invitrogen™

pEF6/V5-His A, B, and C

Catalog no. V961-20

Rev. date: 30 December 2010

Manual part no. 25-0226

MAN0000070

User Manual

Table of Contents

Kit Contents and Storage iv

Introduction 1

Product Overview 1

Methods 2

Cloning into pEF6/V5-His A, B, and C 2

Transfection 6

Creation of Stable Cell Lines 8

Appendix 11

Human EF-1α Promoter 11

pEF6/V5-His Vector 12

pEF6/V5-His/*lac*Z 14

Blasticidin 15

Recipes 16

Accessory Products 17

Technical Support 18

Purchaser Notification 19

References 20

Kit Contents and Storage

Shipping and Storage

pEF6/V5-His vectors are shipped on wet ice. Upon receipt, store vectors at –20°C.

Kit Contents

All vectors are supplied as detailed below. **Store the vectors at –20°C.**

Item	Composition	Amount
pEF6/V5-His A, B, and C	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg
pEF6/V5-His/*lacZ*	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg

Introduction

Product Overview

Description of the System

pEF6/V5-His A, B, and C are 5.8 kb vectors derived from pcDNA™6/V5-His and designed for overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow purification and detection of expressed proteins (see pages 12-13 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human elongation factor 1α-subunit promoter (hEF-1α) for high-level expression across a broad range of species and cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990) (see page 15 for more information).
- Three reading frames to facilitate in-frame cloning with a C-terminal tag encoding the V5 epitope and a polyhistidine metal-binding peptide.
- Blasticidin resistance gene (*bsd*) for selection of stable cell lines.
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g., COS-1, COS-7).

The control plasmid, pEF6/V5-His/*lac*Z, is included for use as a positive control for transfection, expression, purification, and detection in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pEF6/V5-His.

1. Consult the multiple cloning sites described on pages 3-5 to determine which vector (A, B, or C) should be used to clone your gene in frame with the C-terminal V5 epitope and polyhistidine tag.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50 to 100 µg/mL ampicillin (or 50 µg/mL blasticidin).
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and confirm that your gene is in frame with the C-terminal peptide by sequencing.
5. Transfect your construct into the cell line of choice.
6. Test for expression of your recombinant gene by western blot analysis or other functional assay. For antibodies to the V5 epitope or the polyhistidine, C-terminal tag, see page 17.
7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 17 for ordering information).

Methods

Cloning into pEF6/V5-His A, B, and C

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 for Transformation

Many *E. coli* strains are suitable for the growth of this vector including TOP10F´, DH5αF´, JM109, and INVαF´. 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 (see page 17).

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.

Maintaining pEF6/V5-His

To propagate and maintain the pEF6/V5-His vectors, use a small amount of the supplied 0.5 µg/µl stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH5α, JM109, or equivalent. Select transformants on LB plates containing 50 to 100 µg/mL ampicillin (or 50 µg/mL blasticidin). Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 5).

Cloning Considerations

Your insert should contain a Kozak translation initiation sequence 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 (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**

To express your gene as a recombinant fusion protein, you must clone your gene in frame with the C-terminal peptide. The vector is supplied in three reading frames to facilitate cloning. See pages 3-5 to develop a cloning strategy.

If you wish to express your protein WITHOUT the C-terminal peptide, be sure to include a stop codon.

Continued on next page

Cloning into pEF6/V5-His A, B, and C, Continued

Multiple Cloning Site of Version A

Below is the multiple cloning site for pEF6/V5-His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there is a stop codon between the *Spe* I site and the *BstX* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. Download the vector sequence from www.invitrogen.com or contact **Technical Support** (page 18). For details on the hEF-1α promoter, see page 11.

