invitrogen™

pcDNA™3.1/Zeo (+)
pcDNA™3.1/Zeo (–)

For high-level stable and transient expression in mammalian hosts

Catalog nos. V860–20 and V865–20

Version J
12 November 2010
28-0110

User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

pcDNA™3.1/Zeo(+/–) vectors are shipped on wet ice. Upon receipt, store vectors at –20°C.

Kit Contents

Each catalog number contains the following vectors. All vectors are supplied in aliquot detailed below. **Store the vectors at –20°C.**

Catalog nos.	Vector	Quantity	Composition (supplied as)
V860–20	pcDNA™3.1/Zeo(+)	20 µg	40 µl of 0.5 µg/µl pcDNA™3.1/Zeo(+) vector in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0
	pcDNA™3.1/Zeo/CAT control	20 µg	40 µl of 0.5 µg/µl pcDNA™3.1/Zeo/CAT control vector in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0
V865–20	pcDNA™3.1/Zeo(–)	20 µg	40 µl of 0.5 µg/µl pcDNA™3.1/Zeo(–) vector in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0
	pcDNA™3.1/Zeo/CAT control	20 µg	40 µl of 0.5 µg/µl pcDNA™3.1/Zeo/CAT control vector in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0

Accessory Products

Introduction

The following additional products may be used with the pcDNA™3.1/Zeo(+/–) vectors. For more information, visit our web site at www.invitrogen.com, or contact **Technical Support** (page 13).

Item	Quantity	Catalog no.
One Shot® TOP10F´ (chemically competent *E. coli*)	20 x 50 µl	C3030–03
One Shot® TOP10 (chemically competent *E. coli*)	10 reactions	C4040–10
One Shot® TOP10 Electrocompetent *E. Coli*	10 reactions 20 reactions	C4040-50 C4040-52
MAX Efficiency® DH10B™ (chemically competent cells)	1 ml	18297–010
T7 Promoter Primer	2 µg	N560–02
BGH Reverse Primer	2 µg	N575–02
S.N.A.P. Miniprep Kit	100 reactions	K1900–01
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100–03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100–04
Zeocin™ Selection Reagent	1 g 5 g	R250–01 R250–05
Lipofectamine™ 2000 Transfection Reagent	15 ml 1.5 ml	11668–500 11668–019

Introduction

Overview

Introduction

pcDNA™3.1/Zeo(+) and pcDNA™3.1/Zeo(–) are 5.0 kb vectors derived from pcDNA™3.1 and are designed for high-level stable and transient expression in mammalian hosts. pcDNA™3.1/Zeo (+/–) is available with the multiple cloning sites in the forward (+) and reverse (–) orientations to facilitate cloning. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. A control plasmid, pcDNA™3.1/Zeo/CAT, is included for use as a positive control for transfection and expression in your cell line of choice.

Features of pcDNA™3.1/ Zeo (+/–)

pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) contain the following features:

- The human cytomegalovirus immediate-early (CMV) promoter provides high-level expression in a wide range of mammalian cells.
- Multiple cloning sites in the forward (+) and reverse (–) orientations to facilitate cloning of your gene of interest.
- The Zeocin™ resistance gene allows selection in both *E. coli* and mammalian cells in the presence of the antibiotic Zeocin™.
- SV40 early promoter allows episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS–7).

CMV Promoter

pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) vectors contain the human CMV immediate early promoter to allow high-level, constitutive expression of the gene of interest in mammalian cells (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987). Although highly active in most mammalian cell lines, activity of the viral promoter can be down-regulated in some cell lines due to methylation (Curradi *et al.*, 2002), histone deacetylation (Rietveld *et al.*, 2002), or both.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™3.1/Zeo(+/–).

1. Consult the multiple cloning sites (pages 3–4) to design a strategy to clone your gene into pcDNA™3.1/Zeo (+) or pcDNA™3.1/Zeo (–).
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50–100 µg/ml ampicillin or Low Salt LB plates containing 25 µg/ml Zeocin™ (see page 9 for recipe).
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.

