invitrogen™

pEF1/His A, B, and C

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User Manual

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Kit Contents and Storage

Shipping and Storage

pEF1/His vectors are shipped on wet ice. Upon receipt, store vectors at –20ºC.

Contents

20 µg each of pEF1/His A, B, and C are supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

20 µg of pEF1/His/*lac*Z is supplied at 0.5 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 in a total volume of 40 µL.

Introduction

Product Overview

Description of the System

pEF1/His A, B, and C are 6.2 kb vectors designed for overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow for the purification and detection of expressed proteins (see pages 14–15 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- The human elongation factor 1α subunit (hEF-1α) promoter for high-level expression across a broad range of species and cell types (Kim *et al.*, 1990; Mizushima and Nagata, 1990; Uetsuki *et al.*, 1989) (see page 13 for more information).
- Three reading frames to facilitate in-frame cloning with an N-terminal peptide encoding the Xpress™ epitope and a polyhistidine (6xHis) metal-binding tag.
- Neomycin resistance gene for selection of stable cell lines (Southern and Berg, 1982) .
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g., COS7).

The control plasmid, pEF1/His/*lac*Z, is included for use as a positive control for transfection, expression, and detection in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pEF1/His.

1. Consult the multiple cloning sites depicted on pages 3–5 to determine which vector (A, B, or C) should be used to clone your gene in-frame with the N-terminal Xpress™ 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.
3. Analyze transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that the gene is cloned in-frame with the N-terminal peptide.
5. Transfect the construct into the cell line of choice using your own method of transfection. Generate a stable cell line, if desired.
6. Test for expression of your recombinant protein by western blot analysis or functional assay. An antibody to the Xpress™ epitope is available from Invitrogen, see page 17.
7. To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately from Invitrogen (see page 18 for ordering information).

Methods

Cloning into pEF1/His A, B, and C

Before Starting

Diagrams are provided on pages 3–5 to help you ligate your gene of interest in-frame with the N-terminal peptide. General considerations for cloning and transformation are listed below.

General Molecular Biology Techniques

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

Transformation Method

You may use any method of your choice for transformation. Chemical transformation is the most convenient for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintaining pEF1/His

To propagate and maintain the pEF1/His vectors, use the supplied 0.5 µg/µL stock solution in TE, pH 8.0 to transform a recombination deficient (*rec*A) and endonuclease A deficient (*end*A) *E. coli* strain such as TOP10F´, DH5α, or INVαF´ (see page 17 for ordering information). Select transformants on LB plates containing 50 to 100 µg/mL ampicillin. Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 6).

Note

The pEF1/His vectors are fusion vectors. To ensure proper expression of your recombinant fusion protein, clone your gene in frame with the ATG at base pairs 1716–1718. This will create a fusion with the N-terminal polyhistidine tag, Xpress™ epitope, and the enterokinase cleavage site. The vector is supplied in three reading frames to facilitate cloning. See below and pages 3–5 to develop a cloning strategy.

If you wish to clone as close as possible to the enterokinase cleavage site, follow the guidelines below:

1. Digest pEF1/His A, B, or C with *Kpn* I.
2. Create blunt ends with T4 DNA polymerase and dNTPs.
3. Clone the blunt-ended insert in-frame with the lysine codon (AAG) of the enterokinase recognition site.

Following enterokinase cleavage, no vector-encoded amino acid residues will be present in your protein.

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Cloning into pEF1/His A, B, and C, Continued