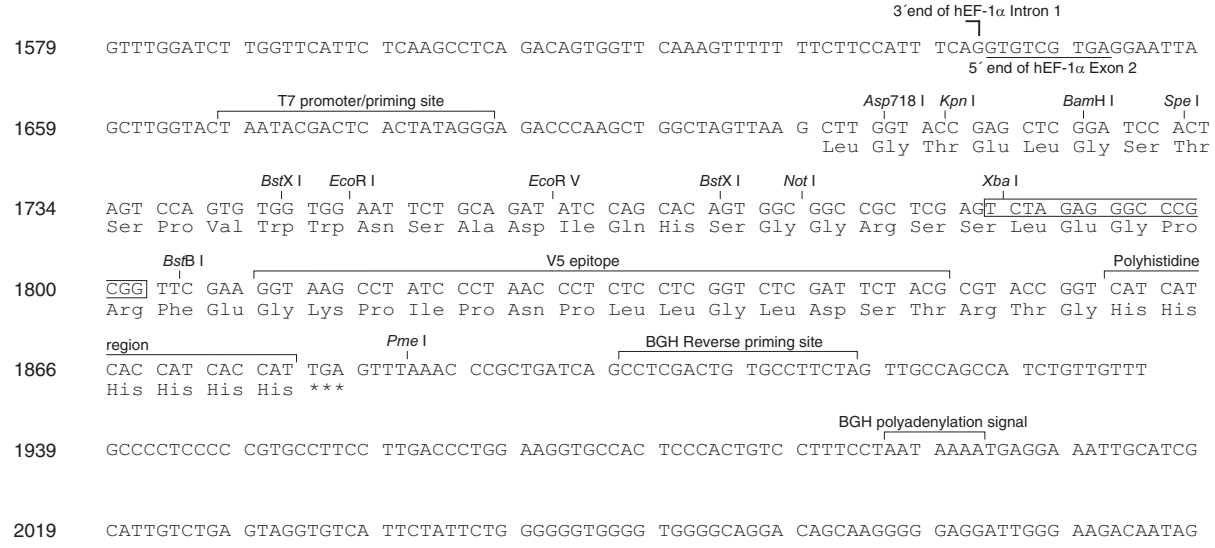
```
                                                                                3´end of hEF-1α Intron 1
1579  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA
                                                                                5´ end of hEF-1α Exon 2

                  T7 promoter/priming site                                  Asp718 I  Kpn I          BamH I     Spe I
1659  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGTTAA GCT TGG TAC CGA GCT CGG ATC CAC
                                                                            Trp Tyr Arg Ala Arg Ile His

                        BstX I   EcoR I              EcoR V           BstX I    Not I                Xba I
1733  TAG TCC AGT GTG GTG GAA TTC TGC AGA TAT CCA GCA CAG TGG CGG CCG CTC GAG TCT AGA GGG CCC
      *** Ser Ser Val Val Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu Glu Ser Arg Gly Pro

      BstB I                          V5 epitope                                                  Polyhistidine
1799  TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC
      Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His

      region              Pme I                    BGH priming site
1865  CAT CAC CAT TGA GT TTAAACCCGC TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG TTGTTTGCCC
      His His His ***

                                                                  BGH polyadenylation signal
1939  CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA TGAGGAAATT GCATCGCATT


2019  GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG
```

Continued on next page

Cloning into pEF6/V5-His A, B, and C, Continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pEF6/V5-His B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. Download the vector sequence from www.invitrogen.com or contact **Technical Support** (page 18). For details on the hEF-1α promoter, see page 11.