Continued on next page

Methods

Cloning into pcDNA™3.1/Zeo (+/–)

Introduction

To recombine your gene of interest into pcDNA™3.1/Zeo (+/–), you will need to ligate your gene of interest into either pcDNA™3.1/Zeo (+) or pcDNA™3.1/Zeo (–). Diagrams of the multiple cloning sites for each vector are provided on pages 3–4.

General Molecular Biology Techniques

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

E. coli Strain

Many *E. coli* strains are suitable for the growth of this vector. For the most efficient selection we highly recommended choosing an *E. coli* strain that does not contain the full Tn5 transposon.

Note: Any *E. coli* strain that contains the complete Tn5 transposable element (i.e DH58F´IQ, SURE, SURE2) encodes the *ble* (bleomycin) resistance gene. These strains will be resistant to Zeocin™.

We recommend that you propagate pcDNA™3.1/Zeo in *E. coli* strains that are recombination deficient (recA) and endonuclease A-deficient (endA) such as TOP10F´ and DH10B (page vi).

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™3.1/Zeo

To propagate and maintain pcDNA™3.1/Zeo (+) or pcDNA™3.1/Zeo (–), we recommend that you use 10 ng of the vector to transform a *rec*A, *end*A *E. coli* strain such as TOP10, TOP10F´ DH5α™, or equivalent using your method of choice. Select transformants on LB plates containing 50–100 µg/ml ampicillin or Low Salt LB plates containing 25 µg/ml Zeocin™ (see page 9 for recipe).

For long-term storage of pcDNA™3.1/Zeo (+/–), be sure to prepare a glycerol stock of your plasmid-containing *E. coli* strain (page 5).

Points to Consider Before Recombining into pcDNA™3.1/ Zeo (+/–)

pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) are nonfusion vectors. Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1990; Kozak, 1991). 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)NN<u>ATG</u>**G**

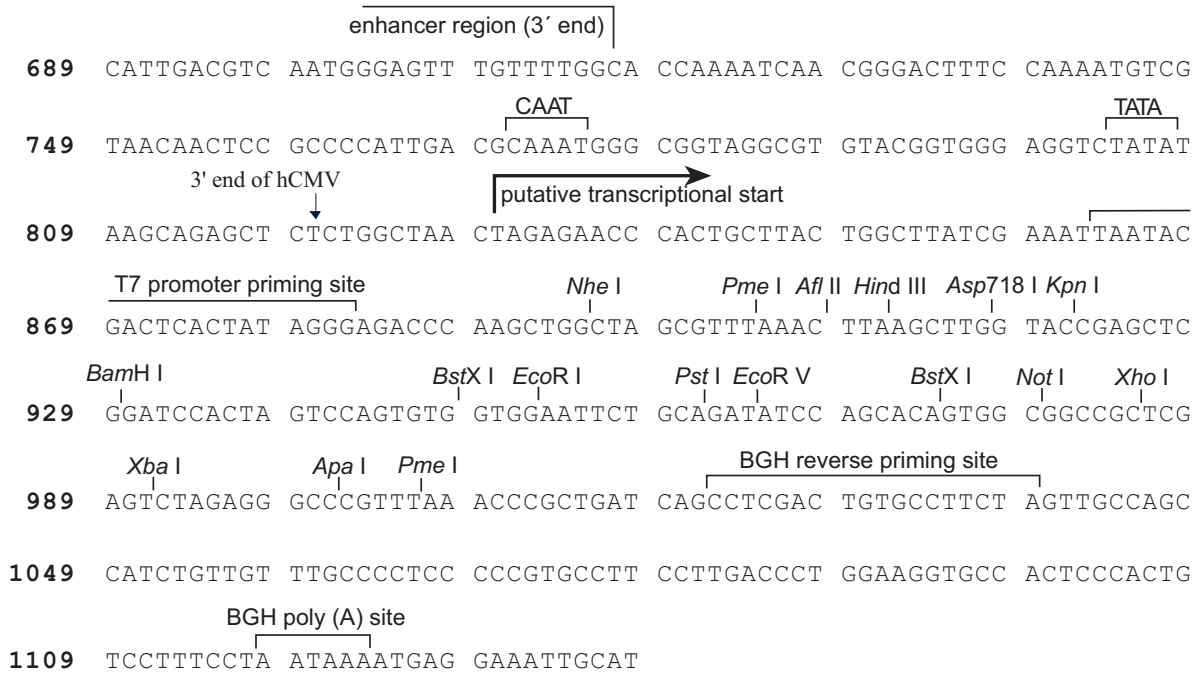
Your insert should also contain a stop codon for proper termination of your gene. Note that the *Xba* I site contains an internal stop codon (TC<u>TAG</u>A).

