pVAX1©

Catalog no. V260-20

Version C
111110
25-0256

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Table of Contents

General Information

Contents	20 µg each of pVAX1© and pVAX1©/*lacZ*, lyophilized in TE, pH 8.0
Shipping/Storage	Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Methods

Overview

Introduction

pVAX1© is a 3.0 kb plasmid vector designed for use in the development of DNA vaccines. The vector was constructed to be consistent with the Food and Drug Administration (FDA) document, "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications", published December 22, 1996 (see **FDA "Points to Consider"** below). Features of the vector allow high-copy number replication in *E. coli* and high-level transient expression of the protein of interest in most mammalian cells (see page 5-6 for more information). The vector contains the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Bovine growth hormone (BGH) polyadenylation signal for efficient transcription termination and polyadenylation of mRNA
- Kanamycin resistance gene for selection in *E. coli*

The control plasmid, pVAX1©/*lac*Z, 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 pVAX1©.

- Consult the multiple cloning site described on page 3 to design a strategy to clone your gene into pVAX1©.
- Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 µg/ml kanamycin.
- Analyze your transformants for the presence of insert by restriction digestion.
- Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the proper orientation.
- Transfect your construct into the mammalian cell line of choice and test for transient expression of your protein of interest.

FDA "Points to Consider"

pVAX1© was constructed by modifying the vector, pcDNA™3.1, to accommodate the following considerations put forth by the FDA Center for Biologics Evaluation and Research (CBER) in its document, "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Diseases Indications" (Docket no. 96N-0400).

- Sequences not necessary for replication in *E. coli* or for expression of recombinant protein in mammalian cells were removed to limit DNA sequences with possible homology to the human genome and to minimize the possibility of chromosomal integration.
- The kanamycin resistance gene was substituted for the ampicillin resistance gene because aminoglycoside antibiotics are less likely to elicit allergic responses in humans.

Cloning into pVAX1©

Introduction

A diagram is provided on the next page to help you design a cloning strategy for ligating your gene of interest into pVAX1©. General considerations for transformation are listed below.

General Molecular Biology Techniques

For help with DNA ligation, *E. coli* transformation, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, please refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) (See **References**, page 11).

E. coli Strain for Transformation

Many *E. coli* strains are suitable for the propagation of this vector, including TOP10, DH5α, and DH10. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A[1]) and endonuclease A deficient (*end*A).

For your convenience, TOP10 is available as chemically competent or electrocompetent cells from Invitrogen.

Description	Reactions	Efficiency	Catalog no.
One Shot® TOP10 Chemically Compentent	20 reactions	1 x 10^9	C4040-03
One Shot® TOP10 Electrocomp™	20 reactions	1 x 10^9	C4040-50
TOP10 Electrocomp™	20 reactions	1 x 10^9	C664-55

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 pVAX1©

To propagate and maintain the pVAX1© plasmid, resuspend the vector in 20 µl sterile water to prepare a 1 µg/µl stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *rec*A[1], *end*A *E. coli* strain like TOP10, DH5α, or equivalent. Select transformants on LB plates containing 50 µg/ml kanamycin. Be sure to prepare a glycerol stock of your plasmid-containing *E. coli* strain for long-term storage (see page 3).

Cloning Considerations

pVAX1© is a nonfusion vector. Your insert must contain a Kozak translation initiation sequence and an initiation codon (ATG) 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 A at position -3 and the G at position +4 are the most critical (shown in bold). The ATG initiation codon is shown underlined.

ANN<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).

