pAc5.1/V5-His A, B, and C

Catalog no. V4110-20

Version C
100401
28-0181

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

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Important Information

Contents

20 µg each of pAc5.1/V5-His A, B, and C, lyophilized in TE, pH 8.0
20 µg of pAc5.1/V5-His/*lacZ*, lyophilized in TE, pH 8.0

Shipping/Storage

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

Product Qualification

The pAc5.1/V5-His and pAc5.1/V5-His/*lacZ* vectors are 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 (see below).

Vector	Restriction Enzymes	Expected Results (bp)
pAc5.1/V5-His A	*Apa* I	5375
	*Bst*E II	No site
	Sac II	No site
pAc5.1/V5-His B	*Apa* I	5379
	*Bst*E II	No site
	Sac II	5379
pAc5.1/V5-His C	*Apa* I	No site
	*Bst*E II	5371
	Sac II	No site
pAc5.1/V5-His/*lacZ*	*Apa* I	No site
	*Bam*H I	3267, 5166
	*Bst*E II	8433
	Kpn I	8433
	Sac II	No site

Purchaser Notification

License Information

This product is licensed under patents assigned to SmithKline Beecham Corporation (SB) for research use only. Customers may not sell or transfer this product to any other person. Customers agree to make no commercial use of this product. For further details, please refer to the licensing agreement. A copy of the licensing agreement is available for downloading from our World Wide Web site (www.invitrogen.com) or by calling Technical Service (see page 13).

Please note that you need a license to practice this technology. If you do not have a signed agreement on file, please use the contact information below to obtain a license agreement:

Licensing Coordinator
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
Tel: 760-603-7200
Fax: 760-602-6500

OR

Licensing Coordinator
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel: (0) 800 5345 5345
Fax: +44 (0) 141 814 6287

Accessory Products

Introduction

The products listed in this section are intended for use with the pAc5.1/V5-His vector and the DES® Constitutive Kits. For more information, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 13).

Products Available Separately

The following products may be used with pAc5.1/V5-His and the DES® Constitutive Kits and are available separately from Invitrogen.

Product	Amount	Catalog no.
Ac5 Forward Primer	2 µg, lyophilized in TE	N621-02
BGH Reverse Primer	2 µg, lyophilized in TE	N575-02
Hygromycin B	1 g	R220-05
Blasticidin S HCl	50 mg	R210-01
Schneider (S2) Cells	1 ml vial, 1 x 10^7 cells/ml	R690-07
Schneider's *Drosophila* Medium	500 ml	11720-034
Calcium Phosphate Transfection Kit	75 reactions	K2780-01

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for detection of C-terminal fusion proteins expressed using pAc5.1/V5-His. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

The amount of antibody supplied is sufficient for 25 westerns.

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991) GKPIPNPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection (Lindner *et al.*, 1997) HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

continued on next page

Accessory Products, continued

Purification of Recombinant Protein

The metal binding domain encoded by the polyhistidine tag allows simple, easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using Invitrogen's ProBond™ Resin (see below). To purify proteins expressed from pAc5.1/V5-His, the ProBond™ Purification System or the ProBond™ resin in bulk are available separately. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Metal-Binding Resin	50 ml	R801-01
(precharged resin provided as a 50% slurry in 20% ethanol)	150 ml	R801-15
ProBond™ Purification System (includes six 2 ml precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification)	6 purifications	K850-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
Purification Columns (10 ml polypropylene columns)	50	R640-50

Methods

Overview

Introduction

pAc5.1/V5-His is a 5.4 kb expression vector designed for use with the *Drosophila* Constitutive Expression System (DES®; Catalog nos. K4110-01 and K5110-01) available from Invitrogen. Upon transfection, the vector allows transient expression of your protein of interest in *Drosophila* cells. When cotransfected with the selection vector, pCoHygro or pCoBlast, included with the appropriate DES® Constitutive Kit, pAc5.1/V5-His allows selection of stable cell lines exhibiting constitutive expression of the protein of interest. The pAc5.1/V5-His vector contains the following elements:

- The *Drosophila* actin 5C (Ac5) promoter for high-level, constitutive expression of the gene of interest in S2 cells (Chung and Keller, 1990)
- Multiple cloning site to facilitate cloning the gene of interest
- C-terminal peptide containing the V5 epitope and polyhistidine (6xHis) tag for detection and purification of your protein of interest (if desired)
- Three reading frames to facilitate in-frame cloning with the C-terminal peptide
- Ampicillin resistance gene for selection of transformants in *E. coli*

The control plasmid, pAc5.1/V5-His/*lacZ*, is included for use as a positive control for transfection and expression.