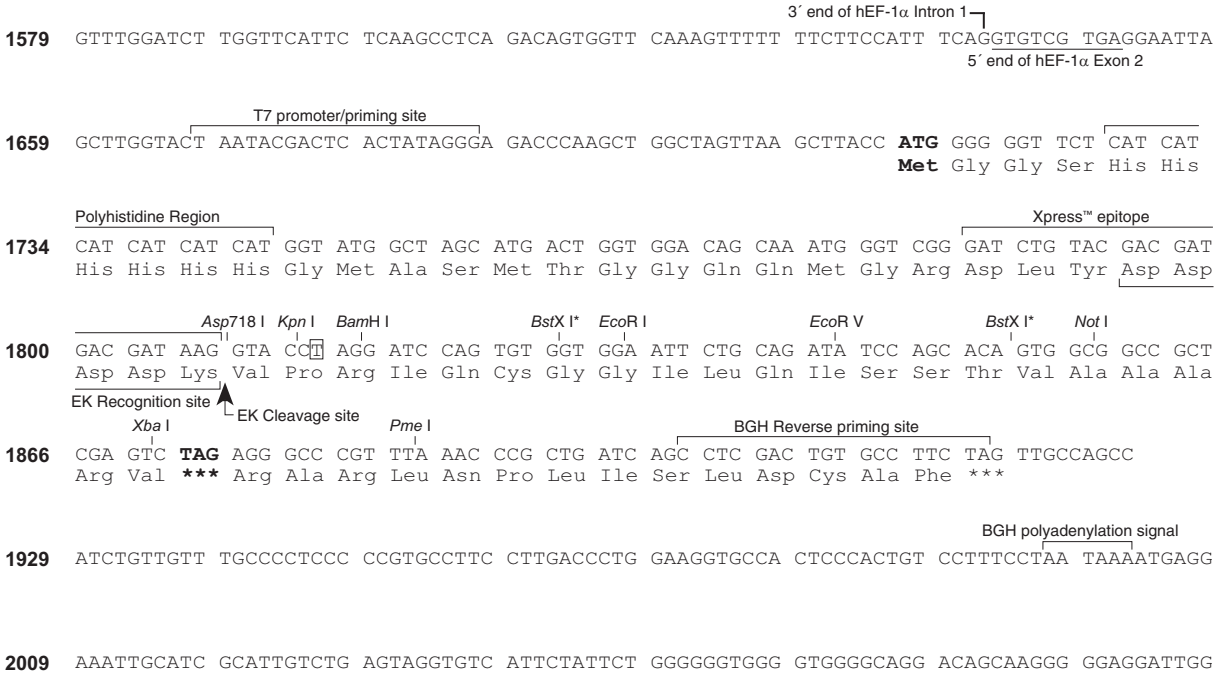
Kozak Sequence for Mammalian Expression

If you will be recombining your entry clone with a destination vector for mammalian expression, 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)NNATG**G**

Multiple Cloning Site of pEF1/His A

Below is the multiple cloning site for pEF1/His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotide indicates the variable region. **Note that there is a stop codon after the *Xba* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The sequence of pEF1/His A is available at www.invitrogen.com or by request from Technical Support (see page 19).**



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

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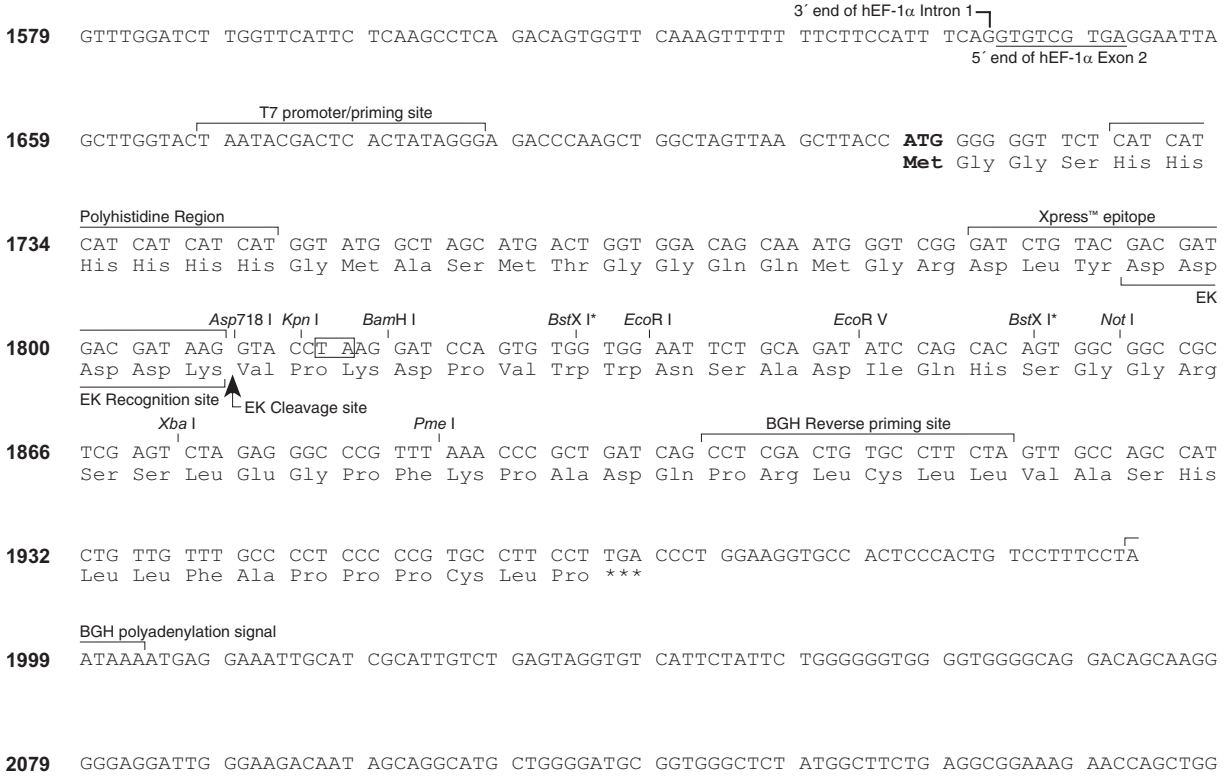
Cloning into pEF1/His A, B, and C, Continued

