pcDNA3.1(+)
pcDNA3.1(-)

Catalog nos. V790-20 and V795-20, respectively

Version I
081401
28-0104

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Table of Contents

Important Information

Contents

pcDNA3.1 is supplied as follows:

Catalog no.	Contents
V790-20	20 µg pcDNA3.1(+), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0
V795-20	20 µg pcDNA3.1(-), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0

Shipping/Storage

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

Product Qualification

Each of the pcDNA3.1 vectors is 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. The table below lists the restriction enzymes and the expected fragments.

Vector	Restriction Enzyme	Expected Fragments (bp)
pcDNA3.1(+)	*Nhe* I	5428
	Pst I	1356, 4072
	Sac I	109, 5319
pcDNA3.1(-)	*Nhe* I	5427
	Pst I	1363, 4064
	Sac I	169, 5258
pcDNA3.1/CAT	*Nhe* I	6217
	Pst I	2145, 4072
	Sac I	109, 6008

Purchaser Notification

Introduction

Use of pcDNA3.1 is covered under a number of different licenses as described below.

CMV Promoter

Use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned and licensed by the University of Iowa Research Foundation and may be used **for research purposes only**. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation. Inquiries for commercial use should be directed to:

Brenda Akins
University of Iowa Research Foundation (UIRF)
214 Technology Innovation Center
Iowa City, IA 52242
Phone: 319-335-4549

BGH Polyadenylation Signal

The bovine growth hormone (BGH) polyadenylation sequence is licensed under U.S. Patent No. 5,122,458 for research purposes only. "Research purposes" means uses directed to the identification of useful recombinant proteins and the investigation of the recombinant expression of proteins, which uses shall in no event include any of the following:

a. any use in humans of a CLAIMED DNA or CLAIMED CELL;

b. any use in human of protein or other substance expressed or made at any stage of its production with the use of a CLAIMED DNA or a CLAIMED CELL;

c. any use in which a CLAIMED DNA or CLAIMED CELL would be sold or transferred to another party other than Invitrogen, its AFFILIATE, or its SUBLICENSEE;

d. any use in connection with the expression or production of a product intended for sale or commercial use; or

e. any use for drug screening or drug development.

Inquiries for commercial use should be directed to:

Bennett Cohen, Ph.D.
Research Corporation Technologies
101 North Wilmot Road, Suite 600
Tucson, AZ 85711-3335
Tel: 1-520-748-4400
Fax: 1-520-748-0025

Methods

Overview

Introduction

pcDNA3.1(+) and pcDNA3.1(-) are 5.4 kb vectors derived from pcDNA3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Multiple cloning sites in the forward (+) and reverse (-) orientations to facilitate cloning
- Neomycin resistance gene for selection of stable cell lines
- Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA3.1/CAT, is included for use as a positive control for transfection and expression in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA3.1.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 to 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 proper orientation.
5. Transfect your construct into the mammalian cell line of interest using your own method of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.

Cloning into pcDNA3.1

Introduction

Diagrams are provided on pages 3-4 to help you design a cloning strategy for ligating your gene of interest into pcDNA3.1. General considerations for cloning and transformation are listed below.

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, 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 including TOP10F′, DH5α™-T1^R, and TOP10. 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.
One Shot® TOP10F′ (chemically competent cells)	21 x 50 µl	C3030-03
Electrocomp™ TOP10F′	5 x 80 µl	C665-55
Ultracomp™ TOP10F′ (chemically competent cells)	5 x 300 µl	C665-03

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 pcDNA3.1

To propagate and maintain pcDNA3.1, we recommend resuspending the 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 TOP10F′, DH5α™-T1^R, TOP10, or equivalent. Select transformants on LB plates containing 50 to 100 µg/ml ampicillin. Be sure to prepare a glycerol stock of your plasmid-containing *E. coli* strain for long-term storage (see page 5).

