein is approximately 120 kDa). For more information about detection of the β-galactosidase fusion protein by western blot, see the **Appendix**, page 11.

Important

Because tetracycline-regulated expression in the T-REx™ System is based on a repression/derepression mechanism, the amount of Tet repressor that is expressed in the host cell line from pcDNA™6/TR will determine the level of transcriptional repression of the Tet operator sequences in your pcDNA™5/TO construct. Tet repressor levels should be sufficiently high to suitably repress basal level transcription. We have varied the ratio of pcDNA™6/TR and pcDNA™5/TO plasmid that we transiently cotransfect into mammalian cells to optimize repression and inducibility of the hybrid CMV/TetO_2 promoter in pcDNA™5/TO. We recommend that you cotransfect your mammalian host cell line with a ratio of **at least 6:1** (w/w) pcDNA™6/TR:pcDNA™5/TO plasmid DNA, but you may want to try varying ratios of pcDNA™6/TR:pcDNA™5/TO plasmid to optimize repression and expression for your particular cell line and your gene of interest.

Cotransfection and Induction with Tetracycline

General guidelines are provided below to cotransfect your pcDNA™5/TO construct (or the control plasmid) and pcDNA™6/TR into your cell line of interest and to induce expression of your protein of interest with tetracycline.

- Use cells that are approximately 60% confluent for transfection.
- Cotransfect your pcDNA™5/TO construct and pcDNA™6/TR at a ratio of 6:1 (w:w) into the cell line of choice using your preferred method. Absolute amounts of plasmid used for transfection will vary depending on the method of transfection and the cell line used.
- After transfection, add fresh medium and allow the cells to recover for 24 hours before induction.
- Remove medium and add fresh medium containing the appropriate concentration of tetracycline to the cells. We recommend that you add tetracycline to a final concentration of 1µg/mL to the cells and incubate the cells for 24 hours at 37°C.
- Harvest the cells and assay for expression of your gene of interest.

Creation of Stable Cell Lines

Introduction

Once you have established that your construct can be inducibly expressed, you may create a stable cell line that inducibly expresses your gene of interest. pcDNA™5/TO contains the hygromycin resistance gene to allow selection of stable lines using hygromycin.

Note

Note that your gene of interest will be constitutively expressed if you transfect your pcDNA™5/TO construct into mammalian host cells prior to transfecting the pcDNA™6/TR plasmid. For more information on selection of stable cell lines using pcDNA™6/TR and blasticidin, refer to the T-REx™ System manual.

Reminder: When generating a stable cell line expressing the Tet repressor (from pcDNA™6/TR), you will want to select for clones that express the highest levels of Tet repressor to use as hosts for your pcDNA™5/TO expression plasmid. Those clones which express the highest levels of Tet repressor should exhibit the most complete repression of basal transcription of your gene of interest.

Hygromycin B

The pcDNA™5/TO vector contains the hygromycin resistance gene (Gritz and Davies, 1983) for selection of stable transfectants with the antibiotic, hygromycin B (Palmer *et al.*, 1987). When added to cultured mammalian cells, hygromycin B acts as an aminocyclitol to inhibit protein synthesis.

Determination of Antibiotic Sensitivity

To generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of hygromycin required to kill your untransfected host cell line. Typically, concentrations between 10 and 400 µg/mL hygromycin are sufficient to kill the untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line.

Note: Before transfecting your host cell line with pcDNA™6/TR, you will need to perform a similar experiment to determine the minimum concentration of blasticidin required to kill the untransfected cell line. Refer to the T-REx™ System manual for information about blasticidin.

- Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates.
- The next day, substitute culture medium with medium containing varying concentrations of hygromycin (e.g., 0, 10, 25, 50, 100, 200, and 400 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 hygromycin that prevents growth within 1–2 weeks after addition of hygromycin.

Continued on next page

Possible Sites for Linearization

To obtain stable transfectants, you may choose to linearize your pcDNA™5/TO construct 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 either the gene of interest or other elements important for mammalian expression. The table below lists unique sites that may be used to linearize your construct prior to transfection. Other restriction sites are possible. **Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.**

Enzyme	Restriction Site (bp)	Location	Supplier
Mun I	162	Upstream of CMV promoter	Many
Nru I	209	Upstream of CMV promoter	Life Technologies
Sap I	3736	Backbone	Many
*Eam*1105 I	4745	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	4967	Ampicillin gene	Many
Ssp I	5549	*bla* promoter	Life Technologies

*Angewandte Gentechnologie Systeme

Selection of Stable Cell Lines

Once you have determined the appropriate hygromycin concentration to use for selection, you can generate a stable cell line expressing pcDNA™6/TR and your pcDNA™5/TO construct.