Multiple Cloning Site of pEF1/His B

Below is the multiple cloning site for pEF1/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. **The sequence for pEF1/His B is available at www.invitrogen.com or by request from Technical Support (see page 19).**




```
1579  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA

1659  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGTTAA GCTTACC ATG GGG GGT TCT CAT CAT
                                                                    Met Gly Gly Ser His His

1734  CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT
      His His His His Gly Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp

1800  GAC GAT AAG GTA CCT AAG GAT CCA GTG TGG TGG AAT TCT GCA GAT ATC CAG CAC AGT GGC GGC CGC
      Asp Asp Lys Val Pro Lys Asp Pro Val Trp Trp Asn Ser Ala Asp Ile Gln His Ser Gly Gly Arg

1866  TCG AGT CTA GAG GGC CCG TTT AAA CCC GCT GAT CAG CCT CGA CTG TGC CTT CTA GTT GCC AGC CAT
      Ser Ser Leu Glu Gly Pro Phe Lys Pro Ala Asp Gln Pro Arg Leu Cys Leu Leu Val Ala Ser His

1932  CTG TTG TTT GCC CCT CCC CCG TGC CTT CCT TGA CCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA
      Leu Leu Phe Ala Pro Pro Pro Cys Leu Pro ***

1999  ATAAAATGAG GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG

2079  GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGCTTCTG AGGCGGAAAG AACCAGCTGG
```

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

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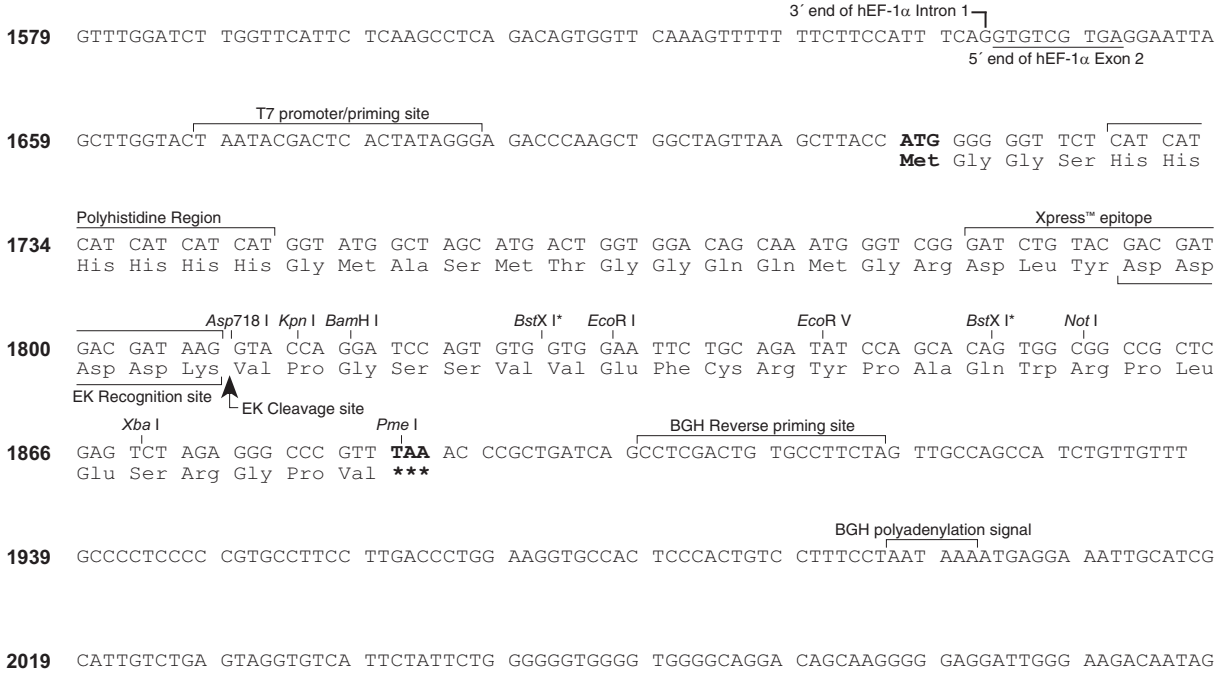
Cloning into pEF1/His A, B, and C, Continued