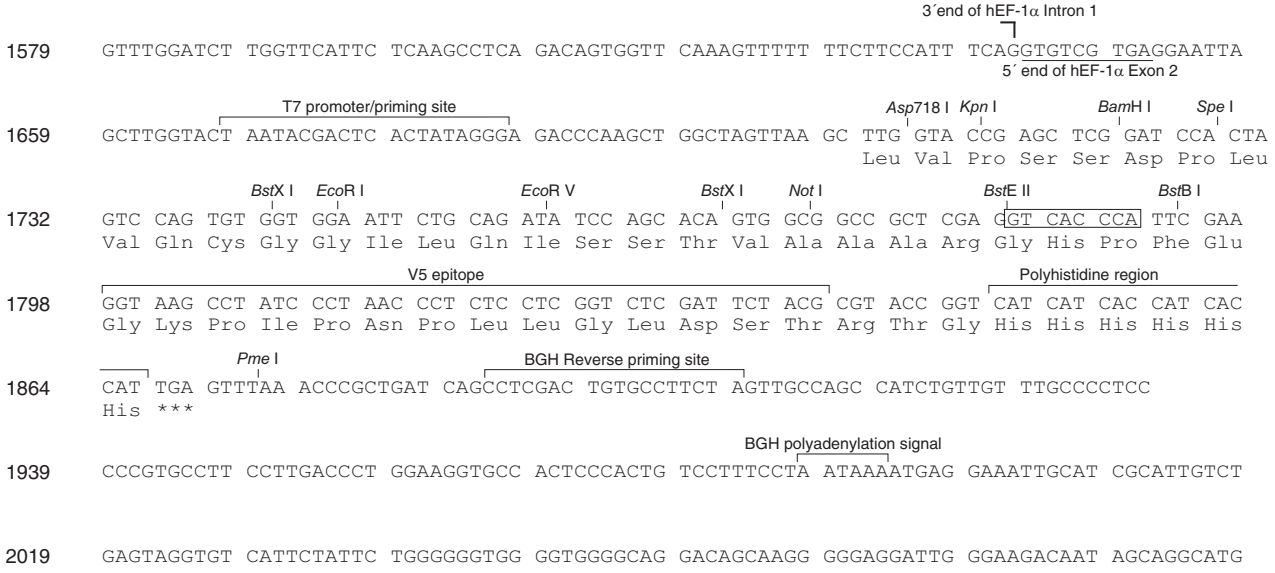


Continued on next page

Cloning into pEF6/V5-His A, B, and C, Continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pEF6/V5-His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. Download the vector sequence from www.invitrogen.com or contact **Technical Support** (page 18). For details on the hEF-1α promoter, see page 11.



Transforming Ligation Mixtures

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F´, DH5α) and select on LB plates containing 50–100 µg/mL ampicillin or 50 µg/mL blasticidin. 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 fused in frame with the V5 epitope and the C-terminal polyhistidine tag. See pages 3-5 for location and sequence of recommended primer binding sites. For ordering information, see page 17. Alternatively, you may design your own primers for sequencing.

Preparing a Glycerol Stock

Once you have identified the correct clone, be sure to 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 in case the glycerol stock dies.

1. Streak the original colony out for single colonies on an LB plate containing 50 µg/mL ampicillin (or blasticidin). 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 (or blasticidin).
3. Grow the culture to mid-log phase (OD600 = 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 confirmed that your construct is in the correct orientation and fused to the C-terminal peptide (if desired), then you are ready to transfect your cell line of choice. We recommend that you include the positive control vector and a mock transfection 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 PureLink™ HiPure Miniprep Kit or the PureLink™ HiPure Midiprep Kit (see page 17 for ordering information).

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 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* (see page 20).

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 Lipofectamine™ 2000 Reagent for mammalian transfection.

Positive Control

pEF6/V5-His/*lacZ* is provided as a positive control vector for mammalian transfection and expression (see page 14). It may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the hEF-1α 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 and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page 17).

Continued on next page

Detecting Fusion Proteins

Several antibodies are available from Invitrogen that can be used to detect expression of your fusion protein from pEF6/V5-His (see page 17).

To detect the fusion protein by Western blot, you will need to prepare a cell lysate from transfected cells. We recommend that you perform a time course to optimize expression of the fusion protein (*e.g.*, 24, 48, 72 hours, etc. after transfection). To lyse cells:

1. Wash cell monolayers (~10^6 cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 mL PBS and pellet the cells at 1,500 × g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (See page 16.).
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Vortex the cell lysate and centrifuge at 10,000 × g for 10 minutes to pellet nuclei. Transfer the post-nuclear lysate to a fresh tube. Assay the lysate for protein concentration.

   Note: Do not use protein assays utilizing Coomassie Blue or other dyes. NP-40 interferes with the binding of the dye with the protein.
6. Add SDS-PAGE sample buffer to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

Note

The C-terminal peptide containing the V5 epitope and the polyhistidine region will add approximately 5 kDa to the size of your protein.

Purification

You will need 5 × 10^6 to 1 × 10^7 of **transfected** cells for purification of your protein on a 2 mL ProBond™ column (or other metal-chelating column). Refer to the manufacturer's instructions before attempting to purify your fusion protein. To prepare cells for lysis, refer to the protocol on page 10.

Creation of Stable Cell Lines

Blasticidin

Blasticidin is used to select stable cell lines transfected with the pEF6/V5-His vectors. See the guidelines below to select stable transfectants. For information on handling and preparing stock solutions of blasticidin, see page 15.

Possible Sites for Linearization

To obtain stable transfectants, you may choose to linearize your vector before transfection. While linearizing your vector may not improve your chances of obtaining stable transfectants, it ensures that the vector does not integrate in a way that disrupts the gene of interest.

The table below lists some unique sites that may be used to linearize your construct prior to transformation. Other restriction sites are possible. To obtain the sequence of any of the pEF6/V5-His vectors and a more extensive restriction list, visit our website (www.invitrogen.com) or call **Technical Support** (see page 18).

Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Restriction Site (bp) (A, B, C)	Location	Supplier
Ssp I	1	Upstream of promoter	Invitrogen (Cat. no. 15458-011)
Aat II	117	Upstream of promoter	Many
Bst1107 I	3746(A) 3750(B) 3742(C)	End of SV40 pA	AGS*, Fermentas, Takara, Boehringer-Mannhiem
Sap I	4004(A) 4008(B) 4000(C)	Backbone	New England Biolabs
Eam1105 I	5015(A) 5019(B) 5011(C)	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	5240(A) 5244(B) 5236(C)	Ampicillin gene	Many
Sca I	5498(A) 5502(B) 5494(C)	Ampicillin gene	Invitrogen (Cat. no. 15436-017)

*Angewandte Gentechnologie Systeme

Continued on next page

Creation of Stable Cell Lines , Continued

Selection in Mammalian Cell Lines

To successfully generate a stable cell line expressing your protein, you need to determine the minimum concentration required to kill your untransfected host cell line. Typically, concentrations between 2 and 10 µg/mL blasticidin are sufficient to kill the untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Seed cells at 60–80% confluency for each time point (~6 time points) and allow the cells to adhere overnight.

   Note: In general, confluent monolayers take about 2–3 times longer to die off when compared to monolayers at 60-80% confluency.
2. The next day, substitute culture medium with medium containing varying concentrations of blasticidin (e.g., 0, 1, 3, 5, 7.5, and 10 µg/mL blasticidin).
3. Feed the cells with selective medium every 3–4 days.
4. Monitor the cells each day. Cells sensitive to blasticidin will round up and detach from the plate. Dead cells will accumulate in the medium.
5. For each time point, harvest the cells and count live cells using trypan blue exclusion. Cell death generally occurs within 7 to 12 days.

Selecting Stable Integrants

Once the appropriate concentration is determined, you can generate a stable cell line with your construct. Colonies can generally be identified in 7 to 10 days with complete selection and expansion in ~2 weeks.

1. Transfect your cells and plate in fresh medium after transfection.
2. 48 hours after transfection, replace medium with medium containing the appropriate concentration of blasticidin.
3. Check cells every day for developing foci.
4. Change medium every 3–4 days until foci are detected (7 to 10 days).
5. Pick and expand foci (1–2 weeks).

Continued on next page

Preparing Cells for Lysis

You will need 5×10^6 to 1×10^7 cells for purification of your protein on a 2 mL ProBond™ column (see ProBond™ Protein Purification manual).

1. Seed cells (from a stable cell line) in five T-75 flasks or 2 to 3 T-175 flasks.
2. Grow the cells in selective medium until they are 80–90% confluent.
3. Harvest the cells by treating with trypsin-EDTA for 2 to 5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 240 × *g* for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at –80°C until needed.

Lysis of Cells

If you are using ProBond™ resin, refer to the ProBond™ Protein Purification manual for details about sample preparation for chromatography.