Continued on next page

Cloning into pcDNA™3.1/Zeo (+/–), Continued

Multiple Cloning Site of pcDNA™3.1/Zeo (+)

Below is the multiple cloning site for pcDNA™3.1/Zeo (+). Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.

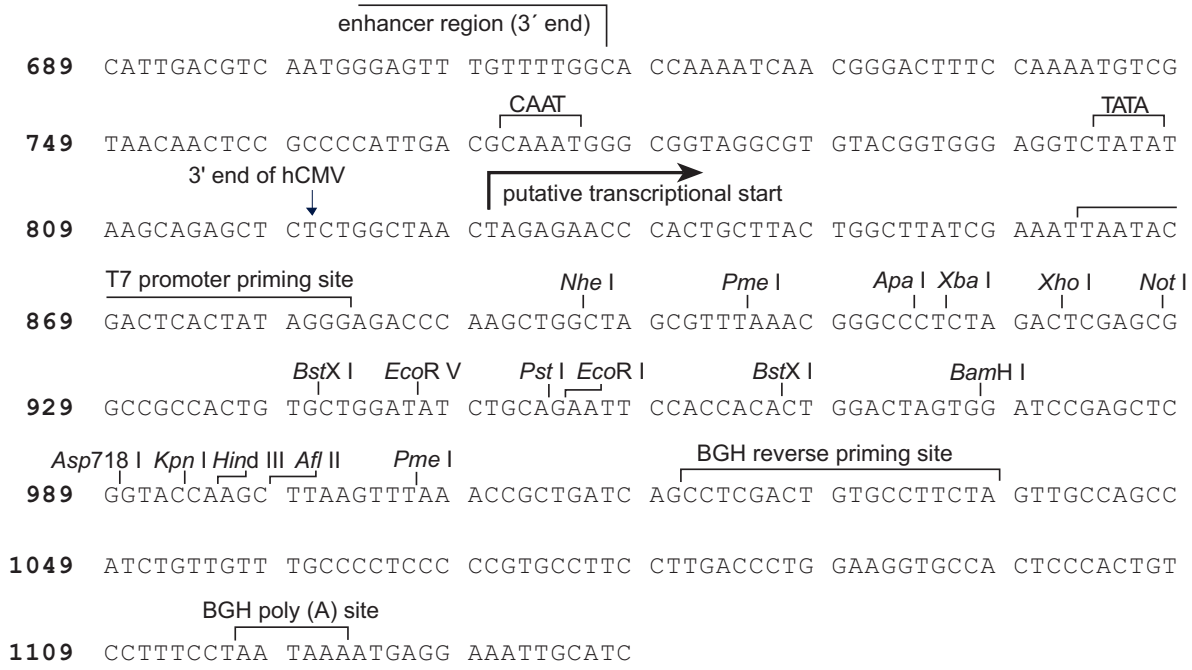


Continued on next page

Cloning into pcDNA™3.1/Zeo (+/–), Continued

Multiple Cloning Site of pcDNA™3.1/Zeo (–)

Below is the multiple cloning site for pcDNA™3.1/Zeo (–). Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.



Continued on next page

E. coli Transformation

1. Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, TOP10F´, DH10B™).
2. Select on LB plates containing 50–100 µg/ml ampicillin or Low Salt LB plates containing 25 µg/ml Zeocin™ (see page 9 for recipe).
3. 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 (page vi) to confirm that your gene is in the correct orientation for expression, and contains an ATG initiation codon and a stop codon. Refer to the multiple cloning sites on pages 3–4 for the sequences and location of the priming sites.

