invitrogen™
by life technologies™

pEF-DEST51 Gateway™ Vector

A destination vector for cloning and expression of C-terminal fusion proteins in mammalian cells

Catalog no. 12285-011

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Manual part no. 25-0428

MAN0000240

User Manual

Table of Contents

Important Information

Shipping and Storage

pEF-DEST51 and pEF/GW-51/*lac*Z are shipped at room temperature. Upon receipt, store at -20°C. Products are guaranteed for six months from date of shipment when stored properly.

Contents

The pEF-DEST51 Gateway™ Vector components are listed below.

Item	Concentration	Volume
pEF-DEST51 Vector	lyophilized in TE, pH 8.0	6 µg
pEF/GW-51/*lac*Z Control Plasmid	lyophilized in TE, pH 8.0	10 µg

Accessory Products

Additional Products

Additional products that may be used with the pEF-DEST51 Gateway™ Vector are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
Gateway™ LR Clonase™ Enzyme Mix	20 reactions	11791-019
One Shot® TOP10 Chemically Competent Cells	10 reactions	C4040-10
	20 reactions	C4040-03
One Shot® TOP10 Electrocompetent Cells	10 reactions	C4040-50
	20 reactions	C4040-52
Lipofectamine™ 2000 Reagent	1.5 ml	11668-019
	0.75 ml	11668-027
Blasticidin	50 mg	R210-01
β-Gal Antiserum*	50 µl	R901-25
β-Gal Assay Kit	100 reactions	K1455-01
β-Gal Staining Kit	1 kit	K1465-01

*Amount supplied is sufficient for 25 Westerns using 10 ml working solution per reaction.

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. 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 Fusion Protein

If your gene of interest is in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use Immobilized Metal Affinity Chromatography (IMAC) to purify your recombinant fusion protein. The ProBond™ Purification System or bulk ProBond™ resin are available separately from Invitrogen. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Nickel-chelating Resin	50 ml	R801-01
	150 ml	R801-15
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
Purification Columns (10 ml polypropylene columns)	50	R640-50

Methods

Overview

Description

pEF-DEST51 is a 7.5 kb vector derived from pEF6/V5-His and adapted for use with the Gateway™ Technology. It is designed to allow high-level, constitutive expression in a variety of mammalian hosts.

Features

pEF-DEST51 contains the following elements:

- Human elongation factor 1α-subunit promoter (hEF-1α) for high-level expression across a broad range of species and cell types (see page 14)
- Two recombination sites, *att*R1 and *att*R2, downstream of the EF-1α promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene located between the two *att*R sites for counterselection
- The *ccd*B gene located between the two *att*R sites for negative selection
- The V5 epitope and 6xHis tag for detection and purification (optional)
- Bovine growth hormone (BGH) polyadenylation sequence for proper termination and processing of the recombinant transcript
- f1 intergenic region for production of single-strand DNA in F plasmid-containing *E. coli*
- SV40 early promoter and origin for expression of the blasticidin resistance gene and stable propagation of the plasmid in hosts expressing the SV40 large T antigen
- EM7 promoter for expression of the blasticidin resistance gene in *E. coli*
- Blasticidin resistance gene for selection of stable cell lines
- The pUC origin for high copy replication and maintenance of the plasmid in *E. coli*
- The ampicillin (*bla*) resistance gene for selection in *E. coli*

For a map of pEF-DEST51, see page 15.

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Overview, continued

The Gateway™ Technology

Gateway™ is a universal cloning technology that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest using Gateway™ cloning technology, simply:

1. Clone your gene of interest into a Gateway™ entry vector to create an entry clone.
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and a Gateway™ destination vector (*e.g.* pEF-DEST51).
3. Transfect your expression clone into the cell line of choice for transient or constitutive expression of your gene of interest.

For more information on the Gateway™ System, refer to the Gateway™ Technology Manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 18).

Using pEF-DEST51

Important

The pEF-DEST51 vector is supplied as a supercoiled plasmid. Although Invitrogen has previously recommended using a linearized destination vector for more efficient recombination, further testing has found that linearization of this vector is **NOT** required to obtain optimal results for any downstream application.