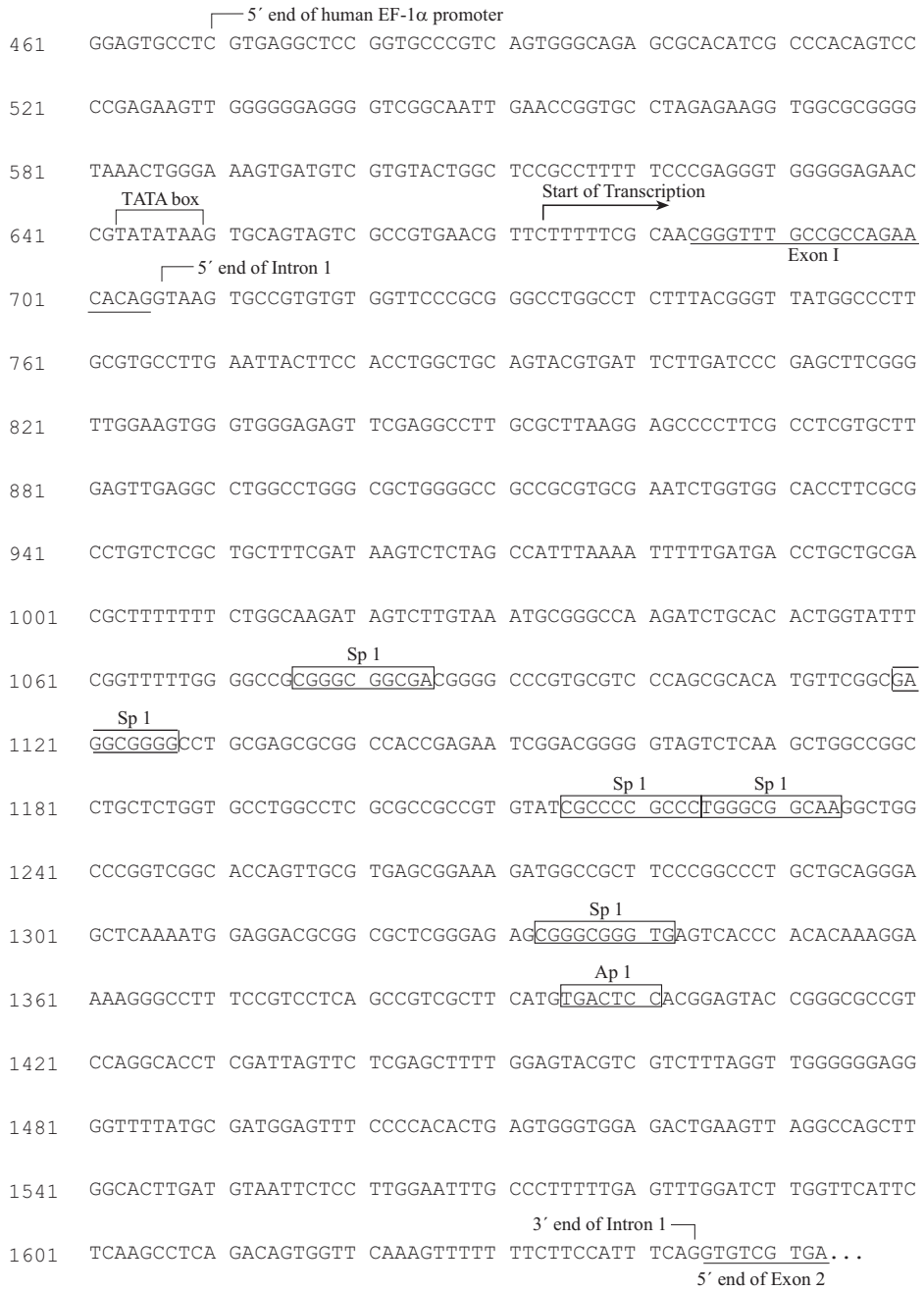
If you are using other metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

Human EF-1α Promoter

Description

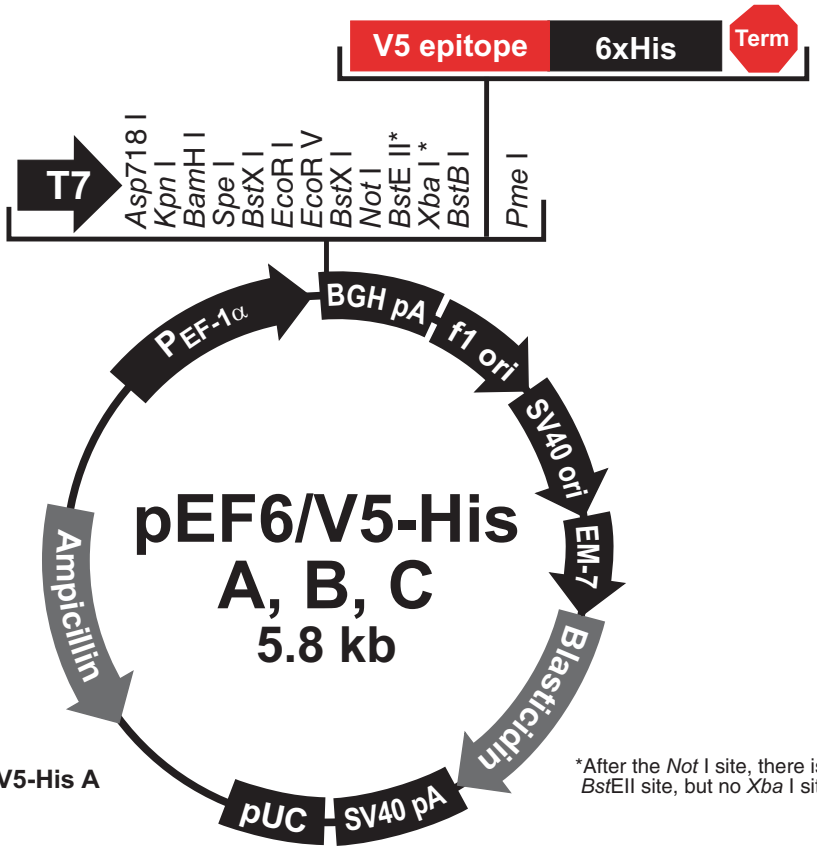
The diagram below shows all the features of the EF-1α promoter used in pEF6/V5-His vectors (Mizushima and Nagata, 1990). Features are marked as per Uetsuki, *et al.*, 1989.