For more information about the DES® Constitutive Kits, pCoHygro, and pCoBlast, refer to the *Drosophila* Expression System manual. The *Drosophila* Expression System manual is supplied with each DES® Constitutive Kit, but is also available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 13).

Description of Ac5 Promoter

The *Drosophila* Ac5 promoter allows high-level, constitutive expression of the gene of interest in *Drosophila* S2 cells. Expression levels are comparable to the induced levels obtained from the metallothionein promoter. The Ac5 promoter is well characterized (Chung and Keller, 1990) and the start of transcription is shown in the diagrams on pages 5-7.

Note

pAc5.1/V5-His is an improved version of pAc5/V5-His. During construction of the original vector, an ATG was inadvertently created upstream of the multiple cloning site. Since it may interfere with correct translation of the cloned gene, this ATG was removed to create pAc5.1/V5-His.

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Overview, continued

Experimental Outline

The table below describes the general steps needed to clone and express your gene of interest. For more details, please refer to the manual and pages indicated.

Step	Action	Source
1	Develop a cloning strategy to ligate your gene of interest into pAc5.1/V5-His A, B, or C in frame with the C-terminal peptide encoding the V5 epitope and the polyhistidine tag (if desired).	Pages 4-7, this manual
2	Transform your ligation reactions into a *rec*A, *end*A *E. coli* strain (e.g. TOP10). Select on LB agar plates containing 50-100 μg/ml ampicillin.	Page 8, this manual
3	Analyze your transformants for the presence of insert by restriction digest.	Page 8, this manual
4	Select a transformant with the correct restriction pattern and sequence it to confirm that your gene is cloned in frame with the C-terminal peptide.	Page 8, this manual
5	Transfect your pAc5.1/V5-His construct into S2 cells.	Page 9, this manual and DES® manual
6	Assay for transient expression of your recombinant protein.	Page 9, this manual and DES® manual
7	To generate stable cell lines, cotransfect your pAc5.1/V5-His construct and pCoHygro or pCoBlast into S2 cells and select for hygromycin resistant clones.	DES® manual
8	Scale up expression for purification.	DES® manual
9	Purify your recombinant protein by chromatography on metal-chelating resin (i.e. ProBond™).	DES® manual

Cloning into pAc5.1/V5-His A, B, and C

Introduction

Diagrams are provided on pages 5-7 to help you clone your gene of interest into pAc5.1/V5-His. General considerations for cloning and transformation are discussed below.

General Molecular Biology Techniques

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

E. coli Strain

Many *E. coli* strains are suitable for the propagation and maintenance of the pAc5.1/V5-His vectors including TOP10 (Catalog no. C610-00) or DH5α™-T1^R. We recommend that you propagate the vectors in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 and DH5α™-T1^R *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot® format from Invitrogen.

Item	Quantity	Catalog no.
One Shot® TOP10 (chemically competent cells)	21 x 50 µl	C4040-03
One Shot® TOP10 Electrocomp™ (electrocompetent cells)	21 x 50 µl	C4040-52
One Shot® DH5α™-T1^R Max Efficiency (chemically competent cells)	21 x 50 µl	12297-016

Transformation Method

You may use any method of your choice for 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 Plasmids

To propagate and maintain the pAc5.1/V5-His and pAc5.1/V5-His/*lacZ* vectors, we recommend resuspending each vector in 20 µl sterile water to prepare a 1 µg/µl stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1^R, or equivalent. Select transformants on LB agar plates containing 50 to 100 µg/ml ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 8).