Multiple Cloning Site of pEF1/His C

Below is the multiple cloning site for pEF1/His C. Restriction sites are labeled to indicate the cleavage site. **Note that there is a stop codon within the *Pme* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. **The sequence for pEF1/His C is available at www.invitrogen.com or by request from Technical Support (see page 19).**




```
1579  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA

1659  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGTTAA GCTTACC ATG GGG GGT TCT CAT CAT
                                                                     Met Gly Gly Ser His His

1734  CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT
      His His His His Gly Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp

1800  GAC GAT AAG GTA CCA GGA TCC AGT GTG GTG GAA TTC TGC AGA TAT CCA GCA CAG TGG CGG CCG CTC
      Asp Asp Lys Val Pro Gly Ser Ser Val Val Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu

1866  GAG TCT AGA GGG CCC GTT TAA AC CCGCTGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT
      Glu Ser Arg Gly Pro Val ***

1939  GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG

2019  CATTGTCTGA GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG GAGGATTGGG AAGACAATAG
```

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

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E. coli Transformation

Transform the ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g., TOP10F´, INVαF´) and select on LB plates containing 50 to 100 µg/mL ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

Sequence your construct with the T7 Forward and BGH Reverse primers (see page 17 for ordering information) to confirm that your gene is fused in-frame with the Xpress™ epitope and the N-terminal polyhistidine tag. Refer to the diagrams on pages 3–5 for the sequences and locations of the priming sites. For your convenience, Invitrogen offers a custom primer service. For more information, visit www.invitrogen.com or call Technical Support (see page 19).

Primer	Sequence
T7 Forward	5′-TAATACGACTCACTATAGGG-3′
BGH Reverse	5′-TAGAAGGCACAGTCGAGG-3′

Preparing a Glycerol Stock

After identifying 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.

1. Streak the original colony 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.

Applying Selective Pressure

Take some (if not all) of the following precautions to prevent your clone from being "overrun" by background contaminants:

- **Use carbenicillin instead of ampicillin.** Carbenicillin is more stable than ampicillin, and allows for a longer period of selective pressure.
- **Increase the antibiotic concentration.** More antibiotic means that your clones will not be overwhelmed by β-lactamase buildup.
- **Periodically refresh plate media.** If you suspect that tubes/plates may be beginning to fail, spin them down, remove the old media, and replenish the wells with fresh LB media plus glycerol and antibiotic.

Streak clones on selective (preferably carbenicillin) LB agar plates. After about 12 hours, isolate colonies for downstream usage. This will isolate your desired clones from potential background contaminants.

Transfection and Analysis

Introduction

Once you have confirmed that your construct is in the correct orientation and fused in-frame with the N-terminal peptide, 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 lipid complexing, decreasing transfection efficiency. We recommend isolating DNA using the PureLink™ HiPure Miniprep Kit, the PureLink™ HiPure Midiprep Kit (see page 17 for ordering information), 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. Precisely follow 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 21).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). For high efficiency transfection in a broad range of mammalian cells, use Lipofectamine™ 2000 Reagent available from Invitrogen (see page 17). For more information on Lipofectamine™ 2000 and other transfection reagents, visit www.invitrogen.com or contact Technical Support (see page 19).

Positive Control

pEF1/His/*lac*Z is provided as a positive control vector for mammalian cell transfection and expression (see page 16), and 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 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 (see page 17 for ordering information) for fast and easy detection of β-galactosidase expression.

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Detecting Fusion Proteins

The Anti-Xpress™ Antibody may be used to detect expression of your fusion protein from pEF1/His (see page 17 for ordering information).

To detect the fusion protein by western blot, 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 below) or other suitable lysis buffer.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Centrifuge at 10,000 × *g* for 10 minutes to pellet nuclei and transfer the post-nuclear lysate to a new 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.