pEF6/V5-His Vector

Map of pEF6/V5-His

The figure below summarizes the features of the pEF6/V5-His vectors. The sequences for pEF6/V5-His A, B, and C are available for downloading from our website (www.invitrogen.com) or from **Technical Support** (see page 18).



*After the *Not* I site, there is a unique *Bst*EII site, but no *Xba* I site in version C.

Comments for pEF6/V5-His A
5818 nucleotides

EF-1α promoter: bases 474-1651
T7 promoter/priming site: bases 1668-1687
Multiple cloning site: bases 1713-1804
V5 epitope: bases 1805-1846
Polyhistidine tag: bases 1856-1873
BGH reverse priming site: bases 1896-1913
BGH polyadenylation sequence: bases 1899-2122
f1 origin of replication: bases 2172-2600
SV40 promoter and origin: bases 2627-2935
EM-7 promoter: bases 2982-3037
Blasticidin resistance gene (ORF): bases 3056-3454
SV40 polyadenylation sequence: bases 3612-3742
pUC origin: bases 4126-4799
Ampicillin resistance gene (ORF): bases 4944-5804 (complementary)

Continued on next page

Features of pEF6/V5-His

pEF6/V5-His A (5818 bp), pEF6/V5-His B (5824 bp), and pEF6/V5-His C (5822 bp) contain the following elements. All features have been functionally tested.

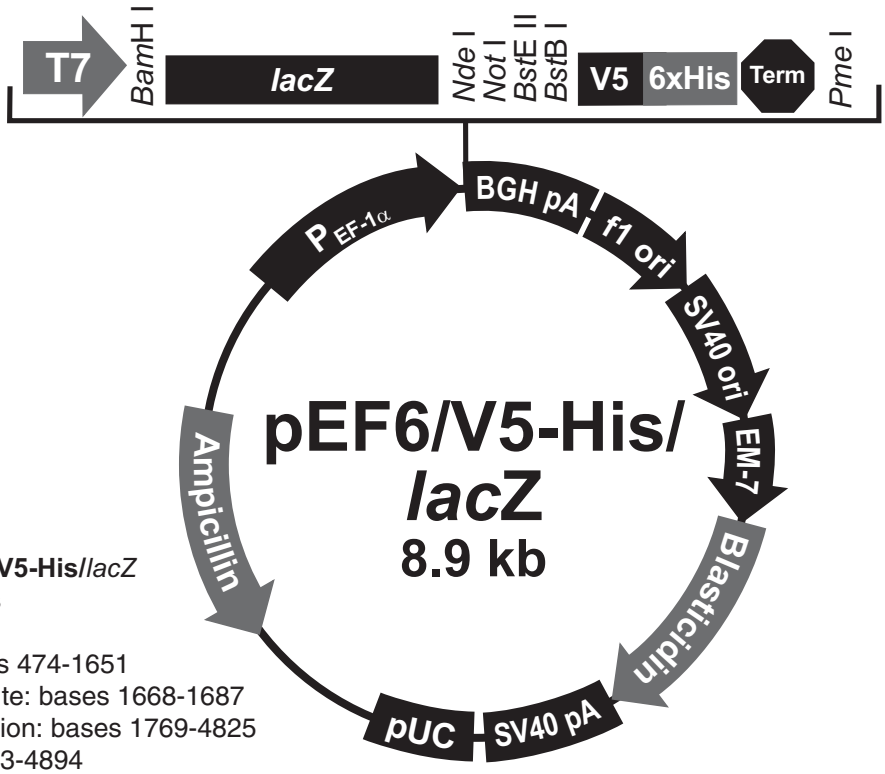
Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Permits overexpression of your recombinant protein in a broad range of mammalian cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990).
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the V5 epitope and polyhistidine C-terminal tag.
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 Antibody, Anti-V5-HRP Antibody or Anti-V5-AP Antibody (Southern *et al.*, 1991) (see page 17 for ordering).
C-terminal polyhistidine tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody, the Anti-His (C-term)-HRP Antibody and the Anti-His(C-term)-AP Antibody (Lindner *et al.*, 1997) (see page 17 for ordering).
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).
f1 origin	Allows rescue of single-stranded DNA.
SV40 early promoter and origin	Allows efficient, high-level expression of the blasticidin resistance gene and episomal replication in cells expressing the SV40 large T antigen.
EM-7 promoter	For expression of the blasticidin resistance gene in *E. coli*.
Blasticidin resistance gene (*bsd*)	Selection of stable transfectants in mammalian cells (Kimura *et al.*, 1994).
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
ColE1 origin (pUC-derived)	High-copy number replication and growth in *E. coli.*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli.*