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Cloning into pAc5.1/V5-His A, B, and C, continued

Cloning Considerations

Consider the following points when designing a strategy to clone your gene of interest into pAc5.1/V5-His.

- Your insert should contain a Kozak translation initiation 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. Note that other sequences are possible, 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)NNATG**G**

- It is possible to clone and express a secreted protein in pAc5.1/V5-His if your protein includes a native signal sequence. If your protein does not have a secretion signal, you may wish to consider using the pMT/BiP/V5-His vector available from Invitrogen for secreted expression. For more information, refer to the DES® manual.
- If you wish to wish to use the V5 epitope and the polyhistidine (6xHis) tag for detection and purification of your recombinant protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. Refer to the diagrams on pages 5-7 to develop a cloning strategy. **Be sure that your gene does not contain a stop codon upstream of the C-terminal peptide.**
- If you do not wish to include the C-terminal peptide, include the native stop codon for your gene of interest.

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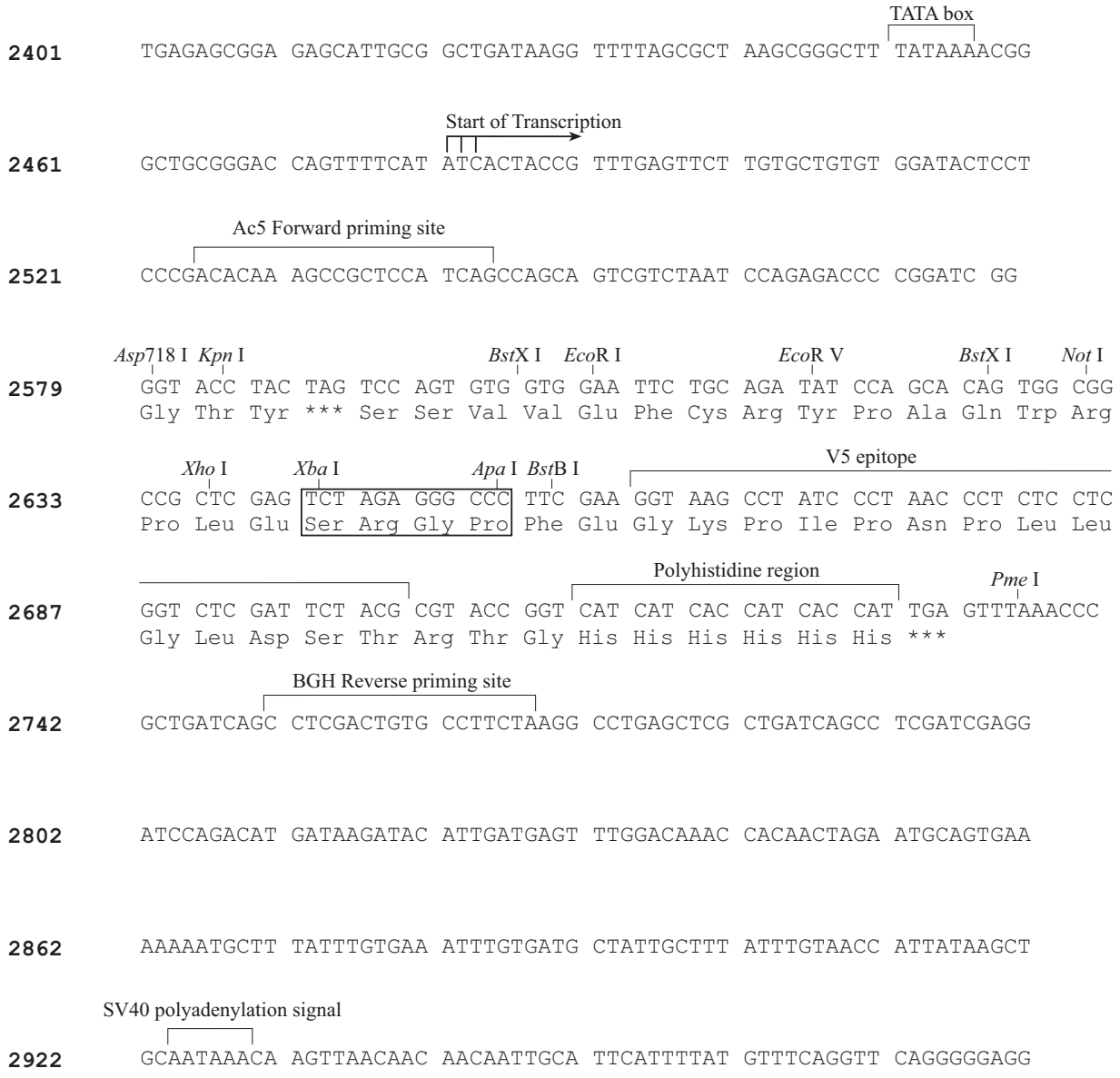
Cloning into pAc5.1/V5-His A, B, and C, continued