Cloning Considerations

pcDNA3.1(+) and pcDNA3.1(-) are nonfusion vectors. Your insert must contain a Kozak translation initiation sequence and an ATG start codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Please note that other sequences are possible (see references above), but the G or A at position -3 and the G at position +4 are the most critical for function (shown in bold). The ATG initiation codon is shown underlined.

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

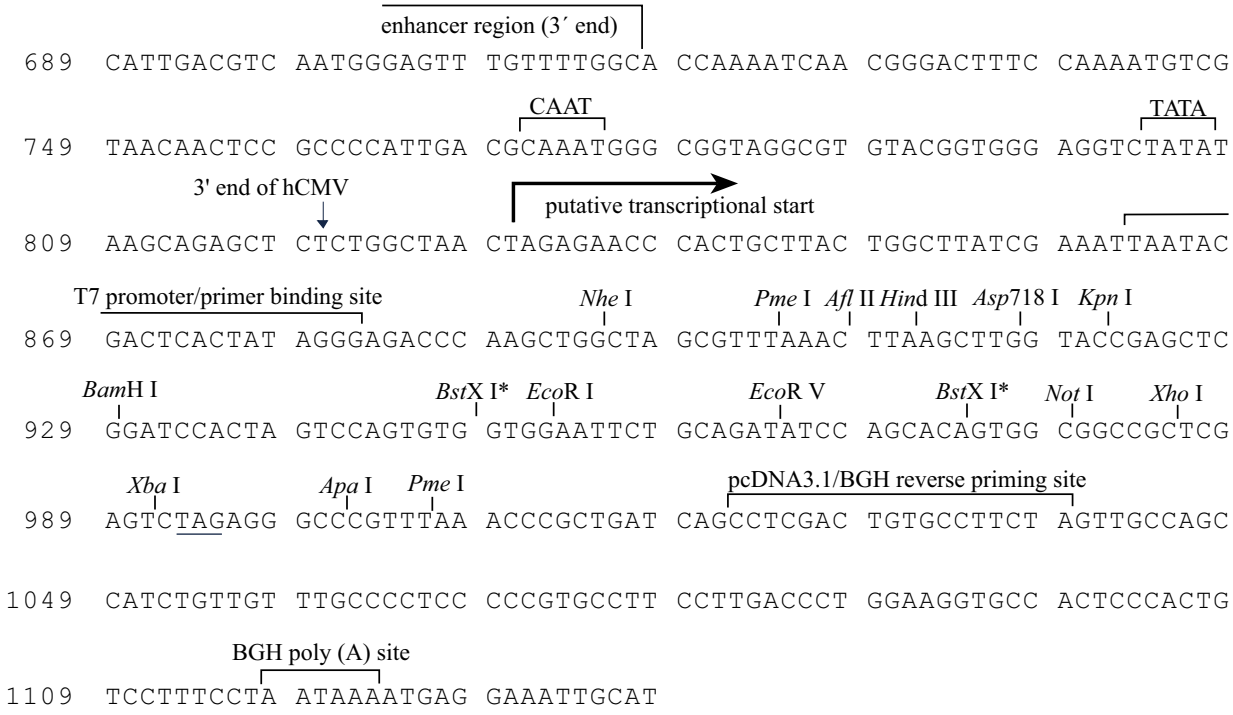
Your insert must also contain a stop codon for proper termination of your gene. Please note that the *Xba* I site contains an internal stop codon (TC<u>TAG</u>A).

continued on next page

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(+)

Below is the multiple cloning site for pcDNA3.1(+). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA3.1(+) is available for downloading from our web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(+), please refer to the **Appendix**, pages 10-11.