pEF6/V5-His/*lacZ*

Map of Control Vector

pEF6/V5-His/*lacZ* is a 8855 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3852 bp *Bam*H I-*Bsm* I fragment containing the EF-1α promoter from pEF1/V5-His to a 4416 bp *Bam*H I-*Bsm* I fragment containing the *lacZ* gene, V5 epitope, polyhistidine tag and blasticidin resistance gene from pcDNA™6/V5-His/*lacZ*.

The figure below summarizes the features of the pEF6/V5-His/*lacZ* vector. The complete nucleotide sequence for pEF6/V5-His/*lacZ* is available for downloading from www.invitrogen.com or by contacting **Technical Support** (page 18).



Comments for pEF6/V5-His/*lacZ*
8855 nucleotides

EF-1α promoter: bases 474-1651
T7 promoter/priming site: bases 1668-1687
LacZ portion of the fusion: bases 1769-4825
V5 epitope: bases 4853-4894
Polyhistidine (6xHis) tag: bases 4947-5170
BGH reverse priming site: bases 4945-4962
BGH polyadenylation signal: bases 4948-5175
f1 origin of replication: bases 5220-5648
SV40 promoter and origin: bases 5675-5983
EM-7 promoter: bases 6030-6085
Blasticidin resistance gene: bases 6104-6502
SV40 polyadenylation signal: bases 6663-6793
pUC origin: bases 7174-7836
Ampicillin resistance gene: bases 7981-8841 (complementary)

Blasticidin

Description

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two blasticidin S deaminase genes: *BSD from Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (e.g., a laboratory coat) when handling blasticidin. Weigh out blasticidin and prepare solutions in a hood.

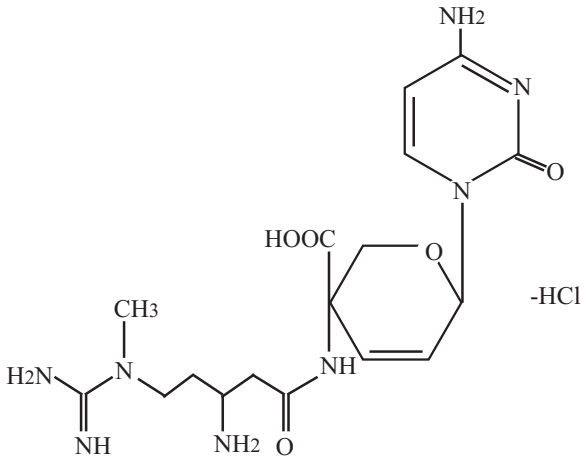
Preparing and Storing Stock Solutions

Blasticidin is soluble in water. Water is generally used to prepare stock solutions of 5 to 10 mg/mL.

- Dissolve blasticidin in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one time use (see last point below) and freeze at –20°C for long-term storage or store at 4°C for short term storage.
- Aqueous stock solutions are stable for 1–2 weeks at 4°C and 6–8 weeks at -20°C.
- pH of the aqueous solution should not exceed 7 to prevent inactivation of blasticidin.
- Do not subject stock solutions to freeze/thaw cycles (do not store in a frost-free freezer).
- Upon thawing, use what you need and discard the unused portion.

Molecular Weight, Formula, and Structure

The formula for blasticidin is $C_{17}H_{26}N_8O_5$-HCl, and the molecular weight is 458.9. The diagram below shows the structure of blasticidin.



Recipe

Cell Lysis Buffer

50 mM Tris
150 mM NaCl
1% Nonidet P-40
pH 7.8

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

1 M Tris base	5 mL
5 M NaCl	3 mL
Nonidet P-40	1 mL

2. Bring the volume to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 mL. Store at room temperature.

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

1 mM PMSF
1 µg/mL Pepstatin
1 µg/mL Leupeptin

Accessory Products

Introduction

The following products may be used with the pcDNA™4/*myc*-His vectors. For details, visit www.invitrogen.com or contact **Technical Support** (page 18).