Multiple Cloning Site of pAc5.1/V5-His A

Below is the multiple cloning site for pAc5.1/V5-His A. The start of transcription can occur at either nucleotide 2481, 2482, or 2483 (Chung and Keller, 1990). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. **The complete sequence of pAc5.1/V5-His A is available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pAc5.1/V5-His A, refer to pages 10-11.



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

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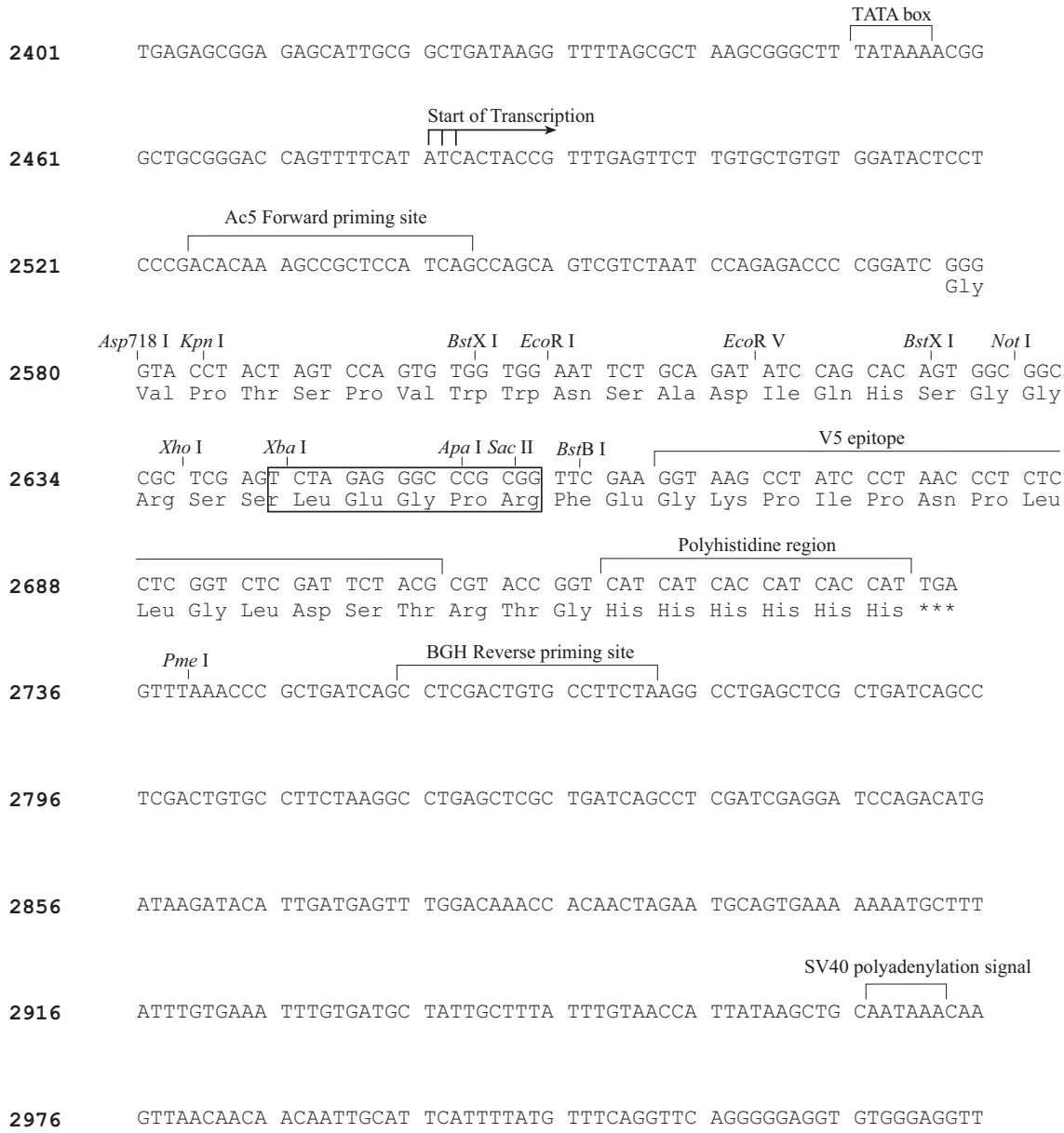
Cloning into pAc5.1/V5-His A, B, and C, continued