Propagating pEF-DEST51

If you wish to propagate and maintain pEF-DEST51, we recommend using Library Efficiency® DB3.1™ Competent Cells (Catalog no. 11782-018) from Invitrogen for transformation. The DB3.1™ *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene.

Note: DO NOT use general *E. coli* cloning strains including TOP10 or DH5α for propagation and maintenance as these strains are sensitive to CcdB effects.

Resuspending pEF-DEST51

Before you perform the LR Clonase™ reaction, resuspend pEF-DEST51 to 50-150 ng/µl in sterile water.

Entry Clone

To recombine your gene of interest into pEF-DEST51, you should have an entry clone containing your gene of interest. For your convenience, Invitrogen offers the pENTR Directional TOPO® Cloning Kit (Catalog no. K2400-20) for 5-minute cloning of your gene of interest into an entry vector. For more information on entry vectors available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 18).

For detailed information on constructing an entry clone, refer to the specific entry vector manual. For detailed information on performing the LR recombination reaction, refer to the Gateway™ Technology Manual.

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Using pEF-DEST51, continued

Points to Consider Before Recombining

Your insert should contain a Kozak consensus 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. 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**

If you wish to include the V5 epitope and 6xHis tag, your gene in the entry clone **should not** contain a stop codon. In addition, the gene should be designed to be in frame with the C-terminal epitope tag after recombination. Refer to the **Recombination Region** on the next page.

If you do NOT wish to include the V5 epitope and 6xHis tag, your gene should contain a stop codon in the entry clone.

Recombining Your Gene of Interest

Each entry clone contains *att*L sites flanking the gene of interest. Genes in an entry clone are transferred to the destination vector backbone by mixing the DNAs with the Gateway™ LR Clonase™ enzyme mix (see page v for ordering information). The resulting recombination reaction is then transformed into *E. coli* and the expression clone selected. Recombination between the *att*R sites on the destination vector and the *att*L sites on the entry clone replaces the *ccd*B gene and the chloramphenicol (Cm^R) gene with the gene of interest and results in the formation of *att*B sites in the expression clone.

Follow the instructions in the Gateway™ Technology Manual to set up the LR Clonase™ reaction, transform *E. coli*, and select for the expression clone.

Note

The presence of the EM7 promoter and the blasticidin resistance gene allows for selection of *E. coli* transformants using blasticidin. For selection, use Low Salt LB agar plates containing 100 µg/ml blasticidin (see page 11 for a recipe). For blasticidin to be active, the salt concentration of the medium must remain low (< 90 mM) and the pH must be 7.0.

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Using pEF-DEST51, continued

Confirming the Expression Clone

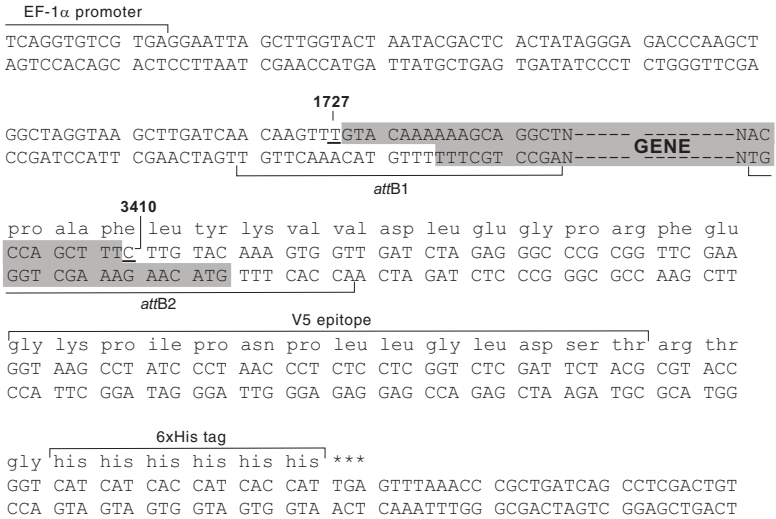
The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccd*B gene will be both ampicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 µg/ml chloramphenicol. A true expression clone will not grow in the presence of chloramphenicol.