Item	Amount	Catalog no.
ProBond™ Purification System	6 × 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Lipofectamine™ 2000 Reagent	0.75 mL	11668-027
Electrocomp™ TOP10F´	5 × 80 µL	C665-55
One Shot® TOP10F´ (chemically competent cells)	21 × 50 µL	C3030-03
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01

Antibodies

If you do not have an antibody specific to your protein, Invitrogen offers the Anti-V5, or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)-conjugated antibodies are available for convenient one-step detection.

Antibody	Epitope	Catalog no.
Anti-V5	Detects a 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		R961-25
Anti-V5-AP		R962-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection) (Lindner et al., 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP		R931-25
Anti-His(C-term)-AP		R932-25

Primers

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details.

Technical Support

Web Resources

Visit the Invitrogen website 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 website (www.invitrogen.com).

Corporate Headquarters:
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:
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:
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

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

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Warranty

Invitrogen (a part of Life Technologies Corporation) 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.
All Invitrogen products are warranted to 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 the Company's liability to only the price 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. The Company reserves the right to select the method(s) used to analyze a product unless the Company 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 the Company 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.
Life Technologies Corporation shall have 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

Limited Use Label License No. 22: Vectors and Clones Encoding Histidine Hexamer

This product is licensed under U.S. and/or foreign patents from Hoffmann-LaRoche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from QIAGEN GmbH, Max-Volmer-Str. 4, D-40724 Hilden, Germany.

Limited Use Label License No. 51: Blasticidin and the Blasticidin Selection Marker

Blasticidin and the blasticidin resistance gene (bsd) are the subject of U.S. patents sold under patent license for research purposes only. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. email: outlicensing@invitrogen.com

Limited Use Label License No. 60: EF-1α Promoter

EF-1alpha promoter products are the subject of U.S. and/or foreign patents, and sold under license for research purposes only. The use of this product for any commercial purpose, including but not limited to, use in any study for the purpose of a filing of a new drug application, requires a license from: Mochida Pharmaceutical Co., Ltd., 7, Yotsuya 1-Chome, Shinjuku-Ku, Tokyo 160, Japan. Tel: 81-3-3225-5451; Fax: 81-3-3225-6091.

References

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).

Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. Molec. 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.

Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M., and Danielsen, M. (1987). Lipofectin: A Highly Efficient, Lipid-mediated DNA-transfection Procedure. Proc. Natl. Acad. Sci. USA *84*, 7413-7417.

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

Goldman, L. A., Cutrone, E. C., Kotenko, S. V., Krause, C. D., and Langer, J. A. (1996). Modifications of Vectors pEF-BOS, pcDNA1, and pcDNA3 Result in Improved Convenience and Expression. BioTechniques *21*, 1013-1015.

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.

Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. (1991). Blasticidin S-Resistance Gene (*bsr*): A Novel Selectable Marker for Mammalian Cells. Exper. Cell Res. *197*, 229-233.

Kimura, M., Takatsuki, A., and Yamaguchi, I. (1994). Blasticidin S Deaminase Gene from *Aspergillus terreus* (*BSD*): A New Drug Resistance Gene for Transfection of Mammalian Cells. Biochim. Biophys. ACTA *1219*, 653-659.

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

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

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

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).

Mizushima, S., and Nagata, S. (1990). pEF-BOS, a Powerful Mammalian Expression Vector. Nucleic Acids Res. *18*, 5322.

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.

Continued on next page

References, Continued

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.

Takeuchi, S., Hirayama, K., Ueda, K., Sakai, H., and Yonehara, H. (1958). Blasticidin S, A New Antibiotic. The Journal of Antibiotics, Series A *11*, 1-5.

Uetsuki, T., Naito, A., Nagata, S., and Kaziro, Y. (1989). Isolation and Characterization of the Human Chromosomal Gene for Polypeptide Chain Elongation Factor-1α. J. Biol. Chem. *264*, 5791-5798.

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.

Yamaguchi, H., Yamamoto, C., and Tanaka, N. (1965). Inhibition of Protein Synthesis by Blasticidin S. I. Studies with Cell-free Systems from Bacterial and Mammalian Cells. J. Biochem (Tokyo) *57*, 667-677.

Notes

invitrogen™

Corporate Headquarters
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**