Primer	Sequence
BGH Reverse	5′-TAGAAGGCACAGTCGAGG-3′
T7 Promoter	5´-TAATACGACTCACTATAGGG-3´

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details, or contact **Technical Support** (page 13).

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.

1. Streak the original colony out on an LB plate containing 50–100 µg/ml ampicillin or Low Salt LB plates containing 25 µg/ml Zeocin™ (see page 9 for recipe) Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1-2 ml of LB containing 50–100 µg/ml ampicillin (page 9) or Low Salt LB plates containing 25 µg/ml Zeocin™ (page 9)
3. Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
4. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
5. 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 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 DNA using the PureLink™ HiPure Miniprep Kit or the PureLink™ HiPure Midiprep Kit (page vi) or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended that you follow the protocol for your cell line *exactly*. 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 & Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner & Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa & Dower, 1988). Invitrogen offers the Lipofectamine™ 2000 Transfection Reagent (page vi) as well as a selection of other transfection reagents for your convenience. For more information on available reagents, visit our web site at www.invitrogen.com or contact **Technical Support** (page 13).

Positive Control

pcDNA™3.1/Zeo/CAT is provided as a positive control vector for mammalian transfection and expression (see page 12). It 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 CMV promoter. A successful transfection will result in positive CAT expression and can be easily assayed (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).

Creation of Stable Cell Lines

Introduction

The pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) 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™ belongs to a family of structurally related bleomycin/phleomycin-type antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong antibacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells. Zeocin™ is not as toxic as bleomycin on fungi. As a broad-spectrum antibiotic Zeocin™ is particularly useful, allowing selection in a number of cell types containing vectors with a Zeocin™ resistance gene.

Zeocin™ Mechanism of Action

The exact mechanism of action of Zeocin™ is not known; however, it is thought to be the same as bleomycin and phleomycin due to its similarity to these drugs and its inhibition by the Sh ble resistance protein (see next section). The copper/glycopeptide complex is selective and involves chelation of copper (Cu^{2+}) by the amino group of the 8-carbox-amide, single nitrogen atoms of both the pyrimidine chromophore and the imidazole moiety, and the carbamoyl group of mannose. The copper-chelated form is inactive. When the antibiotic enters the cell, the copper cation is reduced from Cu^{2+} to Cu^{1+} and removed by sulfhydryl compounds in the cell. Upon removal of the copper, Zeocin™ is activated to bind DNA and cleave it causing cell death (Berdy, 1980). High salt concentrations and acidity or basicity inactivate Zeocin™; therefore, it is necessary to reduce the salt in bacterial medium to 90 mM (5 g/liter) or less and adjust the pH to 7.5 to make sure the drug remains active.

Zeocin™ Applications

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 tissue culture cells and *E. coli* are listed below:

Organism	Zeocin™ Concentration and Selective Medium
E. coli	25-50 µg/ml in low salt LB medium*
Mammalian cells	50-1000 µg/ml (depends on cell line)

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

Continued on next page

Determining Antibiotic Sensitivity

To obtain a stable integrant, you must first determine if the cell line in question can grow as an isolated colony. You may already know this for your cell line. If you do not, seed ~100 cells in a 60 mm plate and feed every 4 days for 10–12 days. Count the number of colonies. Growing in soft agar can help cells to grow when they are diluted; however, some cell lines (e.g. NIH3T3) require plating at a certain density in order to grow properly (see Ausubel, *et al.*, 1990).

Next, determine the minimal concentration of Zeocin™ required to prevent growth of the parental cell line using the protocol below:

1. Plate or split a confluent plate so there are approximately 2.5 × 105 cells per 60–100 mm dish. Prepare 7 plates and add varying concentrations of Zeocin™ (0, 50, 125, 250, 500, 750, and 1000 µg/ml) to each plate.
2. Replenish the selective media every 3–4 days, and observe the percentage of surviving cells.
3. Count the number of viable cells at regular intervals to determine the appropriate concentration of Zeocin™ that prevents growth.