Recombination Region

The recombination region of the expression clone resulting from pEF-DEST51 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pEF-DEST51 by recombination. Non-shaded regions are derived from the pEF-DEST51 vector.
- The nucleotides on either side of the shaded region correspond to bases 1727 and 3410, respectively, of the pEF-DEST51 vector sequence.



Transfection

Introduction

This section provides general information for transfecting your expression clone into the mammalian cell line of choice. We recommend that you include the positive control vector pEF/GW-51/*lac*Z and a mock transfection (negative control) in your experiments to evaluate your results.

Plasmid Preparation

Once you have generated your expression clone, you must isolate plasmid DNA for transfection. 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 plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (*e.g.* HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend 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* (Ausubel *et al.*, 1994).

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). If you wish to use a cationic lipid-based reagent for transfection, we recommend using Lipofectamine™ 2000 Reagent available from Invitrogen (see page v for ordering information). For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 18).

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Transfection, continued

Positive Control

pEF/GW-51/*lac*Z is provided as a positive control vector for mammalian cell transfection and expression (see page 17 for a map) and may be used to optimize recombinant protein expression levels in your cell line. The vector allows expression of a C-terminally tagged β-galactosidase fusion protein that may be detected by Western blot or functional assay.

To propagate and maintain the plasmid:

1. Resuspend the vector in 10 µl sterile water to prepare a 1 µg/µl stock solution. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α, JM109, or equivalent.
2. Select transformants on LB agar plates containing 50-100 µg/ml ampicillin.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

Expression and Analysis

Introduction

Expression of your gene of interest from the expression clone can be performed in either transiently transfected cells or stable cell lines (see page 10 for guidelines to create stable cell lines). A sample protocol is provided below. Other protocols are suitable.

Preparation of Cell Lysates

To lyse cells:

1. Wash cell monolayers (~5 x 10^5 to 1 x 10^6 cells) once with phosphate-buffered saline (PBS, available from Gibco™, Catalog no. 10010-023).
2. Scrape cells into 1 ml PBS and pellet the cells at 1500 x g for 5 minutes.
3. Resuspend in 50 µl Cell Lysis Buffer (see page 12 for a recipe). Other cell lysis buffers are suitable. Vortex.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
5. Centrifuge the cell lysate at 10,000 x g for 10 minutes at +4°C to pellet nuclei and transfer the supernatant 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 (see page 12 for a recipe) 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.

Polyacrylamide Gel Electrophoresis

To facilitate separation and visualization of your recombinant fusion protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Invitrogen. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 18).

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Expression and Analysis, continued

Detecting Recombinant Fusion Proteins

To detect expression of your recombinant fusion protein by Western blot analysis, you may use the Anti-V5 antibodies or the Anti-His(C-term) antibodies available from Invitrogen (see page v for ordering information) or an antibody to your protein of interest. In addition, the Positope™ Control Protein (Catalog no. R900-50) is available from Invitrogen for use as a positive control for detection of fusion proteins containing a V5 epitope or a polyhistidine (6xHis) tag. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 18).

Assay for β-galactosidase

If you use the pEF/GW-51/*lac*Z plasmid as a positive control vector, you may assay for β-galactosidase expression by Western blot analysis or activity assay using cell lysates (Miller, 1972). Invitrogen offers β-Gal Antiserum, the β-Gal Assay Kit, and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page v for ordering information).

Note

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

Purification of Recombinant Fusion Proteins

The presence of the C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows use of a metal-chelating resin such as ProBond™ to purify your fusion protein. The ProBond™ Purification System and bulk ProBond™ resin are available from Invitrogen (see page vi for ordering information). Refer to the ProBond™ Purification System manual for protocols to purify your fusion protein. Invitrogen also offers Ni-NTA Agarose (Catalog no. R901-01) for purification of proteins containing a polyhistidine (6xHis) tag**. Note:** Other metal-chelating resins and purification methods are suitable.