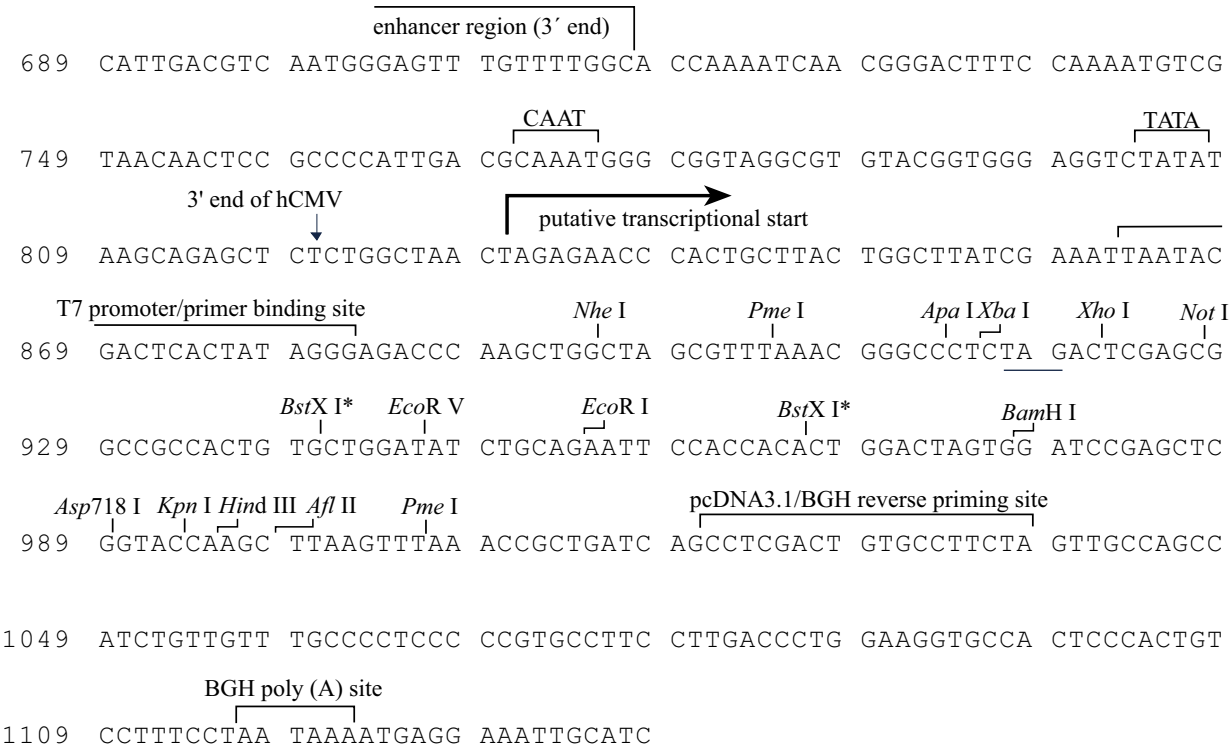
*Please note that there are two *Bst*X I sites in the polylinker.

continued on next page

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(-)

Below is the multiple cloning site for pcDNA3.1(-). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA3.1(-) is available for downloading from our web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(-), please see the **Appendix**, pages 10-11.



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

continued on next page

Cloning into pcDNA3.1, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F´, DH5α™-T1^R, TOP10) and select transformants on LB plates containing 50 to 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 with the T7 Promoter and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and contains an ATG and a stop codon. Please refer to the diagrams on pages 3-4 for the sequences and location of the priming sites. The primers are available separately from Invitrogen in 2 µg aliquots.

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 verified that your gene is cloned in the correct orientation and contains an initiation ATG and a stop codon, 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 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). Invitrogen offers the Calcium Phosphate Transfection Kit (Catalog no. K2780-01) and a large selection of reagents for transfection. For more information, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 13).

Positive Control

pcDNA3.1/CAT is provided as a positive control vector for mammalian transfection and expression (see page 12) and may be used to optimize transfection conditions for your cell line. The gene encoding chloramphenicol acetyl transferase (CAT) is expressed in mammalian cells under the control of the CMV promoter. A successful transfection will result in CAT expression that can be easily assayed (see below).