Multiple Cloning Site of pAc5.1/V5-His B

Below is the multiple cloning site for pAc5.1/V5-His B. The start of transcription can occur at either nucleotide 2481, 2482, or 2483 (Chung and Keller, 1990). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. **The complete sequence of pAc5.1/V5-His B is available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pAc5.1/V5-His B, refer to pages 10-11.



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

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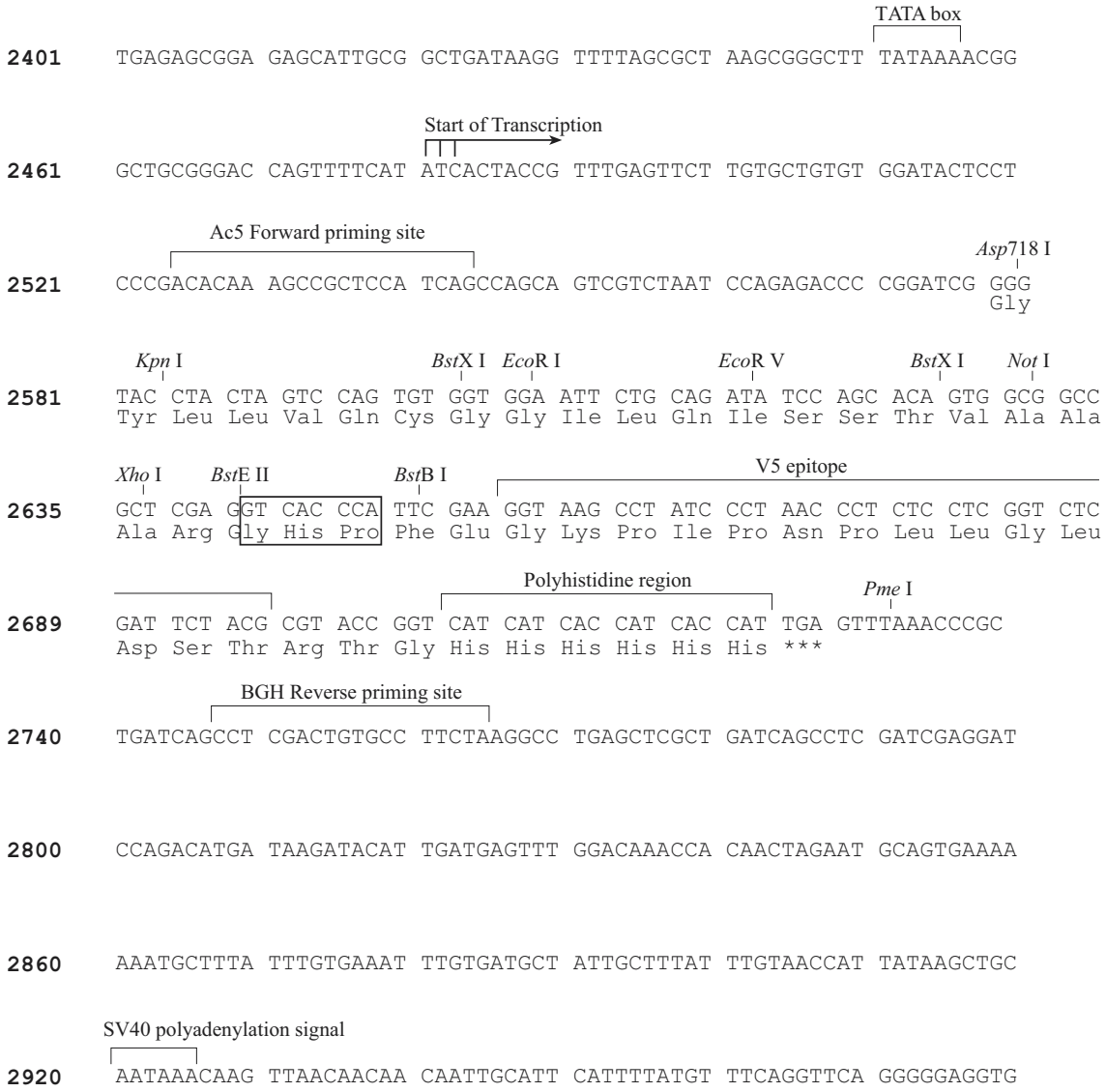
Cloning into pAc5.1/V5-His A, B, and C, continued