Creating Stable Cell Lines

Introduction

The pEF-DEST51 vector contains the blasticidin resistance gene to allow selection of stable cell lines. To create stable cell lines, transfect your construct into the mammalian cell line of choice and select for foci using blasticidin. General guidelines are provided below.

To obtain stable transfectants, we recommend that you linearize your pEF-DEST51 construct before transfection. While linearizing the vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts elements necessary for expression in mammalian cells. To linearize your construct, cut at a unique site that is neither located within a critical element nor within your gene of interest.

Blasticidin

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).

Blasticidin Selection Guidelines

Blasticidin is available separately from Invitrogen (see page v for ordering information). Use as follows:

1. Prepare blasticidin in sterile water and filter-sterilize the solution.
2. Use 2.5 to 10 µg/ml of blasticidin in complete medium.
3. Test varying concentrations of blasticidin on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to blasticidin. Complete selection can take up to 10 days of growth in selective medium.

Refer to the **Appendix**, page 13 for instructions on how to prepare and store blasticidin.

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

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

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic (100 μg/ml ampicillin) if needed.
4. Store at room temperature or at +4°C.

LB agar plates

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

Low Salt LB Medium with Blasticidin

Low Salt LB Medium:
10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 ml. Adjust pH to 7.0 with 1 N NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 psi and 121°C for 20 minutes.
3. Allow the medium to cool to at least 55°C before adding the blasticidin to 100 μg/ml final concentration.
4. Store plates at +4°C in the dark. Plates containing blasticidin are stable for up to 2 weeks.

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Recipes, continued

Cell Lysis Buffer

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

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 up 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.

To prevent proteolysis, you may add 1 mM PMSF, 1 μM leupeptin, or 0.1 μM aprotinin before use.

4X SDS-PAGE Sample Buffer

1. Combine the following reagents:

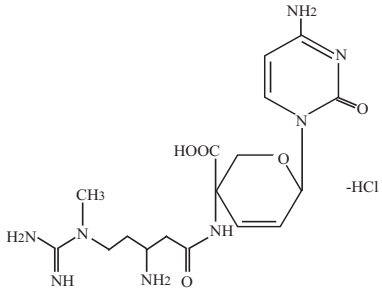
0.5 M Tris-HCl, pH 6.8	5 ml
Glycerol (100%)	4 ml
β-mercaptoethanol	0.8 ml
Bromophenol Blue	0.04 g
SDS	0.8 g

2. Bring the volume to 10 ml with sterile water.
3. Aliquot and freeze at -20°C until needed.

Blasticidin

Molecular Weight, Formula, and Structure

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



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

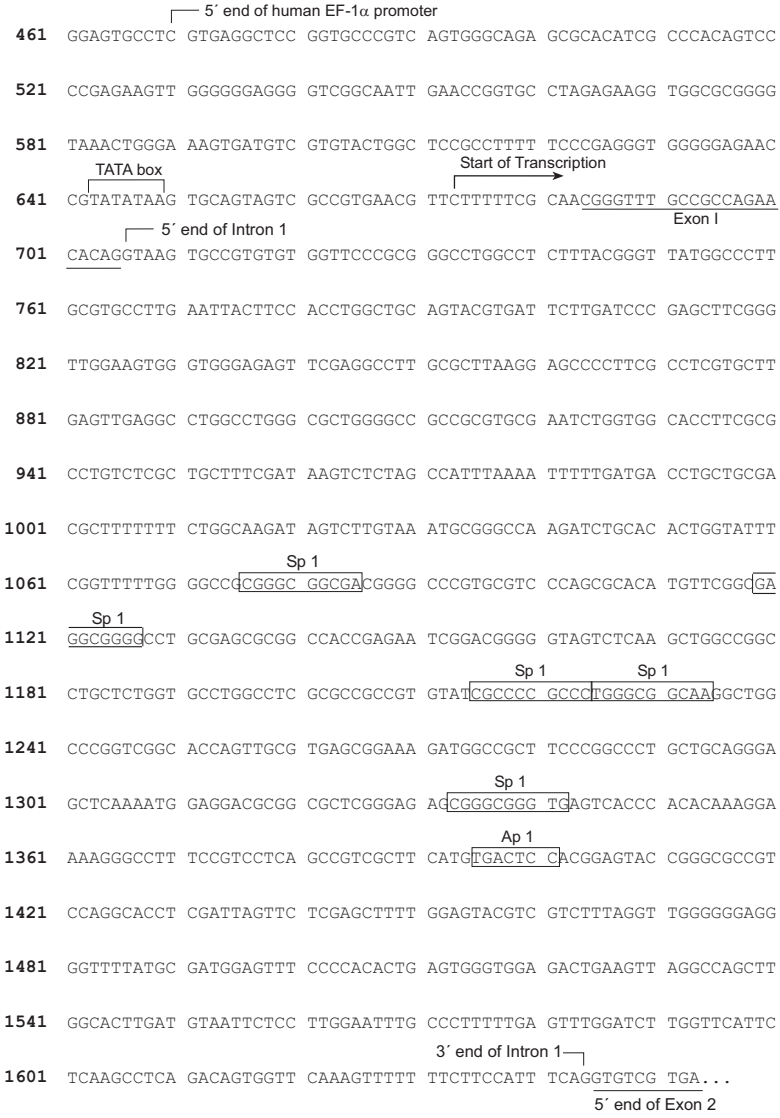
Blasticidin may be obtained separately from Invitrogen (Catalog no. R210-01) in 50 mg aliquots. Blasticidin is soluble in water. Sterile water is generally used to prepare stock solutions of 5 to 10 mg/ml.