Cell Lysis Buffer

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

1. Prepare the solution from the following common stock solutions.

   For 100 mL, combine:

Stock Solution	Volume
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 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

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Note

The N-terminal peptide containing the Xpress™ epitope and the polyhistidine tag will add approximately 3.4 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, see the protocol on page 12.

Creating Stable Cell Lines

Introduction

The pEF1/His vectors contain the neomycin resistance gene to allow for selection of stable cell lines using Geneticin® (G418). Test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines.

General information and guidelines are provided below for your convenience.

Geneticin® Activity

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH) derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982) .

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (see page 17 for ordering information). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g., 100 mM HEPES, pH 7.3).
- Use 100 to 1,000 µg/mL of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label or the certificate of analysis).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take from 3 to 6 weeks of growth in selective medium.

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Creating Stable Cell Lines, Continued

Possible Linearization Sites

To obtain stable transfectants, you may choose to linearize your vector before transfection. While linearizing your vector may not improve the efficiency of transfection, it increases the likelihood that the vector will not integrate in a way that disrupts the gene of interest. The table below lists unique sites that may be used to linearize your construct prior to transfection. Other restriction sites are possible. Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Location	Supplier
Ssp I	Upstream of hEF-1α promoter	Invitrogen*
Aat II	Upstream of hEF-1α promoter	Many
Nru I	Upstream of hEF-1α promoter	Invitrogen*
Mlu I	Upstream of hEF-1α promoter	Invitrogen*
*Bst*1107 I	End of SV40 pA	Roche
*Eam*1105 I	Ampicillin gene	Roche
Pvu I	Ampicillin gene	Invitrogen*
Sca I	Ampicillin gene	Invitrogen*

*see page 17 for ordering information

Selecting Stable Integrants

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

1. Transfect your cells using the appropriate protocol for your cell line. Include a sample of untransfected cells as a negative control.
2. After transfection, wash the cells once with 1X PBS and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent.
4. Replenish selective medium every 3–4 days until Geneticin®-resistant colonies are detected.
5. Pick and expand colonies.

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Preparing Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purifying your protein on ProBond™. You will need 5×10^6 to 1×10^7 cells for purification of your protein on a 2 mL ProBond™ column (see the ProBond™ Protein Purification manual).

1. Seed cells 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 250 × *g* for 5 minutes. Resuspend the cell pellet in PBS.
6. Centrifuge the cells at 250 × *g* for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at −70°C until needed.