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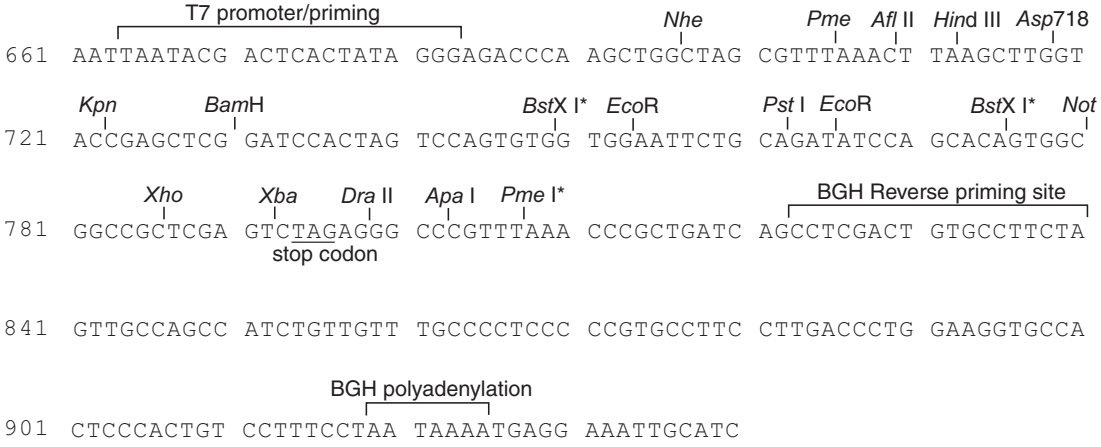
Cloning into pVAX1©, continued

Multiple Cloning Site of pVAX1©

Below is the multiple cloning site for pVAX1©. Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence may be downloaded from our web site (http://www.invitrogen.com)** or from Technical Service (see page 8).



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

Transformation of Ligation Mixture

Transform your ligation mixture into a competent *rec*A[1], *end*A *E. coli* strain (e.g. TOP10, DH5α) and select on LB plates containing 50 µg/ml kanamycin. Select 10-20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the T7 Forward and BGH Reverse primers to confirm that your gene is cloned in the proper orientation for expression and that it contains an ATG and a stop codon. See the diagram above for the sequences and location of the priming sites.

For your convenience, Invitrogen offers the T7 Promoter Primer (Catalog no. N560-02) as well as a custom primer synthesis service. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 8).

Preparing a Glycerol Stock

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

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

Transient Transfection

Introduction

Once you have verified that your gene is cloned in the correct orientation and contains an initiation ATG and a stop codon, then you are ready to transiently transfect your mammalian cell line of choice to check for protein expression. We recommend that you include the positive control vector and a mock transfection (negative control) to evaluate your results.

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipids, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. HeLa), please consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (**Reference** section, page 11).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1987; Felgner *et al.*, 1989), and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). Invitrogen offers the Calcium Phosphate Transfection Kit for mammalian transfection.

Catalog No.	Description	Quantity
K2780-01	Calcium Phosphate Transfection Kit	75 reactions

Positive Control

pVAX1©/*lacZ* is provided as a positive control vector for mammalian transfection and expression (see page 7). It may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the control of the CMV promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see below).

Assay for β-galactosidase Activity

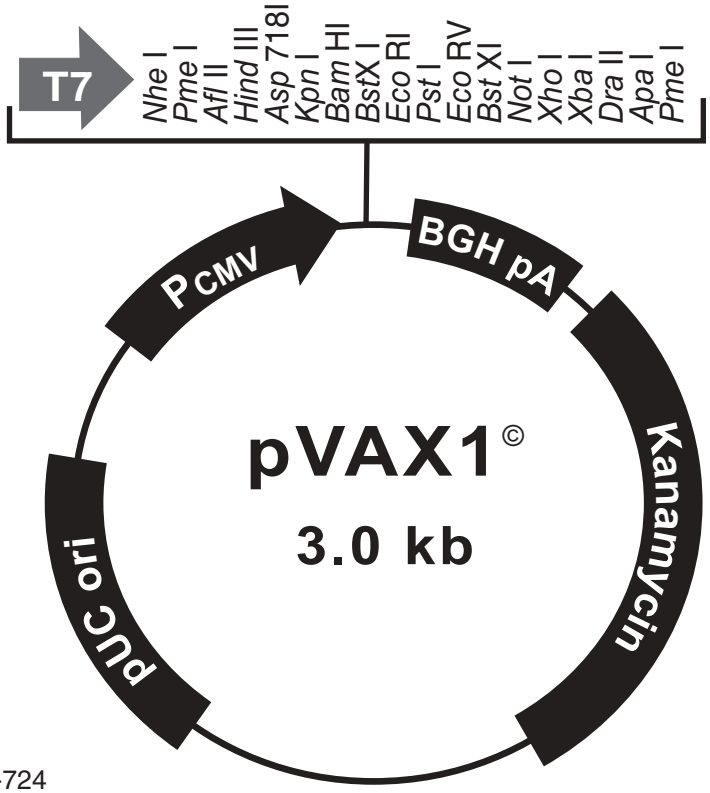
You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit (Catalog no. K1455-01) and the β-Gal Staining Kit (Catalog no. K1465-01) for fast and easy detection of β-galactosidase expression.