- Dissolve blasticidin in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one time use 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 be 7.0 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 store the thawed stock solution at +4°C for up to 2 weeks.
- Medium containing blasticidin may be stored at +4°C for up to 2 weeks.

Human EF-1α Promoter

Description

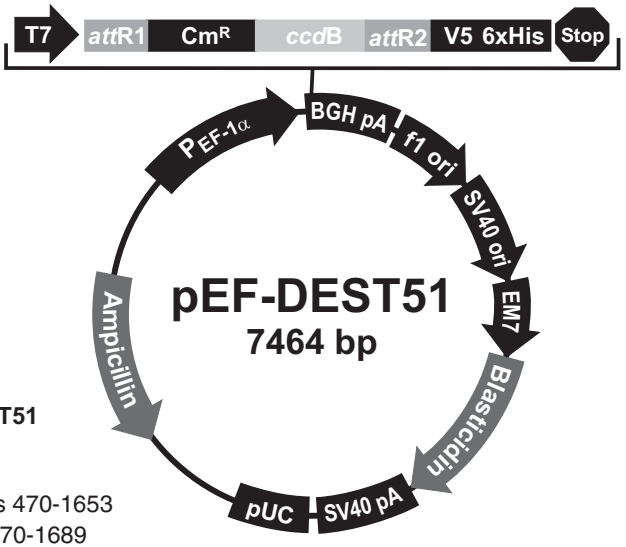
The diagram below shows all the features of the EF-1α promoter used in Invitrogen pEF vectors (Mizushima and Nagata, 1990; Uetsuki *et al.*, 1989).



Map and Features of pEF-DEST51

Map of pEF-DEST51

The map below shows the elements of pEF-DEST51. DNA from the entry clone replaces the region between bases 1727 and 3410. **The complete sequence of pEF-DEST51 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 18).**



Features of pEF-DEST51 7464 nucleotides

EF-1α promoter: bases 470-1653
T7 promoter: bases 1670-1689
*att*R1 recombination site: bases 1720-1844
Chloramphenicol resistance gene: bases 1953-2612
*ccd*B gene: bases 2954-3259
*att*R2 recombination site: bases 3300-3424
V5 epitope: bases 3450-3491
6xHis tag: bases 3501-3518
BGH polyadenylation region: 3544-3771
f1 origin: bases 3817-4245
SV40 early promoter and origin: bases 4250-4594
EM7 promoter: bases 4629-4684
Blasticidin resistance gene: bases 4703-5101
SV40 early polyadenylation region: bases 5259-5389
pUC origin: bases 5772-6445
Ampicillin resistance gene (*bla*): bases 6590-7450 (c)
bla promoter: bases 7451-85 (c)
(c) = complementary strand