Lysing Cells

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

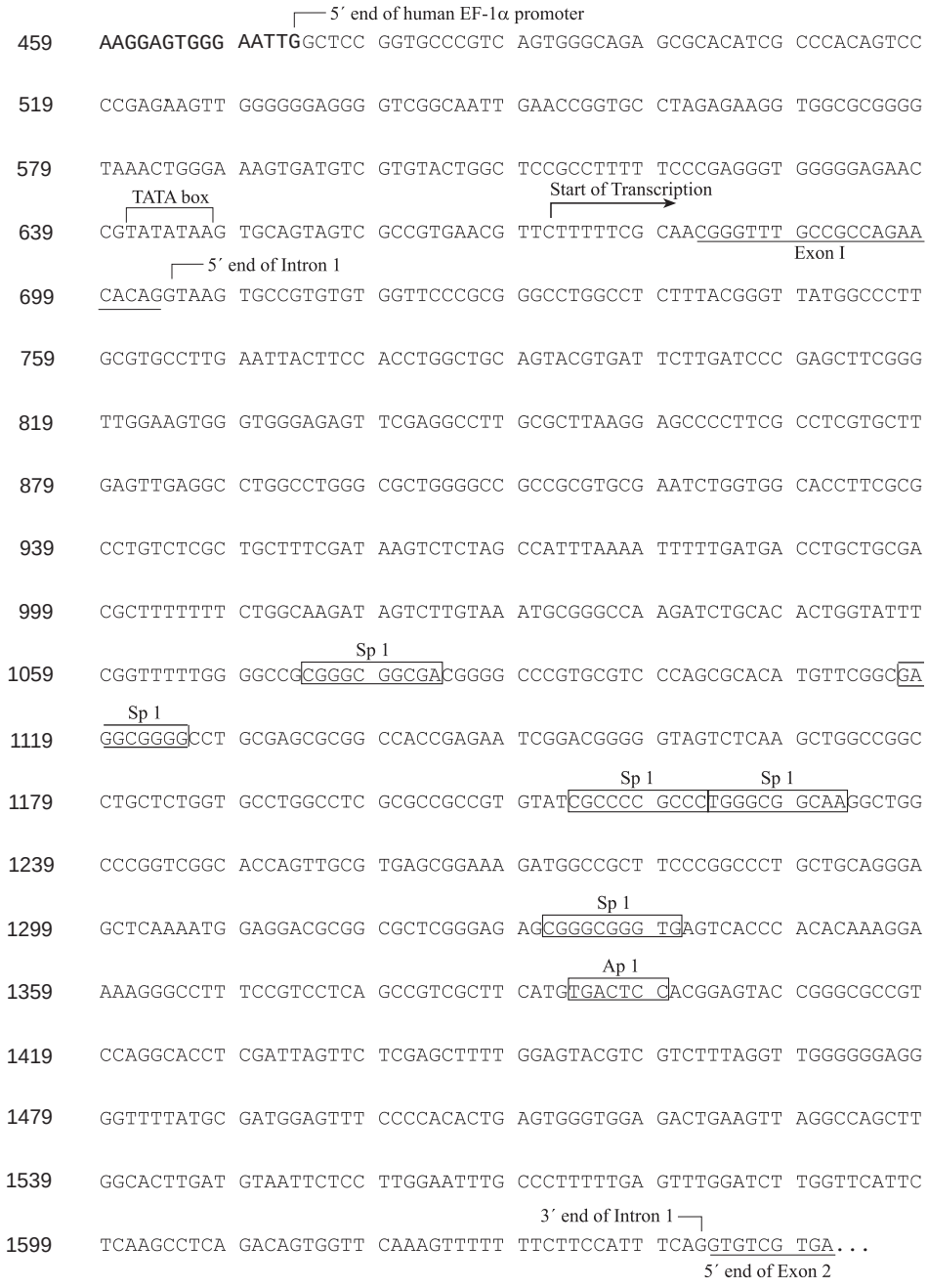
If you are using another metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

Human EF-1α Promoter

Description

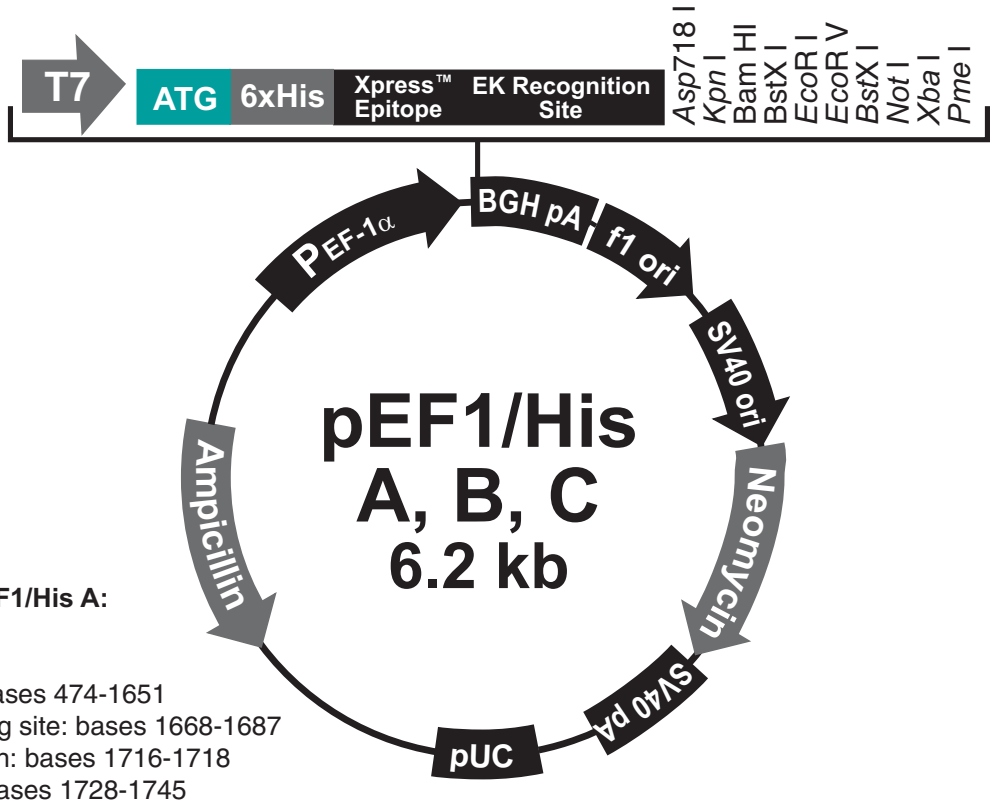
The diagram below shows the features of the hEF-1α promoter used in the pEF1/His vectors (Mizushima and Nagata, 1990). Features are marked as per Uetsuki *et al.*, 1989.