Selection of Stable Integrants

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

1. Transfect cells with your construct using the desired protocol and plate. Remember to include a plate of untransformed 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 Zeocin™ at the pre-determined concentration required for your cell line. Split the cells such that the cells are no more than 25% confluent.
4. Feed the cells with selective medium every 3–4 days until foci can be identified.
5. Pick and expand the foci to test for expression of your recombinant protein.

Appendix

Recipes

LB (Luria-Bertani) Medium

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to ~55°C and add antibiotic, if desired.
4. Store at +4°C.

LB Plates Containing Ampicillin

Follow the instructions below to prepare LB agar plates containing ampicillin.

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes.
3. After autoclaving, cool to ~55°C, add ampicillin to a final concentration of 100 µg/ml and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C, in the dark.

Low Salt LB Medium Containing Zeocin™

For Zeocin™ to be active, the salt concentration of the medium must remain low (< 90 mM) and the pH must be 7.5. You must prepare LB broth and plates using the following recipe. Note the lower salt content of this medium.

Failure to lower the salt content of your LB medium will result in non-selection due to inactivation of the drug.

Low Salt LB Medium:

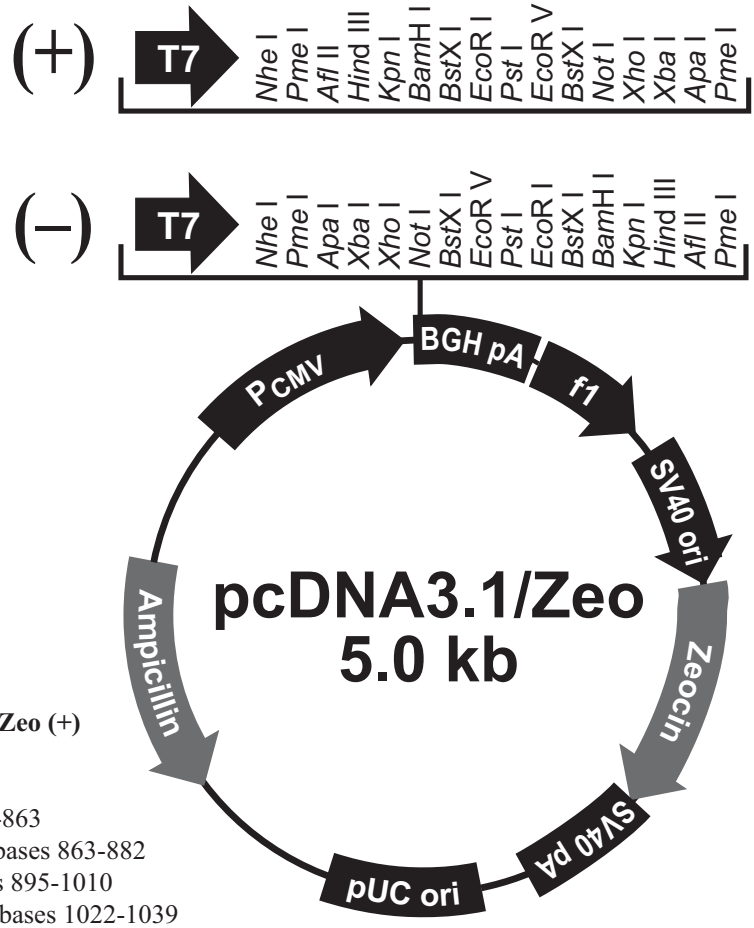
10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 ml. Adjust pH to 7.5 with 1 N 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. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 µg/ml final concentration.
4. Store plates at 4°C in the dark. Plates containing Zeocin™ are stable for 1-2 weeks.

Map of pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) Vectors

Map of pcDNA™3.1/Zeo

The figure below summarizes the features of the pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) vectors. The complete nucleotide sequences for pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) are available for downloading from our web site at www.invitrogen.com or from **Technical Support** (page 13).