Multiple Cloning Site of pAc5.1/V5-His C

Below is the multiple cloning site for pAc5.1/V5-His C. The start of transcription can occur at either nucleotide 2481, 2482, or 2483 (Chung and Keller, 1990). Restriction sites are labeled to indicate the actual cleavage site. The boxed nucleotides indicate the variable region. **The complete sequence of pAc5.1/V5-His C is available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pAc5.1/V5-His C, refer to pages 10-11.



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

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Cloning into pAc5.1/V5-His A, B, and C, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, DH5α™-T1^R) and select on LB agar 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 Ac5 Forward and BGH Reverse primers (Catalog nos. N621-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and is cloned in frame with the C-terminal peptide. The Ac5 Forward and BGH Reverse primers are included in each DES® Constitutive Kit. Please refer to the diagram on pages 5-7 for the sequences and location of the priming sites.

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 µg/ml ampicillin. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin.
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 and Analysis

Introduction

Once you have cloned your gene of interest into pAc5.1/V5-His and have prepared purified plasmid DNA, you are ready to transfect your construct into S2 cells. If you are assaying for transient expression of your gene of interest, you may transfect your pAc5.1/V5-His construct alone into S2 cells. If you wish to generate stable cell lines, you **must** cotransfect your pAc5.1/V5-His construct and pCoHygro or pCoBlast into S2 cells. Note that the pAc5.1/V5-His vector does not contain a resistance marker for selection in *Drosophila* cells. We recommend that you include the pAc5.1/V5-His/*lacZ* positive control vector and a mock transfection (negative control) in your experiments to evaluate your results. Specific guidelines and protocols for transient transfection and generation of stable cell lines can be found in the DES® manual.

Note: Either pCoHygro or pCoBlast is supplied with the appropriate DES® Constitutive Kit. For detailed information about each vector, refer to the DES® manual.

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be clean and free from phenol and sodium chloride. Contaminants will kill the cells, 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

pAc5.1/V5-His/*lacZ* is provided as a positive control vector for *Drosophila* cell transfection and expression (see page 12 for a map) and may be used to optimize transfection conditions for S2 cells. Transfection of pAc5.1/V5-His/*lacZ* results in constitutive β-galactosidase expression. A successful transfection will result in β-galactosidase expression that can be easily assayed by staining with X-gal (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.