pEF1/His A, B, and C Vectors

Map of pEF1/His

The figure below summarizes the features of the pEF1/His vectors. **The sequences for pEF1/His A, B, and C are available at www.invitrogen.com or by request from Technical Support (see page 19).**



Comments for pEF1/His A: 6188 bp

EF-1α promoter: bases 474-1651
T7 promoter/priming site: bases 1668-1687
ATG initiation codon: bases 1716-1718
Polyhistidine tag: bases 1728-1745
Xpress™ epitope: bases 1785-1808
Enterokinase recognition site: bases 1794-1808
Multiple cloning site: bases 1808-1889
BGH reverse priming site: bases 1901-1918
BGH polyadenylation sequence: bases 1904-2131
f1 origin: bases 2177-2605
SV40 promoter and origin: bases 2633-2940
Neomycin resistance gene: bases 30158-3809
SV40 polyadenylation sequence: bases 3983-4113
pUC origin: bases 4496-5169
Ampicillin resistance gene: bases 5314-6174

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pEF1/His A, B, and C Vectors, Continued

Features of pEF1/His

pEF1/His A (6,188 bp), pEF1/His B (6,189 bp), and pEF1/His C (6,187 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Overexpression of 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.
N-terminal polyhistidine tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™.
Xpress™ epitope tag	Allows for the detection of an 8 amino acid epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys) on the recombinant protein with the Anti-Xpress™ Antibody.
Enterokinase cleavage site	Allows for the removal of the N-terminal polyhistidine tag from the recombinant protein using an enterokinase such as EKMax™ Enterokinase (see page 17).
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in-frame with the N-terminal polyhistidine tag.
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 for the rescue of single-stranded DNA.
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen.
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982).
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
pUC origin	High-copy number replication and growth in *E. coli.*
Ampicillin resistance gene (β-lactamase)	Selection of transformants in *E. coli.*

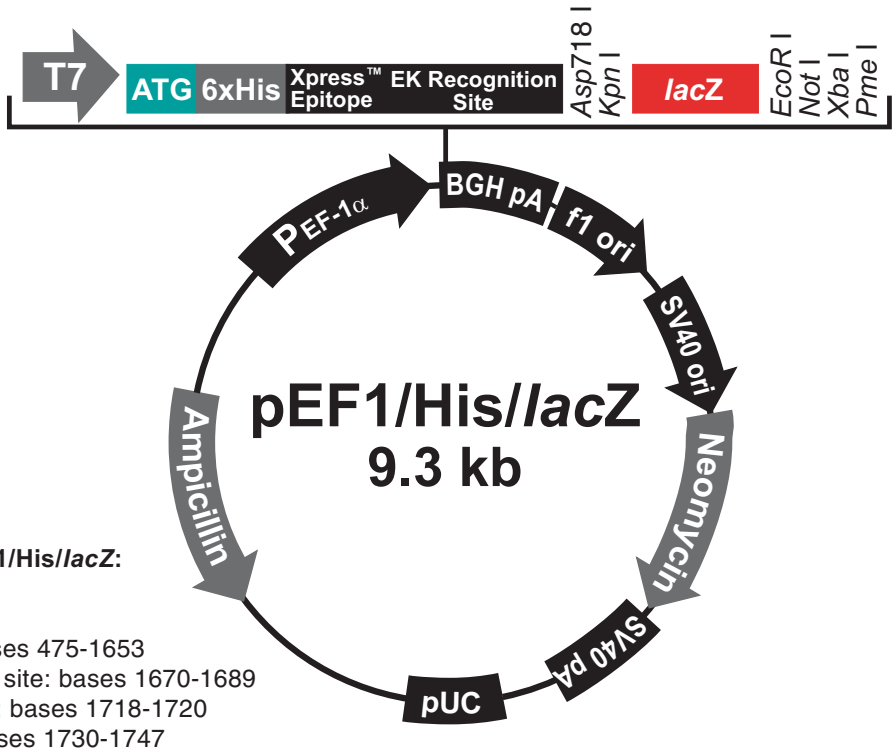
pEF1/His/*lacZ*

Description

pEF1/His/*lacZ* is a 9,254 bp control vector containing the gene for β-galactosidase. pEF1/His/*lacZ* was constructed by ligating a 2,207 bp *Not* I-*Bsm* I fragment containing the neomycin resistance gene from pcDNA™3.1/His A to a 7,047 bp *Not* I-*Bsm* I fragment containing the EF-1α promoter, *lacZ* gene, Xpress™ epitope, and polyhistidine tag from pEF4/His/*lacZ*.