Appendix

pVAX1© Vector

Map of pVAX1©

The figure below summarizes the features of the pVAX1© vector. **The sequence for pVAX1© is available for downloading from our World Wide Web site (http://www.invitrogen.com) or from Technical Service (see page 8).**



Comments for pVAX1©:
2999 bp

CMV promoter: bases 137-724
T7 promoter/priming site: bases 664-683
Multiple cloning site: bases 696-811
BGH reverse priming site: bases 823-840
BGH polyadenylation signal: bases 829-1053
Kanamycin resistance gene: bases 1226-2020
pUC origin: bases 2320-2993

continued on next page

pVAX1© Vector, continued

Features of pVAX1©

pVAX1© (2999 bp) contains 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	Allows insertion of your gene and facilitates cloning
BGH reverse priming site	Permits sequencing through the insert
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
Kanamycin resistance gene	Selection of vector in *E. coli* (Davies and Smith, 1978)
pUC origin (pUC-derived)	High-copy number replication and growth in *E. coli* (Bolivar *et al.*, 1977; Bolivar *et al.*, 1977)

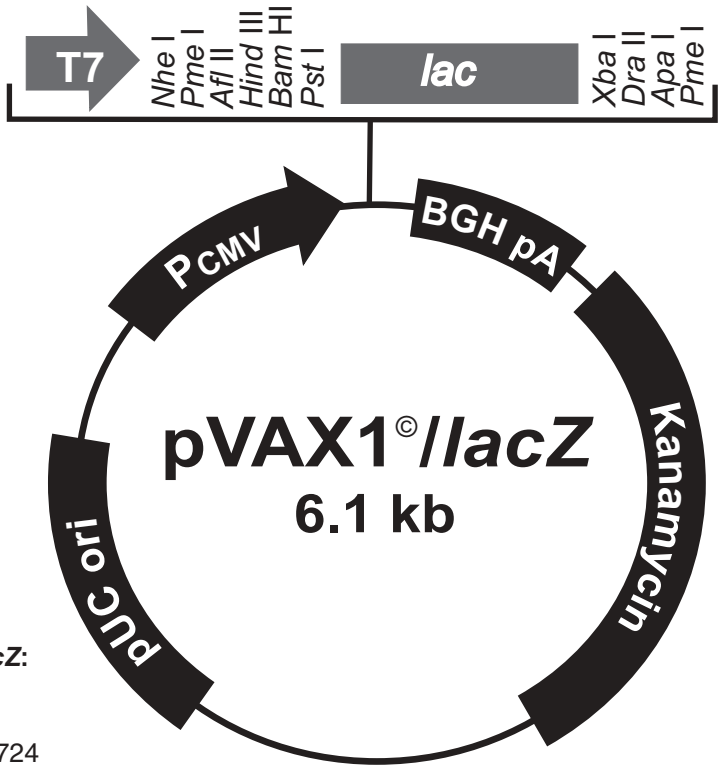
pVAX1©/*lacZ* Map

Description

pVAX1©/*lacZ* is a 6050 bp control vector containing the gene for β-galactosidase. The vector was constructed by cloning a 3.1 kb *Pst* I-*Xba* I fragment containing the *lacZ* gene into the *Pst* I-*Xba* I site of pVAX1©.

Map of Control Vector

The figure below summarizes the features of the pVAX1©/*lacZ* vector. **The complete nucleotide sequence for pVAX1©/*lacZ* is available for downloading from our World Wide Web site (http://www.invitrogen.com) or by contacting Technical Service.** See the next page for more information.



Comments for pVAX1©/*lacZ*: 6050 bp

CMV promoter: bases 137-724
T7 promoter/priming site: bases 664-683
LacZ ORF: bases 773-3829
BGH reverse priming site: bases 3874-3891
BGH polyadenylation signal: bases 3880-4104
Kanamycin resistance gene: bases 4277-5071
pUC origin: bases 5371-6044

Technical Service

World Wide Web

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- Obtain citations for Invitrogen products
- Request catalog and product literature

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Continued on next page

Technical Service, continued

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