Detection and Purification of Recombinant Fusion Proteins

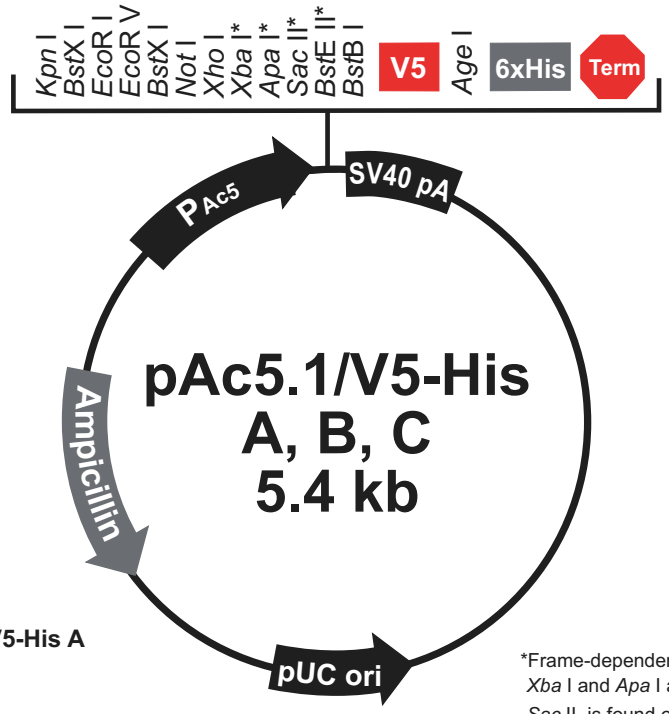
If you have cloned your gene of interest in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use the Anti-V5 antibodies or Anti-His(C-term) antibodies available from Invitrogen to detect expression of your recombinant fusion protein by western blot analysis. The 6xHis tag also allows purification of recombinant protein using metal-chelating resins including ProBond™. Please refer to the DES® manual for more detailed guidelines and instructions to detect and purify your recombinant fusion protein.

Appendix

pAc5.1/V5-His Vector

Map of pAc5.1/V5-His

The figure below summarizes the features of the pAc5.1/V5-His A, B, and C vectors. For a more detailed description of each feature, see the next page. The complete sequences of pAc5.1/V5-His A, B, and C are available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (see page 13).



*Frame-dependent variations.
Xba I and *Apa* I are found only in versions A and B.
Sac II is found only in version B.
*Bst*E II is found only in version C.

Comments for pAc5.1/V5-His A
5375 nucleotides

Ac5 promoter: bases 46-2568
TATA box: bases 2451-2456
Start of transcription: base 2481, 2482, or 2483
Ac5 Forward priming site: bases 2525-2544
Multiple cloning site: bases 2579-2653
V5 epitope: bases 2660-2701
Polyhistidine region: bases 2711-2728
BGH Reverse priming site: bases 2751-2768
SV40 polyadenylation signal bases 2924-2929
pUC origin: bases 3511-4171 (complementary strand)
bla promoter: bases 5177-5275 (complementary strand)
Ampicillin (*bla*) resistance gene (ORF): bases 4316-5176 (complementary strand)

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pAc5.1/V5-His Vector, continued

Features of pAc5.1/V5-His

The features of pAc5.1/V5-His A (5375 bp), pAc5.1/V5-His B (5379 bp), and pAc5.1/V5-His C (5371 bp) are described below. All features have been functionally tested. The multiple cloning site has been tested by restriction enzyme analysis.

Feature	Benefit
Drosophila actin 5C (Ac5) promoter	Permits high-level, constitutive expression of heterologous proteins (Chung and Keller, 1990)
Ac5 Forward priming site	Allows sequencing in the sense orientation
Multiple cloning site	Allows insertion of your gene of interest
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of your recombinant protein with the Anti-V5 Antibodies available from Invitrogen (Southern *et al.*, 1991)
Polyhistidine (6xHis) tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the C-terminal 6xHis tag is the epitope for the Anti-His(C-term) Antibodies available from Invitrogen (Lindner *et al.*, 1997)
BGH Reverse priming site	Permits sequencing of the non-coding strand
SV40 late polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Angelichio *et al.*, 1991)
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)	Permits selection of transformants in *E. coli*