Map of Control Vector

The figure below summarizes the features of the pEF1/His/*lacZ* vector. **The sequence for pEF1/His/*lacZ* is available at www.invitrogen.com or by request from Technical Support (see page 19).**



Comments for pEF1/His/*lacZ*: 9254 bp

EF-1α promoter: bases 475-1653
T7 promoter/priming site: bases 1670-1689
ATG initiation codon: bases 1718-1720
Polyhistidine tag: bases 1730-1747
Xpress™ epitope: bases 1787-1810
Enterokinase recognition site: bases 1796-1810
LacZ ORF: bases 1835-4885
BGH reverse priming site: bases 4966-4983
BGH polyadenylation sequence: bases 4969-5196
f1 origin: bases 5242-5670
SV40 promoter and origin: bases 5698-6006
Neomycin resistance gene: bases 6081-6875
SV40 polyadenylation sequence: bases 7049-7179
pUC origin: bases 7562-8235
Ampicillin resistance gene: bases 8380-9240

Accessory Products

Additional Products

The following additional products may be used with the pEF1/His vectors. For more information, visit www.invitrogen.com or contact Technical Support (see page 19).

Item	Quantity	Cat. no.
Electrocomp™ Kit (TOP10F´)	2 × 20 reactions	C665-11
	6 × 20 reactions	C665-24
One Shot® TOP10F´ Chemically Competent *E. coli*	20 × 50 µL	C3030-03
One Shot® INVαF' Chemically Competent *E. coli*	20 × 50 µL	C2020-03
	40 × 50 µL	C2020-06
Ampicillin	200 mg	11593-027
Carbenicillin	5 g	10177-012
T7 promoter primer	2 µg	N560-02
BGH Reverse primer	2 µg	N575-02
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
Lipofectamine™ 2000 Reagent	1.5 mL	11668-019
β–Gal Assay Kit	1 kit	K1455-01
β–Gal Staining Kit	1 kit	K1465-01
Anti-Xpress™ Antibody	50 µL	R910-25
Geneticin®	1 g	11811023
	5 g	11811031
	25 g	11811098
EKMax™ Enterokinase	250 units	E180-01

Restriction Enzymes

For your convenience, Invitrogen offers an extensive selection of restriction enzymes, including the following:

- *Ssp* I
- *Nru I*
- *Mlu* I
- *Pvu I*
- *Sca* I

Visit www.invitrogen.com for more details.

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Accessory Products, Continued

ProBond™ Resin

Ordering information for ProBond™ resin is provided below.

Item	Quantity	Cat. no.
ProBond™ Purification System	12 mL precharged ProBond™ resin, 6 columns, and buffers for native and denaturing purification	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15

Other Mammalian Expression Vectors

Invitrogen offers a wide variety of mammalian expression vectors utilizing the CMV or EF-1α promoters. Vectors are available with the Xpress™ (N-terminal), *c-myc* (C-terminal), V5 (C-terminal), or polyhistidine epitopes for detection and either the neomycin, blasticidin, or Zeocin™ resistance genes. All vectors utilize the polyhistidine tag for purification. For more information on the mammalian expression vectors available, visit www.invitrogen.com or contact Technical Support (see page 19).

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).

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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.

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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. Mol. Cell. Biol. *7*, 2745-2752.

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

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

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

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.

Kim, D. W., Uetsuki, T., Kaziro, Y., Yamaguchi, N., and Sugano, S. (1990). Use of the Human Elongation Factor 1 Promoter as a Versatile and Efficient Expression System. Gene 91, 217-223.

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.

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. Nuc. 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.

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References, Continued

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

Uetsuki, T., Naito, A., Nagata, S., and Kaziro, Y. (1989). Isolation and Characterization of the Human Chromosomal Gene for Polypeptide Chain Elongation Factor-1 . 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.

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