Comments for pcDNA3.1/Zeo (+)
5015 nucleotides

CMV promoter: bases 209-863
T7 promoter priming site: bases 863-882
Multiple cloning site: bases 895-1010
BGH reverse priming site: bases 1022-1039
BGH polyadenylation signal: bases 1021-1235
f1 origin: bases 1298-1711
SV40 promoter and origin: bases 1776-2101
EM7 promoter: bases 2117-2183
Zeocin™ resistance gene: bases 2184-2558
SV40 polyadenylation: bases 2688-2817
pUC origin: bases 3201-3874 (C)
bla promoter: bases 4880-4978 (C)
Ampicillin (*bla*) resistance gene: bases 4019-4879 (C)

Features of pcDNA™3.1/Zeo (+) and pcDNA™3.1/Zeo (–) Vectors

Features of pcDNA™3.1/ Zeo (+/–)

pcDNA™3.1/Zeo (+) (5015 bp) and pcDNA™3.1/Zeo (–) (5014 bp) contain the following elements. All features have been functionally tested.

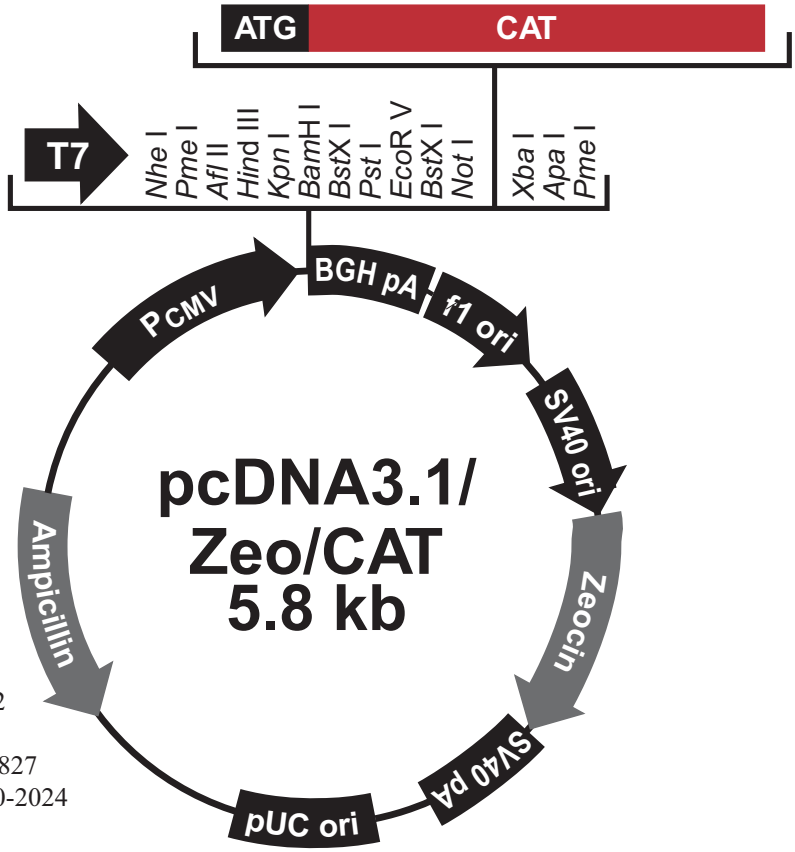
Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
Multiple cloning site in forward or reverse orientation	Allows insertion of your gene and facilitates cloning
BGH reverse priming site	Allows sequencing through the insert in the reverse orientation
Bovine growth hormone (BGH) polyadenylation signal	Permits efficient transcription termination and polyadenylation of mRNA (Goodwin & 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 and episomal replication in cells expressing SV40 large T antigen
EM7 promoter	Permits expression of the Zeocin™ resistance gene in *E. coli*
Zeocin™ resistance gene	Allows selection of transformants in *E. coli* and stable transfectants in mammalian cells (Drocourt, *et al.*, 1990; Mulsant, *et al.*, 1988)
SV40 polyadenylation signal	Permits efficient transcription termination and polyadenylation of mRNA
pUC origin	Permits high-copy number replication and growth in *E. coli*
bla promoter	Permits expression of the ampicillin resistance gene in *E. coli*
Ampicillin resistance gene (β-lactamase)	Allows selection in *E. coli*

Map of pcDNA™3.1/Zeo/CAT

Map of pcDNA™3.1/Zeo/CAT

pcDNA™3.1/Zeo/CAT is a 5803 bp control vector containing the gene for CAT. It was constructed by digesting pcDNA™3.1/Zeo (+) with *Xho* I and *Xba* I and was treated with Klenow. An 800 bp *Hin*d III fragment containing the CAT gene was treated with Klenow and then ligated into pcDNA™3.1/Zeo (+).