1. Cotransfect your pcDNA™5/TO construct and pcDNA™6/TR into the cell line of choice using your preferred method. Include a sample of untransfected cells as a negative control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing hygromycin and blasticidin at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent. If the cells are too dense, the antibiotic will not kill the untransfected cells.
4. Replenish selective medium every 3–4 days until hygromycin-resistant and blasticidin-resistant colonies are detected.
5. Pick at least 40 foci and expand them to test for tetracycline-inducible gene expression.

Note

You may also create stable cell lines by first stably transfecting the pcDNA™6/TR plasmid into your cell line of choice, and then using this cell line as the host for your pcDNA™5/TO construct. If you are using one of the T-REx™ cell lines, simply transfect your pcDNA™5/TO construct into the cells and select for hygromycin-resistant and blasticidin-resistant clones.

Appendix

Accessory Products

Introduction

The products listed below are designed for use with the T-REx™ System.

T-REx™ Cell Lines

For your convenience, Life Technologies has available several mammalian cell lines that stably express the Tet repressor. T-REx™-293 cells, T-REx™-HeLa cells, T-REx™ CHO cells, and T-REx™-Jurkat cells express the Tet repressor from pcDNA™6/TR and should be maintained in medium containing blasticidin. Expression of your gene of interest from pcDNA™5/TO may be assayed by transfection of your pcDNA™5/TO construct into any of the T-REx™ cell lines and induction with tetracycline. Ordering information is provided below.

Cell Line	Source	Catalog No.
T-REx™-293	Human embryonic kidney	R710-07
T-REx™-HeLa	Human cervical adenocarcinoma	R714-07
T-REx™-CHO	Chinese hamster ovary	R718-07
T-REx™-Jurkat	Human lymphocyte	R722-07

Additional Reagents

Many of the reagents used in the T-REx™ System as well as other reagents that may be used in conjunction with the T-REx™ System are available separately from Life Technologies. The amount of antibody supplied is sufficient to detect 25 western blots in a 10 mL working volume. See the table below for ordering information.

Item	Amount	Catalog No.
pcDNA™6/TR	20 µg	V1025-20
pcDNA™4/TO	20 µg	V1020-20
pcDNA™4/myc-His A, B, and C	20 µg each	V1030-20
Blasticidin	50 mg, powder	R210-01
Anti-Xpress™ Antibody	50 µL	R910-25

Detection of β-Galactosidase Fusion Proteins

Introduction

To detect expression of your β- galactosidase fusion protein from pcDNA™5/TO/*lacZ* by western blot, you may use the Anti-Xpress™ Antibody available from Life Technologies (see page vi for ordering information). The antibody should detect a β-galactosidase fusion protein of approximately 120 kDa in size. To perform a western blot, you will need to prepare a cell lysate from transfected cells. A sample protocol is provided below. Other protocols are suitable. Refer to *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) for more information.

Preparation of Cell Lysates

Follow the protocol below to prepare cell lysates.

1. Wash cells (~10^6 cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 mL PBS and pellet the cells at 1500 × *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 on ice or at room temperature.
5. Centrifuge the cell lysate at 10,000 × *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

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

Reagent	Volume	Final Concentration
1 M Tris-HCl	5 mL	50 mM Tris-HCl
5 M NaCl	3 mL	150 mM NaCl
Nonidet P-40	1 mL	1% Nonidet P-40

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.

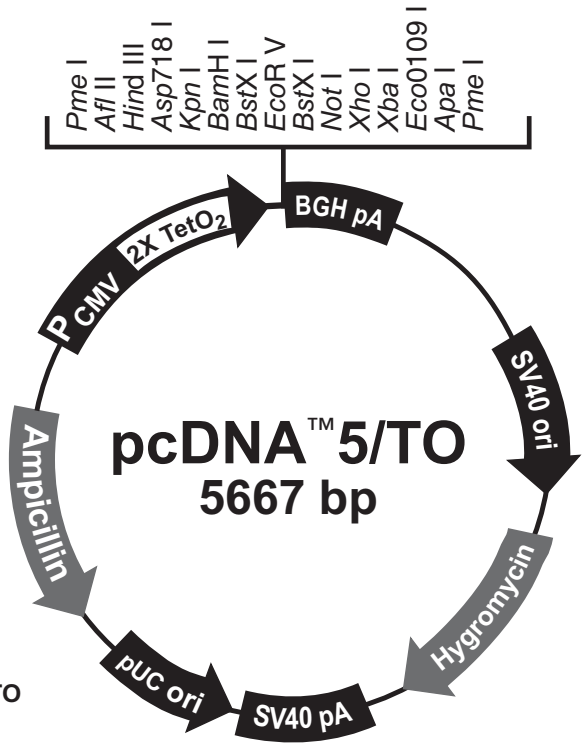
Note: Protease inhibitors may be added at the following concentrations:

1 mM PMSF, 1 µg/mL pepstatin, and 1 µg/mL leupeptin

pcDNA™5/TO Vector

Map of pcDNA™5/TO

The figure below summarizes the features of the pcDNA™5/TO vector. **The complete nucleotide sequence for pcDNA™5/TO is available for downloading from www.lifetechnologies.com or by contacting Technical Support (see page 16).**



Comments for pcDNA™5/TO 5667 nucleotides

CMV promoter: bases 232-958
  TATA box: bases 804-810
  Tetracycline operator (2X $TetO_2$) sequences: bases 820-859
CMV forward priming site: bases 769-789
Multiple cloning site: bases 968-1077
BGH reverse priming site: bases 1089-1106
BGH polyadenylation sequence: bases 1095-1319
SV40 promoter and origin: bases 1820-2128
Hygromycin resistance gene: bases 2185-3210
SV40 early polyadenylation sequence: bases 3340-3470
pUC origin: bases 3853-4526 (complementary strand)
bla promoter: bases 5532-5630 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4671-5531 (complementary strand)

Continued on next page

Features of pcDNA™5/TO

pcDNA™5/TO is a 5667 bp vector that expresses your gene of interest under the control of a hybrid CMV/TetO_2 promoter. The table below describes the relevant features of pcDNA™5/TO. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate early promoter	Allows high-level expression of your gene of interest (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
CMV Forward priming site	Allows sequencing in the sense orientation
Tetracycline operator (O_2) sequences	Two tandem 19 nucleotide repeats which serve as binding sites for Tet repressor homodimers (Hillen and Berens, 1994)
Multiple cloning site	Allows insertion of your gene of interest
BGH Reverse priming site	Allows sequencing of the non-coding strand
Bovine growth hormone (BGH) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
SV40 early promoter and origin	Allows efficient, high-level expression of the hygromycin resistance gene in mammalian cells and episomal replication in cells expressing SV40 large T antigen
Hygromycin resistance gene (expressed from the SV40 early promoter)	Allows selection of stable transfectants in mammalian cells (Gritz and Davies, 1983)
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene
Ampicillin (*bla*) resistance gene (β-lactamase)	Allows selection of transformants in *E. coli*

pcDNA™5/TO/lacZ Vector

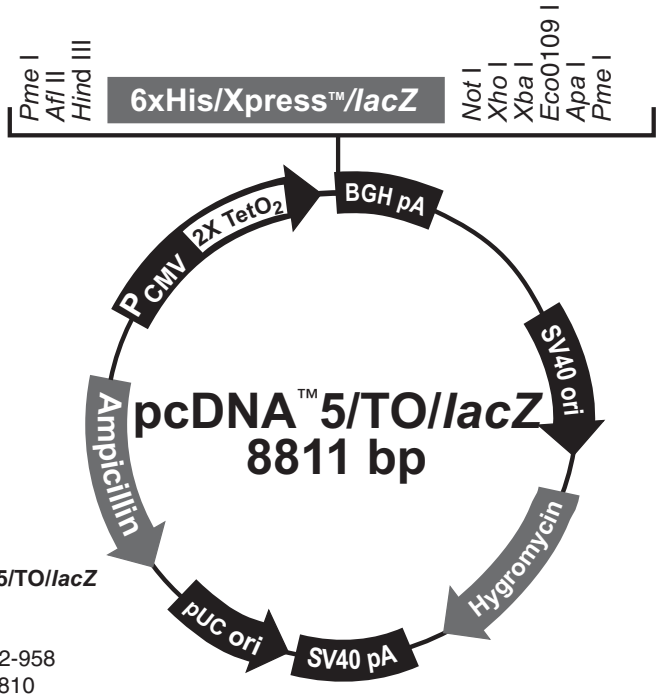
Description

pcDNA™5/TO/*lacZ* is a 8811 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3.1 kb *Hin*d III-*Pst* I fragment containing the *lacZ* gene from pcDNA™3/His/*lacZ* into the *Hin*d III-*Pst* I site of pcDNA™5/TO.