Map and Features, continued

Features of pEF-DEST51

pEF-DEST51 (7464 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Allows expression of recombinant proteins in a broad range of mammalian cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990)
T7 promoter	Allows *in vitro* transcription in the sense orientation
*att*R1 and *att*R2 sites	Allows recombinational cloning of the gene of interest from an entry clone
Chloramphenicol resistance gene	Allows counterselection of expression clones
*ccd*B gene	Allows negative selection of expression clones
V5 epitope	Allows detection of recombinant fusion proteins by the Anti-V5 antibodies (Southern *et al.*, 1991)
C-terminal polyhistidine tag	Allows purification of recombinant proteins on metal-chelating resin such as ProBond™ Allows detection of the recombinant protein by the Anti-His (C-term) antibodies (Lindner *et al.*, 1997)
Bovine growth hormone (BGH) polyadenylation signal	Allows 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 high-level expression of the blasticidin resistance gene and episomal replication in cells expressing the SV40 large T antigen
EM7 promoter	Allows expression of the blasticidin resistance gene in *E. coli*
Blasticidin resistance gene	Allows selection of stable transfectants in mammalian cells (Kimura *et al.*, 1994)
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA
pUC origin	Allows high-copy number replication and growth in *E. coli*
Ampicillin resistance gene	Allows selection of transformants in *E. coli*

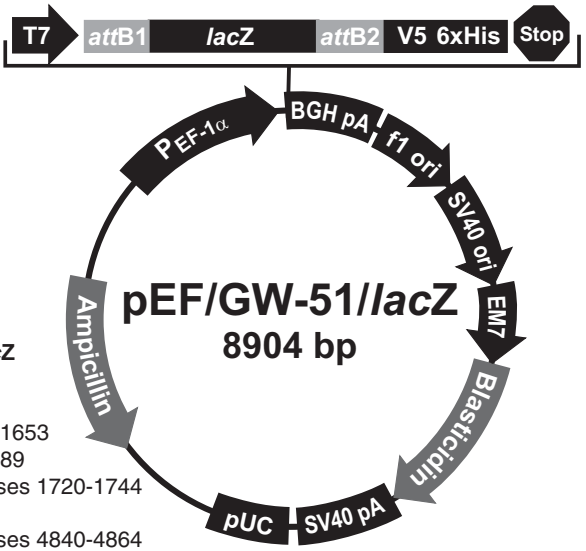
Map of pEF/GW-51/*lacZ*

Description

pEF/GW-51/*lacZ* is an 8904 bp control vector containing the gene for β-galactosidase. pEF/GW-51/*lacZ* was constructed using the Gateway™ LR recombination reaction between an entry clone containing the *lacZ* gene and pEF-DEST51. β-galactosidase is expressed as a fusion to the C-terminal tag. The fusion protein is approximately 121 kDa in size.

Map of pEF/GW-51/*lacZ*

The map below shows the elements of pEF/GW-51/*lacZ*. **The complete sequence of pEF/GW-51/*lacZ* is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 18).**



Features of pEF/GW-51/*lacZ*
8904 nucleotides

EF-1α promoter: bases 470-1653
T7 promoter: bases 1670-1689
*att*B1 recombination site: bases 1720-1744
lacZ ORF: bases 1764-4823
*att*B2 recombination site: bases 4840-4864
V5 epitope: bases 4890-4931
6xHis tag: bases 4941-4958
BGH polyadenylation region: bases 4984-5211
f1 origin: bases 5257-5685
SV40 early promoter and origin: bases 5690-6034
EM7 promoter: bases 6069-6124
Blasticidin resistance gene: bases 6143-6541
SV40 early polyadenylation region: bases 6699-6829
pUC origin: bases 7212-7885
Ampicillin resistance gene (*bla*): bases 8030-8890 (c)
bla promoter: bases 8891-85 (c)
(c) = complementary strand

Technical Service

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, 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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SDS

Safety Data Sheets (SDSs) are available on our website at www.invitrogen.com/sds.

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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Technical Service, continued

Limited Warranty

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