The figure below summarizes the features of the pcDNA™3.1/Zeo/CAT vector. The complete nucleotide sequence for pcDNA™3.1/Zeo/CAT is available by downloading it from our web site at www.invitrogen.com or from **Technical Support** (page 13).



Comments for pcDNA3.1/Zeo/CAT
5803 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
CAT ORF: bases 989-1779
BGH reverse priming site: bases 1810-1827
BGH polyadenylation signal: bases 1810-2024
f1 origin: bases 2086-2499
SV40 promoter and origin: bases 2564-2889
Zeocin™ resistance gene: bases 2972-3346
SV40 polyadenylation: bases 3476-3605
pUC origin: bases 3989-4662 (C)
Ampicillin (*bla*) resistance gene: bases 4807-5667 (C)

Technical Support

Web Resources

Visit the Invitrogen web site at www.invitrogen.com for:

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

Contact Us

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

Corporate Headquarters:
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
Invitrogen Japan
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail:
jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS

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

Certificate of Analysis

Product qualification is described in the Certificate of Analysis (CofA), available on our website by product lot number at www.invitrogen.com/cofa.

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, contact our Technical Support Representatives.

Invitrogen warrants that all of its products will perform according to 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 Support 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.

Purchaser Notification

Introduction

Use of the pcDNA™3.1/Zeo (+/–) vectors is covered under a number of different licenses including those detailed below.

Limited Use Label License No. 358: 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*, Greene Publishing Associates and Wiley-Interscience, New York

Baron, M., Reynes, J. P., Stassi, D., and Tiraby, G. (1992) A Selectable Bifunctional β-Galactosidase: Phleomycin-resistance Fusion Protein as a Potential Marker for Eukaryotic Cells. Gene *114*, 239-243

Berdy, J. (1980) in *Amino Acid and Peptide Antibiotics* (Berdy, J., ed) Vol. IV, Part I, pp. 459-497, CRC Press, Boca Raton, FL

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. Nucleic Acids Res. *15*, 1311-1326

Curradi, M., Izzo, A., Badaracco, G., and Landsberger, N. (2002) Molecular Mechanisms of Gene Silencing Mediated by DNA Methylation. Mol. Cell. Biol. *22*, 3157-3173

Drocourt, D., Calmels, T. P. G., Reynes, J. P., Baron, M., and Tiraby, G. (1990) Cassettes of the *Streptoalloteichus hindustanus ble* Gene for Transformation of Lower and Higher Eukaryotes to Phleomycin Resistance. Nucleic Acids Res. *18*, 4009

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

Felgner, P. L. a., 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. Nucleic Acids Res. *15*, 8125-8148

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

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

Mulsant, P., Tiraby, G., Kallerhoff, J., and Perret, J. (1988) Phleomycin Resistance as a Dominant Selectable Marker in CHO Cells. Somat. Cell Mol. Genet. *14*, 243-252

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

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

Perez, P., Tiraby, G., Kallerhoff, J., and Perret, J. (1989) Phleomycin Resistance as a Dominant Selectable Marker for Plant Cell Transformation. Plant Mol. Biol. *13*, 365-373

Continued on next page

References, Continued

Rietveld, L. E., Caldenhoven, E., and Stunnenberg, H. G. (2002) In vivo Repression of an Erythroid-Specific Gene by Distinct Corepressor Complexes. EMBO J. *21*, 1389-1397

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

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

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

©1996–2008, 2010 Invitrogen Corporation. All rights reserved.
For research use only. Not intended for any animal or human therapeutic or diagnostic use.

invitrogen™

Corporate Headquarters
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at **www.invitrogen.com**