Note: The *lacZ* gene is fused to an N-terminal peptide containing an ATG initiation codon, a polyhistidine (6xHis) tag, and the Xpress™ epitope. The size of the β-galactosidase fusion protein is approximately 120 kDa in size.

Map of pcDNA™5/TO/*lacZ*

The figure below summarizes the features of the pcDNA™5/TO/*lacZ* vector. **The complete nucleotide sequence for pcDNA™5/TO/*lacZ* is available for downloading from www.lifetechnologies.com or from Technical Support (see page 16).**



Comments for pcDNA™5/TO/*lacZ*
8811 nucleotides

CMV promoter: bases 232-958
  TATA box: bases 804-810
  Tetracycline operator (2X $TetO_2$) sequences: bases 820-859
CMV forward priming site: bases 769-789
Polyhistidine (6xHis) tag: bases 999-1016
Xpress™ epitope: bases 1056-1079
LacZ ORF: bases 1104-4154
BGH reverse priming site: bases 4235-4252
BGH polyadenylation sequence: bases 4241-4465
SV40 promoter and origin: bases 4966-5275
Hygromycin resistance gene: bases 5332-6357
SV40 early polyadenylation sequence: bases 6487-6617
pUC origin: bases 7000-7673 (complementary strand)
bla promoter: bases 8676-8774 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 7815-8675 (complementary strand)

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com.**

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

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.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

References

Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989). Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. J. Biol. Chem. *264*, 8222-8229.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985). A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. Cell *41*, 521-530.

Goodwin, E. C., and Rottman, F. M. (1992). The 3´-Flanking Sequence of the Bovine Growth Hormone Gene Contains Novel Elements Required for Efficient and Accurate Polyadenylation. J. Biol. Chem. *267*, 16330-16334.

Gossen, M., and Bujard, H. (1992). Tight Control of Gene Expression in Mammalian Cells by Tetracycline-Responsive Promoters. Proc. Natl. Acad. Sci. USA *89*, 5547-5551.

Gritz, L., and Davies, J. (1983). Plasmid-Encoded Hygromycin-B Resistance: The Sequence of Hygromycin-B-Phosphotransferase Gene and its Expression in *E. coli* and *S. Cerevisiae*. Gene *25*, 179-188.

Hillen, W., and Berens, C. (1994). Mechanisms Underlying Expression of Tn*10* Encoded Tetracycline Resistance. Annu. Rev. Microbiol. *48*, 345-369.

Hillen, W., Gatz, C., Altschmied, L., Schollmeier, K., and Meier, I. (1983). Control of Expression of the Tn*10*-encoded Tetracycline Resistance Genes: Equilibrium and Kinetic Investigations of the Regulatory Reactions. J. Mol. Biol. *169*, 707-721.

Kozak, M. (1987). An Analysis of 5´-Noncoding Sequences from 699 Vertebrate Messenger RNAs. Nucleic Acids Res. *15*, 8125-8148.

Kozak, M. (1991). An Analysis of Vertebrate mRNA Sequences: Intimations of Translational Control. J. Cell Biology *115*, 887-903.

Kozak, M. (1990). Downstream Secondary Structure Facilitates Recognition of Initiator Codons by Eukaryotic Ribosomes. Proc. Natl. Acad. Sci. USA *87*, 8301-8305.

Miller, J. H. (1972). Experiments in Molecular Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987). Negative and Positive Regulation by a Short Segment in the 5´-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. Molec. Cell. Biol. *7*, 4125-4129.

Palmer, T. D., Hock, R. A., Osborne, W. R. A., and Miller, A. D. (1987). Efficient Retrovirus-Mediated Transfer and Expression of a Human Adenosine Deaminase Gene in Diploid Skin Fibroblasts from an Adenosine-Deficient Human. Proc. Natl. Acad. Sci. U.S.A. *84*, 1055-1059.

Continued on next page

References, continued

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Yao, F., Svensjo, T., Winkler, T., Lu, M., Eriksson, C., and Eriksson, E. (1998). Tetracycline Repressor, tetR, Rather than the tetR-Mammalian Cell Transcription Factor Fusion Derivatives, Regulates Inducible Gene Expression in Mammalian Cells. Hum. Gene Ther. *9*, 1939-1950.

©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Notes

Notes

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.invitrogen.com/support or email techsupport@invitrogen.com

www.lifetechnologies.com

life technologies™