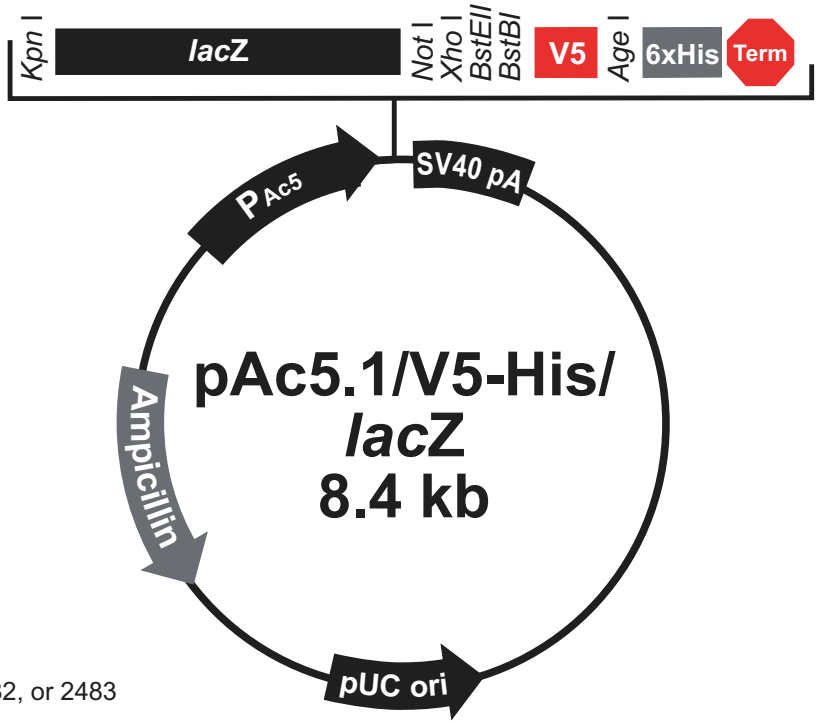
pAc5.1/V5-His/*lacZ* Vector

Description

pAc5.1/V5-His/*lacZ* is a 8433 bp control vector expressing β-galactosidase. The plasmid was constructed by ligating a 3.2 kb *Kpn* I-*Age* I fragment containing the *lacZ* gene and the V5 epitope in frame with the polyhistidine tag into pAc5.1/V5-His B.

Map of pAc5.1/V5-His/*lacZ*

The figure below summarizes the features of the pAc5.1/V5-His/*lacZ* vector. **The complete nucleotide sequence for pAc5.1/V5-His/*lacZ* is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 13).**



Comments for pAc5.1/V5-His/*lacZ*
8433 nucleotides

Ac5 promoter: bases 46-2568
TATA box: bases 2451-2456
Start of transcription: base 2481, 2482, or 2483
Ac5 Forward priming site: 2525-2544
β-galactosidase portion of fusion: bases 2634-5690
V5 epitope: bases 5718-5759
Polyhistidine region: bases 5769-5786
BGH Reverse priming site: bases 5809-5826
SV40 polyadenylation signal: bases 5982-5987
pUC origin: bases 6569-7229 (complementary strand)
bla promoter: bases 8235-8333 (complementary strand)
Ampicillin (*bla*) resistance gene (ORF): bases 7374-8234 (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

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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.

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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

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References

Angelichio, P. L., Beck, J. A., Johansen, H., and Ivey-Hoyle, M. (1991). Comparison of Several Promoters and Polyadenylation Signals for Use in Heterologous Gene Expression in Cultured *Drosophila* Cells. Nucleic Acids Res. *19*, 5037-5043.

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).

Chung, Y. T., and Keller, E. B. (1990). Positive and Negative Regulatory Elements Mediating Transcription from the *Drosophila melanogaster* Actin 5C Distal Promoter. Mol. Cell. Biol. *10*, 6172-6180.

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.

Lindner, P., Bauer, K., Krebber, A., Nieba, L., Kremmer, E., Krebber, C., Honegger, A., Klinger, B., Mocikat, R., and Pluckthun, A. (1997). Specific Detection of His-tagged Proteins With Recombinant Anti-His Tag scFv-Phosphatase or scFv-Phage Fusions. BioTechniques *22*, 140-149.

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

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

Southern, J. A., Young, D. F., Heaney, F., Baumgartner, W., and Randall, R. E. (1991). Identification of an Epitope on the P and V Proteins of Simian Virus 5 That Distinguishes Between Two Isolates with Different Biological Characteristics. J. Gen. Virol. *72*, 1551-1557.