Assay for CAT Protein

You may assay for CAT expression by ELISA assay, western blot analysis, fluorometric assay, or radioactive assay (Ausubel *et al.*, 1994; Neumann *et al.*, 1987). If you wish to detect CAT protein using western blot analysis, you may use the Anti-CAT Antiserum (Catalog no. R902-25) available from Invitrogen. Other kits to assay for CAT protein using ELISA assay are available from Roche Molecular Biochemicals (Catalog no. 1 363 727) and Molecular Probes (Catalog no. F-2900).

Creation of Stable Cell Lines

Introduction

The pcDNA3.1(+) and pcDNA3.1(-) vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin®). We recommend that you test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

Geneticin® Selective Antibiotic

Geneticin® Selective Antibiotic 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® Selective Antibiotic is available from Invitrogen (Catalog no. 10486-025). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100 to 800 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 (see below). 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 up to 3 weeks of growth in selective media.

Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing your gene of interest from pcDNA3.1, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. We recommend that you test a range of concentrations to ensure that you determine the minimum concentration necessary for your host cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Geneticin® (0, 50, 100, 200, 400, 600, 800 µg/ml Geneticin®).
3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Geneticin® that prevents growth within 2-3 weeks after addition of Geneticin®.

continued on next page

Creation of Stable Cell Lines, continued

Possible Sites for Linearization of pcDNA3.1(+)

Prior to transfection, we recommend that you linearize the pcDNA3.1(+) vector. Linearizing pcDNA3.1(+) will decrease the likelihood of the vector integrating into the genome in a way that disrupts the gene of interest or other elements required for expression in mammalian cells. The table below lists unique restriction sites that may be used to linearize your construct prior to transfection. **Other unique 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
Bgl II	12	Upstream of CMV promoter	Invitrogen, Catalog no. 15213-028
Mfe I	161	Upstream of CMV promoter	New England Biolabs
*Bst*1107 I	3236	End of SV40 polyA	AGS*, Fermentas, Takara, Roche Mol. Biochemicals
*Eam*1105 I	4505	Ampicillin gene	AGS*, Fermentas, Takara
Pvu I	4875	Ampicillin gene	Invitrogen, Catalog no. 25420-019
Sca I	4985	Ampicillin gene	Invitrogen, Catalog no. 15436-017
Ssp I	5309	*bla* promoter	Invitrogen, Catalog no. 15458-011

*Angewandte Gentechnologie Systeme

Possible Sites for Linearization of pcDNA3.1(-)

The table below lists unique restriction sites that may be used to linearize your pcDNA3.1(-) construct prior to transfection. **Other unique 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
Bgl II	12	Upstream of CMV promoter	Invitrogen, Catalog no. 15213-028
Mfe I	161	Upstream of CMV promoter	New England Biolabs
*Bst*1107 I	3235	End of SV40 polyA	AGS*, Fermentas, Takara, Roche Mol. Biochemicals
*Eam*1105 I	4504	Ampicillin gene	AGS*, Fermentas, Takara
Pvu I	4874	Ampicillin gene	Invitrogen, Catalog no. 25420-019
Sca I	4984	Ampicillin gene	Invitrogen, Catalog no. 15436-017
Ssp I	5308	*bla* promoter	Invitrogen, Catalog no. 15458-011

*Angewandte Gentechnologie Systeme

continued on next page

Creation of Stable Cell Lines, continued

Selection of Stable Integrants

Once you have determined the appropriate Geneticin® concentration to use for selection in your host cell line, you can generate a stable cell line expressing your gene of interest.

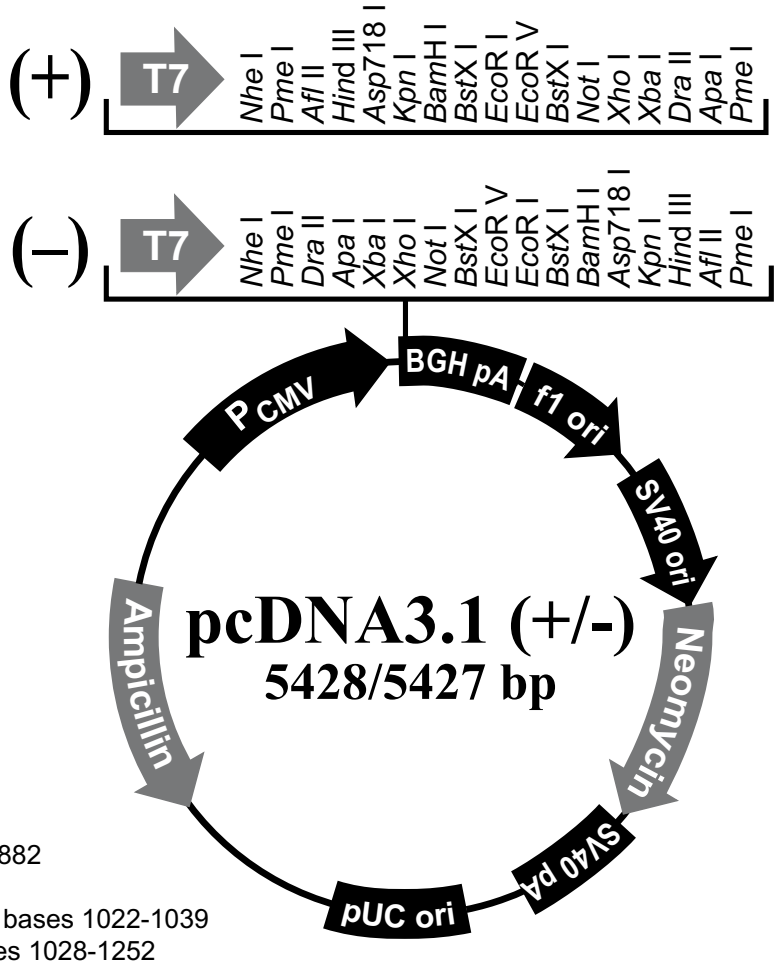
1. Transfect your mammalian host cell line with your pcDNA3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA3.1/CAT plasmid as a positive control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
4. Feed the cells with selective medium every 3-4 days until Geneticin®-resistant foci can be identified.
5. Pick and expand colonies in 96- or 48-well plates.

Appendix

pcDNA3.1 Vectors

Map of pcDNA3.1(+) and pcDNA3.1(-)

The figure below summarizes the features of the pcDNA3.1(+) and pcDNA3.1(-) vectors. **The complete sequences for pcDNA3.1(+) and pcDNA3.1(-) are available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (see page 13).** Details of the multiple cloning sites are shown on page 3 for pcDNA3.1(+) and page 4 for pcDNA3.1(-).



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

continued on next page

pcDNA3.1 Vectors, continued

Features of pcDNA3.1(+) and pcDNA3.1(-)

pcDNA3.1(+) (5428 bp) and pcDNA3.1(-) (5427 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits 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 forward or reverse orientation	Allows insertion of your gene and facilitates cloning
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 resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 early 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*

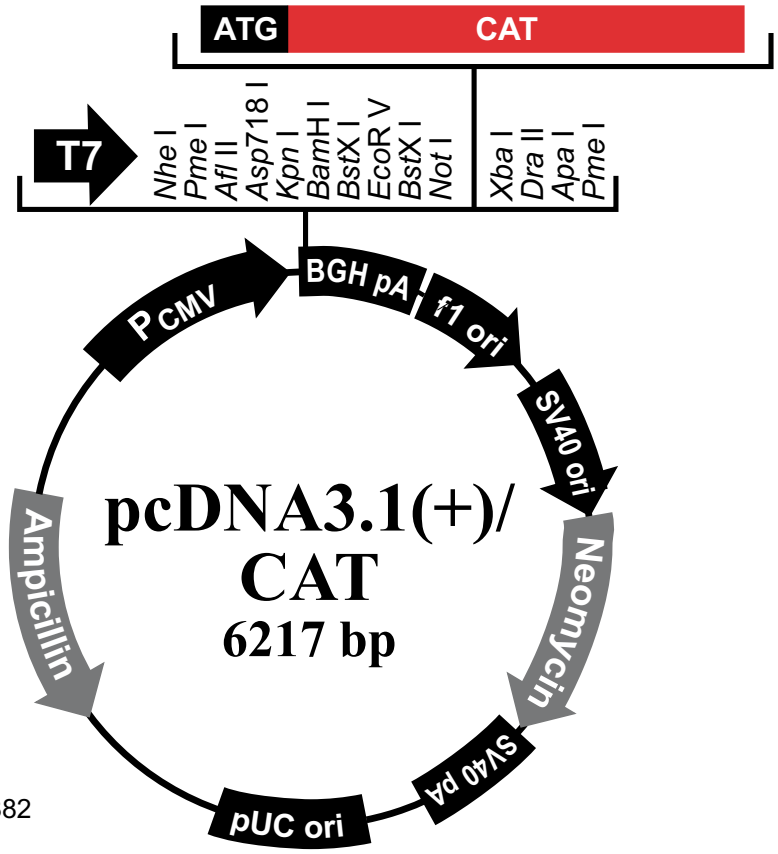
pcDNA3.1/CAT

Description

pcDNA3.1/CAT is a 6217 bp control vector containing the gene for CAT. It was constructed by digesting pcDNA3.1(+) with *Xho* I and *Xba* I and treating with Klenow. An 800 bp *Hin*d III fragment containing the CAT gene was treated with Klenow and then ligated into pcDNA3.1(+).

Map of Control Vector

The figure below summarizes the features of the pcDNA3.1/CAT vector. **The complete nucleotide sequence for pcDNA3.1/CAT is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 13).**



Comments for pcDNA3.1(+)/CAT
6217 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
CAT ORF: bases 1027-1686
pcDNA3.1/BGH reverse priming site: bases 1811-1828
BGH polyadenylation sequence: bases 1817-2041
f1 origin: bases 2087-2515
SV40 early promoter and origin: bases 2520-2863
Neomycin resistance gene (ORF): bases 2925-3719
SV40 early polyadenylation sequence: bases 3893-4023
pUC origin: bases 4406-5076 (complementary strand)
Ampicillin resistance gene (ORF): bases 5221-6081 (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):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact us

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

United States Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
E-mail: eurotech@invitrogen.com

MSDS Requests

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

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

continued on next page

Technical Service, continued

Emergency Information

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

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

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

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.

Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. Mol. Cell. Biol. *7*, 2745-2752.

Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. Nuc. Acids Res. *15*, 1311-1326.

Felgner, P. L., Holm, M., and Chan, H. (1989). Cationic Liposome Mediated Transfection. Proc. West. Pharmacol. Soc. *32*, 115-121.

Felgner, P. L., and Ringold, G. M. (1989). Cationic Liposome-Mediated Transfection. Nature *337*, 387-388.

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.

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

Kozak, M. (1991). An Analysis of Vertebrate mRNA Sequences: Intimations of Translational Control. J. Cell Biol. *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.

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. Mol. Cell. Biol. *7*, 4125-4129.

Neumann, J. R., Morency, C. A., and Russian, K. O. (1987). A Novel Rapid Assay for Chloramphenicol Acetyltransferase Gene Expression. BioTechniques *5*, 444-447.

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

Shigekawa, K., and Dower, W. J. (1988). Electroporation of Eukaryotes and Prokaryotes: A General Approach to the Introduction of Macromolecules into Cells. BioTechniques *6*, 742-751.

Southern, P. J., and Berg, P. (1982). Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter. J. Molec. Appl. Gen. *1*, 327-339.

Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C., and Axel, R. (1977). Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells. Cell *11*, 223-232.